Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

Harper Adams University

The Impact of Post-Slaughter Natural Antioxidants Application on the Physical and Chemical Characteristics of Broiler Chicken Meat

By

Jamal Sadeek Khurshid

(BSc & MSc)

A thesis submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy by Harper Adams University.

September 2016

Director of studies: Prof. Frank Vriesekoop

Supervisor: Dr Annette Creedon

Ph.D. **2016**

Declaration

This Thesis has been written by me. As a part of my degree this research is original and has not been published before. All information has been written by means of references.

Jamal Sadeek Khurshid

Abbreviations

A•	Antioxidant Radical
a*	Red/green coordinate
AA	L-Ascorbic Acid,
ABTS	2,2' Azino-Bis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)
AH	Antioxidant
ANOVA	Analysis of Variance
AO	Antioxidant
a_w	Water Activity
b*	Yellow/blue coordinate
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxy Toluene
CD	Conjugated Dienes
CH_3	Methyl Group
CL	Cooking Loss
CO_2	Carbon Dioxide
COOH	Carboxylic Acid Group
CT	Conjugated Trienes
DHA	Docosahexaenoic Acid
DPPA	Diphenylphosphoryl Azide
DPPH	2, 2-Diphenyl-1-Picrylhydrazyl
DT	Dipping Time
DW	Dry Weight
DW	Deionised Water
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
Fe^{2+}	Ferrous Iron
Fe^{3+}	Ferric
FL	Fluorescent Light,
FRAP	Ferric Reducing/Antioxidant Power
GAE	Gallic Acid Equivalents
GC	Gas Chromatograph
GGR	Ginger
H	Hydrogen Atom
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulfuric Acid
HTHO	High-Temperature & High-Oxygen
KOH	Potassium Hydroxide
L*	Lightness
LOD	Limit of Detection
LOQ	Limit of Quantitation
LTLO	Low-Temperature & Low-Oxygen
MDA	Malondialdehyde
ML	Meat Layer
MUFA	Monounsaturated Fatty Acid
N_2	Nitrogen
Na_2HPO_4	Sodium Phosphate Dibasic
O_2	Oxygen

Abbreviation (continued)

OH•	Hydroxyl Radicals
ORAC	Oxygen Radical Absorbance Capacity
PE	Polyethylene
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
PVC	Polyvinyl Chloride Film
R•	Lipid Free Radicals
RH	Fatty Acid
RO•	Alkoxy Radical
ROO•	Peroxyl Free Radical
ROOH	Lipid Hydroperoxide
ROOR	Non-Radical
ROS	Rosemary
SED	Standard Error of Differences of Means
SFA	Saturated Fatty Acid
SFS	Sunflower Seeds
SRB	Small Red Bean
TBARS	2-Thiobarbituric Acid Reactive Substances
TBHQ	Tertiary Butyl Hydroquinone
TCA	Trichloroacetic Acid
TEAC	Trolox Equivalent Antioxidant Capacity
TEP	1,1,3,3–Tetra-Ethoxypropane
Toc	A-Tocopherol.
UFA	Unsaturated Fatty Acid
UV	Ultra Violet
WB	Weight Basis
WHC	Water Holding Capacity

Abstract

Experiments assessing the efficiency of natural antioxidants rosemary (ROS), small red bean (SRB), sunflower seeds (SFS) and ginger (GGR) extracts compared to the synthetic antioxidant (BHT) on characteristics of chicken meat were studied. The initial experiment evaluated the effect of the addition of antioxidants at 10 and 20 mg to lipids extracted from different tissues (breast, thigh, adipose and skin tissue) during the Schaal oven test 62.8 ^{o}C. Antioxidants at 10 and 20 mg significantly reduced TBARS, CD and CT values in all extracted lipids compared to the non-treated samples. However, no significant differences were found between both levels. Among the antioxidants, ROS significantly reduced TBARS values in fat from all the chicken portions over 7 days.

In the subsequent experiments, the impact of antioxidants on physical and chemical characteristics of raw and freshly cooked meat was investigated. In addition, the impact of the application of natural antioxidants on warmed-over characteristics in cooked chicken meat was evaluated. Raw meat samples treated with SFS had the lowest TBARS values, while in meat freshly cooked, ROS treatment had the lowest TBARS values. The addition of antioxidants reduced the degradation of phospholipids and formation of CDs and CTs in both raw and freshly cooked meats compared to the non-treated samples. Antioxidant treatments had a significantly lower drip loss and pH values, but did not affect cooking loss and shear force. Natural antioxidants significantly increased the colour stability and yielded more fatty acids in chicken meat. Natural antioxidants significantly reduced ($p \leq 0.05$) the formation of TBARS, CD and CT in both LTLO and HTHO samples, while their effect was much greater in LTLO samples compared to HTHO meat samples. Reheating process had a significant an effect on TBARS, CD and CT accumulation of most treatments either cooked by LTLO or HTHO methods ($p \leq 0.05$). In conclusion, the results suggest that natural antioxidants may have the ability to inhibit the lipid oxidation and enhance meat quality. The impact of natural antioxidants was similar to that of BHT.

Acknowledgments

I would like to warmly thank all people who supported me. Especially I would like to thank my directors of study **Prof. Frank Vriesekoop** and supervisor **Dr Annette Creedon** for their assistance and guidance.

I would express my thanks to all staff of Regional food academy specially Clare Hutchinson and princess Margret Laboratories especially Amjad Ali for their assistance.

Special thanks to all staff of international student services both Mrs Sarah Estibeiro, Miss Jo Rawlinson and Mrs Anne-Marie Cambiotti for their support in terms of language and at the same time guidance.

I would like to thank Faccenda Company for providing chicken carcass and many thanks also to the Ministry of Higher Education and Scientific Research, Kurdistan Regional Government for funding this project.

My deepest gratitude to my wonderful parents, brothers, sisters and all my family for their continuous support and encouragement throughout my PhD studies.

Finally, I would like to express my deep and sincere gratitude to my gorgeous wife ***Payman Mohammed*** for her love, care and moral support which always motivated and encouraged me to complete my PhD studies and to accomplish my goals. This dissertation is dedicated to my lovely wife and sons "***Shah and Nor***"

Thank you again...

Jamal S. Khurshid

Table of Contents

List of Tables **Page No.**

List of Figures **Page No.**

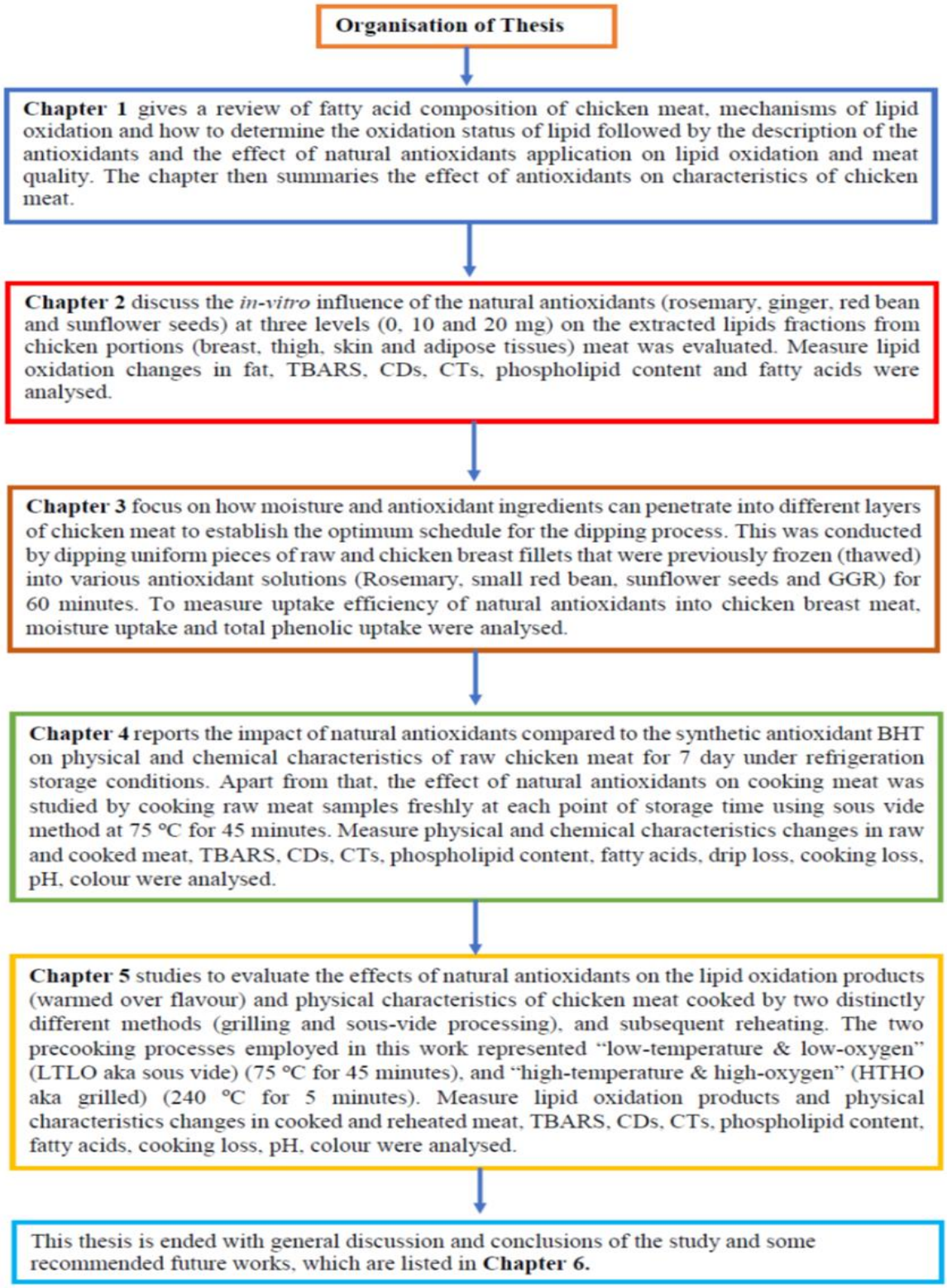

Figure 1. Structure diagram of thesis organisation

Chapter 1

Literature Review

1.0 Introduction

Autoxidation of lipids is of primary concern in many lipid-rich foods because it negatively affects the rheological characteristics of meat (Byrne et al., 2001), through the formation of hydroperoxides, malondialdehyde, 4-hydroxynonenal, and volatile compounds during the lipid oxidation process which are responsible for undesirable tastes, flavours and discolouration of meat (Frankel, 1980; Min and Boff, 2002) and cause a reduction in the nutritional value (Min et al., 2008). The discolouration of meat during storage takes place by oxidation of oxymyoglobin, resulting in the appearance of an undesirable colour in meat (Nerin et al., 2006). This formation of abnormal colour and off-odours in meat is due to susceptible lipids and proteins being attacked by free radicals (Nanke et al., 1998; Ahn et al., 2001). Unsaturated fatty acids are considered more prone to oxidation (Min and Ahn, 2008). Poultry meat is susceptible to oxidation due to the particularly high proportion of polyunsaturated fatty acids compared to most other meat types (Hayes, 2000). Moreover, meat processing methods such as cooking can accelerate the oxidation rate in beef, pork and chicken (Min et al., 2008); grinding and deboning meat also plays a role in the lipid oxidation progress (Laak, 1994). This is because these processes disrupt the cell membranes and facilitate the lipid compounds to come into direct contact with oxygen and compounds that have the ability to catalyse meat lipid oxidation (Bragagnolo, 2009). Lipid oxidation has an association with warmed-over flavour (Erickson, 2002). Warmed-over flavour is defined as unpleasant odours and flavours (Estevez et al., 2009). Warmed over flavour is commonly described as a grassy, cardboard-like, rancid, stale, and painty that occurred in cooked, refrigerated and reheated meat (Pegg and Shahidi,

2007; Colindres and Brewer, 2010). Several studies have reported that warmed over flavour is strongly correlated with the development of thiobarbituric acid reactive substances (TBARS) in meat (Lanari et al., 1995; Byrne et al., 2001; Nute, 2009). Hence, in the study conducted by Sato et al. (1971) reported that the warmed-over flavour is rapidly produced in cooked meat during the post-cooking storage time. The process of lipid oxidation can be terminated or minimised by the presence of free-radical scavenging compounds (e.g. antioxidants) (Erickson, 2002; Velasco and Williams, 2011). The use of antioxidants to prevent and delay the oxidation of lipids in meat and meat-derived products has been the focus of a number of studies (Erickson, 2002; Mielnik, et al., 2003; Naveena et al., 2004). Some reports have focused on the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) and have been employed in various food industries to retard and reduce lipid oxidation (Erickson, 2002; Velasco and Williams, 2011). More recently, the focus of research has been on the use of natural antioxidants. This is mainly because consumers prefer, and place increasing demands on, foods containing natural antioxidants instead of synthetic ones because of the perceived health benefits, improved nutritional value and the enhanced quality of meat (Velasco and Williams, 2011). A further reason for the move towards natural antioxidants is to avoid reported carcinogenic side effects associated with long-term consumption of some synthetic antioxidants (Gharavi et al., 2007). Several studies have pointed out that rosemary, small red bean, sunflower and ginger contained several compounds that have high antioxidant activity (Shan et al., 2005; Luthria et al., 2006; Amakura et al., 2013). Hence, given the move towards the role of natural antioxidants, there is a little research focussing on the impact of these natural antioxidants on broiler chicken meat. Although information about the effect of extraction of natural antioxidants from plant origins such rosemary and ginger on the oxidation of lipids, degradation and sensory properties of meat is well documented, there is no research

related to investigate the impact of the application of both small red bean and sunflower seeds to chicken meats on their meat quality. Furthermore, most of the research has demonstrated the efficiency of sunflower seed extract and small red bean in *in vitro* tests. Until now, to our knowledge, application of this antioxidant to chicken meat post-slaughter has not been well-documented. Therefore, to explore and evaluate the effectiveness of small red bean and sunflower seeds in chicken meat is important. Hence, this study conducted to evaluate the effect of post-slaughter natural antioxidants application on the physical and chemical characteristics of raw and cooked chicken meat.

1.1 Fatty Acid Composition of Chicken Meat

The lipids in meat are present at various locations, such as subcutaneous (directly under the skin); intermuscular fat surrounding the muscle cells, including cell membranes); and intramuscular (fat surrounding muscle tissue, but within cuts of meat and as such is responsible of the marbling in specific cuts) (Huff-Lonergan, 2010). However, meat contains various types of lipid compounds. There are two noteworthy types of lipids present in meat, namely neutral lipids (triglycerides) and polar lipids (phospholipids) (Belitz et al., 2009). Triglycerides represent the greatest proportion of lipids. All triglycerides are composed of one molecule of glycerol and three fatty acids that are connected via ester bonds (Figure 1.1). Triglycerides are typically synthesised as either visceral fat that surrounds vital organs as a protective layer, or as adipose fat that is laid down when excess nutrients are converted as an energy dense stores. The adipose stores can then be accessed to provide energy to the body through the lipolysis process. Lipolysis is the breakdown of triglyceride into free fatty acids and glycerol (Belitz et al., 2009).

Phospholipids are lipids that are found in cell membranes and the membranes of organelles where they play an important role in the fluidity of the membrane and functioning of cells. Phospholipids are composed of two fatty acids bound via ester bonds

to a glycerol. The glycerol is also bound to a phosphate group that links the lipid moiety to a polar molecule such as choline (Figure 1.1). Hence, structurally phospholipids are composed of a hydrophilic head group (the polar moiety) and a hydrophobic tail (the lipid moiety). (Erickson, 2002).

Fatty acids (FA) are formed of a hydrocarbon chain with a methyl group (CH_3) at one side and a carboxylic acid group (COOH) at the other end. The vast majority of fatty acids consists on an even number of carbons, due to the principal means of synthesis (Belitz et al., 2009). The relative frequency of fatty acids of varying length varies from source to source. For example, chocolate, cottonseed and coconut that contain a high level of short chain fatty acids; while flaxseed, soybean oil, fish, milk and meat contain a high level of long chain fatty acid (Abedi and Sahari, 2013). Fatty acids predominantly exist in a saturated form (all carbon carries the maximum number of hydrogens that can surround them while in a chain of carbons); these are referred to a saturated fatty acids (SFA). Alternatively, some fatty acids might contain one or more double bonds between carbons in the chain; these are referred to as monounsaturated (MUFA) containing a single double carbon bond; or polyunsaturated fatty acids (PUFA) containing multiple double carbon bonds. Depending on the position of double bonds the unsaturated fatty acids can be assigned a specific nomenclature (e.g. n-3, n-6 or n-9), with the numbers indicating the first carbon in a double bond that is closest to the methyl end of the fatty acid (O'Keefe, 2002).

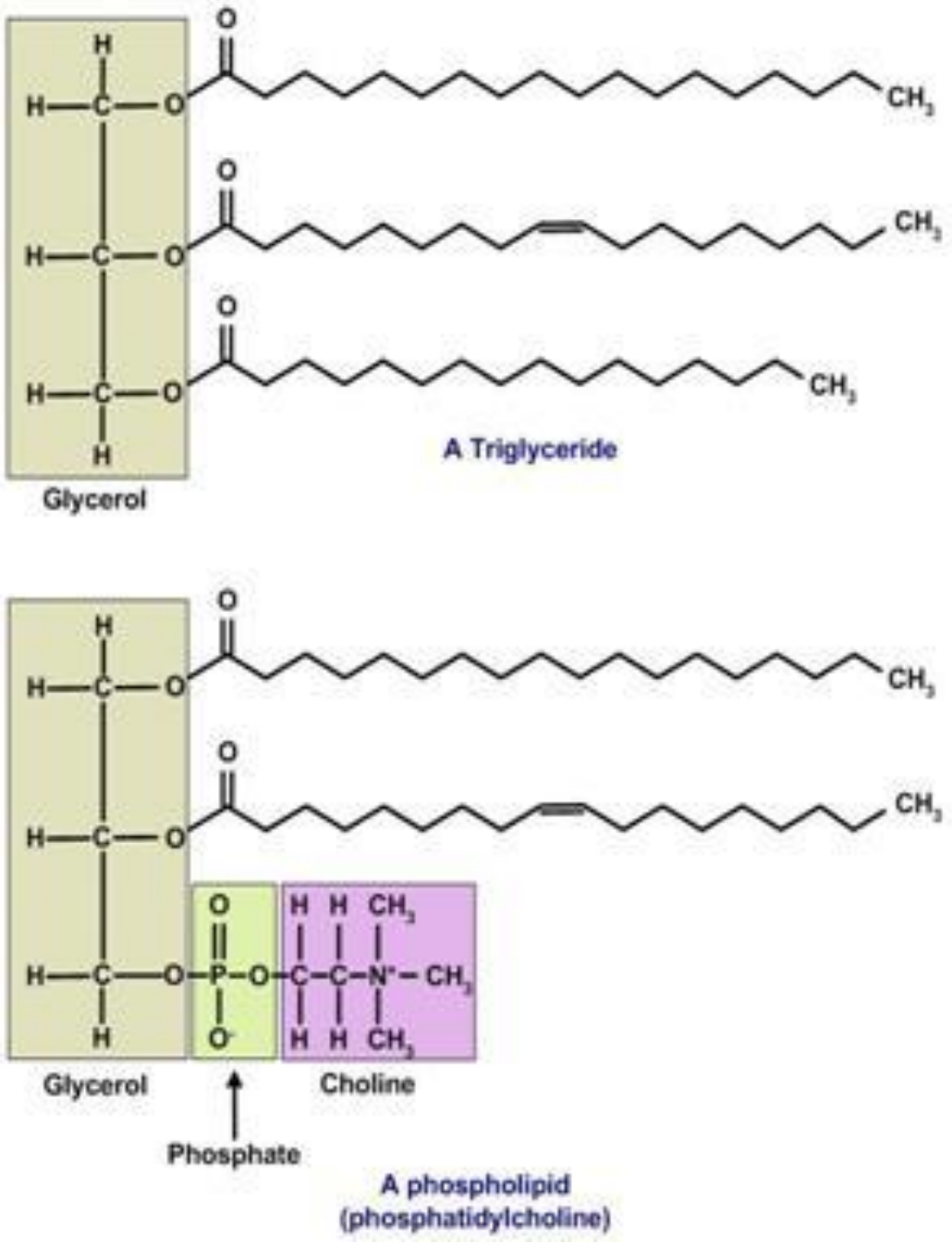

Figure 1. 1 Structures of the main lipid compounds. Top, triglycerides; Bottom, phospholipids. (Adapted from Blake, 2010).

In general, fat of food stuff and particularly meat is made out of a mix of different sorts of lipids and at different quantities. The combination of which is commonly referred to as fatty acid profile. The typical fatty acid profile of chicken muscle and tissues are shown in Table 1.1. Both saturated and monounsaturated fatty acids make up the main proportion

of fatty acids in chicken tissues, while polyunsaturated unsaturated fatty acids are present in much lower quantities (Waldroup and Waldroup 2005; Feddern et al., 2010; Zhang et al., 2013; Ahmed et al., 2015).

The highest levels of SFA are typically found in breast, thigh and drumstick meat with values of 35.57, 33.32 and 31.26 g/100 g fat respectively; while skin and adipose tissue typically contain a lower proportion of SFA (Table 1.1). Palmitic acid (C16:0) is the most prominent SFA in all types of chicken tissue. The amount of MUFA can vary from 39.00 g/100 g fat in the skin tissue to 46.17 g/100 g fat in thigh muscle, with oleic acid being the most abundant among the monounsaturated fatty acids, closely followed by palmitoleic acid (Table 1.1) (Waldroup and Waldroup, 2005; Feddern et al., 2010; Zhang et al., 2013; Ahmed et al., 2015). Skin tissue tends to be rich in PUFAs at 30.6 g/100 g fat compared to the amount of PUFA in thigh meat, adipose tissue. The most abundant PUFA is linoleic acid (C18:2 n-6), which ranges from 14.30 g/100 g fat in breast meat to 28.23 g/100 g in skin tissue.

As mentioned before, the principal lipid components are triglycerides and phospholipids. In the two main types of chicken tissue (breast and thigh), the triglycerides carry the largest proportion of fatty acids (Table 1.2). Relatively the highest proportion of SFAs are typically found in the phospholipid portion of breast tissue followed thigh tissue with values of 33.7 and 32.6 % respectively; while the triglycerides from thigh tissue typically contain a lower proportion of SFAs of 18.76 % followed by breast tissue of 21.1 % (Table 1.2). Palmitic acid (C16:0) is the most prominent SFA among both phospholipids and triglycerides from breast and thigh tissue (Betti et al., 2009). The amount of MUFA can vary from 25.1 % in phospholipid thigh tissue to 29.3 % in breast tissue; while the proportion triglycerides in MUFA are very similar to 48.5 % in breast tissue and 49.1 % in thigh tissue with oleic acid being the most abundant among the MUFAs, closely

followed by cis-vaccenic acid (Table 1.2) (Betti et al., 2009). The highest proportion of PUFAs are typically found in the phospholipid portion of thigh tissue followed breast tissue with values of 42.3 and 36.9 %, respectively; while the triglycerides from breast tissue typically contain a lower proportion of PUFAs 30.3 % (Table 1.2). Linoleic acid (C18:2 n-6) followed by arachidonic acid (C20.4 n-6) is the most prominent PUFAs among both phospholipids and triglycerides from breast and thigh tissue (Betti et al., 2009).

Table 1.1 Fatty acid composition (g/100 g fat) in chicken muscle and fat tissue. (Adapted from Waldroup and Waldroup 2005; Feddern et al., 2010; Zhang et al., 2013; Ahmed et al., 2015)

Structure	Name	Breast	Thigh	Drumstick	Skin	Adipose
C14:0	Myristic	0.4	1.1	0.9	0.5	1.9
C14:1 n-5	Myristoleic	-	0.3	-	-	-
C15:0	Pentadecanoic	-	-	0.0	-	-
C16:0	Palmitic	24.0	25.0	23.6	23.5	21.5
C16:1 n-7	Palmitoleic	4.4	6.2	5.7	4.2	5.0
C17:1	Heptadecaenoic	-	0.2	-	-	-
C18:0	Stearic	10.7	6.1	6.3	6.1	7.2
C18:1 n-9	Oleic	37.4	39.2	38.7	34.8	39.8
C18:2 n-6	Linoleic	14.3	15.6	15.4	28.2	19.9
C18:3 n-3	α-linolenic	1.4	1.7	-	2.4	1.8
C20:0	Arachidic	0.4	1.1	0.4	-	-
C20:1 n-9	Eicosenoic	0.9	0.1	0.8	-	-
C20:2 n-6	Eicosadienoic	-	0.2	0.1	-	-
C20:3 n-6	Eicosatrienoic	-	0.2	-	-	-
C20:3 n-3	Eicosatrienoic	1.2	-	-	-	-
C20:4 n-6	Arachidonic	2.2	1.0	0.2	-	-
C20:5 n-3	EPA[1]	0.2	0.2	-	-	-
C22:1 n-9	Erucic	-	-	0.7	-	
C22:5 n-3	DPA[2]	-	-	-	-	-
C22:6 n-3	DHA[3]	1.2	1.7	-	-	-
C24:1 n-9	Nervonic	1.3	0.2	-	-	-
SFA[4]		35.6	33.3	31.3	30.2	23.4
MUFA[5]		43.9	46.2	44.0	39.0	44.8
PUFA[6]		19.2	20.5	17.2	30.6	21.7
∑n-3 PUFA[7]		2.7	3.5	-	2.4	1.8
∑n-6 PUFA[8]		17.7	17.0	15.7	28.2	19.9

[1]Eicosapentaenoic acid EPA cis, [2]Docosapentaenoic acid DPA, [3]Docosahexaenoic acid DHA; [4]SFA, saturated fatty acids; [5]MUFA, monounsaturated fatty acids; [6]PUFA, polyunsaturated fatty acids. ∑ n-3 PUFA[7] = C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22.5 n-3 + C22.6 n-3. ∑n-6 PUFA[8] = C18:2 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6.

Table 1.2 Fatty acid composition (mg/100 g meat) of phospholipids and triglycerides in chicken breast and thigh meat. (Adapted from Betti et al., 2009)

Structure	Name	Breast		Thigh	
		PL[1]	TAG[2]	PL[1]	TAG[2]
C14:0	Myristic	1.0	5.5	1.1	14.5
C16:0	Palmitic	66.6	239.4	63.7	588.7
C16:1n-7	Palmitoleic	1.9	45.3	2.7	165.3
C18:0	Stearic	33.1	62.1	60.3	91.3
C18:1 n-7	Cis-vaccenic	17.3	44.6	16.5	111.7
C20:0	Arachidic	-	1.3	-	2.5
C18:1 n-9	Oleic	68.3	617.5	76.8	1548.0
C18:2 n-6	Linoleic	68.6	361.8	101.2	977.5
C18:3 n-3	α-linolenic	2.8	70.7	5.6	195.0
C18:3 n-6	Gamma-linolenic	-	1.9	-	5.0
C20:2 n-6	Eicosadienoic	1.2	2.4	1.6	5.4
C20:3 n-6	Eicosatrienoic	-	1.3	2.0	2.4
C20:4 n-6	Arachidonic	20.6	2.2	30.6	4.8
C20:5 n-3	EPA[3]	3.1	0.4	2.2	1.2
C22:4 n-6	Adrenic	-	-	3.6	-
C22:1 n-9	Erucic	-	1.0	-	-
C22:5 n-3	DPA[4]	8.7	1.1	9.0	2.1
C22:6 n-3	DHA[5]	5.0	ND	6.3	ND
SFA[6]		100.7	308.3	125.1	697.0
MUFA[7]		87.4	708.3	96.0	1825.0
PUFA[8]		110.1	441.7	162.1	1193.0
∑n-3[9]		19.6	72.2	23.1	198.3
∑n-6[10]		90.4	369.5	139.1	990.1

[1] Phospholipids, [2]Triglycerides; [3]Eicosapentaenoic acid EPA cis, [4]Docosapentaenoic acid DPA, [5]Docosahexaenoic acid DHA; [6]SFA, saturated fatty acids; [7]MUFA, monounsaturated fatty acids; [8]PUFA, polyunsaturated fatty acids. [9]∑ n-3 PUFA= C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22.5 n-3 + C22.6 n-3. [10]∑n-6 PUFA[8] = C18:2 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6.

1.2 Lipid Oxidation

Fat and fatty acid composition in meat are considered important for consumers due to their health benefit and nutritional value (Wood et al., 2008). However, some of these components are susceptible to oxidation during processing such as cooking, grinding and possible post-cooking storage periods (Sampaio et al., 2012; Humada et al., 2014). Sensitivity to oxidation is mainly dependent on the composition of lipids. Lipids that contain high levels of unsaturated fatty acids are more prone to oxidation compared to lipids high in saturated fatty acids (Min et al., 2008). Unsaturated fatty acids are

susceptible to oxidation by photo-oxidation, enzymatic oxidation and autoxidation (Gordon, 2001; Yanishlieva-Maslarova, 2001; Zhuang et al., 2002). Photo-oxidation is produced when lipids are exposed to light. However, photo-oxidation of lipids in meat is not considered a significant issue as suitable protection can reduce the absorption of light unless the meat is exposed to fluorescent light or direct sunlight, which might occur when meat is presented in a retail display cabinet (Gordon, 2001). During enzymatic lipid oxidation, enzymes such as lipoxygenases are a major initiator of oxidation process in lipids (Min and Ahn, 2005), which have ability to catalyse the oxygenation of fatty acids containing one or more double bonds (MUFAs and PUFAs respectively) and produce hydroperoxides (Shahdi and Wanasundara, 2002). However, enzymatic lipid oxidation in cooked meat is not considered a significant issue, due to the thermal inactivation of the most meat-related lipoxygenases at 60 ^{o}C (Yanishlieva-Maslarova, 2001).

Lipid autoxidation is of primary concern in many lipid-rich foods, which negatively affects the physical and chemical characteristics of meat (Cortinas et al., 2004; Naveena et al., 2008; Selani et al., 2011), its nutritional value (Byrne et al., 2001; Min et al., 2008), and it produces undesirable tastes, flavours and discolouration of meat (Frankel, 1980; Min and Boff, 2002). Mechanisms of lipid oxidation and the development of rancidity can be categorized into three main steps (Figure 1.2).

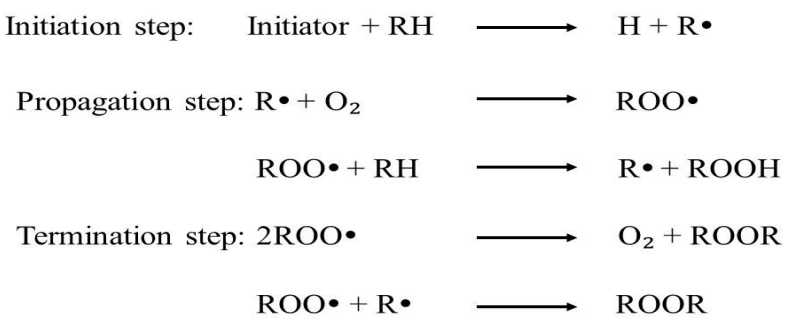

Figure 1. 2 Mechanisms of lipid oxidation (Adapted from Erickson, 2002). Where: RH is a fatty acid, H is a hydrogen atom, R• is a lipid free radicals, ROO• is a peroxyl free radical, ROOH is lipid hydroperoxide and ROOR is non-radical.

In the initiation step, lipid free radicals (R•) are formed after a hydrogen atom is removed from an allylic methylene group of unsaturated fatty acids (RH) in the presence of initiators such as heating, iron, transition metals or free radicals. Lipid free radicals (R•) react with molecular oxygen which exists in the atmosphere and this leads to the production of peroxyl free radical (ROO•). Meanwhile, the latter promotes oxidation by reacting with unsaturated fatty acid and obtaining a hydrogen atom. The results of this reaction are the formation of the primary products, lipid hydroperoxide (ROOH) and a new lipid free radical. This lipid hydroperoxide is odourless and tasteless (Estevez et al., 2009). Although hydroperoxide has no effect on the meat quality further decomposition causes off-odours and off-flavours (Erickson, 2002). The hydroperoxide decomposes and generates secondary products such as pentanal, hexanal, 4-hydroxynonenal, and malonaldehyde which do have noticeable off-odours and flavours. These compounds are extremely volatile and detectable at very low levels (parts per billion), and are also considered indicators of rancidity and warmed over-flavour (Estevez et al., 2009). During the termination step, two peroxyl free radicals interact with each other and produce non-radical products such as lipid peroxide (ROOR). Lipid oxidation development in chicken

meat post-slaughtering is affected by several factors such as processing, storage conditions, oxygen availability, temperature, lipid composition, anti-oxidative content and the presence of pro-oxidants (Min and Ahn, 2005; Ma et al., 2007).

1.3 Factors Affecting Lipid Oxidation

Lipid oxidation in meat usually begins immediately post slaughter, which can be facilitated through various factors. The degree of processing is associated with **intrinsic** factors such as animal species, fatty acid composition, pH, meat content, and the presence of metals and **extrinsic** factors such as storage time and conditions, processing operations and conditions, light and exposure to oxygen (Min and Ahn, 2005).

1.3.1 The Influence of Fatty Acid Composition on Lipid Oxidation.

The rate of lipid oxidation is mainly influenced by the molecular geometry, position and number of the double bonds in the fatty acids (Nawar, 1996). During the propagation step, hydrogen atoms that are adjacent to the double bonds are most prone to abstraction. For that reason, fatty acids that carry multiple double bonds in their structure (i.e. polyunsaturated fatty acids) are more susceptible to oxidation compared to monounsaturated and unsaturated fatty acids (Huang et al., 2012). In addition, oxidation more readily occurs in *cis* compared to *trans* isomers of unsaturated fatty acids (Nawar, 1996). The fat and its composition in muscle tissue depend on animal species, genetic origin, diet, and muscle position. For example, chicken thigh meat has higher levels of lipids and unsaturated fatty acids compared to the beef and pork meat; and chicken thigh meat contains lower levels of lipids than in chicken breast meat (Erickson, 2002). The rate of fatty acid oxidation is more related to fat composition than the amount of fat (Min et al., 2008). For instance, the oxidation rate is relative to the amount of unsaturated fatty acids and degree of unsaturation in individual fatty acids (Estevez et al., 2009).

1.3.2 The Influence of Storage Conditions on Lipid Oxidation

The impact of storage conditions such as: time in storage, temperature, oxygen, light and type of packaging on acceptability properties of meat have been well documented (Nam and Ahn, 2002; Sanchez-Escalante et al., 2011; Muela et al., 2010; Zhou et al., 2010; Haile et al., 2013). Throughout the storage time, meat can undergo several undesirable changes such as lipid and protein oxidation, which can negatively affect the sensorial and rheological characteristics of meat (Frankel, 1980; Byrne et al., 2001; Min and Boff, 2002; Leygonie et al., 2012). Changes in the flavour profile are often observed when meat is stored for extended periods. These flavours are usually described as undesirable "off" flavours or rancid flavours (Vieira et al., 2009), which are strongly correlated with the generation of various volatile compounds that are associated with lipid oxidation (Erickson, 2002).

The storage (chilly and frozen) temperature is considered an important factor that can affect the quality and the shelf life of meat (Zhou et al., 2010). The longer meat and its products are exposed to elevated storage temperatures; the more the lipid is prone to oxidation (Flavia et al., 2014). McKee (2007) reported that pork meat kept at temperature above 7 ^{o}C had a higher off-odour and discolouration as compared to those kept at -4, 0 or 3 ^{o}C. Low temperature can protect the properties of meat and prolongs its shelf life (Sebranek, 1995). This is probably due to the combined reduction in enzymatic activity and associated hydrolytic reactions and a reduction in the rate of chemical reactions that yield oxidative rancidity (Zhou et la., 2010). Low temperature storage such as freezing and refrigeration is widely used to limit oxidative deterioration of meat and hence increase its shelf life (Miller, 1994; Zhou et al., 2010; Leygonie et al., 2012). Frozen storage reduces the rate of lipid oxidation more than refrigerated storage (Miller, 1994). While freezing has the ability to reduce the rate of lipid oxidation, however it does not arrest the

process (Leygonie et al., 2012). The lipid oxidation can be minimised if the products stored at temperature -18 ^{o}C, well packaged and minimal the fluctuation of temperature (Sebranek, 1995). The development of lipid oxidation could be prevented in full just if oxygen will be totally wiped out and stored at low temperature -55 ^{o}C (Zhou et al., 2010).

1.3.3 The Influence of Light and Water Activity (a_w) on Lipid Oxidation

Light is considered pro-oxidative factor that may affect the display life of meat (Gordon, 2001). Light-exposure can stimulate and increase the rate of lipid oxidation in meat by photo-oxidation (Gordon, 2001; Yanishlieva-Maslarova, 2001; Zhuang et al., 2002). Photo-oxidation occurs when lipids are exposed to fluorescent light or direct sunlight (254-546 nm) (Anderson and Skibsted, 1992), which might occur when meat is presented in a retail display cabinet (Gordon, 2001). The rate of lipid oxidation is mostly dependant on lighting conditions such as wavelength range, light intensity and meat-surface exposed to the light (Anderson and Skibsted, 1992; Min and Boff, 2002). Erickson (2002) identified that the longer meat is exposed to light, the higher its susceptibility to lipid oxidation and discolouration. This due to the ability of light to stimulate haem-containing proteins and activating them as sensitizers. Photoactivated sensitizers can react directly with oxidisable products and yield free radicals that can promote oxidation (Decker, 2002). Huang et al. (2005) reported that in the presence of light and photosensitizers, singlet oxygen can be formed and react with unsaturated fatty acid and ultimately produce hydroperoxides (Reische et al., 2002). For instance, the exposure to light will reduce the redness of ham meat over time (Haile et al., 2013). Furthermore, Sanchez-Escalante et al. (2011) found that beef meat stored under light sources such as standard fluorescent (TF/36 w) and low-UV (L36 w; 254 nm) lamps had a higher amount of TBARS and metmyoglobin than those stored at dark conditions. The discolouration of meat during

storage is related to the oxidation of oxymyoglobin (ferrous Fe^{2+}) to metmyoglobin (ferric Fe^{3+}) which turns meat colour from red to brown (Mancini and Hunt, 2005).

Water activity (a_w) of raw meat (at 25°C) is 0.99 and 0.6 for frozen meat at -18°C. At a range of water activity between 0.6-0.8, haem pigments initiate the oxidation of lipid (Rogers, 2007). Freeze-dried meat was found to have a greater lipid oxidation at a_w ranged from 0-0.33 and stored at 49 °C as compared to those stored at 25 °C and $a_w < 0.33$, while the degradation of protein solubility was higher when meat stored at 49 °C and a_w ranged from 0.33-0.66 compared to $a_w < 0.33$ and > 0.66, respectively (Sun et al., 2002). Laack (1994) reported that the oxidation of meat during the frozen storage time is associated with a lowering in the water activity.

1.3.4 The influence of Meat Myoglobin Content on Lipid Oxidation

Lipid oxidation is also strongly correlated with myoglobin content (Min and Ahn, 2009; Thiansilkul et al., 2011). Min and Ahn (1998; 2009) showed that myoglobin contains several compounds such as ferryl-myoglobin, hematin and free ionic iron that have the ability to catalytically promote lipid oxidation. More specifically, both haem and non-haem iron have the ability to decompose the lipid hydroperoxide and generate off-odours and off-flavours in meat (Erickson, 2002). Thiansilakul et al. (2011) found that the addition of myoglobin to fish meat accelerated the oxidation rate of lipids as evidenced by higher thiobarbituric acid reactive substances (TBARS), peroxide value (PV) and volatile compounds. Oxidation of myoglobin occurred during the frozen storage and the consequent formation the amount of metmyoglobin which was probably produced during the combination of fluctuation of temperature and defrosting. Leygonie et al. (2012) reported that both freezing and defrosting of meat caused a rapid increase of thiobarbituric acid reactive substances (TBARS) which this attributed to the disruption of cell

membranes during the formation of a large crystal of ice and leading to release existing pro-oxidants particularly the haem iron.

1.3.5 The Influence of Non-Meat Additives on Lipid Oxidation

Lipid oxidation is affected by sodium chloride (Rhee and Ziprin, 2001). The addition of salt to meat and meat product has been commonly used to enhance the flavour, shelf-life, tenderness, drip loss and juiciness of meat (Min and Ahn, 2005). It has been reported that sodium chloride can act as a pro-oxidant and accelerate lipid oxidation in meat and meat products, however the accelerating rate is dependent on its concentration (Rhee and Ziprin, 2001; Min and Ahn, 2005). Rhee and Ziprin (2001) reported that the rate of lipid oxidation increased with elevating levels of sodium chloride in beef and chicken. Their study also found that non-haem iron increased with increasing sodium level in both types of meat. This was supported by the findings of Min and Ahn (2005) who reported that sodium chloride supports the release of ionic iron from haem proteins, which in turn induces autoxidation in meat. Moreover, addition of sodium chloride to ground pork meat increased both lipid oxidation products (TBARS and lipid peroxide) with increasing the concentration. Their study also found that sodium chloride reduced the activities of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase in pork meat, which could be responsible for the acceleration of lipid oxidation in the meat tissue (Lee et al., 1997). In the study conducted by Rhee et al. (1983) found that the application of sodium chloride at levels of 0, 0.5, 1, 2 or 3 % (w/w) to raw ground beef and stored at 4 °C for 3 or 6 days increased TBARS values with increasing salt concentration up to 2 %. When the level of salt increased from 2 to 3 % it resulted in decreased TBARS values. This suggests that sodium chloride is responsible for inducing the oxidation of lipid in meat and meat products, which could have limited the shelf-life of meat and its products.

1.3.6 The Influence of Molecular Oxygen on Lipid Oxidation

Oxygen is one of the major factors that has a negative effect on lipid oxidation in both raw and cooked meat (Min and Ahn, 2005). In the presence of oxygen, lipids that contain high levels of unsaturated fatty acids are prone to oxidation (Min et al., 2008). Unsaturated fatty acids are susceptible to oxidation by reacting with molecular oxygen that exists in the atmosphere and this leads to the production of unpleasant flavour and volatile compounds (Estevez et al., 2009). Oxygen can rapidly promote oxidation of meat during processing such as cooking, grinding and deboning (Min et al., 2008). This is because cooking, grinding and deboning processes disrupt the cell membranes in muscle tissue and facilitate the lipid compounds to come into direct contact with oxygen and compounds that have the ability to catalyse meat lipid oxidation (Bragagnolo, 2009). Min and Ahn (2005) suggested that the potential for lipid oxidation in meat depends more on the availability of oxygen and its concentration in modified atmospheres. As such, turkey breast meat stored under aerobic conditions was found to have a higher lipid oxidation compared to those stored in the absence of oxygen under refrigerated conditions over a two-week period (Nam and Ahn, 2002). Jääskeläinen et al. (2016) found that the beef meat stored at higher oxygen modified atmospheres had a higher amount of volatile compounds compared to those stored under vacuum condition. When oxygen is present, myoglobin is also prone to oxidation and produce metmyoglobin. Thus, metmyoglobin is formed when the ferrous iron (Fe^{2+}) in the myoglobin is oxidised and is converted to the ferric (Fe^{3+}) state (Mancini and Hunt, 2005). The formation of metmyoglobin can take place slowly after oxygen penetration into the meat commences (Feiner, 2006). Metmyoglobin in beef meat stored at 4 °C under high oxygen level increases with increasing storage time (Faustman and Cassens, 1991). Several studies have demonstrated that packaging meat in modified atmospheres containing different mixtures of gas can inhibit the oxidation of lipid (O'Grady et al., 2000; Fraqueza and Barreto, 2011).

According to the results reported by O'Grady et al. (2000), meat stored at the following air mixtures (60 % O_2 + 20 % N_2 + 20 % CO_2) and (80 % O_2 + 20 % CO_2) had the highest level of lipid oxidation when compared to those stored at a much lower oxygen level of (20% O_2 + 60% N_2 + 20% CO_2). Min and Ahn (2005) argued that cooked turkey meat stored under vacuum condition was only slightly affected by the presence of pro-oxidants such as haemoglobin, sodium chloride, lipid content and free ionic iron.

1.3.7 The Influence of Metals on Lipid Oxidation

Various metals such as (Fe, Cu, Mn, Cr, Ni, V, Zn, Al) are considered major factors accelerating the lipid oxidation process. Metals can promote oxidation by two mechanisms. Metals can react with hydroperoxide ROOH and form peroxy radicals and alkoxy radicals ROO• and RO• (Erickson, 2002). Metals also have the ability to react with unsaturated fatty acid (RH) directly, resulting in the formation of alkyl free radicals (R•) (Reische et al., 2002). Authors such as Frankel (1980) and Min and Boff (2002) showed that metals can interact with oxygen and produce singlet oxygen; this oxygen later promotes the oxidation of lipids. It was observed that singlet oxygen has the ability to promote photo-oxidation of unsaturated fatty acid and produce hydroperoxides (Reische et al., 2002).

1.3.8 The Influence of Heating and Reheating on Lipid Oxidation

Lipids in meat are susceptible to oxidation during meat processing, cooking and post-cooking storage (Min et al., 2008). Meats with elevated levels of polyunsaturated fatty acids are more prone to develop off odours and warmed-over flavour owing to oxidation of these polyunsaturated fatty acids (Hayes, 2008). Warmed over flavour is rapidly produced in cooked meat during the post-cooking storage time (Mielnik et al., 2006). The onset of warmed over flavour is argued to be due to thermal processes that disrupt the cell membranes and release pro-oxidants (Min and Ahn, 2005); the latter leading to increased

lipid oxidation. As mentioned before, Bragagnolo (2009) reported that the cooking process facilitates the lipid compounds to react with oxygen and compounds that have the ability to catalyse meat lipid oxidation. The warmed-over flavour can be determined by measuring volatile compounds, thiobarbituric acid reactive substances (TBARS) and sensory analysis (rancid, grassy, cardboard-like, rancid, stale, and painty characteristics) (Lanari et al., 1995; Byrne et al., 2002; Nute, 2009). As mentioned before, during the oxidation of PUFAs different volatile compounds can be generated in meat products (Estevez et al., 2009). These oxidation products of PUFAs in meat have been identified as acids, alcohols, aldehydes, ketones, sulphur-containing compounds and pyrazines (Byrne et al., 2002; Jääskeläinen et al., 2016). Among them, are oxidation products that commonly used as indicators of warmed-over flavour are particularly volatile aldehydes such as propanal, hexanal and 4-hydroxy-2 nonenal (Estevez et al., 2009). One of the major oxidation products attributed to the warmed-over flavour is hexanal, which is predominantly derived from the oxidation of linoleic and arachidonic fatty acids (Meynier et al., 1998). It has been found that increasing warmed-over flavour in reheated, cooked chicken meat correlates with increasing amounts of hexanal (Kerler and Grosch, 1997). Mielnik et al. (2006) investigated cooked turkey meat to determine the volatile compounds contributing warmed-over flavour and found that hexanal, followed pentanal, contributed significantly to the development of warmed-over flavour and TBARS during storage. The incidence of warmed over flavour has been shown to coincide with the development of thiobarbituric acid reactive substances (TBARS) (Lanari et al., 1995; Byrne et al., 2001; Nute, 2009). St. Angelo et al. (1987) pointed out that both hexanal and 2,3-octanedione had a strong negative correlation with sensory evaluation scores in cooked beef and reheated. Byrne et al. (2001) also reported that polyunsaturated fatty acids decreased after warmed-over flavour developed; while Lanari et al. (1995) and Nute (2009) found that warmed over flavour becomes noticeable when TBARS values range

between 0.6 to 2 mg MDA/kg beef meat. It was reported that TBARS increased significantly in cooked meat (Min et al., 2008). The development of warmed over flavour is a significant issue and causes undesirable sensory changes in meat. The most noticeable sensorial attribute that indicates warmed-over flavour in first-cooked and then-refrigerated chicken meat is a decrease in the meaty sensorial attribute and an increase rancid, roasted and sulphur/rubber sensorial attributes (Byrne et al., 2002). It can be concluded that lipid oxidation is considered to be of concern and induces unpleasant attributes in meat and its products can be measured by TBARS. These products are highly linked with warmed over flavour.

1.3.9 The Influence of pH on Lipid Oxidation

The pH value of breast and thigh chicken meat is approximately 5.7 and 6.3 respectively (Gong et al., 2010), however, the pH value is variable and heavily depends on post-slaughter conditions (Feiner, 2006). Chicken meat has a higher pH than turkey meat, which is reported to be linked to higher levels of myoglobin in chicken meat than in turkey meat (Saucier et al., 2000). Several studies have been suggested that the lipid oxidation is linked to pH of meat (Love, 1987; Buaneow et al., 2008; Sharedeh, et al., 2015). Lipid oxidation products (TBARS) increased in meat after adjusting pH to 5.0 compared to those at pH 6.0 or 7.0 (Love, 1987). Similar findings were reported by Sharedeh, et al. (2015), who found that lipid oxidation in beef meat significantly increased with decreasing pH from 6.5 to 4.3, the highest formation of TBARS was detected at pH 4.3. This is argued to be due to pH decreases that can release pro-oxidants from iron containing proteins (ferritin) such as iron. This iron can react with hydrogen peroxide and produce hydroxyl radicals (OH•); the later leading to accelerate the lipid oxidation (Sharedeh, et al., 2015). It was shown that the oxidation process occurs more at low pH values in comparison with higher pH values.

1.4 Phospholipids Oxidation

Phospholipid are known as a natural component of the fat in many foods (Gordon, 2001). The major constituents of phospholipids in meat are phosphatidylcholine and phosphatidylethanolamine, while phospholipids components that present in minor amounts are phosphatidylserine and sphingomyelin (Toldra, 2006). The proportion of phospholipids, as the percentage of total fat content in muscle tissue is about 40 % (Ruiz et al., 2009). However, chicken contains approximately ⅔ of its phospholipids in the chicken breast fat and ⅓ of its phospholipids in the leg fat (Pikul, 1984; 1985). The proportion of phospholipids in red meat is higher than in white muscle tissue (Chamul, 2007). Phospholipids, similar to triglycerides, are prone to oxidation, however, phospholipids are more susceptible (Igene et al., 1980). Igene et al. (1980) reported that the oxidation process in muscle tissue typically initiates in the phospholipids. The sensitivity of phospholipids to the oxidation process would be due to the structural phospholipids (i.e. those in cell membranes) that contain high levels of PUFAs (Ruiz et al., 2009), particularly PUFAs that possess three or more double bonds, which are more prone to oxidation than PUFAs with two double bonds (Mottram, 1998). The breakdown of phospholipids and generation of volatile compounds that correlated with warmed-over flavour have been reported (Meynier et al., 1998; Carr, 2007). Warmed-over flavours can be generated during the cooking process and post-cooking storage time (Erickson, 2002 and Pearson and Gray, 2009). Furthermore, among the phospholipids fractions, as mentioned before, phosphatidylethanolamine appears to play a major role in the development of warmed-over flavour; while phosphatidylcholine has only a minor role to play in the development of warmed over flavour (Pearson and Gray, 2009). This could be due to the ability of phosphatidylethanolamine to trap ferrous ion and precluding it from oxidised to ferric ion. This would be responsible for oxidisability of phosphatidylethanolamine (Kawakatsu, et al., 1984). Mottram et al. (1998) reported that

warmed over flavour compounds slightly increased when triglycerides have been removed from cooked beef, while these compounds were markedly increased after removing both triglycerides and phospholipids. Similar findings were shown in pork meat after removed total intramuscular lipids (Huang et al., 2010)

1.5 Colour Oxidation

Colour is one of the attributes of most interest to consumers in assessing and purchasing meat, because colour is the first noticeable quality attribute and as such it is often used to evaluate meat quality either in the home or at retail (Velasco and Williams, 2011). The variation of meat colour from different animal species is due to the presence of pigments. The main pigments responsible for giving meat colour are haemoglobin and myoglobin. The degree of meat colour is predominantly dependent upon the concentration and chemical state of component within these compounds and light reflectance (Totosaus et al., 2007).

Myoglobin is the main pigment present in meat and meat products. However, haemoglobin is a pigment present at low concentration. Myoglobin is a major globular protein and recognized as a rich source of iron. The iron structure and its chemistry have an effect on the interaction and alteration of colour (Guid and Castigliego, 2010). Meat myoglobin undergoes oxidation by interaction with oxygen and forms oxymyoglobin (Fe^{2+}), which gives meat its desirable bright red colour. Discolouration of meat under storage conditions is related to the oxidation of oxymyoglobin (ferrous Fe^{2+}) to metmyoglobin (ferric Fe^{3+}) which turns meat colour to brown (Mancini and Hunt, 2005). In general, meat colour is strongly affected by amount of myoglobin, although it is also influenced by storage conditions, pH, oxygen available, and temperature. Colour of meat has high association with pH value of meat. Hence, lightness of breast meat colour decreases with an increase in pH (Fletcher et al., 2000). As mentioned before,

discolouration of meat through a period of refrigeration is related to producing metmyoglobin (Laak, 1994). At low pH, the formation of methaemoglobin and deoxyhaemoglobin increased rapidly during the autoxidation of haem pigment in the meat (Richards and Hultin, 2000). Oxidation of myoglobin in fish meat at pH 6.0 was combined with reducing the redness and increasing the metmyoglobin content (Thiansilakul et al., 2011).

1.6 Oxidation Status Determination

At the commencement of the oxidation process unsaturated fatty acids undergo oxidative degradation and results in the formation of lipid hydroperoxides and conjugated dienes, which are referred to as primary lipid oxidation products (Gordon, 2001). During the subsequent stages of the lipid oxidation process elevated levels of secondary oxidation products, but relatively low levels of primary oxidation products can be observed (Estevez et al., 2009). The progress of lipid oxidation in meat can be monitored by following the progressive development of conjugated dienes, conjugated trienes and TBARS values. Conjugated dienes are indicators of primary oxidation; while conjugated trienes and TBARS value are indicators of secondary oxidation (Mensink and Plat, 2002; Gordon, 2001; Estevez et al., 2009).

1.6.1 Formation of Primary Lipid Oxidation Products

The principal site of oxidation in (poly)unsaturated fatty acids are the double bonds (Feiner, 2006). During the early stages of the oxidation process of polyunsaturated fatty acids, the double bonds migrate along the carbon chain and yield unconjugated dienes, which are relatively unstable compounds (Figure 1.3). These unconjugated dienes will chemically stabilize, yielding conjugated dienes (Estevez et al., 2009). The latter can be

measured and quantified by using spectrophotometric UV measurement at wavelength 232 nm (Pegg, 2005).

1.6.2 Formation of Secondary Lipid Oxidation Products

As an index of lipid oxidation, monitoring of secondary lipid oxidation products is often more appropriate instead of monitoring primary lipid oxidation products. Primary lipid oxidation products are less stable compared to secondary lipid oxidation products, and are colourless, odourless and tasteless (Estevez et al., 2009). Further decomposition of primary lipid oxidation products (such as fatty acid hydroperoxides) leads to the generation of secondary lipid oxidation products such as malondialdehyde and pentanal, hexanal, 4-hydroxynonenal, which do have noticeable and distinctive off-odours and flavours (Erickson, 2002). These secondary lipid oxidation compounds are extremely volatile and observable at very low levels (parts per billion) and considered indicators of rancidity and warmed over flavour (Estevez et al., 2009). Malondialdehyde (MDA) is an oxidation product produced from the breakdown of polyunsaturated fatty acids particularly those with three or more double bonds in their structure which can be measured by the TBARS test utilizing thiobarbituric acid as a reagent. The amount of this product can be measured by reacting one mole of MDA with two moles of thiobarbituric acid to produce a pink condensation product which absorb light at a wavelength range of 532-538 nm and the values are expressed as mg of MDA/kg sample. Byrne et al. (2001) reported that secondary lipid oxidation products provided a strong correlation with negative sensory attributes of meat.

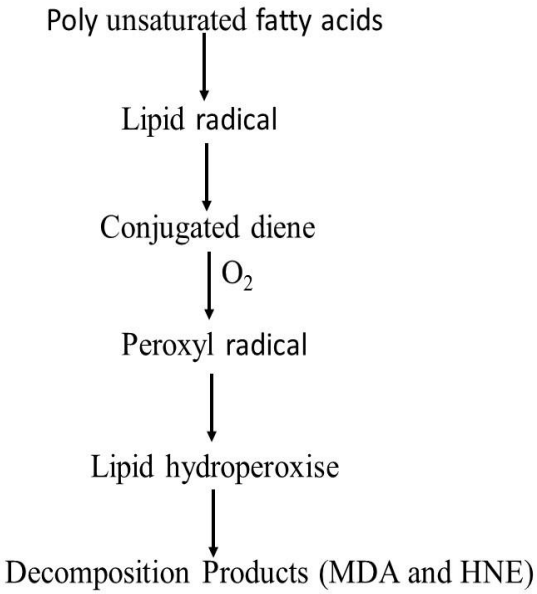

Figure 1. 3 Formation of primary and secondary lipid oxidation products. MDA is a malondialdehyde and HNE is a 4-hydroxynonenal (Adapted from Mensink and Plat, 2002).

Conjugated trienes are a by-product of secondary lipid oxidation products. The presence of conjugated trienes in fat is indicative of advanced lipid oxidation, where fatty acids containing three or more double bonds in their structure have undergone the conjugation process of multiple diene moieties (Wrolstad et al., 2005). Conjugated trienes are formed from the reduction of hydroperoxides produced from the PUFA oxidation as illustrated in Figure 1.4 (Gordon, 2001). Conjugated trienes have a substantial absorbance at 268 nm, which can be utilised to quantitate them. Foods containing high levels of polyunsaturated fatty acids are more likely to accumulate high levels of conjugated trienes when the lipids oxidise (Pegg, 2005).

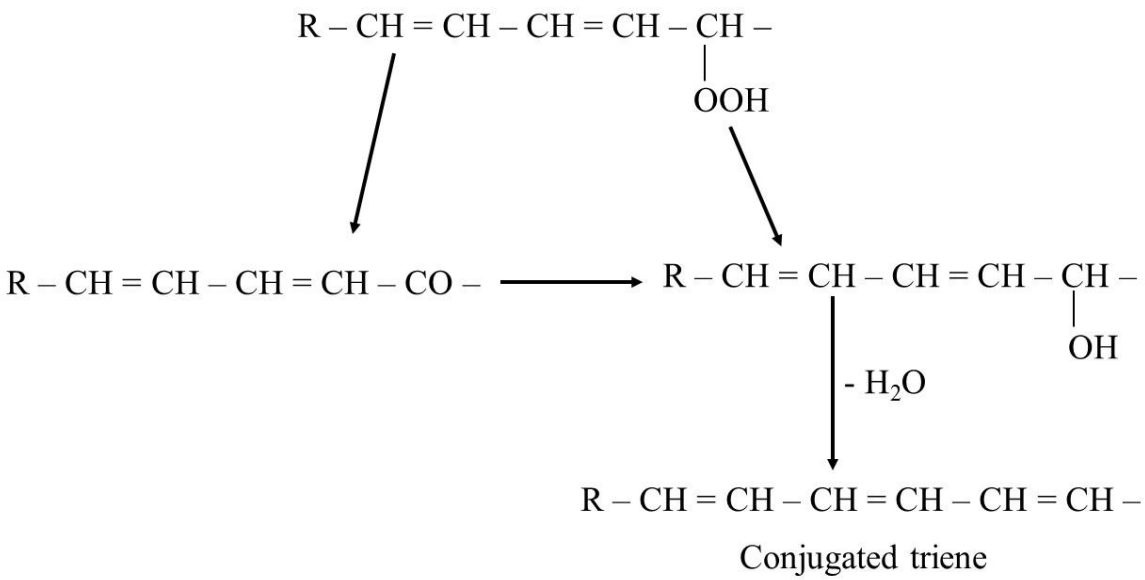

Figure 1. 4 Formation of conjugated triene from polyunsaturated fatty acids (Gordon, 2001).

Understanding, measuring and controlling of lipid oxidation in meat and meat products are of primary importance, because consumer demand for good quality products has increased within a world of rapid development of innovative processes and products (Bostsoglou et al., 1994). Several methods, such as vacuum packaging, freezing and refrigeration have been used to control and enhance the oxidative stability of food products. These methods can reduce lipid oxidation, but do not terminate the oxidation process (Laack, 1994). This inability to completely halt the lipid oxidation process is most likely due to the reality that trace amounts of oxygen are retained in the food during these processes which still leads to autoxidation. Therefore, it is of interest to also utilise chemical additives (natural or synthetic) to reduce the oxidation process and protect the quality of meat and its products. These additives are commonly known antioxidants.

1.7 Antioxidant Capacity Determination

The capacity of antioxidants cannot be determined directly but rather by the impact of a particular antioxidant on an oxidant (Gordon, 2001; Huang et al., 2005; Apak et al., 2013**).** There are various methods available to measure antioxidant capacity (Gordon, 2001). However, these methods vary from each other by using different substrate, oxidant, and conditions of reaction and means of quantification (Huang et al., 2005). The methods of measuring antioxidant capacity can be categorised based on the chemical reaction into two main groups: hydrogen atom transfer based methods and single electron transfer based methods (Gordon, 2001; Wu et al., 2004; Huang et al., 2005; Charles, 2013; Apak et al., 2013).

The mechanisms of hydrogen atom transfer based methods determine the capacity of antioxidants by the capability of antioxidant to quench free radicals such as peroxyl radicals by donating a hydrogen atom. Generally, these methods utilise antioxidants, synthetic free radicals and florescent probes (Huang et al., 2005; Apak et al., 2013), where the competition kinetics that occurs during the reaction between both antioxidants and fluorescent probe with peroxyl free radicals are observed and the antioxidant capacity can be quantified accordingly. Common hydrogen atom transfer based methods include: oxygen radical absorbance capacity (ORAC); crocin bleaching method; total radical trapping antioxidant parameter (TRAP); and β-carotene bleaching method. Among these, the ORAC method is widely applied for antioxidant quantification in research studies, food laboratories, and clinical applications (Charles, 2013), and is compatible to determine the capacity of both lipophilic and hydrophilic antioxidants (Wu et al., 2004).

Electron transfer reaction based methods are known colourimetric methods that can determine the capacity of antioxidant through the change in colour that occurred during the reduction of oxidising agents (oxidant) in the presence of antioxidants. The resultant

colour intensity is predominantly dependent upon the concentration of antioxidants (Huang et al., 2005; Charles, 2013; Apak et al., 2013). The most commonly employed electron transfer reaction based methods include electron acceptors such as: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid in the Trolox-equivalent antioxidant capacity (TEAC) method; 2,2-diphenyl-1-picrylhydrazyl (DPPH); a mixture of phosphomolybdate and phosphotungstate used in the Folin-Ciocalteu reagent; ferric ions in the ferric reducing antioxidant power (FRAP); and cupric reducing antioxidant capacity (Hall, 2001; Huang et al., 2005; Shan et al., 2005; Ghasemzadeh et al., 2010; Singh et al., 2014).

It has been suggested that these methods are suitable for screening only antioxidants and not useful for measuring the antioxidant capacity in food (Gorden, 2001). Hence, the antioxidant capacity can be measured by quantifying the amount of primary and secondary lipid oxidation products through methods such as TBARS values, peroxide value, conjugated dienes and trienes and fatty acids composition (Antolovich et al., 2002). To measure these oxidation products, oxidation substrates such as oil, animal fats and meat have been used (Khan and Shahidi, 2001: Zhang et al., 2010; Tavasalkar et al., 2012; Naveena et al., 2013; El-Shourbagy and El-Zahar, 2014). The following chapter (materials and methods) will provide more details about the above methods will be used in this work, TBARS values (section 2.2.3.4.1); conjugated dienes and conjugated trienes (section 2.2.3.4.2) and fatty acids (section 2.2.3.4.4).

1.8 Antioxidants

1.8.1 Definition of Antioxidants

Antioxidants are chemical compounds that have the ability (at low concentrations) to delay and impede the oxidation process by scavenging the free radicals, binding metal and/or quenching singlet oxygen that would otherwise enable the oxidation process (Reische et al., 2002; Huang et al., 2005). Antioxidants are used to protect against the oxidation-induced deterioration of rheological characteristics, rancidity and discolouration of food, including meat as a result of oxidation (Velasco and Williams, 2011). Antioxidants can occur naturally in foods or can be added during the manufacture of products. The role of antioxidants is not only to maintain the quality of the meat, but also to extend its shelf life and protect the nutritional value (Reische et al., 2002). The incorporation of the natural antioxidants in the diet has potential health benefits for consumers because they have the ability to reduce the attack of reactive oxygen species on the components of cells such as DNA, proteins, and membrane lipids in order to protect them (Su et al., 2007).

1.8.2 Classification of Antioxidants

1.8.2.1 Synthetic Antioxidants

Synthetic antioxidants are compounds that are chemically synthesized with the purpose to produce highly effective antioxidants that remain stable under a wide range of pHs, processing conditions, often at a lower cost compared to natural antioxidants (Fasseas et al., 2008; Rowe et al., 2007; Karre et al., 2013). These compounds have been widely used for inhibition the lipid oxidation in food for a very long time (Che Man and Tan, 1999; Sasse et al., 2009). The introduction of synthetic compounds into food industries were started in the 1940s, and BHA, BHT and gallates were the first compounds introduced

for preservation purposes (Kraybill et al., 1949; Gearhart and Stuckey, 1955; Yanishlieva-Maslarova, 2001).

There are various synthetic antioxidants that have been approved for application in food include butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), propyl gallate (PG), octyl and dodecyl gallate, ascorbyl palmitate, ethoxyquin, and tertiary butyl hydroquinone (TBHQ) (Che Man and Tan, 1999; Middleton et al., 2001; Martinez et al., 2013; Taghvaei, et al., 2014; Javidipour et al., 2015). Among these, BHT, BHA, PG and TBHQ have been extensively employed in various food industries (Che Man and Tan, 1999; Reische et al., 2002; Sasse et al., 2009; Carocho and Ferreira, 2013). Despite the many positive attributes, several reports have pointed out that the application of synthetic antioxidants in food have negative attributes (Chen et al., 1992; Kahl and Kappus, 1993; Reische et al., 2002; Cordova et al., 2011).

*1.8.2.2 Natural A**n**tioxidants*

More recently, the focus of research has been on the use of natural antioxidants. This is mainly because consumers prefer, and place increasing demands on, foods containing natural ingredients instead of synthetic ones because of the perceived health benefits (Fasseas et al., 2008), improved nutritional value and the enhanced quality of meat (Velioglu et al., 1998; Velasco and Williams, 2011), and a greater general ecological and environmental awareness. In addition, the natural sources used as antioxidants have been used widely whether in folk medicine or employed as traditional food ingredients and additives in various recipes (Chan et al., 2011; Shahidi and Ambigaipalan, 2015). A further reason for the move towards natural antioxidants is to avoid reported carcinogenic side effects associated with long-term consumption of some synthetic antioxidants (Altmann et al., 1986; Van, 1986; Chen et al., 1992; Gharavi, et al., 2007).

The term “natural antioxidants” means that these antioxidative compounds are obtained from natural sources such as vegetables, fruits, seeds, spices and herbs. Most natural antioxidants are phenolic compounds, flavonoids, and also some vitamins that contain a suitable molecular structure that is chemically able to scavenge free radicals, reduce ferric /antioxidant power (FRAP) and chelate metal (Shan et al., 2005; Velasco and Williams 2011; Liang et al., 2012). Phenolic compounds in plants have various biological functions such as antioxidant, anti-inflammatory, anti-cancer and anti-bacterial properties (Manthey and Grohmann, 2001). Antioxidants obtained from natural sources have the ability to enhance meat quality, minimize unwanted changes that occur during storage and retain nutritional value (Velasco and Williams, 2011). As such, natural antioxidants can perform similar or better than synthetic antioxidants (Selani et al., 2011; Naveena et al., 2008)

1.8.3 Mechanisms of Antioxidants' Action

The ability of antioxidants to retard oxidation is mediated through three different mechanisms: [1] scavenging of free-radical; [2] quenching singlet oxygen; and [3] chelating metals (Reische et al., 2002). The antioxidant substances can retard lipid oxidation in the initiation and propagation steps by reacting with lipid free radicals and free radicals to form stable and non-free-radical products (Huang et al., 2005). As mentioned before in section 1.8.1, Reische et al. (2002) reported that antioxidants donate hydrogen atoms to the peroxy- and oxy- free radicals, which are produced during the autoxidation propagation phase. Furthermore, antioxidants have the ability to react directly with lipid radicals that are formed during the initiation phase and convert these into non-radical products as illustrated in Figure 1.5. On the other hand, Huang et al. (2005) reported that the antioxidant radical (A•) formed can react with lipid but only very slowly.

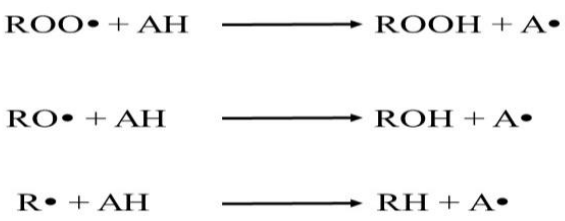

Figure 1. 5 Mechanisms of controlling lipid oxidation by antioxidants (Adapted from Reische et al., 2002). Where: AH is antioxidant, ROO• is a peroxyl free radical, A• is antioxidant radical, ROOH is lipid hydroperoxide, and RO• is alkoxy radical, R• is a lipid free radicals, and RH is a fatty acid.

The ability of antioxidants to retard oxidation is their ability to quench singlet oxygen. Singlet oxygen is a form of oxygen which possess a high energy and highly reactive (Frankel, 1980), which can be generated either chemically, photo-chemically, or through the reduction of hydroperoxides (Min and Boff, 2002). As mentioned before in section 1.2 and 1.3.3, singlet oxygen can be formed by various means: in the presence of light and photosensitizers (Huang et al., 2005). It has been shown that the generation of a singlet oxygen is at the source of photo-oxidation of unsaturated fatty acid and ultimately produce hydroperoxides (Reische et al., 2002). Antioxidants could quench or inactivate singlet oxygen by both physical and chemical means (Decker, 2002). Quenching can be defined as a process of transferring and reducing energy or stopping a chemical reaction between a molecule in a chemically exited state (i.e. singlet oxygen) and a receptive recipient molecule (i.e. PUFA) (Decker, 2002), By physical means singlet oxygen can be quenched by absorbing or shifting its excitation energy to antioxidant molecules producing endo-peroxides (Huang et al., 2005). β-carotene is considered an excellent quenchers of singlet oxygen, which has ability to receive the energy from singlet oxygen (Decker, 2002). Singlet oxygen can be quenched by chemical means particularly in the presence of tocopherol, ascorbic acid and β-carotene. For instance, tocopherol can

inactivate singlet oxygen by chemical means in reactions that lead to produce tocopherol epoxides and peroxides (Decker, 2002).

Yet another mechanism by which antioxidants are believed to act is by chelating metals (Reische et al., 2002). Some metals that can occur in two (or more) distinct oxidation stages, and the more oxidised stage can induce lipid oxidation through a redox-based interaction in which the metal becomes reduced and the lipid becomes oxidised (Decker, 2002). Antioxidants can prevent oxidation of lipids through reducing the metal's redox potential and preventing the interaction of metals with the lipid's hydroperoxide by forming a metal-antioxidant complex. Furthermore, several non-conventional antioxidants, such as citric acid and phosphoric acid, have the ability to inactivate metals (Reische et al., 2002). These compounds can reduce the ability of metals to oxidize lipids through binding metals through their multiple carboxylic acid groups and as such forming an organometallic complex.

1.8.4 Phenolic Compounds

Phenolic compounds are defined as substances that possess an aromatic ring with one or more hydroxyl groups (Johnson, 2001). The main phenolic compounds from plants are phenolic acids, flavonoids and, tannins, and complex compounds such as diferuloylmethane (curcumin) (Figure 1.6) (Han et al., 2007).

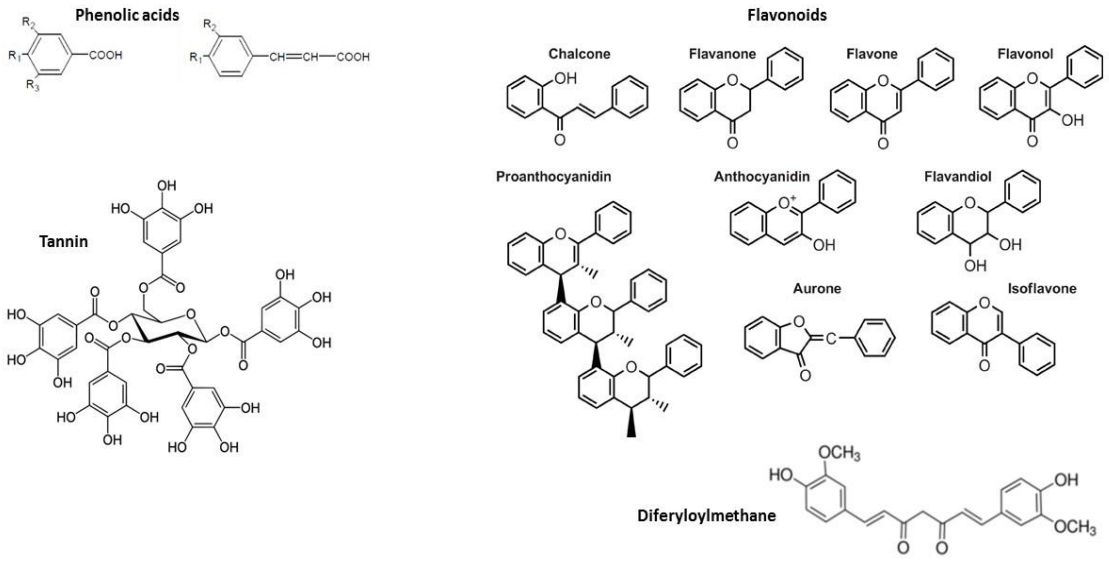

Figure 1. 6 Chemical structures of the main phenolic compounds

Phenolic acids are a major class of phenolic compounds (Ghasemzadeh et al., 2010), which are classified into two main groups, i.e. hydroxybenzoic and hydroxycinnamic acids. These compounds can be found in various natural sources such as vegetables, fruits, seeds, spices and herbs. Several studies have identified the phenolic compounds in rosemary, ginger, small red bean and sunflower seed extracts (Luthria et al., 2006; Lin et al., 2008; Hernandez-Hernandez et al., 2009; Baker et al., 2012; Amakura et al., 2013; Varakumar et al., 2017). The main phenolic compounds isolated from these extractions are shown in Table 1.3. Of these compounds, some are present as a lipophilic or hydrophilic form and other compounds are clearly amphiphilic (e.g. naringin and catechin) (Baker et al., 2012; Varakumar et al., 2017). The major phenolic compounds in rosemary, sunflower seeds and ginger extracts are lipophilic. Indicating that many of these compounds can be found in their essential oils (Hernandez-Hernandez et al., 2009; Baker et al., 2012; Amakura et al., 2013; Varakumar et al., 2017), which would be likely to have a more lipophilic antioxidant capacity. In contrast, small red bean contains more hydrophilic antioxidative compounds (Luthria et al., 2006; Lin et al., 2008). Wu et al. (2004) determined the capacity of antioxidants based on lipophilic and hydrophilic

compounds in ginger and small red bean and found that ginger had three times the lipophilic antioxidant capacity compared to its hydrophilic antioxidant capacity; while small red bean had an almost 40 times higher hydrophilic antioxidant capacity compared to its hydrophilic antioxidant capacity. Lipophilic compounds (e.g. carnosic acid) extracted from rosemary have a higher antioxidant activity than hydrophilic compounds (e.g. rosmarinic acid) according to the DPPA and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging test (Erkan et al., 2008). Moreover, predominant phenolic compounds in ginger that act as antioxidants are zingiberene, (Baker et al., 2012) and 6-gingerol (Nile and Park, 2015). In the latter study, it was found that lipophilic compound (e.g. 6-gingerol) had a higher antioxidant capacity according to the DPPH, FRAP and H_2O_2 tests than the other lipophilic compound (e.g. 6-shogaol). These results indicate that the antioxidant capacity of lipophilic compounds is stronger than hydrophilic compounds and that the antioxidant capacity of particular compounds can be different from the others regardless of their hydrophilicity.

Table 1. 3 Antioxidant compounds isolated from the extraction of natural sources

Species and systematic names		**Phenolic compounds**
Rosemary (*Rosmarinus officinalis*)	Hydrophilic	rosmarinic acid
	Lipophilic	carnosic acid, carnosol, rosmanol, crsimaritin, genkwanin (Hernandez-Hernandez et al., 2009; Baker et al., 2012).
Small red bean (*Phaseolus vulgaris*)	Hydrophilic	Ferulic acid, cyanidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-(6-malonyl) glucoside, pelargonidin 3-*O*-(6-malonyl) glucoside, cyanidin, kaempferol 3-*O* xylosylglucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-(6-*O*-malonyl) glucoside, kaempferol 3-*O*-(malonyl) glucoside, pelargonidin. (Luthria et al., 2006; Lin et al., 2008).
	Lipophilic	*p*-coumaric acid, sinapic acid, kaempferol (Luthria et al., 2006; Lin et al., 2008).
Sunflower seeds (*Helianthus annuus*)	Hydrophilic	4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid (Amakura et al., 2013).
	Lipophilic	caffeic acid, methyl caffeoate, chlorogenic acid, methyl chlorogenate, 3,5-di-*O*-caffeoylquinic acid, and eriodictyol 5-*O*-β-d-glucoside, benzyl alcohol β-d-apiofuranosyl-(1-6)-β-d-(4-*O*-caffeoyl) glucopyranoside. (Amakura et al., 2013).
Ginger (*Zingiber officinale*)	Amphiphilic	naringin, catechin (Baker et al., 2012; Varakumar et al., 2017)
	Lipophilic	gingerol, zingiberene, α-curcumene, α-farnesene, ß-sesquiphellandrene, shogaol, 6-gingerol, 6-shogaol, 8-gingerol and10-gingerol. (Baker et al., 2012; Varakumar et al., 2017)

1.8.5 Extraction of Phenolic Compounds

As mentioned before, antioxidants can be roughly divided into two groups: lipophilic and hydrophilic antioxidants (Wu et al., 2004). In most instances, both types can be found in the same source (Table 1.3). Extraction efficiency of phenolic compounds from different sources depends more on the solvent polarity (Akowuah et al., 2005), with many hydrophilic phenolic compounds being extracted at a greater efficiency in a water-based solvent; while lipophilic phenolic compounds being extracted at greater efficiency in lipophilic solvents (Puangsombat and Smith, 2010). Extraction of antioxidant compounds such as phenolic acids and flavonoids from plants has been conducted in several studies (Tavassoli and Djomeh 2011; Rodríguez-Rojo et al., 2012; Do et al 2014; Shah et al., 2014). Total phenolic content extraction yield and antioxidant activity is strongly associated with the type of solvent used. For instance, the extraction of phenolic compounds from ginger was higher when polar solvents such as methanol and acetone were used compared a non-polar solvent (chloroform) Ghasemzadeh et al. (2010). In addition, polar solvents such as methanol has been used to extract polyphenols from several plants that have a lower molecular weight; while for the extracting of higher molecular weight flavanols, aqueous acetone is more efficient (Dai et al., 2010).

The yield of extraction from plants depends more on technique, solvent type, pH, heating process, times of extraction and composition of the sample (Do et al., 2014). Hence, to obtain the highest yield of substances and recover antioxidants from natural sources, several techniques have been used such as soxhlet extraction (Tavassoli and Djomeh 2011), microwave assisted extraction, conventional solvent extraction, ultrasound assisted extraction (Rodríguez-Rojo et al., 2012) and supercritical fluid extraction (Shah et al., 2014). The yield of rosemary extraction by ethanol was higher in the microwave-assisted extraction method than by ultrasound or extraction with organic solvents

(Rodríguez-Rojo et al., 2012). Moreover, a variety of solvents either independently or in combination have been used to prepare the extraction from plants. Typically used solvents include ethanol, methanol, dimethyl sulfoxide, acetone and hexane (Shah et al., 2014), ethyl acetate and chloroform (Hall, 2001). Application of a specific solvent and plant-solvent ratios appropriate for recovering all phenolic compounds from all plants have not been elucidated. However, according to Shah et al. (2014), ethanol is frequently used for extraction purposes, because it is a permitted solvent for additives and non-toxic for human consumption. The combination of water and organic solvent have been recommended in a range of research studies; this may facilitate the recovery of compounds that are soluble in solvent or in water (Sutivisedsak et al., 2010; Dai et al., 2010; Puangsombat and Smith, 2010). Hence, utilization of a specific ratio of aqueous solvents depends predominantly on the type of plant, or the target compounds. As mentioned before, beans contain a considerably higher amount of hydrophilic antioxidant compounds than lipophilic (Wu et al., 2004). Therefore, in order to extract both hydrophilic and lipophilic fractions the correct ratio of ethanol and water is required Accordingly, Sutivisedsak et al. (2010) showed that extracts from several varieties of beans using aqueous ethanol, a ratio of ethanol: water at (50:50 v/v) had higher total phenolic compounds compared to 100 % ethanol or 100 % water. Solubility of phenolic compound either in water or solvent depends on the –OH and –COOH groups (Rodríguez-Rojo et al., 2012). Anwar and Przybylski (2012) found that the amount of total phenolic compounds and highest free radical scavenging properties was observed in 80% ethanol extract. The highest reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was found in an 80 % methanol extract followed by an 80 % ethanol extract (Shirin and Jamuna, 2010). Moreover, the amount of phenolic content correlated with the temperature, which at a higher extraction temperature had a higher total phenolic content (Sutivisedsak et al., 2010). However, the heating methanol extract of peanut hulls

at 185 ^{o}C for 30 min reduced the antioxidant activity by approximately 5 %, which may be due to the alterations of phenolic structure which decrease its ability to donate hydrogen (Hall, 2001). Furthermore, the increment of pH from 3 to 9 reduced the effectiveness of the methanol extract of peanut hulls, while at pH 7.0 antioxidant activities remained at around 80 %, at pH 9.0 it was entirely lost (Hall, 2001).

1.9 The Effect of Natural Antioxidants Application on Lipid Oxidation and Meat Quality

Rosemary, ginger, small red bean and sunflower seed extract contain elevated total levels of phenolic. These can slow down the rate of lipid oxidation, bind metallic compounds and scavenge free radical. The following sections will provide an overview of the natural antioxidant sources used in this work.

1.9.1 Rosemary

The rosemary plant *(Rosmarinus officinalis)* belongs to the Lamiaceae family. This plant is widely used in folk medicine and employed as a food ingredient and additive in various recipes and formulations due to its flavour or its health benefit (Yanishlieva-Maslarova, 2001; Berdahl and McKeague, 2015). The antioxidant properties of rosemary are well known and as such rosemary is considered a protector of lipid oxidation, metal chelator and free radical scavenger (Yanishlieva-Maslarova, 2001). The application of rosemary has also been well documented as a natural antioxidant in meat and meat products either used in either an unprocessed form or in extracted form (Chen et al., 1999; Mielnik et al., 2003; Rojas and Brewer, 2007). Extensive work has observed that rosemary and rosemary extract can inhibit the autoxidation of lipids and development of off-odour and off-flavour (including warmed-over flavour) in meat and meat products at concentrations from 0.02 to 1 % (McCarthy et al., 2001; Ahn et al., 2002; Mielnik et al., 2003; Nissen, et al., 2004; Ahn et al., 2007; Abou-Arab et al., 2010; Colindres and Brewer, 2011; Lara et al., 2011;

Naveena et al., 2013). The effectiveness of the rosemary antioxidants is variable in terms of reducing the rancidity and maintaining the quality of meat. These most likely depend on the application methods being employed (Table 1.4).

Rosemary slows down the oxidation of lipids during the storage period by reducing 2-thiobarbituric acid (TBARS value) and hexanal formation when applied to beef and mechanically deboned turkey meat (Ahn et al., 2002; Mielnik et al., 2003; Ahn et al., 2007). Rosemary also reduced the peroxide values in ostrich, chicken and buffalo meat (Abou-Arab et al., 2010; Naveena et al., 2013). TBARS values did not significantly ($p > 0.05$) differ from control when adding rosemary at 0.02 % to beef and pork patties (Rojas and Brewer, 2007). Several studies have demonstrated that the effect of rosemary was concentration dependent (Ahn et al., 2002; Mielnik et al., 2003; Naveena et al., 2013). For instance, cooked beef pre-treated with different concentrations of rosemary in the range of 0.02, 0.05 and 0.10 % (w/w) reduced the formation of both TBARS and hexanal by approximately 25, 36 and 46 %, and 51, 62 and 73 %, respectively (Ahn et al., 2002). McCarthy et al. (2001) found that the addition of rosemary extract at 0.10 % to ground beef and pork and packaged in oxygen permeable film reduced TBARS values by approximately 50 % compared to control samples. However, in the study conducted by Baker et al. (2012), the application of antioxidant at lower dose of 0.05 % was found to have more antioxidant activity than both application levels (0.075 and 0.10 %) respectively in lamb meat. Furthermore, the formation of TBARS and PV values were decreased ($p \leq 0.05$) in samples containing a combination of L-ascorbic acid + rosemary and α-tocopherol + rosemary compared with individual antioxidants (Abou-Arab et al., 2010). Several studies found the effects of rosemary as natural antioxidant more efficient than synthetic preservatives (McCarthy et al., 2001; Colindres and Brewer, 2011; Lara et al., 2011). Lara et al. (2011) compared the natural antioxidant activities of rosemary extracts with synthetic antioxidants (BHT) in cooked pork patties, for which monitoring

TBARS and hexanal methods were used. The authors observed that the reduction of lipid oxidation in rosemary treatment was higher than synthetic antioxidant treatments. According to McCarthy et al. (2001), raw and cooked pork patties samples containing rosemary extract 0.10 % had a greater reduction of lipid oxidation than those containing BHA/BHT (0.01 %). Treated pork patties with 30 mg of rosemary extract /100 g meat before cooking reduced TBARS and hexanal by approximately 90.7 and 94.1 %, followed by BHT 76.3 % and 87.0 %, respectively (Lara et al., 2011).

The effectiveness of antioxidants to reduce the discoloration of meat has been reported by Ahn et al. (2007), who found that beef meat samples treated with oleoresin rosemary had significantly higher lightness (L*), and yellowness (b*) values, but less redness (a*) value compared to the control. However, the variation of colour in meat may influence the perception of the consumer. Rosemary extract at 0.10 % was found more effective in protecting the redness of pork raw meat at day 9 of storage compared to the negative control but did not differ from control in cooked meat over storage time (McCarthy et al., 2001). According to the study conducted by Colindres and Brewer (2011), rosemary extract and storage time had no effect on (L*), and (b*) values in cooked, frozen and reheated beef patties over 6 months of frozen storage time. Whilst rosemary had the ability to protect red colour (a*) value up to 3 months and no change was observed after 6 months. These authors also mentioned that after the addition 0.2 g/kg of oleoresin rosemary extract reduced grassy, rancid odour and beef flavour of cooked, frozen and reheated beef patties compared to control. Over 6 months of the frozen storage period, rosemary and BHA had better preservation of beef flavour than BHT. The addition of rosemary markedly reduced the formation of metmyoglobin in ostrich and buffalo meat compared to the control over storage time (Abou-Arab et al., 2010; Naveena et al., 2013).

Table 1.4 Effect of applications of rosemary and ginger as natural antioxidants on the quality of meat and meat products

Treatment	Concentration	Meat products	Fat %	Storage conditions	Results	Reference
Rosemary extract	0.10 %	Raw and cooked pork patties	-	Packaged in oxygen permeable film, 4 °C, FL (616 lux), 9 days	Reduced TBARS values by approximately 50 % compared to control samples and was found to be higher effective than (BHA/BHT) (0.01 %). The redness a* values in rosemary treatment >BHA/BHT.	McCarthy et al. (2001)
Oleoresin rosemary	0.02, 0.05 and 0.10 %	Cooked, ground beef		4 °C for 3 days	Reduced TBARS value and hexanal content (up to 25, 36 and 46 %) and (up to 51, 62 and 73 %)	Ahn et al., (2002)
Guardian rosemary extracts GP flavour guard LO	0.2, 0.5 and 0.8 g/kg	Mechanically deboned turkey meat	15.30 %	Transparent PE cups, −25 °C, 7 months	Reduced both TBARS, hexanal values and total volatiles and the reduction was increased with increased doses. Biolox HT-W was more effective than ascorbic acid.	Mielnik et al. (2003)
Herbalox W, Stabiloton WS Biolox HT-W	0.8, 1.6 and 2.4 g/kg					
Rosemary extract	200 ppm	Cooked pork patties	25%	Packaged in PE film, 4.5 °C in the dark, 10 days	TBARS values and hexanal values were reduced, and the effectiveness of antioxidants was in order: rosemary > grape skin > tea > coffee > control and maintained sensory eating quality	Nissen, et al. (2004)
Oleoresin rosemary (Herbalox)	1 %	Cooked beef	18%	Packaged in sterile plastic bags and stored at 4 °C for 9 days	TBARS and hexanal values were reduced ($p \leq 0.05$) in samples contained oleoresin rosemary. Decreased the redness and increased the lightness and yellowness compared to control.	Ahn et al. (2007)

Table 1. 4 (continued) Effect of applications of rosemary and ginger as natural antioxidants on the quality of meat and meat products

Treatment	Concentration	Meat products	Fat %	Storage conditions	Results	Reference
Rosemary (Herbalox Seasoning HT-25)	0.02 %	Ground beef and pork	30 %	Vacuum packaged, -18 °C, 4 months	TBARS values, sensory attributes in meat treated with rosemary were not significantly differed (P>0.05) compared to the control	Rojas and Brewer (2007)
Rosemary powder	0.05 %	Ostrich steaks	1.5 %	Packaged in plastic film under vacuum, 4 °C, 21 days	The formation of TBARS and PV values were decreased ($P\leq$ 0.05) in samples contained a combination of L-ascorbic acid + rosemary and α-tocopherol + rosemary. α-tocopherol + rosemary had a significant lower formation of metmyoglobin.	Abou-Arab et al. (2010)
Oleoresin rosemary	0.2 g/kg	Beef patties	0.15 g/kg WB	Packaged in PVC film, −18 ◦C in the dark, 6 months	Rosemary reduced TBARS value cooked and reheated meat and observed more antioxidant activity than BHT and BHA.	Colindres and Brewer (2011)
Rosemary extract	30 mg/100 g meat	Cooked pork patties		PE/ethylvinylalcohol, 70 % N+30 % CO2, 4 °C, 600 lx, 6 days	Rosemary was reduced TBARS of 90.7 followed BHT 76.3 %, and lemon balm extracts 74.8 % and hexanal 94.1, 87.0, 85.4 %. Rosemary treatment had a lower protein oxidation, with lower pH and less cooking loss, higher a* values observed in rosemary treatments than control and BHT.	Lara et al. (2011)
Rosemary extract	0.05, 0.075, 0.1%	Lamb patties		PE film, 4 °C, 7 days	TBARS was reduced significantly in meat treated with rosemary extract compared to the control, 0.05 % had a highest antioxidant effect.	Baker et al. (2012)
Carnosic acids	22.5 and 130 ppm	Cooked chicken and buffalo patties		4 °C, 9 days for raw and 28 for cooked	Reduced TBARS values in raw and cooked meat, 130 mg had a highest reduction effect. Reduced the formation PV reduced while free fatty acids were reduced only in cooked buffalo meat. Reduced metmyoglobin formation in raw buffalo meat compared to the control.	Naveena et al. (2013)

Table 1. 4 (continued) Effect of applications of rosemary and ginger as natural antioxidants on the quality of meat and meat products

Treatment	Concentration	Meat products	Fat %	Storage conditions	Results	Reference
Ginger extract	1 ml of extract / 10 g meat	Raw and cooked pork patties		4 ^{o}C for 7 days and - 18 ^{o}C, 6 months	TBARS values were reduced significantly in raw and cooked pork meat treated with ginger extract stored under both storage conditions.	El-Alim et al. (1999)
Ginger extract	0.5, 0.75 and 1 %	Lamb patties		PE film, 4 ^{o}C, 7 days	TBARS and metmyoglobin formation was reduced significantly in meat treated with ginger extract at different concentrations compared to the control, 0.5 % had a highest antioxidant effect.	Baker et al. (2012)
Ginger extract	3 % extract	Raw chicken meat		4 ^{o}C, 9 days	Reduced the formation of free fatty acids, peroxide and TBARS values over 9 days of storage period	Singh et al. (2014)

PVC, Polyvinyl chloride film; PE, polyethylene; FL, fluorescent light; WB, weight basis; PV, peroxide value

1.9.2 Ginger

Ginger (*Zingiber officinale*) is one of the plants which have been widely used as a spice and flavouring agent in food processing and traditional medicine (Chan et al., 2011). Ginger has been shown to contain a total phenolic content of 3.17 mg gallic acid equivalent (GAE)/g dry weight (Wu et al., 2004). The antioxidant capacity of ginger extracts correlates strongly with total phenolic content (Chan et al., 2011). The antioxidant compounds that are found in ginger are both lipophilic and hydrophilic in nature, with values of 218.67 and 69.44 µmol trolox equivalents/g, respectively (trolox is a measure for lipophilic antioxidants) (Wu et al., 2004). Indicating a significantly greater proportion of lipophilic antioxidant power over the hydrophilic ones in ginger. Ginger extract has been tested as a natural antioxidant *in vivo* and *in vitro* experiments and also in various meat post slaughter (El-Alim et al., 1999; Naveena et al., 2004; Ghasemzadeh et al., 2010; Maizura et al., 2011). Phenolic compounds in ginger have an ability to scavenge free radical, inhibit lipid peroxidation and possess high ferric reducing power (Chan et al., 2011). The free radical scavenging capacity significantly increased approximately 75 % with increasing the concentration of ginger from 0.50 to 0.75 % (Kishk and El Sheshetawy, 2010). The ability of the antioxidants in ginger (*Zingiber officinale*) extract to scavenge the free radical *in vitro* reached 79 % with a ferric reducing/antioxidant power (FRAP) to 26.2 µmol Fe (II)/g compared to the turmeric and kesum antioxidant capacity (Maizura et al., 2011).

In the study conducted by Ghasemzadeh et al. (2010), extract from ginger has a strong free radical scavenging activity by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test which reached to 31.45 to 58.22 % to the commercial antioxidants BHT and α-tocopherol of 96.21 and 89.57 %, respectively. The values of ferric reducing antioxidant potential in the ginger extract were markedly lower than α-tocopherol but at the same time higher

than BHT. Incorporating ginger at a concentration of 3 % into raw chicken meat emulsion had higher 1,1-diphenyl-2-picrylhydrazyl (DPPH % inhibition) radical scavenging and 2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid (ABTS % inhibition) than control samples (Singh et al., 2014). Ginger demonstrated the antioxidant activity due to retarding the lipid oxidation in meat (El-Alim et al., 1999; Naveena et al., 2004; Baker et al., 2012). El-Alim et al. (1999) reported that adding ginger extract to raw pork patties significantly reduced the TBARS value at 4 ^{o}C for 7 days and at-18 ^{o}C for 6 months. This study also found that it did not inhibit the formation of peroxide in raw pork patties under refrigeration storage. In contrast, the peroxide value ranged from 0.18 to 0.70 milliequivalents /kg meat compared to the control which ranged from 0.55 to 4.60 milliequivalents /kg meat over 6 months of frozen storage. Lipid oxidation reduced in Muscovy duck breast meat after being submerged in the ginger extract for 14 days at 5 ^{o}C (Tsai et al., 2012). The formation of peroxide and TBARS were inhibited significantly in cooking pork patties supplemented with ginger extract at a concentration of 1 ml/10 g meat and stored in both refrigeration and frozen storage (El-Alim et al., 1999). According to the results reported by Baker et al. (2012), TBARS values were found to be significantly reduced in lamb meat patties treated with ginger extract at 0.50, 0.75 and 1 % (TBARS value ranged 0.34-0.98 mg MDA/kg meat in treated samples and 0.88-2.89 mg MDA/kg in control), while the concentration of extract at a level of 0.50 % ginger extract was found to have more impact on the TBARS over 7 days of storage time. Ginger extracts have an effect on reducing autoxidation in sunflower oil stored either during heating or storage time (Al-dalain et al., 2011); sunflower oil treated with ginger extract at a concentration of 400 ppm was found to have a lower peroxide, TBARS, conjugated diene and conjugated triene values than control and BHT treatment over 8 weeks of storage at ambient temperature, while no significant difference was found among antioxidants after being heated at 180 ^{o}C for 18 hr. The oxidation of lipid and myoglobin

to metmyoglobin reduced in lamb patties either treated with ginger at a level 0.50 % alone or combined with sodium lactate over 150 days at -18 ^{o}C (Baker et al., 2013). Ginger supplementation at a concentration of 3 % to raw chicken meat emulsion reduced the formation of free fatty acids, peroxide and TBARS values over 9 days of storage time (Singh et al., 2014).

In addition, application of ginger extracts can enhance physical and sensory properties of meat. For instance, Naveena and Mendirratta (2004) found that marinating buffalo meat chunks in ginger extract at 0, 3, 5 and 7 % v/w for 2 days at 4 ^{o}C enhanced the cooking meat yield and significantly decreased shear force values, water-holding capacity and enhanced sensory evaluation attributes flavour, juiciness, tenderness acceptability score. In another study conducted by Naveena and Mendirratta (2001), the addition of ginger at 1, 3 and 5 % v/w to post-chilled spent hen breast meat chunks increased moisture content, cooking yield, total pigments, and water holding capacity, while it reduced the shear force value, however higher concentration had more effectiveness. These authors also found the addition of ginger to pre-and post-chilled spent hen breast meat chunks and cooked in a gas tandoor oven enhanced the appearance, flavour, juiciness, tenderness score compared to samples without ginger extract. Ginger extracts were found to have an effect on pH value in chicken meat according to work published by Goswami et al. (2014) who pointed out that chicken meat treated with ginger at 4 % had a higher pH value compared to the control samples over 6 days of storage time. A marked increase was observed in the pH value of cooked spent hen breast meat chunks with increasing the ginger concentrations from 1% to 5 % v/w (Naveena and Mendirratta, 2001).

1.9.3 Sunflower Seeds

The Sunflower plant (*Helianthus annuus*) belongs to the family Asteraceae (Fan and Michael Eskin, 2015). Sunflower seeds are a rich source of the phenolic compounds

which have been shown to have antioxidant capacity (Liang et al., 2013). The average phenolic and tocopherols compounds in sunflower is 15.97 and 0.211 mg/g DM, respectively. Phenolic compounds separated by HPLC from sunflower seeds are rosmarinic acid, chlorogenic acid, myricetin and rutin, while the most abundant phenol shown is chlorogenic acid 10.46 mg/g DM (Zilic et al., 2010). These authors also reported that phenolic compounds varied among three varieties of sunflower such as Petunia, Allium and Albarte. According to the results of Chen and Ho (1997), the action of caffeic acid as an antioxidant is stronger than chlorogenic acid. These authors also reported that the antioxidant capacity of natural source extracts correlated strongly with their hydroxyl groups. Acid or ring groups of phenolics are responsible for chelating metals (Decker, 2002). In view of this, rosmarinic acid was found to have the strongest DPPH scavenging activity due to rosmarinic acid possessing four hydroxyl groups (Chen and Ho, 1997). However, revisable results were reported by Zilic et al. (2010) who claimed that rosmarinic acid in sunflower samples negatively correlated with chlorogenic acid.

Sunflower extract was found to possess an elevated ability to scavenge free-radical activity *in vitro* experiments at 0.16 mg/g and a very strong reducing power at 1 mg/g (Kosinska and Karamac, 2006). There is a strong correlation between the results of ABTS, DPPH and Fe^{3+}/ ferricyanide and phenolic content, this is due to a higher amount of phenolic compounds such as 5-*O*-caffeoylquinic acid, dicaffeoylquinic acid and caffeoyl-dimethoxycinnamoylquinic acid (Karamac et al., 2012). Sunflower seeds were found to have a stronger DPPH scavenging activity than sunflower kernel. However, the kernel had the greatest amount of phenolic compounds (Zilic et al., 2010).

The activities of antioxidants may depend more on phenol than total phenolic compounds. Sterols have shown to play an important role in inhibiting polymerization and thermal degradation of oil during the frying applications, while tocopherols have the ability to

donate hydrogen and serve as radical-trapping antioxidants (Hall, 2001). Most of the research has demonstrated the efficiency of sunflower seed extract in *in vitro* tests, until now, to our knowledge, application of this antioxidant to chicken meat post-slaughter has not been well-documented. However, sunflower has been used in poultry diet as a potential source of proteins, fibre, and amino acids, e.g. cysteine and methionine (Tsuzuki et al., 2003). Therefore, there is a need to conduct this study to assess the efficiency of sunflower seed application in chicken meat.

1.9.4 Small Red Bean

Small red bean (*Phaseolus vulgaris*) is one of the common beans belonging to the family Leguminosae. The cultivation and consumption of this bean has increased throughout the world, due to its potential source of proteins, fat and crude fibre (Siddiq et al., 2010), its capacity to reduce the risk of coronary heart disease, diabetes, and obesity, to decrease serum cholesterol concentration, anti-proliferative effects against human ovarian cancer cells (SK-OV-3), human colon cancer cells (SW480), tongue cancer cells (CAL 27), and hepatocarcinoma cells (Hep G2) (Zou and Chang, 2014).

Small red bean is considered one of the leguminous seeds that possesses high antioxidant capacity. Among 20 kinds of food, small red bean found to have the highest total phenolic content of 11.85 mg GAE/g dry weight basis (Wu et al., 2004). Among various beans, the small red bean had a highest total content of flavonoid and kaempferol 3-*O*-glucoside (Lin et al., 2008). Luthria et al. (2006) pointed out that small red bean contains ferulic acid of 17.4 mg/100g. Several studies have evaluated the antioxidant capacity of small red bean extract as a natural antioxidant in *in vitro* experiment (Chou et al., 2003; Zou and Chang, 2014). Small red bean had a strong free radical scavenging activity against DPPH and ABTS radicals, with the IC50 of 0.128 and 0.036 mg/ml, respectively (Zou and Chang, 2014). *In vitro* experiment reported by Chou et al. (2003) showed that the

extract from red bean has a strong free radical scavenging activity at a dosage of 0.62 to 10 mg/ml, and the metal chelating impact of red bean extract at a dosage of 0.25 mg/ml reached 81.7 % compared to the commercial antioxidants (BHT and α-Tocopherol). In their study, they also found that the reducing power increased with increments in the concentration of red bean extract. The application of small red bean as a source of antioxidant to chicken meat post-slaughter has not been well-documented. Therefore, it is important to conduct this study to assess the efficiency of small red bean application in chicken meat.

1.10 Summaries from Literature Review

- Lipid, phospholipid and protein oxidation is a primary concern in meat products. It can lead to the development of off odours, off flavours and discoloration of meat, reduction in shelf-life, loss of quality and a decrease in nutritional value. The presence of lipids and phospholipids that contain high levels of unsaturated fatty acids makes poultry more susceptible to oxidation process. Moreover, phospholipid-like lipids polyunsaturated fatty acids undergoes oxidation by a free radical mechanism.
- Application of antioxidants to reduce the lipid oxidation and protect the meat quality is possible, but little information about the effect of rosemary and ginger on a characteristic of chicken meat exists and no information exists on the application of small red bean and sunflower seeds to chicken meat.
- Rosemary and ginger extract at concentrations of 0.02-5 % can inhibit the autoxidation of lipids and development of off-odour and off-flavour (warmed-over flavour) in meat and meat products
- Antioxidant supplementation to meat reduces the peroxide, TBARS, conjugated diene and conjugated triene values and increase meat shelf life.

1.11 Hypothesis:

This study aimed to evaluate these following hypotheses

- The post-slaughter application of natural antioxidants can improve the stability of lipid and chicken meat quality as measured by instrumental analyses
- The use of natural antioxidants can limit the degree of warmed-over character in chicken meat
- The post-slaughter application of natural antioxidants can perform similarly to the synthetic antioxidants in broiler chicken meat

1.12 Objectives:

- To evaluate the effect of post-slaughter application of natural antioxidants at different levels on the oxidative stability of fat from various portions of chicken carcasses under accelerated storage at 62.8 ^{o}C.
- To investigate the impact of post-slaughter application of natural antioxidants on physic and chemical characteristics of raw and freshly cooked meat.
- To determine the effect of natural antioxidants application on the degree of warmed-over character in chicken meat.
- To evaluate the efficiency of natural antioxidants to reduce the lipid oxidation products in comparison with synthetic antioxidants in broiler chicken meat
- To assess the diffusion of an antioxidant solution into the different layers of chicken fillets by dipping process.

Chapter 2

The Impact of Natural Antioxidants on the Oxidative Stability of Fat from Broiler Chicken Meat.

2.1 Introduction

Lipid oxidation can cause deterioration and reduce the quality of meat and meat-derived products (Estevez et al., 2009). As mentioned previously (section 1.8), the process of lipid oxidation can be halted or delayed by the presence of free-radical scavenging compounds (e.g. antioxidants). In order to assess the progression of any chemical process, or events that influence these chemical processes, their relatively slow progression can be sped up by employing accelerated storage conditions at elevated temperatures (Khan and Shahidi, 2001; Zhang et al., 2010; Tavasalkar et al., 2012; El-Shourbagy and El-Zahar., 2014). Khan and Shahidi (2001) and Tavasalkar et al. (2012) have demonstrated the oxidative stability and potential shelf life of lipids under standardised accelerated storage conditions (i.e. Schaal oven test). For practical purposes, it has been suggested that accelerated storage is considered a very useful tool to identify and characterise a new antioxidant and determine its activity (Tavasalkar et al., 2012). In order to estimate the activities of potential antioxidants, oils and fats have been used as oxidation substrates (Khan and Shahidi, 2001: Zhang et al., 2010; Tavasalkar et al., 2012; El-Shourbagy and El-Zahar, 2014). El-Shourbagy and El-Zahar (2014) studied the capacity of rosemary, peanut skins, pomegranate fruits and BHA in ghee at 63 ^{o}C for 21 days. In order to monitor oxidation of oil and evaluate shelf life, several chemicals, instrumental and sensory evaluation techniques have been used. These techniques can also be applied to estimate the efficiency of antioxidants in various lipid systems (Che Man and Tan, 1999). Chemical and instrumental techniques used include, measuring fatty acid degradation, phospholipid

content, conjugated dienes, conjugated trienes and TBARS value. Conjugated dienes are indicators of primary oxidation products; while conjugated trienes and TBARS are indicators of secondary oxidation products (Estevez et al., 2009).

Most of these studies have focused on using oil as an oxidation substrate and evaluating the antioxidant capacity of the extracts. To our knowledge, the use of fat from chicken meat as an oxidation substrate for evaluating the antioxidant activity of natural extracts has not been reported. The efficacy of natural antioxidants in pure fat extracted from chicken meat has not yet been evaluated. Such knowledge could encourage to apply these antioxidants to other products that contain high levels of fat to reduce the lipid oxidation process. Therefore, this study was carried out to evaluate the effects of natural antioxidants such as: rosemary, ginger and small red bean and sunflower seeds, plus the synthetic antioxidant (BHT) on the oxidative stability of fat from various portions of chicken carcasses under accelerated storage at 62.8 ^{o}C.

2.2 Materials and Methods

2.2.1 Raw Materials

2.2.1.1 Chicken Carcasses

Freshly slaughtered, whole chicken carcasses of Ross breed were obtained from a single supplier in the UK (Faccenda Group Ltd., Brackley, UK) for all experiments, where all animals came from the same stock and were fed the same base diet (Table 2.1). Slaughter age ranged from 36-41 days with an average carcass weight of 1.7 kg.

Table 2.1 Ingredients and chemical composition (%) of broiler diet.

Ingredients (%)	**Starter**	**Grower**	**Finisher**	**Withdrawal**
Wheat	54.00	48.00	55.00	55.00
Barley	0.00	8.00	8.00	9.00
Soya	31.50	28.00	20.50	20.00
Rapeseed	4.00	6.20	6.75	6.75
Fishmeal	4.00	0.00	0.00	0.00
Biscutmeal	0.00	2.80	2.70	2.70
Vegetable Oil	3.25	4.00	3.70	4.00
Vits/Mins/Amino Acids/Enzyme	3.25	3.00	3.35	2.55
Chemical composition (%)				
Protein	24.00	21.00	17.90	17.60
Fat	6.30	8.00	8.50	8.70
Fibre	3.00	3.50	3.50	3.50
Ash	5.30	4.80	4.40	4.30

2.2.1.2 Sources of Natural Antioxidants

Ground rosemary (*Rosmarinus officinalis*), ginger (*Zingiber officinale* Roscoe), dry small red bean seeds (*Phaseolus vulgaris*) and sunflower seeds (*Helianthus annuus*) were obtained from various commercial sources. Dried and ground rosemary and ginger were obtained from Gekruid vof (Hoogstraten, Belgium), small red bean seeds from Bob's Red Mill (USA) and sunflower seeds from Suma (Elland, UK).

2.2.1.3 Chemicals Used

Table 2. 2 Chemicals used.

Chemical	Grade / Purity	Supplier	
		Name	Address (town, country)
Sodium sulphite	≥98%,	Sigma-Aldrich	Gillingham, UK
Ethanol, Absolute	Assay (99.99% GC)	Sigma-Aldrich	Gillingham, UK
Gallic acid	97.5-102.5% (titration)	Sigma-Aldrich	Gillingham, UK
Butylated hydroxytoluene	≥99%, Food Grade, kosher	Sigma-Aldrich	Gillingham, UK
2-thiobarbituric acid	≥98%,	Sigma-Aldrich	Gillingham, UK
L-ascorbic acid	reagent grade, crystalline	Sigma-Aldrich	Gillingham, UK
Sulphuric acid	ACS reagent, 95.0-98.0%	Sigma-Aldrich	Gillingham, UK
Disodium phosphate dibasic	BioXtra, ≥99.0%	Sigma-Aldrich	Gillingham, UK
Hydrogen peroxide	Laboratory reagent grade, Assay > 30% (w/v)	Sigma-Aldrich	Gillingham, UK
Tridecanoic acid	Analytical standard	Sigma-Aldrich	Gillingham, UK
cis-4,7,10,13,16,19-Docosahexaenoic methyl ester	≥ 98% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl all-cis-7,10,13,16,19-docosapentaenoate	Analytical standard	Sigma-Aldrich	Gillingham, UK
Methyl myristate	≥99% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl palmitoleate	≥99% (capillary GC), liquid	Sigma-Aldrich	Gillingham, UK
Methyl palmitate	≥99% (capillary GC)	Sigma-Aldrich	Gillingham, UK
Methyl palmitoleate	≥ 99% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl stearate	≥99% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl oleate	99%	Sigma-Aldrich	Gillingham, UK
Methyl linoleate	≥99% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl linolenate	≥99% (GC)	Sigma-Aldrich	Gillingham, UK
Methyl arachidonate	≥99% (GC)	Sigma-Aldrich	Gillingham, UK
cis-5,8,11,14,17-Eicosapentaenoic methyl ester	Analytical standard	Sigma-Aldrich	Gillingham, UK

Table 2.2 (Continued) Chemicals used:

Chemical	**Grade / Purity**	**Supplier**	
		Name	Address (town, country)
2,2,4-trimethylpentane (iso-octane)	99%, for spectroscopy, Acros Organics	Fisher Scientific	Loughborough, UK
Potassium hydroxide	99.98%, (trace metal basis), Acros Organics	Fisher Scientific	Loughborough, UK
Ammonium molybdate(VI) tetrahydrate	Analytical reagent grade	Fisher Scientific	Loughborough, UK
Chloroform	HPLC grade	Fisher Scientific	Loughborough, UK
Methanol	HPLC gradient grade, (Assay 99.99%)	Fisher Scientific	Loughborough, UK
Hexane	99%, pure, mixture of isomers, Acros Organics	Fisher Scientific	Loughborough, UK
Ethanol, Absolute	Analytical reagent grade, (Assay 99.99% GC)	Fisher Scientific	Loughborough, UK
Trichloracetic acid	99+%, ACS reagent, Acros Organics	Fisher Scientific	Loughborough, UK
Glacial acetic acid	Analytical reagent grade, 99.83%	Fisher Scientific	Loughborough, UK
Malonaldehyde bis(diethyl acetal),1,1,3,3-tetra-ethoxypropane	97%, Acros Organics	Fisher Scientific	Loughborough, UK
Acetone	Analytical reagent grade, 99.99%	Fisher Scientific	Loughborough, UK
Hydrochloric acid	Analytical reagent grade (37%)	Fisher Scientific	Loughborough, UK
Petroleum ether	Laboratory reagent grade (40-60^{o}C)	Fisher Scientific	Loughborough, UK
Folin-Ciocalteau	Phenol reagent	Fisher Scientific	Loughborough, UK
Sodium carbonate anhydrous	Analytical reagent grade, (Assay 99.96%)	Fisher Scientific	Loughborough, UK

2.2.2 Preparation of Natural Antioxidant Extracts

The extraction of natural antioxidants from rosemary (ROS), small red bean (SRB), sunflower seeds (SFS) and ginger (GGR) was optimised and analysed to determine the anti-oxidative capacity of the extracts. The ROS and GGR were supplied as powders, whereas the SRB and SFS were supplied whole. Hence, SRB and SFS were freeze dried (Edwards Modulyo freeze dryer, Sussex, UK) and then ground to a powder in a commercial coffee grinder (DeLonghi KG49, Treviso, Italy), after which the SRB and SFS powders were passed through an 80 and 20 mesh sieve respectively.

Antioxidant extracts of SFS, GGR and ROS, were produced employing aqueous ethanol (absolute ethanol: water at 80:20 v/v) according to the method described by Selani et al. (2011). Ten grams of the powdered samples were accurately weighed and mixed with 100 ml aqueous ethanol. The mixture was gently shaken in an orbital shaker (HS 501 digital, IKA labortechnik, Staufen, Germany) for 48 h in a dark place at ambient temperature (20 ^{o}C). The extracts were passed through a cheesecloth before being filtered through Whatman® No. 1 filter paper. The filtrates were then concentrated in a water bath at 60 °C to remove excess solvent until a volumetric reduction of 90 % was obtained. The concentrated extracts were stored at -20 ^{o}C, until analyses for calibration and subsequent use. Antioxidant extracts from SRB were also obtained using aqueous ethanol, but at an equal ethanol: water (50:50 v/v) ratio instead. SRB contains a considerably higher amount of hydrophilic antioxidant compounds than lipophilic (see section 1.7.4 for more detail). Accordingly, to extract both hydrophilic and lipophilic fractions the correct ratio of ethanol and water is required (Wu et al., 2004). The same extraction protocol as described above was used to obtain the antioxidant extract from the SRB. For BHT extract, 10 gm of BHT powder directly dissolved in 100 ml of absolute ethanol. The plant and BHT extracts were obtained analysed for total phenolic content (section 2.2.2.1).

2.2.2.1 Total Phenolic Measurement in Plant Extracts

Total phenolics were assessed in extracts from various plants (section 2.2.1) by the Folin-Ciocalteau reagent as described by Singleton and Rossi (1965). The total phenolic content was expressed as milligrams Gallic acid equivalent (mg of GAE / 100 g of sample) based on sample dry weight. Half a ml (0.5 ml) of appropriate extracts and standard solutions were transferred and mixed with 30 ml of distilled water in a 50ml test tube. To that, 2.5 ml of Folin–Ciocalteu reagent were added, after 1 min 7.5 ml of 20 % sodium carbonate (w/v) was added and the volume adjusted to 50 ml using deionised water. All samples were incubated with the standard for 2 h at 23 ℃ to allow the reaction colour to develop. Absorbance at 760 nm was recorded after colour development against a blank using spectrophotometry (Beckman, DU640 spectrophotometer, Fullerton, CA).

A calibration curve was prepared using gallic acid solution 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml. This was conducted by weighing 100 mg of gallic acid in 100 ml volumetric flask and adding 10 ml of ethanol to dissolve, followed by adding distilled water up to mark to produce (1 mg/ml) stock solution. 0, 1, 2, 3, 4, 5 and 6 ml of this solution was transferred and then distilled water was added to volume 10 ml to produce 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg gallic acid / ml extraction (Figure 2.1). After vortexing for 15 sec, 0.5 ml of each concentration was placed in a test tube and the same procedure above applied for analysis. The total phenolic content was calculated from the standard curve and then expressed as mg Gallic acid equivalent (GAE) / 1 ml extract, followed by the conversion of this value to mg GAE / 100 g of dry weight. The amount of phenolic content in ROS, SRB, GGR, and SFS extracts and BHT is reported in Appendix (Figure A1).

$$x = (y + 0.022)/1.0975$$

$$\text{mg total phenolic content /100 g dry weight} = \left[\frac{x\,(mg)\;X\;V}{\text{weight of sample (g)}}\right] \text{x}100$$

Where x is the unknown amount of phenolics content in sample (mg/ml) is taken from the standard calibration curve (Figure 2.1), y is the absorbance of meat sample, V, is the dilution factor. Figure 2.1 shows the strong correlation between Gallic acid concentrations and absorbance (R^2 = 0.9883), however, the line went through all points on a graph was not straight. This could be due to the random errors or variance of triplicate measurements or could be due to the interference of the environment with the measurement process which cannot be possible to control these errors.

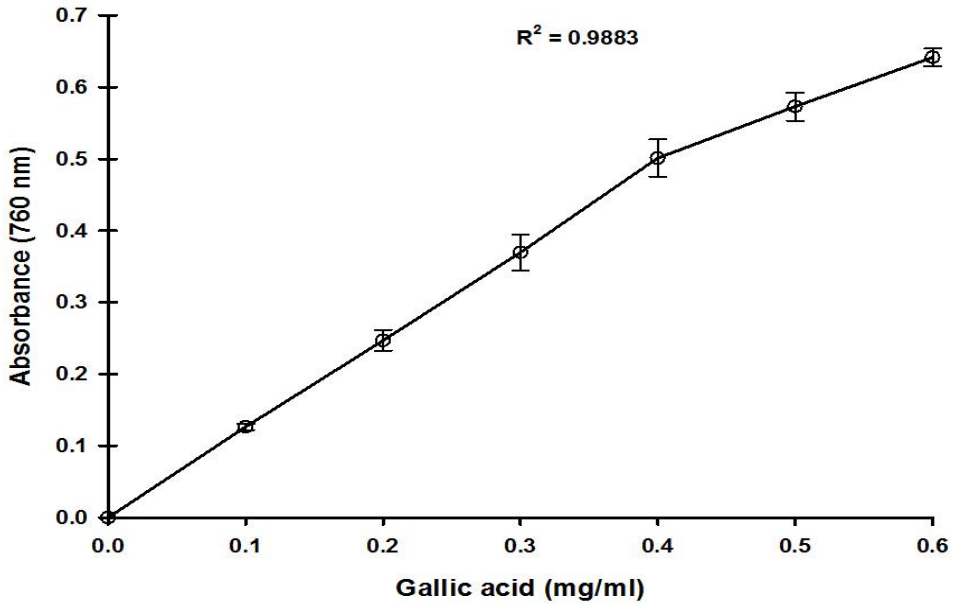

Figure 2. 1 Gallic acid calibration curves employed to determine the total phenolic content in plant extracts (Means ± SED; n=3).

2.2.3 Preparation of Fat Extract

2.2.3.1 Portioning and Grinding of Chicken Carcasses

The chicken carcasses were manually deboned and portioned into: breast meat; thigh meat; adipose tissue; and skin. Dry matter (section **2.2.3.1.1**) and total lipid content (section **2.2.3.1.2**) of each tissue was analysed before extraction process and data is

presented in Appendix (Table A1.1). The chicken meat samples were ground separately in a Mainca blender (Mainca Ltd., Horton, Berkshire, UK) in the presence of dry ice (approximately 200 g dry ice / kg tissue) to avoid oxidation and then freeze dried. The freeze-dried samples were finely pulverised in a commercial coffee grinder (DeLonghi KG49, Treviso, Italy).

2.2.3.1.1 Dry Matter (DM)

Dry matter of raw meat samples was determined using a freeze dryer (Edwards Modulyo freeze dryer, Sussex, UK). Approximately 40 g of ground chicken meat was accurately weighed in a plastic pot and kept at -20 ºC overnight. The frozen samples were then freeze-dried for 72 h at -40 ºC and, the weight of samples was then recorded. The dry matter (DM) was calculated and measured as g / kg raw meat using the following equation.

$$\text{DM g/kg raw meat} = \left[\frac{\text{weight of dried samples (g)}}{\text{Initial weight of raw meat (g)}}\right] x\ 1000$$

2.2.3.1.2 Lipid (Ether Extracts) in Meat Samples

Total lipid content was determined using an ether extraction of the meat samples according to the solvent method described by FOSS (1987) using a Soxhlet system (HT 1043 extraction apparatus, FOSS, Warrington, UK). Approximately 1 g of dried meat sample was weighed and then placed in cellulose extraction thimbles (Whatman ®, Maidstone, UK), after which the thimbles were plugged from the top with pure cotton wool 100 % (Wilko cotton wool pleat, Nottinghamshire, UK) followed by recording the weight of empty extraction cups. 25 ml of 40-60 ºC petroleum ether was added to the extraction cups, and all samples were then extracted by immersing thimbles into a solvent for 30 min of continuous reflux at 150 ºC. The time was counted from the point that dripping was observed from all of the condensers. The thimbles were then lifted out from

the solvent and rinsed for 30 min followed by closure of the taps on Soxhlet units and the condensed solvent was allowed to collect for 15 min. The petroleum ether was then evaporated by opening the evaporation valve on the Soxhlet units for 5 min, followed by removing the extraction cups and placing in the fume cupboard for 30 min to evaporate further solvents and cool them down. The weight of extraction cups containing the fat was recorded and the fat was calculated as follows:

$$\text{Fat (Ether Extract)g/kg DM} = \left[\frac{\text{Fat weight (g)}}{\text{Initial weight of sample (g)}}\right] \text{x } 1000$$

2.2.3.2 Fat Extraction from Chicken Portions

Fat was extracted from the dried chicken tissue samples with chloroform: methanol (2:1 v/v) mixture as described by Folch et al. (1957). One gram of ground sample was homogenised with 20 ml of extraction solvent. The mixture was gently shaken (HS 501 digital, IKA labortechnik, Staufen, Germany) for 25 min at ambient temperature (~23 ^{o}C). The homogenates were filtered by Whatman No. 1 filter paper. The remaining solids were extracted twice more with the same solvent volume, following which the filtrates containing the lipids were combined and concentrated under vacuum in a rotary evaporator (BuchiRotavapor R II, Flawil, Switzerland) at 40 °C to remove the excess solvent. The lipid extracts were stored at -20 ^{o}C until subsequent use.

2.2.3.3 Experimental Design and Sample Preparation

This experiment was consisted of a 6 x 3 x 3 factorial design plus added control with five different types of antioxidants plus control (ROS, SRB, SFS, GGR, BHT and CON) combined with three levels of antioxidants (0, 10 and 20 mg) applied to the fat extracted from 4 portions of chicken carcasses (breast, thigh, adipose and skin tissues) and all samples were stored at 62.8 ^{o}C for three storage times (0, 3 and 7 days).

The preparation of fat extracted from breast, thigh, adipose and skin tissues was conducted as described in section 2.2.3.2 and each portion was conducted and treated separately. Fat samples of each portion were treated by adding extracts from ROS, SRB, SFS, and GGR at concentrations of 0, 10 and 20 mg equivalent total phenolic /100 g fat. In addition to the four natural antioxidant extracts, this study also used the synthetic antioxidant BHT at 0, 10 and 20 mg equivalent total phenolics as an example of a powerful artificial antioxidant. Each treatment was conducted in triplicate. All samples were vortexed for 1 min to facilitate uniform distribution of the antioxidant and stored under schaal oven conditions for 7 days.

2.2.3.4 Fat Storage and Sampling

Treated and non-treated fat samples with various antioxidant extracts (section 2.2.3.3) were subjected to the Schaal oven test (Guzman et al., 2011). The control and treated samples were placed in an oven (Binder, Tuttlingen, Germany) after the temperature was set at 62.8 ^{o}C for up to one week in order to produce oxidation compounds and measuring the oxidative stability of lipids during the storage times. This test is a standard method utilised for assessing fats and oils at 62.8 ^{o}C. Fat samples were periodically sampled at day 0, 3 and 7 and samples were analysed for thiobarbituric acid reactive substances, phospholipid content, conjugated dienes, conjugated trienes and fatty acids following 0, 3 and 7 days of storage.

2.2.3.4.1 Thiobarbituric Acid Reactive Substances (TBARS) Determination

TBARS is a secondary lipid oxidation product generated from the decomposition of hydroperoxides and considered a good indicator of oxidation process (Hayes, 2000; McCarthy et al., 2001; Bax et al., 2012). An increase of TBARS in meat is indicative of advanced lipid oxidation (McCarthy et al., 2001; Selani et al., 2011; Bax et al., 2012; Naveena et al., 2013). The presence of TBARS is responsible for off-odours and off-

flavours which have a negative effect on sensory properties of meat (Byrne et al., 2001), decreases the shelf-life and nutritional values (Byrne et al., 2001; Sampaio et al.,2012).

TBARS value was determined in extracting fat samples in triplicate according to the method described by Ke and Woyewoda (1979). Ten milligrams of extracted fat were weighed into a 10-ml test tube to which 5 ml of the TBA work solution was added. TBA work solution was prepared approximately 30 min before use by mixing, 180 ml TBA stock solution (prepared by dissolving 2.88 g of 2-thiobarbituric acid in 50 ml deionised water in a 500 ml volumetric flask and made up to volume with glacial acetic acid and placed on a magnetic stirrer until dissolved), with 120 ml chloroform and 15 ml sodium sulphite solution (prepared by dissolving 18.91 g of sodium sulphite in 500 ml deionised water). All samples were vortexed for 15 sec before being incubated in a water bath at 95 ^{o}C for 45 min. The tubes were rapidly cooled down by tap water and 2.5 ml of trichloracetic acid solution (prepared by dissolving 22.87 g of trichloracetic acid in 500 ml deionised water) was added. After thorough mixing by inverting ten times, all samples were centrifuged (Rotina 46R HettichZentrifugen, Tuttlingen, Germany) at 2500 g for 10 min at 4 ^{o}C. The absorbance (at 538 nm) of the resulting supernatant was determined against a blank, which contained all chemical solutions except a sample, by spectrophotometer (Beckman, DU640 spectrophotometer, Fullerton, CA).

A standard curve was prepared by dissolving 220 mg of 1,1,3,3–tetra-ethoxypropane (TEP) in 100 ml of deionised water to produce (2.2 mg TEP / ml) working solution. Then serial dilutions were prepared by pipetting aliquots of 0, 0.02, 0.075, 0.15, 0.30, 0.45 and 0.600 ml of working TEP standard solution into test tubes and made up to 0.600 ml by adding deionized water (DW) to produce (0, 0.044, 0.165, 0.330, 0.660, 0.990 and 1.320 mg TEP equivalents / 0.600 ml extraction). All samples were then vortexed for 15 sec. 0.010 ml was pipetted from each dilution which contained (0.00073 – 0.022 mg TPE /

0.010 ml) (Figure 2.2) and placed in 10 ml test tube and the same procedure was applied as used for sample analysis, except the TEP work solution was used instead of a sample. For constructing standard curve, the concentrations of (TEP) were plotted against the absorbance of TEP (Figure 2.2). From the standard curve, the amount of TBARS in fat samples was determined and expressed as mg of malondialdehyde equivalents / kg fat by utilising the following equations:

$$x = (y + 0.0094)/124.27$$

$$\text{mg TBARS (MDA)/kg fat meat} = \left[\frac{x\ (mg)}{\text{weight of fat sample (g)}}\right] \text{x1000}$$

Where x is the unknown amount of MDA in fat (mg/g) is taken from the standard calibration curve (Figure 2.2). y, is the absorbance of fat chicken sample, and 1000 is the dilution factor used to obtain the results of TBARS in mg MDA/kg fat.

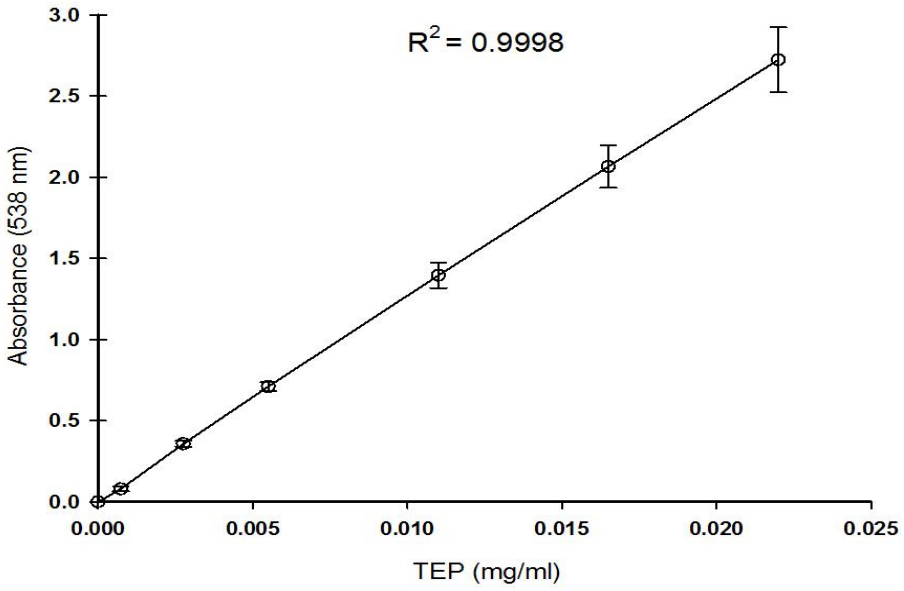

Figure 2. 2 Standard curve of 1,1,3,3–tetra-ethoxypropane (TEP) for determination of TBARS (mg MDA) (Means ± SED; n=3).

2.2.3.4.2 Conjugated Dienes and Conjugated Trienes Determination

The conjugated dienes (CD) and conjugated trienes hydroperoxides (CT) formation in the lipids were determined according to the method mentioned by Pegg (2005). Twenty-five milligrams of lipid extract were weighed and dissolved in 25 ml of 2, 2, 4-trimethylpentane (iso-octane) and the contents then homogenized by vortex for 15 sec. The homogenate sample was transferred to a quartz cuvette, 1 cm path length and the absorbance was determined against a blank by spectrophotometer (Beckman, DU640 spectrophotometer, Fullerton, CA) at 232 nm and 268 nm for CD and CT respectively. Each sample was assessed in triplicate. The concentration of CD and CT formed in fat was calculated by utilising the molar extinction coefficient of 25,200 $M^{-1}cm^{-1}$. The concentration of both CD and CT were calculated and reported as micromole/g by utilising the following equation:

C_{CD} and C_{CT} = A_{232} and A_{268} / (E x L)

CD value = [C_{CD} and C_{CT} x (2.5 x 10 4)] / W

Where: $C_{CD\ and}$ C_{CT} is CD and CT concentration (mmol/ml).

$A_{232\ and}$ A_{268} is the absorbance measured at both wavelengths 233 for CDs and 268 for CTs, E is the molar extinction coefficient for CD and CT 2.525 x 10^4 M^{-1} cm^{-1}. L is the length of the light path in cm (1 cm). 2.5 x 10^4 is a factor that includes the volume of iso-octane (25 ml) used to dissolve the oil sample and a unit conversion (1000 µmol/mmol) to report the results as micromole, W is weight of sample in g.

2.2.3.4.3 Phospholipid Content Determination

The phospholipid content was determined by using an adjusted procedure according to Anderson and Davis (1982). Twenty-five milligrams of extracted fat was dissolved in 0.5 ml chloroform, of which 0.025 ml were transferred into digestion tubes and heated in a

block heater at 38 °C until dry. To this, 0.45 ml H_2SO_4 (4.45 M) (4.45 M of H_2SO_4 was prepared by mixing 24.97 ml of H_2SO_4 with 100 ml of DW) was added and vortexed after which all samples were heated in a block heater (Maplelab Scientific BH-603, Dalang, China) at 200 °C for 25 min. The tubes were then cooled and 0.150 ml H_2O_2 (30 %) was added. The samples were mixed and heated again for a further 30mins before 3.9 ml of deionised water was added and the mixture cooled. Then 0.5 ml of ammonium molybdate (VI) tetrahydrate solution 2.5 % (2.5 g dissolved in100 ml DW) and 0.5 ml of ascorbic acid solution 10 % (10 g dissolved in 100 ml DW) were added. The samples were mixed well and incubated in a block heater at 100 °C for 10 min. The tubes were cooled and the absorbance determined against a blank that contained all chemicals except samples by a spectrophotometer (Beckman, DU640 spectrophotometer, Fullerton, CA) at 820 nm.

A standard curve was prepared by dissolving 125 g sodium phosphate dibasic in 50ml deionised water to produce 2.5 mg/ml stock solution. From this solution 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2 ml of the solution were placed in 10 ml test tubes followed by adding 10 ml of deionised water to produce a calibration standard curve at concentrations of 0, 0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 mg of sodium phosphate / ml extract. After mixing all samples by vortex for 15 sec, 0.025 ml from each concentration, which contained (0.00125-0.01250 mg sodium phosphate dibasic / 0.025 ml extraction) was placed in digestion tubes and the same procedure above applied for analysis. The phosphorus content values were calculated from a standard curve, which was prepared from sodium phosphate dibasic (Na_2HPO_4) and the results were expressed as g of phospholipids / 100 g of fat. The total phospholipids contents of fat were calculated using the following formula:

$$x = (y + 0.0021)/76.855$$

$$\text{g phosphorus /100 fat} = \left[\frac{(x\ mg\ X\ V)/1000}{\text{weight of fat sample (g)}}\right] \text{x } 100$$

Total phospholipids contents (g/100 g fat) = phosphorus content (g) x 25

Where x is the unknown amount of phosphorus in the lipid sample (mg/ml) and is taken from the standard calibration curve (Figure 2.3). Y is the absorbance of meat sample. V is the dilution factor. 100 is a dilution factor used to obtain the results of phosphorus in g/100 g fat. The number 25 is derived from dividing the molecular weight of phosphoglyceride (754) by the molecular weight of phosphorus (31).

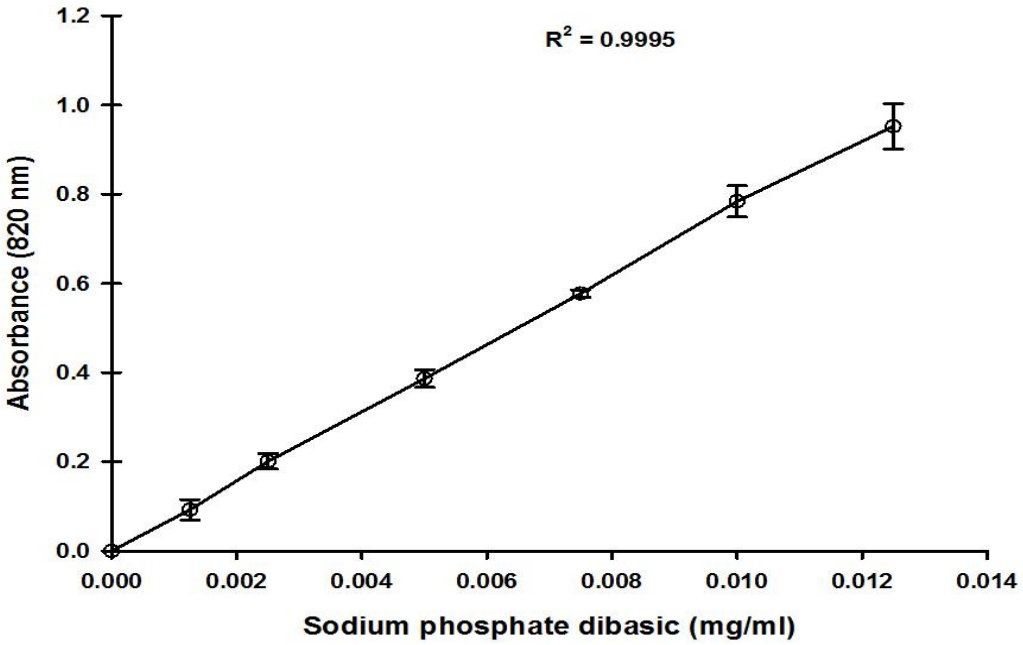

Figure 2. 3 Standard calibration curve of sodium phosphate dibasic for determination of phospholipid content (Means ± SED; n=3).

2.2.3.4.4 Fatty Acids Determination

Fatty acids were determined according to the method outlined by O'Fallon et al. (2007) with slight modification. Forty milligrams of extracted fat or 0.5 g of dried meat was accurately weighed into 15 ml Kimax test tubes. To these samples 1.0 ml of internal standard (4 mg of C13:0/ml of methanol) was added, followed by 0.7 ml of 10 M KOH

in water (10 M KOH was prepared by dissolving 56 g of KOH in 100 ml of deionised water), and 5.3 ml of methanol to facilitate saponification. The tubes were incubated for 1.5 h at 55 °C in a water bath with shaking vigorously by hand every 20 min for 5 sec. The tubes were cooled to ambient temperature and 0.58 ml of 12 M H_2SO_4 was added. The solution of 12 M H_2SO_4 was prepared by dissolving 67.33 ml of concentrated H_2SO_4 and made up to 100 ml by adding deionised water. After mixing by inversion, the samples were then incubated for a further 1.5 h at 55 °C in a water bath with shaking vigorously by hand every 20 min for 5 sec to facilitate the formation of methyl esters of the liberated free fatty acids. After cooling tubes to room temperature, 3 ml of hexane were added and vortexed, after which the tubes were centrifuged (Rotina 46R Hettich Zentrifugen, Germany) at 2500 g for 10 min. The solvent layer containing the fatty acid methyl esters was transferred by glass pipette and placed into a GC vial. The fatty acid methyl esters were assessed by Gas Chromatography (Hewlett Packard HP 6890) equipped with a flame ionization detector utilising a capillary column (CP-SIL88, 100 m × 250 µm × 0.20 µm). The initial oven temperature was 70 °C, held isothermally for 2 min, before increasing by 8 °C/m to 110 °C, held isothermally at 110 °C for 4 min, then a programmed temperature increase of 5 °C/min to 170 °C and a further increase of 4 °C/min to 225 °C and finally held isothermally for 15 min. Hydrogen was used as the carrier gas at a flow rate of 2.1 ml/min, and the pressure of the column head was 29.59 psi. The temperature of the injector and detector were set at 250 °C. One microliter of fatty acid methyl esters was injected in split mode at a 100:1 ratio. Fatty acid identification was conducted by comparison of retention times of fatty acid methyl esters from known standards. The following formula calculated individual fatty acids.

$$\text{Calculation individual fatty acid (g/100 g fat)} = \frac{\text{Weight of Standard (mg) x fatty acid (\%)}}{\text{internal standard area (\%) x weight of sample (g)}}$$

∑ SFA = sum of C14:0, C16:0, C18:0 and C20:0

∑ MUFA = sum of C16:1 n-7 and C18:1 n-9

∑ PUFA= sum of C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3, C22:5 n-3 and C22:6 n-3

∑ n-3 PUFA sum of C18:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3

∑ n-6 PUFA= sum of C18:2 n-6 and C20:4 n-6

2.2.3.4.4.1 Determination of GC accuracy

The accuracy of GC method was determined by calculating the linearity of the calibration curve, limit of quantitation (LOQ) and limit of detection (LOD). For calculating the linearity of the standard curve, serial dilutions 1:60, 1:36, 1:22 and 1:13 of individual fatty acids were prepared in GC vials by dissolving correct amount of each fatty acids that ranged from 15.00 to 104.10 mg in 1ml of hexane as shown in Table 2.3. Each dilution was prepared in triplicates and run all samples through the GC. To construct the calibration curve, the concentrations of each fatty acid were plotted against the set of data for each of the fatty acids obtained from GC as shown in Figure 2.4. For determination of both LOD and LOQ, steyx/slope method was applied according to the method described by Thummaluru and Gurrala (2016). Limit of quantitation and limit of detection were calculated as per the following formulas:

$$\text{LOD mg/ml} = \left[\frac{\text{steyx}}{\text{slope}}\right] \text{x } 3.3$$

$$\text{LOQ mg/ml} = \left[\frac{\text{steyx}}{\text{slope}}\right] \text{x}10$$

Table 2. 3 Linearity of calibration of illustrated fatty acids method and determination of Limit of Quantitation (LOQ) and Limit of Detection (LOD)

Fatty acid	LOQ (mg/ml)	LOD (mg/ml)	Linearity range mg/ml	Linearity Calibration (R^2 value)
Myristic acid	1.04	0.34	0.16-34.00	1.0000
Palmitic acid	2.11	0.69	0.48-104.10	1.0000
Palmitoleic acid	5.20	1.71	0.15-33.41	0.9993
Stearic acid	8.31	2.74	0.32-68.11	0.9996
Oleic acid	8.58	2.83	0.32-68.81	0.9995
Linoleic acid	8.16	2.69	0.16-34.75	0.9984
Linolenic acid	5.04	1.66	0.16-34.83	0.9994
Arachidic acid	8.11	2.67	0.32-68.60	0.9996
Arachidonic acid	5.07	1.67	0.17-36.11	0.9994
Eicosapentaenoic acid	0.85	0.28	0.15-33.33	0.9993
Docosahexaenoic acid	5.72	1.88	0.16-34.63	0.9992

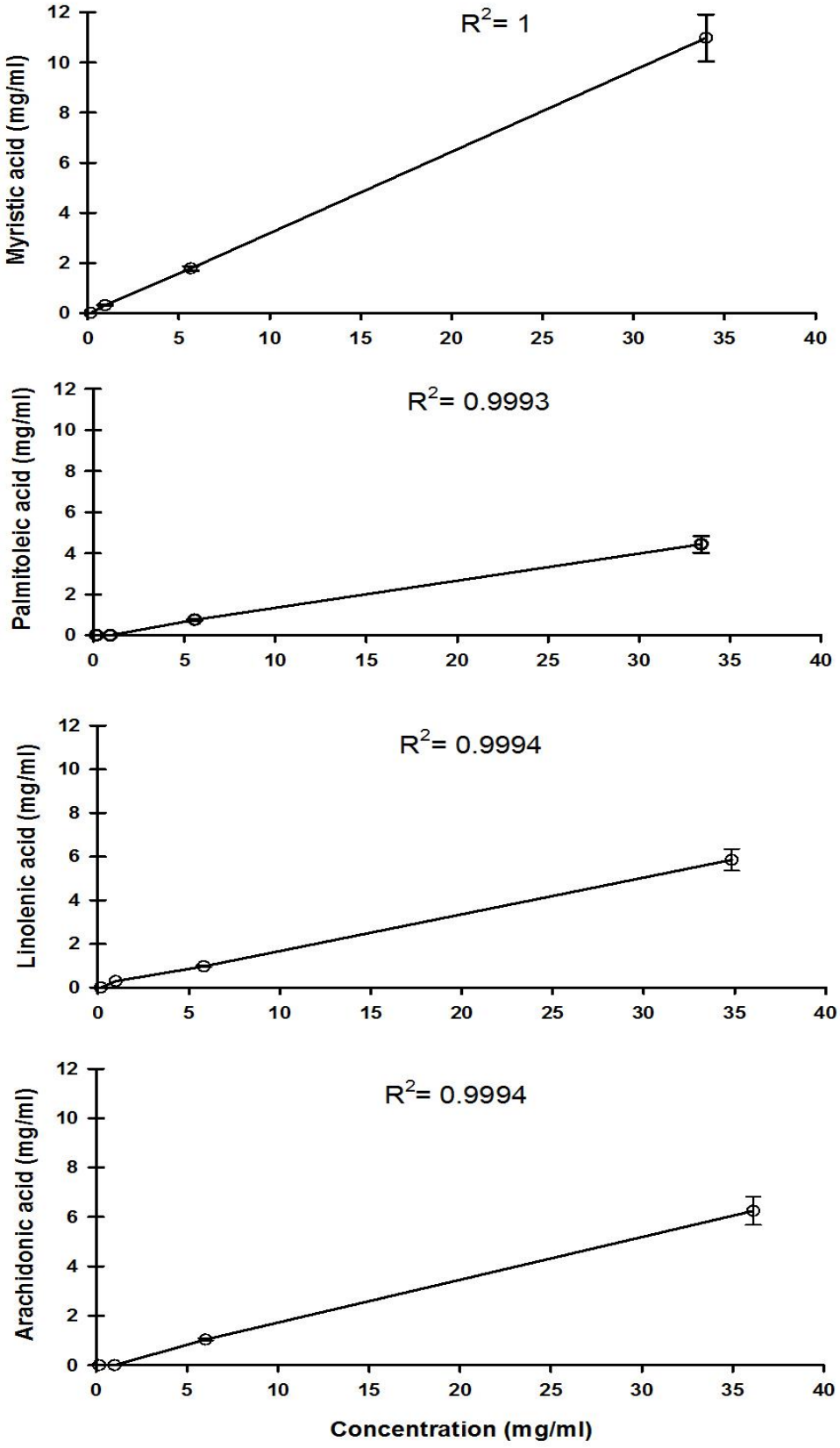

Figure 2. 4 Standard calibration curve of fatty acids for determination the linearity (Means ± SED; n=3).

2.2.4 Statistical Analysis

Four separate factorial plus added control of 6 x 3 x 3 was used for analysing TBARS, CD, CT, phospholipid content and fatty acids of fat extracted from the four portions of chicken carcass over schaal oven test for 7 days. Each portion was conducted and analysed separately, and each experiment was conducted in triplicate (n = 3). In this experiment five different antioxidants were used to decrease the rancidity in fat samples and each of the antioxidant had two levels (10 mg and 20 mg) and a control treatment (without any added antioxidant dose) was included and all samples were stored at 0, 3 and 7 days. The control treatment represented (0) level for three factors and factorial plus added control structure was applied by using the equation (Antioxidants /(level x storage time)). The model expanded to the following: (**Antioxidant + Antioxidant**. **Level** + **Antioxidant**. **Storage time + Antioxidant**. **Level**. **Storage time)**

In which

Antioxidant: represented the main effect of any antioxidant at any level and storage time.

Antioxidant. **Level**: represented the comparison between 0 level and two antioxidant levels (10 and 20 mg) (averaged over the storage time and different antioxidants)

Antioxidant. **Storage time**: represented overall differences between antioxidants and storage time (averaged over 0, 10 and 20 mg levels)

Antioxidant. **Level**. **Storage time:** represented the interaction between Antioxidant x Level x Storage time.

All data obtained from the various treatments were analysed by general treatment structure (no blocking), using the GenStat statistical software (Edition 17th, VSN International Ltd). Tukey's HSD test was used to identify the significant differences

between means, and the significance level of all data was set at $p \leq 0.05$. The null (H_0) and alternative (H_1) hypothesis for each dependent variable were set as:

Null hypothesis (H_0):

There was no significant effect of natural antioxidants application, application level, accelerate storage time and interaction between them on oxidative stability of fat from different portions of chicken carcasses (H_0: $\mu = 0$; $p > 0.05$).

Alternative hypothesis (H_1)

There was a significant effect of natural antioxidants application, application level, storage time and interaction between them on oxidative stability of fat from different portions of chicken carcasses (H_1: $\mu \neq 0$; $p \leq 0.05$).

2.3 Results

2.3.1 Effect of Natural Antioxidants on the Formation of Thiobarbituric Acid-Reactive Substances (TBARS) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions

2.3.1.1 Chicken Breast Fat

The addition of antioxidants to the lipid fraction of chicken breast meat showed a significant ($p < 0.001$) reduction in the formation of TBARS compared to the non-treated samples. The lowest TBARS values were found in samples treated with BHT followed by ROS, SRB, SFS and GGR (Appendix Table A2). However, the addition of antioxidants at both lower and higher levels had a lower TBARS values compared to the "zero" level. A significant ($p \leq 0.05$) difference was found only between both levels of GGR extract that lower level awarded a greater protection against an increase of TBARS compared to the higher level (Figure 2.5). Regardless of antioxidant levels, the TBARS values in none and treated samples significantly increased ($p < 0.001$) at day 3 of storage and declined at day 7. Samples treated with antioxidants were found to have significantly lower ($p < 0.001$) TBARS values compared to the non-treated samples for 7 days (Figure 2.6). An interaction was found between antioxidant x level x storage time ($p < 0.001$) for TBARS values due to the increase the TBARS value in all treatments up to 3 days and decreased afterward at day 7 (Table 2.4). The addition of natural antioxidants at lower and higher level awarded a greater protection against oxidative rancidity compared to the non-treated samples during 7 days of storage time. However, none of the natural antioxidants performed significantly better than the synthetic antioxidant (BHT treatment) within the first 3 day period. Only the addition of the ROS extract at the lower level and over a 7 day period awarded a greater protection against oxidative rancidity compared to the BHT treatment.

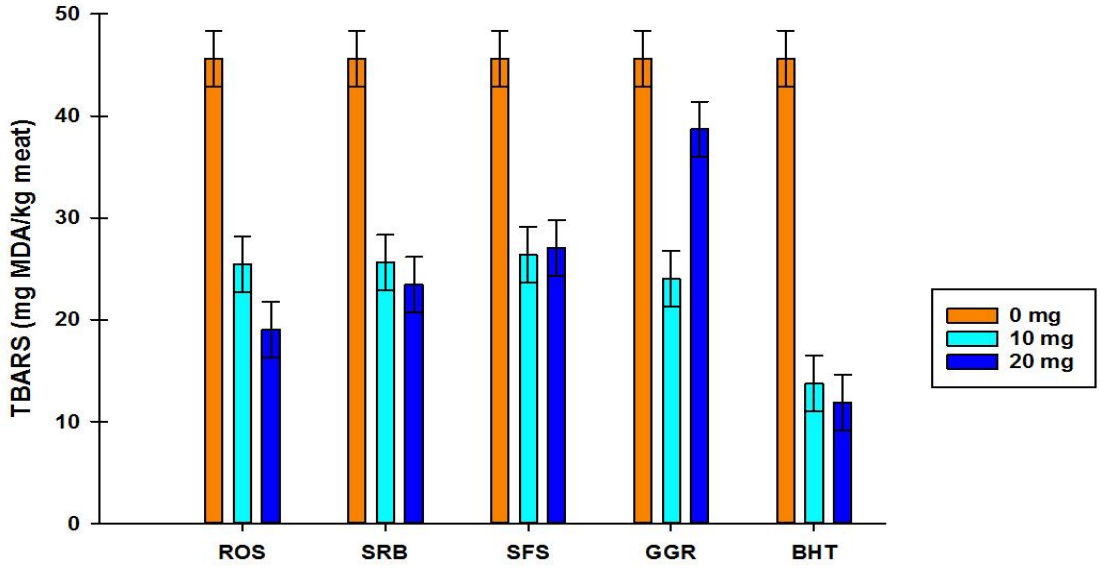

Figure 2.5 Effect of different levels of natural antioxidants on TBARS values in fat from chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

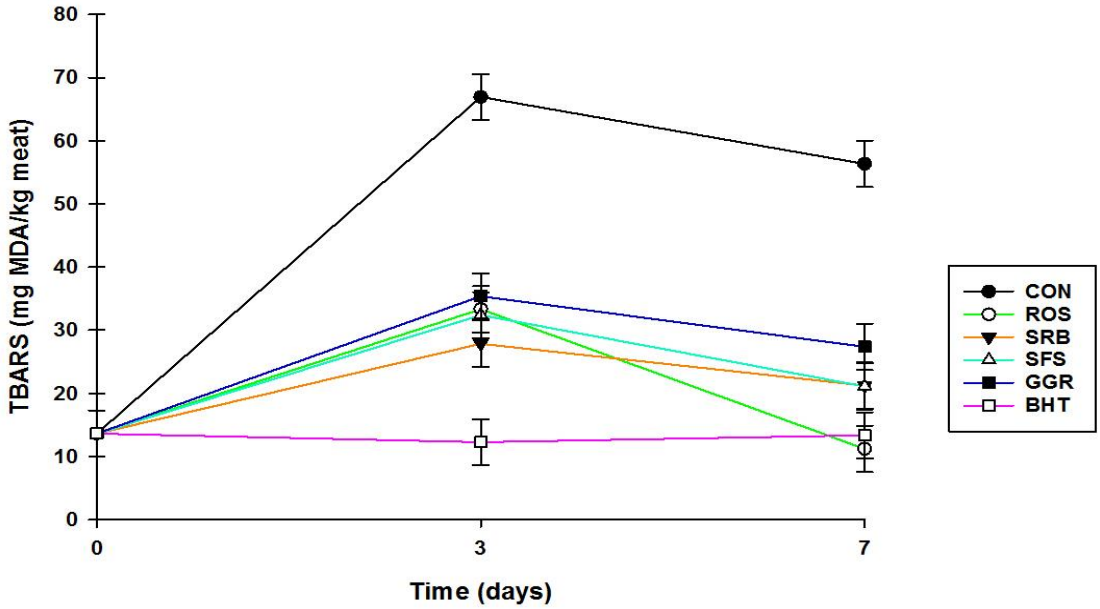

Figure 2.6 Effect of natural antioxidant application on TBARS values in fat from chicken breast meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

Table 2.4 Effect of natural antioxidant extracts at different levels on TBARS values (mg MDA/kg fat) in fat from chicken portions during the accelerated storage time at 62.8 °C.

Tissue	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
Breast	0	13.63^{ab}										
	3	66.93^{g}	12.73^{a}	11.84^{a}	41.52^{ef}	25.12^{abcd}	32.96^{cdef}	22.74^{abcd}	29.79^{bcde}	35.00^{de}	25.02^{abcd}	45.72^{ef}
	7	56.34^{fg}	14.71^{ab}	11.98^{a}	9.42^{a}	12.99^{a}	18.32^{abc}	24.14^{abcd}	23.04^{abcd}	19.19^{abcd}	23.09^{abcd}	31.66^{cde}
Thigh	0	25.35^{bcdefg}										
	3	57.27^{j}	7.28^{a}	17.91^{abcd}	22.57^{abcde}	36.10^{efghi}	39.58^{ghi}	38.94^{fghi}	26.70^{bcdefg}	21.95^{abcde}	43.75^{hij}	35.01^{efghi}
	7	48.50^{ij}	16.08^{abcd}	14.40^{abc}	22.71^{abcde}	21.43^{abcde}	27.04^{bcdefg}	20.79^{abcde}	30.17^{defgh}	12.44^{ab}	23.22^{bcdef}	30.05^{cdefgh}
Adipose	0	41.84^{def}										
	3	74.96^{i}	25.77^{abc}	26.19^{abc}	38.66^{bcde}	30.55^{abcd}	71.08^{hi}	59.86^{gh}	42.82^{def}	37.00^{abcde}	40.74^{def}	39.49^{cde}
	7	75.10^{i}	23.69^{a}	24.94^{ab}	44.89^{def}	46.14^{efg}	72.33^{hi}	84.80^{i}	54.87^{fg}	50.30^{efg}	54.87^{fg}	46.56^{efg}
Skin	0	12.95^{abcdef}										
	3	33.87^{g}	8.56^{abcdef}	7.90^{abcd}	9.38^{abcdef}	6.91^{abc}	8.19^{abcde}	10.07^{abcdef}	14.73^{bcdef}	16.38^{cdef}	12.93^{abcdef}	15.93^{cdef}
	7	54.57^{h}	4.70^{a}	5.74^{ab}	8.04^{abcde}	9.93^{abcdef}	16.94^{def}	16.24^{cdef}	17.81^{ef}	15.71^{cdef}	18.03^{f}	16.59^{cdef}

Mean values with different small superscript letters presented within each row and column of each tissue differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.1.2 Chicken Thigh Fat

Similar to the chicken breast fat, the results show that the addition of antioxidants to the thigh fat before processing significantly reduced TBARS values ($p < 0.001$) Among the treatments with antioxidants, the lowest TBARS value was found in BHT treated samples followed by SFS, ROS, SRB, GGR treatments and the non-treated control (Appendix Table A2). Regardless of the storage time, the TBARS values of the thigh fat treated with 10 and 20 mg of antioxidant extracts were significantly lowered as compared to "zero" level. Only significant differences were found between 10 and 20 mg of SFS ($p = 0.002$) and a significant inhibition of TBARS values was found in samples treated with a higher level (20 mg) compared to the lower level (10 mg) (Figure 2.7). Regardless of the level of antioxidants, the TBARS values in all treated or non-treated samples (with the exception of BHT) increased at day 3 and decreased thereafter. However, higher level oxidation of lipid was detected in non-treated samples (Figure 2.8). The results show that a significant antioxidant x level x storage time interactions were observed for the TBARS values of thigh fat ($p < 0.001$), due to the increase the TBARS value in all treatments up to 3 days and decreased the values over time. On day 0, all natural antioxidants (except GGR at 10 mg) significantly ($p < 0.001$) reduced the formation of TBARS values. Among natural antioxidants, SFS at 20 mg was found to have the lowest TBARS values. At day 7, the addition of natural antioxidants showed a significant reduction in the formation of TBARS compared to the non-treated samples. SFS at 20 mg was found to have the lowest TBARS values of 12.44 mg MDA/kg. Only the addition of SFS at 20 mg and ROS at 10 mg extract over a 7 day period awarded similar protection against oxidative rancidity compared to the BHT treatment (Table 2.4).

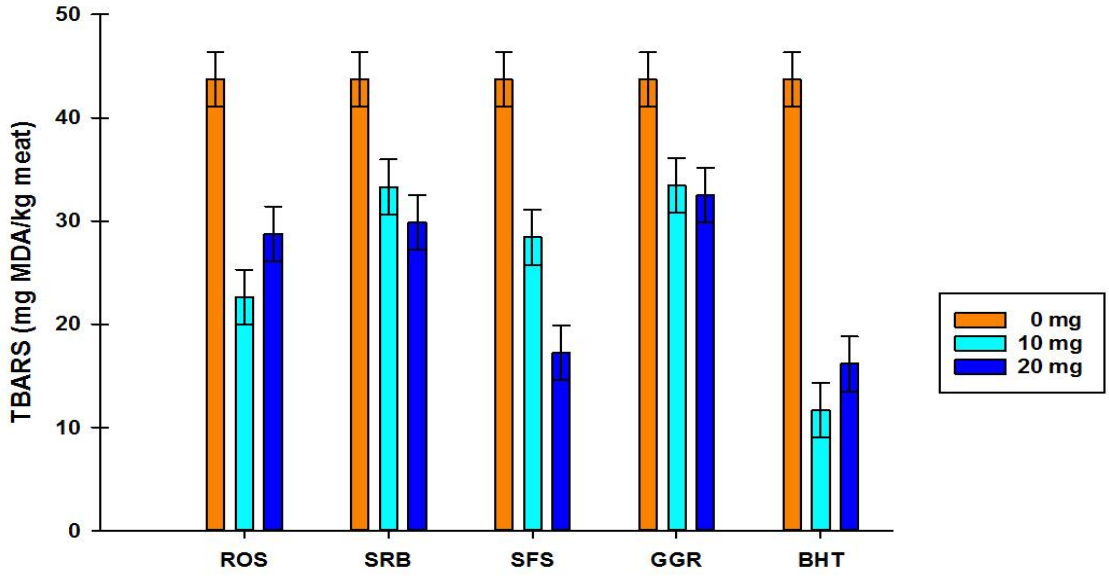

Figure 2.7 Effect of different levels of natural antioxidants on TBARS values in fat from chicken thigh meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

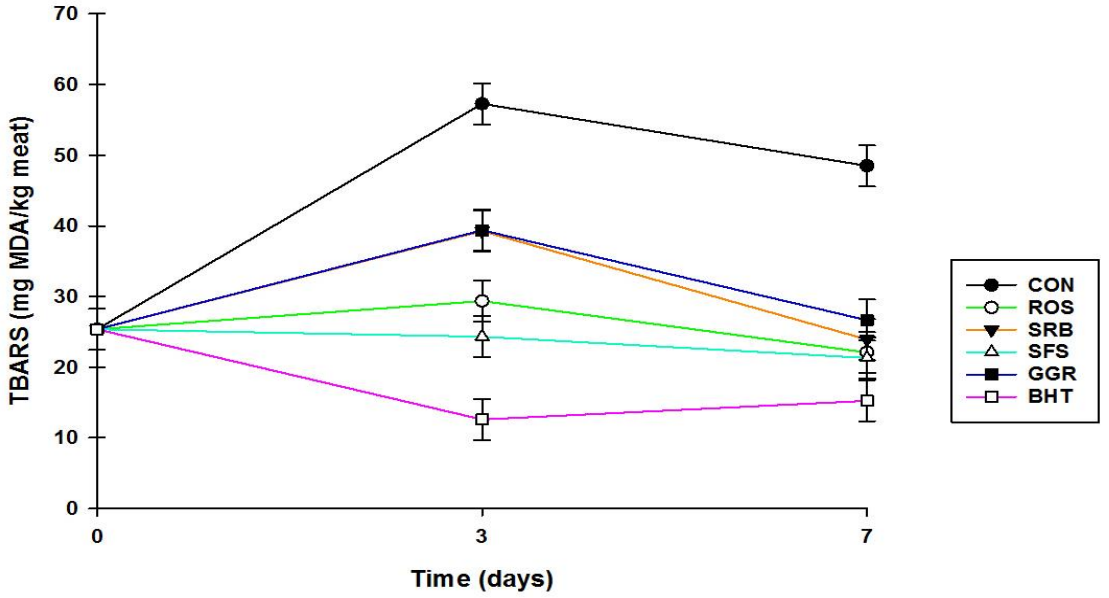

Figure 2.8 Effect of natural antioxidant application on TBARS values in fat from chicken thigh meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.1.3 Chicken Adipose Fat

The addition of antioxidants to fat from adipose tissue significantly declined the lipid oxidation compared to the non-treated samples The BHT treatment had significantly lower ($p < 0.001$) lipid oxidation with a mean value of 25.15 mg MDA/kg meat compared to the ROS, GGR, SFS, non-treated samples and SRB (Appendix Table A2). No significant differences were found between 10 and 20 mg of each antioxidant, although they were significantly differed from non-treated samples "zero" level. Among natural antioxidants, ROS at both 10 and 20 mg had the lowest TBARS values (Figure 2.9). Regardless of antioxidant levels, the TBARS value of non-treated and treated fat samples increased over storage time, showing a rapid increase up to 3 days with no discernible changes over 7 days. Over storage time, all treated samples (with exception SRB) were found to have the lowest TBARS value compared to non-treated samples and BHT followed by ROS were found to have the lowest formation of TBARS at each interval of storage time (Figure 2.10). The results presented in Table 2.4 shows that a significant interaction between antioxidant x level x storage time ($p < 0.001$). All natural antioxidants (with the exception of SRB at both levels) effectively reduced the lipid oxidation during the storage time compared to the non-treated samples. Among the natural antioxidants, ROS and SFS at higher levels were found to have the lowest TBARS values compared to SRB, GGR, SFS and non-treated samples. The addition of natural extracts at the lower and higher level on day 3 of storage time awarded similar protection against oxidative rancidity compared to the BHT. Subsequently, none of the natural antioxidants performed significantly more than a synthetic antioxidant (BHT treatment).

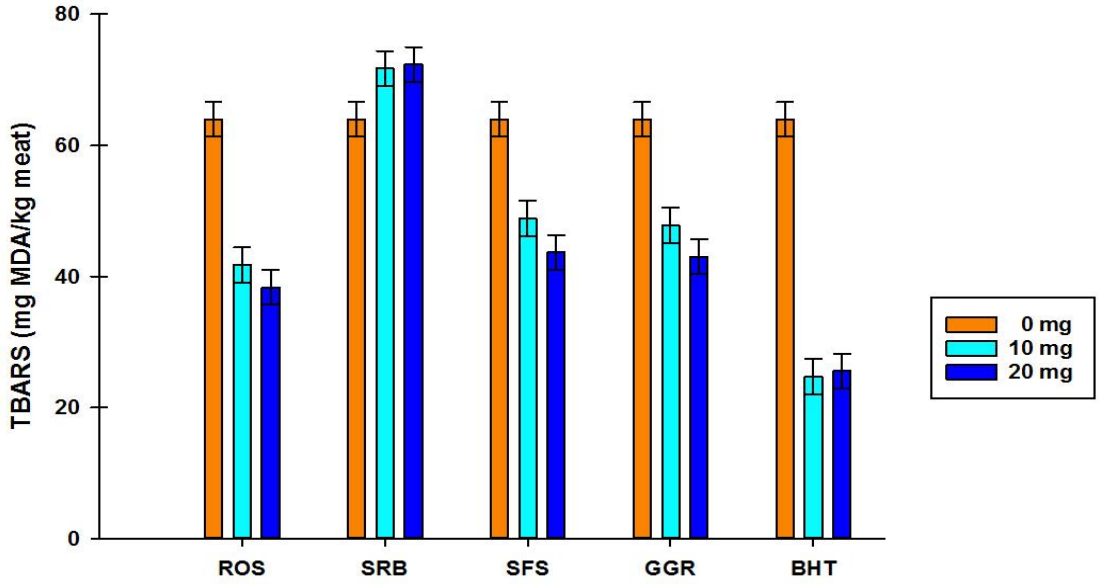

Figure 2.9 Effect of different levels of natural antioxidants on TBARS values in fat from chicken adipose tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

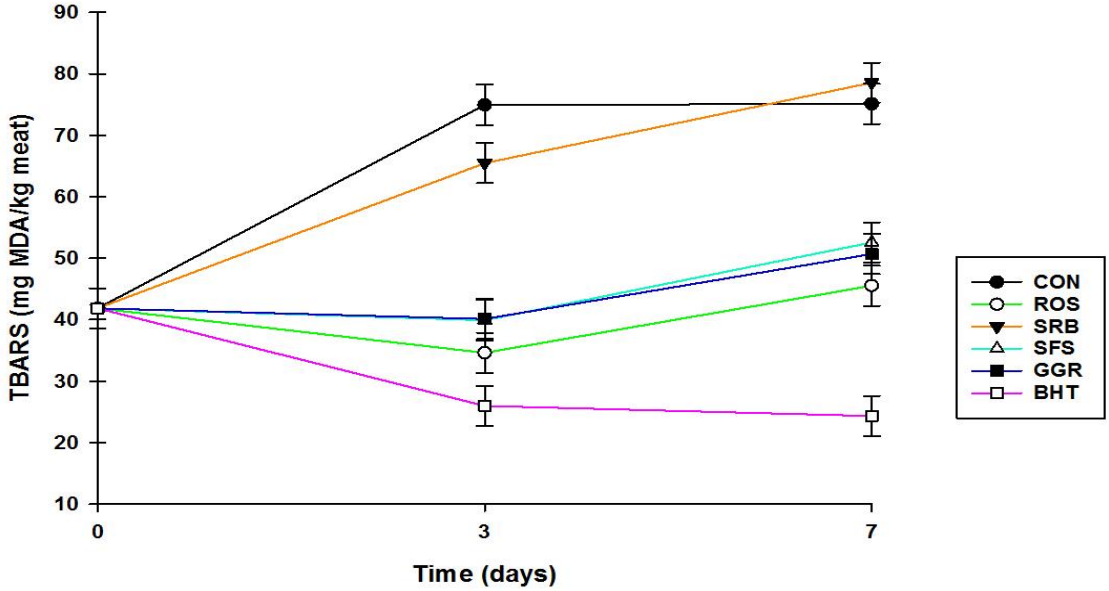

Figure 2.10 Effect of natural antioxidant application TBARS values in fat from chicken adipose tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.1.4 Chicken Skin Fat

The application of antioxidants significantly reduced the formation of TBARS in skin fat, while samples treated with BHT and ROS provided greater protection against oxidative rancidity compared to the non-treated samples (Appendix Table A2). No significant differences were found between 10 and 20 mg of each antioxidant, although they were significantly differed from non-treated samples "zero" level. BHT and ROS at both levels were found to have the lowest formation of TBARS (Figure 2.11). Regardless of the antioxidant levels, TBARS content significantly increased ($p \leq 0.05$) in non-treated of fat from skin tissue over 3 and up to 7 days' storage time from 12.95 to 33.87 and 54.57 mg MDA/kg respectively. While, antioxidant treatments were found to have a lower TBARS values compared to the non-treated samples over storage time. The antioxidants that found to have the lowest TBARS values for 7 days, were BHT and ROS (Figure 2.12). A significant difference was found between antioxidant x level x storage time ($p < 0.001$) for TBARS in the skin fat sample (Table 2.4). The TBARS content increased in non-treated samples of fat from skin tissue over 3 and up to 7 days of storage time. Natural antioxidant supplementation at both concentrations significantly lowered the TBARS values as compared to the non-treated samples over storage time. No significant differences were found among natural antioxidant supplementation at either level over 7 days. The addition of natural extracts at the lower and higher levels on day 3 of the storage time awarded similar protection against oxidative rancidity compared to the BHT. Subsequently, most of the natural antioxidants (with the exception of ROS at 10 and 20 mg) did not perform significantly better than the synthetic antioxidant (BHT treatment) (Table 2.4).

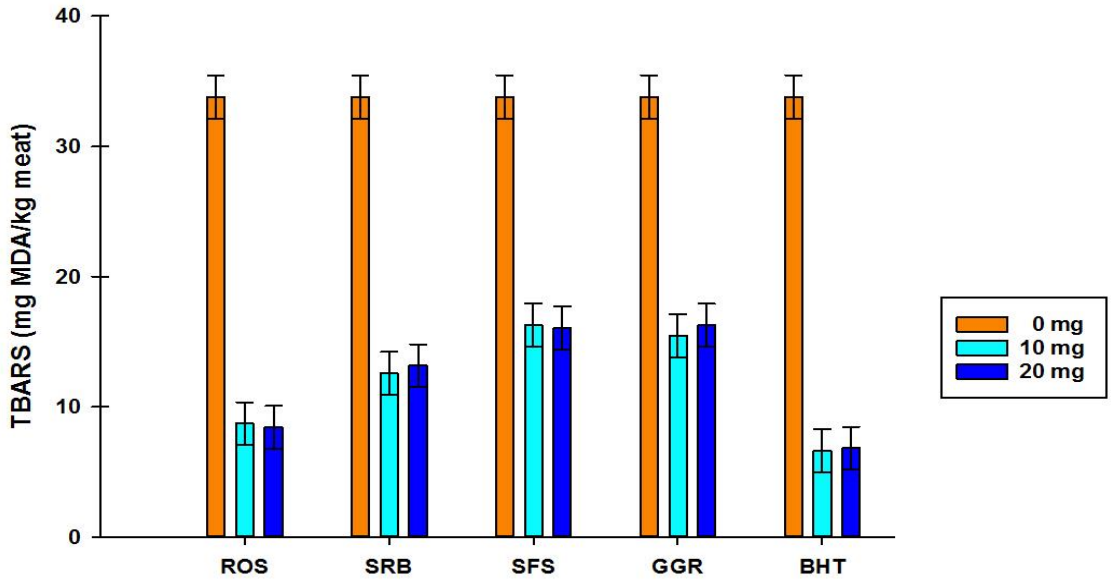

Figure 2.11 Effect of different levels of natural antioxidants on TBARS values in fat from chicken skin tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

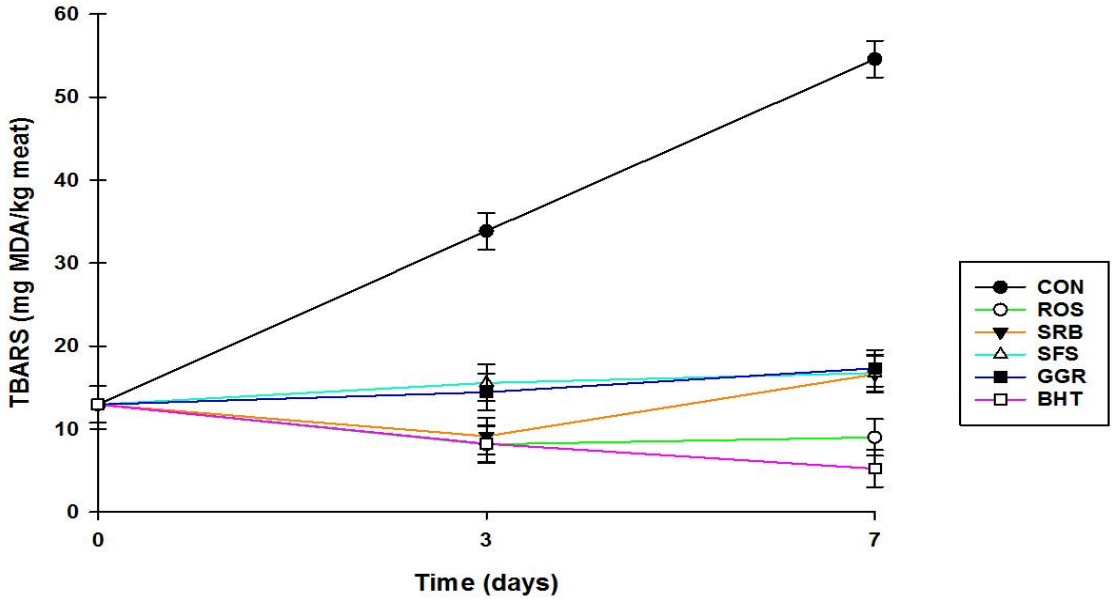

Figure 2.12 Effect of natural antioxidant application on TBARS values in fat from chicken skin tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.2 Effect of Natural Antioxidants on the Degradation of Phospholipids in Fat Extracted from Chicken Portions during Accelerated Storage Conditions

2.3.2.1 Chicken Breast Fat

In fat from breast chicken meat, phospholipid content was significantly affected by antioxidant supplementation ($p = 0.005$). BHT treatment was found to have the lowest content of phospholipid with a mean value of 46.71 g/100 g fat (Appendix Table A2). No significant differences were found between "zero" level and 10 mg of each antioxidant, while samples treated with 20 mg yielded markedly a lower phospholipid content than non-treated samples "zero" level (Figure 2.13). Over storage time, the content of phospholipid was decreased at day 3 and slightly increased at day 7, while a significant reduction of phospholipid was found in fat treated with BHT at day 3, with no discernible changes over 7 days (Figure 2.14). No significant differences ($p = 0.475$) were found between antioxidant, level and storage time for phospholipid content (Table 2.5).

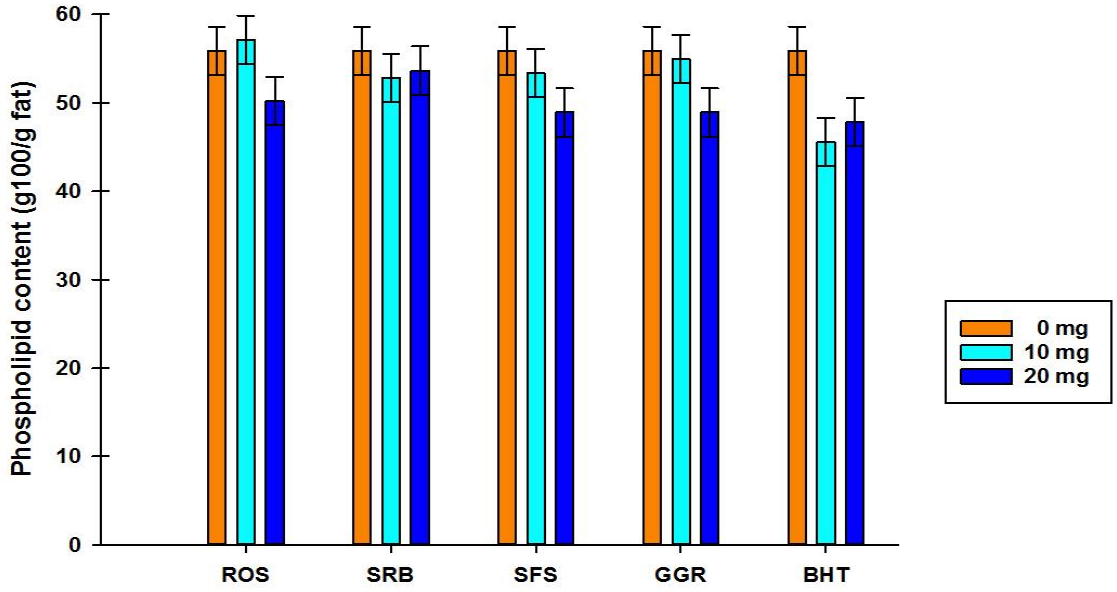

Figure 2.13 Effect of different levels of natural antioxidants on phospholipid content in fat from chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

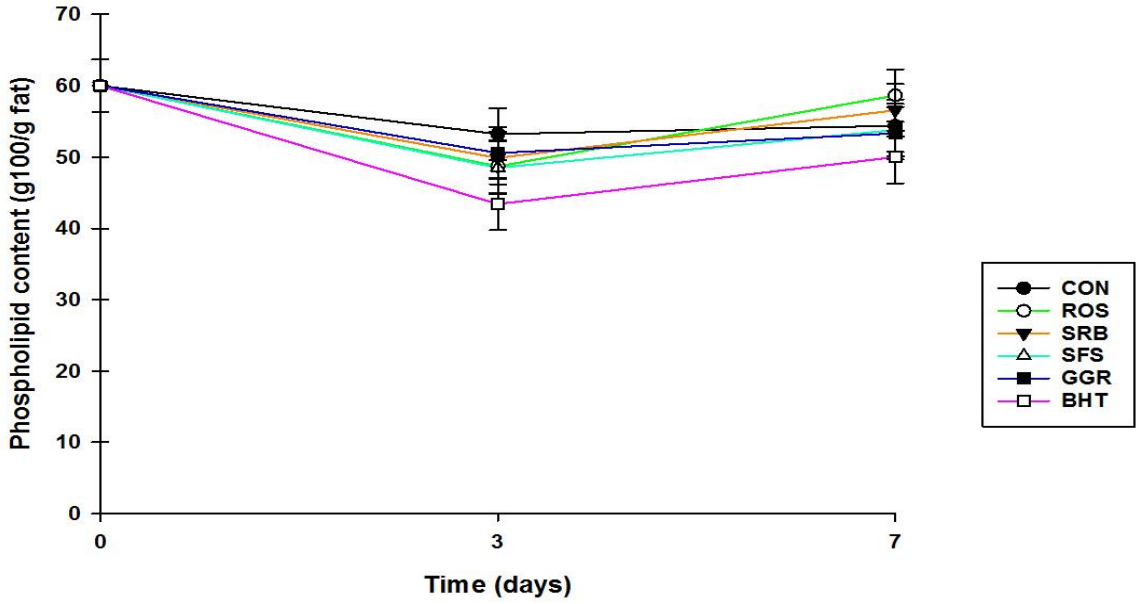

Figure 2.14 Effect of natural antioxidant application on phospholipid content in fat from chicken breast meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

Table 2.5 Effect of natural antioxidant extracts at different levels on phospholipid content (g100/g fat) in fat from chicken portions during the accelerated storage time at 62.8 °C.

Tissue	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
Breast	0	59.99										
	3	53.22	43.88	42.19	53.14	44.23	50.85	48.92	50.67	46.32	51.35	49.79
	7	54.39	46.50	53.50	61.14	56.12	54.75	58.37	56.09	51.49	58.62	48.03
Thigh	0	34.46										
	3	24.86	25.14	27.06	26.68	28.27	29.02	28.41	26.80	27.46	29.42	29.66
	7	25.82	28.21	29.23	27.99	30.44	32.25	34.29	31.48	30.93	30.69	31.62
Adipose	0	4.68^{bcd}										
	3	2.90^{ab}	2.89^{ab}	3.27^{ab}	2.68^{a}	3.13^{ab}	2.99^{ab}	2.92^{ab}	2.93^{abc}	2.94^{ab}	2.51^{a}	3.71^{abc}
	7	3.51^{abc}	5.28^{cd}	4.83^{bcd}	3.94^{abcd}	4.71^{bcd}	4.06^{abcd}	4.03^{abcd}	3.94^{abcde}	3.57^{abc}	5.75^{d}	3.48^{abc}
Skin	0	8.05										
	3	5.01	4.75	5.21	5.43	4.97	6.44	4.61	5.15	5.89	4.66	5.56
	7	5.26	5.96	6.09	6.27	4.79	5.82	5.90	6.91	5.87	6.14	5.37

Mean values with different small superscript letters presented within each row and column of each tissue differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.2.2 Chicken Thigh Fat

In fat from thigh meat, treated and non-treated samples was not significantly affected by the application of antioxidants and interaction between antioxidant x level x storage time ($p > 0.05$) (Appendix Table A2). No significant difference was found between antioxidant levels ($p = 0.842$). Regardless of the antioxidant levels, phospholipid content in all samples (treated and non-treated) was decreased over 3 days and slightly increased thereafter at day 7. All natural antioxidants yielded more phospholipid content than non-treated samples at day 3 and 7 of storage times. The natural antioxidants that yielded significantly higher amount of phospholipid content at day 3 and 7, were SRB and GGR compared to the non-treated samples (Figure 2.15). There was no significant interaction ($p = 0.979$) between antioxidant x level x storage time (Table 2.5).

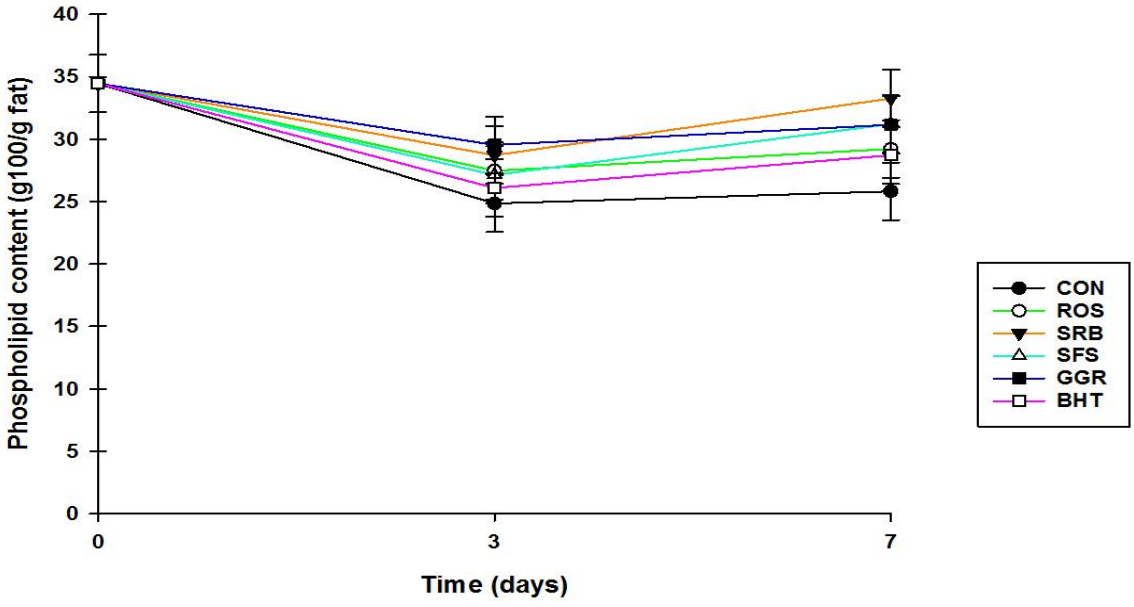

Figure 2.15 Effect of natural antioxidant application on phospholipid content in fat from chicken thigh meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.2.3 Chicken Adipose Fat

In the fat from adipose tissue, phospholipid was not affected by natural antioxidant supplementation ($p = 0.158$) (Appendix Table A2). No significant differences were found between antioxidant levels and non-treated samples ($p = 0.295$). The results reported in Figure 2.16 show that the effect of antioxidants on phospholipid content was depended on the storage time. Hence, none of the natural antioxidants yielded more phospholipid content than non-treated samples within the first 3 days. At day 7, antioxidant treatments were found to have a higher amount of phospholipid content than non-treated samples. The only natural antioxidant that provided a greater protection against a decrease in phospholipid content at day 7 was BHT and GGR. The results also show that a significant interaction ($p = 0.007$) between antioxidant x level x storage time (Table 2.5). The phospholipid content of non-treated control was reduced over 7 days of accelerated storage. However, a significant reduction of phospholipid content was shown in samples treated with ROS and GGR extract at the lower application dose at day 3 compared to non-treated samples at day 0 of storage. None of the natural antioxidants provided protection against a reduction in phospholipid content within the first 3 days. The only natural antioxidant that provided a greater protection against a decrease in phospholipid content at day 7 was GGR at the lower application dose. Moreover, no significant differences were found between both levels (10 and 20 mg) of each antioxidant (with the exception of GGR extract). The impact of natural antioxidants at both levels was most similar to that of BHT.

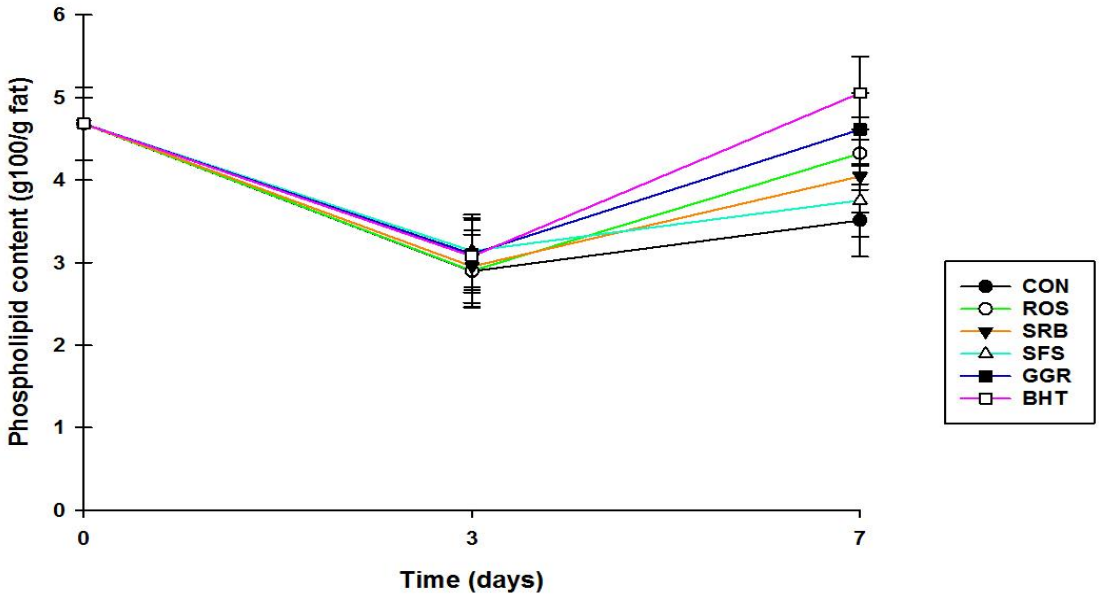

Figure 2.16 Effect of natural antioxidant application on phospholipid content in fat from chicken adipose tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.2.4 Chicken Skin Fat

Similar to the chicken adipose fat, the results show that the addition of antioxidants to the skin fat before processing did not have any effect on phospholipid content ($p = 0.333$) (Appendix Table A2). No significant difference was found between antioxidants at different levels of application and non-treated samples ($p = 0.267$). Accelerated storage conditions significantly reduced the phospholipid content in non-treated samples and samples treated with ROS, SFS, GGR, SRB and BHT over 3 days, found no effect at day 7. None of the natural antioxidants awarded a significant protection against a decrease in phospholipid content over 7 days as compared to the non-treated and BHT samples (Figure 2.17). There was no significant interaction ($p = 0.374$) between antioxidant x level x storage time (Table 2.5).

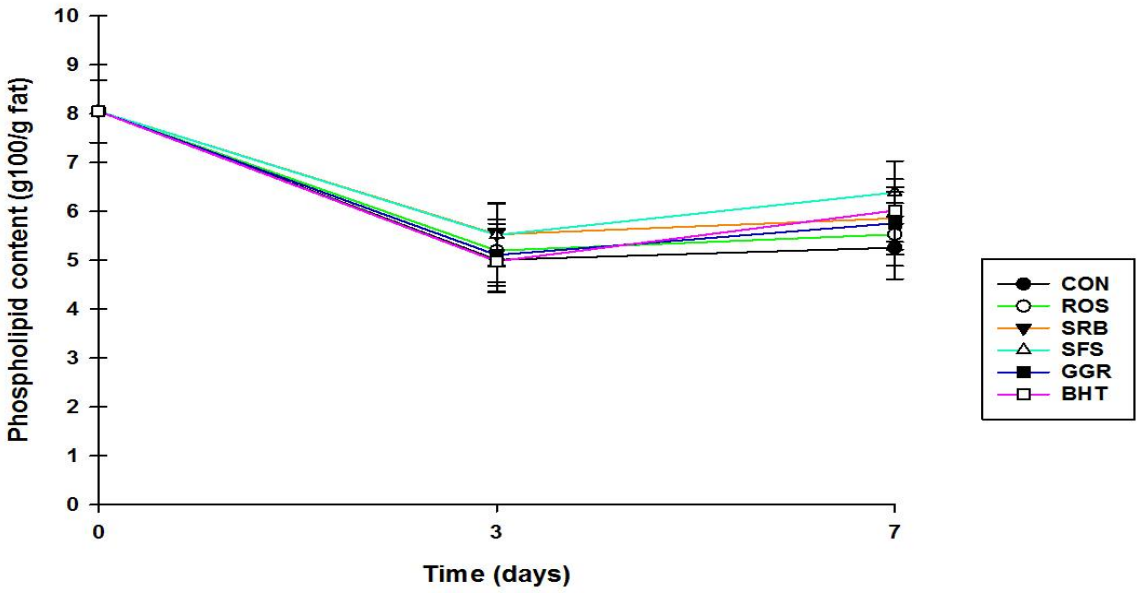

Figure 2.17 Effect of natural antioxidant application on phospholipid content in fat from chicken skin tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.3 Effect of Natural Antioxidants on the Formation of Conjugated Dienes (CD) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions.

2.3.3.1 Chicken Breast Fat

Conjugated dienes (CD) in chicken breast fat were significantly affected by antioxidant application ($p < 0.001$) (Appendix Table A2). The CD values of non-treated samples were significantly higher compared to the sample treated with antioxidants with mean values of 54.95, 40.52, 39.92, 38.49, 35.59 and 34.69 µmol/g fat for non-treated samples, GGR, ROS, SRB, SFS and BHT, respectively). The results shown in Figure 2.18 show that both levels of natural antioxidants were significantly reduced the formation of CDs in fat from breast chicken meat compared to "zero" level. The highest reduction was found in samples treated with 20 mg followed by 10 mg and "zero" level respectively. Figure 2.19 shows that there was a significant difference between antioxidant and storage time ($p < 0.001$). The CD values of non-treated samples at day

0 was 44.64 µmol/g fat. After the fat samples were subjected to the accelerated storage time, the CD values significantly increased by over 40 % to 62.66 µmol/g fat at day 3 of storage and slightly declined toward the end of storage to 57.55 µmol/g fat at day 7. The CDs in breast fat samples were significantly reduced by the addition of natural antioxidants compared to the non-treated samples over time. No significant differences were found among natural antioxidant during the 7 days of storage time. The addition of natural extracts to fat from breast meat on days 3 and 7 awarded similar protection against oxidative rancidity compared to the synthetic antioxidants. Table 2.6 shows that there was no significant interaction between antioxidant x level x storage time ($p = 0.145$).

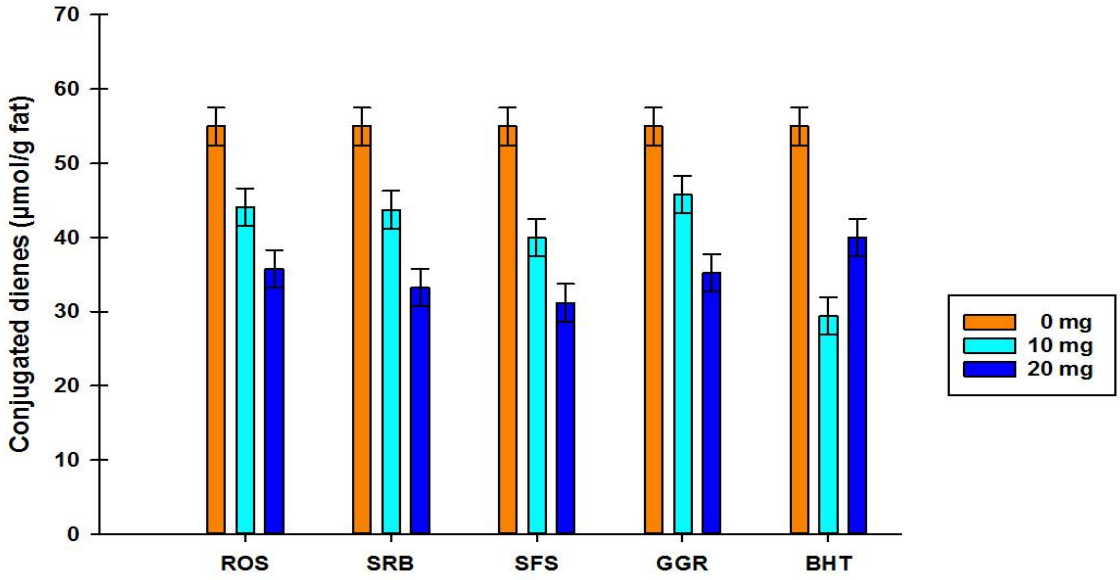

Figure 2.18 Effect of different levels of natural antioxidants on conjugated dienes in fat from chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

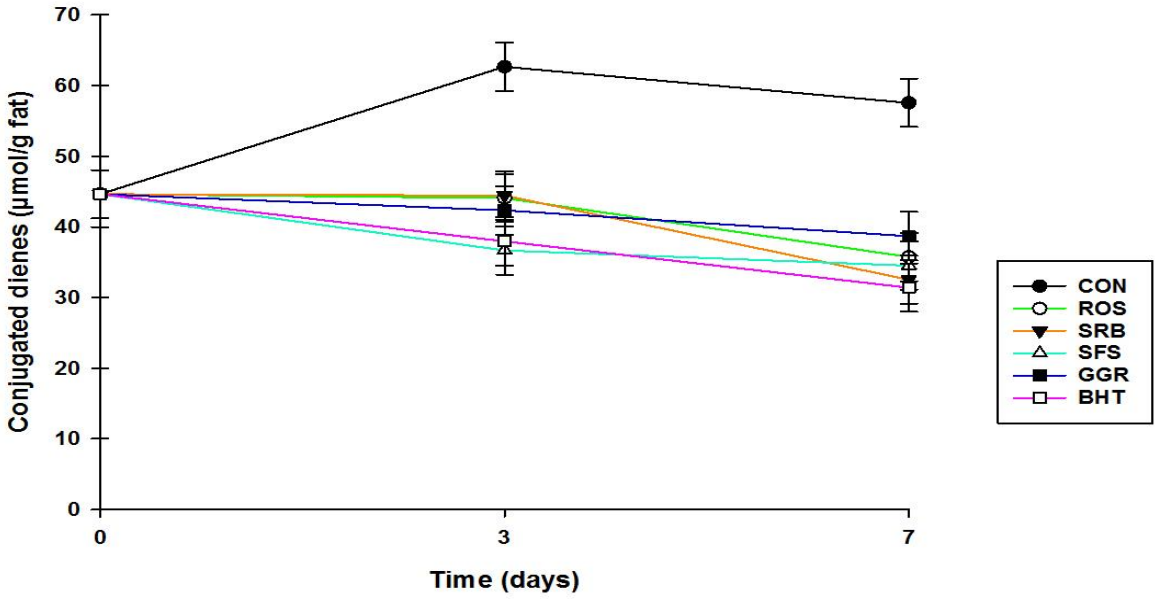

Figure 2.19 Effect of natural antioxidant application on conjugated dienes in fat from chicken breast meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

Table 2.6 Effect of natural antioxidant extracts at different levels on conjugated dienes (µmol/g fat) in fat from chicken portions during the accelerated storage time at 62.8 °C.

Tissue	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
Breast	0	44.64										
	3	62.66	36.08	39.87	46.17	41.99	49.36	39.53	40.29	33.06	47.29	37.40
	7	57.55	22.69	40.13	41.94	29.56	38.11	26.97	39.72	29.33	44.34	33.06
Thigh	0	20.34										
	3	29.29	29.32	33.66	29.16	31.03	28.27	23.61	28.22	23.88	27.20	29.72
	7	30.07	35.90	36.78	30.63	33.37	30.17	31.12	30.95	28.85	30.09	32.49
Adipose	0	7.55^{a}										
	3	13.68^{def}	13.83^{def}	13.13^{def}	14.94^{fg}	17.37^{h}	13.77^{def}	15.32^{fgh}	13.81^{def}	13.95^{def}	12.03^{cd}	14.08^{efg}
	7	10.63^{bc}	10.28^{bc}	12.12^{cde}	14.31^{efg}	16.42^{gh}	10.56^{bc}	9.36^{ab}	10.47^{bc}	10.10^{bc}	10.71^{bc}	9.61^{ab}
Skin	0	8.83^{a}										
	3	16.32^{ghi}	20.96^{k}	27.07^{i}	17.05^{ghi}	17.58^{hij}	13.51^{def}	11.16^{bc}	13.27^{cde}	12.77^{bcd}	15.50^{fgh}	18.21^{ij}
	7	29.14^{i}	19.49^{jk}	28.19^{i}	15.02^{efg}	16.52^{ghi}	12.11^{bcd}	10.64^{ab}	12.37^{bcd}	11.61^{bcd}	13.10^{cde}	15.76^{gh}

Mean values with different small superscript letters presented within each row and column of each tissue differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.3.2 Chicken Thigh Fat

In thigh fat, the CD values were found significantly lower in non-treated samples compared to the sample treated with antioxidants ($p < 0.001$) (Appendix Table A2). Antioxidants at both levels were found to have higher CD values compared to non-treated samples ($p = 0.007$). However, none of the natural antioxidants awarded any protection against a reduction of CD values compared to the non-treated samples. BHT treatment contained 20 mg had a higher content of CDs compared to any other treatments (Figure 2.20). Figure 2.21 shows that the CD values of all samples were significantly increased at day 3 with no discernible changes over 7 days. None of the natural antioxidants inhibited the formation of CDs in thigh fat samples during the 7 days. However, the natural antioxidants from SFS and SRB provided very little protection against oxidation at day 3, while did not affect the CDs at day 7. While at face value the natural antioxidants provided a slightly better protection against changes in CD compared to BHT. No significant interaction ($p = 0.288$) was found between antioxidant x level x storage time (Table 2.6).

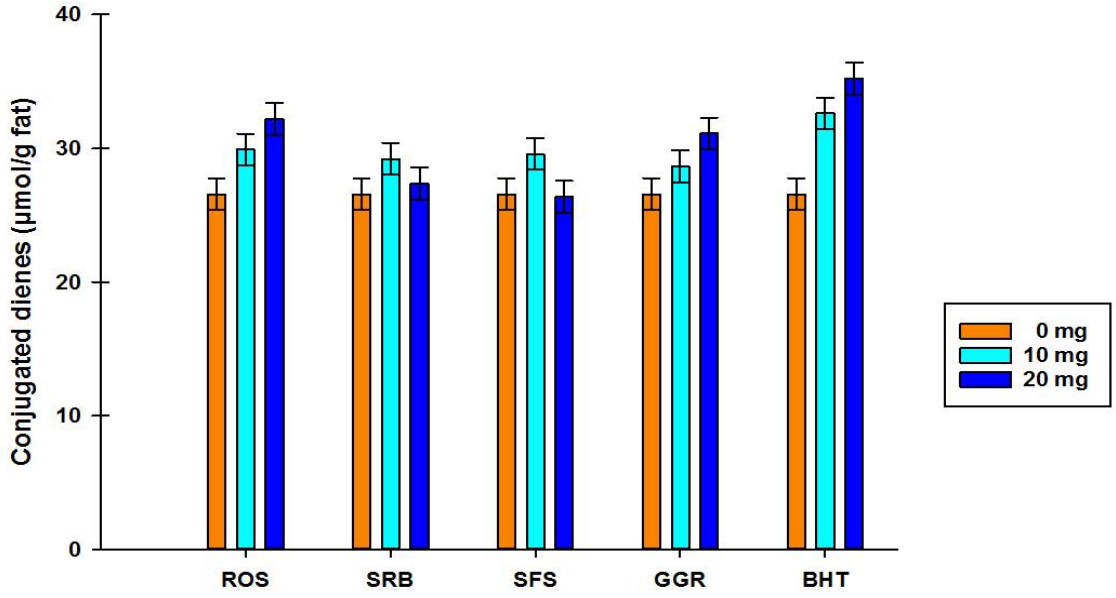

Figure 2.20 Effect of different levels of natural antioxidants on conjugated dienes in fat from chicken thigh meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

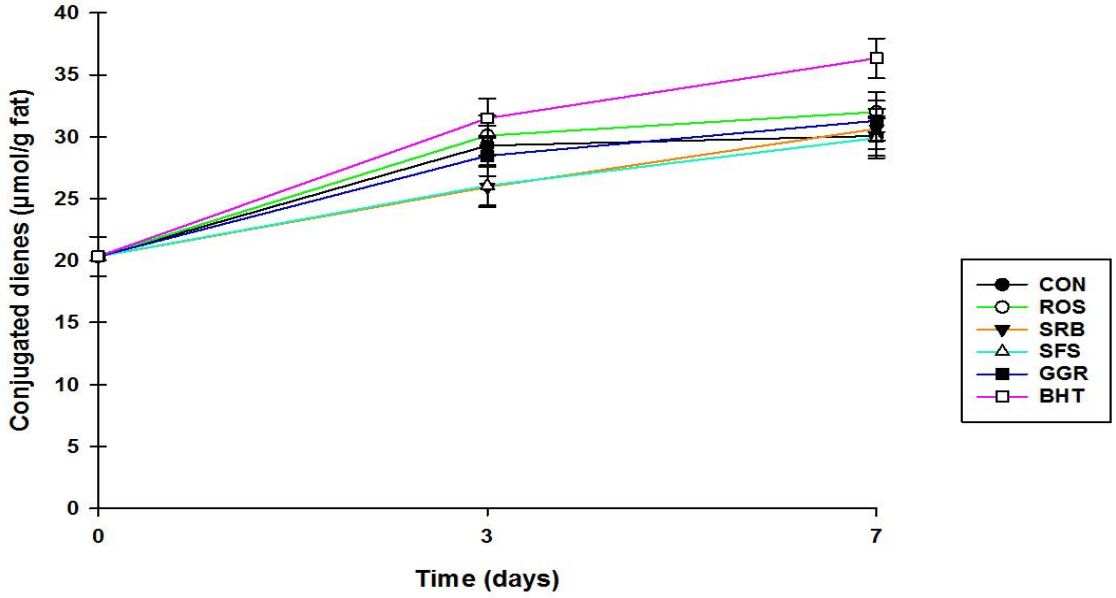

Figure 2.21 Effect of natural antioxidant application on conjugated dienes in fat from chicken thigh during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.3.3 Chicken Adipose Fat

In fat from adipose tissue, the CD values were found significantly higher in samples treated with ROS with mean value of 15.76 µmol/g fat compared to the other treatments, regardless of antioxidants and storage time ($p < 0.001$) (Appendix Table A2). All antioxidant at both levels was found to have significantly higher content of CDs compared to the non-treated samples ($p < 0.001$). A significant difference was found only between both levels of ROS and lower level provided a greater protection against formation of CD (Figure 2.22). Regardless of natural antioxidant levels, the CD values in all samples were significantly increased at day 3 compared to day 0 and significantly decreased thereafter at day 7. The CD values were significantly higher in samples treated with ROS treatment than any other treatments at day 3 and 7 of storage time (Figure 2.23). The results reported in Table 2.6 show a significant interaction between antioxidant x level x storage time ($p < 0.05$). In all instances (treated or non-treated) the peak in CDs occurred at day 3, with markedly lower CD values at day 7 compared to day 3. None of the natural antioxidants awarded any protection against an increase in CDs at day 3. However, samples treated with ROS had a markedly higher level of CD at both times and at both levels of application. The only natural antioxidants that provided a decrease in CD at day 7 were SRB and GGR at the higher application dose. The natural antioxidants and BHT was found to have similar effects on CD content in adipose fat.

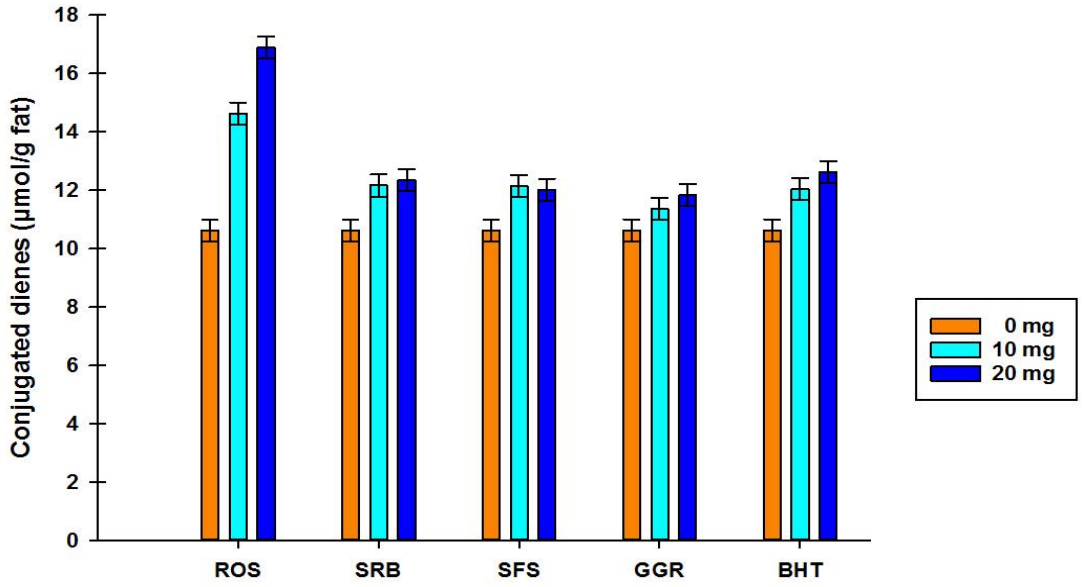

Figure 2.22 Effect of different levels of natural antioxidants on conjugated dienes in fat from chicken adipose tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

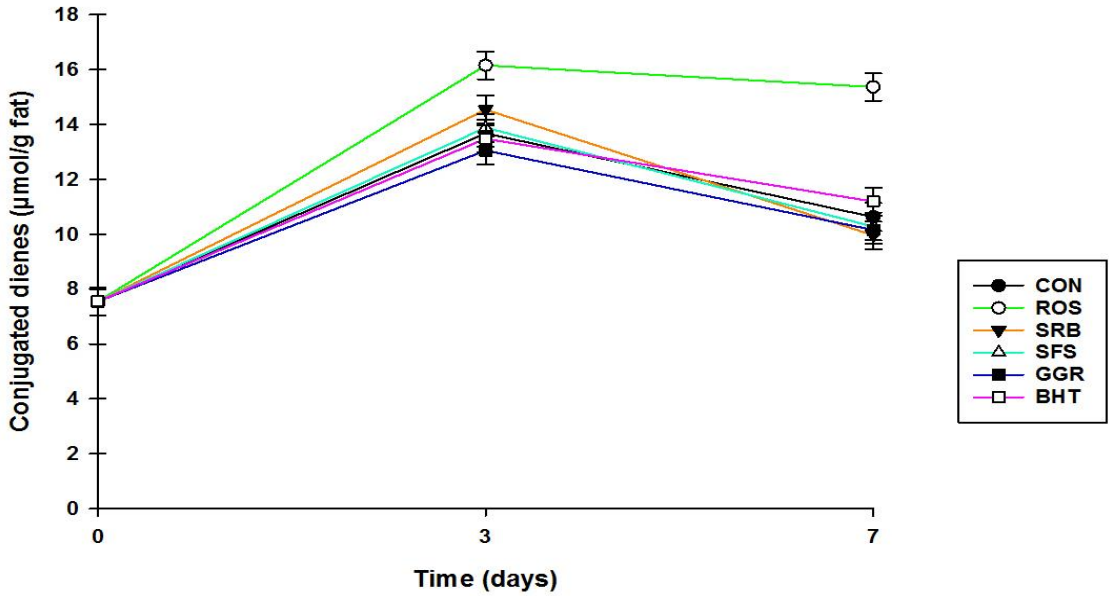

Figure 2.23 Effect of natural antioxidant application on conjugated dienes in fat from chicken adipose tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

3.3.3.4 Chicken Skin Fat

In fat from skin tissue, all antioxidants (with exception BHT) significantly reduced the formation of CD compared to the non-treated samples with mean values of 11.86, 12.51, 15.64, 16.54, 18.10 and 23.93 µmol/g fat for SRB, SFS, GGR, ROS, non-treated samples and BHT, respectively (Appendix Table A2). All antioxidants (with the exception of BHT) at both levels were significantly differed from non-treated samples ($p \leq 0.05$), while significantly the highest reduction of CD was found in samples treated with SRB and SFS at both levels compared to the non-treated samples (Figure 2.24). The results shown in Figure 2.25 observed that the CD values were increased significantly in non-treated samples with storage time, while in treated samples significant increase of CD was found at day 3, with no significant changes at day 7. The antioxidants that significantly reduced the formation of CD at day 3, were SRB and SFS. At day 7, all natural antioxidants were significantly reduced the CD values compared to the non-treated samples, while the highest reduction was found in SRB and SFS treatment. A significant ($p < 0.001$) interaction between antioxidant, level and storage time was found for CD values (Table 2.6). The CD values of non-treated samples were significantly increased ($p < 0.001$) over storage time, while in treated samples an increase of CD formation was found up to 3 days and decreased over time. All natural antioxidants with the exception of ROS at both levels and GGR at 20 mg awarded discernible protection against an increase in CDs at day 3. Samples treated with ROS at both levels and GGR at higher levels had a markedly higher level of CD at day 3 of storage. All natural antioxidants provided a decrease in CDs at day 7, while natural antioxidants that provided the highest decrease in CDs at day 7 were SRB and SFS at the lower and higher application dose. The natural antioxidants were performed similar to that of BHT.

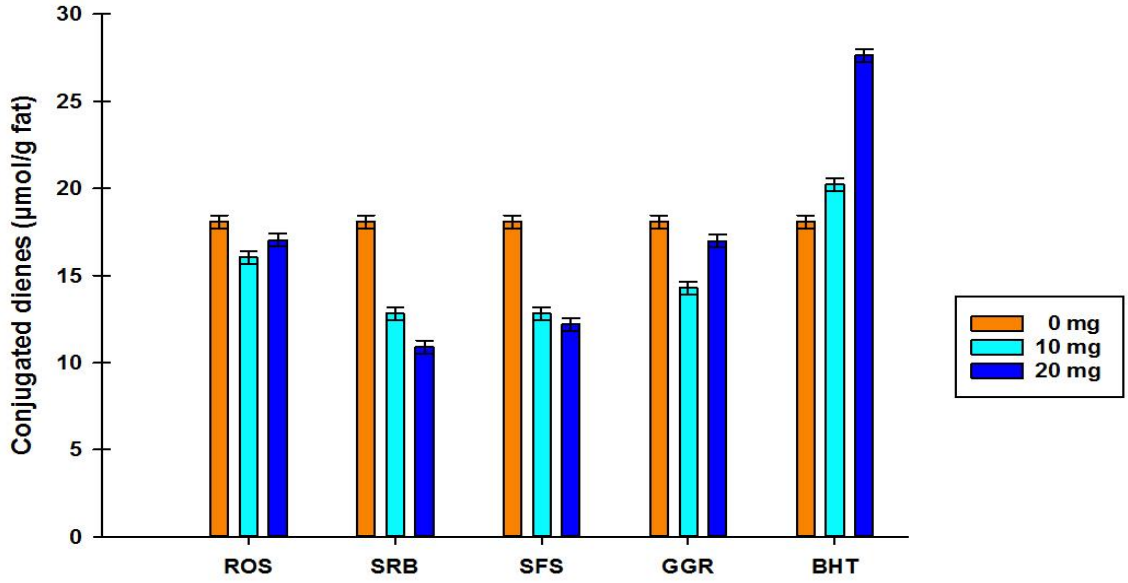

Figure 2.24 Effect of different levels of natural antioxidants on conjugated dienes in fat from chicken skin tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

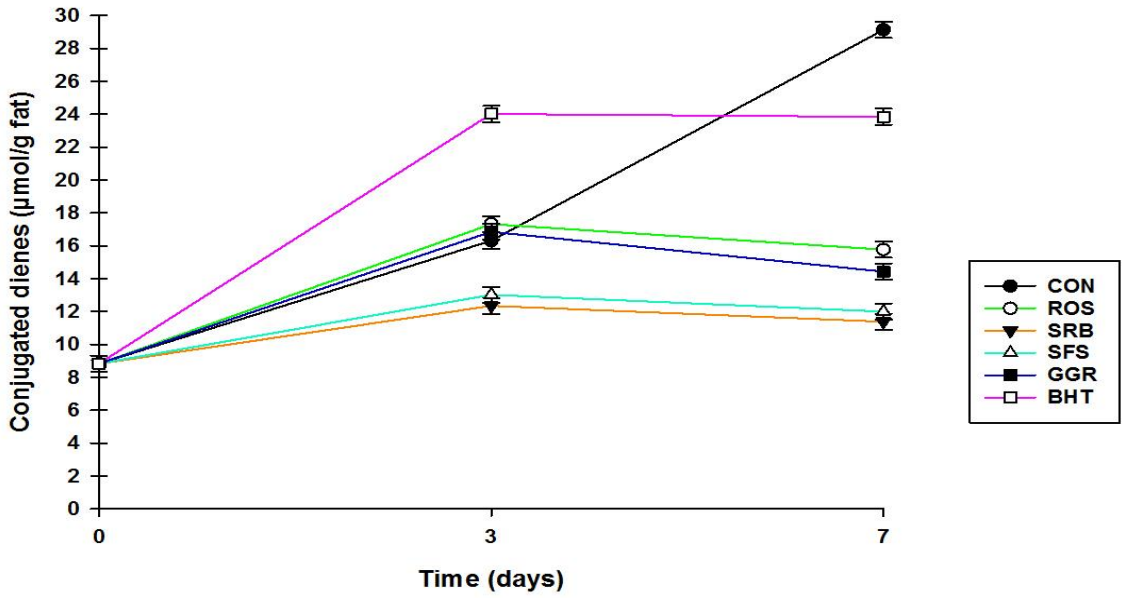

Figure 2.25 Effect of natural antioxidant application on conjugated dienes in fat from chicken skin tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.4 Effect of Natural Antioxidants on the Formation of Conjugated Trienes (CT) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions

2.3.4.1 Chicken Breast Fat

The application of antioxidants significantly reduced ($p < 0.001$) the formation of CTs in chicken breast fat compared to the untreated samples with mean values of 9.58, 12.03, 13.97, 14.50, 14.89 and 21.76 µmol/g fat for BHT, SFS, ROS, SRB, GGR and non-treated samples, respectively) (Appendix Table A2). Antioxidants at both levels (10 and 20 mg) significantly reduced the formation of CT in fat samples compared to the non-treated samples ($p < 0.001$). The natural antioxidants at a higher level of application were found to have the lowest formation of CT compared to lower and non-treated samples. Among them, SFS at the lower and higher application dose had the lowest formation of CT. BHT at 10 mg significantly reduced more CT formation than 20 mg and non-treated samples (Figure 2.26). Regardless of antioxidant levels, the CT values in non-treated samples at 0 day was 19.17 µmol/g fat. After the fat samples were subjected to the accelerated storage time, the CT values increased by 35 % to 25.88 µmol/g fat at day 3 of storage time and declined afterward to 20.23 µmol/g fat at the end of the storage time. The application of natural antioxidants was significantly reduced the formation of CTs compared to the non-treated samples at day 3 and 7 of storage time ($p < 0.001$). BHT and SFS treatments were found to have the lowest CT values compared to the non-treated samples during the storage time (Figure 2.27). The natural antioxidant that provided the highest reduction in CTs over time was SFS. The impact of the natural antioxidants was similar to that of BHT particularly at the end of storage (Figure 2.27). There was no significant interaction ($p = 0.449$) between antioxidant x level x storage time (Table 2.7).

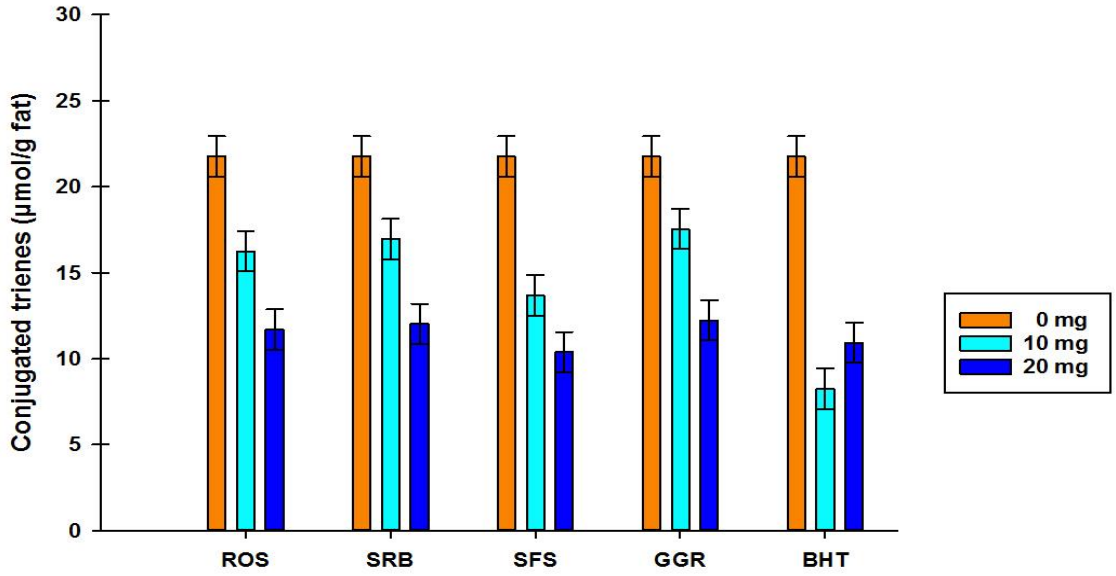

Figure 2.26 Effect of different levels of natural antioxidants on conjugated trienes in fat from chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

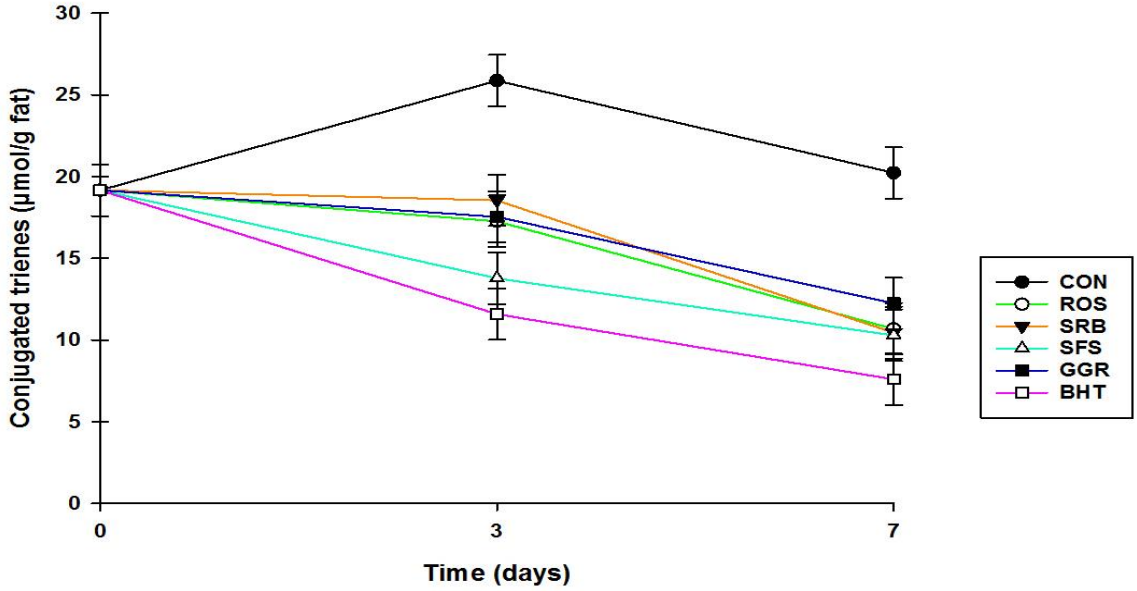

Figure 2.27 Effect of natural antioxidant application on conjugated trienes in fat from chicken breast meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

Table 2.7 Effect of natural antioxidant extracts at different levels on conjugated trienes (µmol/g fat) in fat from chickens' portions during the accelerated storage time at 62.8 °C.

Tissue	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
Breast	0	19.17										
	3	25.88	11.00	12.16	19.21	15.32	21.26	15.84	15.36	12.19	20.34	14.74
	7	20.23	5.47	9.71	13.29	8.06	12.68	8.20	12.00	8.57	14.76	9.72
Thigh	0	9.83										
	3	12.14	10.73	12.31	11.28	13.15	12.09	10.21	10.74	9.70	11.00	12.91
	7	12.98	14.15	13.82	13.64	14.00	13.72	14.17	13.35	12.96	13.18	14.50
Adipose	0	1.16^{abc}										
	3	$3.24b^{cdefg}$	$4.42d^{efg}$	$4.02d^{efg}$	$4.41d^{efg}$	5.56^{fg}	4.04^{defg}	4.22^{defg}	4.75^{efg}	4.66^{efg}	3.14^{bcdef}	5.63^{g}
	7	0.92^{ab}	0.79^{ab}	2.02^{abcd}	2.53^{abcde}	3.49^{cdefg}	1.47^{abc}	0.64^{a}	1.03^{abc}	0.81^{ab}	0.91^{ab}	0.44^{a}
Skin	0	2.69^{a}										
	3	4.06^{b}	7.08^{g}	8.97^{h}	5.94^{ef}	6.03^{f}	5.17^{def}	3.89^{b}	4.28^{bc}	3.90^{b}	5.71^{ef}	7.46^{g}
	7	4.41^{bcd}	5.08^{cde}	8.35^{h}	3.69^{b}	3.83^{b}	2.58^{a}	2.19^{a}	2.39^{a}	2.27^{a}	3.57^{b}	5.28^{def}

Mean values with different small superscript letters presented within each row and column of each tissue differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.4.2 Chicken Thigh Fat

The conjugated triense (CTs) in thigh fat treated with was not significantly affected by supplementation of antioxidants ($p > 0.05$) (Appendix Table A2). The addition of natural extracts at the lower and higher level statistically did not provide protection against oxidative rancidity compared to the non-treated samples. All antioxidants (with exception SRB and SFS) at 20 mg were found to have significantly higher content of CT than zero level (Figure 2.28). Moreover, CTs values were increased in all samples either treated or non-treated over 7 days of storage. The addition of natural extracts over a 7 day period statistically did not provide any protection against oxidative rancidity compared to the non-treated samples. While at face value the natural antioxidants provided a slightly better protection against changes in CT compared to the synthetic antioxidant BHT (Figure 2.29). The results present in Table 2.7 show that there was no significant interaction ($p = 0.311$) between antioxidant x level x storage time.

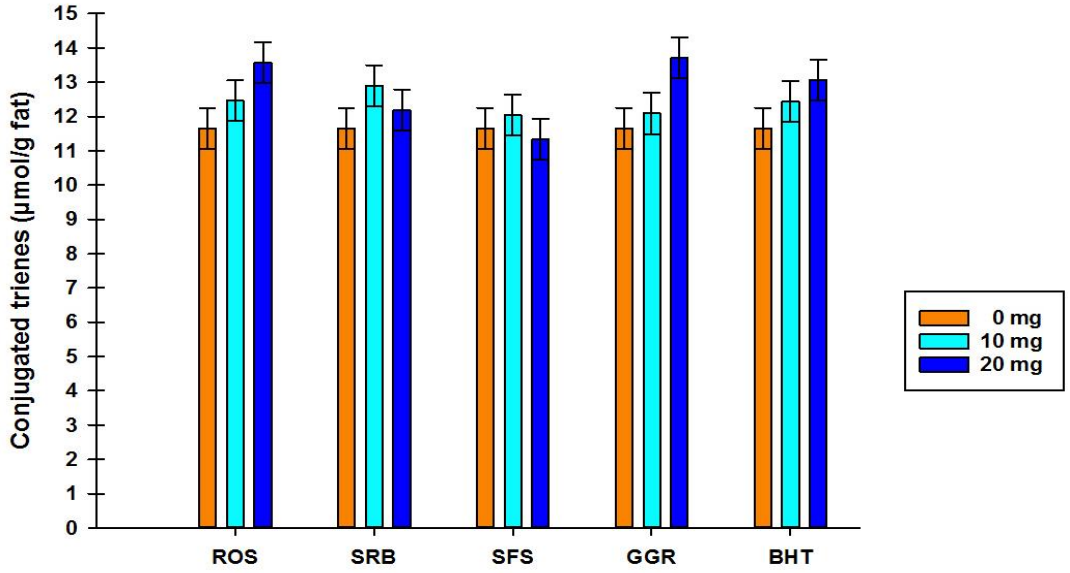

Figure 2.28 Effect of different levels of natural antioxidants on conjugated trienes in fat from chicken thigh meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

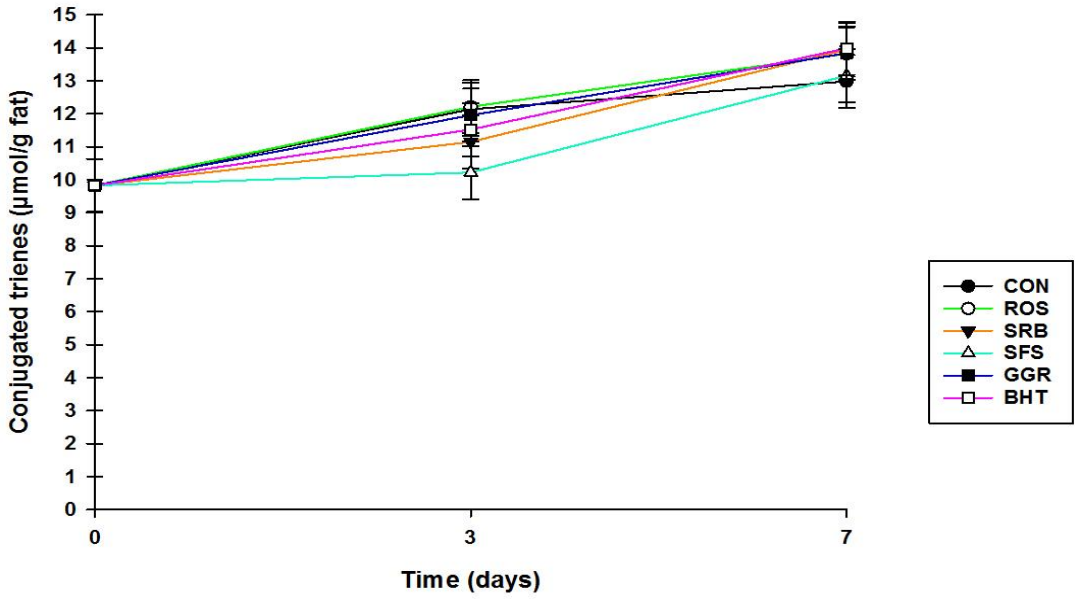

Figure 2.29 Effect of natural antioxidant application on conjugated trienes in fat from chicken thigh meat during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.4.3 Chicken Adipose Fat

In fat from adipose tissue, the CT values were found significantly higher in samples treated with ROS with mean value of 4.00 μmol/g fat compared to the other treatments, regardless of the level of antioxidants and storage time ($p < 0.001$) (Appendix Table A2). ROS at higher level was found to have significantly ($p = 0.054$) higher content of CT compared to the non-treated and treated samples (Figure 2.30). Figure 2.31 shows that the formation of the CT was significantly increased ($p < 0.001$) over the first 3 days and markedly declined at day 7. The addition of natural extracts statistically did not provide protection against oxidative rancidity compared to the non-treated samples for 7 days. The interactions between antioxidant, level and storage time are shown in Table 2.7. The peak in CTs occurred at day 3, with markedly lower CT values at day 7 compared to day 3. None of the natural antioxidants awarded any protection against an increase in CTs at day 3. However, samples treated with ROS had a markedly ($p = 0.014$) higher level of CTs at both times and at both levels of application. The only natural antioxidants that provided a decrease in CT at day 7 were SRB and GGR at the higher application dose. However, statistically they were not significant differed from non-treated samples. The impact of the natural antioxidants on reduction of CT content was comparable to that of BHT.

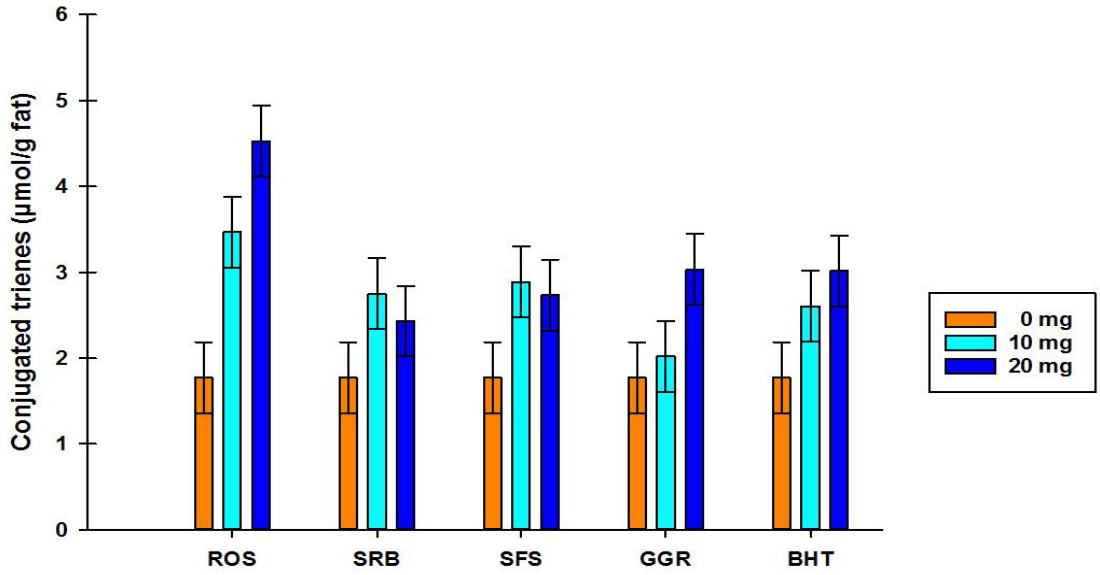

Figure 2.30 Effect of different levels of natural antioxidants on conjugated trienes in fat from chicken adipose tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

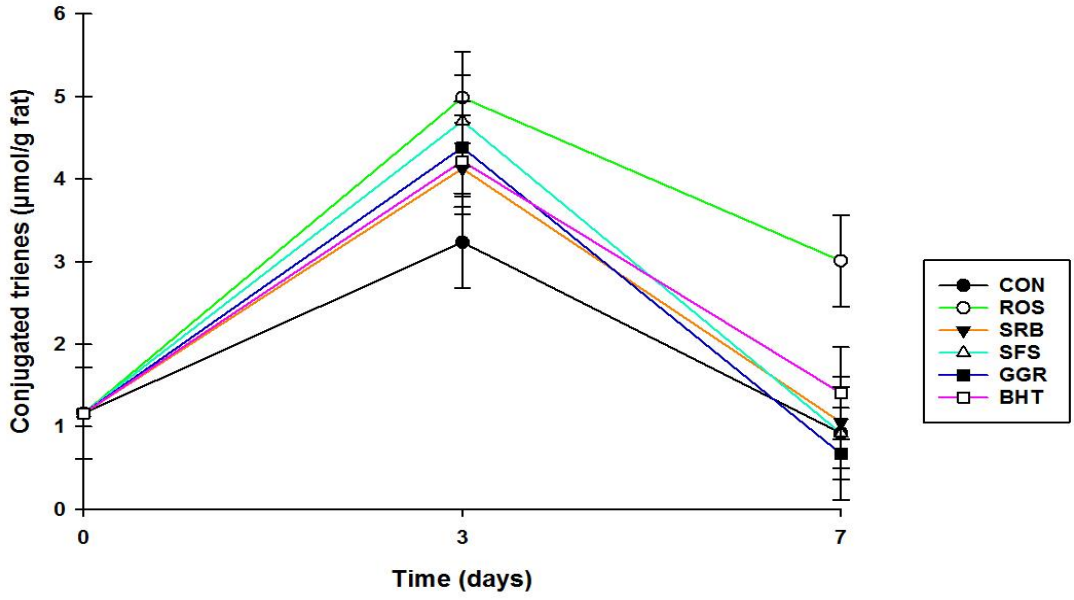

Figure 2.31 Effect of natural antioxidant application on conjugated trienes in fat from chicken adipose tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.4.4 Chicken Skin Fat

In fat from skin tissue, only SFS significantly reduced the formation of CT compared to the non-treated samples with mean values of 3.21 and 3.72 μmol/g fat for SFS and non-treated samples, respectively (Appendix Table A2). All antioxidants at both levels (with exception of SRB at 20 mg and SFS at 10 and 20 mg) significantly differed from non-treated samples ($p < 0.001$). However, only SRB at 20 mg and SFS at both levels awarded discernible protection against an increase in CT compared to the non-treated samples (Figure 2.32). The results presented in Figure 2.33 show that the CT values in non-treated samples were increased significantly at day 3 compared to 0 day, but did not differ thereafter at day 7. In contrast, the peak in CTs in treated samples occurred at day 3, with markedly lower CT values at day 7 compared to day 3. None of the natural antioxidants significantly reduced the formation of CT at day 3, while at day 7 SRB, SFS and ROS were significantly reduced the formation of CT in skin fat samples. The impact of the natural antioxidants was more effective compared to that of BHT. Furthermore, the interaction between antioxidant, level and storage time are listed in Table 2.7. The results show that storage time was found to have a significant ($p < 0.001$) effect on CT content in untreated samples, with a 30 + % increase over 7 days. In all instances (treated or non-treated) the peak in CTs occurred at day 3, with markedly lower CT values at day 7 compared to day 3. The only natural antioxidants that provided a decrease in CT at day 3 were SRB and SFS at the higher application dose. At day 7, all natural antioxidants at both levels, with the exception GGR at the higher dose, awarded higher protection against an increase in CTs. Natural antioxidants that had the lowest formation of CTs was SRB and SFS at the higher application dose.

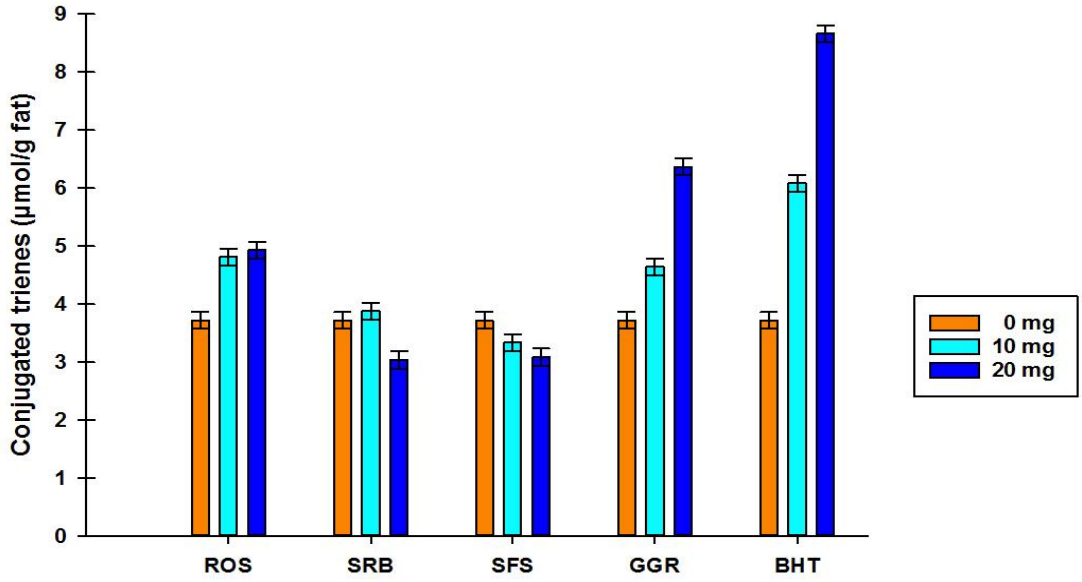

Figure 2.32 Effect of different levels of natural antioxidants on conjugated trienes in fat from chicken skin tissue (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

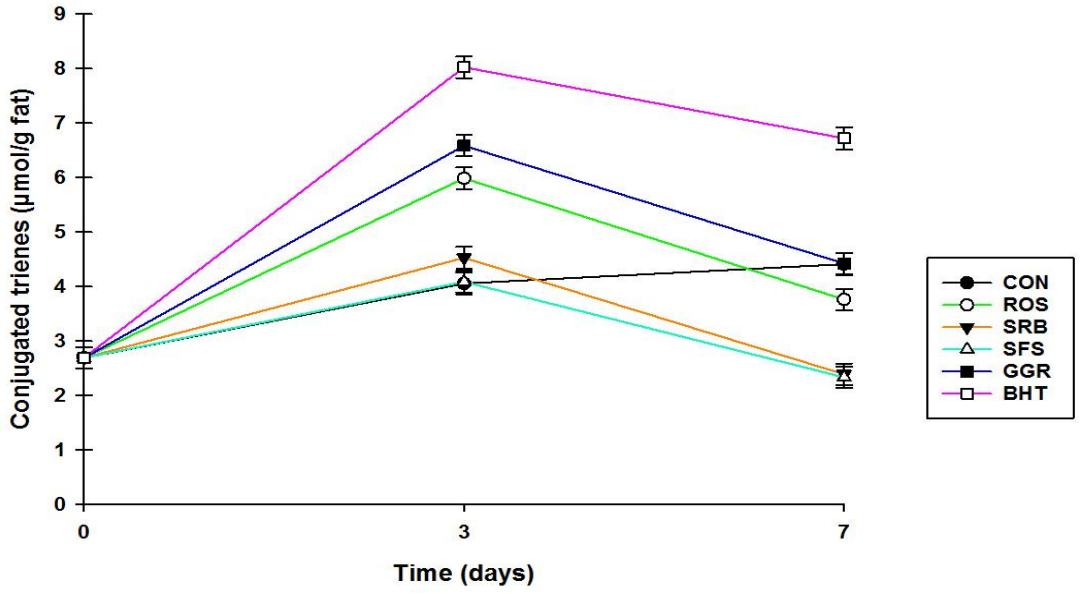

Figure 2.33 Effect of natural antioxidant application on conjugated trienes in fat from chicken skin tissue during the accelerated storage time (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

2.3.5 Effect of Natural Antioxidants on Fatty Acid Profile in Fat Extracted from Chicken Portions during Accelerated Storage Conditions

2.3.5.1 Chicken Breast Fat

In fat from the breast meat, all saturated fatty acids were significantly affected by antioxidants application ($p \leq 0.05$). SFS treatment was found to have significantly a higher amount of SFAs than SRB, GGR and BHT, while none of the natural antioxidants awarded any protection against a decrease in SFAs compared to the non-treated samples (Appendix Table A3). Regardless of storage time, a significant difference was found between antioxidant levels for all SFAs (with exception C14:0) ($p \leq 0.05$). SFS at 20 mg had a significantly higher amount of all SFAs than SRB at 10 and 20 mg, GGR at 10 mg and BHT at 20 mg, respectively, none of the antioxidants yielded a significantly higher amount of SFAs as compared to the non-treated samples. A significant difference was found between antioxidants and storage time ($p < 0.001$). During the storage time, none of the natural antioxidants significantly reduced the degradation of SFAs compared to the non-treated samples for 7 days. The content of C14:0, C16:0, C18:0, C20:0 and total SFAs in treated or non-treated was decreased with time. However, a significant reduction of C16:0 and total saturated fatty acids (SFAs) was found in untreated samples at day 7 of storage compared to 0 day, with a 10 + % decrease over 7 days of storage time (Appendix Table A3). A significant interaction ($p \leq 0.05$) was found between antioxidant x level x storage time for fatty acid C16:0, C18:0 and sum of SFAs (Table 2.7). The content of C16:0, C18:0 and the sum of SFAs in untreated and treated samples were decreased with storage time. None of the natural antioxidants awarded any protection against the decrease in SFAs at day 3. However, samples treated with SRB had a higher reduction of SFAs at both times

and at both levels of application. The only natural antioxidants that provided protection against degradation of SFAs at day 7 were ROS, SFS and GGR at the higher application dose.

For mono-unsaturated fatty acids (MUFAs), the application of antioxidants significantly affected the C18:1 n-9 and the sum of MUFAs ($p \leq 0.05$). SFS treatment was found to have significantly a higher amount of MUFAs than SRB, GGR and BHT, while none of the natural antioxidants awarded any protection against a decrease in MUFAs compared to the non-treated samples (Appendix Table A3). There was a significant difference between levels of antioxidants for C18:1 n-9 and the sum of MUFAs ($p \leq 0.05$). Hence, SFS at 20 mg dose had significantly higher C18:1 n-9 and the sum of MUFAs compared to the SRB and BHT at 20 mg and GGR at 10 mg. Furthermore, the results also show that there was a significant difference between antioxidants and storage time for all MUFAs ($p \leq 0.05$). Over storage time, the content of C18:1 n-9 and sum of MUFAs reduced during the storage time. The highest reduction was found in non-treated samples. None of the natural antioxidants yielded a significantly higher amount of C18:1 n-9 and the sum of MUFAs for 12 days. The results also show that a significant AO x L x ST interaction ($p \leq 0.05$) was found for C18:1 n-9 and sum MUFA. The content of C18:1 n-9 and the sum of MUFAs for 12 days in all samples was decreased with storage time. None of the natural antioxidants awarded any protection against the decrease in MUFAs at day 3. However, samples treated with SFS at 20 mg yielded a higher amount. The only natural antioxidants that provided protection against degradation of MUFAs at day 7 were ROS, SFS and GGR at the higher application dose. The impact of the natural antioxidants was more than that of BHT.

With respect to polyunsaturated fatty acids (PUFAs), the natural antioxidants that provided a greater protection against oxidation of C18:2 n-6, C18:3 n-3 and PUFAs was SFS, however, statistically was not differ from non-treated samples (Appendix Table A3). The application of natural extracts at lower or higher application dose statistically did not impose any protection for PUFAs compared to the non-treated; however, SFS at higher level had a higher content of C18:2 n-6, C18:3 n-3 and PUFAs (Appendix Table A3). Regardless of the antioxidant levels, none of the natural antioxidants markedly yielded more PUFAs content than non-treated samples over time. The accelerated storage conditions had a significant effect on C18:2 n-6, C18:3 n-3, C22:6 n-3 and the sum of PUFAs in non-treated samples, SRB, GGR and BHT at day 7compared to day 0 (Appendix Table A3). A significant antioxidant x level x storage time interactions ($p \leq 0.05$) were found for C18:2 n-6, C20:4 n-6, C20:5 n-3, C22:6 n-3 and the sum of PUFAs (Table 2.8). Indicating that PUFAs in both treated at both levels and non-treated decreased over storage time and the highest reduction of PUFAs was observed after 7 days of storage. The application of natural extracts at the lower or higher application dose statistically did not impose any protection for PUFAs compared to the non-treated samples over 7 days of the period. However, all natural antioxidants, with the exception of SRB at both levels and GGR at 10 mg, yielded more PUFAs compared to the non-treated samples. The application of natural antioxidants performed similar to the synthetic antioxidant (BHT treatment) over 7 days of storage.

Antioxidant supplementation had a significant ($p < 0.001$) effect on ∑n-3 and n-6 PUFA. Samples treated with ROS extract had the highest n-3 PUFA with mean value of 2.35 g/100 g fat. In contrast, SFS awarded greater protection against the reduction

of n-6 PUFA with a mean value of 14.03 g/100 g fat (Appendix Table A3). SFS at 20 mg was found to have a higher amount of n-3 and n-6 compared to any other treatments (Appendix Table A3). The amount of long chain n-3 and n-6 PUFA in all samples was significantly decreased with storage time, while a significant reduction of n-3 in samples treated with BHT at day 7 was found as compared to day 0. With respect to ∑n-6, the amount of n-6 was markedly decreased in all treatments with exception SFS at day 7 (Table A2.6). The results presented in Table 2.8 show a significant interaction between three main factors was observed for ∑n-6 PUFA ($p = 0.016$) due to the increase the reduction of n-6 with time of storage. A significant reduction of n-6 was found in samples treated with SRB at both levels and GGR at lower application dose at day 7 of storage. The addition of the SFS extract at a higher application dose and over a 7-day period awarded a greater protection against oxidation of ∑n-6 PUFAs. The natural antioxidants performed similar to the synthetic antioxidant (BHT) over 7 days of storage (Table 2.8).

Table 2.8 Effect of natural antioxidant extracts at different levels on fatty acid profile (g of fatty acids/100 g of fat) in fat from the chicken breast tissue during the accelerated storage time at 62.8 ^{o}C

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C14:0	0	0.21										
	3	0.19	0.21	0.20	0.23	0.22	0.21	0.21	0.21	0.20	0.20	0.21
	7	0.16	0.16	0.16	0.18	0.18	0.16	0.14	0.20	0.20	0.15	0.20
C16:0	0	12.48^{fg}										
	3	12.09^{defg}	12.53^{fg}	11.19^{bcdef}	12.33^{efg}	12.15^{defg}	11.96^{defg}	12.08^{defg}	12.08^{defg}	12.84^{g}	12.13^{defg}	12.17^{defg}
	7	11.05^{abcde}	11.10^{abcde}	10.28^{bcdef}	11.29^{bcdef}	11.97^{defg}	10.93^{abcd}	9.74^{a}	11.23^{bcdef}	12.12^{defg}	10.15^{ab}	11.58^{cdefg}
C18:0	0	4.30^{ef}										
	3	4.27^{ef}	4.29^{ef}	3.85^{bcd}	4.30^{ef}	4.13^{def}	4.16^{def}	4.12^{def}	4.17^{def}	4.36^{f}	4.21^{ef}	4.16^{def}
	7	4.00^{bcdef}	3.84^{bcd}	3.65^{ab}	3.98^{bcde}	4.10^{def}	4.01^{cdef}	3.38^{a}	4.01^{cdef}	4.12^{def}	3.72^{abc}	3.95^{bcde}
C20:0	0	0.23										
	3	0.23	0.24	0.21	0.24	0.23	0.23	0.23	0.23	0.25	0.22	0.23
	7	0.20	0.21	0.18	0.18	0.23	0.19	0.18	0.21	0.22	0.19	0.21
∑ SFA	0	17.46^{ef}										
	3	17.02^{cdef}	17.48^{ef}	15.64^{bcd}	17.35^{def}	16.94^{cdef}	16.78^{cdef}	16.86^{cdef}	16.93^{cdef}	17.87^{f}	17.01^{cdef}	16.99^{cdef}
	7	15.65^{bcd}	15.49^{bc}	14.47^{ab}	15.84^{bcde}	16.69^{cdef}	15.54^{bc}	13.61^{a}	15.88^{bcde}	16.86^{cdef}	14.46^{ab}	16.13^{bcdef}
C16:1 n-7	0	1.31										
	3	1.38	1.60	1.41	1.44	1.45	1.46	1.41	1.42	1.53	1.47	1.44
	7	1.34	1.39	1.19	1.37	1.44	1.21	1.22	1.38	1.51	1.18	1.50
C18:1 n-9	0	24.68^{def}										
	3	23.47^{bcdef}	25.11^{ef}	22.55^{abcdef}	24.14^{def}	24.20^{def}	23.64^{bcdef}	24.05^{cdef}	23.87^{cdef}	25.73^{f}	23.81^{bcdef}	24.06^{cdef}
	7	21.23^{abcd}	22.07^{abcde}	20.27^{ab}	21.94^{abcde}	23.75^{bcdef}	20.57^{abc}	19.32^{a}	21.74^{abcde}	24.15^{def}	19.07^{a}	23.06^{bcdef}
∑ MUFA	0	25.99^{def}										
	3	24.86^{bcdef}	26.71^{ef}	23.96^{abcdef}	25.58^{cdef}	25.65^{def}	25.10^{bcdef}	25.46^{cdef}	25.28^{cdef}	27.26^{f}	25.27^{bcdef}	25.50^{cdef}
	7	22.57^{abcd}	23.46^{abcdef}	21.47^{ab}	23.31^{abcde}	25.19^{bcdef}	21.78^{abc}	20.54^{a}	23.12^{abcde}	25.67^{def}	20.26^{a}	24.56^{bcdef}
C18:2 n-6	0	13.19^{def}										
	3	12.69^{cdef}	13.41^{ef}	11.95^{abcde}	13.01^{def}	13.05^{def}	12.78^{cdef}	12.91^{cdef}	12.94^{cdef}	13.94^{f}	12.86^{cdef}	12.97^{cdef}
	7	11.54^{abcd}	11.81^{abcde}	10.92^{ab}	11.81^{abcde}	12.72^{cdef}	11.25^{abc}	10.32^{a}	11.84^{abcde}	13.03^{def}	10.46^{a}	12.33^{bcdef}

Table 2.8 (continued)

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C18:3 n-3	0	1.58										
	3	1.53	1.67	1.48	1.60	1.62	1.56	1.63	1.58	1.70	1.55	1.59
	7	1.35	1.41	1.30	1.40	1.55	1.30	1.23	1.37	1.57	1.19	1.47
C20:4 n-6	0	1.14^{abcd}										
	3	1.22^{d}	1.12^{abcd}	0.95^{abc}	1.20^{cd}	1.10^{abcd}	1.16^{bcd}	1.09^{abcd}	1.14^{abcd}	1.13^{abcd}	1.19^{bcd}	1.14^{abcd}
	7	1.23^{d}	1.02^{abcd}	1.03^{abcd}	1.13^{abcd}	1.09^{abcd}	1.28^{d}	0.89^{a}	1.18^{bcd}	0.92^{ab}	1.23^{d}	1.03^{abcd}
C20:5 n-3	0	0.13^{e}										
	3	0.12b^{cde}	0.12^{bcde}	0.09^{abc}	0.12^{abcde}	0.11^{abcde}	0.11^{abcde}	0.09^{abcd}	0.11^{abcde}	0.11^{abcde}	0.11^{abcde}	0.13^{cde}
	7	0.13^{e}	0.10^{abcde}	0.11^{abcde}	0.11^{abcde}	0.10^{abcde}	0.13^{de}	0.09^{ab}	0.11^{abcde}	0.11^{abcde}	0.13^{de}	0.09^{a}
C22:5 n-3	0	0.47										
	3	0.45	0.42	0.36	0.45	0.41	0.44	0.41	0.43	0.43	0.45	0.43
	7	0.46	0.38	0.39	0.44	0.42	0.48	0.44	0.45	0.40	0.46	0.40
C22:6 n-3	0	0.25^{c}										
	3	0.25^{abc}	0.24^{ab}	0.19^{a}	0.30^{bc}	0.29^{bc}	0.30^{bc}	0.24^{ab}	0.24^{ab}	0.24^{ab}	0.24^{ab}	0.22^{ab}
	7	0.23^{ab}	0.22^{ab}	0.22^{ab}	0.26^{abc}	0.22^{ab}	0.29^{bc}	0.18^{a}	0.25^{abc}	0.22^{ab}	0.27^{ab}	0.23^{ab}
∑ PUFA	0	16.76^{def}										
	3	16.36^{cdef}	16.97^{ef}	15.03^{abcd}	16.68^{def}	16.58^{cdef}	16.36^{cdef}	16.37^{cdef}	16.45^{cdef}	17.54^{f}	16.38^{cdef}	16.48^{cdef}
	7	14.94^{abcd}	14.94^{abcd}	13.98^{ab}	15.15^{bcde}	16.10^{cdef}	14.72^{abc}	13.16^{a}	15.20^{bcde}	16.24^{cdef}	13.73^{ab}	15.55^{bcde}
∑ n-3	0	2.44										
	3	2.45	2.45	2.13	2.47	2.43	2.41	2.38	2.37	2.47	2.34	2.37
	7	2.17	2.11	2.03	2.20	2.30	2.20	1.95	2.19	2.29	2.04	2.19
∑ n-6	0	14.32^{efg}										
	3	13.91^{defg}	14.52^{fg}	12.91^{bcdef}	14.21^{efg}	14.15^{defg}	13.94^{defg}	14.00^{defg}	14.09^{defg}	15.07^{g}	14.04^{defg}	14.11^{defg}
	7	12.77^{abcde}	12.83^{abcde}	11.95^{abc}	12.95^{bcdef}	13.81^{defg}	12.52^{abcd}	11.21^{a}	13.02^{bcdef}	13.95^{defg}	11.69^{ab}	13.36^{cdef}
Total FA	0	60.21^{def}										
	3	58.23^{cdef}	61.16^{ef}	54.63^{abcde}	59.61^{def}	59.17^{cdef}	58.23^{cdef}	58.70^{cdef}	58.66^{cdef}	62.66^{f}	58.67^{cdef}	58.98^{cdef}
	7	53.17^{abcd}	53.89^{abcde}	49.92^{ab}	54.30^{abcde}	57.98^{cdef}	52.04^{abc}	47.31^{a}	54.21^{abcde}	58.77^{cdef}	48.44^{a}	56.25^{bcdef}

Mean values with different small superscript letters presented within each row and column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.5.2 Chicken Thigh Fat

In fat from thigh meat, no significant differences were found between non-treated samples and natural antioxidant treatments for all SFAs, while natural antioxidants significantly yielded more all individual SFAs compared to that of BHT ($p \leq 0.05$). Antioxidant supplementation at different levels had a significant ($p < 0.001$) effect on all SFAs (with exception of C14:0 and C20:0 ($p \leq 0.05$). Hence, BHT at both levels had a significantly higher reduction, while none of the natural antioxidants provided protection against the degradation of any of the SFAs (Appendix Table A4). The accelerated storage conditions caused a decrease in all SFAs over 7 days. A significant reduction of all SFAs (with the exception of C20:0) was shown in non-treated samples at the end of the storage time. None of the natural antioxidants provided protection against the degradation of any of the SFAs over 7 days ($p > 0.05$). The impact of the natural antioxidants was similar to that of BHT.

For MUFA, application of antioxidants had a significant an effect on all MUFAs ($p < 0.001$). BHT was found to have significantly higher amount of all MUFAs than any other treatments (Appendix Table A4). Antioxidant supplementation at different levels had a significant effect on all MUFAs ($p < 0.001$). Hence, BHT at both levels had significantly higher reduction, while none of the natural antioxidants at both levels provided protection against the degradation of any of the MUFAs (Appendix Table A4). Furthermore, a significant difference was found between antioxidant and storage time for all MUFAs ($p \leq 0.05$). The content of all MUFAs in all samples was decreased with storage time, while the highest reduction of all MUFAs was found in non-treated samples, with a 10 % decrease at the end of the storage time. None of the natural antioxidants provided a protection against the degradation of all individual MUFAs and total MUFAs for 7 days

($p > 0.05$). The natural antioxidants provided a slightly better protection against changes in MUFAs compared to BHT (Table A1.4). No significant interaction was found between antioxidant, level and storage time ($p > 0.05$) (Table 2.9).

With respect to the PUFAs, none of the natural antioxidants provided protection against the degradation of fatty acids such as C18:2 n-6, C18:3 n-3 and total PUFAs compared to the non-treated samples. However, the natural antioxidants provided a slightly better protection against changes in PUFAs compared to BHT. All antioxidants significantly reduced the degradation of C22:6 n-3 compared to the non-treated samples, but no significant differences were found between antioxidant treatments. Moreover, the addition of natural extracts at both the lower and higher levels did not statistically provide protection against oxidation of PUFAs compared to the non-treated samples. The natural antioxidants at both levels and times provided a slightly better protection against changes in PUFAs compared to BHT (Appendix Table A4). A significant difference was found between antioxidant and storage time for all PUFAs ($p \leq 0.05$) (Appendix Table A4). Hence, accelerated storage conditions caused a decrease in C18:2 n-6, C18:3 n-3 and total PUFAs content in non-treated samples, with a 10 % decrease at day 7 of the storage time. During the storage time, a significant difference between antioxidant and non-treated samples were shown for C20:4 n-6 and C22:6 n-3. Antioxidants that yielded significantly more C22:6 n-3 content was ROS and BHT at day 7 of storage compared to the non-treated samples, while SRB treatment yielded more C20:4 n-6 than non-treated samples (Appendix Table A4). No significant interaction was found between antioxidant, level and storage time ($p > 0.05$) (Table 2.9).

For both ∑n-3 and ∑n-6, none of the natural antioxidants at lower and higher levels of application provided protection against the degradation of ∑n-3 and ∑n-6 compared to the non-treated samples. However, the natural antioxidants provided a slightly better

protection against changes in ∑n-3 and ∑n-6 PUFAs compared to BHT as highest degradation was detected in samples treated with BHT. The amount of ∑n-3 and n-6 was affected by the storage time ($p > 0.05$) (Appendix Table A4). A significant reduction was shown at the end of the storage time compared to that at day 0. None of the natural antioxidants awarded a significant protection against oxidation of ∑n-3 and ∑n-6 PUFAs over 7 days. However, natural treatments yielded more ∑n-3 and ∑n-6 PUFAs. The only natural antioxidants provided marked a protection against decrease of ∑n-3 and n-6 PUFAs was SFS at lower application dose at the end of storage time. No significant interaction ($p > 0.05$) was found between antioxidant, level and storage time (Table 2.9).

Table 2.9 Effect of antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from the chicken thigh tissue during the accelerated storage time at 62.8 ^{o}C.

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C14:0	0	0.38										
	3	0.35	0.34	0.33	0.33	0.36	0.36	0.35	0.37	0.34	0.35	0.34
	7	0.34	0.33	0.33	0.37	0.35	0.37	0.36	0.39	0.37	0.38	0.37
C16:0	0	18.49										
	3	17.11	16.28	15.59	16.59	17.08	17.45	16.76	17.48	16.71	17.05	16.42
	7	16.72	16.20	16.11	17.74	17.20	17.65	16.72	18.15	17.45	17.88	17.31
C18:0	0	5.47										
	3	5.11	4.85	4.69	5.01	5.16	5.26	5.05	5.21	5.00	5.11	4.93
	7	5.00	4.94	4.85	5.28	5.16	5.28	5.07	5.44	5.24	5.34	5.14
C20:0	0	0.35										
	3	0.34	0.32	0.32	0.34	0.35	0.35	0.32	0.36	0.33	0.32	0.32
	7	0.31	0.31	0.30	0.32	0.34	0.34	0.32	0.35	0.34	0.36	0.32
∑SFA	0	24.92										
	3	23.11	22.00	21.02	22.47	23.14	23.63	22.68	23.64	22.58	23.02	22.19
	7	22.56	21.95	21.68	23.93	23.27	23.85	22.68	24.54	23.62	24.19	23.35
C16:1 n-7	0	2.62										
	3	2.39	2.28	2.17	2.32	2.38	2.45	2.33	2.49	2.33	2.50	2.2
	7	2.35	2.22	2.22	2.49	2.41	2.46	2.31	2.56	2.44	2.51	2.43
C18:1 n-9	0	39.04										
	3	35.51	34.29	32.84	35.19	35.99	37.06	35.14	37.27	35.25	36.12	34.27
	7	35.04	33.75	33.79	37.09	35.99	36.96	34.70	38.21	36.31	37.61	36.16
∑MUFA	0	41.66										
	3	37.90	36.58	35.01	37.52	38.37	39.51	37.47	39.76	37.58	38.62	36.57
	7	37.39	35.97	36.01	39.58	38.41	39.42	37.01	40.77	38.75	40.12	38.59
C18:2 n-6	0	20.69										
	3	18.90	18.14	17.41	18.50	19.04	19.52	18.60	19.69	18.75	19.08	18.23
	7	18.67	18.28	17.96	19.82	19.16	19.68	18.55^{de}	20.31	19.44	19.94	19.24

Table 2.9 (continued)

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C18:3 n-3	**0**	2.90										
	3	2.64	2.55	2.38	2.55	2.64	2.71	2.62	2.72	2.60	2.63	2.53
	7	2.56	2.47	2.47	2.76	2.67	2.77	2.58	2.83	2.68	2.80	2.70
C20:4 n-6	**0**	0.69										
	3	0.61	0.62	0.63	0.64	0.68	0.65	0.65	0.65	0.64	0.67	0.66
	7	0.65	0.68	0.65	0.73	0.70	0.70	0.70	0.69	0.69	0.69	0.68
C22:5 n-3	**0**	0.22										
	3	0.21	0.21	0.20	0.21	0.22	0.22	0.22	0.21	0.21	0.22	0.22
	7	0.22	0.23	0.22	0.23	0.23	0.23	0.24	0.23	0.23	0.24	0.22
C22:6 n-3	**0**	0.22										
	3	0.20	0.24	0.20	0.24	0.27	0.20	0.22	0.17	0.19	0.20	0.22
	7	0.23	0.28	0.29	0.35	0.33	0.33	0.29	0.30	0.31	0.27	0.27
∑PUFA	**0**	24.73										
	3	22.35	21.52	20.62	21.90	22.58	23.11	22.09	23.27	22.20	22.59	21.65
	7	22.10	21.66	21.30	23.54	22.75	23.38	22.07	24.06	23.04	23.66	22.84
∑n-3	**0**	3.34										
	3	3.04	3.00	2.78	3.01	3.12	3.13	3.06	3.10	3.00	3.05	2.97
	7	3.02	2.98	2.98	3.34	3.23	3.33	3.10	3.37	3.22	3.30	3.20
∑n-6	**0**	21.38										
	3	19.51	18.76	18.04	19.14	19.72	20.18	19.25	20.34	19.39	19.75	18.89
	7	19.32	18.96	18.61	20.55	19.86	20.38	19.26	21.00	20.13	20.63	19.92
Total FA	**0**	91.31										
	3	83.56	80.33	76.85	82.12	84.36	86.45	82.46	86.84	82.55	84.44	80.62
	7	82.29	79.86	79.27	87.41	84.76	86.99	82.05	89.68	85.72	88.25	85.05

.

2.3.5.3 Chicken Adipose Fat

In adipose fat, antioxidants at different levels and storage times were found to have an effect on the fatty acid profiles (Appendix Table A5). None of the natural antioxidants at different levels provided protection against the degradation of SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 compared to the non-treated samples. However, the natural antioxidants provided a slightly better protection against changes in these fatty acids compared to BHT as highest degradation was detected in samples treated with BHT (Appendix Table A5). All fatty acids in both treated and non-treated samples were significantly decreased ($p \leq 0.05$) with increasing accelerating storage time, while the highest reduction was shown at day 12 compared to day 0. Over storage time none of the natural antioxidants significantly inhibited ($p > 0.05$) the degradation of individual fatty acids in fat from adipose tissue (Appendix Table A5). The results of interaction between antioxidant x level x storage time are presented in Table 2.10. The results show that the amount of all fatty acids in non-treated samples of adipose fat significantly decreased ($p \leq 0.05$) as the accelerated storage increased, while a significant reduction of 12% was found at the end of the storage time compared to day 0. None of the natural antioxidants at both levels provided protection against a decrease in all SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 at day 3. At day 7, all natural antioxidants inhibited the reduction of all SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 compared to the non-treated samples. The natural antioxidants that provided significant protection against a decrease of all fatty acids at day 7, were ROS, SRB, GGR at the lower application doses and SFS at the higher application dose. The natural antioxidants at both time and application dose provided better protection against changes in fatty acids compared to BHT (Table 2.10 and Appendix Table A5).

Table 2.10 Effect of antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from the chicken adipose tissue during the accelerated storage time at 62.8^{o}C.

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C14:0	0	0.44^{cde}										
	3	0.42^{bcde}	0.38^{abcde}	0.37^{abcd}	0.41^{bcde}	0.39^{abcde}	0.36^{ab}	0.34^{a}	0.41^{abcde}	0.38^{abcde}	0.39^{abcde}	0.36^{abc}
	7	0.39^{abcde}	0.38^{abcde}	0.37^{abcde}	0.44^{cde}	0.43^{bcde}	0.44^{de}	0.40^{abcde}	0.41^{abcde}	0.41^{bcde}	0.44^{d}	0.37^{abcde}
C16:0	0	19.66^{egh}										
	3	19.07^{defg}	16.52^{abc}	16.25^{ab}	18.13^{bcdefg}	17.69^{abcdef}	16.64^{abc}	15.72^{a}	18.13^{bcdefg}	17.64^{abcde}	17.40^{abcd}	16.02^{ab}
	7	17.28^{abcd}	19.12^{defg}	16.62^{abc}	19.91^{fg}	19.28^{defg}	19.68^{efg}	18.61^{cdefg}	19.13^{defg}	20.11^{g}	19.95^{g}	18.59^{cdefg}
C18:0	0	5.24^{fgh}										
	3	5.08^{efgh}	4.40^{abcd}	4.28^{abc}	4.83^{cdefgh}	4.69^{abcdefg}	4.46^{abcd}	4.16^{a}	4.79^{bcdefgh}	4.66^{abcdef}	4.63^{abcde}	4.21^{ab}
	7	4.62^{abcde}	5.09^{efgh}	4.40^{abcd}	5.29^{gh}	5.14^{efgh}	5.24^{fgh}	4.93^{defgh}	5.14^{efgh}	5.39^{h}	5.32^{h}	4.95^{defgh}
C20:0	0	0.53^{ab}										
	3	0.53^{ab}	0.46^{ab}	0.45^{ab}	0.48^{ab}	0.49^{ab}	0.46^{ab}	0.44^{ab}	0.50^{ab}	0.49^{ab}	0.48^{ab}	0.45^{ab}
	7	0.48^{ab}	0.46^{ab}	0.45^{ab}	0.54^{b}	0.53^{ab}	0.54^{b}	0.46^{ab}	0.47^{ab}	0.51^{ab}	0.54^{b}	0.42^{a}
∑ SFA	0	26.13^{ef}										
	3	25.35^{def}	21.97^{abc}	21.54^{ab}	24.09^{bcdef}	23.48^{abcdef}	22.13^{abc}	20.82^{a}	24.06^{bcdef}	23.38^{abcde}	23.12^{abcd}	21.25^{ab}
	7	22.99^{abcd}	25.27^{def}	22.04^{abc}	26.41^{f}	25.62^{def}	26.14^{ef}	24.61^{cdef}	25.37^{def}	26.63^{f}	26.50^{f}	24.51^{cdef}
C16:1 n-7	0	2.99^{fg}										
	3	2.87^{defg}	2.51^{abc}	2.45^{ab}	2.72^{bcdefg}	2.67^{abcdef}	2.51^{abc}	2.36^{a}	2.74^{bcdefg}	2.66^{abcdef}	2.63^{abcde}	2.44^{ab}
	7	2.61^{abcd}	2.82^{cdefg}	2.48^{ab}	2.98^{fg}	2.88^{defg}	2.96^{efg}	2.71^{bcdefg}	2.82^{cdefg}	2.92^{defg}	3.01^{g}	2.73^{bcdefg}
C18:1 n-9	0	42.73^{efg}										
	3	41.47^{defg}	36.11^{abc}	35.01^{ab}	39.22^{bcdefg}	38.32^{abcdef}	36.16^{abc}	34.08^{a}	39.33^{bcdefg}	38.29^{abcdef}	37.78^{abcde}	34.89^{ab}
	7	37.56^{abcd}	41.51^{defg}	36.07^{abc}	42.97^{fg}	41.57^{defg}	42.45^{defg}	40.25^{cdefg}	41.28^{defg}	42.89^{fg}	43.33^{g}	40.46^{cdefg}
∑ MUFA	0	45.72^{efg}										
	3	44.34^{defg}	38.62^{abc}	37.45^{ab}	41.94^{bcdefg}	40.99^{abcdef}	38.66^{abc}	36.44^{a}	42.12^{bcdefg}	40.95^{abcdef}	40.41^{abcd}	37.32^{ab}
	7	40.17^{abcd}	44.33^{defg}	38.55^{abc}	45.95^{fg}	44.45^{defg}	45.41^{defg}	42.96^{cdefg}	44.10^{defg}	45.82^{fg}	46.34^{g}	43.19^{cdefg}

Table 2.10 (continued)

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C18:2 n-6	0	20.55^{fg}										
	3	19.89^{defg}	17.32^{abc}	16.98^{abc}	18.84^{bcdefg}	18.37^{abcdef}	17.34^{abc}	16.42^{a}	19.02^{bcdefg}	18.48^{abcdefg}	18.17^{abcde}	16.80^{ab}
	7	17.96^{abcd}	19.92^{defg}	17.37^{abc}	20.65^{fg}	19.85^{defg}	20.33^{efg}	19.16^{cdefg}	19.86^{defg}	20.62^{fg}	20.75^{g}	19.33^{cdefg}
C18:3 n-3	0	3.16^{fg}										
	3	3.06^{defg}	2.71^{abcd}	2.63^{ab}	2.91^{bcdefg}	2.79^{abcde}	2.68^{abc}	2.53^{a}	2.93^{bcdefg}	2.85^{abcdefg}	2.83^{abcdef}	2.63^{ab}
	7	2.78^{abcde}	3.01^{cdefg}	2.65^{abc}	3.16^{fg}	3.05^{defg}	3.16^{fg}	2.90^{bcdefg}	3.00^{cdefg}	3.10^{efg}	3.20^{g}	2.92^{bcdefg}
C20:4 n-6	0	0.19^{abc}										
	3	0.18^{abc}	0.16^{abc}	0.17^{abc}	0.17^{abc}	0.17^{abc}	0.17^{abc}	0.16^{ab}	0.17^{abc}	0.18^{abc}	0.18^{abc}	0.16^{abc}
	7	0.17^{abc}	0.17^{abc}	0.14^{a}	0.19^{abc}	0.18^{abc}	0.19^{bc}	0.16^{ab}	0.21^{c}	0.20^{bc}	0.20^{bc}	0.15^{ab}
C22:5 n-3	0	0.28^{bcd}										
	3	0.25^{bcd}	0.33^{cde}	0.22^{bcd}	0.28^{bcd}	0.19^{b}	0.22^{bc}	0.33^{cde}	0.35^{de}	0.28^{bcd}	0.35^{de}	0.32^{bcd}
	7	0.23^{abc}	0.25^{abc}	0.41^{e}	0.32^{bcd}	0.26^{bcd}	0.29^{bcd}	0.27^{bcd}	0.32^{a}	0.31^{bcd}	0.19^{b}	0.28^{bcd}
∑ PUFA	0	24.17^{fg}										
	3	23.38^{efg}	20.55^{abcd}	20.00^{abc}	22.20^{bcdefg}	21.52^{abcdef}	20.41^{abcd}	19.43^{a}	22.47^{bcdefg}	21.79^{abcdefg}	21.52^{abcdef}	19.91^{ab}
	7	21.14^{abcde}	23.34^{efg}	20.57^{abcd}	24.32^{g}	23.34^{efg}	23.97^{fg}	22.49^{bcdefg}	23.07^{defg}	24.23^{g}	24.34^{g}	22.68^{cdefg}
∑ n-3	0	3.43^{f}										
	3	3.31^{cdef}	3.05^{abcd}	2.85^{a}	3.19^{abcdef}	2.99^{abc}	2.89^{ab}	2.85^{a}	3.29^{cdef}	3.13^{abcdef}	3.17^{abcdef}	2.95^{abc}
	7	3.01^{abc}	3.25^{bcdef}	3.05^{abcde}	3.48^{f}	3.31^{cdef}	3.45^{f}	3.17^{abcdef}	3.00^{abc}	3.41^{def}	3.39^{def}	3.20^{abcdef}
∑ n-6	0	20.74^{fg}										
	3	20.07^{defg}	17.49^{abc}	17.15^{abc}	19.01^{bcdefg}	18.54^{abcdef}	17.51^{abc}	16.58^{a}	19.19^{bcdefg}	18.66^{abcdefg}	18.34^{abcde}	16.96^{ab}
	7	18.13^{abcd}	20.09^{defg}	17.51^{abc}	20.84^{fg}	20.03^{defg}	20.52^{efg}	19.32^{bcdefg}	20.07^{defg}	20.82^{fg}	20.95^{g}	19.48^{cdefg}
Total FA	0	96.02^{fg}										
	3	93.08^{defg}	81.12^{abc}	78.99^{ab}	88.23^{bcdefg}	85.98^{abcdef}	81.20^{abc}	76.69^{a}	88.65^{bcdefg}	86.12^{abcdef}	85.06^{abcde}	78.47^{ab}
	7	84.29^{abcd}	92.94^{defg}	81.16^{abc}	96.69^{fg}	93.41^{defg}	95.52^{efg}	90.06^{cdefg}	92.53^{defg}	96.68^{fg}	97.18^{g}	90.39^{cdefg}

Mean values with different small superscript letters presented within each row and column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.3.5.4 Chicken Skin Fat

In fat from skin tissue, none of the natural antioxidants at different levels provided any protection against the degradation of all fatty acids compared to the non-treated samples. However, the natural antioxidants provided significantly better protection against changes in all fatty acids compared to BHT as highest degradation was detected in samples treated with BHT (Appendix Table A6). Moreover, all individual SFAs, MUFAs and PUFAs in non-treated samples and samples treated with natural antioxidant extracts were not significantly changed during the storage time ($p > 0.05$). A significant reduction of all fatty acids was shown in BHT treatment ($p \leq 0.05$) (Appendix Table A6). There was a significant interaction ($p \leq 0.05$) between antioxidant x level x storage time for all individual fatty acids with exception C14:0 (Table 2.11). All fatty acids were reduced with increasing storage time, while a significant reduction was shown in samples treated with BHT at both levels at day 3 of storage time. None of the natural antioxidants at both time and application dose performed significantly better than non-treated samples. However, natural antioxidants yielded more fatty acids than non-treated samples and BHT (Table 2.11).

Table 2.11 Effect of antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from the chicken skin tissue during the accelerated storage time at 62.8 ºC.

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C14:0	0	0.44										
	3	0.43	0.37	0.37	0.41	0.43	0.43	0.43	0.43	0.44	0.44	0.44
	7	0.43	0.43	0.38	0.44	0.43	0.45	0.43	0.43	0.44	0.45	0.42
C16:0	0	19.71^{c}										
	3	18.71^{abc}	17.42^{a}	17.08^{a}	18.62^{abc}	19.63^{c}	20.03^{c}	19.67^{c}	19.37^{bc}	19.78^{c}	19.81^{c}	19.83^{c}
	7	19.32^{bc}	19.52^{c}	17.71^{ab}	19.95^{c}	19.78^{c}	19.66^{c}	19.66^{c}	19.55^{c}	19.95^{c}	20.06^{c}	19.55^{c}
C18:0	0	5.52^{c}										
	3	5.22^{abc}	4.85^{a}	4.76^{a}	5.21^{abc}	5.49^{c}	5.60^{c}	5.49^{c}	5.39^{bc}	5.52^{c}	5.54^{c}	5.56^{c}
	7	5.40^{bc}	5.42^{c}	4.93^{ab}	5.58^{c}	5.52^{c}	5.49^{c}	5.49^{c}	5.44^{c}	5.59^{c}	5.60^{c}	5.43^{c}
C20:0	0	0.43^{abcd}										
	3	0.43^{abcd}	0.37^{abc}	0.34^{a}	0.43^{abcd}	0.4^{abcd}	0.44^{bcd}	0.44^{bcd}	0.42^{abcd}	0.44^{bcd}	0.46^{cd}	0.43^{bcd}
	7	0.45^{cd}	0.40^{abcd}	0.36^{ab}	0.46^{d}	0.41^{abcd}	0.40^{abcd}	0.41^{abcd}	0.38^{abcd}	0.39^{abcd}	0.42^{abcd}	0.37^{abc}
∑ SFA	0	26.37^{c}										
	3	25.02^{bc}	23.25^{ab}	22.79^{a}	24.92^{abc}	26.23^{c}	26.77^{c}	26.29^{c}	25.88^{c}	26.44^{c}	26.51^{c}	26.54^{c}
	7	25.87^{c}	26.05^{c}	23.66^{ab}	26.71^{c}	26.42^{c}	26.26^{c}	26.26^{c}	26.06^{c}	26.64^{c}	26.81^{c}	26.05^{c}
C16:1 n-7	0	2.85^{c}										
	3	2.69^{bc}	2.49^{ab}	2.41^{a}	2.68^{bc}	2.80^{c}	2.86^{c}	2.81^{c}	2.79^{c}	2.85c	2.84^{c}	2.84^{c}
	7	2.78^{c}	2.81^{c}	2.53^{ab}	2.89^{c}	2.86^{c}	2.85^{c}	2.83^{c}	2.79^{c}	2.87c	2.88^{c}	2.81^{c}
C18:1 n-9	0	42.09^{c}										
	3	39.92^{abc}	37.20^{a}	36.37^{a}	39.82^{abc}	42.01^{c}	42.85^{c}	42.14^{c}	41.40^{bc}	42.22c	42.23^{c}	42.33^{c}
	7	41.19^{bc}	41.70^{c}	37.81^{ab}	42.68^{c}	42.27^{c}	42.05^{c}	42.02^{c}	41.70^{c}	42.58c	42.91^{c}	41.67^{c}
∑ MUFA	0	44.26^{d}										
	3	42.61^{bcd}	39.68^{ab}	38.78^{a}	42.51^{abcd}	44.81^{d}	45.71^{d}	44.95^{d}	44.19^{d}	45.06^{d}	45.07^{d}	45.17^{d}
	7	43.97^{cd}	44.52^{d}	40.34^{abc}	45.57^{d}	45.13^{d}	44.90^{d}	44.86^{d}	44.48^{d}	45.45^{d}	45.78^{d}	44.48^{d}

Table 2.11 (continued)

Fatty acid	Time (d)	Controls			Treatment with Natural Antioxidants							
		CON	BHT		ROS		SRB		SFS		GGR	
		T0	T10	T20	T10	T20	T10	T20	T10	T20	T10	T20
C18:2 n-6	0	20.70^{c}										
	3	19.77^{abc}	18.48^{a}	18.04^{a}	19.80^{abc}	20.85^{c}	21.35^{c}	21.01^{c}	20.72^{c}	21.13c	21.04^{c}	21.06^{c}
	7	20.53^{bc}	20.79^{c}	18.79^{ab}	21.21^{c}	20.97^{c}	20.93^{c}	20.92^{c}	20.75^{c}	21.25c	21.28^{c}	20.75^{c}
C18:3 n-3	0	3.07^{cd}										
	3	2.98^{bcd}	2.77^{ab}	2.65^{a}	3.00^{bcd}	3.16^{d}	3.21^{d}	3.17^{d}	3.13^{d}	3.17d	3.18^{d}	3.17^{d}
	7	3.11^{d}	3.11^{d}	2.81^{abc}	3.20^{d}	3.18^{d}	3.14^{d}	3.14^{d}	3.06^{cd}	3.13d	3.18^{d}	3.12^{d}
C20:4 n-6	0	0.27^{a}										
	3	0.26^{a}	0.26^{a}	0.26^{a}	0.28^{a}	0.29^{a}	0.30^{a}	0.29^{a}	0.30^{a}	0.30a	0.29^{a}	0.29^{a}
	7	0.30^{a}	0.28^{a}	0.26^{a}	0.28^{a}	0.26^{a}	0.27^{a}	0.28^{a}	0.28^{a}	0.28a	0.28^{a}	0.28^{a}
C22:6 n-3	0	0.22^{bcd}										
	3	0.18^{bc}	0.20^{bcd}	0.16^{abc}	0.23^{bcd}	0.39^{d}	0.22^{bcd}	0.27^{bcd}	0.33^{cd}	0.19^{bc}	0.30^{bcd}	0.19^{bc}
	7	0.22^{bcd}	0.23^{bcd}	0.24^{bcd}	0.21^{bcd}	0.04^{a}	0.04^{a}	0.28^{bcd}	0.24^{bcd}	0.00^{a}	0.21^{bcd}	0.12^{ab}
∑ PUFA	0	24.26^{d}										
	3	23.20^{abcd}	21.71^{ab}	21.11^{a}	23.30^{bcd}	24.69^{d}	25.09^{d}	24.74^{d}	24.48^{d}	24.80^{d}	24.81^{d}	24.71^{d}
	7	24.16^{cd}	24.41^{d}	22.09^{abc}	24.91^{d}	24.41^{d}	24.34^{d}	24.62^{d}	24.32^{d}	24.66^{d}	24.94^{d}	24.27^{d}
∑ n-3	0	3.39^{de}										
	3	3.16^{bcd}	2.97^{ab}	2.81^{a}	3.23^{bcde}	3.55^{e}	3.44^{de}	3.44^{de}	3.46^{de}	3.37^{cde}	3.48^{de}	3.36^{cde}
	7	3.33^{cde}	3.42^{de}	3.05^{abc}	3.51^{e}	3.26^{bcde}	3.23^{bcde}	3.50^{e}	3.38^{cde}	3.22^{bcde}	3.48^{de}	3.33^{cde}
∑ n-6	0	20.97^{c}										
	3	20.04^{abc}	18.74^{a}	18.30^{a}	20.08^{abc}	21.15^{c}	21.65^{c}	21.30^{c}	21.02^{c}	21.43^{c}	21.33^{c}	21.35^{c}
	7	20.83^{bc}	21.07^{c}	19.05^{ab}	21.50^{c}	21.23^{c}	21.21^{c}	21.20^{c}	21.03^{c}	21.53^{c}	21.56^{c}	21.03^{c}
Total FA	0	95.66^{d}										
	3	90.82^{bcd}	84.64^{ab}	82.68^{a}	90.80^{bcd}	95.74^{d}	97.57d	95.98^{d}	94.55^{d}	96.30^{d}	96.40^{d}	96.42^{d}
	7	93.99^{cd}	95.05^{d}	86.10^{abc}	97.28^{d}	96.05^{d}	95.60^{d}	95.82^{d}	94.95^{d}	96.84^{d}	97.63^{d}	94.89^{d}

Mean values with different small superscript letters presented within each row and column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

2.4 Discussion

2.4.1 Effect of Natural Antioxidants on the Formation of Thiobarbituric Acid-Reactive Substances (TBARS) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions

Autoxidation of lipids can be determined by quantification of the secondary lipid oxidation products. For instance, TBARS are a secondary lipid oxidation product produced from the breakdown of polyunsaturated fatty acids, particularly, those with three or more double bonds in their structure which can be measured by the TBARS test utilizing thiobarbituric acid as a reagent (Estevez et al., 2009). However, the profile of fat from the breast, thigh meat, skin, and adipose tissue varied with respect to fatty acids (especially unsaturated fatty acids). The application of natural antioxidants significantly reduced the oxidative deterioration of fats extracted from the four portions (Figures 2.6, 2.8, 2.10 and 2.12 and TableA2). Among natural antioxidant treatments, it was observed that the ROS extract reduced lipid oxidation more in fat from breast meat, skin and adipose tissue as compared to non-treated samples, while in thigh fat the lowest reduction of TBARS values was found in the SFS treatment at each interval of storage time. This is probably associated with efficiency of compounds and the chemical structure of natural extracts. Zilic et al. (2010) reported that natural sources which contain several compounds that exert anti-oxidative functions tend to retard lipid oxidation. These compounds are mainly phenolic compounds such as the chlorogenic, caffeic, ferulic and rosmarinic acids (Velasco and Williams, 2011). The phenolic compounds can retard oxidation by scavenging free-radicals, quenching singlet oxygen and chelating metals (Velasco and Williams, 2011; Karre et al., 2013). However, BHT is well-known as a potent antioxidant, which can retard lipid oxidation in meat (Selani et al., 2011) and meat products (Banerjee

et al., 2012). The effectiveness of ROS, SRB, SFS, and GGR extracts were comparable to BHT. These results clearly show that autoxidation of fat decreased in the presence of both natural antioxidants and BHT (Table 2.4). Similar results were observed by Baker (2011) who found that the value of TBARS in olive oil stored for 12 days at 65 ^{o}C was significantly reduced in samples treated with GGR extract at 0.50, 0.75 and 1 % and ROS extract at 0.05, 0.075 and 0.1 %.

In addition, no significant difference was found between either level of the most natural or the synthetic antioxidants after applying them to the different extracted fat ($p > 0.05$), while a significant difference was found between each antioxidant level and the "zero" level (Figures 2.5, 2.7, 2.9 and 2.11). These results suggested that the optimum level of natural and synthetic antioxidants that can inhibit the formation of lipid oxidation in fat from various portions of the chicken carcass is probably between 10 and 20 mg total phenolics per 100 g fat. Lau and King (2003) found that lipid oxidation was lower in dark poultry meat with added grape seed extract at 1 % compared to 2 %. Naveena et al. (2008) reported that the addition of pomegranate seed and rind powder extract containing total phenolics up to 10 mg total phenolics to 100 g of chicken patties meat reduced TBARS values significantly during 12 days. However, the conditions of their experiment were different from our study. The addition of natural extracts at the lower and higher levels over a 7-day period awarded a greater protection against oxidative rancidity compared to the non-treated samples (Table 2.4). Furthermore, increased TBARS values in fat is considered a good indicator of decomposition of hydroperoxides and generates secondary lipid oxidation products (Hayes, 2000; McCarthy et al., 2001; Bax et al., 2012). The increase of TBARS values in fat samples of breast or thigh meat over 3 days and which subsequently decreased at the end of the storage time (Figures 2.6, 2.8 and Table 2.4), this agreed with results shown by McCarthy et al. (2001) who reported that TBARS

values in cooked pork patties increased significantly over 6 days and declined up to 9 days of storage. The breakdown of malondialdehyde might cause the decrease of TBARS value following a continuous storage time and produce volatile compounds during the on-going exposure to heat and oxygen (Bax et al., 2012). Furthermore, oxidation of lipids throughout the storage time occurred more rapidly up to 3 days of storage in control samples of fat from breast meat than in fat from skin tissue, thigh meat, and adipose (Figures 2.6, 2.8, 2.10 and 2.12). There could be two reasons for the rapid formation of TBARS during the 3 days of storage. The first could be related to the higher concentration of phospholipids in breast meat compared to thigh meat (Table 2.5). The second could be linked to the large amount of long-chain fatty acids such as C20:4 n-6, C22:5 n-3, C20:5 n-3 and C22:6 n-3 (Table 2.8). Similar results were reported by Sampaio et al. (2012) who showed that lipid oxidation and degradation in cooked breast meat occurred quicker than in cooked thigh meat. Hence, chicken meat contains high levels of unsaturated fatty acids and is more susceptible to oxidation than pork, beef and lamb (Hayes, 2000). Min and Ahn (2005) reported that phospholipids have a high level of polyunsaturated fatty acids and, therefore, plays a major role in increasing rancidity. It was pointed out that the thermal process facilitates the lipid compounds' reaction with oxygen and other catalysts (Ahn and Kim, 1998). Nevertheless, these findings clearly observed that the employ of natural antioxidant extracts could be effective in inhibiting chicken fat against oxidation of lipid at accelerated storage conditions.

2.4.2 Effect of Natural Antioxidants on the Degradation of Phospholipids in Fat Extracted from Chicken Portions during Accelerated Storage Conditions

Phospholipids are known to be susceptible to thermal degradation (Jayasena et al., 2013) and have been linked to the development of warmed-over flavours (Igene and Pearson, 1979; Igene, et al., 1980; Roldan et al., 2014). The content of phospholipids in the current study was within the range of those reported by Pikul et al. (1985) and Alasnier et al. (2000), but higher than those published by Soyer et al. (2010) and lower than those reported by Pikul et al. (1984). Moreover, supplementation with either natural or synthetic antioxidants impeded the degradation of phospholipid content in fat from all portions with (exception breast fat) by means of yielding a higher phospholipid content than the equivalent control. In thigh fat, natural antioxidants yielded more phospholipid content than non-treated samples at day 3 and 7 of storage times. The natural antioxidants that yielded significantly higher amount of phospholipid content at day 3 and 7, were SRB and GGR compared to the non-treated samples (Figure 2.15 and Table A2). In fat from adipose and skin, phospholipid degradation was reduced at day 7 of storage time compared to non-treated samples (Figures 2.16, 2.17 and Table A2). These results indicated that phospholipids can be protected by adding antioxidants to fat as a result of low level of degradation of phospholipids in fat samples were detected during the accelerated storage time at 62.8 ^{o}C. The underpinning mechanism by antioxidants in protecting phospholipids from thermal decomposition is not well known. This mechanism could be similar to the protective mechanism as it applies to individual fatty acids since phospholipids are considered to have elevated levels of unsaturated fatty acids. Furthermore, no significant differences were found between "zero" level and both 10 and 20 mg of each antioxidant. These results suggest that the phospholipid content did not affect by antioxidant application doses. Moreover, the phospholipid content of fat from

breast meat, thigh meat, skin tissue and adipose tissue was significantly influenced ($p \leq 0.05$) by accelerating storage condition (Figures 2.14, 2.15, 2.16 and 2.17). Several reports have been published regarding the effect of storage time on the phospholipid content. For example, Soyer et al. (2010) showed that phospholipid content significantly decreased in fat from chicken breast and thigh meat frozen for 6 months. Phospholipids degradation is associated with endogenous and exogenous factors such as saturation degree of fatty acids, enzymes, metals, storage time, temperature, oxidation reaction and oxygen (Erickson, 2000; Soyer et al., 2010; Wang et al., 2011). To that effect, Wang et al. (2011) observed that phospholipid content in duck muscle declined after exposure to different heating methods such as boiling water for 30 min and roasting at 90 ^{o}C for 1 hr, hydrolysis of phospholipids was greater in roasted meat than in boiled meat. Yoshida et al. (2005) showed that the phospholipids decreased significantly in oils from peanut seeds unroasted and roasted for 6, 12, 20 and 30 min in a microwave. The cause of lowering phospholipids could be associated with changes the chemical composition during the oxidative deterioration of lipid. In addition, the higher degradation of phospholipids in fat from breast and thigh meat than fat from skin and adipose tissue due to the higher content of phospholipids detected in the breast and thigh meat, which is expected to show high degradation of phospholipids. Ma et al. (2007) pointed out that phospholipid is a rich source of polyunsaturated fatty acids, which is highly susceptible to oxidative deterioration. It can be explained that the rate of phospholipid degradation is associated with the relative presence of polyunsaturated fatty acids. Therefore, the elevated levels of oxidative deterioration in fat from breast and thigh meat are likely to be due to high levels of polyunsaturated fatty acids in phospholipid fraction.

2.4.3 Effect of Natural Antioxidants on the Formation of Conjugated Dienes (CDs) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions.

Conjugated dienes (CDs) are a good indicator of primary lipid oxidation products, which are produced through the oxidation of polyunsaturated fatty acids that have two double bonds in their structure (Feiner, 2006; Estevez et al., 2009). CDs are considered to be associated with warmed over flavour (Byrne et al., 2001). CD values found in extracted fat in the current study were within the range of those shown in previous work (Hawang et al., 2013), which found that the CD values in raw patties ranged from 57-78 µmol/g lipid. Furthermore, the fat extracted from chicken breast and skin portions is more stable with regards to CDs under thermal conditions when antioxidants are added (Table A2, Figures 2.19 and 2.25). The application of natural antioxidants significantly ($p \leq 0.05$) reduced the formation of CD in fat from breast meat compared to the non-treated samples, but no significant differences was found between natural antioxidant treatments and BHT (Figures 2.19 and Table A2). In skin, the antioxidants that significantly reduced the formation of CD at day 3, were SRB and SFS. At day 7, all natural antioxidants significantly reduced the CD values compared to the non-treated samples, while the highest reduction was found in SRB and SFS treatments (Figure 2.25 and Table A2). It appears that incorporation of the natural antioxidants in fat has the ability to retard oxidation level and enhance the stability of fat against autoxidation. The ability of antioxidants to retard oxidation in fat from the breast meat and increase its stability could be associated with low levels of unsaturated fatty acids. The results agree with the previous studies outlined by Al-dalain and Al-fraihat (2011) who highlighted that addition of natural and synthetic antioxidants to sunflower oil retarded the increments of the CDs during the heating period.

Furthermore, the results show that both levels of natural antioxidants were significantly reduced the formation of CDs in fat from breast and skin tissues compared to "zero" level (Figures 2.18 and 2.24). The highest inhibition of CDs was found in fat samples of breast tissue treated with 20 mg followed by 10 mg and "zero" level respectively (Figure 2.18). These results are in agreement with Poiana (2012), who found that CD values were reduced significantly with application levels of grape seeds extracts into sunflower oil and stored under heating storage conditions. Similar findings were found in skin fat, while SRB and SFS at both levels awarded discernible protection against an increase in CDs compared to the non-treated samples (Figure 2.24). These results suggest that the inclusion of SRB and SFS into the breast and skin fat effectively reduced the CDs formation (Figure 2.18 and 2.24). This could be due to active compounds in SRB and SFS extracts which are considered a rich source of phenolic compounds (Luthria et al., 2006; Lin et al., 2008; Amakura et al., 2013; Ye et al., 2015) and whose anti-oxidative functions tend to retard lipid oxidation. No significant differences between some antioxidant levels indicated that increasing the concentration did not have any effect on lipid oxidation. Similar findings observed by Choe et al. (2011), found that the inhibition of CDs in raw pork meat was not affected by increasing the concentrations of pumpkin leaf extracts over time. In addition, the difference between meat portions in response to the antioxidant levels was attributed to the disparity in chemical composition, mainly, phospholipids and polyunsaturated fatty acids. Moreover, the addition of natural extracts at the lower and higher level awarded similar protection against oxidative rancidity compared to the BHT, which could be attributed to the efficiency of compounds and the chemical structure of natural antioxidants.

In addition, a significant increase in CD content progressed in fat samples of breast meat and adipose tissue up to 3 days and decreased thereafter (Figures 2.19 and 2.23). These

results were similar to other reports that described an initial increase in CDs followed by a decline during extended storage of chicken breast meat, pork and vegetable lipid sources (Juntachote et al., 2007; Teets et al., 2008). Erickson (2002) reported that an elevated content of the CDs is a strong indicator of auto-oxidation. The formation and reduction of CDs are affected by several factors, such as processing and storage conditions, oxygen availability, and temperature (Min and Ahn, 2005). CDs are produced concurrently to the formation of lipid hydroperoxides from polyunsaturated fatty acids (Estevez et al., 2009). In the early stages of lipid oxidation, the decomposition of hydroperoxides occurs slowly (Erickson, 2002), meaning that there is an initial accumulation of hydroperoxides and CDs (Gordon, 2001). However, ultimately, the rate of CDs degradation becomes more rapid than the rate of CDs formation, which accounts for the reduction in CDs following an initial accumulation. Over time, secondary lipid oxidation products such as pentanal, hexanal, 4-hydroxynonenal, and TBARS are formed (Mensink and Jogchum, 2002).

2.4.4 Effect of Natural Antioxidants on the Formation of Conjugated Trienes (CTs) in Extracted Fat from Chicken Portions during Accelerated Storage Conditions

Conjugated trienes (CT) are formed from oxidation of polyunsaturated fatty acids, particularly those that have three or more double bonds in their structure (Wrolstad et al., 2005). The presence of conjugated trienes (CT) in fat is indicative of advanced lipid oxidation (Gordon, 2001). The application of natural antioxidants was significantly reduced the formation of CTs in fat from breast tissue compared to the non-treated samples, while BHT and SFS treatments were found to have the lowest CT values compared to the non-treated samples during the storage time (Figure 2.27 and Table A2). In skin fat, none of the natural antioxidants significantly reduced the formation of CT at day 3, while at day 7 SRB and SFS were significantly reduced the formation of CT in

skin fat samples (Figure 2.33). It appears that incorporation of the natural antioxidants in fat has the ability to retard oxidation levels and enhance the stability of fat against autoxidation. The fat extracted from chicken breast and skin portions are more stable under thermal conditions when antioxidants are added to them could be due to the phenolic compounds present in natural antioxidant extracts that could inhibit the oxidation of polyunsaturated fatty acids and decrease the formation of CTs to lower levels compared to the non-treated samples. These results are in agreement with the previous study outlined by Al-dalain and Al-fraihat, (2011) who observed that the addition of natural and synthetic antioxidants to sunflower oil retarded the increments of CTs during the heating period while the lowest accumulation of CTs was detected in samples containing BHT. The effect of application at different levels was only shown in fat samples from breast and skin tissues (Figures 2.26 and 2.32). Hence, the natural antioxidants at a higher level of application were found to have the lowest formation of CT in fat from breast tissue compared to lower and non-treated samples. Among them, SFS at the lower and higher application dose had the lowest formation of CT. These results are in agreement with Poiana (2012), who found that the CT values were reduced significantly with application levels of grape seeds extracts into sunflower oil and stored under heating storage conditions. Similar results were shown in fat from skin tissue, SRB at 20 mg and SFS at both levels awarded discernible protection against an increase in CT compared to the non-treated samples (Figure 2.32). Hence, these results indicate that the addition of natural antioxidants at the lower and higher levels to fat extracted from breast and skin could provide a greater protection against oxidative rancidity compared to the non-treated samples and similar to BHT, as lower formation of CTs was shown.

In addition, the CT values in all fat samples were significantly influenced by storage at elevated temperatures (Figure 2.27, 2.29, 2.31 and 2.33). The level of CTs in non-treated

samples of thigh and skin fat increased progressively over storage time; while the formation of CTs in breast and adipose fat reached its highest level on day 3 of the accelerated storage time which declined towards the end of storage. The increase in CTs in fat under the accelerated storage is likely due to the decomposition of conjugated dienes; which, as primary oxidation products, undergo further oxidation and produce CTs as secondary lipid oxidation products. The discrepancy in the continued formation of the CTs over time in non-treated samples of breast and adipose tissue could be due to the ongoing degradation of the CTs, facilitated by compounds with a prominent presence in breast and adipose tissue, into the form no longer detectable by the CTs detection system used. Among the raw cuts of chicken meat (non-treated), the fat from breast tissue contained the highest levels of CTs, followed by the fat from thigh and adipose tissue, with the fat from raw skin containing the lowest levels of CTs. This could be attributed to the high amount of phospholipid in fat from the breast meat.

2.4.5 Effect of Natural Antioxidants on Fatty Acid Profile in Fat Extracted from Chicken Portions during Accelerated Storage Conditions

The fatty acid composition in the current study was similar to those reported by Sampaio et al. (2012) and higher than those reported by Cortinas et al. (2004) and Mariutti et al. (2011). The evaluation of the fatty acid composition in fat from different portions of chicken meat subjected to an accelerated storage time can provide valuable information about the changes which occur to each fatty acid. Measuring fatty acid content is an important factor in evaluating the nutritional quality of meat and its products (Andres et al., 2014). According to the literature, the stability of fatty acid content in meats increases after it has been supplemented with antioxidants since the existing antioxidants act as hydrogen donors to supply electrons to reduce the hydrolysis of unsaturated fatty acids

(Cortinas et al., 2004; Sampaio et al., 2012). In the present study, extracts of natural sources and BHT were used as antioxidants in fat derived from different portions of chicken meat. Such natural antioxidants are well documented to have many anti-oxidative phytochemicals (Trindade et al., 2010; Velasco and Williams, 2011; Sampaio et al., 2012). The results showed that the application of natural extracts at the lower and higher levels into fat from breast meat, thigh meat and skin tissue over a 7 day period did not award a significantly greater protection against oxidative rancidity compared to the non-treated samples (Tables 2.8, 2.9 and 2.11). In contrast, in skin fat, natural antioxidants at both levels provided a significant protection against a decrease in all SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 at day 7. Hence, the natural antioxidants that provided significant protection against a decrease of all fatty acids at day 7, were ROS, SRB, GGR at the lower application doses and SFS at the higher application dose (Table 2.10). These findings were similar to the data reported by Sampaio et al. (2012) who found that the SFAs, MUFAs and PUFAs were inhibited from degradation in breast and thigh meat supplemented with BHT, oregano plus sage, oregano plus sage plus 5 % honey, and oregano plus sage plus 10 % honey and stored at 4 ^{o}C for 4 days. The results from their studies also suggested that the degradation of fatty acids, either saturated or unsaturated, was lower in natural than in synthetic antioxidant treatments. In the study conducted by Trindade et al. (2010), they found that ROS extract supplemented either alone or mixed either with BHT/BHA or oregano extract reduced the hydrolysis of most fatty acids in beef burgers after being submitted to different irradiation doses and stored for 90 days. The results of our experiment indicated that fatty acid composition can be protected by adding natural antioxidants to fat and results in a low level of degradation of SFAs, MUFAs and PUFAs in all chicken portion fat samples during the storage time at 62.8^{o}C. Huang et al. (2005) illustrated that antioxidant substances can retard lipid oxidation in

initiation and propagation steps by reacting with lipid free radicals and free radicals produced by the oxidation of unsaturated fatty acids to form stable and non-radical products. Reische et al. (2002) reported that antioxidants donate hydrogen atoms carried in their structure to the peroxy and oxy free radicals which are produced in the propagation phase. Also, antioxidants have the ability to react directly with lipid radicals formed in the initiation phase and convert them into non-radical products. In our study, the effect of natural antioxidants on the rate of oxidation varied in fat from different portions of chicken and depended mostly on the type and level of antioxidant that was being used to stabilize the fatty acid from oxidation. For instance, using SFS at 10 or 20 mg extract was more active in chicken breast and thigh fat in the oxidative stabilization of individual fatty acids and the SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 PUFAs content than other treatments. Adipose fat supplemented with ROS extract at 10 mg had the lowest reduction of most individual and the sum of SFAs, MUFAs, and PUFAs. In contrast, GGR extract at 10 mg was observed to be more efficient in skin fat as it reduced the degradation of the most individual and the sum of SFAs, MUFAs, PUFAs content (Appendix Table A3, 4, 5 and 6). These differences between meat portions in response to the natural and synthetic antioxidants was attributed to the disparity in chemical composition, particularly, phospholipids and polyunsaturated fatty acids. Consequently, according to these results, antioxidants behaved differently in different fat samples. Most of the natural antioxidants when added to the fat extracted from four chicken portions performed similarly to the synthetic antioxidant (BHT treatment) over the 7 day storage period. This may be due to the natural antioxidants containing many phenolic compounds which may have the ability to scavenge free radicals similar to phenolic compounds in synthetic antioxidant structures. It appeared that the fat supplemented with antioxidants

and stored at high temperature can protect long chain fatty acids from oxidation and further decomposition.

Several studies have found that antioxidant activity was observed more at higher concentration than the lower concentration (Mielnik et al., 2006; Rojas and Brewer, 2007; Carpenter et al., 2007). However, in our study, the effect of the level mostly depended on the type of antioxidant added. The lowest level of degradation was detected in the SFS extract treatment at 20 mg in breast fat. In contrast, thigh fat treated with sunflower seed extract at 10 mg had the highest value of all fatty acids. This could be related to a higher concentration of phospholipids in breast meat than in thigh meat. Therefore, high levels of antioxidants might be needed to improve the oxidative stability of fat. No significant differences were found between the 10 and 20 mg addition of antioxidants. These observations indicate that applying antioxidants at a higher level could become a pro-oxidative (Lau and King, 2003). In addition, the results showed that accelerated storage induced a decline of fatty acid content that influenced all three families of fatty acids SFAs, MUFAs and PUFAs in different proportions (Tables 2.8, 2.9, 2.10 and 2.11). Unsaturated fatty acids were more susceptible to oxidation than saturated fatty acids, due to the ability of free radicals to attack easily and react with unsaturated fatty acids double bonds and produce short-chain aldehydes (Huang et al., 2013). Our results indicate that higher temperature (62.8 ^{o}C) results in accelerated degradation of SFAs content in all chicken portions. A decrease of saturated fatty acids is associated with the breakdown of a large amount of fatty acid C16:0 which was the most predominant SFAs followed by C18:0 in fat from all cuts. Similar results were shown by Sampaio et al. (2012) who found that the saturated fatty acids were decreased in cooked breast and thigh meat during the storage time of 4 days. In the current study, the highest degradation of SFA in fat samples stored at 62.8 ^{o}C for 7 days was probably due to the thermal process. Additionally, fat

stored at a high temperature and for a long time can undergo various reactions including autoxidation and thermolytic reactions which could result in greater changes to the fatty acid profile (Erickson, 2002). Similar results were found by Cortinas et al. (2004), who observed that the content of SFAs rapidly reduced after cooking thigh meat compared to raw meat. Moreover, the content of MUFAs gradually declined throughout the storage time in fat from all cuts. This could be due to susceptible lipids being attacked by free radicals. The results suggest that the oxidative degradation of MUFAs occurred after subjecting fat samples to oven temperature (62.8 ^{o}C). This may be due to lipid oxidation which appears to be stimulated by a thermal process. The results are in agreement with those reported by Sampaio et al. (2012) who found that the mono-unsaturated fatty acids decreased in cooked chicken breast and thigh meat over 4 days of storage. Cortinas et al. (2004) also observed that the content of MUFAs declined in cooked thigh meat compared to raw meat. In addition, after subjecting fat samples from all cuts to an oven temperature of 62.8 ^{o}C, accelerated storage had an effect on the PUFA content over 7 days. These results agree with those reported by Cortinas et al. (2004) who found that the content of PUFA declined in cooked thigh meat compared to raw meat. Similar results were reported by Sampaio et al. (2012) who found that the polyunsaturated fatty acids decreased in breast and thigh meat over 4 days of storage at 4 ^{o}C. The decrease in PUFA content was mainly due to oxidation degradation of polyunsaturated fatty acids mainly C18:2 n-6 and C18:3 n-3 into primary and secondary oxidation products. The content of n-3 and n-6 in samples of all cuts without antioxidants decreased over 7 days of storage. However, the reduction was greater in $\sum$n-3 compared to $\sum$n-6 PUFAs for all cuts except adipose fat. Erickson (2002) reported that n-3 PUFAs oxidized faster than n-6 PUFAs, due to the effect of location of methylene-interrupted double bonds on the rate of oxidation.

2.5 Conclusions

The effect of natural and synthetic antioxidants on lipid oxidation outlined that in the non-treated samples (without antioxidant added), during storage, had low stability against oxidation deterioration. Adipose tissue was more susceptible to lipid oxidation followed by breast, thigh and skin tissue. The addition of ROS, SRB, SFS, GGR extracts and BHT to fat from portions of chicken has a positive effect on delaying the oxidative deterioration throughout the storage, as evidenced by reducing the degradation of fatty acids and phospholipids, lower formation of CD, CT and TBARS values. Among natural treatments, the application of ROS at the lower and higher level over a 7 day period provided greater protection against oxidative rancidity compared to the non-treated samples. No significant difference was found between antioxidants at level 10 and 20 mg total phenolic/100 g. However, the addition of 10 mg to thigh, skin and adipose tissue had lower lipid oxidation, while breast was more resistant to the oxidation process when 20 mg was added. These results suggest that the optimum level of natural and synthetic antioxidants needed to inhibit the rate of lipid oxidation is probably between 10 and 20 mg total phenolic/100 g fat. The addition of natural extracts (10 and 20 mg) on day 3 of the storage time provided a protection similar to BHT, against oxidative rancidity. It seems that the use of these antioxidants extracted from natural sources could be considered as an efficient way to protect the nutritional value of meat and extend its shelf life. Application of natural and synthetic antioxidants to raw and freshly cooked meat during the storage time would be necessary to understand whether antioxidants would have any positive impact on physical and chemical characteristics of broiler chicken meat. Therefore, further investigation is required to evaluate the efficiency of natural and synthetic antioxidant application to raw and freshly cooked meat during the storage time.

Chapter 3:

Uptake Efficiency of Natural Antioxidants into Chicken Breast Meat.

3.1 Introduction

A number of different approaches can be employed to apply antioxidants to a piece of meat (Naveena et al., 2008; Lee et al., 2010). These approaches include wet applications such as soaking or dipping (with or without tumbling), injection or rubbing on as a dry application. For this study the choice was made to use dipping (without tumbling) in order to apply the various antioxidants to chicken meat. The basis of any wet application process is the capacity of animal muscles to retain water when penetrated into muscle (Nunez-Gonzalez, 2010). The dipping process involves submerging meat for a predetermined time into a liquid containing additives to facilitate absorption of water and additives and to increase water retention (Yusop et al., 2010). The diffusion rate of a solution into the meat, and its retention, is dependent on the gross characteristics of the meat (i.e. fat content) (Post and Heath, 1982; Huff-Lonergan and Lonergan, 2005); the content and location of connective tissue (Hansen, et al., 2008); and on the muscle myofibril content (Nunez-Gonzalez, 2010). Muscle myofibrillar proteins are principally responsible for water retention by meat (Nunez-Gonzalez, 2010). The process of dipping meat in any solution will cause the uptake of water and increase the weight of a piece of meat. More importantly, the process of dipping can alter the structure of muscle tissue to increase tenderness, juiciness and to enhance the flavour of meat (Lemos et al., 1999; Alarcon-Rojo, 2010). Several studies have shown the progress of uptake of model active ingredients into different layers of meat, using phosphate, sodium chloride and a fluorescent dye to monitor the diffusion (Xiong and Kupski, 1999a; Xiong and Kupski, 1999b; Alarcon-Rojo, 2010).

Although several studies have been carried out to evaluate the effect of the dipping process with antioxidants on characteristics of chicken meat (Naveena and Mendirratta, 2001; Naveena et al., 2004; Vaithiyanathan et al., 2011); the amount of moisture uptake and penetration of antioxidant ingredients is not well documented. In particular, the kinetic process that contributes in terms of penetration of the antioxidant solution into the core of the chicken breast fillet during immersion is not well understood. Such knowledge is of practical importance since it can help to establish the optimum schedule for the dipping process and gives an indication of the diffusion of an antioxidant solution into the different meat layers. Therefore, this study aimed to determine the readiness by which antioxidant ingredients penetrate chicken breast fillets using the dipping method (soaking).

3.2 Materials and Methods

3.2.1 Raw Materials

Chicken carcasses, sources of natural antioxidants and chemicals used was described in Chapter 2 section 2.2.1.

3.2.2 Experimental Design and Preparation of Samples

This experiment comprised of a 6 x 4 factorial design which included six experimental antioxidant treatments (ROS, SRB, SFS, GGR, BHT and CON) and three dipping times (5, 20, 30 and 60 min). With respect to the preparation of meat samples, fresh whole carcasses of chicken meat were obtained and divided into two batches, half batches of the chicken carcasses were initially stored at -20 ^{o}C for at least two months prior to being thawed to 4 ^{o}C under refrigeration temperature (4 ^{o}C), and a half of whole chicken carcasses were used on the same day of conducting experiments as raw samples. After removing the breast fillets and all visual fat, the breast fillets (raw and thawed meat) were cut into uniform sized pieces, dimensions length 4 cm, width 2 cm, and thickness 2 cm, and weighing 23.59 ± 0.68 g each before dipping. The uniform pieces of raw and thawed breast fillets were dipped into an appropriate antioxidant solutions for 5, 20, 30 and 60 min: four of these treatments were treated with natural antioxidants ROS, SRB, SFS and GGR (200 mg equivalent total phenolics dissolved in 1 litre of deionised water (DW)). One batch of raw and thawed chicken breast meat was treated with butylated hydroxytoluene (BHT) as an example of a powerful artificial antioxidant (200 mg equivalent total phenolics dissolved in 1 litre of DW), and one batch was treated with DW as a control (CON). At each time interval of dipping process, the samples were removed from the extraction and dried using a paper towel. All samples were immediately frozen

at -20 ^{o}C for three days in the freezer to aid in slicing before measuring phenolic penetration (Figure 3.1).

3.2.3 Antioxidant Extraction from Meat Slices

In order to measure the penetration of phenolic content into different layers of chicken fillets. Four slices in dimensions ranging from 4-5 mm thickness were removed from the top side of raw and thawed dipped meat samples (section 3.2.1) as illustrated in Figure 3.1. The phenolic content was extracted according to the method described by Naveena et al. (2008) with slight modifications. To obtain the phenolic content, 2 g of individual slices were weighed and homogenized (Silverson Machines Ltd., Chesham, UK) in 10 ml of aqueous acetone (acetone: water at 70:30 (v/v)) for 30 sec at high speed. The mixture was gently shaken in an orbital shaker (HS 501 digital, IKA labor Technik, Staufen, Germany) for 4 h in the dark at ambient temperature (~23 ^{o}C) and then refrigerated for 24 h. The extracts were filtered through Whatman® No. 1 filter paper.

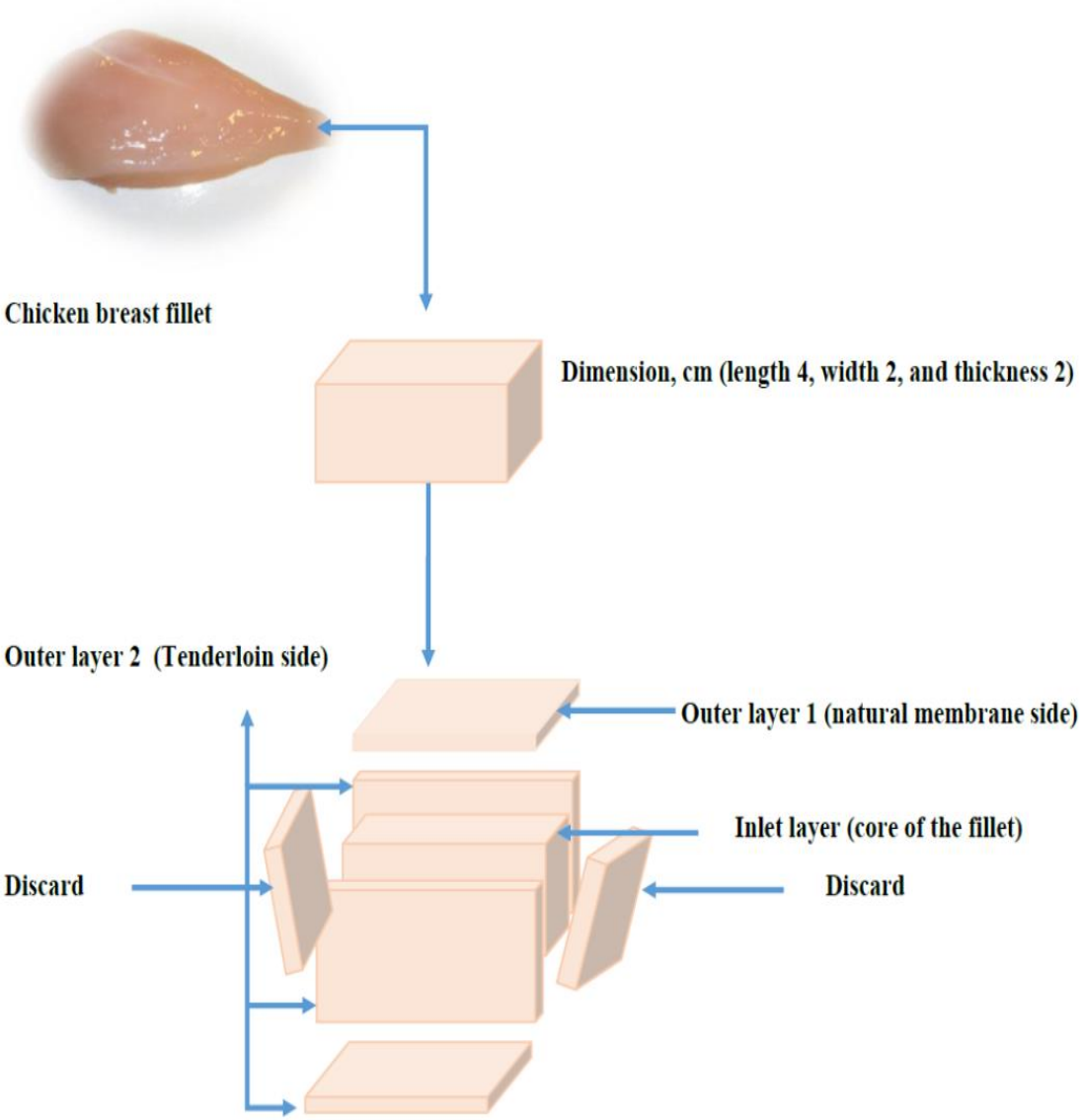

Figure 3. 1 Illustration of the sample diffusion for monitoring total phenolic penetration into the chicken breast fillet.

3.2.4 Total Phenolic Content Determination in Meat Extraction

The total phenolic content of extraction obtained from both raw and thawed meats (section 3.2.2) was measured according to the method described in section 2.2.2.1.

3.2.5 Moisture Uptake Analysis

Portions of chicken breast meat were cut into a cuboid shape of approximately 4 x 2 x 2 cm^3 which equated to approximately 23.59 g of meat in weight. One side of the cuboid retained the fascial membrane while the remaining lengthwise sides were cut through the muscle tissue and dipped in separate solutions containing antioxidant extracts. Following the dipping, the meat samples were re-weighed. Each treatment was conducted in triplicate. Moisture uptake was calculated as per the following formula:

$$\text{Moisture uptake (\%)} = \left[\frac{\text{weight of dipped meat (g)} - \text{weight of meat before dipping (g)}}{\text{weight of meat before dipping (g)}}\right] \text{x } 100$$

3.2.6 The Rate of Phenolic Uptake in Raw and Thawed Meat

The rate of phenolic uptake in meat samples was calculated as per the following formula:

$$\text{Rate of Phenolic Uptake} = \frac{(\text{Phenolic content in dipped sample (g)} - \text{Phenolic content in control sample(g)})}{\text{Time of dipping (min)}}$$

3.2.7 Statistical Analysis

The data of dipping raw and thawed chicken meat were conducted and analysed separately. This experiment was conducted utilising full factorial design of 6 x 4 x 3, where the three factors were the antioxidant treatments (ROS, GGR, SFS, SRB, BHT and

non-treated), dipping times (5, 20, 30 and 60 minutes) and chicken breast fillet layers (Membrane layer, tenderloin layer and the core of fillets). The interaction between antioxidant x dipping time x meat layer was also assessed. Penetration of total phenolic content was analysed by using general analysis of variance (ANOVA). The experiment was conducted in triplicate (n = 72). While moisture uptake was analysed by using two-way analysis of variance (ANOVA). Tukey's HSD test was used to identify the significant differences between means, and the significance level of all data was set at $p \leq 0.05$. The null (H_0) and alternative (H_1) hypothesis for each dependent variable were set as:

Null hypothesis (H_0):

There was no significant effect of natural antioxidants application, dipping time, meat layer and the interaction between them on penetration of antioxidant ingredients into raw and thawed chicken breast fillets (H_0: $\mu = 0$; $p > 0.05$)

Alternative hypothesis (H_1)

There was a significant effect of natural antioxidants application, dipping time, meat layer and the interaction between them on penetration of antioxidant ingredients into raw and thawed chicken breast fillets (H_1: $\mu \neq 0$; $p \leq 0.05$).

3.3 Results

3.3.1 Moisture Uptake

3.3.1.1 The Moisture Uptake in Raw Meat

The uptake of moisture due to dipping raw chicken breast meat into various antioxidant solutions was significantly affected by antioxidant solutions (Appendix Table B1). The moisture uptake was found higher in SRB followed by ROS, non-treated samples, SFS and GGR respectively (Appendix Table B1). Regardless of antioxidants, the moisture uptake was significantly affected by dipping samples into antioxidants solution and deionised water for 60 min. From 5 min, onwards, the moisture uptake was increased significantly ($p < 0.001$) in raw meat samples with increasing dipping time (Appendix Table B1). Moreover, a significant AO x DT interaction ($p = 0.019$) was found for moisture uptake (Figure 2.2), indicating that the effect of antioxidant on moisture uptake in raw meat samples is depending on the dipping time. The uptake of moisture in raw meat samples due to dipping samples into various antioxidant solutions increased as the dipping time progressed. No significant differences were found between treatments over the first 20 min of the dipping process, from 30 min onwards the highest absorption of moisture was observed in raw meat samples dipped in SRB followed by ROS solution. Moisture uptake in raw breast samples treated with SFS and GGR from 30 min onwards was markedly less than the non-treated samples.

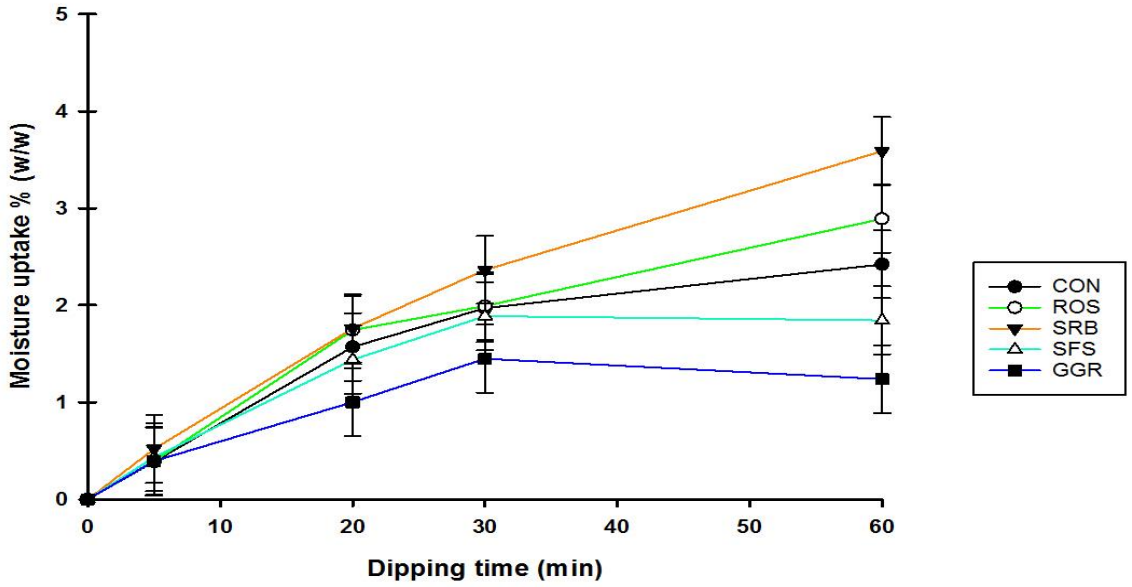

Figure 3. 2 Effect of natural antioxidants and dipping time interaction on moisture uptake of raw chicken fillets (Means ± SED; n = 3). Non-treated control (CON), Rosemary extract (ROS), Small Red Bean extract (SRB), Sun Flower Seed extract (SFS), ginger extract (GGR).

3.3.1.2 The Moisture Uptake in Thawed Meat

In chicken breast meat samples that were previously frozen and thawed, the moisture uptake was not significantly affected by antioxidant ($p = 0.588$) (Appendix Table B1). Regardless of antioxidant effects, the dipping time causes a similar increase in moisture uptake as was observed for raw chicken breast samples (Appendix Table B1). Hence, the uptake of moisture due to dipping chicken breast meat into various antioxidant solutions in thawed meat samples increased significantly as the dipping time progressed ($p < 0.001$). At 60 min, moisture uptake in thawed breast samples treated with natural antioxidants was highest. The thawed chicken breast meat samples showed limited overall diversion with regards to weight compared to the non-treated samples and each other during the entire dipping process especially when compared to the raw breast meat

samples (Appendix Table B1). No significant (p = 0.286) interaction was found between antioxidant and dipping time for the moisture uptake in thawed samples (Figure 3.3)

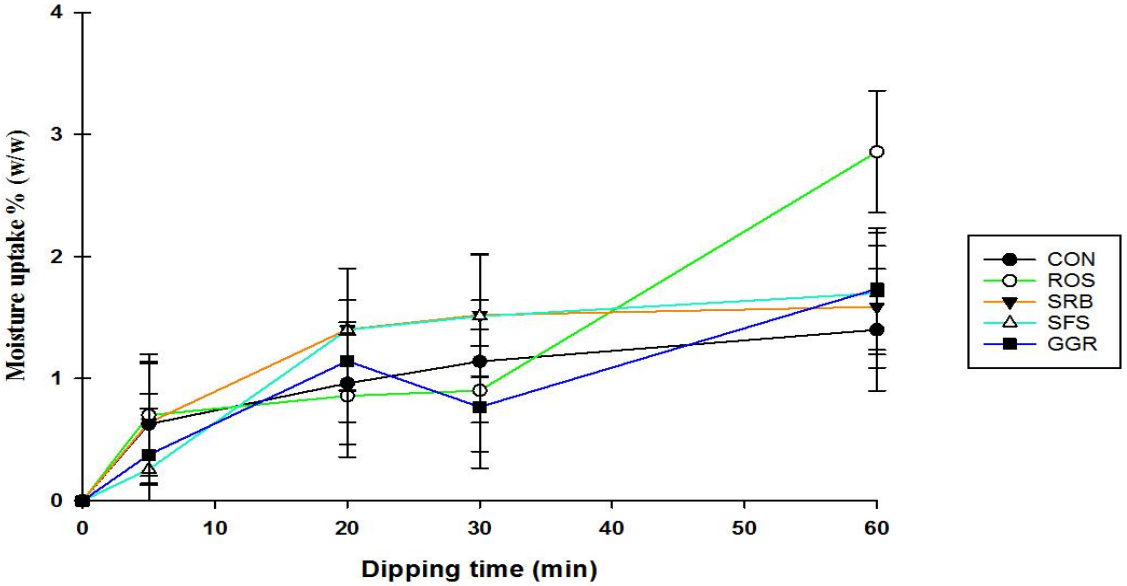

Figure 3. 3 Effect of natural antioxidants and dipping time interaction on moisture uptake of thawed chicken fillets (Means ± SED; n = 3). Non-treated control (CON), Rosemary extract (ROS), Small Red Bean extract (SRB), Sun Flower Seed extract (SFS), ginger extract (GGR).

3.3.2 Total Phenolic Content

After the moisture uptake was determined (section 3.3.1) the penetration of phenolic compounds into the sequential layers of raw and thawed chicken breast fillets was also monitored by assessing total phenolic content expressed as mg GAE/100 g meat (Table 3.1 and 3.2).

3.3.2.1 Raw Chicken Breast Fillets

3.3.2.1.1 The Penetration of Phenolic Content into Raw Meat

The total phenolic in raw meat was affected significantly by antioxidant, meat layer, dipping time and interaction between them (Appendix Table B2). The phenolic content

was found significantly higher in raw meat samples dipped in antioxidant solutions compared to the non-treated samples, while highest absorption was shown in samples dipped into ROS solution. The penetration of phenolic compounds into both membrane and tenderloin sides of breast fillets were markedly higher compared to the core of fillet ($p < 0.001$). The uptake of phenolic content due to dipping chicken breast meat into various antioxidant solutions in raw meat samples was affected significantly by dipping time, while the highest phenolic content was found at 20 min (Appendix Table B2). A significant AO x ML interaction was appeared for phenolic content, indicating that penetration of phenolic content into ML of chicken meat is depending on the AO solutions (Figure 3.4). The phenolic content of ROS, SRB, SFS, GGR and BHT was limited to the outer layer only (there was only a minor accumulation of phenolic content into the core). Both layers of raw breast fillets dipped in the ROS solution had the highest total phenolic uptake compared to any other treatments (Figure 3.4). A significant AO x DT interaction ($p < 0.001$) was found for phenolic content (Figure 3.5), due to the rapid increase in the total phenolic penetration in all treatments (with exception ROS) up to 20 min of dipping time and decreased over 60 min. Over dipping time meat samples exposed to ROS had a highest phenolic content compared to any other treatments (Figure 3.5). The results presented in Figure 3.6 also show a significant interaction ($p < 0.001$) between ML x DT for phenolic content due to the increase the penetration of phenolic content into three meat layers (membrane, tenderloin sides and core) over 20 min and decreased thereafter. The absorption of phenolic content was lower through the core of the fillets compared to the membrane, and tenderloin sides. A significant AO x ML x DT interaction ($p = 0.035$) was found for total phenolic content (Table 3.1). The results show that when meat samples were exposed to deionised water (control), the phenolic content of the outer layers (membrane and tenderloin sides) decreased while little change in total phenolic content

in the core of the fillets could be observed over time. The membrane side of raw chicken breast tissue that is directly covered by a membrane showed very little variation from the control when dipped in a variety of antioxidant solutions, with the exception of the ROS dipping solution. Over time, the raw samples exposed to the ROS dipping solution doubled its total phenolic content. The same observations were made on the tenderloin side outer layer of the raw chicken breast tissue. In addition, total phenolic content in the core of chicken breast fillets dipped into antioxidant solutions and deionised water was not significantly affected by dipping time. The penetration of ROS phenolic compounds into both membrane and tenderloin sides of breast fillets were markedly higher compared to the BHT.

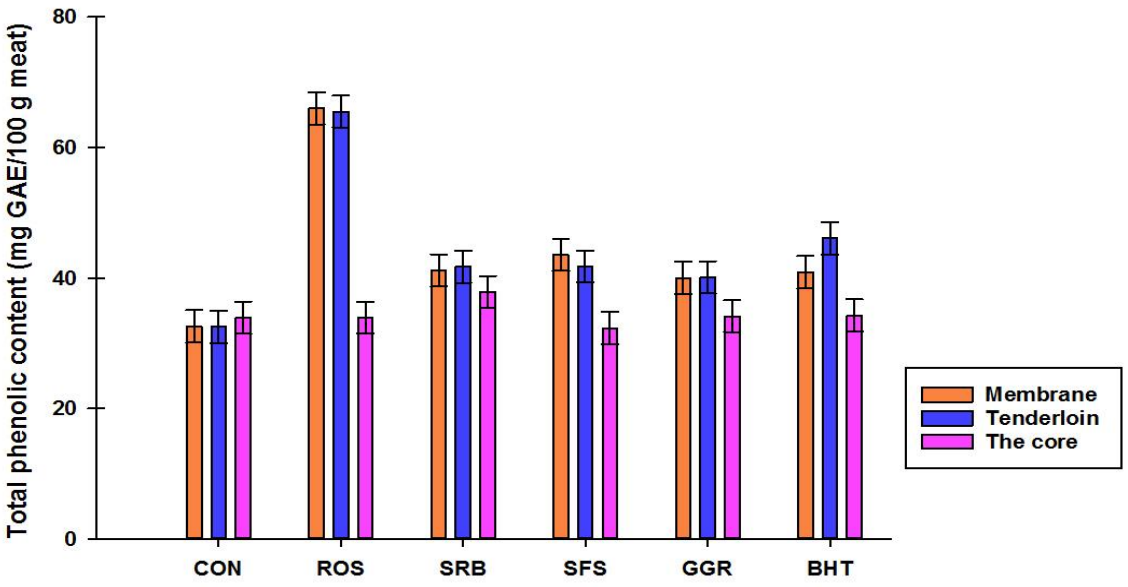

Figure 3. 4 Effect of natural antioxidants and meat layer interaction on total phenolic content of raw chicken fillets (Means ± SED; n = 3).

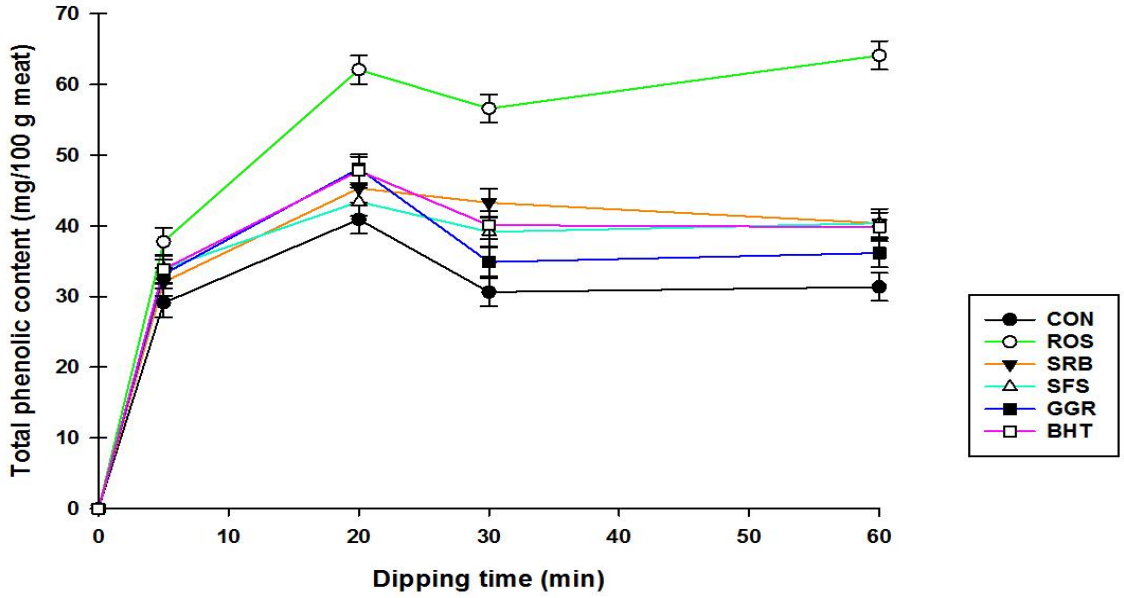

Figure 3.5 Effect of natural antioxidants and dipping time interaction on total phenolic content in raw chicken fillets (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

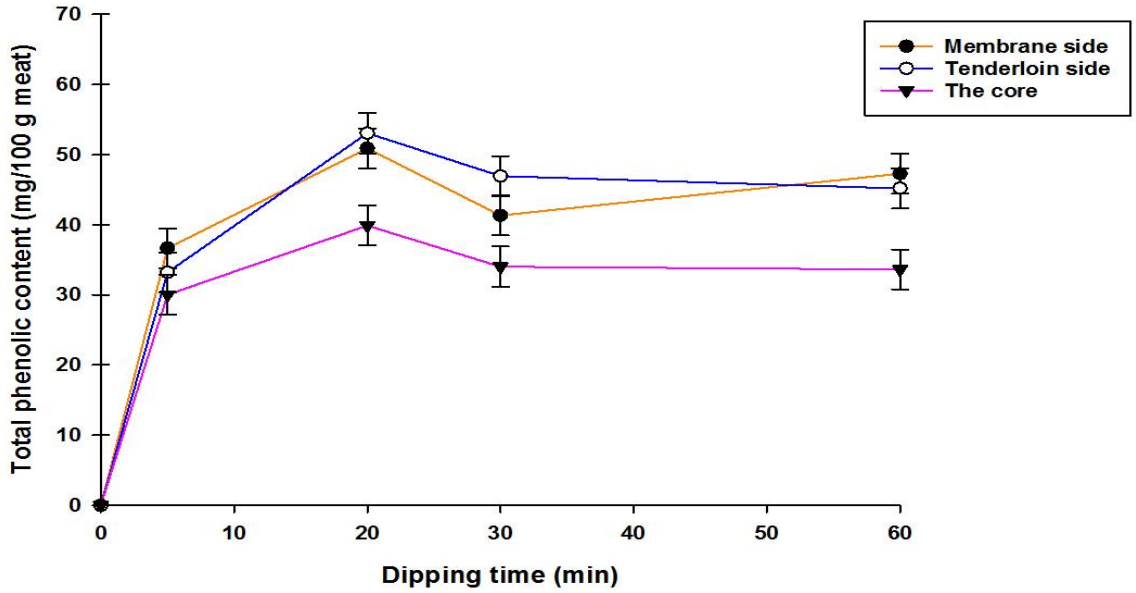

Figure 3.6. Effect of meat layer and dipping time interaction on total phenolic content in raw chicken fillets (Means ± SED; n = 3).

Table 3.1 Effect of natural antioxidants and dipping time on total phenolic content (mg GAE/100 g meat) in different layers of raw breast chicken meat.

Meat layer	Treatment	Dipping Time (minutes)			
		5	20	30	60
Membrane side	CON	28.38^{a}	41.66^{ab}	28.24^{a}	32.05^{a}
	ROS	42.15^{ab}	72.13^{de}	67.44^{bcde}	82.35^{e}
	SRB	37.40^{a}	44.83^{abc}	39.27^{a}	43.20^{abc}
	SFS	43.11^{abc}	46.61^{abcd}	40.79^{ab}	43.76^{abc}
	GGR	36.48^{a}	49.71^{abcd}	33.05^{a}	40.87^{ab}
	BHT	32.54^{a}	50.34^{abcd}	39.30^{a}	41.43^{ab}
Tenderloin side	CON	29.74^{a}	40.60^{ab}	30.32^{a}	29.41^{a}
	ROS	39.99^{ab}	71.74^{def}	73.46^{ef}	76.87^{f}
	SRB	30.01^{a}	49.72^{abcde}	46.80^{abc}	40.45^{ab}
	SFS	29.22^{a}	48.28^{abcd}	45.77^{abc}	43.80^{abc}
	GGR	31.94^{ab}	52.73^{abcdef}	38.99^{ab}	36.74^{ab}
	BHT	38.57^{ab}	55.31^{bcdef}	46.44^{abc}	43.96^{abc}
The core of the fillet	CON	29.17^{a}	40.53^{a}	33.32^{a}	32.65^{a}
	ROS	31.09^{a}	42.40^{a}	28.98^{a}	33.12^{a}
	SRB	28.74^{a}	41.43^{a}	43.79^{a}	37.50^{a}
	SFS	29.45^{a}	35.37^{a}	30.91^{a}	33.55^{a}
	GGR	31.13^{a}	41.88^{a}	32.65^{a}	30.88^{a}
	BHT	30.55^{a}	37.78^{a}	34.60^{a}	34.14^{a}

Mean values with different small superscript letters presented within each row of each treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different small superscript letters presented within each column of each meat layer differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

3.3.2.1.2 The Rate of Phenolic Uptake in Raw Meat

The rate of phenolic uptake through the 4 to 5 mm slices of outer layers (membrane and tenderloin) to the core of raw meat after dipping into ROS, SRB, SFS, GGR and BHT solutions was calculated (section 3.2.6) and results are presented in Figure 3.7. The results show that the rate of uptake was higher through the tenderloin side of the raw fillets compared to the membrane side (Figures 3.7A and B). Overall, the rate of uptake was the highest over a 20-min period for all products. The uptake rate of ROS, SRB, SFS, and GGR were limited to the outer layer only (there was only a minor accumulation of phenolic content into the core); while the phenolics in SRB continued to be taken up well past 20 min (in a manner similar to BHT) (Figure 3.7). The uptake of ROS (and BHT) at the tenderloin side was immediate (positive rate of uptake at 5 mins); while the rate of uptake for the SRB, SFS and GGR was negative for the first 5 min. The highest rate of uptake was noticed over the first 20 min with exception non-treated samples and that the rate of uptake slowed after that.

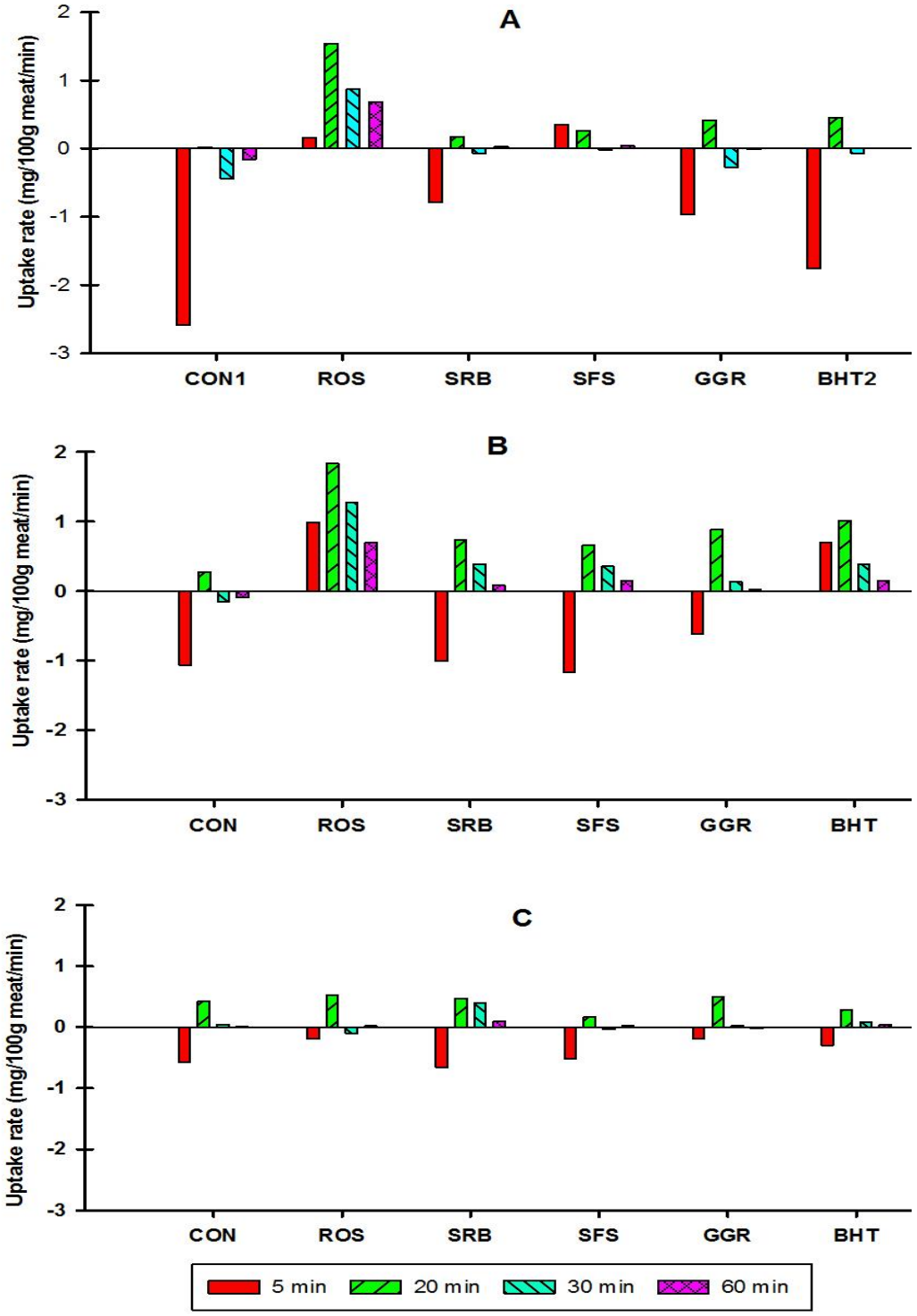

Figure 3. 7 Effect of dipping time on the rates of phenolic uptake of raw chicken fillets after dipping into antioxidant solutions. ***A****, membrane side;* ***B****, tenderloin side;* ***C****, core of the fillets. Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract*

3.3.2.2 Thawed Chicken Breast Fillets

3.3.2.2.1 The Penetration of Phenolic Content into Thawed Meat

A similar trend was observed in thawed samples as was observed in raw meat. The total phenolic in thawed meat samples was significantly affected by main factors antioxidant, meat layer, dipping time and interaction between them (Appendix Table B2). The phenolic content was significantly higher ($p < 0.001$) in meat samples exposed to the different antioxidant solutions compared to the non-treated samples with mean values of 98.22, 84.54, 76.48, 73.70, 62.09 and 54.33 mg GAE/ 100 g meat for ROS, GGR, BHT, SFS, SRB and non-treated samples, respectively (Appendix Table B2). Regardless of the antioxidants and dipping time, the rate of diffusion of phenolic compounds into the meat layers of thawed samples increased in order of: tenderloin side > membrane side > the core of fillets ($p < 0.001$) (Appendix Table B2). The uptake of phenolic content due to dipping chicken breast meat into various antioxidant solutions in thawed samples significantly increased ($p < 0.001$) at each interval of dipping time (Appendix Table B2). A significant AO x ML interaction ($p < 0.001$) was found for phenolic content (Figure 3.8). The phenolic content was higher in the membrane side of thawed samples dipped in all antioxidant solutions followed by tenderloin side and the core of fillets. In addition, meat layers were found to have a significantly higher uptake of phenolic content than those dipped into deionised water (control). Samples dipped in the ROS solution had the highest total phenolic uptake compared to any other treatments. (Figure 3.8). A significant AO x DT interaction was found for phenolic content ($p < 0.001$), due to the increase of phenolic content in all thawed meat samples dipped into various antioxidant solutions as the dipping time progressed. All dipped thawed meat samples had significantly the highest total phenolic penetration at 60 min. At 60 min, thawed meat samples had the

highest total phenolic for those dipped in GGR followed by BHT, ROS, SFS, SRB and non-treated samples, respectively (Figure 3.9). The results presented in Figure 3.10 also show a significant interaction ($p < 0.001$) between ML x DT for phenolic content due the increase of phenolic content of the outer layers (membrane and tenderloin sides) and the core of the fillets over time. The high absorption was found in the membrane side, followed tenderloin sides and the core of fillets over time. There was a significant ($p < 0.001$) interaction between AO x ML x DT (Table 3.2). The results show that, when meat samples exposed to deionised water (control), the phenolic content of the outer layers (membrane and tenderloin sides) decreased while little change in total phenolic content in the core of the fillets could be observed over time. Thawed meat samples were found to have a significantly higher rate of penetration of antioxidants compared to the samples dipped into deionised water (control). The rate of diffusion of phenolic compounds into the meat layers of thawed samples increased in order of: tenderloin side > membrane side > the core of fillets. Thawed breast fillets dipped in the ROS solution had the highest total phenolic uptake compared to any other treatments over 30 min of dipping time. In addition, all dipped thawed meat samples with the exception of SRB had the highest total phenolic penetration at 60 min. At 60 min, thawed meat samples had the highest total phenolic for those dipped in GGR followed by ROS, BHT, SFS, SRB and non-treated samples, respectively. In the core of thawed meat samples dipped into GGR solution, a significant accumulation of phenolic compounds was detected as compared to the non-treated samples. The penetration of phenolic compounds from natural antioxidants into the membrane and tenderloin sides was higher compared to the BHT (Table 3.2).

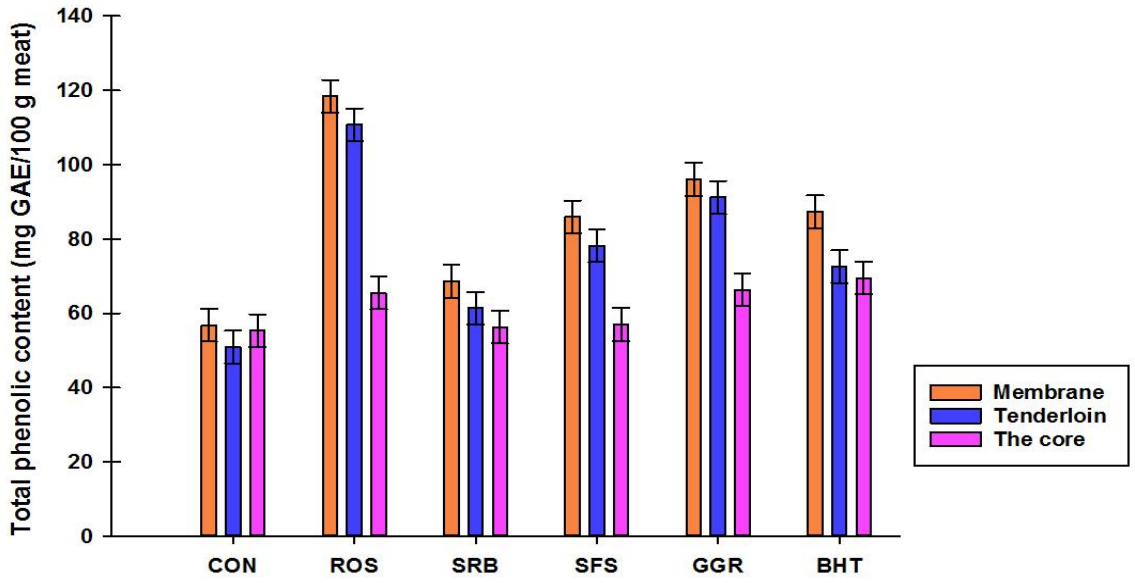

Figure 3.8 Effect of natural antioxidants and meat layer interaction on total phenolic content of thawed chicken fillets (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract

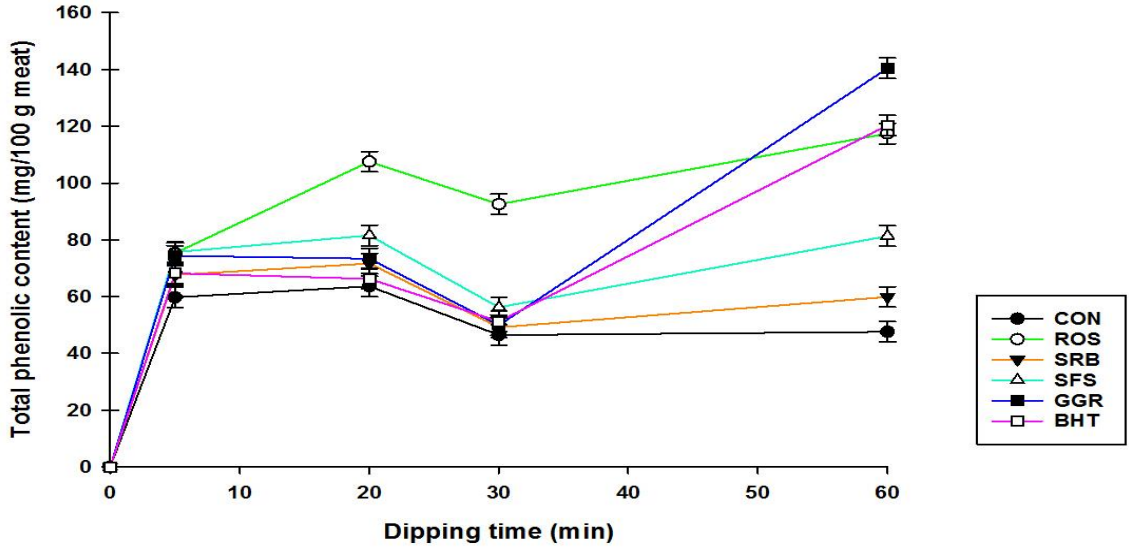

Figure 3.9 Effect of natural antioxidants and dipping time interaction on total phenolic content in thawed chicken fillets (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract

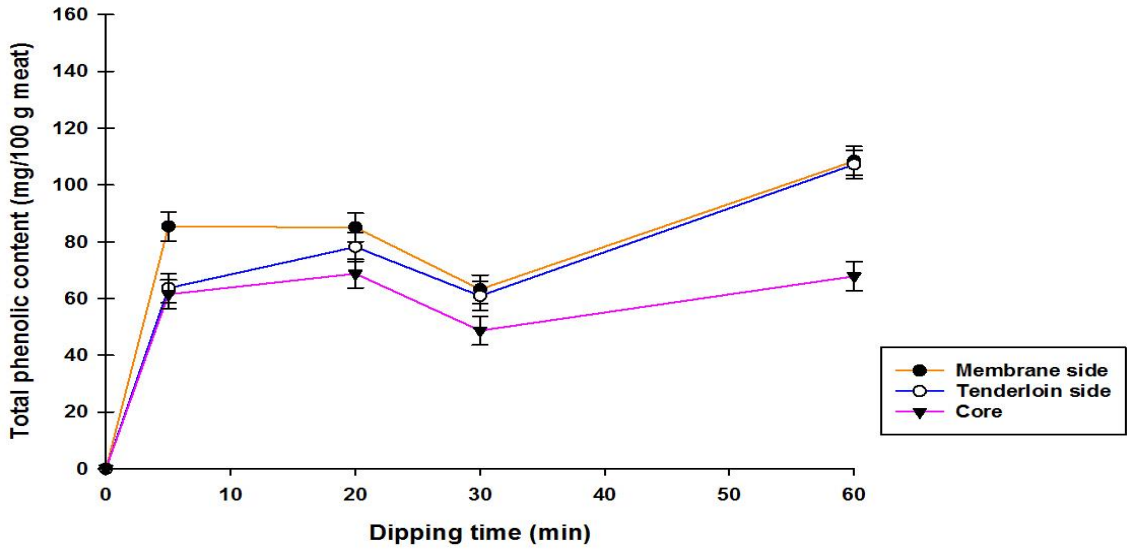

Figure 3.10 Effect of meat layer and dipping time interaction on total phenolic content in thawed chicken fillets (Means ± SED; n = 3).

Table 3.2 Effect of natural antioxidants and dipping time on total phenolic content (mg GAE/100 g meat) in different layers of thawed chicken meat.

Meat layer	Treatment	Dipping Time (minutes)			
		5	20	30	60
Membrane side	CON	62.35^{abc}	64.26^{abc}	50.56^{a}	49.97^{a}
	ROS	92.90^{bcd}	138.75^{efg}	106.30^{def}	135.78^{efg}
	SRB	86.81^{abcd}	73.43^{abcd}	51.46^{a}	62.91^{abc}
	SFS	89.77^{abcd}	88.59^{abcd}	62.56^{abc}	102.83^{cde}
	GGR	99.82^{cde}	76.00^{abcd}	54.24^{ab}	154.16^{g}
	BHT	80.78^{abcd}	69.44^{abcd}	53.96^{ab}	145.18^{fg}
Tenderloin side	CON	59.85^{abc}	57.28^{abc}	42.93^{a}	43.48^{a}
	ROS	75.81^{abcd}	114.67^{ef}	115.13^{ef}	137.45^{f}
	SRB	54.71^{abc}	71.62^{abcd}	50.05^{ab}	69.03^{abcd}
	SFS	70.46^{abcd}	84.69^{cde}	58.65^{abc}	98.98^{de}
	GGR	58.25^{abc}	75.20^{abcd}	51.56^{abc}	179.76^{g}
	BHT	62.75^{abc}	65.70^{abcd}	47.12^{a}	114.76^{ef}
The core of the fillet	CON	56.93^{abcd}	69.24^{abcde}	45.85^{ab}	49.30^{abc}
	ROS	57.30^{abcd}	69.17^{bcde}	56.26^{abcd}	79.13^{def}
	SRB	61.41^{abcd}	70.02^{bcde}	45.95^{ab}	47.63^{abc}
	SFS	66.83^{abcde}	71.39^{cde}	47.35^{abc}	42.30^{a}
	GGR	65.12^{abcde}	69.04^{bcde}	43.96^{a}	87.40^{ef}
	BHT	60.92^{abcd}	63.49^{abcde}	52.66^{abc}	101.02^{f}

Mean values with different small superscript letters presented within each row of each treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different small superscript letters presented within each column of each meat layer differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

3.3.2.2.2 The Rate of Phenolic Uptake of Thawed Meat

With regards to the rate of phenolic uptake in chicken breast meat samples that were previously frozen (thawed), the rate of ROS, SRB, SFS, and GGR was limited to the outer layer (membrane and tenderloin side) (Figures 3.11A and B), only with a minor accumulation of phenolic content in the core (Figure 4.4C). The results also showed that the rate of uptake was higher through the membrane side of the fillets compared to the tenderloin side (Figures 3.11A and B). Overall, the rate of phenolic uptake through the membrane side was the highest over 5-min period followed by the 20-min period for all products. Although the rate of phenolic uptake through the membrane side dipped into GGR solution was highest over 5 min, the highest rate of phenolic uptake was detected in samples dipped into ROS solution over time (Figure 3.11A). Similar to findings observed in membrane side, the highest rate of phenolic uptake was also noticed in the tenderloin side dipped into ROS (Figure 3.11 B).

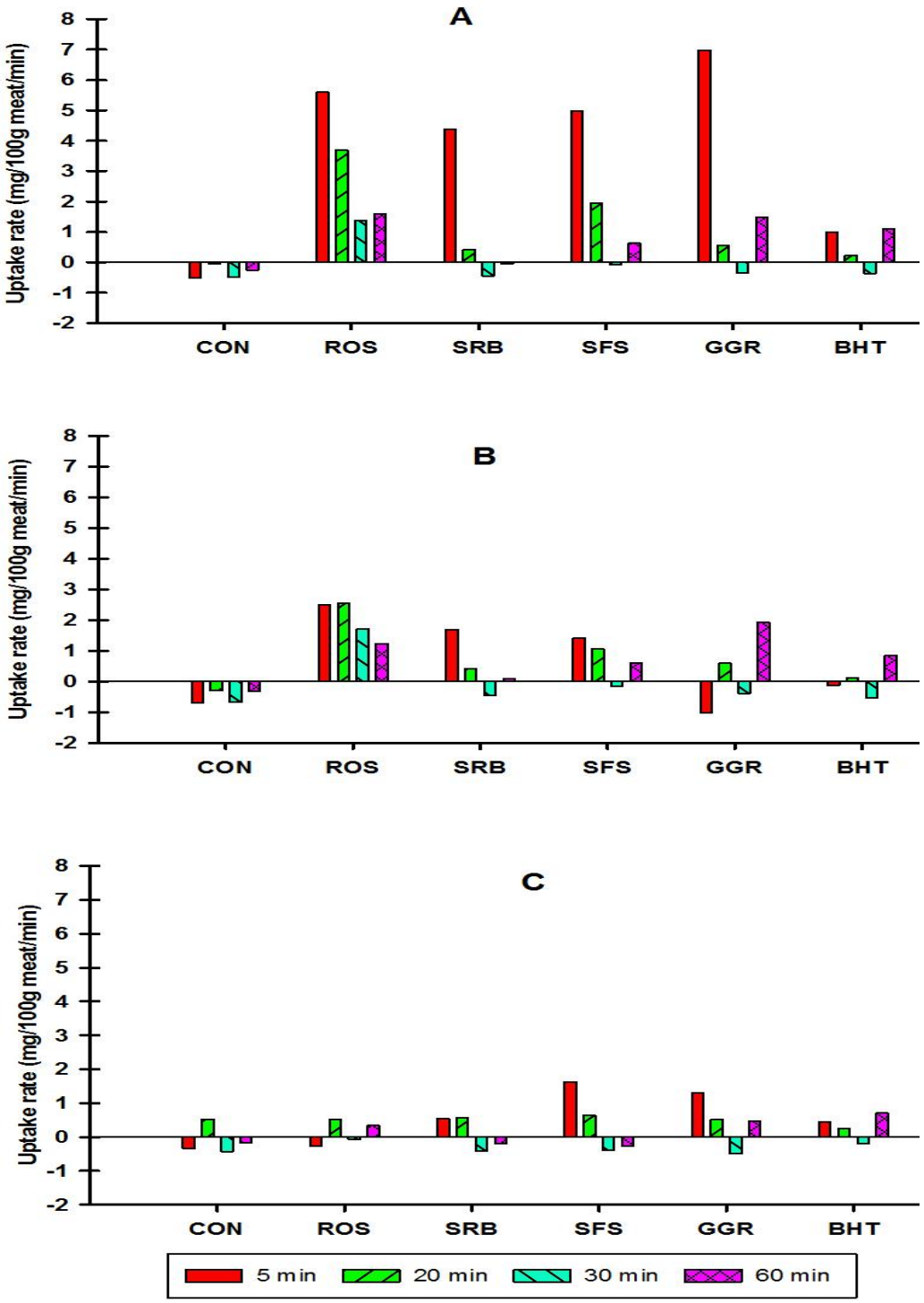

Figure 3. 11 Effect of dipping duration on the rates of phenolic uptake in thawed chicken fillets after dipping into antioxidant solutions. ***A****, membrane side;* ***B****, tenderloin side;* ***C****, core of the fillets. Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract*

3.4 Discussion

3.4.1 Moisture Uptake

Moisture content is one of the most important indicators to evaluate the meat quality. Hosseini and Mehr. (2014) found a high correlation between moisture content and solution uptake. Dipping chicken meat in the GGR solution leads to increased moisture content which that enhanced hydrophilic properties (Naveena and Mendirratta, 2001). Similar findings were observed in the current study when the dipping process increased moisture uptake of chicken breast fillets (Figures 3.2, 3.3 and Table B1). The percentage of moisture uptake in chicken meat was similar to those reported by Yusop et al. (2010), who found that the moisture uptake by chicken breast fillets dipped for 30, 60, 120 and 180 min ranged from 2.63-2.92 %. In this study, the moisture uptake was dependent on the dipping time (Figures 3.2 and 3.3) in accordance with results previously published by Yusop et al. (2010). These results suggest that the uptake of antioxidant solution into the chicken meat is a dynamic process during which compound gradually diffuse into the meat tissue. The highest level of absorption of moisture was observed in raw meat samples dipped in the SRB followed by ROS solution over dipping time (Figure 3.2). This is probably associated with their phenolic compounds. Both had hydrophilic compounds that can easily penetrate into muscle tissue. Moisture uptake in raw breast meat samples treated with SFS and GGR from 30 min onwards was markedly less than the non-treated samples (Figure 3.2). This could be due to the phenolic compounds that had higher levels of lipophilic compounds hinder to penetrate through the muscle. Furthermore, the greatest moisture uptake observed in raw meat could be attributed to the ability of meat structure to absorb and retain water during and after dipping process.

Furthermore, the reduction of moisture uptake in thawed samples could be due to inducing severe distortions and excessive damage to chicken muscle fibre structure during the freezing periods which then limits the ability of meat to take up and ultimately retain moisture. Meat stored under freezing temperature develops structural damage through the formation of ice crystal (Aidani et al., 2014). It was suggested that damage that takes place in cell walls due to the freezing process could reduce the ability of meat to reabsorb water and increase exude of water from meat as drip loss (James and James, 2010)).

3.4.2 Total Phenolic Content

The penetration of antioxidant compounds into the sequential layers of chicken breast fillets was monitored in order to determine the amount of total phenolic content. Several studies have used phosphate, sodium chloride and fluorescent dyes to monitor the diffusion of marinade into different layers of meat (Xiong and Kupski, 1999a, Xiong and Kupski, 1999 b; Alarcon-Rojo, 2010). However, the principal aim of this study was to establish the appropriate time of exposure to an antioxidant containing solution for subsequent experiments. In the current study, the penetration level of total phenolic into chicken breast fillets mostly depended on antioxidant type and dipping time (Tables 3.2, 3.3 and Table B2). Since the penetration rate of total phenolic into the core of the fillet was lower than what was found in the outer layers (Figures 3.4, 3.8 and Table B2); it was determined that the uptake of various natural antioxidants is not instantaneous. It is evident that the antioxidant solution that overcomes the physical barriers or restrictions in the chicken muscles was limited to approximately 4 to 5 mm on each side during dipping time. Moreover, phenolic content was markedly penetrated into the outer layers

(membrane and tenderloin sides) compared to the core of the fillets over time (Figures 3.6 and 3.10). Similar findings were reported by Xiong and Kupski (1999a) who found that the diffusion rate of phosphate into the chicken meat was depth dependent, where the rate of phosphate uptake was higher at the outer layer, followed at progressively lower rates to the middle and inner layers when chicken breast fillets were dipped with different concentrations of phosphates. Interestingly, samples treated with natural antioxidants were found to have a higher total phenolic content in comparison with non-treated samples (Tables 3.2, 3.3 and Table B2). The penetration of antioxidant solution and total phenolic into meat was apparently produced by an increased capillary force that can deliver water into the core of muscle and expand muscle fibres (Offer and Trinick, 1983). The ability of meat to absorb any solution was found to be correlated with dipping time (Lemos et al., 1990). Furthermore, penetration of a particular solution into chicken meat is dependent on the meat structure, with chicken breast having a greater ability to absorb a solution than the thigh or drumstick (Post and Heath, 1982), which is probably due to the higher amount of connective tissue in thigh and drumstick meat (Min and Ahn, 2012). Xiong and Kupski (1999a) found increasing signs of swollen fibres in the chicken muscle tissue based on results obtained by microscopic analysis after meat irrigated with different phosphate marinades caused a quick extension of the myofibril matrix of the muscle and disintegration of the actomyosin complex. Naveena and Mendiratta (2004a) pointed out that samples dipped into ginger extract at 5 %, for 48 h had a lower muscle fibre diameter compared to the non-treated samples with mean values 60.76 and 57.66 microns, for non-treated samples and ginger treatment, respectively. Ginger extract was markedly caused an extensive degradation of fibres and layers of connective tissue around muscle fibres (Naveena and Mendiratta, 2004b). Naveena and Mendiratta, (2001; 2004b) reported that

the ginger extract added to buffalo meat enhanced cooking meat yield, sensory acceptability, flavour, juiciness, tenderness, and decreased significantly meat shear force values, which could be due to decrease the degradation of protein and bind more water. Furthermore, in our study, rapid penetration of total phenolic in raw meat and thawed meat was at 20 min and 60 min, respectively (Tables 3.2, 3.3 and Table B2). These results were in contrast to those reported by Xiong and Kupski (1999b) who found that a rapid penetration of phosphate was detected at 5 min of dipping time. Moreover, according to the results, fillets of thawed and raw meat dipped in ROS solution was found to have a higher total phenolic content over dipping duration (Figures 3.5 and 3.9). However, the mechanisms behind this are not well documented but could be due to the molecular weight of phenolic compounds existing in ROS, which could have a high diffusivity. This result suggests that the ROS solution penetrated into the chicken fillets more rapidly while the diffusion in the deep layer of fillets was much slower. The dynamic process that contributed in term of penetration of the antioxidant solution into the core of the chicken breast fillet during immersion could be due to capillary forces. The higher phenolic uptake in the thawed meat samples compared to the raw samples (Tables 3.2, 3.3 and Table B2), could be associated with high diffusion of phenolic content into the thawed meat and ability of meat to retain a high amount of antioxidant compounds.

3.4.3 The Rate of Phenolic Uptake

The uptake rate of phenolic content in samples dipped into ROS, SRB, SFS, GGR and BHT solution was limited to the outer layers (membrane and tenderloin side) in both raw and thawed meat samples (there was only a minor accumulation of phenolic content into the core) (Figures 3.7 and 3.11). These results clearly show that the diffusion of

antioxidant solutions into the chicken fillets was limited. These findings are in agreement with those reported b Xiong and Kupski (1999a) that a higher diffusion rate of phosphate into the chicken meat was found in the outer layers than that in the core of chicken fillets. In raw meat, the rate of phenolic uptake by samples dipped into antioxidant solutions in raw meat was highest at 20 min. In contrast, the highest rate uptake of phenolic content in thawed meat was shown in the first 5 min. Although the rate of phenolic uptake through the membrane side dipped into GGR solution was highest over 5 min in thawed meat, the highest rate of phenolic uptake was detected in both raw and thawed meat samples dipped into ROS solution over time (Figure 3.7 and 3.11). As mentioned before, this could be due to the molecular weight of phenolic compounds existing in ROS which could have a high diffusivity.

3.5 Conclusions

All antioxidants were capable of improving moisture uptake as raw and thawed chicken fillets dipped in natural antioxidant solution absorbed moisture with increasing dipping time. Breast fillets dipped in SRB and ROS solution were observed to have a higher moisture uptake than the other treatments over time. The results also suggested that dipping raw and thawed meat into various antioxidant solutions had a positive effect on the penetration of phenolic content. The penetration of phenolic was highest in both raw, and thawed chicken breast fillets dipped in ROS solution. Rapid absorption of phenolic content in raw meat was in the first 20 min and the thawed meat was at 60 min. Penetration of phenolic content in raw and thawed meat was greater in the outer layers (membrane and tenderloin side) followed by the core of the fillets.

Chapter 4:

The Impact of Post-Slaughter Natural Antioxidant Application on the Physical and Chemical Characteristics of Broiler Chicken Meat

4.1 Introduction

Lipid autoxidation is of primary concern in many lipid-rich foods and negatively affect the sensorial characteristics of meat (Frankel, 1980; Byrne et al., 2001; Min and Boff, 2002), and is also known to cause reductions in nutritional value (Min, Nam et al., 2008). Many of these negative effects are due to the formation of hydroperoxides, malondialdehyde, 4-hydroxynonenal, and volatile compounds (Erickson, 2002; Domínguez et al., 2014). The processing of meat, such as cooking, can accelerate the rate of lipid oxidation in beef, pork and chicken (Min et al., 2008); grinding and deboning of meat also promotes lipid oxidation (Laack, 1994). This is because cooking, grinding and deboning processes disrupt the cell membranes and facilitate the lipid compounds to come into direct contact with oxygen and compounds that have the ability to catalyse meat lipid oxidation (Bragagnolo, 2009).

While minimising the processing of meat just prior to consumption limits the events that lead to oxidation of lipids; in a fast-moving society with ready-meals and pre-prepared foods for convenience, processing meats well-ahead of consumption means that controlling the possible onset of autoxidation is of utmost importance. Lipid and phospholipid oxidation can be effectively controlled using packaging materials and/or antioxidants. The use of antioxidants to prevent and delay autoxidation of lipids in meat

and meat-derived products during the storage time has been the focus of a number of studies (McCarthy et al., 2001; Ahn et al., 2002; Mielnik et al., 2003; Nissen et al., 2004).

The antioxidant capacity of naturally sourced extracts correlates strongly with their total phenolic content (Shan et al., 2005; Rusaczonek et al., 2007; Velasco and Williams 2011). The active compounds that act as antioxidants in natural sources are phenolic in nature such as phenolic acids, phenolic diterpenes, flavonoids and volatile oils (Velasco & Williams 2011). Several studies have pointed out that rosemary, red bean, ginger and sunflower are a rich sources of antioxidant compounds, while ROS was found to have the highest antioxidant compounds (Shan et al., 2005; Amakura et al., 2013; Baker et al., 2013). Although information about the effect of extraction of natural antioxidants from plant origins such ROS and GGR on the oxidation of lipids of meat is well documented, to our knowledge there were no research related to investigate the impact of SRB and SFS as natural antioxidants on quality of raw and freshly cooked chicken meat. Therefore, the current study was carried out to evaluate the effects of extract of ROS, SRB, SFS, and GGR as natural antioxidants compared to the synthetic antioxidant BHT on the physical and chemical characteristics of chicken meat.

4.2 Materials and Methods

4.2.1 Raw Materials

Chicken carcasses, sources of natural antioxidants and chemicals used was described in Chapter 2 section 2.2.1.

4.2.2 Experimental Design and Sample Preparation

This experiment was designed as factorial design consisted of a 6 x 3 with five antioxidant treatments plus control (ROS, GGR, SFS, SRB, BHT, and non-treated control), and three storage times (0, 3 and 7 days). For the preparation of meat samples, raw meat samples were prepared as per the same procedure discussed in section 3.2.2. Six experimental treatments were prepared as mentioned in a previous Chapter 3 (section 3.2.2) by dipping raw breast fillets into an appropriate antioxidant solution for 20 min as a rapid absorption of phenolic content in raw meat was found in the first 20 min as discussed in Chapter 3. All samples were then packaged in polyethylene bags (transmission rate of oxygen = 51000 cm^3/m^2. 24h. bar). Each treatment was conducted in triplicate. All samples were stored at 4 ^oC for 7 days. Following refrigerated storage at 0, 3, and 7 days, the samples were taken to be analysed as a raw meat and at the same time, samples were taken at each point of storage time and cooked by sous vide method.

4.2.2.1 Sous-Vide - "Low-Temperature & Low-Oxygen" (LTLO)

Chicken breast fillets samples were taken during storage time (section 4.2.2) and packaged in a plastic vacuum bag (65 Micron Vacuum Pouches 160 x 300) and the air was evacuated (Tre Spade V33 Auto Vacuum Sealer, Torino, Italy). The samples were then cooked in a water bath (Grant water bath, Shepreth, England) at 75 ^oC for 45 min.

After the internal temperature of the meat reached 71 ^{o}C, a digital calibrated thermometer (Therma 20 thermometer, UK) was used to monitor the internal temperature. The packages were removed from the water bath, and cooled with tap water to an ambient temperature of 23 ^{o}C. Raw and cooked meat samples were analysed following 0, 3 and 7 days of storage for chemical and physical parameters.

4.2.3 Chemical Parameters Analysis

4.2.3.1 Thiobarbituric Acid Reactive Substances (TBARS) Determination

TBARS value was determined in meat samples according to the method described by Buege and Aust (1978). Approximately 0.5 g of finely chopped meat was weighed and put in a 10 ml test tube to which 2.5 ml of TBA stock solution was added. A litre of TBA stock solution contained 3.75 g thiobarbituric acid (TBA), 150 g trichloroacetic acid (TCA), and HCl at a final concentration of 0.25 M. Samples were then vortexed for 15 sec before being incubated in a water bath at 95 ^{o}C for 15 min until the development of a pink colour. The tubes were rapidly cooled down in a bath of tap water and centrifuged (Rotina 46R, Hettich Zentrifugen, Germany) at 2500 g for 10 min at 4 ^{o}C. The supernatant was transferred to a cuvette and the absorbance determined by spectrophotometer (Beckman, DU640 spectrophotometer, Fullerton, CA) at 532 nm against a blank containing 0.5 ml of deionised water and 2.5ml TBA stock solution.

A standard curve was prepared by dissolving 31 mg of 1,1,3,3–tetra-ethoxypropane (TEP) in 1000 ml of deionised water to produce (0.031 mg TEP / ml) working solution. Then serial dilutions were prepared by pipetting aliquots of 0, 1, 2, 3, 4, 5 and 6 ml of working TEP standard solution into 50 ml test tubes and filled up to mark by adding deionized water (DW) to produce (0, 0.00062, 0.00124, 0.00186, 0.00248, 0.0031 and 0.00372 mg

TEP equivalents / ml extraction). All samples were then vortexed for 15 sec. Half ml was pipetted from each dilution and placed in 10 ml test tube. The same procedure was applied as used for sample analysis, except the TEP work solution was used instead of a sample. For constructing standard curve, the concentrations of (TEP) were plotted against the absorbance of TEP (Figure 2.5). From the standard curve, the amount of TBARS in meat samples was determined and expressed as mg of malondialdehyde equivalents / kg raw meat by utilising the following equations:

$$x = (y - 0.0013)/126.22$$

$$\text{mg TBARS (MDA)/kg raw meat} = \left[\frac{x\ (mg)}{\text{weight of meat sample (g)}}\right] \text{x}1000$$

Where x is the unknown amount of MDA in meat samples (mg/g), and is taken from standard calibration curve (Figure 4.1), y is the absorbance of meat sample, 1000, is the dilution factor used to obtain the results of TBARS in mg MDA/kg meat.

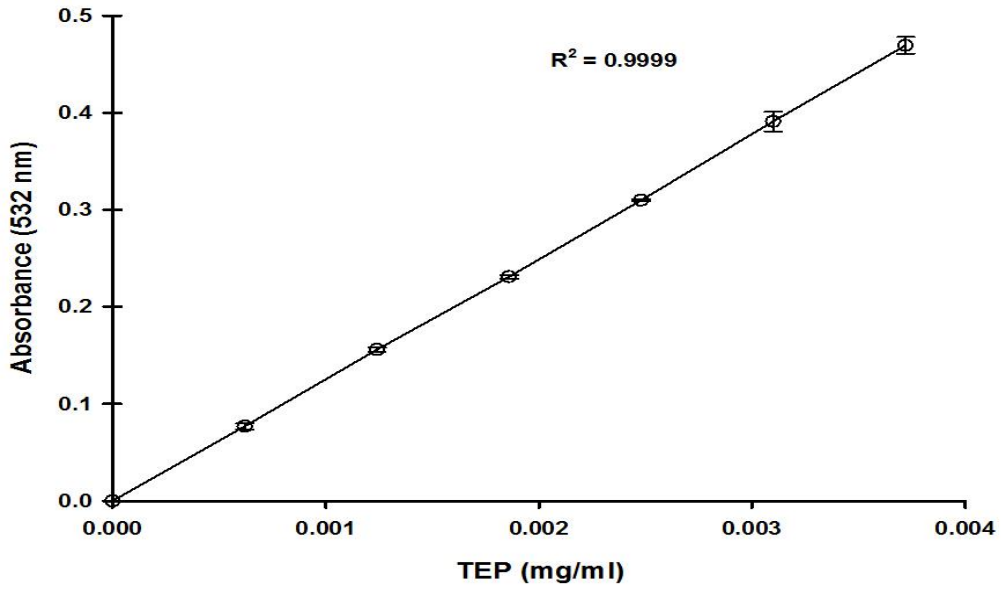

Figure 4. 1 Standard curve of 1,1,3,3–tetra-ethoxypropane (TEP) for determination of TBARS (mg MDA) (Means ± SED; n = 3).

4.2.3.2 Conjugated Dienes and Conjugated Trienes Determination

The conjugated dienes (CD) and conjugated trienes hydroperoxides (CT) formation in the lipids were determined according to the procedure described in section 2.2.3.4.2.

4.2.3.3 Phospholipid Content Determination

The phospholipid content was determined according to the procedure described in section 2.2.3.4.3.

4.2.3.4 Fatty Acids Determination

Fatty acids were determined according to the method described in section 2.2.3.4.4.

4.2.4 Physical Parameters Analysis

4.2.4.1 Colour Measurement

Meat colour stability of each treatment was evaluated by measuring meat parameters such as L* (lightness), a* (redness) and b* (yellowness) using a CR-400 Chroma meter measuring head with data processor DP-400 (Konica Minolta sensing, Inc., Japan). Measurements were taken at four points on the surface area of chicken breast fillets, after placing in a white tray, using D65 illuminator at 2° standard observer angle after calibration with a calibration plate.

4.2.4.2 pH Value

Approximately 5 g of the ground raw and cooked meat were weighed and mixed with 20 ml of deionized water and homogenised (Silverson Machines Ltd., Chesham, UK) for 30 sec at high speed and pH values of samples were assessed with pH meter (Jenway, Stone, Staffordshire) after calibration with buffer solutions at pH 4.0 and 7.0. Each sample was evaluated in triplicate throughout the storage time.

4.2.4.3 Drip Loss

Drip loss of breast fillets was determined according to the method described by Honikel (1998). Approximately 80 g weighed of raw chicken breast was weighed and placed into a net bag and then suspended in an airtight plastic container (520 ml Round Tamper Evident Container 9 3 mm x 118 mm, UK), and stored at refrigeration temperature (4 ^{o}C) for 24 h. After that, meat samples were removed from the container and dried using paper towel. The same samples were used for next time during the storage time. Subsequently, the samples were reweighed and drip loss was calculated using the following formula:

$$\text{Drip loss (\%)} = \left[\frac{\text{Initial weight of raw meat (g)} - \text{ final weight of meat (g)}}{\text{Initial weight of raw meat (g)}}\right] \text{x } 100$$

4.2.4.4 Cooking Loss

Approximately 100 g of raw meat was weighed before a thermal processing and cooked until the internal temperature reached 71 ^{o}C. After the cooked meat, had been cooled to ambient temperature (23 ^{o}C), meat samples were dried with paper towel and reweighed. Subsequently, cooking loss was measured by the following formula:

$$\text{Cooking loss (\%)} = \left[\frac{\text{Initial weight of raw meat} - \text{ weight of cooked meat}}{\text{Initial weight of raw meat (g)}}\right] \text{x} 100$$

4.2.4.5 Textural Analysis

The texture of the cooked meat was evaluated by using a texture analyser (TA. XT plus,TA.HD.Plus. Stable Micro Systems, UK) equipped with 30 kg load cell. The crosshead speed was set at 10 mm and test speed 2 mm/s. After refrigeration of meat at 4 ^{o}C, cooked meat samples were cut into uniform pieces with dimensions 1 cm^2 by using twin blade sample preparation cutting tool (Stable Micro Systems sample preparation

tool, UK). The meat samples were then sheared perpendicular to the fibres. For cutting the sample a Warner Bratzler blade set with 'Rectangular slot blade' (HDP/WBR) was used. The shear force of meat was evaluated by measuring the peak force (N) (Honikel, 1998).

4.2.5 Statistical Analysis

The data of raw and sous vide cooked chicken meat were analysed separately. For each raw and cooked meat, full factorial design of a 6 x 3 was utilised where the two factors were the six antioxidant treatments (ROS, GGR, SFS, SRB, BHT, and control), and three storage times (0, 3 and 7 days). The interaction between antioxidant x storage time also was assessed. All parameters were analysed using two-way analysis of variance (ANOVA). The experiment was conducted in triplicate (n = 3). When the main factors and interactions was significant, Tukey's HSD test was used to identify the significant differences between means and the significance level of all data was set at $p \leq 0.05$. The null (H_0) and alternative (H_1) hypothesis for each dependent variable were set as:

Null hypothesis (H_0):

There was no significant effect of natural antioxidants application, storage time and interaction between them on physical and chemical properties of raw and freshly cooked chicken breast fillets (H_0: $\mu = 0$; $p > 0.05$)

Alternative hypothesis (H_1)

There was a significant effect of natural antioxidants application, storage time and interaction between them on physical and chemical properties of raw and freshly cooked chicken breast fillets (H_1: $\mu \neq 0$; $p \leq 0.05$).

4.2 Results

4.2.1 Effect of Natural Antioxidants on Thiobarbituric Acid-Reactive Substances (TBARS)

Lipid oxidation (TBARS) values in chicken breast meat were affected significantly by the cooking process for 7 days ($p \leq 0.05$) (Appendix Table C2). Hence, TBARS values were found to be significantly higher ($p \leq 0.05$) in sous vide cooked samples than in raw meat. The application of the natural antioxidants provided ($p \leq 0.05$) protection against oxidative rancidity in both raw and cooked meats compared to the non-treated control (CON) (Tables 4.1 and Appendix Table C2). In raw meat samples, all natural antioxidants provided a decrease in TBARS values, while among natural antioxidants SFS was found to have significantly lower ($p = 0.012$) TBARS compared to the non-treated samples over 7 days (Table 4.1). The impact of natural antioxidants was similar to that of the synthetic antioxidant BHT. After samples were cooked by sous vide, natural antioxidants caused a similar decrease in TBARS values as was observed in raw meat (Table 4.1 and Appendix Table C2). A significant higher ($p < 0.001$) protective effect was observed in samples treated with ROS followed by GGR, BHT, SRB and SFS with a resulting decrease in TBARS compared to the non-treated samples. The reduction of TBARS values in all cooked meat samples treated with antioxidants with exception SFS was above 45% (Table 4.1 and Appendix Table C2). In addition, storage time was found to have a significant effect on TBARS values in raw meat samples and cooked meat ($p \leq 0.05$). In all instances the peak in TBARS values in raw and cooked meat samples occurred at day 3, with markedly lower TBARS values at day 7 compared to day 3 (Table 4.1 and Appendix Table C2). No significant AO x ST interaction ($p = 0.468$) was found for TBARS value

in raw meat samples (Table 4.1). In contrast, there was a significant AO x ST interaction ($p < 0.001$) found for TBARS in cooked meat samples (Table 4.1 and Figure 4.2), due to the increase of TBARS values in non-treated and treated samples with storage time. However, all meat samples supplemented with antioxidants were found to have lower lipid oxidation compared to the non-treated samples throughout the storage time. At day 0, the application of antioxidants provided a greater protection against oxidation compared to the non-treated samples. However, with the exception of ROS, SFS and GGR, the natural antioxidants from SRB provided very little protection against oxidation at day 3. A higher protective effect was observed only in samples treated with GGR and ROS with a resulting decrease in TBARS values over storage time. The natural antioxidants that provided a greater protection against an increase of TBARS values compared to the BHT, was ROS at day 0 and 3 of storage, while GGR performed more in reducing lipid oxidation than to the BHT over 7 days.

Table 4.1 Effect of natural antioxidants application on lipid oxidation TBARS values (mg MDA/kg meat) of raw and sous-vide processed chicken breast meat following storage at 4°C.

		Antioxidants (AO)							p value		
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Raw meat	0	0.35	0.35	0.31	0.33	0.33	0.35				
	3	0.62	0.44	0.47	0.38	0.46	0.49	0.07	0.012	<0.001	0.468
	7	0.53	0.38	0.35	0.33	0.43	0.33				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Sous vide	0	3.16	0.99	1.92	1.69	1.12	1.16				
	3	7.14	2.61	3.73	6.73	3.66	3.53	0.38	<0.001	<0.001	<0.001
	7	5.68	2.52	3.01	4.17	1.65	2.25				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

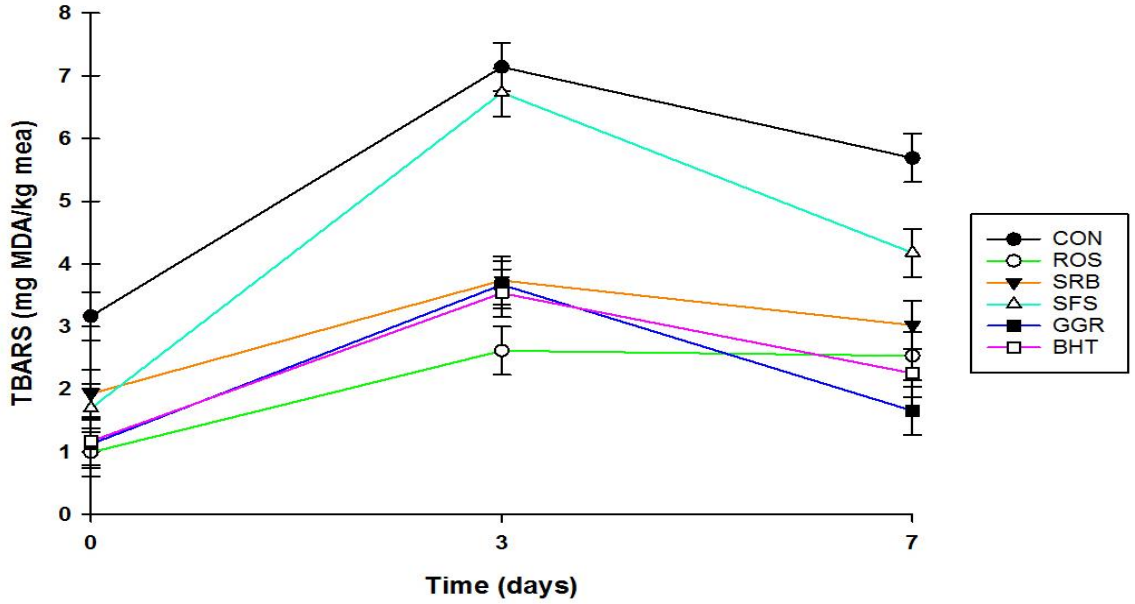

Figure 4.2 Effect of natural antioxidants application and storage time interaction on TBARS values of sous-vide processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

4.2.2 Effect of Natural Antioxidants on Conjugated dienes (CDs)

Conjugated dienes (CDs) values were significantly increased in cooked meat samples as compared to that in raw meat (Appendix Table C2). In raw meat, the application of natural antioxidants was found to have a significant effect on CD values in raw but did not have any effect on cooked meat (Table 4.2 and Appendix Table C2). In raw meat, all antioxidants were awarded a greater protection against an increase in CDs as compared to the non-treated samples ($p < 0.001$), but no significant differences were found between antioxidant treatments (Table 4.2 and Appendix Table C2). Regardless of the antioxidants, storage time was found to have a significant ($p < 0.001$) effect on CD content in raw and cooked meat. Hence, the CD values were increased over 7 days of storage.

However, a significant increase of CDs content was found in raw samples at 7 days compared to day 0 of storage (Table 4.2 and Appendix Table C2). There was a significant AO x ST interaction (p = 0.034) for CDs in raw meat samples (Table 4.2 and Figure 4.3). The formation of CD in all raw meat samples increased with increasing storage time. However, all antioxidants provided a significant decrease in CDs compared to the non-treated samples over 7 days of storage time. The only natural antioxidants that markedly reduced the formation of CDs at day 7 compared to any other antioxidant treatments was SFS. The effect of natural antioxidants compared to the BHT was similar over storage time (Figure 4.3). In cooked meat, no significant interaction (p = 0.407) was shown between antioxidant and storage time for CDs (Table 4.2).

Table 4.2 Effect of natural antioxidants on Conjugated Dienes (µmol/g fat) of raw and sous-vide processed chicken breast meat following storage at 4°C.

		Antioxidants (AO)							p value		
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Raw meat	0	35.59	32.44	25.88	26.03	24.93	23.70				
	3	47.43	31.93	29.50	31.25	27.86	30.31	3.01	<0.001	<0.001	0.034
	7	49.10	46.36	44.36	39.62	44.60	45.01				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Sous vide	0	46.11	44.68	45.57	44.63	43.51	42.48				
	3	54.68	48.14	48.69	52.69	57.50	52.07	3.24	0.104	<0.001	0.407
	7	56.12	53.80	48.56	54.98	55.24	53.73				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

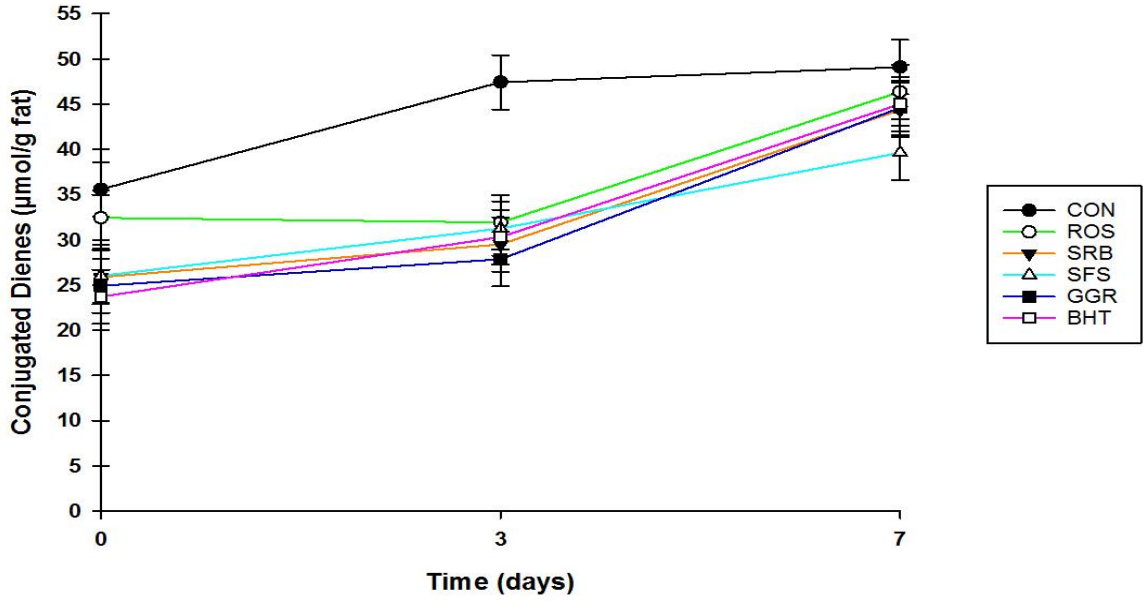

Figure 4.3 Effect of interaction of natural antioxidants application and storage time on Conjugated Dienes of raw chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

4.2.3 Effect of Natural Antioxidants on Conjugated Trienes (CTs)

Conjugated trienes (CTs) was higher in cooked chicken breast meat than in raw meat ($p \leq 0.05$). Hence, a significant formation of CTs was found in cooked meat compared to the raw meat (Appendix Table C2). The use of natural antioxidants significantly reduced the formation of CT in raw, but did not have any effect on CT of cooked meat (Table 4.3 and Appendix Table C2). In raw meat, all natural antioxidants provided a greater protection against an increase in CT values compared to the non-treated samples, while no significant differences were found between antioxidant treatments. The effect of natural antioxidants as compared to the BHT was similar (Table 4.3 and Appendix Table C2). Storage time was found to have a significant effect on CTs content in raw and cooked meat ($p < 0.001$). The CT content in raw and cooked samples of breast meat significantly increased over 7 days of storage. However, values of CT were higher in cooked meat than

those in raw meat over 7 days (Appendix Table C2). Table 4.3 shows that there was no significant AO x ST interaction for CTs in raw and cooked meat (p = 0.555; p = 0.393).

Table 4.3. Effect of natural antioxidants application on Conjugated Trienes (µmol/g fat) of raw and sous-vide processed chicken breast meat following storage at 4 °C.

		Antioxidants								p value	
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Raw meat	0	16.13	15.64	12.48	12.16	13.65	11.76	2.13	0.003	<0.001	0.555
	3	21.84	16.26	14.83	15.36	14.02	15.4				
	7	25.06	23.2	23.07	20.35	24.2	22.74				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Sous vide	0	24.97	20.92	22.23	20.93	21.53	20.96	1.71	0.056	<0.001	0.393
	3	26.05	23.53	24.73	24.73	28.51	27.14				
	7	29.84	28.15	27.35	28.93	29.43	29.61				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

4.2.4 Effect of Natural Antioxidants on Phospholipid Content

Phospholipid content in chicken breast meat samples was significantly affected after the cooking process ($p \leq 0.05$). Hence, following the sous vide treatment of chicken meat the phospholipid degradation was significantly higher compared to the raw meat samples with mean values of 41.24 and 40.13 g/100 g fat for raw and cooked meat, respectively (Appendix Table C3). The effect of antioxidants on phospholipid content in raw meat was not significant, regardless of the storage time (p = 0.348) (Tables 4.4 and Appendix Table C3). In contrast, a significant effect of antioxidant supplementation on the phospholipid content in cooked meat was found ($p < 0.001$). Hence, natural antioxidants provided a greater protection against a decrease in phospholipid content. The highest phospholipid

content was found in ROS treatment followed by SRB, SFS, GGR and BHT respectively. Furthermore, the application of natural antioxidants awarded a better protection against a decrease of phospholipids compared to the BHT (Table 4.4 and Appendix Table C3). Moreover, a significant effect of storage time was seen in phospholipid content in both raw and cooked meat ($p \leq 0.05$). The resulting phospholipid content decreased over 7 days of storage and a significant reduction of phospholipid content was found at the end of the storage time compared to day 0 (Table 4.4 and Appendix Table C3). A significant AO x ST interaction ($p = 0.004$) was found for phospholipid content in raw meat (Table 4.4 and Figure 4.4), due to the decrease phospholipid content with storage time. Over storage time, all antioxidants yielded more phospholipid content at day 7 of storage compared to the non-treated samples. Among antioxidant treatments, the antioxidant that had markedly reduced the degradation of phospholipids only at day 7 was SFS. Figure 4.5 shows that a significant AO x ST interaction ($p < 0.001$) for phospholipid content in cooked meat indicating that the effect of antioxidants on phospholipid content was depending on the storage time. All natural antioxidants provided a greater protection against a decrease in phospholipid content over storage time as the higher reduction of phospholipid content was found in non-treated samples. The highest phospholipid content was found in ROS treatment followed by SFS, SRB, and GGR respectively. Furthermore, the application of natural antioxidants awarded a better protection against a decrease of phospholipids compared to the BHT over time.

Table 4.4 Effect of natural antioxidants application on phospholipid content (g/100 g fat) of raw and sous-vide processed chicken breast meat following storage time (ST) at 4 °C.

		Antioxidants (AO)								p value	
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Raw meat	0	45.16	43.64	42.49	41.37	43.36	43.80	1.03	0.348	<0.001	0.004
	3	41.85	42.15	41.07	41.48	41.27	41.59				
	7	37.53	38.41	37.85	41.65	38.10	39.50				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Sous vide	0	40.39	42.10	41.41	40.43	41.64	40.84	0.58	<0.001	<0.001	<0.001
	3	38.93	42.07	42.50	41.06	39.48	39.32				
	7	35.36	40.71	38.88	40.03	38.55	38.61				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

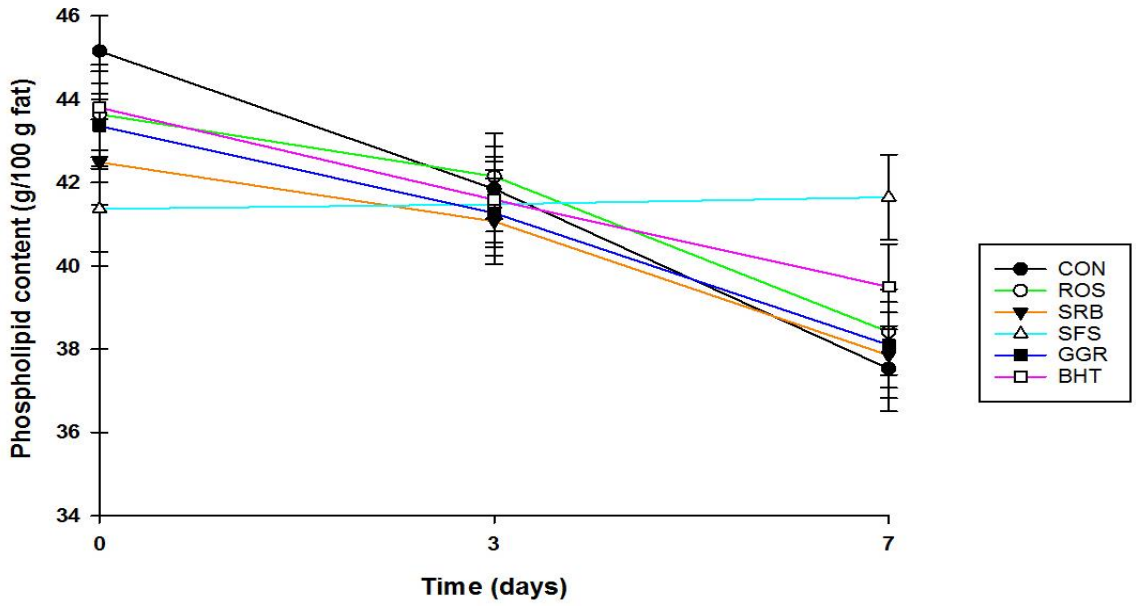

Figure 4.4 Effect of interaction of natural antioxidants application and storage time on phospholipid content of raw chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

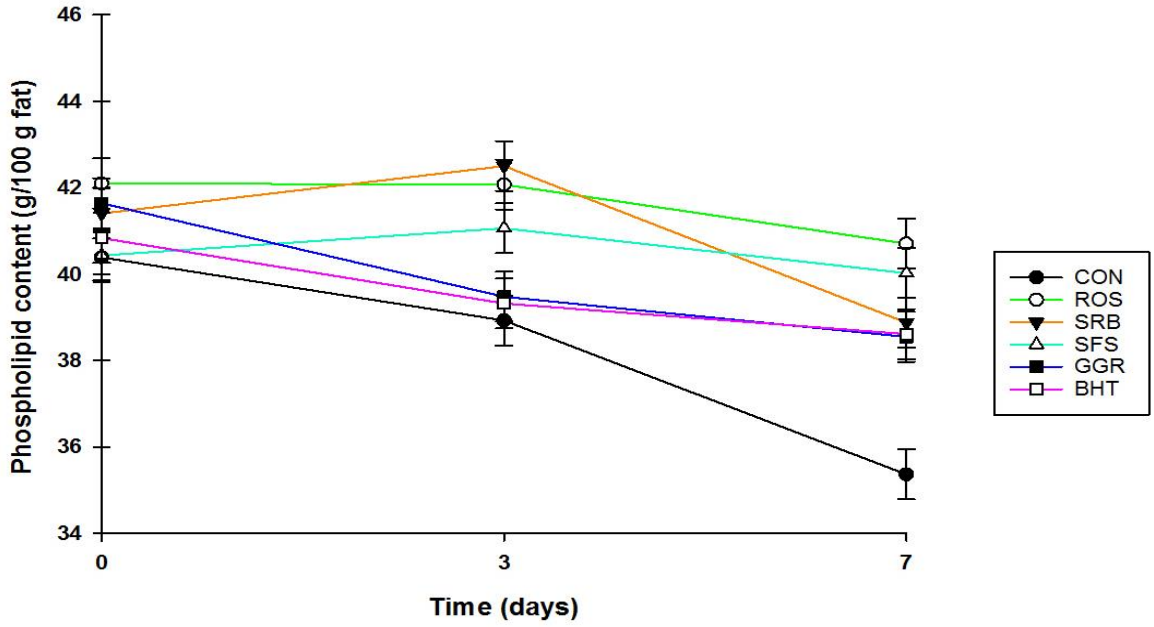

Figure 4.5 Effect of interaction of natural antioxidants application and storage time on phospholipid content of sous-vide processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

4.2.5 Effect of Natural Antioxidants on pH Values

The pH values in all treatments significantly increased after the cooking process (Appendix Table C3). Antioxidant supplementation had a significant an effect on the pH values in raw meat ($p = 0.004$), but did not have any effect on the pH values in cooked meat ($p = 0.155$). In raw meat, the natural antioxidants that had significantly lower pH values were ROS and GGR than the non-treated samples (Appendix Table C3). Storage time also statistically ($p > 0.05$) did not affect the pH values in both raw and cooked chicken meat (Tables 4.5 and Appendix Table C3). Table 4.5 shows that there was no significant interaction between both antioxidant and storage time for pH values in raw and cooked meat ($p = 0.725$; $p = 0.920$).

Table 4.5 Effect of natural antioxidants application on pH values of chicken breast meat during the storage time (ST) at 4 °C.

		Antioxidants (AO)							p value		
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Raw meat	0	5.95	5.69	5.89	5.94	5.79	5.86				
	3	5.96	5.79	5.91	5.92	5.79	5.77	0.09	0.004	0.223	0.725
	7	6.00	5.91	5.92	5.90	5.82	5.89				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Sous vide	0	6.17	6.06	6.05	6.07	6.17	6.07				
	3	6.15	6.06	6.08	6.03	6.00	6.01	0.08	0.155	0.348	0.920
	7	6.11	6.07	6.04	6.00	6.11	6.01				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

4.2.6 Effect of Natural Antioxidants on Cooking Loss

The proportion of cooking loss in treated and non-treated samples was significantly ($p = 0.013$) affected by the addition of natural antioxidants (Tables 4.6 and Appendix Table C4). However, a significant protection against an increase of cooking loss was found in samples treated with BHT with mean value of 20.51% compared to the non-treated samples of 23.42 %. Regardless of antioxidant, storage time had a significant ($p = 0.015$) an effect on cooking loss (Table 4.6 and Appendix Table C4). Hence, the proportion of cooking loss was increased at day 3, but significantly decreased at the end of storage time compared to day 3. No significant AO x ST interaction ($p = 0.628$) was found for cooking loss (Table 4.6).

4.2.7 Effect of Natural Antioxidants on Texture (Shear Force)

The shear force values of cooked meat were not significantly ($p = 0.541$) affected by natural antioxidant supplementation (Tables 4.6 and Appendix Table C4). However, meat samples

treated with natural antioxidants had lower shear force values compared to the non-treated samples. In addition, storage time had a significant effect on the shear force ($p < 0.001$). The shear force was significantly increased at day 3 and decreased at the end of storage time (Tables 4.6 and Appendix Table C4). There was no significant interaction ($p = 0.481$) between antioxidant and storage time for shear force (Table 4.6).

Table 4.6 Effect of natural antioxidants application on cooking loss and shear force of chicken breast meat following time (ST) at 4 °C.

		Antioxidants (AO)							p value		
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Cooking Loss (%)	0	23.24	23.29	21.91	22.8	20.67	19.91				
	3	24.37	22.86	23.77	23.15	23.87	20.64	1.31	0.013	0.015	0.628
	7	22.64	21.2	21.81	21.35	20.92	20.99				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
Shear force (N)	0	16.23	14.5	15.21	14.92	15.59	16.07				
	3	18.45	18.64	17.5	17.89	16.86	17.56	1.02	0.541	<.001	0.481
	7	17.39	17.18	15.8	17.64	17.84	16.77				

SED, standard error of differences of means; $p \leq 0.05$ is significantly different according to the Tukey's HSD test; $p > 0.05$ is not significant different according to the Tukey's HSD test.

4.2.8 Effect of Natural Antioxidants on Drip Loss /Water Holding Capacity

The application of natural antioxidant significantly reduced the drip loss ($p \leq 0.05$) (Appendix Table C4). The antioxidants that provided a greater protection against a reduction of drip loss were ROS and SRB compared to the non-treated samples with mean values of 1.76, 191 and 2.44 % for ROS, SRB and non-treated samples, respectively. Storage time significantly increased the drip loss with increasing storage time ($p < 0.001$) (Appendix Table C4). Figure 4.5 shows a significant interaction between antioxidant and storage time ($p = 0.023$), due to the increase of drip loss in meat samples with increasing time. All natural antioxidant treatments were found to have a significantly lower drip loss than that in non-treated, but did not significantly differ from each other at day 0. A similar effect of natural antioxidants was shown on drip loss at day 3. Among natural antioxidants, ROS and SRB treatments had the lowest significant drip loss at day 3. On day 7, the only natural antioxidant that markedly reduced the drip loss compared to the non-treated samples was ROS with values of 2.51 %. The drip loss of all raw chicken meat (treated or non-treated) significantly increased with storage time. However, the highest proportion of drip loss was found in the non-treated samples compared to treated samples. Moreover, the impact of natural antioxidants on drip loss was similar to that in BHT.

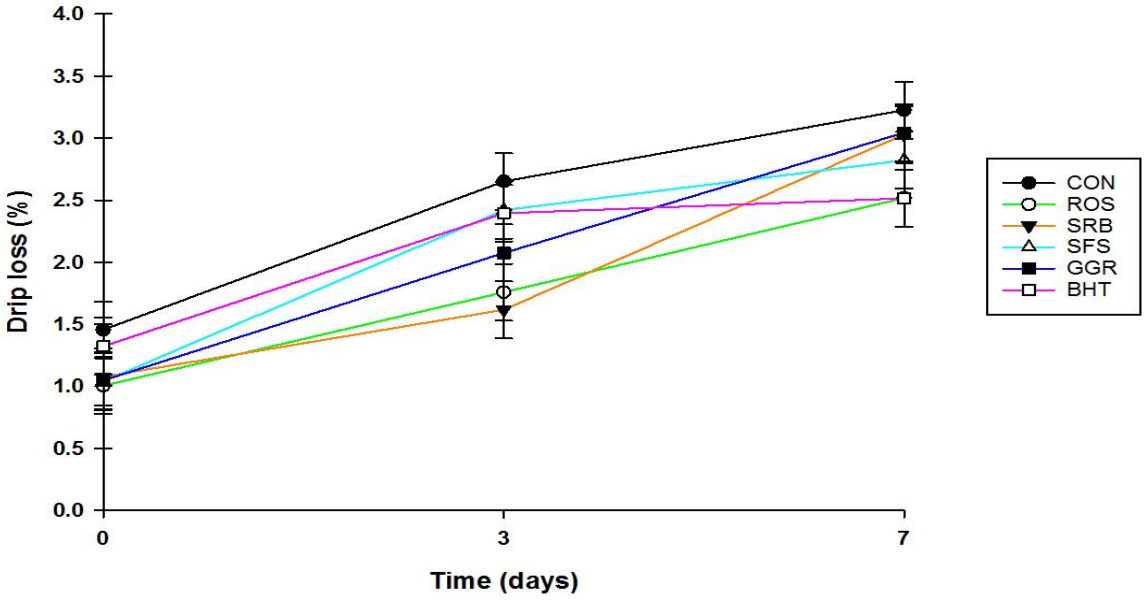

Figure 4.6 Effect of interaction of natural antioxidants application and storage time on drip loss of raw chicken breast meat (% (w/w)) (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

4.2.9 Effect of Natural Antioxidants on Colour

The lightness (L*) values in raw chicken meat were not significantly affected by the application of natural antioxidants ($p = 0.609$) (Tables 4.7 and Appendix Table C4). Storage time had a significant ($p = 0.026$) effect on the lightness values. The lightness values were significantly decreased from 61.01 to 59.58 at day 3 of storage and slightly increased toward the end of storage time to 59.60 (Tables 4.7 and Appendix Table C4). There was no significant interaction ($p = 0.233$) between antioxidant and storage time (Table 4.7). With respect to the redness (a*) values, the redness (a*) values of raw chicken meat were significantly ($p < 0.001$) affected by the application of natural antioxidants (Tables 4.7 and Appendix Table C4). The antioxidants that provided a greater stability of redness (a*) values were GGR and ROS compared to the non-treated samples (Tables 4.7 and Appendix Table

C4). The effect of storage time on redness values was not significant (p = 0.091). No significant interaction (p = 0.624) was found between the antioxidant and storage time (Table 4.7).

For the yellowness (b*) values, the b* value was affected significantly (p < 0.001) by antioxidants application and storage time (Tables 4.7 and Appendix Table C4). GGR and ROS had significantly higher b* values compared to the non-treated samples. The yellowness values were markedly increased over storage time. However, the highest increase was shown at day 3 (Tables 4.7 and Appendix Table C4). No significant interaction (p = 0.654) was found between the antioxidant and storage time (Table 4.7).

Table 4.7 Effect of natural antioxidants application on L, a* and b* values of chicken breast meat during the storage time (ST) at 4 ^{o}C.*

		Antioxidant (AO)							**p value**		
Variable	**ST (d)**	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
L* value	0	59.26	62.97	60.73	61.71	60.55	60.85				
	3	60.24	59.43	57.98	59.24	60.15	60.41	1.41	0.609	0.026	0.233
	7	58.33	59.16	60.92	60.75	58.86	59.57				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
a* value	0	2.17	3.09	1.90	2.18	3.26	2.23				
	3	2.35	2.67	1.63	2.37	3.18	2.59	0.38	<.001	0.091	0.624
	7	2.18	3.21	2.48	2.89	3.24	2.60				
	ST (d)	**CON**	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**	**ST**	**AO x ST**
b* value	0	5.89	9.54	5.96	6.01	9.43	7.47				
	3	7.48	10.31	7.37	7.64	10.93	7.98	0.80	<.001	<.001	0.654
	7	7.21	10.51	8.22	8.55	10.35	7.55				

SED, standard error of differences of means; p ≤ 0.05 is significantly different according to the Tukey's HSD test; p > 0.05 is not significant different according to the Tukey's HSD test.

4.2.10 Effect of Natural Antioxidants on Fatty Acid Composition

In raw meat, all antioxidants yielded higher content of all fatty acids, while a significant effect of antioxidants was found on C14:0, C18:0, C18: 2 n-6, C20:4 n-6, PUFAs, n-3 and n-6 (Table 4.8 and Appendix Table C5). The antioxidants that significantly yielded these fatty acids was GGR compared to the non-treated samples. All fatty acids in raw meat samples were declined over storage time, but significant reduction ($p \leq 0.05$) was found in C20:0 content (Table 4.8 and Appendix Table C5). A significant interaction was found between antioxidant and storage time for C20:0 in raw meat ($p < 0.001$). The amount of C20:0 was higher in all treated samples (with exception BHT) compared to the non-treated samples over 3 days, but antioxidants did not award any protection over 7 days (Table 4.8). There was a significant interaction between antioxidants and storage time for ∑ n-3 ($p = 0.038$), indicating the effect of antioxidants on ∑n-3 is depending on the storage time (Table 4.8). All antioxidants (with exception of BHT) had a higher amount of n-3 compared to the non-treated samples over storage time.

After raw meat subjected to the sous vide cooking process, the fatty acids in chicken breast samples were significantly affected by the cooking process, which resulted in a decrease of all fatty acids after cooking compared to that in raw meat (Table 4.9 and Appendix Table C6). The application of antioxidants had a significant effect on C20:0 fatty acid ($p = 0.002$). However, none of the antioxidants awarded significant protection against a reduction of C20:0 compared to that in non-treated samples. BHT was found to have a higher amount of C20:0 of 0.25 g/kg DM compared to the GGR treatment of 0.20 g/kg DM. The storage time had a significant impact on content of C14:0, C16:0, C18:0 and the sum of SFA in cooked samples ($p \leq 0.05$). All these fatty acids were decreased over 7 days of storage time, while a significant reduction was found at day 3 compared to day 0 (Table 4.9 and Appendix Table

C6). A significant AO x ST interaction ($p < 0.001$) was shown for C20:0 in cooked meat (Table 4.9), indicating that the effect of antioxidant on C20:0 is depending on the storage time. The amount of this fatty acid in BHT treatment was significantly higher than that in non-treated samples at day 3 days of storage time, but thereafter none of antioxidants provided any protection against reduction of C20:0 at day 7. With respect to the MUFAs, a significant effect of antioxidant on C16:1 n-7 in cooked meat was shown ($p = 0.007$), but did not significantly affect the C18:1 n-9 and total content of MUFAs ($p > 0.05$) (Table 4.9 and Appendix Table C6). The amount of C16:1 n-7 was highest in BHT, but did not significantly differ from SFS, ROS and non-treated samples, only was significantly different from SRB and GGR treatment. The interaction between antioxidant and storage time for all MUFAs was not significant ($p > 0.05$). The application of antioxidants had a significant effect on the C18:2 n-6, C20:4 n-6, C22:6 n-3 and total of PUFAs ($p \leq 0.05$). The antioxidants that provided a greater protection against degradation of C18:2 n-6 and total PUFAs was SFS compared to the non-treated samples, while SRB treatment was found to have the highest C22:6 n-3 content of 0.51 g/kg DM, but statistically did not differ from the non-treated samples. A significant ($p = 0.016$) effect of storage time was found only on content of C18:3 n-3 at day 7 compared to day 0 with a + 18 % decrease over 7 days of storage time (Table 4.9 and Appendix Table C6). A significant AO x ST interaction ($p = 0.041$) was found for C20:4 n-6 in cooked samples (Table 4.9). None of the antioxidants provided any protection against reduction of these fatty acids compared to the non-treated samples over 7 days of storage time. Over storage time, all samples (treated or non-treated) statistically were not changed with time. The results also showed that there was a significant effect of antioxidants on the ∑n-3 and ∑n-6 PUFA content in cooked chicken breast meat ($p \leq 0.05$). SFS treatment had the highest content of ∑n-3 and ∑n-6 PUFA with mean values

of 3.17 and 12.82 g/kg DM for ∑n-3 and ∑n-6 PUFA, respectively. Regarding the storage time, ∑n-3 PUFA content in cooked meat significantly reduced over storage time, highest reduction was found at day 7 compared to the day 0. No significant AO x ST interaction for both ∑n-3 and ∑n-6 ($p = 0.222$; $p = 0.334$) was detected in cooked meat (Table 4.9).

Table 4. 8 Effect of natural antioxidants application on fatty acid composition (g of fatty acid/kg DM) of raw chicken breast meat during the storage time (ST) at 4 °C.

Fatty acid	ST (d)	Antioxidants (AO)								p value	
		CON	BHT	ROS	SRB	SFS	GGR	SED	AO	ST	AO x ST
Total F. A	0	56.75	62.73	62.06	73.15	64.24	63.68	7.48	0.073	0.286	0.109
	3	53.19	50.83	50.00	57.56	65.59	75.95				
	7	52.77	65.92	67.31	60.88	58.54	63.39				
C14:0	0	0.28	0.28	0.26	0.33	0.29	0.32	0.04	0.026	0.394	0.112
	3	0.20	0.25	0.22	0.25	0.29	0.39				
	7	0.22	0.33	0.32	0.27	0.27	0.29				
C16:0	0	12.73	13.86	13.99	16.72	14.25	14.36	1.82	0.091	0.134	0.094
	3	11.01	11.34	11.14	12.41	14.07	16.73				
	7	11.21	15.89	14.74	13.22	12.58	13.50				
C18:0	0	5.36	5.85	5.94	6.11^{ab}	6.09	5.88	0.55	0.049	0.382	0.071
	3	5.16	5.15	5.06	5.73^{ab}	6.49	7.41				
	7	5.55	5.58	5.76	5.39^{ab}	5.50	5.70				
C20:0	0	0.20^{a}	0.33^{bcd}	0.26^{abc}	0.38^{d}	0.27^{abcd}	0.27^{abcd}	0.03	0.069	<.001	<.001
	3	0.23^{abc}	0.22^{ab}	0.27^{abcd}	0.26^{abc}	0.27^{abcd}	0.33^{cd}				
	7	0.29^{abcd}	0.26^{abc}	0.24^{abc}	0.24^{abc}	0.20^{a}	0.19^{a}				
∑ SFA	0	18.58	20.32	20.45	23.54	20.90	20.83	2.32	0.073	0.228	0.076
	3	16.61	16.96	16.68	18.65	21.12	24.87				
	7	17.27	22.06	21.05	19.10	18.55	19.69				
C16:1 n-7	0	1.76	1.67	1.80	2.20	1.81	2.10	0.46	0.336	0.052	0.235
	3	1.37	1.34	1.18	1.26	1.58	2.07				
	7	1.25	2.80	2.01	1.82	1.51	1.92				
C18:1 n-9	0	21.96	24.28	23.54	29.19	24.93	24.98	3.50	0.186	0.141	0.204
	3	20.40	18.84	17.54	21.70	25.04	28.03				
	7	19.94	26.24	25.87	23.04	21.98	24.69				
∑ MUFA	0	23.73	25.95	25.33	31.40	26.74	27.08	3.92	0.210	0.126	0.218
	3	21.77	20.18	18.72	22.96	26.61	30.10				
	7	21.20	29.04	27.88	24.86	23.49	26.61				
C18:2 n-6	0	11.24	12.71	12.43	14.31	12.89	12.29	1.46	0.019	0.965	0.115
	3	11.37	10.33	11.05	12.36	13.82	16.01				
	7	10.75	11.53	14.30	13.13	12.74	13.18				
C18:3 n-3	0	1.46	1.71	1.59	1.74	1.65	1.58	0.30	0.118	0.738	0.398
	3	1.37	1.22	1.36	1.62	1.92	2.11				
	7	1.36	1.51	1.99	1.76	1.72	1.80				
C20:4 n-6	0	0.25	0.40	0.45	0.42	0.41	0.40	0.06	0.013	0.092	0.074
	3	0.30	0.41	0.48	0.36	0.39	0.60				
	7	0.38	0.38	0.37	0.37	0.29	0.36				

Table 4.8 (continued)

Fatty acid	ST (d)	Antioxidants (AO)						SED	p value		
		CON	BHT	ROS	SRB	SFS	GGR		AO	ST	AO x ST
C20:5 n-3	0	0.28	0.25	0.27	0.28	0.27	0.27	0.06	0.687	0.867	0.899
	3	0.20	0.29	0.29	0.26	0.29	0.34				
	7	0.26	0.29	0.29	0.29	0.25	0.31				
C22:5 n-3	0	0.74	0.84	0.97	0.72	0.82	0.80	0.14	0.185	0.025	0.326
	3	0.96	0.90	0.96	0.81	0.94	1.28				
	7	0.94	0.64	0.88	0.81	0.93	0.85				
C22:6 n-3	0	0.48	0.55	0.58	0.75	0.57	0.42	0.12	0.795	0.989	0.389
	3	0.61	0.54	0.46	0.54	0.50	0.65				
	7	0.63	0.47	0.54	0.55	0.57	0.59				
∑ PUFA	0	14.44	16.46	16.28	18.21	16.60	15.76	1.73	0.012	0.998	0.068
	3	14.81	13.70	14.60	15.96	17.86	20.98				
	7	14.31	14.82	18.38	16.91	16.49	17.09				
∑ n-3	0	2.96^{a}	3.34	3.40^{ab}	3.48^{ab}	3.31^{ab}	3.07^{a}	0.33	0.028	0.544	0.038
	3	3.14^{ab}	2.96	3.07^{a}	3.23^{ab}	3.66^{ab}	4.37^{b}				
	7	3.18^{ab}	2.92	3.71^{ab}	3.41^{ab}	3.47^{ab}	3.55^{ab}				
∑ n-6	0	11.49^{ab}	13.11	12.88	14.73	13.29	12.69	1.46	0.014	0.980	0.096
	3	11.67^{ab}	10.74	11.53	12.72	14.20	16.61				
	7	11.13^{a}	11.91	14.67	13.50	13.02	13.55				

Mean values with different small superscript letters presented within each row and column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table 4. 9 Effect of natural antioxidants application on fatty acid composition (g of fatty acid/kg DM) of sous vide chicken breast meat during the storage time (ST) at 4 °C.

Fatty acid	ST (d)	Antioxidants (AO)							p value		
		CON	BHT	ROS	SRB	SFS	GGR	SED	AO	ST	AO x ST
	0	55.63	64.96	58.37	57.29	70.37	52.22				
T. FA	3	52.21	50.17	54.20	57.64	56.90	50.77	6.72	0.319	0.025	0.504
	7	48.45	59.14	54.47	53.08	48.81	51.02				
	0	0.30^{ab}	0.31^{ab}	0.26^{ab}	0.25^{ab}	0.35^{b}	0.22^{ab}				
C14:0	3	0.25^{ab}	0.21^{a}	0.22^{a}	0.25^{ab}	0.24^{ab}	0.22^{ab}	0.03	0.065	0.001	0.051
	7	0.20^{a}	0.31^{ab}	0.23^{ab}	0.21^{a}	0.22^{a}	0.21^{a}				
	0	12.74	15.28	12.86	12.64	15.22	11.19				
C16:0	3	11.41	11.69	11.22	12.51	12.43	11.01	1.60	0.223	0.024	0.567
	7	11.37	13.53	12.15	11.20	10.42	11.28				
	0	5.43	5.60	5.27	5.53	6.01	5.02				
C18:0	3	5.07	4.68	4.98	5.34	5.31	5.08	0.38	0.608	0.019	0.523
	7	5.02	5.21	5.10	5.22	4.81	5.03				
	0	0.17^{ab}	0.27^{cd}	0.22^{abcd}	0.24^{abcd}	0.25^{abcd}	0.19^{abcd}				
C20:0	3	0.22^{abcd}	0.20^{abcd}	0.23^{abcd}	0.21^{a}	0.29^{d}	0.17^{abc}	0.02	0.002	0.358	<.001
	7	0.23^{abcd}	0.27^{a}	0.20^{abcd}	0.21^{abcd}	0.16^{a}	0.16^{ab}				
	0	18.63	21.45	18.60	18.66	21.84	16.62				
∑SFA	3	16.95	16.78	16.66	18.31	18.27	16.49	2.00	0.320	0.017	0.533
	7	16.82	19.21	17.68	16.63	15.61	16.53				
	0	1.81	2.80	1.82	1.52	1.99	1.27				
C16:1 n-7	3	1.31	1.79	1.26	1.67	1.66	1.10	0.41	0.007	0.051	0.486
	7	1.62	2.15	1.69	1.12	1.17	1.46				
	0	21.27	25.99	22.24	22.01	27.59	19.95				
C18:1 n-9	3	19.87	19.80	20.77	22.41	22.14	18.71	2.99	0.290	0.038	0.499
	7	19.05	23.38	20.88	19.93	17.87	19.64				
	0	23.08	28.79	24.05	23.53	29.58	21.22				
∑MUFA	3	21.17	21.59	22.03	24.08	23.79	19.81	3.33	0.222	0.036	0.503
	7	20.68	25.53	22.57	21.04	19.05	21.10				
	0	10.66	11.58	12.17	11.86	15.01	11.14				
C18:2 n-6	3	10.93	9.11	12.13	11.84	11.60	11.25	1.44	0.034	0.062	0.318
	7	8.21	11.27	11.03	12.05	10.95	10.34				
	0	1.49	1.54	1.65	1.51	2.17	1.43				
C18:3 n-3	3	1.48	1.05	1.58	1.60	1.52	1.43	0.24	0.090	0.016	0.178
	7	0.97	1.43	1.39	1.54	1.36	1.30				
	0	0.32^{abc}	0.22^{a}	0.29^{abc}	0.25^{ab}	0.31^{abc}	0.23^{ab}				
C20:4 n-6	3	0.33^{abc}	0.22^{a}	0.27^{abc}	0.25^{ab}	0.23^{ab}	0.30^{abc}	0.03	0.001	0.528	0.041
	7	0.39^{c}	0.22^{a}	0.26^{abc}	0.24^{ab}	0.35^{bc}	0.24^{ab}				

Mean values with different small superscript letters presented within each row and column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table 4.9 (continued)

Fatty acid	ST (d)	Antioxidants (AO)							p value	
		CON	BHT	ROS	SRB	SFS	GGR	SED	AO	ST	AO x ST
C20:5 n-3	0	0.27	0.29	0.29	0.25	0.27	0.24	0.023	0.272	0.233	0.521
	3	0.23	0.27	0.23	0.26	0.27	0.25				
	7	0.26	0.27	0.28	0.24	0.27	0.25				
C22:5 n-3	0	0.70	0.69	0.83	0.75	0.75	0.81	0.058	0.061	0.598	0.928
	3	0.69	0.70	0.78	0.79	0.73	0.77				
	7	0.69	0.77	0.77	0.82	0.74	0.80				
C22:6 n-3	0	0.47	0.39	0.50	0.49	0.45	0.52	0.042	0.006	0.705	0.612
	3	0.42	0.45	0.53	0.51	0.50	0.48				
	7	0.43	0.44	0.48	0.52	0.50	0.47				
∑ PUFA	0	13.91	14.71	15.72	15.10	18.95	14.38	1.717	0.041	0.058	0.321
	3	14.08	11.80	15.51	15.26	14.84	14.48				
	7	10.95	14.39	14.22	15.40	14.16	13.39				
∑ n-3	0	2.93	2.91	3.27	3.00	3.63	3.01	0.258	0.013	0.025	0.222
	3	2.82	2.47	3.11	3.16	3.01	2.93				
	7	2.35	2.90	2.93	3.12	2.86	2.81				
∑ n-6	0	10.99	11.80	12.46	12.11	15.30	11.38	1.464	0.049	0.068	0.334
	3	11.26	9.33	12.40	12.10	11.83	11.55				
	7	8.60	11.49	11.29	12.29	11.30	10.58				

4.3 Discussion

4.3.1 Effect of Natural Antioxidants on Thiobarbituric Acid-Reactive Substances (TBARS)

TBARS values in raw and cooked meat in the current study were similar to those shown previously (Min et al., 2008; Selani et al., 2011). However, the magnitude of the change in TBARS values after cooking and storage differs among reports in chicken meat (Cortinas et al., 2004; Naveena et al., 2008; Selani et al., 2011). This variation could be associated with several factors such as the fatty acid profile of the meat, cooking process and storage circumstance (time, temperature, package and antioxidant contents such as vitamin E). TBARS values were significantly higher in sous vide cooked samples than in raw meat (Appendix Table C2). This increase in lipid oxidation has previously been attributed to thermal processes, which are known to disrupt the cell membranes and release pro-oxidants (Min et al., 2008; Selani et al., 2011; Naveena et al., 2013). Cross et al. (1987) reported that cooking related oxidation of meat is linked to the breakdown of haem proteins, which yield pro-oxidants. This study shows that supplementation with natural antioxidants significantly reduces TBARS values in both raw and sous vide cooked meat ($p \leq 0.05$) (Table 4.1 and Appendix Table C2). Raw meat samples had significantly lower TBARS when treated with SFS extract ($p = 0.012$) compared to the non-treated sample control (Table 4.1 and Appendix Table C2). This could be due to phenolic compounds in SFS that can inhibit the formation of TBARS in raw meat. In order to assess the practical effectiveness of the natural antioxidants they were compared to a powerful synthetic antioxidant (BHT). There were no significant differences found between natural antioxidants and BHT, indicating that the natural antioxidants have a

similar protective characteristic in raw chicken meat to that of synthetic ones (Table 4.1 and Appendix Table C2). In cooked meat, all treated samples had significantly lower TBARS values over 7 days. Among the treatments, ROS extract imparted significantly lower TBARS values compared to the non-treated samples (Table 4.1 and Figure 4.2). The application of ROS as a natural antioxidant either in meat and meat products used directly or in extracted form has been well documented (Chen et al., 1999; Yanishlieva-Maslarova et al., 2001; Mielnik et al., 2003; Rojas and Brewer 2007). In this work, ROS retarded lipid oxidation during the storage time by reducing TBARS values when applied to turkey meat following mechanical deboning (Mielnik et al., 2003). This data shows that there are no significant differences between natural antioxidants and synthetic treatments in either raw or cooked meats which suggested that the application of natural extracts was as powerful as the BHT treatment, which is supported by previous studies (McCarthy et al., 2001; Naveena et al., 2008; Selani et al., 2011).

Furthermore, the value of TBARS in all samples either before or after cooking increased at day 3 of storage and decreased at day 7 (Table 4.1 and Appendix Table C2). These results are in agreement with McCarthy et al (2001) who reported that TBARS values in cooked pork patties increased significantly up to 6 days and declined up to 9 days of storage. The reduction of TBARS value following a continuous storage time might be caused by a breakdown of malondialdehyde and produce volatile compounds during the on-going exposure to heat and oxygen (Bax et al., 2012). All meat samples supplemented with antioxidants were found to have lower levels of lipid oxidation throughout the storage time, compared to the non-treated samples. These results are in agreement with those reported by Naveena et al. (2013) who found that raw chicken patties treated with either natural or synthetic antioxidants markedly reduced the formation of TBARS

compared to the non-treated over 9 days of refrigeration storage. In the current study, TBARS values in all raw samples, with or without antioxidants added, ranged from 0.31 to 0.62 mg MDA/kg, which suggested only small oxidative deterioration changes in the breast meat. The most extensive oxidation of lipids occurred in cooked breast meat (0.99–7.14 mg MDA/kg meat) where these values were significantly different ($p \leq 0.05$) from raw samples.

4.3.2 Effect of Natural Antioxidants on Conjugated Dienes (CDs)

Conjugated dienes (CDs) are a good indicator of primary lipid oxidation products (Estevez et al., 2009). The presence of conjugated dienes (CDs) in meat indicates that polyunsaturated fatty acids that have two double bonds in their structure underwent an oxidation process (Feiner, 2006; Estevez et al., 2009). CDs have been associated with warmed over flavour in pork meat (Byrne et al., 2001). Conjugated dienes (CDs) values found in meat in the current study were lower than those shown in previous work (Hwang et al., 2013), who found that the CD values in raw chicken patties ranged from 57-78 µmole/g lipid. In this study, CDs were monitored to evaluate the effectiveness of natural antioxidants in delaying lipid oxidation in cooked and raw chicken meat. The average CD values of sous vide cooked chicken breast samples were significantly higher ($p \leq 0.05$) than that in raw meat (Appendix Table C2). The higher values of CD suggest that the unsaturated fatty acids and phospholipids became unstable during the cooking process. This is most likely due to the decomposition of polyunsaturated fatty acids during the sous vide cooking process which yield elevated amounts of conjugated dienes. During the early stages of the oxidation process of polyunsaturated fatty acids, the double bonds migrate along the carbon chain and yield unconjugated dienes, which are relatively

unstable compounds (Estevez et al., 2009). These unconjugated dienes then chemically stabilised by being converted to conjugated dienes. Hence, the elevated presence of CDs indicates the decomposition of polyunsaturated fatty acids. Similar findings were found by Farhoosh et al. (2012) who found a significant increase in CDs in olive oil samples subjected to a heating process compared to the non-treated samples. In this study, the use of natural antioxidants significantly reduced ($p \leq 0.05$) the formation of conjugated dienes in raw meat samples; there were no significant differences between natural antioxidants and the high-performing synthetic BHT (Table 4.2 and Appendix Table C2). Our results indicate that incorporation of natural antioxidants in chicken meat have the ability to retard oxidation levels and enhance its stability against autoxidation. These results are in agreement with those observed by Choe et al. (2011), who showed that addition of natural antioxidants to ground pork reduced the formation of CDs compared to the non-treated over a 10 day time. Similar findings were reported by Hwang et al. (2011) who found that addition of ganghwayakssuk extract to raw chicken patties reduced the formation of CDs over 10 days at 4 ^{o}C. Furthermore, in the current study, storage time was found to have a significant effect on the formation of the CDs in both raw and cooked meat (Table 4.2 and Appendix Table C2). Along with increased storage time, mean values of CD increased in raw and cooked meat at each interval of storage time. Higher CDs were also noticed in cooked meat over storage time. This could be due to thermal processes that can breakdown the polyunsaturated fatty acids and result in an increase in CD values over time. These results are in agreement with those reported by Lee et al. (2010) who found an increase in CDs over 7 days in ground pork stored at 4 ^{o}C. The formation of CDs depends on the oxygen uptake during the storage time (Sultana et al., 2007).

4.3.3 Effect of Natural Antioxidants on Conjugated Trienes (CTs)

Conjugated trienes (CTs) are a secondary lipid oxidation products. The formation of CT occurs when polyunsaturated fatty acids containing three or more double bonds in their structure undergo an oxidation process that yields conjugated diene moieties, which then extend and incorporate an additional double bond (Wrolstad et al., 2005). The presence of conjugated trienes in meat is indicative of advanced lipid oxidation (Wrolstad et al., 2005; Ali et al., 2009; Al-Dalain et al., 2011). In this study, the sous vide cooked meat caused a marked increase of CT values compared to those in raw meat (Appendix Table C2). These results are in agreement with those reported by Ali et al. (2009), who found that frying significantly increased the formation of CTs in kebabs compared to non-fried samples. The use of natural antioxidants reduced the formation of CTs in raw meat compared to the non-treated samples immediately after the dipping time (Table 4.3 and Appendix Table C2). The protective influence of natural antioxidants with regards to CTs formation was still present post cooking breast chicken meat immediately after dipping time, but was essentially absent following any length of storage time. It seems that the application of natural antioxidants into breast meat could have the ability to retard oxidation and enhance the stability of fat against autoxidation. These results are in agreement with those reported by Sultana et al. (2007), who found the addition of corncob extract to corn oil reduced the formation of CTs. In addition, storage time was found to have an effect on the formation of CTs in both raw and cooked meat (Table 4.3 and Appendix Table C2). Along with increased storage time, mean values of CT increased in raw and cooked meat throughout the 7 days of storage time. Higher CTs were also noticed in cooked meat over storage time (Table 4.3 and Appendix Table C2). This could be due to thermal processes that can breakdown polyunsaturated fatty acids and result in an

increase in CTs over time. Moreover, the higher formation of CTs in cooked meat might be attributed to the oxygen uptake during the storage time (Sultana et al., 2007).

4.3.4 Effect of Natural Antioxidants on Phospholipid Content

Phospholipids are known to be susceptible to thermal degradation (Jayasena et al., 2013) and have been linked to the development of warmed-over flavours in reheated meats (Igene and Pearson, 1979; Igene et al., 1980; Roldan et al., 2014). In the current study, chicken breast meat cooked by sous vide at 75 for 45 min was found to have significantly higher phospholipid degradation compared to the raw meat (Appendix Table C3). These results are in agreement with Wang et al. (2011) who observed that phospholipid content in duck muscle declined after exposure to different heating methods such as boiling in water for 30 min or roasting at 90 ^{o}C for 1 hr. The increase phospholipids degradation is associated with changes in the chemical composition during the oxidative deterioration of lipids, heating and enzymatic degradation (Jayasena et al., 2013).

The addition of antioxidants to chicken breast meat had a significant protective effect on phospholipid content in cooked meat, but did not affect in raw meat (Table 4.4 and Appendix Table C3). Among the sous-vide cooked samples, the highest phospholipid content was found in ROS treated samples followed by SRB, SFS, GGR treatments and the non-treated control. Hence, the effectiveness of the natural antioxidants in inhibition of phospholipid degradation was greater compared to BHT, which supports the notion that natural antioxidants are worthwhile contenders in the replacement of synthetic antioxidants. As mentioned before, the underpinning mechanism by antioxidants in protecting phospholipids from thermal decomposition is not well known. This mechanism could be similar to the protective mechanism as it applies to individual fatty acids since

phospholipids are considered to have elevated levels of unsaturated fatty acids. Moreover, a significant effect of storage time was seen in phospholipid content in both raw and cooked meat ($p \leq 0.05$). The resulting phospholipid content decreased over 7 days of storage and a significant reduction of phospholipid content was found at the end of the storage time compared to day 0 (Table 4.4 and Appendix Table C3). The phospholipid content in treated and non-treated samples of raw and cooked meat significantly decreased over 7 days of storage potentially indicating an enzymatic and thermal degradation. However, the highest reduction of phospholipids was shown in non-treated samples compared to those treated with natural antioxidants (Figures 4.4 and 4.5). This could be attributed to the structural phospholipids that contain high levels of polyunsaturated fatty acids predominantly those PUFAs that have three or more double bonds, which they increase the sensitivity of phospholipid to degradation under storage conditions and heating process. Similar findings were reported by Alasnier et al. (2000) and Soyer et al. (2010), who revealed a marked reduction in phospholipids in raw breast and thigh chicken meat without antioxidants added during a storage time. The decrease of phospholipids related to its decomposition and forming a complex with protein or carbohydrate (Takagi and Yoshida, 1999).

4.3.5 Effect of Natural Antioxidants on pH Values

In the meat industry, pH is considered an important parameter that has an effect on the meat quality (Feiner, 2006). The pH of meat is known as an impact on the rate of lipid oxidation in chicken (Ozer and Saricoban, 2010). Thiansilakul et al. (2011) found a correlation between lipid oxidation and pH value in chicken patties under refrigeration temperature. At pH >7.0, lipid oxidation (TBARS) in chicken, pork, beef and lamb

muscles was found to be higher than pH<7.0 (Tichivangana & Morrissey, 1985). The pH values of raw and cooked meat ranged from 5.69-6.00 and 6.00-6.17, respectively, which corresponded to those reported in a previous study where the pH of raw meat ranged from 5.61-5.87 and cooked meat ranged from 5.92-6.03 (Naveena et al., 2013).

The pH values significantly increased after the cooking process (Appendix Table C3). These results were similar to those published by Naveena et al. (2008; 2013), who demonstrated that the pH of meat increased after a cooking process. Antioxidant supplementation had a significant an effect on the pH values in raw meat ($p = 0.004$), but did not have any effect on the pH values in cooked meat ($p = 0.155$). In raw meat, the antioxidants that had significantly lower pH values were ROS and GGR treatments than the non-treated samples (Table 4.5 and Appendix Table C3). This could be attributed to the acid compounds that present in ROS and GGR extracts that may have caused a drop-in pH in breast chicken meat. Hence, ROS and GGR extracts were found to have a servals acid compounds such as carnosic acid, rosmarinic acid in rosemary extracts and cinnamic acid, salicylic acid in the ginger extract (Hernandez-Hernandez et al., 2009; Ghasemzadeh et al., 2010). The application of natural antioxidants such as Nutrox and Meliox significantly reduced the pH values in pork patties compared to that in non-treated samples. However, in the study conducted by Selani et al. (2011), found that a range of antioxidants (such as: grape peel and seed extract, sodium erythorbate, citric acid) did not have any effect on the pH values in either raw or cooked chicken meat over 9 days of storage. These results suggest that the effect of natural antioxidants on pH values in chicken meat is depending more on the type of natural antioxidants being used.

4.3.6 Effect of Natural Antioxidants on Drip Loss /Water Holding Capacity

Water-holding capacity is shown to be inversely proportional to drip loss (Grossi, et al., 2014). Water holding capacity can be described as water remaining in meat following a storage time or a cooking process (Aaslyng et al., 2003), which is predominantly dependent upon the ability of myofibrillar protein to bind and retain water (Wang et al., 2009). Proteins, like triglycerides and phospholipids, undergo-oxidation by a free radical mechanism (Grossi et al., 2014). According to the results reported by Wang et al. (2009), sarcoplasmic and myofibrillar protein solubility in chicken meat decreased with an increase in time. In the current study, samples supplemented with natural antioxidants had a significantly lower drip loss compared to the non-treated samples over time (Figure 4.6). Among antioxidants, ROS treatment was found to have the lowest drip loss compared to any other treatments (Tables 4.6 and Appendix Table C4). It seems that the reduction of drip loss in meat samples treated with natural antioxidants performed better than BHT. This could be attributed to inhibition of meat protein degradation by phenolic compounds that leads to increase the ability of meat to retain water**.** The drip loss of raw chicken meat significantly increased with storage time. The non-treated samples had a greater drip loss compared to the other treatments (Figure 4.6 and Appendix Table C4). Increasing drip loss in chicken meat during storage is likely to be due to greater oxidation of meat protein, which could reduce the ability of proteins to retain water. These findings were in agreement with Maqsood et al. (2015), who found that drip loss increased progressively in camel meat under refrigeration temperature with increasing storage time.

4.3.7 Effect of Natural Antioxidants on Cooking Loss

Cooking loss is known total loss of water that occurs in meat during the cooking process (Roldan et al., 2015) and has been linked to the thermal process (Aaslyng et al., 2003), which can denature and oxidize protein (Wang et al., 2009). Thus, reducing the ability of the meat protein to retain water and its structure (Aaslyng et al., 2003).

The proportion of cooking loss in meat samples was significantly ($p = 0.013$) affected by the addition of antioxidants (Table 4.6 and Appendix Table C4). However, a significant protection against an increase of cooking loss was found in samples treated with BHT with mean value of 20.51% compared to the non-treated samples of 23.42 %. No significant differences were found among natural antioxidants and non-treated samples. However, natural antioxidants were found to have the lower cooking loss than that in non-treated samples. The increase in cooking loss in natural antioxidants and non-treated samples could be attributed to degradation of meat proteins caused by cooking temperature at 75 ^{o}C for 45 min. The increase of cooking loss in meat depends on the cooking temperature and cooking duration (Roldan et al., 2013). Consequently, the cooking process can cause the oxidation and denaturation of meat proteins (Wang et al., 2009). The latter leads to reduced ability of the meat protein to retain water in its structure by capillary forces (Aaslyng et al., 2003). These results were consistent with those reported by Naveena et al. (2004), when supplementing buffalo meat chunks with ginger (5% w/w) and papain (0.2 % w/w) did not significantly affect the cooking loss compared to the non- treated samples, while meat samples treated with cucumis (2% w/w) resulted in a significantly lower cooking loss compared to the non-treated samples. This could be related to antioxidant compounds and their capacity in cucumis to protect proteins from denaturation and retaining more moisture.

4.3.8 Effect of Natural Antioxidants on Texture (Shear Force)

The shear force values of cooked meat were not significantly affected by antioxidant (natural or synthetic) supplementation ($p > 0.05$) (Table 4.6 and Appendix Table C4). Our results disagree with those reported by Naveena and Mendirratta (2001), who found that the addition of ginger reduced the shear force, especially at higher concentrations. These differences between this study and ours may be due to the concentration of antioxidants or dipping time used which could decrease the shear force.

4.3.9 Effect of Natural Antioxidants on Colour

Colour is one of the attributes of most interest to consumers in assessing and purchasing meat because the colour is a primary sensory characteristic, it is often used to evaluate meat quality either in the home or at retail (Velasco and Williams, 2011). The discolouration of meat during storage time takes place by oxidation of oxymyoglobin, resulting in the appearance of an undesirable colour in meat (Nerin et al., 2006). The addition of antioxidants to meat and its products can inhibit the discolouration of meat (Lee et al., 2010). The results obtained from our study show that the lightness (L*) values of raw chicken meat were not significantly affected by antioxidant treatment (Table 4.7 and Appendix Table C4). However, antioxidants provided a greater stability with respect to discoloration as a higher L* values were found in antioxidant treatments than non-treated samples. Similar findings were observed by Selani et al. (2011), that observed application of antioxidants to raw breast meat did not have any effect on lightness (L*) values as compared to the non-treated samples. There was a significant ($p < 0.001$) effect of natural antioxidants on redness (a*) values (Appendix Table C4). The antioxidants that provided a greater stability of redness (a*) values were GGR and ROS compared to the

non-treated samples (Table 4.7 and Appendix Table C4). This finding indicates that the application of natural antioxidants inhibited the discoloration of chicken meat compared to the non-treated samples. The effectiveness of antioxidants to reduce the discoloration of meat has been reported by Ahn et al. (2007), who found that redness (a*) values in beef meat samples treated with oleoresin rosemary was significantly higher than non-treated samples.

For the yellowness (b*) values, the b* value was affected significantly ($p < 0.001$) by antioxidants application (Table 4.7 and Appendix Table C4). GGR and ROS had significantly higher b* values compared to the non-treated samples. Hence, the higher in yellowness (b*) values of chicken meat treated with GGR and ROS extracts might be caused by the application of plant extracts which presented a dark green and orange colour. Similar findings were demonstrated by Choe et al. (2011), who found the highest b* values in pork meat treated with various levels of barley leaf and lotus powder compared with the non-treated samples. The reduction of discolouration in chicken meat could be related to antioxidant compounds that have the ability to reduce the oxidation of oxymyoglobin and formation metmyoglobin.

4.3.10 Effect of Natural Antioxidants on Fatty Acid Composition

The total fatty acids, SFAs, MUFAs and PUFAs content in the current study were similar to those reported by Mariutti et al. (2011), and higher than those reported by Cortinas et al. (2004) who found that the amount of SFAs, MUFAs and PUFAs in breast meat was 6.24, 9.13 and 3.48 g/kg DM. Measuring fatty acid content is an important factor in evaluating the nutritional quality of meat and its products (Mariutti, et al., 2011). The thermal process can potentially cause hydrolysis of fatty acids in meat (Cortinas et al.,

2004; Alfaia et al., 2010). The cooking process caused a decrease in all individual fatty acids and SFAs, MUFAs and PUFAs in all meat samples (Appendix Table C5 and C6). These results suggest that the majority of SFAs, MUFAs and PUFAs in meat remains relatively unstable after the cooking process. The possible explanation for this is more likely to be due to the high temperature used for cooking which can induce lipid oxidation. Hence, the PUFAs in chicken meat were more susceptible to oxidation degradation followed by MUFAs and SFAs (11.04, 10.20 and 9.98 %, respectively). These results indicate that the cooking process reduced all individual fatty acids and influenced all three families of fatty acids SFAs, MUFAs and PUFAs in different proportions. It seems that unsaturated fatty acids were more susceptible to oxidation than saturated fatty acids. Sensitivity to oxidative processes mainly depends on the composition of lipids. Lipids containing high levels of unsaturated fatty acids are considered to be more prone to oxidation compared to lipids high in saturated fatty acids (Min et al., 2008). Thus, free radicals can easily attack and react with unsaturated fatty acid double bonds, and produce short-chain aldehydes (Huang, et al., 2013). Cortinas et al. (2004) observed that the cooking of thigh chicken meat caused reduction of all individual and the sum SFAs, MUFAs and PUFAs compared to the raw meat.

According to the literature, the stability of fatty acid content in meat increased after it was supplemented with antioxidants, since the existing antioxidants act as hydrogen donors to supply electrons in order to reduce the hydrolysis of unsaturated fatty acids (Cortinas et al., 2004; Sampaio et al., 2012). In our study, the results showed that there was a significant impact of antioxidants on fatty acid composition in raw (Table 4.8 and Appendix Table C5). In raw meat, all antioxidants yielded higher content of all fatty acids, while a significant effect of antioxidants was found on C14:0, C18:0, C18: 2 n-6, C20:4

n-6, PUFAs, n-3 and n-6 (Table 4.8 and Appendix Table C5). The antioxidants that significantly yielded these fatty acids was GGR compared to the non-treated samples. After raw meat subjected to sous vide cooking process, the antioxidants that provided a greater protection against degradation of C18:2 n-6, total PUFAs, ∑n-3 and ∑n-6 was SFS compared to the non-treated samples (Table 4.9 and Appendix Table C6). These results were in agreement with Sampaio et al. (2012) who found that natural antioxidant treatments (oregano, plus sage, oregano, plus sage plus 5 % honey, and oregano plus sage plus 10 % honey) had significantly higher contents of all fatty acids compared to the non-treated samples. In contrast, in the study conducted by Pateiro et al. (2014), that observed the fatty acid composition of pig meat treated with natural antioxidant extract and BHT did not significantly differ from non-treated samples over storage time.

The content of fatty acids in all raw and cooked samples gradually declined throughout the storage time (Tables 4.8 and 4.9). Treated samples were found to have a lower degradation of fatty acids compared to non- treated samples which is likely to be due to susceptible lipids being attacked by free radicals. The decrease of all individual and the sum of SFAs, MUFAs and PUFAs proportion after 7 days of refrigeration is consistent with the data of earlier studies. In the study conducted by Sampaio et al. (2012) all individuals and the sum of SFAs, MUFAs and PUFAs proportions decreased in breast and thigh chicken meat after 96 hr of storage at 4 ^{o}C. Similar findings were shown by Mariutti et al. (2011), who found the amount of SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 PUFAs decreased in raw and grilled chicken patties stored at -18 ^{o}C for 90 days. The reduction of PUFAs content was mainly due to oxidation degradation of polyunsaturated fatty acids, mainly C18:2 n-6 and C18:3 n-3 into primary and secondary oxidation products.

4.4 Conclusions

Lipid oxidation products in chicken breast meat were affected significantly by the cooking process for 7 days. Hence, the results of this study suggest that chicken meat after cooking was more prone to lipid oxidation during the storage time as evidenced by highest degradation of phospholipid and fatty acids and formation of TBARS, CDs and CTs being found in cooked meat. The application of natural antioxidants to chicken breast meat prior to the cooking process provided a higher protection with regards to meat lipid stability of chicken breast meat stored under refrigeration temperature. All natural antioxidants were found to have lower values of TBARS, CD and CT compared to the non-treated samples, samples treated with SFS were more resistant to oxidation as evidenced by the lower TBARS, CD and CT formation observed. After meat subjected to the cooking process, natural antioxidants significantly reduced the formation of TBARS values and degradation of phospholipid content compared to the non-treated samples, ROS treatment had the lowest TBARS values and highest phospholipid content. A significant effect of antioxidants, particularly GGR extract was found on C14:0, C18:0, C18: 2 n-6, C20:4 n-6, PUFAs, n-3 and n-6 in raw meat, while in cooked meat, SFS treatment yielded more C20:00 and C16:1 n-7, C18:2 n-6, ∑PUFA, ∑n-3 and ∑n-6 PUFA compared to any other treatments. The application of natural extracts had significantly increased the stability of meat colour as evidenced by highest redness values being found in meat samples. Natural antioxidants particularly ROS and GGR significantly reduced the pH values. Drip loss and cooking loss of chicken meat markedly enhanced by the application of antioxidants.

The shear force was not significantly affected by antioxidant supplementation. Furthermore, storage time had a significant effect on all physical and chemical parameters

(with exception pH and a* values) in breast chicken meat samples. The results also show that natural antioxidants performed similar to the synthetic antioxidant BHT. It can be concluded that the stability of chicken meat could improve by the application of natural antioxidants. Because of this, utilising these antioxidants extracted from natural sources should be considered as an efficient way to protect physicochemical characteristics and the nutritional value of meat.

Chapter 5:

The Impact of the Post-Slaughter Application of Natural Antioxidants on the Characteristics of Chicken Meat Cooked by Different Methods and Following Reheating.

5.1 Introduction

Results presented in chapter 4 demonstrated that using plant extracts as a source of natural antioxidants in raw meat and cooked meat reduced the lipid oxidation products. In addition, the supplementation of meats with natural antioxidants was found to reduce the degradation of fatty acids and in particular, the degradation of phospholipids, which gives an indication that treating chicken meat with antioxidants before cooking could slow down the autoxidation of cooked meat during the storage time and enhance meat quality. The use of antioxidants to prevent and delay the oxidation of lipids in meat and meat-derived products during the storage time has been the focus of a number of studies (McCarthy et al., 2001; Ahn et al., 2002; Sampaio et al., 2012; Karre et al., 2013; Packer et al., 2015). Sampaio et al. (2012) found that the shelf life of cooked chicken meat increased when natural antioxidants were added prior to processing. They also found that the protection against lipid oxidation and degradation in the cooked breast and thigh was enhanced. Furthermore, cooked pork patties containing rosemary extract at 0.2 % (w/w) and stored under high-oxygen conditions at 4 ^{o}C for 9 days was found to have lower thiobarbituric acid reactive substance (TBARS) compared to an untreated control (McCarthy et al., 2001). Ahn et al. (2002) investigated the influence of rosemary extract (0.02 % (w/w)) in cooked ground beef meat, and found that this significantly decreased

the TBARS value, hexanal content and warmed-over flavour score in cooked ground beef meat throughout the storage time.

The cooking process can lead to an increase in the decomposition of fatty acid composition (Mariutti et al., 2011); formation of cholesterol-linked oxidation products (Khan et al., 2015); and the production of secondary lipid oxidation products and volatile compounds (Dominguez et al., 2014), which could have an effect on consumer acceptance. Several studies have demonstrated that the rate of lipid oxidation is more related to cooking methods in beef (Alfaia et al., 2010), foal meat (Dominguez et al., 2014; 2015), and in chicken meat (Pikul, 1985). Pikul (1985) evaluated the impact of cooking methods and reheating process on lipid oxidation in chicken meat, and showed a significant increase in lipid oxidation products in samples that were first cooked, then cooled, and subsequently reheated. Lipid oxidation has an association with warmed-over flavour, which reduces meat quality by imparting a rancid flavour and grassy odour in cooked, frozen and reheated meat (Colindres and Brewer 2011). There is a large variety of precooked chicken meat on the market where it is up to the customer to merely reheat the meat prior to consumption. In various meat products, this is implicated with the phenomenon of warmed-over characteristics. Development of warmed-over flavour in cooked meat is a significant issue and causes undesirable sensory changes in pre-cooked meat (Lanari et al., 1995; Byrne et al., 2001; Ahn et al., 2002; Mielnik et al., 2006; Hayes, 2008; Colindres and Brewer 2011). Meat quality and its shelf life could be enhanced by means that delay or otherwise inhibit the onset of lipid oxidation such as the use of antioxidants. The activity of natural antioxidants for preventing the oxidation of lipid and changes which occurs in cooked meat and reheated is not well documented. Hence, this study was carried out to evaluate the effects of natural antioxidants on the lipid oxidation

products (warmed over flavour) and physical characteristics of chicken meat cooked by two distinctly different methods (grilling and sous-vide processing), and subsequent reheating. The two precooking processes employed in this work represented “low-temperature & low-oxygen” (LTLO aka sous vide) (Vaudagna et al., 2002), and “high-temperature & high-oxygen” (HTHO aka grilled) (Campo et al., 2006). In order to take into account the fact that, the reheating process for ready-to-eat foods typically occurs sometime after the initial cooking process; the influence of time before reheating was also investigated. The reheating process was facilitated in a fan-forced oven, the type typically installed in many households. To monitor the development rancidity and the oxidation state in cooked chicken meat, changes of phospholipids and fatty acid and analysis the formation of lipid oxidation products were used.

5.2 Materials and Methods

5.2.1 Raw Materials

Chicken carcasses, sources of natural antioxidants and chemicals used was described in Chapter 2 section 2.2.1.

5.2.2 Experimental Design and Sample Preparation

This experiment designed as full factorial consisted of a 6 x 2 x 4 with six antioxidant treatments (ROS, GGR, SFS, SRB, BHT, and control), with two reheating (before and after reheating) and four storage times (0, 4, 8 and 12 days). For preparation of meat samples, raw meat samples were prepared as per the same procedure discussed in section 3.2.2 and treated all samples as per the same procedure discussed in section 4.2.2. After preparation of samples, samples divided into two batches, half batches of chicken breast samples were cooked by sous vide cooking process: "low-temperature & low-oxygen" (LTLO) as described in section 4.2.2.1 and a half batches of chicken breast samples were cooked by the grilled method "high-temperature & high-oxygen" (HTHO).

5.2.1.1 Grilled Method - "High-Temperature & High-Oxygen" (HTHO)

Chicken breast fillets were cooked using the contact grill (Buffalo, Bristol, UK) at 240 ^{o}C for 5 min on both sides, until the internal temperature of the meat reached 71 ^{o}C. After the internal temperature of the meat reached 71 ^{o}C, a digital calibrated thermometer (Therma 20 thermometer, UK) was used to monitor the internal temperature. Cooked samples were allowed to cool down at room temperature to an ambient temperature of 23 ^{o}C. All samples then then packaged in polyethylene bags (transmission rate of oxygen = 51000 cm^3/m^2. 24h. bar). Meat cooked samples were stored at 4 ^{o}C and periodically

sampled at 0, 4, 8 and 12 days. Not reheated cooked meat samples were also taken at each point of storage time and reheated at 160 ^{o}C for 10 min in a convection oven (Binder, Tuttlingen, Germany). Both cooked and reheated samples were analysed at day 0, 4, 8 and 12 of storage for physical and chemical parameters.

5.2.1.2 Chemical and Physical Analysis

Chemical parameters such as TBARS, conjugated dienes and trienes, phospholipid content and fatty acids were analysed according to the method described in Chapter 2, sections 4.2.3.1, 2.2.3.4.2, 2.2.3.4.3 and 2.2.3.4.4. Physical parameters such as colour measurement, pH value, cooking loss and texture analysis were analysed according to the procedures as described in sections 4.2.4.1, 4.2.4.2, 4.2.4.4 and 4.2.4.5.

5.2.3 Statistical Analysis

The data for LTLO and HTHO cooked chicken meat were conducted and analysed separately. A 6 x 2 x 4 factorial design was used with antioxidant treatments (ROS, SRB, SFS, GGR, BHT and non-treated), reheating process (before and after reheating), and the storage times (0, 4, 8 and 12 days) as main factors and interaction between them. The data obtained from the various treatments were analysed by general analysis of variance (ANOVA), using the GenStat statistical software (Edition 17th, VSN International Ltd). The experiment was conducted in triplicate (for each cooking method, n = 48). The significance level of all data was set at $p \leq 0.05$. The null (H_0) and alternative (H_1) hypothesis for each dependent variable were set as:

Null hypothesis (H_0):

There was no significant effect of natural antioxidants application, reheating process, storage time and interaction between them on physical and chemical properties of chicken meat cooked by different methods (sous vide and grilled methods) (H_0: $\mu = 0$; $p > 0.05$)

Alternative hypothesis (H_1):

There was a significant effect of natural antioxidants application, reheating process, storage time and interaction between them on physical and chemical properties of chicken meat cooked by different methods (sous vide and grilled methods) (H_1: $\mu \neq 0$; $p \leq 0.05$).

5.3 Results

5.3.1 Effect of Natural Antioxidants on Thiobarbituric Acid-Reactive Substances (TBARS)

5.3.1.1 TBARS in Sous-Vide (LTLO) Processed Chicken Meat

The influence of the initial cooking process on the TBARS values was much greater in the HTHO (grilled) processed chicken meat compared to the LTLO (sous vide) breast meat, with an initial TBARS value of 13.39 and 6.81 mg MDA/kg meat, respectively (Appendix Table D2). A significant ($p < 0.001$) an effect of antioxidant, reheating process and storage time and interaction between three factors on TBARS values was found in LTLO samples. Regardless of the reheating process and storage time, all antioxidants provided a greater protection against an increase of TBARS value ($p < 0.001$), while among them, ROS awarded significantly lower amount of TBARS with mean value of 3.57 mg MDA/kg meat compared to the any other treatments. The effectiveness of natural antioxidants compared to the synthetic antioxidant BHT to inhibit the formation of TBARS in LTLO samples were ROS > BHT > GGR > SFS > SRB > non-treated samples respectively. The reheating process significantly reduced the formation of TBARS with mean values of 7.33 and 6.29 mg MDA/kg meat for samples before reheating and after reheating process. TBARS values in LTLO samples significantly increased ($p < 0.001$) with increasing storage time (Appendix Table D2).

A significant AO x RH interaction ($p < 0.001$), indicating that the effect of antioxidants on TBARS values in LTLO samples is depending on heating process. All Natural antioxidants (with exception SRB) were significantly reduced TBARS values in both before and reheated meat samples compared to the non-treated control (Figure 5.1). A

significant interaction ($p < 0.001$) between AO x ST, due to the increase the TBARS values in all samples with time, but treated samples (with exception SRB) were found to have higher inhibition of TBARS values compared to the non-treated samples at each interval. Among antioxidants, ROS was found to have the lowest formation of TBARS over time. There was a significant interaction ($p < 0.001$) between reheating process and storage time, due to the increase of TBARS values in samples before and after reheating over time, but marked formation of TBARS were shown in the samples before reheating (Figure 5.3). The results of interaction between AO x RH x ST are presented in Table 5.1. A significant increased ($p < 0.001$) of TBARS values was found in non-treated samples at day 0, 4 and 8 and decreased at day 12. The application of antioxidants provided a greater protection against an increase of TBARS values in all samples either before or after reheating process compared to the non-treated samples during 12 days. Before reheating samples, GGR was found to reduce the high level of TBARS value by up to 3.02 mg MDA/kg (i.e. a reduction of 59.44 %) at day 0, while the lowest amount of TBARS was shown in samples treated with ROS at day 4 and GGR at day 8 of storage. On day 12, the TBARS values were significantly lower in ROS treatment with values of 6.24 mg MDA/kg vs. 10.33, 15.53 and 17.17 mg MDA/kg meat for GGR, non-treated and SRB, respectively. Interestingly, over storage time, the natural antioxidant influence was comparable with BHT as natural antioxidants reduced TBARS value by up to 1.00 - 11.34 mg MDA/kg (i.e. a reduction of 26-72 %) compared to the BHT that reduced TBARS by up to 0.85-9.66 mg MDA/kg (i.e. a reduction of 17-77 %). After reheating LTLO samples for 12 days, the results show that natural antioxidants that awarded a greater reduction against an increase of TBARS compared to the non-treated samples, was ROS treatment which was more resistant to reheating process.

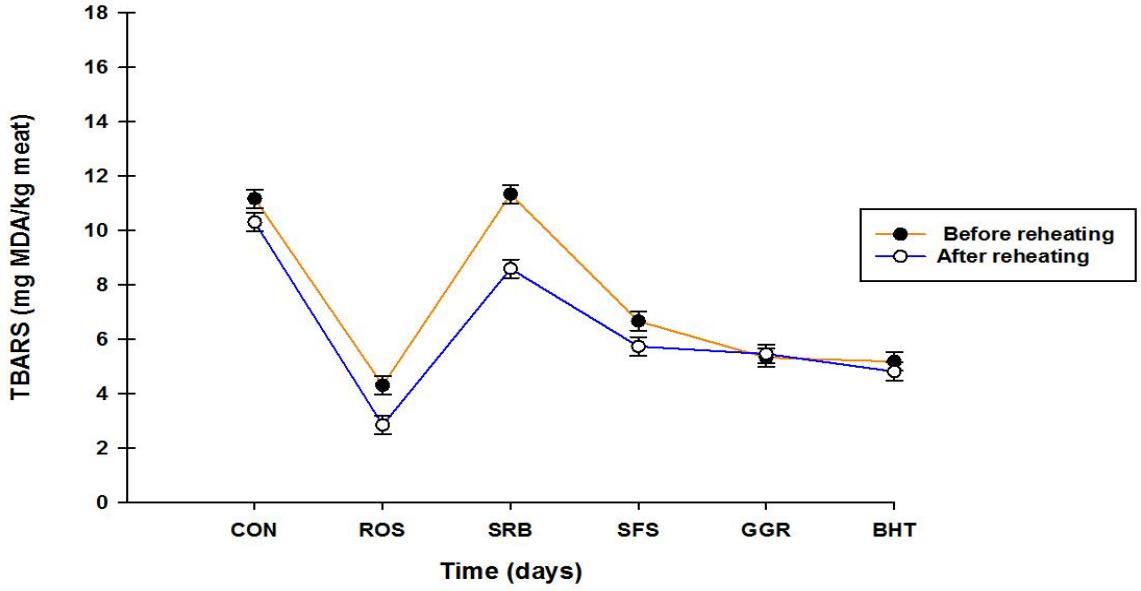

Figure 5. 1 Effect of interaction of natural antioxidants application and reheating process on lipid oxidation (TBARS) of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).

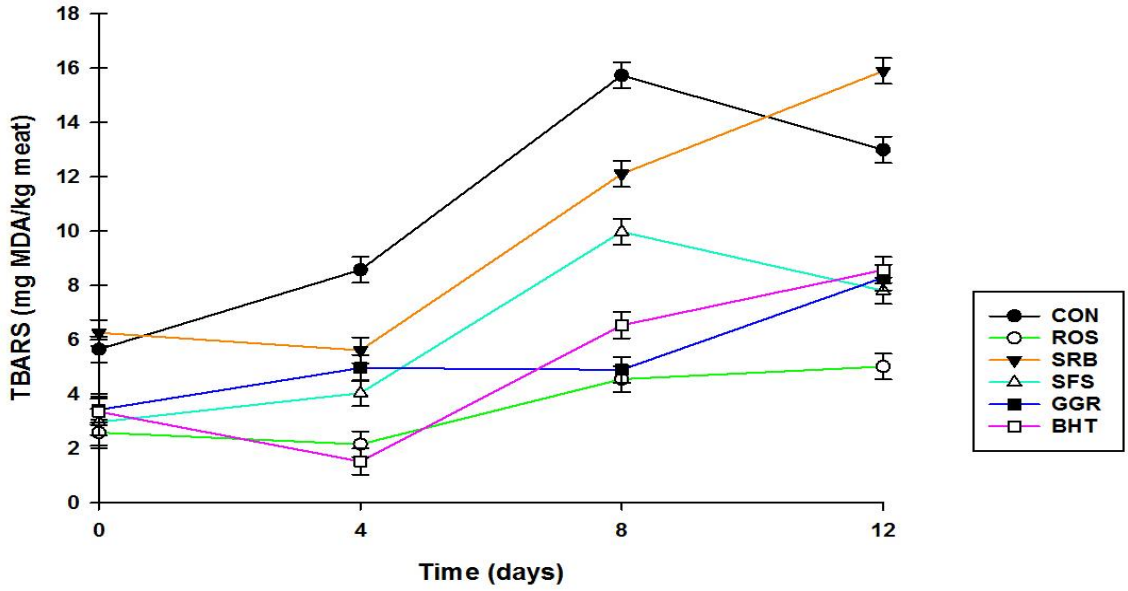

Figure 5. 2 Effect of interaction of natural antioxidants application and storage time on lipid oxidation (TBARS) of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

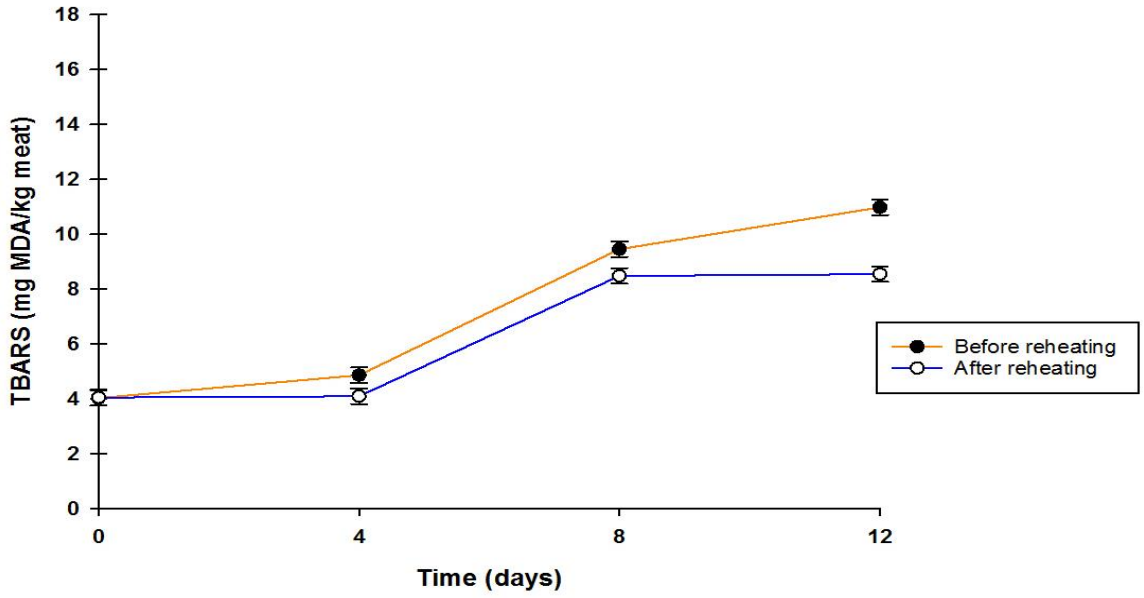

Figure 5. 3 Effect of interaction of reheating process and storage time on lipid oxidation (TBARS) of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).

Table 5.1 Effect of natural antioxidants application on the degree of rancidity as expressed in TBARS (mg MDA/kg meat), comparing a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method for raw chicken breast meat during storage at 4 ^{o}C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	5.08^{Acd}	6.21^{Ade}	4.23^{Bbc}	2.47^{Aa}	2.82^{Aa}	2.34^{Aa}	7.21^{Ae}	5.30^{Acd}	2.76^{Aa}	3.18^{Ba}	2.06^{Aa}	4.78^{Ac}
4		8.26^{Bd}	8.90^{Bd}	1.92^{Aa}	1.11^{Aa}	2.10^{Aa}	2.20^{Aa}	6.15^{Abc}	5.07^{Abc}	6.32^{Bc}	1.75^{Ac}	4.41^{Bb}	5.51^{Abc}
8		15.83^{Cf}	15.65^{Df}	6.17^{Cc}	6.89^{Bc}	6.06^{Bbc}	3.05^{Aba}	14.83^{Bf}	9.39^{Bd}	11.67^{De}	10.61^{Dde}	4.49^{Bab}	5.29^{Abc}
12		15.54^{Cef}	10.46^{Cd}	8.38^{Dbcd}	8.76^{Bcd}	6.24^{Bb}	3.79^{Ba}	17.17^{Cf}	14.63^{Ce}	8.23^{Cbcd}	7.38^{Cbcd}	10.33^{Cd}	6.25^{Ab}
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	14.78^{Ad}	16.09^{Ad}	4.69^{Aab}	6.94^{ABbc}	6.25^{Aabc}	3.02^{Aab}	6.95^{Abc}	10.35^{Ac}	6.75^{Abc}	5.77^{Aab}	10.02^{Ac}	2.50^{Aa}
4		15.42^{Ad}	15.23^{Ad}	4.44^{Aab}	3.76^{Aa}	7.53^{Abc}	10.70^{Bc}	5.11^{Aab}	7.48^{Abc}	8.76^{ABc}	9.44^{Ac}	7.66^{Abc}	9.81^{Bc}
8		25.41^{Cef}	26.75^{Bf}	9.04^{Ba}	9.91^{BCa}	19.25^{Bbc}	18.09^{Cbc}	23.82^{Cdef}	21.97^{Bcde}	21.68^{Ccde}	20.53^{Bbcd}	20.78^{Cbcd}	17.09^{Cb}
12		20.37^{Bdef}	24.30^{Bf}	13.82^{Cabc}	10.67^{Ca}	16.32^{Bbcd}	18.72^{Ccde}	16.69^{Bcd}	22.98^{Bef}	11.59^{Bab}	21.23^{Bdef}	14.14^{Babc}	17.96^{Ccde}

Mean values with different small superscript letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each cooking method/treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.1.2 TBARS in Grilled (HTHO) Processed Chicken Meat

The protective influence of natural antioxidants on high-temperature/high-oxygen (HTHO) processed chicken meat was also observed with respect to the previously discussed LTLO samples. The TBARS values in grilled processed breast meat were affected significantly by antioxidant, reheating process, storage time and interaction between three factors ($p < 0.001$) (Appendix Table D2). Hence, regardless of reheating process and storage time, the application of natural antioxidants significantly decreased the accumulation of TBARS in cooked samples compared to the non-treated control ($p < 0.001$). The effectiveness of natural antioxidants compared to the synthetic antioxidant (BHT) to inhibit the formation of TBARS in HTHO samples was BHT > ROS > GGR > SFS > SRB > non-treated samples, respectively (Appendix Table D2). The reheating process significantly caused an increase of TBARS values compared to those before subjecting to reheating process ($p < 0.001$). Furthermore, the TBARS values in cooked meat samples increased from 7.84 to 19.53 mg MDA/kg meat up to 8 days and declined at day 12 of storage time to 17.40 mg MDA/kg meat, respectively. There was a significant effect of AO x RH interaction ($p < 0.001$) on TBARS values. Hence, antioxidants application awarded higher protection against an increase of TBARS values in both before and after reheating samples compared to the non-treated samples. Reheating process significantly increased the formation of TBARS values in non-treated samples, SRB and SFS ($p < 0.001$) (Figure 5.4). A significant AO x ST interaction was found for TBARS, due to the increase of TBARS values in all treated and non-treated samples with storage time. The application of natural antioxidants significantly inhibited the formation of TBARS values compared to the non-treated samples. Over 12 days, natural antioxidants had reduced TBARS values, about 6 to 67 % compared to the non-treated

samples. The results also showed that the efficiency of these antioxidants was similar to the BHT as the reduction of TBARS was 32 - 71 % (Figure 5.5). A significant RH x ST interaction was found for TBARS values ($p < 0.001$), indicating that the effect of reheating process on TBARS is depending on the storage time. Hence, the reheating process significantly increased TBARS at day 4 and 12 of storage time (Figure 5.6). A significant interaction ($p < 0.001$) was found between three main factors (AO x RH x ST) (Table 5.1). As shown in Table 5.1 the TBARS values in all samples increased over 8 days of storage time and decreased at day 12 of storage time. Natural antioxidant treatments were found to have the highest inhibition level of TBARS values compared to the non-treated samples. Among natural antioxidants, GGR and ROS treatments after reheating were found to have the lowest lipid oxidation compared to the non-treated samples at day 0, while at day 4, SRB treatment either before or after reheating process had the lowest values of TBARS. At day 8, GGR and ROS after reheating awarded the highest protection against an increase of TBARS values. No significant differences were found among natural antioxidants (with exception SRB after reheating) at day12 of storage time. Furthermore, over 12 days, natural antioxidants had reduced TBARS values, about 6 - 67 % compared to the non-treated samples. The results also showed that the efficiency of these antioxidants was similar to the BHT as the reduction of TBARS was 32 - 71 %.

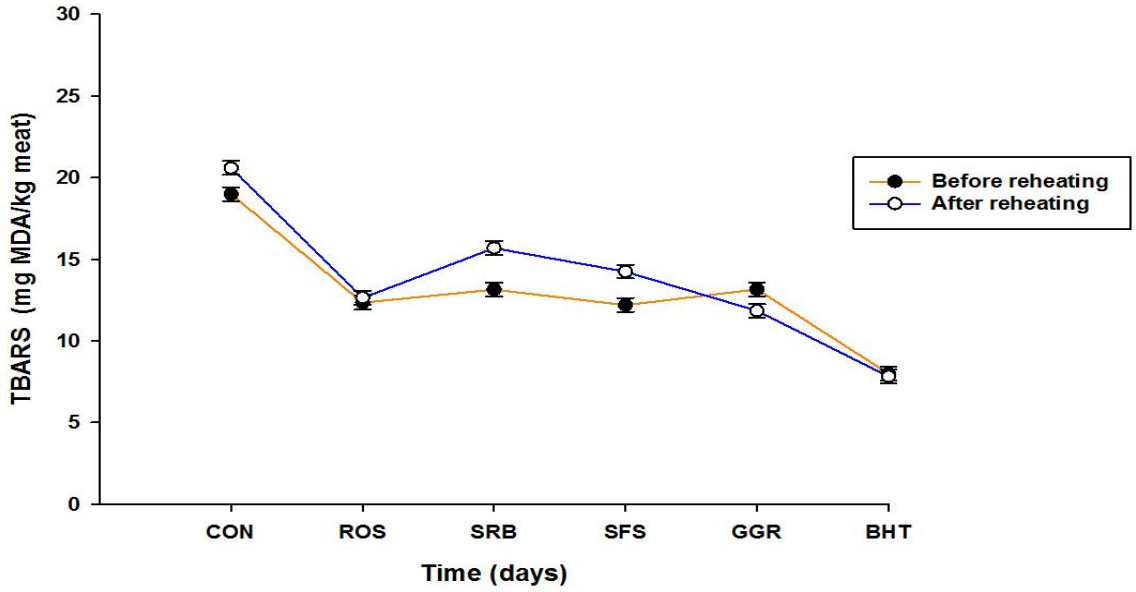

Figure 5.4 Effect of interaction of natural antioxidants application and reheating process on lipid oxidation (TBARS) of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

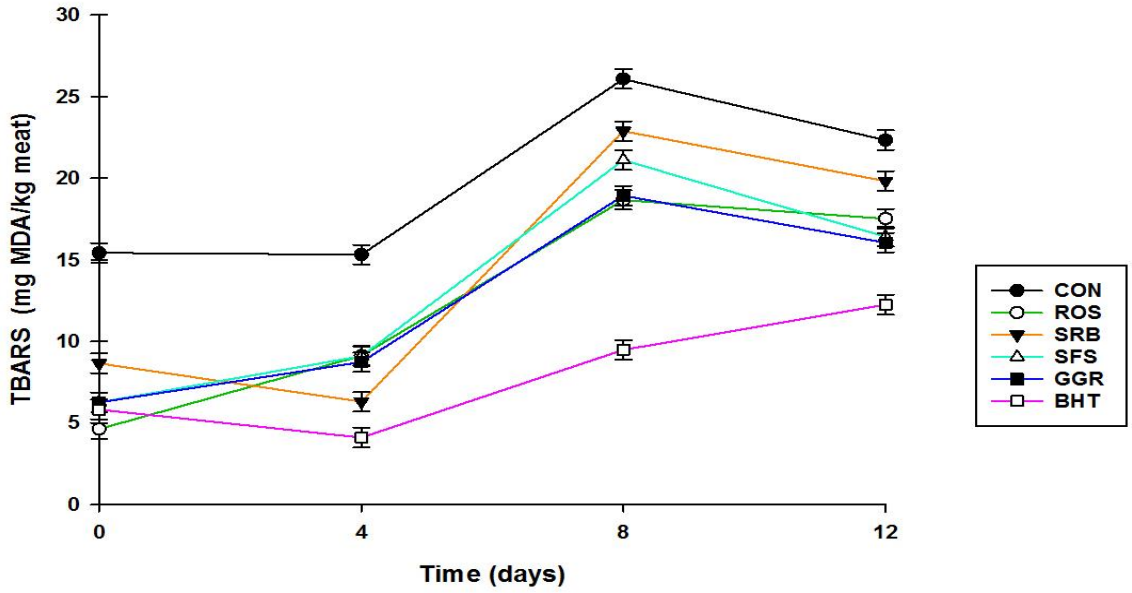

Figure 5.5 Effect of interaction of natural antioxidants application and storage time on lipid oxidation (TBARS) of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

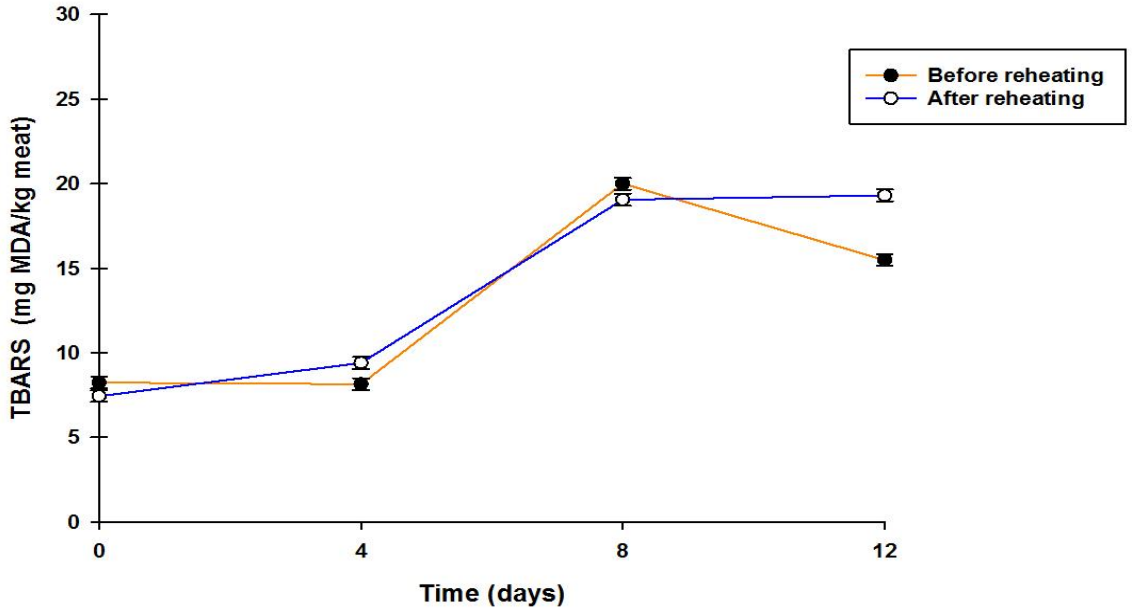

Figure 5.6 Effect of interaction of reheating process and storage time on lipid oxidation (TBARS) of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

5.3.2 Effect of Natural Antioxidants on Phospholipid Content

5.3.2.1 Phospholipids in Sous-Vide (LTLO) processed chicken meat

The influence of the initial cooking process on phospholipid content was much greater in the HTHO (grilled) breast meat compared to the LTLO (sous vide) breast meat with an initial phospholipid content of 44.62 and 45.06 g/100 g fat, respectively (Appendix Table D2). The phospholipid content in LTLO samples was significantly affected by antioxidants ($p < 0.001$). Supplementation with either natural or synthetic antioxidants impeded the degradation of phospholipid content compared to the non-treated control, but there were no significant differences between antioxidant treatments (Appendix Table D2). The reheating process significantly caused a reduction of phospholipid content compared to those before reheating process ($p < 0.001$). It was found that the phospholipid content in cooked meat samples significantly decreased ($p < 0.001$) throughout the storage time, the highest reduction was found on day 8, with no significant changes at day 12 (Appendix Table D2). A significant AO x RH interaction ($p = 0.016$) was found for phospholipid content (Figure 5.7). The phospholipid content was significantly reduced after reheating process compared to those before reheating. Natural antioxidants were found to have a higher phospholipid content compared to the non-treated samples (Figure 5.7). A significant interaction between antioxidant and storage time was found ($p < 0.001$), due to the decrease of the phospholipid content in all LTLO samples with storage time. Natural antioxidants provided a great protection against phospholipid degradation at day 0 and 4 of storage time, while at day 8 and 12 of storage time only ROS and SRB treatments had significantly the highest content of phospholipid compared to the non-treated samples (Figure 5.8). No significant interaction was found between reheating

process and storage time ($p = 0.274$). There was a significant interaction ($p < 0.001$) between three main factors (AO x RH x ST) for phospholipid content (Table 5.2). The phospholipid content in all LTLO meat samples either before or after reheating were susceptible to the oxidation process and decreased with storage time, while the highest reduction of phospholipids was shown in the samples after reheating compared to those before reheating process for 12 days. Supplementation with either natural or synthetic antioxidants impeded the degradation of phospholipid content in LTLO samples compared to the non-treated samples during the storage time. Before reheating process, samples treated with either ROS or SRB had the highest content of phospholipid compared with any other samples on day 0. At day 4, the treatment with GGR and SFS yielded a higher phospholipid content than the equivalent control with mean values 50.99 and 47.36 g/100 g fat respectively vs. 44.26 g/100 g fat for non-treated control. The remaining natural antioxidants (ROS and SRB), plus BHT provided a similar but minor protection against phospholipid degradation at 45.49, 44.34, and 45.96 g/100 g fat respectively. At day 8, samples treated with all natural antioxidants (with the exception of the samples treated with ROS extract) provided a better protection against phospholipids compared to the non-treated samples, while similar to BHT treatment with mean values 49.32, 48.80, 48.30, 46.18, 45.54 and 50.92 g/100 g fat for SRB, GGR, SFS, ROS, CON and BHT, respectively. No significant differences were found between treatments before reheating at day 12 of the storage time. After reheating process, a significant difference was only found at day 4 of storage between SRB and SFS, non-treated and BHT treatments, respectively. Natural antioxidants provided a similar protection to phospholipids compared with BHT that yielded a higher phospholipid content (Table 5.2).

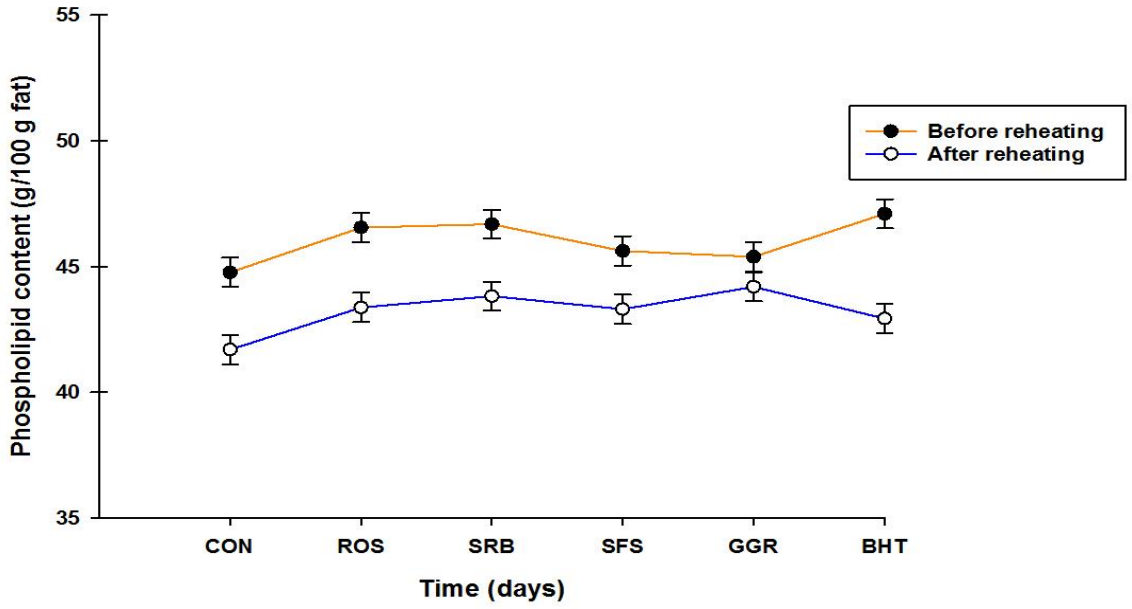

Figure 5.7 Effect of interaction of natural antioxidants application and reheating process on phospholipid content of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).

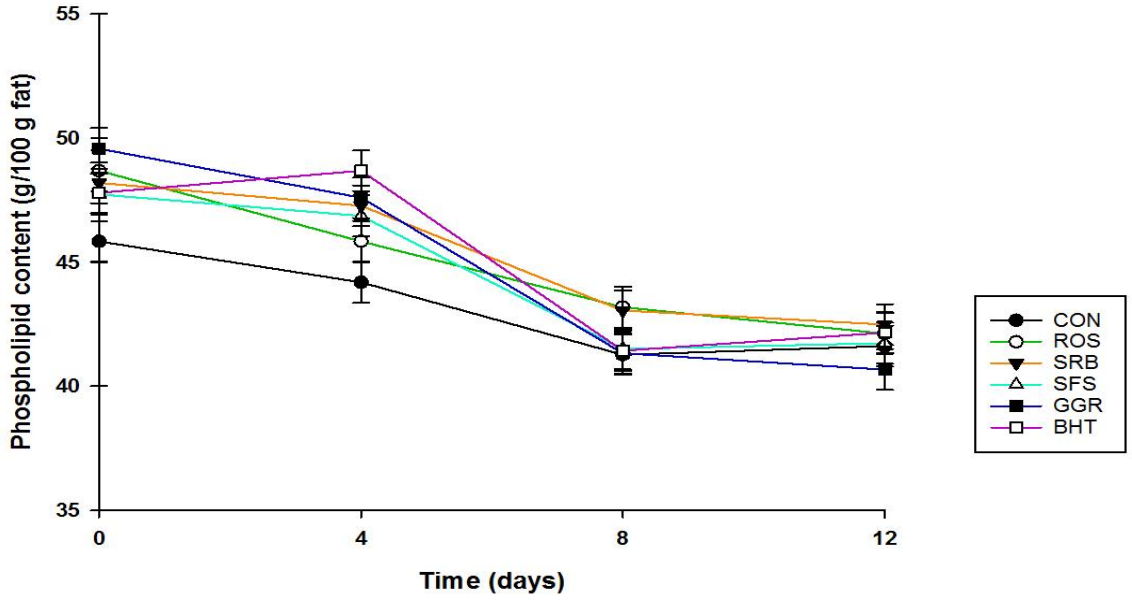

Figure 5.8 Effect of interaction of natural antioxidants application and storage time on phospholipid content of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

Table 5.2 Effect of natural antioxidants application on phospholipid content (g/100 g fat) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	47.42^{Cbc}	42.22^{Ba}	49.64^{BCcd}	44.73^{Cab}	51.89^{Bd}	44.41^{Bab}	52.06^{Bd}	42.18^{Aa}	48.12^{Abcd}	42.66^{Aa}	48.16^{ABbcd}	41.63^{Ba}
4		44.26^{ABde}	40.31^{Aab}	45.96^{Aef}	38.14^{Aa}	45.49^{Aef}	41.97^{ABbcd}	44.34^{Ade}	43.92^{Acde}	47.36^{Af}	40.34^{Aab}	50.99^{Bg}	41.04^{ABabc}
8		45.54^{Bab}	43.89^{Ca}	50.92^{Cc}	43.12^{BCa}	46.18^{Aab}	43.71^{ABa}	49.32^{ABbc}	43.21^{Aa}	48.30^{Abc}	43.40^{Aa}	48.80^{ABbc}	42.97^{Ba}
12		42.84^{Aabcde}	39.35^{Aa}	46.45^{ABe}	41.19^{Babcd}	45.50^{Acde}	40.52^{Aabc}	45.24^{Abcde}	41.77^{Aabcde}	45.44^{Acde}	40.07^{Aab}	46.40^{Ade}	38.37^{Aa}
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	45.55^{Babcd}	42.91^{Aa}	49.97^{Cde}	45.77^{Babcde}	55.84^{Cf}	43.18^{ABab}	49.60^{Bde}	44.03^{Aabc}	50.76^{Bef}	48.08^{Abcde}	48.51^{Acde}	46.76^{Aabcde}
4		43.69^{ABab}	41.34^{Aa}	45.34^{Aab}	40.84^{Aa}	44.28^{Aab}	42.74^{Aab}	47.87^{Bb}	44.55^{Aab}	46.66^{ABab}	42.63^{Aab}	44.37^{Aab}	43.85^{Aab}
8		44.67^{Bab}	42.36^{Aa}	49.05^{BCb}	45.99^{Bab}	46.59^{Bab}	45.89^{Bab}	45.38^{ABab}	43.28^{Aa}	46.07^{Aab}	45.61^{Aab}	45.34^{Aab}	45.91^{Aab}
12		41.86^{Aabcd}	37.55^{Aa}	46.56^{ABd}	42.77^{Abcd}	46.06^{Bcd}	41.69^{Aabcd}	43.21^{Abcd}	41.15^{Aabc}	45.83^{Abcd}	43.75^{Abcd}	46.19^{Acd}	40.88^{Aab}

Mean values with different small superscript letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each cooking method/treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.2.2 Phospholipids in Grilled (HTHO) Processed Chicken Meat

The protective influence of natural antioxidants on high-temperature/high-oxygen (HTHO) processed chicken meat was also examined. The natural antioxidants, plus BHT awarded a greater protection against phospholipid degradation compared to the non-treated control, but there were no significant differences between antioxidant treatments. Natural antioxidants provided similar protection against phospholipids compared to BHT. (Appendix Table D2). The reheating process significantly reduced phospholipid content compared to those before reheating process ($p < 0.001$). The storage time had an effect on phospholipid content of HTHO samples. It was found that the phospholipid content in cooked meat samples significantly decreased ($p < 0.001$) throughout the storage time, the highest reduction was found on day 8 of storage time with no significant changes at day 12 (Appendix Table D2). No significant interaction was found between antioxidant and reheating process for phospholipid content ($p = 0.120$). The results presented in Figure 5.9 show that the effect of interaction between antioxidant and storage time was significant ($p = 0.004$). The phospholipid content in both treated and untreated samples was decreased with increasing storage time, while all natural antioxidants were found yield more phospholipid content compared to the non-treated control at each point of storage time. A significant interaction between reheating process and storage time was found for phospholipid content in HTHO samples ($p < 0.001$). The phospholipid content was markedly reduced in samples after reheating at each interval of storage time compared to those before reheating process (Figure 5.10). A significant AO x RH x ST interaction was found for phospholipid content ($p < 0.001$) (Table 5.2). The treatment with ROS and SFS yielded a higher phospholipid content than the equivalent control with mean values 55.84 and 50.76 g/100 g fat respectively vs. 45.55 g/100 g fat for the non-

treated control. The remaining natural antioxidants (SRB and GGR), plus BHT provided a similar but minor protection against phospholipid degradation at 49.60, 48.50 and 49.97 g/100 g fat respectively at day 0 of storage, while at day 4, 8 and 12, no significant differences were found between treated and non-treated samples when samples of chicken breast meat were stored under refrigerated conditions each interval storage time days. Natural antioxidants provided better protection against phospholipids compared to non-treated samples and similar to BHT at each interval of storage time. After HTHO samples were subjected to the reheating process, SFS treatment was found to have a higher phospholipid content compared to the non-treated samples at day 0 of the storage time. At days 4 and 8 of refrigeration, antioxidants did not have any effect on the phospholipid content. At day 12, the highest phospholipid content was found in SFS with values of 43.75 vs 37.55 g/100 g fat for non-treated samples (Table 5.2).

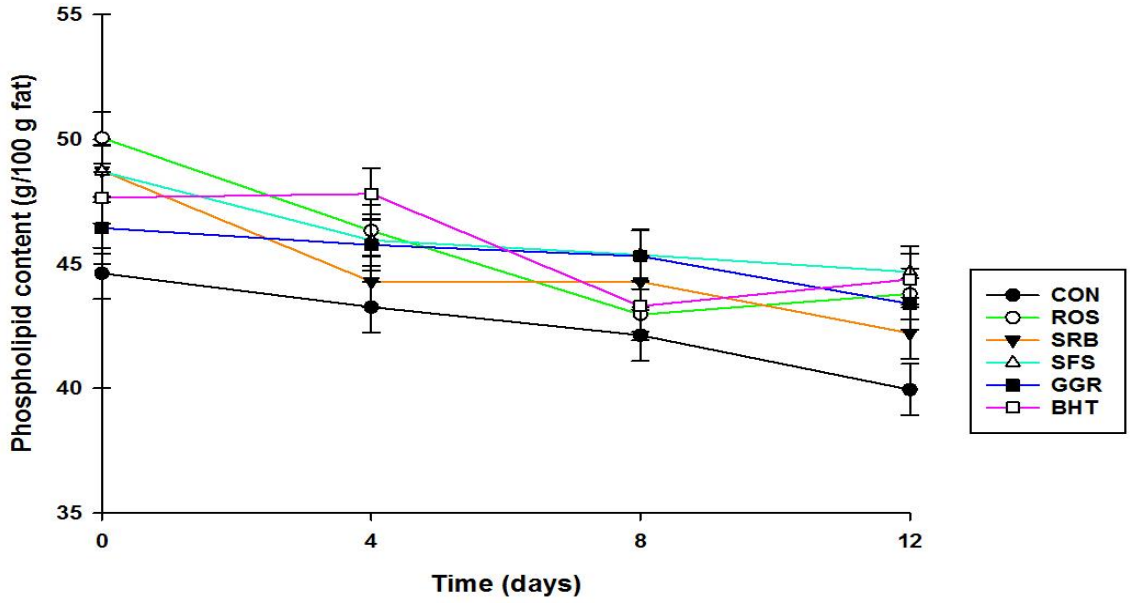

Figure 5.9 Effect of interaction of natural antioxidants application and storage time on phospholipid content of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

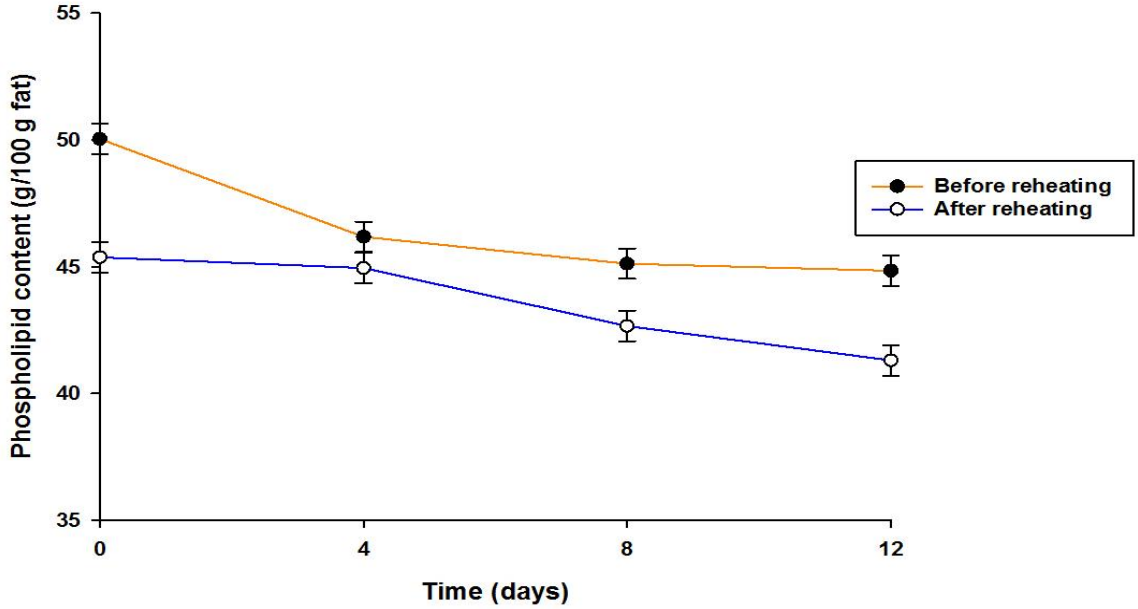

Figure 5.10 Effect of interaction of reheating process and storage time on phospholipid content of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

5.3.3 Effect of Natural Antioxidants on Conjugated Dienes (CDs)

5.3.3.1 Conjugated Dienes (CDs) in Sous-Vide (LTLO) Processed Chicken Meat

The average CDs in LTLO samples was 27.91 µmol/g fat which significantly lower ($p < 0.001$) than that in HTHO samples with mean value of 43.07 µmol/g fat (Appendix Table D2). Regardless of the reheating process and storage time, the effectiveness of natural antioxidants compared to the synthetic antioxidant BHT to inhibit the formation of CDs in LTLO samples were BHT > GGR > ROS > SRB > SFS > non-treated samples, with mean values 25.49, 27.07, 27.08, 28.49, 28.58 and 30.70 µmol/g fat respectively. The CD values in LTLO samples were not significantly affected by reheating process ($p = 0.137$). The storage time was found to have a significant effect on the CD values in LTLO samples ($p < 0.001$). The CD values were markedly increased with increasing time of storage, being at its highest therefore towards the end of storage time (Appendix Table D2). No significant interaction was found between antioxidant and reheating process for CD values ($p = 0.130$). Figure 5.11 shows a significant AO x ST interaction for CD values in LTLO samples ($p = 0.027$). The CD values in all samples increased with increasing storage time. However, non-treated samples were affected more compared to the samples treated with antioxidants (natural and BHT). The natural antioxidants that reduced the formation of CD in LTLO samples for 12 days compared to the non-treated control, were BHT, ROS and GGR (Figure 5.11). There was a significant interaction between reheating process and storage time ($p < 0.001$). The CD values in LTLO samples after reheating were significantly higher at day 0 and 4 of storage time, while at day 8, samples before reheating was found to have the highest CD values, with no significant changes toward the end of storage time (Figure 5.12). The results presented in Table 5.3 also show that

the interaction between antioxidant, reheating process and storage time was significant for CD values ($p = 0.043$). Before reheating LTLO samples, antioxidant supplementation reduced CDs in LTLO samples compared to the non-treated samples (Table 5.3). No significant differences were observed between treated and non-treated samples for up to 8 days of the storage time. At day 12, the CD values were significantly reduced in ROS treatments with mean values of 25.60 vs. 31.52 µmol/g fat for non-treated. The most natural antioxidants were found to have lower CD values compared to the BHT. Moreover, CD values were increased significantly in non-treated and SFS up to 8 days of storage and decreased at the end of the storage time. After reheating LTLO samples, no significant differences were observed between non-treated and most treated samples (with the exception of the samples treated with SRB extract) up to 8 days. At day 12, GGR treatment was found to have the lowest formation of CDs with values of 26.29 µmol/g fat compared to 32.90 µmol/g fat observed in non-treated samples. The reheating process had an effect on CDs in samples treated with SRB and ROS at days 4 and 12 (Table 5.3).

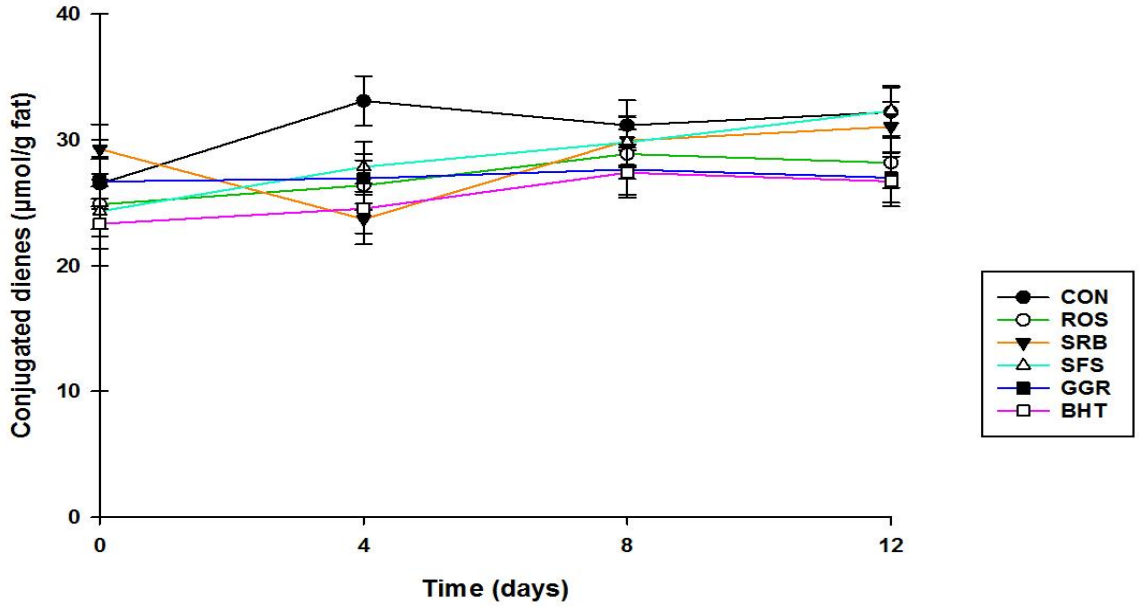

Figure 5.11 Effect of interaction of natural antioxidants application and storage time on conjugated dienes of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

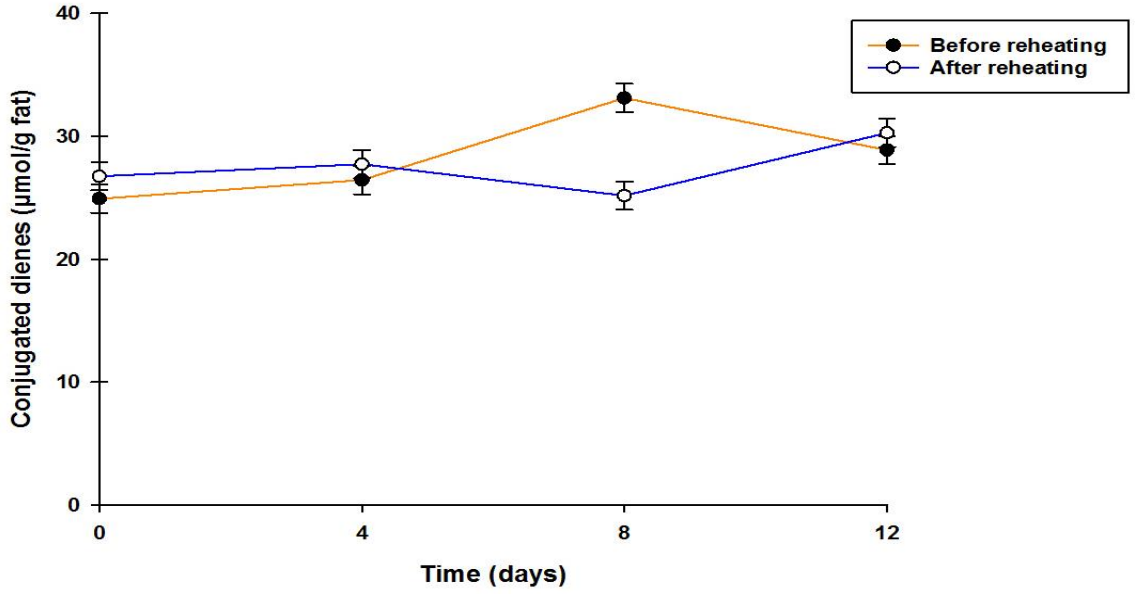

Figure 5.12 Effect of interaction of reheating process and storage time on conjugated dienes of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).

Table 5.3 Effect of natural antioxidants application on conjugated dienes (μmol/g fat) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	27.26^{Aa}	25.76^{Aa}	22.36^{Aa}	24.29^{ABa}	23.24^{Aa}	26.52^{Aa}	26.56^{Aa}	31.99^{Ca}	23.21^{Aa}	25.43^{Aa}	26.88^{Aa}	26.5^{ABa}
4		32.55^{Bb}	33.68^{Cb}	23.42^{Aab}	25.65^{ABab}	24.45^{Aab}	28.34^{Aab}	29.31^{Ab}	18.08^{Aa}	25.75^{ABab}	29.98^{Ab}	23.22^{Aab}	30.66^{Bb}
8		35.22^{Ca}	27.12^{ABa}	30.94^{Aa}	23.86^{Aa}	32.71^{Aa}	25.04^{Aa}	34.41^{Aa}	25.49^{Ba}	34.06^{Ba}	25.56^{Aa}	31.37^{Aa}	23.94^{Aa}
12		31.52^{Bcd}	32.9^{BCd}	26.50^{Aab}	26.92^{Babc}	25.60^{Aa}	30.75^{Abcd}	31.75^{Acd}	30.34^{Cabcd}	30.18^{ABabcd}	34.47^{Ad}	27.72^{Aabc}	26.29^{Aab}
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	55.91^{Bd}	47.01^{Abcd}	48.64^{Abcd}	36.60^{Babc}	52.37^{Acd}	26.44^{Aa}	59.47^{Bd}	46.99^{Cbcd}	56.52^{Bd}	32.04^{Aab}	60.07^{Bd}	47.43^{ABbcd}
4		55.80^{Bd}	46.21^{Abcd}	48.31^{Abcd}	39.28^{Babcd}	41.81^{Aabcd}	37.62^{Aabc}	38.79^{Aabcd}	51.79^{Ccd}	41.24^{Aabcd}	32.89^{Aab}	28.47^{Aa}	48.96^{Bbcd}
8		43.76^{Aab}	60.83^{Bc}	32.69^{Aa}	32.79^{ABa}	35.35^{Aab}	36.18^{Aab}	39.13^{Aab}	40.75^{Bab}	35.52^{Aab}	47.18^{Bb}	39.35^{Aab}	37.00^{Aab}
12		64.81^{Cd}	68.72^{Cd}	37.86^{Aabc}	28.81^{Aa}	40.16^{Abc}	31.42^{Aab}	33.67^{Aab}	30.13^{Aab}	59.93^{Bd}	30.78^{Aab}	34.33^{Aab}	45.65^{ABc}

Mean values with different small superscript letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each cooking method/treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.3.2 Conjugated Dienes (CDs) in Grilled (HTHO) Processed Chicken Meat

A similar trend was seen in HTHO samples as supplementation with either natural or synthetic antioxidants significantly reduced the formation of CDs in all HTHO samples compared to the non-treated control. The antioxidants that had the lowest formation of CD in HTHO was ROS. Natural antioxidants that performed similarly to BHT was ROS and SFS (Appendix Table D2). The CD values were significantly affected by reheating process ($p < 0.001$). Hence, HTHO samples after reheating significantly decreased compared to those before reheating (Appendix Table D2). The storage time had a significant effect on the CD values in HTHO ($p < 0.001$). The CD values were significantly decreased at day 4 compared to day 0, with no significant changes thereafter. A significant interaction ($p < 0.001$) was found between antioxidant and reheating process for CD values (Figure 5.13). Treated samples either before and after reheating process with natural antioxidants were found to have the lowest lipid oxidation compared to the non-treated control. However, the CD values were found higher in ROS, SFS and BHT samples before reheating process compared to those after reheating (Figure 5.13). A significant AO x ST interaction was found for CD values in HTHO samples ($p < 0.001$). The CD values in untreated samples increased with increasing storage time, while treated samples with natural antioxidants were provided a greater protection against an increase of CD values (Figure 5.14). There was significant interaction between reheating process and storage time ($p < 0.001$). The CD values in HTHO samples before reheating were significantly higher at day 0 and 12 days of storage time, while at day 8, samples after reheating was found to have the highest CD values (Figure 5.15). The results listed in Table 5.3 also show that a significant AO x RH x ST interaction was found for CD values in HTHO samples ($p < 0.001$). Supplementation with either natural or synthetic

antioxidants did not reduce the formation of CDs in all HTHO samples before reheating (with the exception of the samples treated with GGR extract) compared to the non-treated samples up to 8 days (Table 5.3). In contrast, on day 12, the lowest CDs formation was detected in SRB and GGR treatments compared to the non-treated samples with values of 33.67, 34.33 vs. 64.81 µmol/g fat for non-treated samples. No significant differences were found between natural antioxidants and BHT at each interval of storage, which shows that natural antioxidants performed similarly to BHT. After reheating HTHO samples, antioxidants reduced the accumulation of CDs in reheated samples compared to the non-treated samples over storage, while a significant reduction was observed on day 8 and 12 of storage time. No significant differences were found between both natural and BHT antioxidant treatments over 12 days.

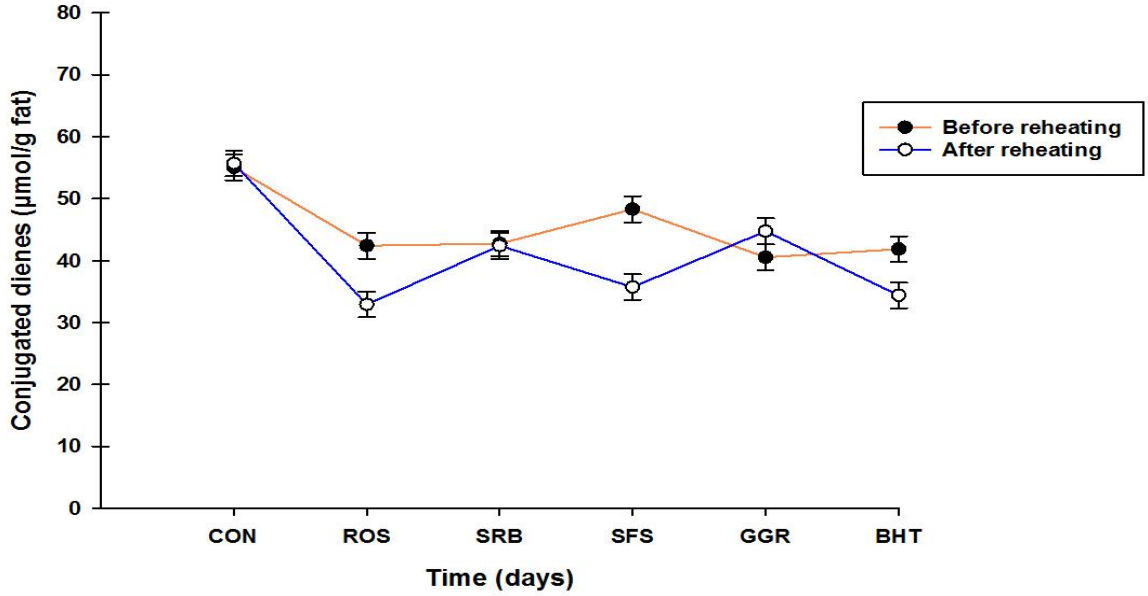

Figure 5.13 Effect of interaction of natural antioxidants application and reheating process on conjugated dienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

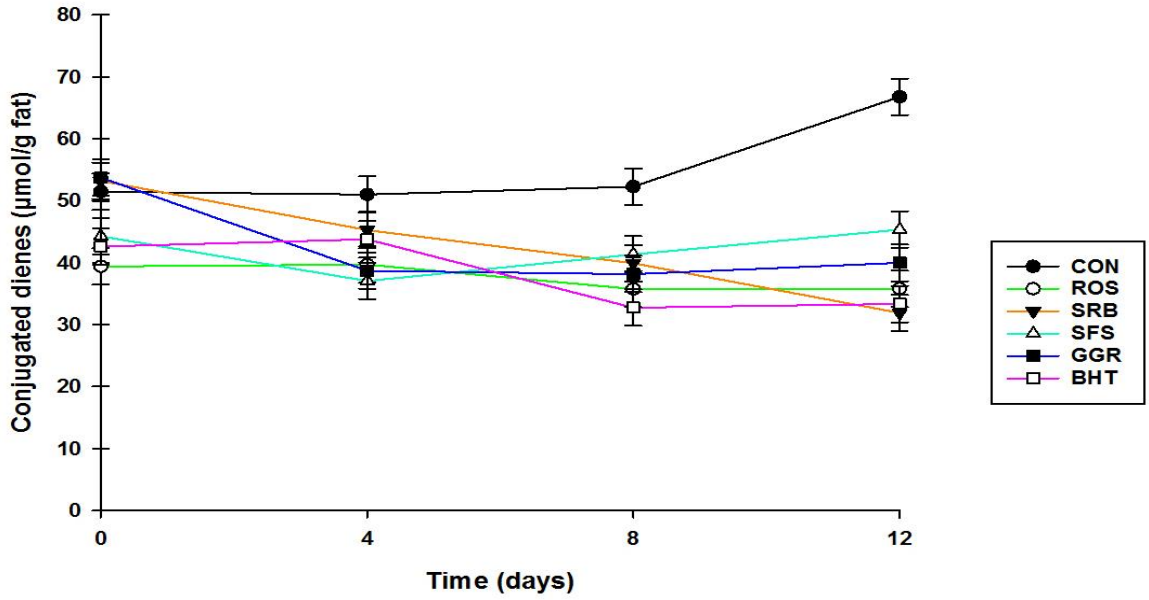

Figure 5.14 Effect of interaction of natural antioxidants application and storage time on conjugated dienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

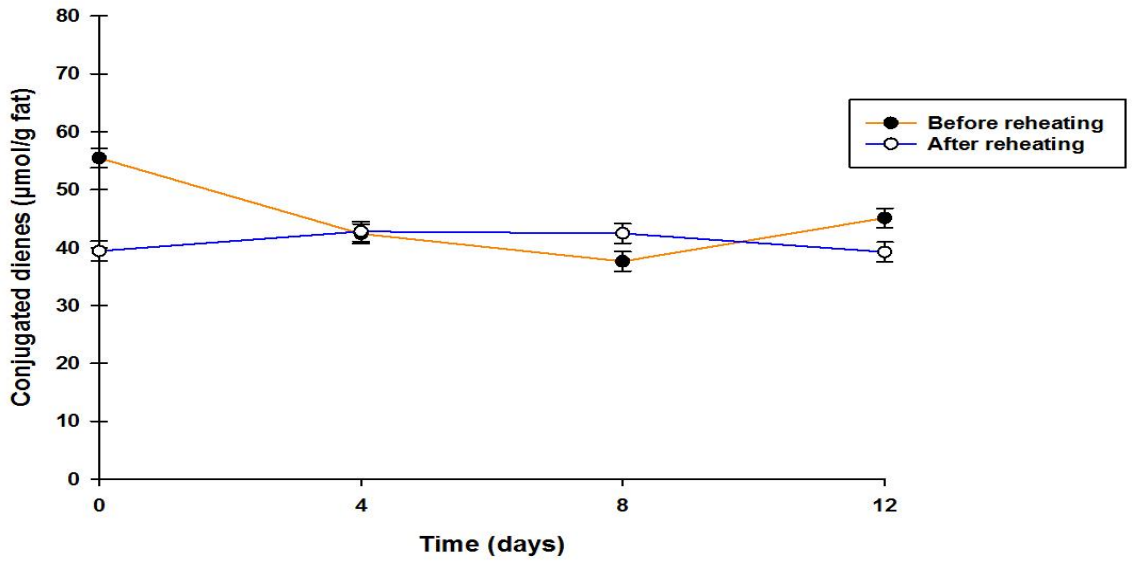

Figure 5.15 Effect of interaction of reheating process and storage time on conjugated dienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

5.3.4 Effect of Natural Antioxidants on Conjugated Trienes (CTs)

5.3.4.1 Conjugated Trienes (CTs) in Sous-Vide (LTLO) Processed Chicken Meat

The influence of the initial cooking process on the CT values was much greater in the HTHO (grilled) breast meat compared to the LTLO (sous vide) breast meat, with an initial CT value of 19.17 and 13.28 µmol/g fat, respectively (Appendix Table D3). Regardless of the storage time and reheating process, the effectiveness of natural antioxidants and the synthetic antioxidant BHT to inhibit the formation of CTs in LTLO samples were BHT > SRB > GGR > SFS > ROS > non-treated samples, respectively. The CT in LTLO samples was not affected significantly by reheating process ($p = 0.422$). Storage time significantly increased the CT values over 4 days, found no effect at day 8 and 12 (Appendix Table D3). Figure 5.16 shows a significant AO x RH interaction was found

for CT values ($p = 0.040$). All natural antioxidants before subjecting to reheating process were significantly reduced the formation of CT compared to the non-treated samples, while after reheating process only SRB extracted significantly inhibited the formation of CT compared to non-treated samples (Figure 5.16). No significant difference was found between antioxidant and storage time for CT values ($p = 0.350$). The results presented in Figure 5.17 show that a significant interaction was found between reheating process and storage time ($p < 0.001$). The CT values were found significantly higher in LTLO samples before reheating at day 8 compared to those after reheating process, while the CT values were therefore significantly higher in samples after reheating process (Figure 5.17). A significant AO x RH x ST interaction ($p = 0.004$) was found for CT values in LTLO samples (Table 5.4). Application of antioxidants to LTLO samples provided a higher protection compared to the non-treated samples for 12 days, despite statistically not differing. The results also showed that there was a significant effect of storage time on the formation of CTs in non-treated samples. In addition, after reheating LTLO samples, on day 4 of storage, SRB extract had the lowest CT value of 9.44 µmol/g fat compared to non-treated samples with a value of 17.22 µmol/g. At day 12 of storage, the lower formation of CTs was found in samples treated with GGR with values of 12.10 µmol/g fat vs. 15.34 µmol/g fat for non-treated. The results of natural antioxidants were similar to BHT as no significant differences were detected between both.

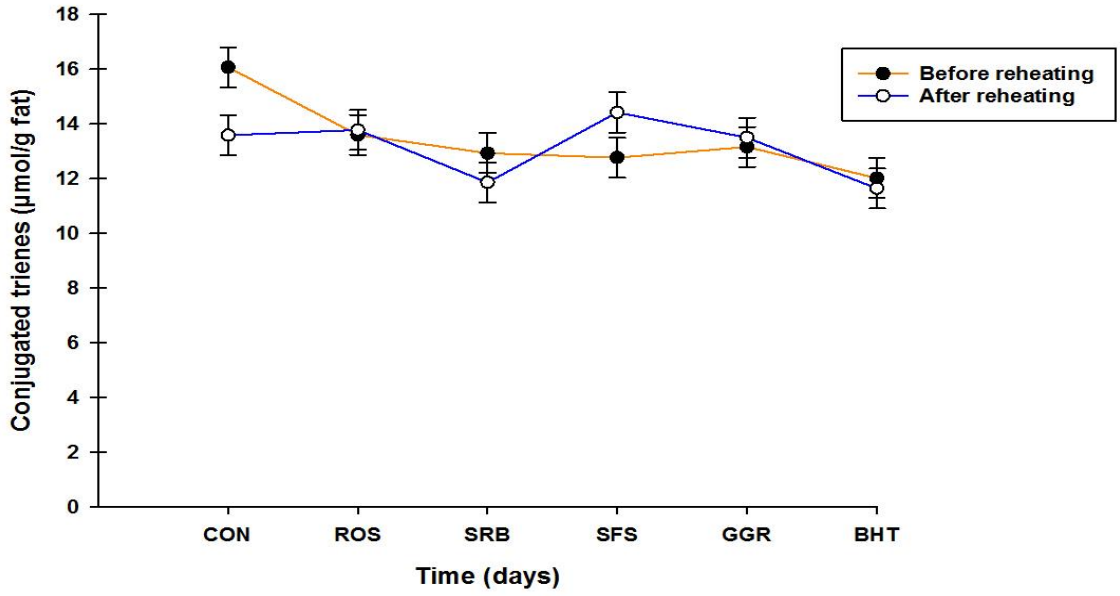

Figure 5.16 Effect of interaction of natural antioxidants application and reheating process on conjugated trienes of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

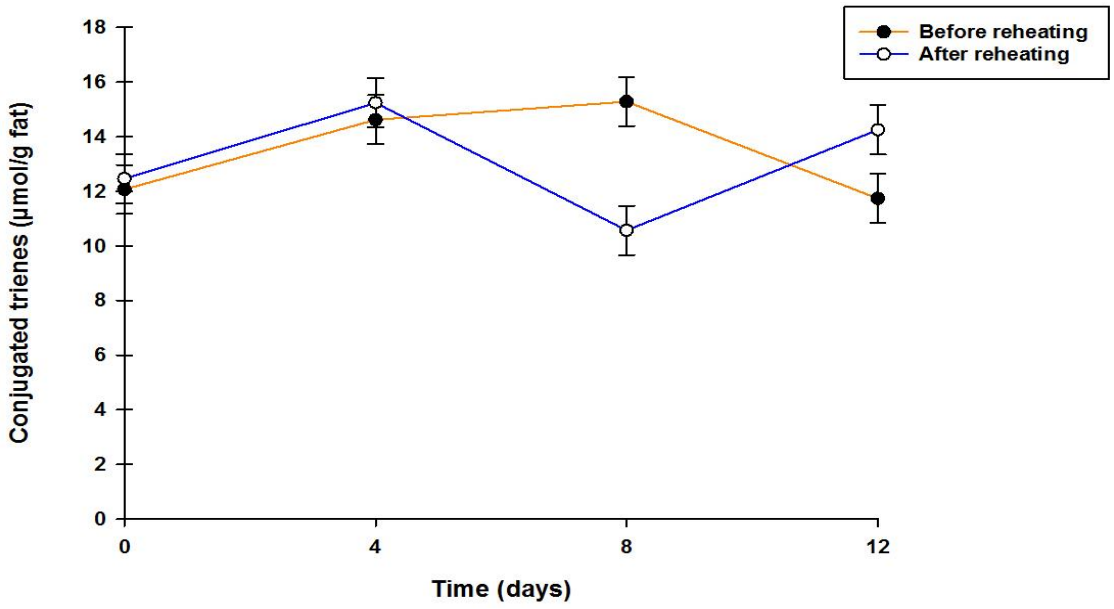

Figure 5.17 *Effect of interaction of reheating process and storage time on conjugated trienes of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).*

Table 5.4 Effect of natural antioxidants application on conjugated trienes (μmol/g fat) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	16.43^{Ba}	10.38^{Aa}	10.99^{Aa}	11.72^{Ba}	11.81^{Aa}	11.70^{Aa}	10.32^{Aa}	15.08^{Ca}	10.08^{Aa}	12.28^{Aa}	12.77^{Aa}	13.62^{Ba}
4		17.38^{Bb}	17.22^{Cb}	12.96^{Aab}	13.81^{Cab}	14.44^{Aab}	16.85^{Aab}	15.91^{Aab}	9.44^{Aa}	13.34^{ABab}	16.96^{Aab}	13.68^{Aab}	17.16^{Cb}
8		17.51^{Bb}	11.40^{ABab}	13.42^{Aab}	9.00^{Aa}	16.49^{Aab}	11.79^{Aab}	14.43^{Aab}	9.54^{Aa}	15.59^{Bab}	10.59^{Aab}	14.23^{Aab}	11.11^{Aab}
12		12.96^{Aabc}	15.34^{BCcd}	10.69^{Aa}	12.04^{Bab}	11.65^{Aa}	14.80^{Abcd}	11.06^{Aa}	13.39^{Babc}	12.08^{ABab}	17.83^{Ad}	11.97^{Aa}	12.10^{ABab}
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	31.81^{Bcd}	21.27^{Aabc}	27.26^{Bbcd}	13.87^{Bab}	29.44^{Bcd}	10.86^{ABa}	37.20^{Bd}	25.01^{Cabcd}	31.77^{Ccd}	10.87^{ABa}	34.16^{Bcd}	23.20^{ABabcd}
4		29.89^{Bc}	23.09^{Aabc}	29.07^{Bbc}	17.58^{Cabc}	23.05^{ABabc}	19.33^{Babc}	20.28^{Aabc}	30.00^{Dc}	20.58^{Babc}	$15.39B^{Cab}$	11.88^{Aa}	29.58^{Bc}
8		12.97^{Aab}	25.19^{Ac}	10.10^{Aa}	9.80^{Aa}	10.72^{Aa}	10.47^{ABa}	11.76^{Aa}	13.08^{Bab}	10.57^{Aa}	18.68^{Cb}	12.05^{Aa}	13.22^{Aab}
12		30.62^{Bc}	39.20^{Bd}	10.07^{Aa}	10.83^{Aab}	13.65^{ABab}	7.30^{Aa}	9.55^{Aa}	7.62^{Aa}	30.31^{Cc}	7.49^{Aa}	9.64^{Aa}	18.87^{Ab}

Mean values with different small superscript letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each cooking method/treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.4.2 Conjugated Trienes (CTs) in Grilled (HTHO) Processed Chicken Meat

The protective influence of natural antioxidants on high-temperature/high-oxygen (HTHO) processed chicken meat was also observed (Appendix Table D3). Regardless of the storage time and reheating process, the effectiveness of natural antioxidants and synthetic antioxidant BHT to inhibit the formation of CTs in HTHO samples were ROS > BHT > SFS > GGR > SRB > non-treated samples, with mean values 15.60, 16.07, 18.21, 19.07, 19.31 and 26.75 µmol/g fat respectively. The CT values were significantly affected by reheating process ($p < 0.001$). Hence, HTHO samples after reheating significantly decreased compared to those before reheating (Appendix Table D3). The storage time had a significant effect on the CT values in HTHO ($p < 0.001$). The CT values were significantly decreased with increasing storage time ($p < 0.001$) (Appendix Table D3). Figure 5.18 shows a significant interaction was found between antioxidant and reheating process for CT values ($p < 0.001$). Treated samples either before and after reheating process were found to have the lowest lipid oxidation compared to the non-treated control. The CT values were significantly higher in ROS, SFS and BHT samples before reheating process compared to those after reheating (Figure 5.18). A significant AO x ST interaction was found for CT values in HTHO samples ($p < 0.001$). The CT values in all samples decreased at day 8 of storage time and increased therefore at day 12. Treated samples with natural antioxidants were provided a greater protection against an increase of CT values over time, while the highest reduction of CT by natural antioxidants was found at day 12 (Figure 5.19). There was a significant interaction between reheating process and storage time ($p < 0.001$). The CT values in HTHO samples before reheating were significantly higher at day 8 of storage time compared to those after reheating (Figure 5.20). The results reported in Table 5.4 show that a significant AO x RH x ST

interaction for CT values in HTHO samples ($p < 0.001$). The values of CT in treated samples of HTHO was not significantly different from non-treated samples at day 0. On day 4, GGR treatment had significantly lower CT values compared to the non-treated samples. Although the CTs of meat samples were not affected by treatments on day 8, samples treated with antioxidants provided a higher protection. At day 12, lower CTs were detected in SRB and GGR treatments with values of 9.55 and 9.64 µmol/g fat vs. 30.31, and 30.62 µmol/g fat for SFS treatment and non-treated samples respectively. The natural antioxidants influence on CTs, particularly SRB, were similar to those treated with BHT. Furthermore, the influence of the storage time on CT values was much greater in non-treated samples compared to those treated with antioxidants over 12 days. After reheating HTHO samples, CTs were not affected by treatments after the first 4 days of storage. Whilst, on days 8 and 12, natural antioxidants reduced CT values significantly and among natural antioxidants ROS was found to have the lowest formation of CTs. The results of natural antioxidants were found to be similar to those treated with BHT.

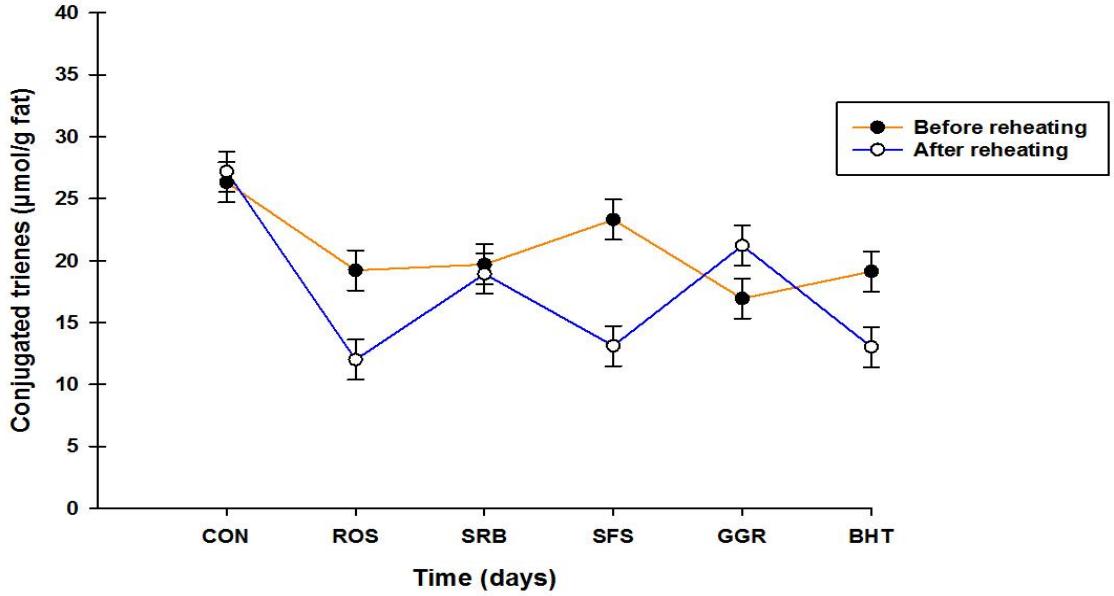

Figure 5.18 Effect of interaction of natural antioxidants application and reheating process on conjugated trienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

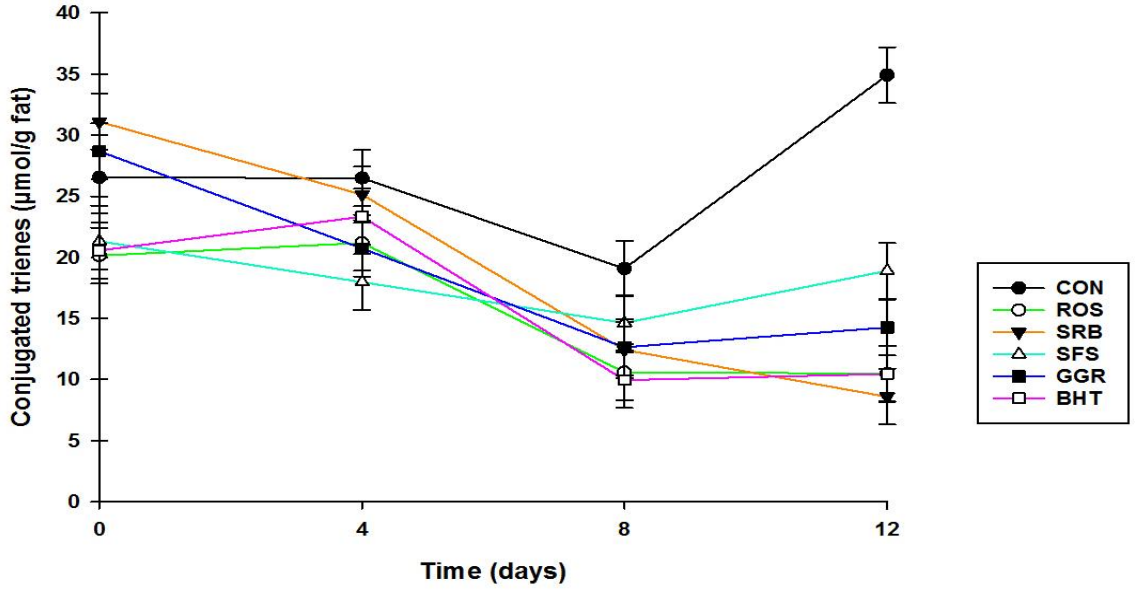

Figure 5.19 Effect of interaction of natural antioxidants application and storage time on conjugated trienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

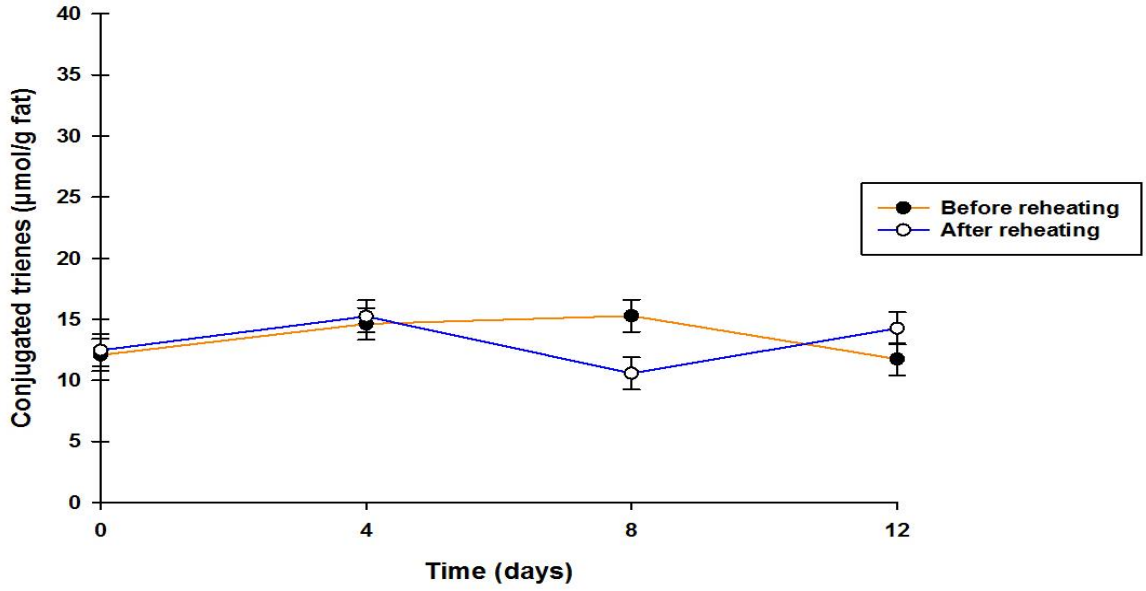

Figure 5. 20 Effect of interaction of reheating process and storage time on conjugated trienes of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

5.3.5 Effect of Natural Antioxidants on Texture (Shear Force)

The effect of antioxidants on the texture of cooked breast meat as indicated by shear force for 12 days of storage time are presented in Appendix Table D3. In general, samples cooked by the HTHO method had a higher shear force value than those cooked by LTLO method. The shear force of LTLO samples was not significantly affected by antioxidant supplementation, reheating process and interaction between three factors ($p > 0.05$). Whilst, shear force of LTLO samples was affected by storage time ($p = 0.001$). Hence, shear force significantly decreased in LTLO samples at day 4 and 12 of storage time compared to day 0 (Appendix Table D3). No significant interaction was found between three factors for shear force of LTLO samples ($p > 0.05$) (Table 5.5)

With respect to the HTHO samples, the results also showed that antioxidants had a significant ($p = 0.022$) an effect on shear force of HTHO samples (Appendix Table D3).

Hence, the effectiveness of natural antioxidants and the synthetic antioxidant BHT to enhance the meat texture (shear force) in HTHO samples was ROS > BHT > non-treated > GGR >SFS > SRD, respectively. The reheating process did not have any effect on the shear force of HTHO samples ($p = 0.818$). The shear force was significantly affected by storage time ($p = 0.004$). The shear force was decreased at day 12 compared to the day 0 (Appendix Table D3). No significant interaction was found between three factors for shear force ($p > 0.05$) (Table 5.5).

Table 5.5 Effect of natural antioxidants application on shear force of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	12.83	16.43	11.87	9.46	14.33	14.13	15.43	12.96	12.47	14.14	15.02	16.07
4		11.40	12.80	13.80	12.83	12.39	12.86	15.42	10.68	11.55	11.35	10.41	12.50
8		11.11	14.02	12.46	13.63	12.05	14.11	12.69	12.51	11.47	13.55	12.34	13.36
12		10.91	10.23	11.41	10.28	11.56	13.56	11.05	12.83	10.49	9.42	12.98	15.15
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	14.12	13.04	13.17	12.30	11.89	12.13	17.25	14.22	14.24	12.36	13.02	12.90
4		11.88	13.57	13.15	12.02	12.65	12.42	13.12	13.00	12.47	13.11	11.79	15.03
8		15.15	13.37	13.64	13.12	13.63	15.33	13.70	14.11	17.29	15.20	14.16	13.53
12		12.77	11.90	13.54	12.50	10.97	13.07	12.27	16.02	14.70	13.29	13.09	14.50

.

5.3.6 Effect of Natural Antioxidants on pH

5.3.6.1 pH in Sous-Vide (LTLO) Processed Chicken Meat

The influence of the initial cooking process on TBARS values was much greater in the LTLO (sous vide) compared to the HTHO (grilled) breast meat (Appendix Table D3). The antioxidant supplementation was found to have a significant effect on pH values of LTLO ($p < 0.001$). The pH values in natural antioxidants compared to the synthetic antioxidant BHT in LTLO samples were SFS > non-treated > SRB > GGR > BHT > ROS, respectively. (Appendix Table D3). Reheating process was found to have an effect on the pH values in LTLO samples ($p \leq 0.05$). Reheated samples were found to have the lowest pH values than those before reheating process. pH values of LTLO samples were significantly affected by storage time which pH values significantly increased ($p < 0.001$) with increasing storage time (Appendix Table D3). No significant interaction was found between antioxidant and reheating process for pH values ($p = 0.451$). A significant AO x ST interaction was found ($p < 0.001$). The natural antioxidants that found to have a lower pH values for 12 days compared to the non-treated samples was ROS (Figure 5.21). Figure 5.22 shows a significant interaction between RH x ST for pH values in LTLO samples ($p = 0.003$). The pH values in samples before and after reheating were found significantly increased at day 12 of storage time compared to day 0. At day 12, samples before reheating had higher pH values compared to those after reheating (Figure 5.22). A significant AO x RH x ST interaction was found for pH values ($p < 0.001$) (Table 5.6). Samples treated with antioxidants had lower pH values over 12 days of storage. However, significant differences were found only between ROS and non-treated samples on day 8 with pH values 6.09 vs. 6.42 respectively. No significant differences were found between

natural antioxidants and BHT. The pH values in all treatments, with the exception of non-treated samples and GGR, significantly increased with increasing storage time. Furthermore, after reheating LTLO samples, most of the meat samples that underwent antioxidant treatments had lower pH values than non-treated samples following storage over 12 days. Significantly higher pH values were found in GGR on day 8 with values of 6.47 vs. 6.20 for non-treated. Over storage time, reheating samples significantly reduced the pH values in non-treated samples only at day 8 of storage. A similar trend was observed in GGR treatment on day 8 as pH values increased after the reheating process.

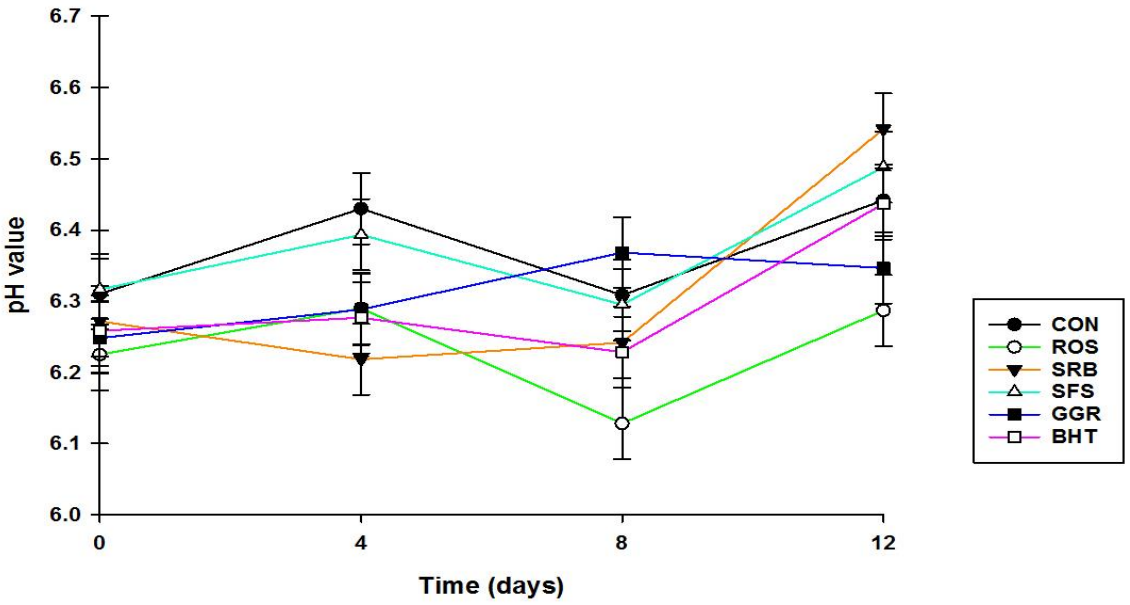

Figure 5.21 Effect of interaction of natural antioxidants application and storage time on pH values of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

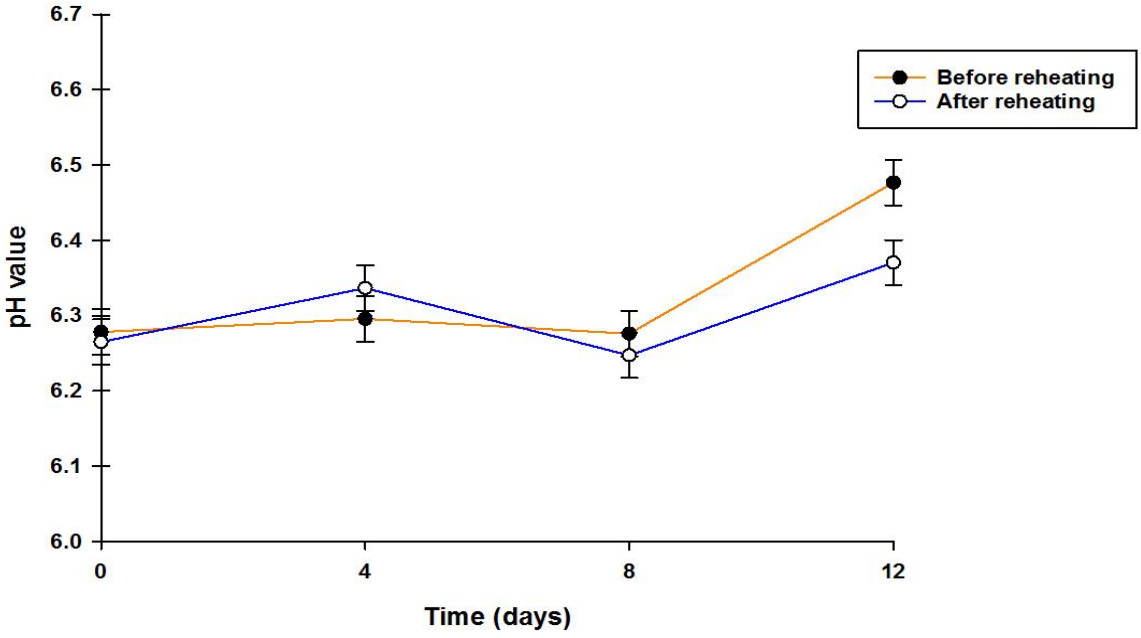

Figure 5.22 Effect of interaction of reheating process and storage time on pH values of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3).

Table 5.6 Effect of natural antioxidants application on pH values of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Sous Vide**	6.33^{Ab}	6.29^{ABab}	6.30^{Aab}	6.21^{Aab}	6.27^{Bab}	6.18^{Aa}	6.24^{Aab}	6.31^{ABab}	6.31^{Aab}	6.32^{ABb}	6.22^{Aab}	6.28^{ABab}
4		6.41^{Aab}	6.45^{Bb}	6.26^{Aab}	6.29^{Aab}	6.27^{Bab}	6.31^{Aab}	6.13^{Aa}	6.31^{ABab}	6.36^{Aab}	6.42^{Bab}	6.34^{Aab}	6.24^{Aab}
8		6.42^{Acd}	6.20^{Aab}	6.26^{Aabc}	6.19^{Aab}	6.09^{Aa}	6.17^{Aab}	6.31^{Abcd}	6.17^{Aab}	6.31^{Abcd}	6.28^{Abcd}	6.27^{Aabc}	6.47^{Bd}
12		6.42^{Aabc}	6.46^{Babc}	6.51^{Babc}	6.36^{Aabc}	6.31^{Bab}	6.26^{Aa}	6.62^{Bc}	6.46^{Babc}	6.61^{Bbc}	6.37^{ABabc}	6.38^{Aabc}	6.31^{ABab}
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**Grilled**	6.24	6.23	6.35	6.10	6.24	6.20	6.19	6.21	6.25	6.15	6.25	6.24
4		6.33	6.18	6.24	6.18	6.32	6.24	6.20	6.20	6.26	6.14	6.32	6.17
8		6.32	6.24	6.20	6.17	6.19	6.17	6.22	6.19	6.10	6.18	6.14	6.14
12		6.29	6.45	6.29	6.31	6.17	6.39	6.20	6.31	6.17	6.26	6.31	6.28

Mean values with different small superscript letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each cooking method/treatment differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.6.2 pH in Grilled (HTHO) Processed Chicken Meat

The protective influence of natural antioxidants on high-temperature/high-oxygen (HTHO) processed chicken meat was also observed (Appendix Table D3). The pH values of HTHO samples were significantly affected by the application of antioxidants ($p = 0.003$). The values of pH in SFS and SRB were found to have significantly lower than that in non-treated samples, while the remaining antioxidants ROS, GGR and BHT were not significantly different from non-treated samples. The pH values were not significantly affected by reheating process ($p = 0.170$). Regardless of the antioxidants and reheating process, pH values significantly increased at day 12 of storage time compared to the day 0, 4 and 8 ($p < 0.001$) (Appendix Table D3). No significant interaction was found between antioxidant and reheating process for pH values in HTHO samples ($p = 0.168$). The interaction between antioxidant and storage time was not significant shown for pH values ($p = 0.578$). A significant RH x ST interaction was found for pH values ($p < 0.001$). The pH values in samples before reheating were significantly higher than those before reheating at day 0 and 4 of storage time, while at day 12, the highest values of pH were shown in samples after reheating (Figure 5.23). No significant AO x RH x ST interaction was found for pH values ($p = 0.149$) (Table 5.6).

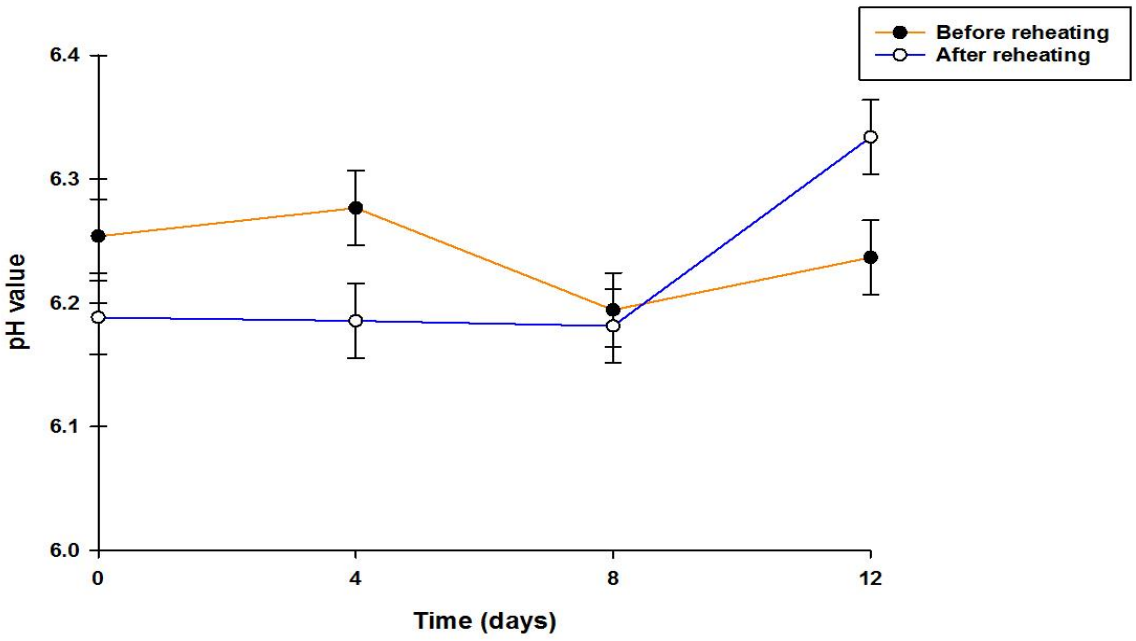

Figure 5.23 Effect of interaction of reheating process and storage time on pH values of grilled (HTHO) processed chicken breast meat (Means ± SED; n = 3).

5.3.7 Effect of Natural Antioxidants on Cooking Loss (CL)

Figure 5.24 shows that all samples (with exception SRB and SFS) cooked by the HTHO method had a significantly higher cooking loss than those cooked by LTLO method. The application of antioxidants had a significant effect on the cooking loss of LTLO and HTHO samples ($p \leq 0.05$). Cooking loss of LTLO samples treated with ROS was found to have a significantly lower cooking loss compared to the other treatments. On the contrary, all samples treated with antioxidants, other than those treated with SFS extract, and cooked by the HTHO method had a lower cooking loss than non-treated control. Among natural antioxidant treatments, ROS treatment was found to have the lowest cooking loss. These findings indicate that the effect of natural antioxidants, particularly ROS on cooking loss was comparable to the BHT.

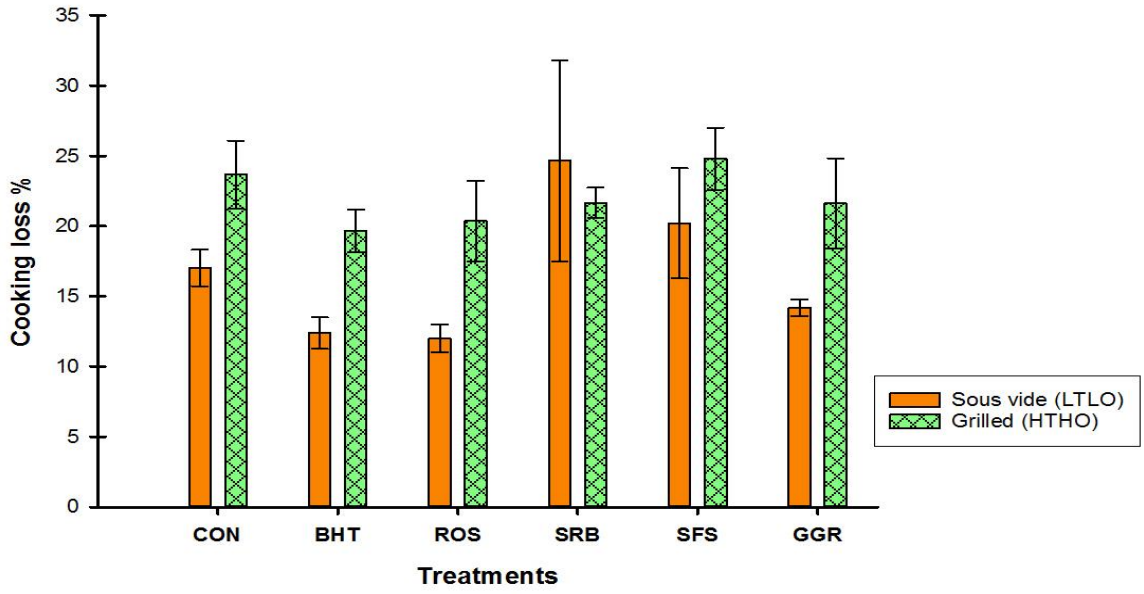

Figure 5.24 Effect of natural antioxidants application on cooking loss of chicken breast meat cooked by a 'Low Temperature & Low Oxygen' (Sous Vide) and a 'High Temperature & High Oxygen' (Grilled) cooking methods for raw chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

5.3.8 Effect of Natural Antioxidants on Colour

5.3.8.1 Colour in Sous-Vide (LTLO) Processed Chicken Meat

The effect of natural antioxidants compared to the non-treated and BHT on lightness (L*), redness (a*) and yellowness (b*) in cooked chicken breast meat during the storage time are listed in Tables 5.7 and Appendix Table D4. Application of natural antioxidants and storage time had a significant effect on lightness (L*) values ($p < 0.001$). The natural antioxidants that found to have significantly lower lightness values was ROS followed by SRB, GGR, SFS, BHT and non-treated samples, respectively. No significant differences were found between before and after reheating LTLO samples ($p = 0.946$). For the storage time, the lightness (L*) values of LTLO samples were increased up to 4 days of storage

time and significantly decreased thereafter ($p < 0.001$) (Appendix Table D4). The interaction between antioxidant x reheating process and between reheating process x storage time was not significantly found for L* values ($p = 0.597$; $p = 0.435$). There was a significant effect of antioxidant x storage time interaction ($p = 0.017$) on lightness (L*) values in LTLO samples (Figure 5.25). The lightness (L*) values were decreased at day 8 and increased thereafter at day 12 of storage time. All natural antioxidants (with exception SRB) were found to have significantly lower lightness values compared to the non-treated samples for 12 days of storage time, while ROS treatment had the lowest lightness (L*) values as compared to any other treatments. No significant AO x RH x ST interaction ($p = 0.590$) was found for lightness values (Table 5.7).

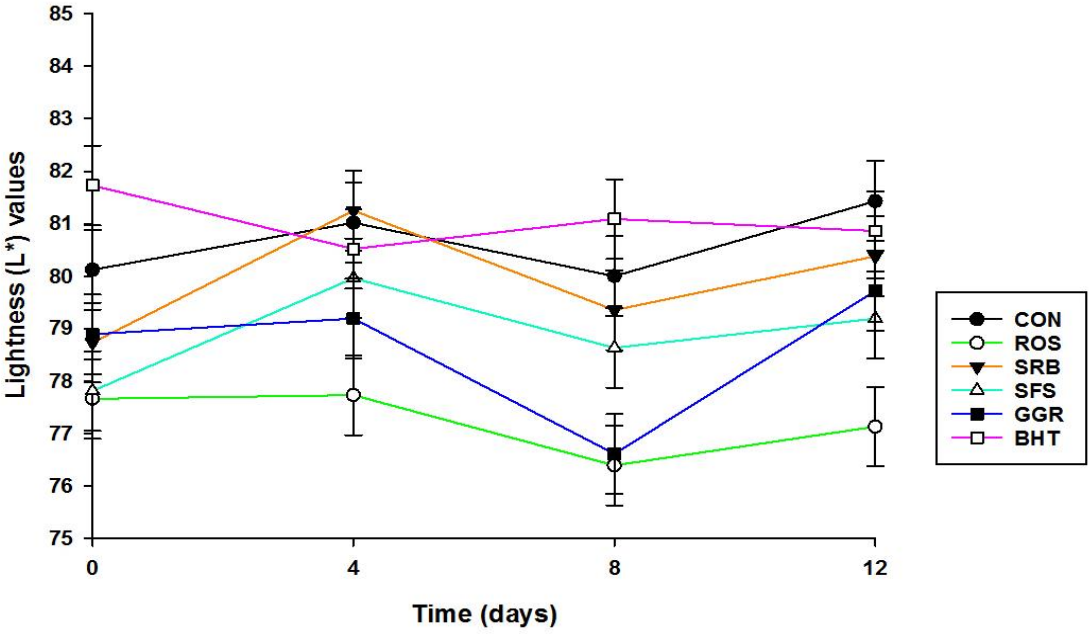

Figure 5.25 Effect of interaction of natural antioxidants application and storage time on lightness of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.

With respect to the redness (a*) value, antioxidant supplementation and storage time had significantly an effect on the redness (a*) values in LTLO samples ($p > 0.001$) (Appendix Table D4). Non-treated samples were found to have the highest a* values compared to any other treatments with mean values of 0.11, -0.16, -0.70, -0.81, -0.96 and -1.40 for non-treated samples, SFS, SRB, GGR, BHT and ROS, respectively. Reheating process did not have any effect on a* values ($p = 0.283$). The results also show that the storage time significantly reduced the redness value in LTLO meat samples up to day 4 and increased thereafter over 12 days (Appendix Table D4). No significant interaction ($p = 0.937$) was found between three factors for a* values in LTLO cooked samples (Table 5.7).

For yellowness (b*) values, the yellowness (b*) values were affected by antioxidant supplementation, reheating process and storage time ($p < 0.001$). Overall, regardless of the storage time and reheating process, yellowness b* values were found to have significantly higher ($p < 0.001$) in GGR treatment followed by ROS, SRB, non-treated, SFS and BHT, respectively (Appendix Table D4). After reheating LTLO cooked samples, b* value was significantly higher than in samples before reheating process ($p = 0.009$). The results also indicated that the storage time had a significant effect on the b* values in sous vide cause an increase of b* value up to day 8 and decreased thereafter at day 12 (Appendix Table D4). Only a significant AO x ST interaction ($p = 0.026$) was found for b* value of LTLO cooked samples (Figure 5.26). Over storage time, significant changes of b* values were shown only in samples treated with GGR compared to the non-treated samples (Figure 5.26). In addition, b* values were found significantly lower in both BHT and SFS at day 0 and 4, with no significant changes at day 8 and 12 of storage time. No

significant interaction (p = 0.974) was found between antioxidant, reheating process and storage time for b* values in LTLO samples (Table 5.7).

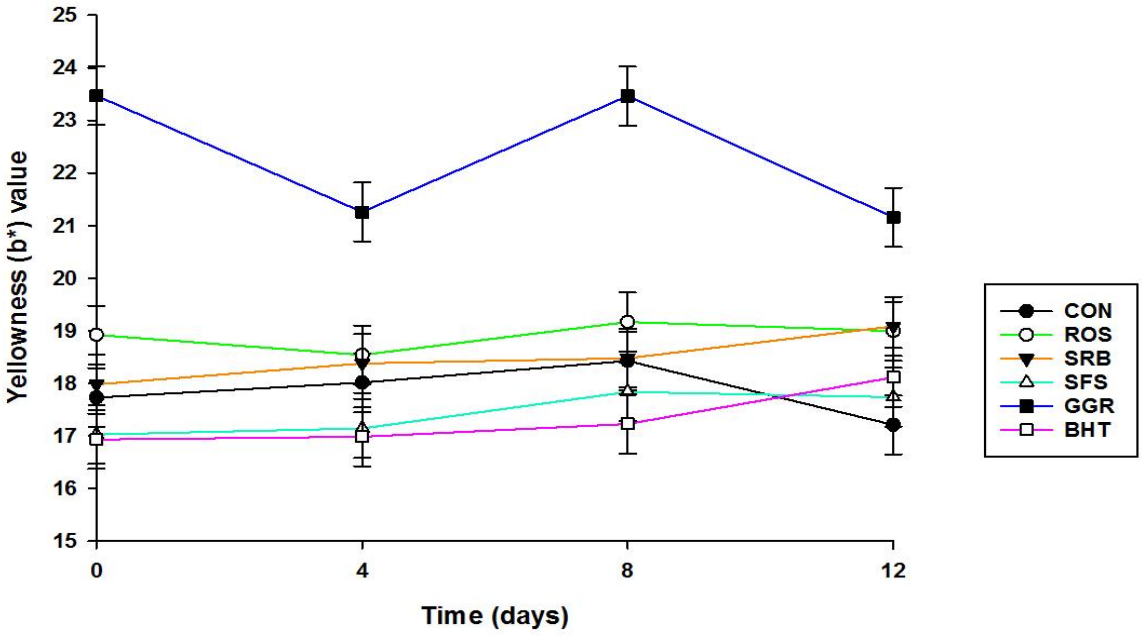

Figure 5.26 Effect of interaction of natural antioxidants application and storage time on yellowness (b) values of sous-vide (LTLO) processed chicken breast meat (Means ± SED; n = 3). Non-treated control (CON); butylated hydroxytoluene (BHT); Rosemary extract (ROS), Small Red Bean extract (SRB); Sun Flower Seed (SFS) extract; ginger (GGR) extract.*

Table 5.7 Effect of natural antioxidants application on L, a* and b* values of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 °C.*

Time (d)		Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**L* value**	81.33	78.91	82.10	81.37	76.94	78.38	78.60	78.86	78.28	77.33	79.08	78.69
4		80.82	81.22	80.13	80.91	77.54	77.92	81.01	81.48	79.84	80.08	78.93	79.45
8		80.58	79.41	80.59	81.59	77.67	75.11	79.04	79.66	78.77	78.49	76.18	77.04
12		81.38	81.49	80.45	81.26	77.38	76.87	80.39	80.37	78.78	79.61	79.74	79.69
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**a* value**	-0.24	0.10	-1.20	-1.26	-1.47	-1.67	-0.07	-0.73	0.11	-0.49	-0.94	-1.46
4		-0.18	-0.21	-1.13	-1.13	-1.38	-1.87	-1.16	-1.58	-0.61	-0.36	-0.88	-1.49
8		0.05	0.65	-0.84	-1.26	-1.40	-1.22	-0.32	-0.58	0.20	0.30	0.17	-0.20
12		0.03	0.70	-0.08	-0.80	-1.26	-0.59	-0.52	-0.63	0.17	-0.58	-1.00	-0.70
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**b* value**	17.32	18.13	16.76	17.11	18.60	19.24	17.36	18.60	17.10	16.95	22.60	24.34
4		17.25	18.78	16.63	17.33	18.35	18.73	17.96	18.80	16.80	17.49	21.21	21.31
8		18.31	18.54	17.20	17.26	19.16	19.18	18.36	18.60	18.15	17.52	23.70	23.23
12		17.01	17.41	17.85	18.38	18.97	19.01	18.86	19.31	17.27	18.20	21.30	21.02

5.3.8.2 Colour in Grilled (HTHO) Processed Chicken Meat

The lightness (L*) values in HTHO samples were not affected by antioxidants application ($p = 0.606$). A significant effect of reheating process was shown on L* values ($p = 0.001$) (Appendix Table D4). Samples before reheating were found to have significantly lower values of L* compared to those before after reheating process. Furthermore, storage time was found to have a significant an effect on L* values in HTHO samples ($p = 0.026$). The L* values were decreased up to 8 days, but significantly increased, therefore at day 12 compared to day 4 of storage time (Appendix Table D4). For redness (a*) values, antioxidant application was found to have significant an effect on redness values ($p = 0.017$). All natural antioxidants awarded a greater protection against the reduction of redness of HTHO samples compared to the non-treated samples, while GGR was found to have a higher a* values compared to any other treatments (Appendix Table D4). The reheating process significantly affected the redness values of HTHO ($p = 0.008$). Hence, a* values were found lower in samples before reheating compared to those after reheating process. Moreover, the redness values were not affected by storage time ($p = 0.663$) (Appendix Table D4). With respect to the yellowness (b*) values in HTHO samples, the effect of antioxidants on yellowness (b*) values in HTHO was not significant ($p = 0.105$) (Appendix Table D4). Whilst, the b* values were significantly affected by reheating process ($p = 0.019$). Reheated samples were found to have a higher yellowness values compared to those before reheating process. b* values were significantly increased ($p < 0.001$) at day 8 compared to day 0 and 4 and slightly declined at day 12 of storage time (Appendix Table D4). No significant AO x RH x ST interaction was found for lightness (L*), redness (a*) and yellowness (b*) values in HTHO ($p > 0.05$) (Table 5.8).

Table 5.8 Effect of natural antioxidants application on L, a* and b* values of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.*

Time (d)		**Controls**				**Natural Antioxidant Treatments**							
		CO	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**L* value**	81.94	84.88	83.87	84.88	83.38	84.46	85.25A	84.86	84.28	85.22	84.11	84.40
4		84.39	84.81	83.06	84.75	82.94	83.39	82.61A	84.46	82.04	84.27	81.72	83.97
8		82.60	84.21	85.27	84.68	83.53	85.30	83.08A	85.09	81.91	82.32	83.69	85.20
12		85.11	84.36	84.07	83.91	84.43	84.33	85.24A	84.89	84.09	85.41	84.24	84.97
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**a* value**	-0.80	-1.82	-0.98	-1.55	-0.98	-1.79	-1.37	-1.89	-1.73	-2.54	-1.28	-1.46
4		-1.93	-1.85	-1.32	-0.46	-1.48	-1.49	-1.39	-0.70	-1.04	-2.06	0.02	-1.84
8		-2.42	-2.16	-1.73	-0.96	-0.15	-1.55	-0.47	-1.55	-1.22	-1.19	-0.21	-2.05
12		-2.44	-1.83	-1.40	-1.38	-0.95	-2.06	-1.11	-0.91	-1.84	-1.00	0.31	-1.25
(d)		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
0	**b* value**	15.75	15.00	14.12	15.25	14.81	15.12	15.06	15.27	14.62	15.60	14.99	14.21
4		14.50	15.46	14.65	14.17	14.72	15.70	14.93	14.44	14.64	16.06	14.11	15.42
8		17.30	16.61	16.18	14.77	14.85	16.31	14.99	15.47	15.67	17.24	15.67	16.64
12		15.79	16.69	15.23	16.24	15.64	16.80	14.90	15.38	16.11	15.72	15.01	16.25

5.3.9 Effect of Natural Antioxidants on Fatty Acids

5.3.9.1 Fatty Acids in Sous-Vide (LTLO) Processed Chicken Meat

The fatty acid composition of the cooked chicken meat expressed as g/kg DM is shown in Tables 5.9 and 5.10. The influence of the initial cooking process on fatty acid composition was much greater in the HTHO breast meat compared to the LTLO breast meat (Appendix Table D5). Accordingly, LTLO samples were found to have a higher content of all fatty acids than those in HTHO samples. The application of antioxidants awarded a greater protection against a decrease of all saturated fatty acids (with exception C20:0), while GGR treatment yielded SFAs significantly ($p \leq 0.05$) more than non-treated samples, but did not differ from other antioxidant treatments (Appendix Table D5). Furthermore, SFAs in LTLO were not significantly affected by reheating process ($p > 0.05$). Storage time had a significant effect on all SFAs in LTLO samples ($p \leq 0.05$). All SFAs were decreased when storage time increased, while a significant reduction was found at day 12 compared to the day 4 (Appendix Table D5). A significant AO x RH interaction was found for C20:0 ($p = 0.026$). The content of C20:0 was significantly higher in ROS and SRB after reheating process, while in GGR and BHT treatment the highest amount of C20:0 was shown before reheating process. A significant interaction was found between antioxidant and storage time for all SFAs ($p \leq 0.05$). The content of SFAs in non-treated samples reduced significantly with time of storage, while all natural antioxidant treatments were awarded a greater protection against a decrease of SFAs at each interval of storage time. At day 0, GGR was found to have a higher content of SFAs, while SRB treatment had the highest content of SFAs at day 4. At day 8 and 12, SFS yielded significantly more amount of SFAs than any other treatments. Natural

antioxidants provided a higher protection compared to the non-treated samples and BHT as the highest reduction of SFAs were detected in non-treated and samples treated with BHT. A significant RH x ST interaction was found for all SFAs in LTLO samples ($p \leq 0.05$). The content of SFAs significantly reduced in samples after reheating process at day 0, with no significant differences between before and after reheating meat samples at day 4, 8 and 12 o storage time. The effect of interaction between three main factors are shown in Table 5.9. Treatment with natural antioxidants significantly reduced the decomposition of fatty acids in LTLO samples ($p \leq 0.05$). Saturated fatty acids (SFAs) in treated LTLO samples had a higher protection particularly at day 12, SFS had significantly the highest ($p \leq 0.05$) amount of C14:0, C16:0 and total SFAs compared to the non-treated sample, but antioxidant treatments did not have an effect on both fatty acids C18:0 and C20:0 ($p > 0.05$). Natural antioxidants provided higher protection compared to the BHT as the highest reduction of SFAs were detected in non-treated and samples treated with BHT. Similar findings were shown in samples treated with antioxidants after cooking samples were reheated.

The amount of monounsaturated fatty acids (with exception C16:1 n-7) in LTLO samples was significantly affected by antioxidant supplementation ($p \leq 0.05$). The natural antioxidants that provided a marked protection against a decrease of C18:1 n9c, C18:1 n9t and total of MUFAs was SFS compared to the non-treated samples. However, all natural antioxidants yielded more MUFAs than non-treated samples and BHT. Reheating process was no affected the amount of MUFAs in LTLO samples ($p > 0.05$). Storage time was significantly affected the content of all MUFAs ($p \leq 0.05$). The amount of all MUFAs decreased up to 8 days of storage time and slightly increased thereafter at day 12, while significant reduction was found at day 8 compared to day 0 and 4 of storage time

(Appendix Table D5). No significant interaction was found between antioxidant and reheating process for all MUFAs ($p > 0.05$). A significant interaction was found between antioxidant and storage time for all MUFAs ($p \leq 0.05$). The content of MUFAs in non-treated samples reduced significantly with time of storage, while all natural antioxidant treatments were awarded greater protection against a decreased of MUFAs at each interval of storage time. At day 0, GGR was found to have a higher content of MUFAs, while SRB and ROS treatments were found to have the highest content of MUFAs at day 4. At day 8 and 12, SFS yielded significantly more amount of MUFAs than any other treatments. Natural antioxidants provided higher protection compared to the non-treated samples and BHT. No significant interaction ($p > 0.05$) was found between reheating process and storage time for all MUFAs (Appendix Table D5). A significant AO x RH x ST interaction ($p \leq 0.05$) was found for all MUFAs in LTLO samples (Table 5.9). During the storage time, natural antioxidants ROS and GGR had the lowest degradation of MUFAs compared to the non-treated samples before reheating. A higher reduction of MUFAs was shown in BHT treatment compared to the natural antioxidant treatments. After samples were reheated, ROS and SFS had a higher amount of MUFAs at day 12 of storage time compared to the non-treated samples.

The amount of all PUFAs (with exception C22:5 n-3) in LTLO was significantly affected by the natural antioxidant ($p \leq 0.05$) (Appendix Table D5). The natural antioxidants that protected PUFAs from degradation were GGR, ROS and SFS compared to the non-treated samples (Appendix Table D5). The reheating process was found to have a significant effect on C20:4 n-6 and C20:5 n-3 in LTLO samples ($p \leq 0.05$). Hence, the amount of these fatty acids was higher in samples after reheating than those before reheating (Appendix Table D5). Furthermore, all PUFAs (with exception C22:6 n-3)

significantly affected by storage time ($p \leq 0.05$). The content of PUFAs was significantly decreased with storage time, significant reduction was found at day 12 compared to day 4 of storage time. No significant AO x RH interaction was found for all PUFAs in LTLO samples ($p > 0.05$). A significant AO x ST interaction was found for all PUFAs with exception C20.5 n-3 and C22:5 n-3 ($p \leq 0.05$) (Appendix Table D5). The amount of PUFAs in all samples (treated or untreated) was decreased over storage time. No significant differences were found between treated and non-treated samples at day 0, 4 and 8 of storage time, while at day 12, ROS awarded a greater protection against a decreased of PUFAs compared to the non-treated samples (Appendix Table D5). Table 5.9 shows a significant AO x RH x ST interaction ($p \leq 0.05$) for all PUFAs with exception C22.5 n-3. Over storage time no significant differences were found between antioxidant treatments and non-treated samples for C18:2 n-6, C18:3 n-3 and total PUFAs at all intervals of storage time, while only the significant differences were found between GGR before reheating process compared to the SFS after reheating process at day 0 of storage time (Table 5.9). The amounts of C20:4 n-6 and C22.5 n-3 was significantly higher only in GGR treatment after reheating process compared to the con-treated samples at day 0 and 8 of storage time, but no significant differences were found between treated and no-treated samples at day 4 and 12 of storage times. The natural antioxidants that yielded more content of C22:6 n-3 were found in samples treated with GGR and ROS compared to non-treated samples at day 0, while at day 4, 8 and 12 of storage time the significant effect of antioxidants on PUFAs did not found (Table 5.9).

The amount of ∑n-3 and ∑n-6 PUFAs in LTLO samples was significantly affected by the application of natural antioxidants ($p = 0.002$) (Appendix Table D5). The natural antioxidants that protected ∑n-3 and ∑n-6 PUFAs from degradation were GGR, ROS and

SFS compared to the non-treated samples. The reheating process did not affect the ∑n-3 and ∑n-6 PUFAs in LTLO samples ($p > 0.05$). Furthermore, both ∑n-3 and ∑n-6 PUFAs significantly affected by storage time ($p < 0.001$). The content of ∑n-3 and ∑n-6 PUFAs was significantly decreased with storage time, while a significant reduction was found at day 12 compared to day 0 and 4 of storage time (Appendix Table D5). No significant AO x RH interaction was found for ∑n-3 and ∑n-6 PUFAs in LTLO samples ($p > 0.05$). A significant AO x ST interaction was found for ∑n-3 and ∑n-6 PUFAs ($p \leq 0.05$). The amount of n-3 and n-6 PUFAs in all samples (treated or untreated) were decreased over storage time. No significant differences were found between treated and non-treated samples at day 0, 4 and 8 of storage time, while at day 12, ROS awarded a greater protection against a decreased of n-3 and n-6 PUFAs compared to the non-treated samples (Appendix Table D5). The results also show that a significant AO x RH x ST interaction was found for ∑n-3 and ∑n-6 PUFAs (Table 5.9). No significant differences were found between antioxidant treatments and non-treated samples for both for ∑n-3 and ∑n-6 PUFAs at each interval of storage time, while only the significant differences were found between GGR before reheating process compared to the SFS after reheating process at day 0 of storage time (Table 5.9). The impact of natural antioxidants on all fatty acids was similar to that of BHT.

Table 5.9 Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 ºC.

Fatty acid	Time (d)	Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C14:0	0	0.46^{Bab}	0.39^{Dab}	0.43^{ABab}	0.36^{Aab}	0.38^{BCab}	0.42^{Aab}	0.34^{Aab}	0.35^{Aab}	0.42^{Aab}	0.28^{Aa}	0.56^{Bb}	0.33^{Aab}
	4	0.38^{ABa}	0.33^{Ca}	0.50^{Ba}	0.38^{Aa}	0.33^{ABa}	0.5^{Aa}	0.37^{Aa}	0.46^{Aa}	0.48^{Aa}	0.31^{Aa}	0.28^{Aa}	0.48^{Ba}
	8	0.30^{ABa}	0.28^{Ca}	0.35^{ABa}	0.34^{Aa}	0.30^{Aa}	0.28^{Aa}	0.38^{Aab}	0.34^{Aa}	0.33^{Aa}	0.50^{Bb}	0.40^{ABab}	0.32^{Aa}
	12	0.25^{Aa}	0.25^{Aa}	0.29^{Aab}	0.31^{Aabc}	0.41^{Cbc}	0.42^{Abc}	0.36^{Aabc}	0.33^{Aabc}	0.43^{Ac}	0.43^{Bc}	0.41^{ABbc}	0.31^{Aabc}
C16:0	0	19.30^{Aab}	17.31^{Cab}	19.35^{ABab}	16.39^{Aab}	16.64^{ABab}	19.15^{Aab}	15.49^{Aab}	15.64^{Aab}	17.78^{Aab}	13.48^{Aa}	23.18^{Bb}	14.8^{Aab}
	4	16.61^{Aa}	15.39^{Ba}	21.68^{Ba}	17.74^{Aa}	14.72^{ABa}	22.32^{Aa}	17.39^{Aa}	20.53^{Aa}	20.73^{Aa}	14.11^{Aa}	13.29^{Aa}	20.18^{Ba}
	8	13.91^{Aa}	13.14^{Aa}	15.5^{ABa}	16.05^{Aa}	13.81^{Aa}	13.41^{Aa}	16.77^{Aa}	15.62^{Aa}	15.09^{Aa}	22.52^{Bb}	17.36^{ABa}	14.82^{Aa}
	12	11.97^{Aa}	12.21^{Aa}	13.56^{Aab}	14.16^{Aab}	17.98^{Bb}	18.66^{Ab}	15.81^{Aab}	15.11^{Aab}	18.39^{Ab}	18.87^{Bb}	17.29^{ABab}	14.75^{Aab}
C18:0	0	6.81^{Bab}	6.67^{Cab}	7.02^{ABab}	6.39^{Aab}	6.82^{Bab}	6.41^{Aab}	5.81^{Aa}	5.98^{Aa}	6.39^{Aab}	5.32^{Aa}	8.17^{Ab}	6.59^{Ba}
	4	6.58^{ABa}	5.96^{Ba}	7.46^{Ba}	6.95^{Aa}	6.09^{ABa}	7.96^{Aa}	6.32^{Aa}	7.63^{Aa}	7.39^{Aa}	5.88^{ABa}	5.78^{Aa}	7.48^{Ca}
	8	5.59^{ABab}	5.25^{Aa}	6.40^{ABab}	6.30^{Aab}	5.75^{Aab}	5.81^{Aab}	6.96^{Abc}	6.28^{Aab}	6.07^{Aab}	8.02^{Cc}	7.05^{Abc}	6.50^{Babc}
	12	5.30^{Aa}	5.71^{ABab}	5.44^{Aab}	5.73^{Aab}	6.83^{Bab}	6.94^{Ab}	6.10^{Aab}	6.09^{Aab}	6.92^{Ab}	6.68^{Bab}	6.37^{Aab}	5.46^{Aab}
C20:0	0	0.43^{Aab}	0.43^{Bab}	0.46^{Aab}	0.37^{Aab}	0.41^{Aab}	0.46^{Aab}	0.33^{Aa}	0.35^{Aab}	0.44^{Aab}	0.30^{Aa}	0.53^{Bb}	0.33^{Aa}
	4	0.38^{Aa}	0.37^{ABa}	0.49^{Aa}	0.40^{Aa}	0.31^{Aa}	0.49^{Aa}	0.4^{Aa}	0.53^{Aa}	0.44^{Aa}	0.35^{Aa}	0.32^{Aa}	0.47^{Ba}
	8	0.34^{Aa}	0.35^{Aa}	0.40^{Aa}	0.37^{Aa}	0.36^{Aa}	0.32^{Aa}	0.38^{Aa}	0.33^{Aa}	0.37^{Aa}	0.56^{Bb}	0.39^{ABa}	0.34^{Aa}
	12	0.31^{Aa}	0.32^{Aab}	0.31^{Aab}	0.30^{Aa}	0.39^{Aab}	0.48^{Ab}	0.36^{Aab}	0.38^{Aab}	0.41^{Aab}	0.42^{ABab}	0.38^{ABab}	0.35^{Aab}
C16:1n-7	0	2.46	2.16	2.37	1.88	1.45	2.73	2.05	1.59	2.37	1.79	2.59	1.26
	4	1.90	1.71	2.83	2.14	1.59	2.62	2.45	2.33	2.51	1.44	1.42	2.25
	8	1.65	1.44	1.21	1.75	1.42	1.29	1.37	1.80	1.80	2.78	1.70	1.40
	12	1.04	0.96	1.45	1.62	1.92	2.25	1.69	1.94	2.29	2.28	1.89	2.00
C18:1 n-9c	0	33.07^{Aab}	30.22^{Cab}	34.99^{ABab}	27.28^{Aab}	30.00^{ABab}	33.56^{Aab}	25.61^{Aab}	28.22^{Aab}	30.40^{Aab}	20.98^{Aa}	41.93^{Bb}	25.37^{Aab}
	4	28.63^{Aa}	24.95^{Ba}	38.57^{Ba}	31.19^{Aa}	26.91^{ABa}	40.25^{Aa}	29.60^{Aa}	37.61^{Aa}	36.67^{Aa}	24.95^{ABa}	22.35^{Aa}	36.94^{Ba}
	8	23.73^{Aa}	21.30^{Aa}	26.77^{ABa}	27.86^{Aa}	$23.52^{A}a$	21.66^{Aa}	30.63^{Aab}	25.09^{Aa}	26.30^{Aa}	39.62^{Cb}	29.98^{ABab}	26.28^{Aa}
	12	20.37^{Aab}	19.15^{Aa}	22.80^{Aabc}	23.20^{Aabc}	31.98^{Bbc}	33.75^{Ac}	27.46^{Aabc}	26.74^{Aabc}	32.07^{Abc}	32.49^{BCc}	29.70^{ABabc}	24.89^{Aabc}

Table 5.9 (Continued) Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 °C.

Fatty acid	Time (d)	Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C18:1 n-9t	0	2.41^{Aa}	2.13^{Ca}	2.49^{ABa}	2.14^{Aa}	2.08^{ABa}	2.62^{Aa}	2.04^{Aa}	2.17^{Aa}	2.28^{Aa}	1.85^{Aa}	2.74^{Ba}	2.06^{Aa}
	4	2.06^{Aa}	2.04^{BCa}	2.67^{Ba}	2.26^{Aa}	2.05^{ABa}	2.58^{Aa}	2.35^{Aa}	2.57^{Aa}	2.57^{Aa}	1.96^{Aa}	1.75^{Aa}	2.54^{Ba}
	8	1.92^{Aa}	1.87^{Ba}	1.97^{ABa}	2.13^{Aa}	1.88^{Aa}	1.78^{Aa}	2.16^{Aa}	1.93^{Aa}	1.92^{Aa}	2.84^{Bb}	2.10^{ABa}	1.95^{Aa}
	12	1.70^{Aa}	1.59^{Aa}	1.76^{Aa}	1.89^{Aa}	2.20^{Ba}	2.37^{Aa}	2.02Aa	2.07Aa	2.36^{Aa}	2.39^{ABa}	2.19^{ABa}	2.00^{Aa}
C18:2 n-6	0	20.19^{Aab}	18.84^{Cab}	21.30^{Bab}	17.75^{Aab}	21.20^{Aab}	18.87^{ABab}	16.39^{Aab}	17.82^{Aab}	18.77^{ABab}	13.67^{Aa}	27.57^{Ab}	17.45^{Aab}
	4	18.04^{Aa}	15.05^{Ba}	23.01^{Ba}	19.66^{Aa}	16.70^{Aa}	24.63^{Ba}	17.52^{Aa}	24.28^{Aa}	23.87^{Ba}	16.27^{ABa}	15.17^{Aa}	23.68^{Ba}
	8	15.14^{Aab}	12.87^{Aa}	17.81^{ABabc}	17.10^{Aab}	14.94^{Aab}	13.96^{Aab}	20.40^{Abc}	16.28^{Aab}	16.32^{Aab}	23.92^{Cc}	20.40^{Abc}	17.91^{Aabc}
	12	13.77^{Aab}	11.68^{Aa}	13.74^{Aab}	14.48^{Aabc}	20.71^{Abc}	21.48^{ABc}	16.86^{Aabc}	15.88^{Aabc}	19.72^{ABbc}	19.65^{BCbc}	18.98^{Aabc}	15.83^{Aabc}
C18:3 n-3	0	3.19^{Aab}	2.91^{Cab}	3.31^{Bab}	2.61^{Aab}	3.16^{Aab}	2.91^{ABab}	2.37^{Aab}	2.65^{Aab}	2.85^{ABab}	1.84^{Aa}	4.49^{Bb}	2.50^{Aab}
	4	2.74^{Aa}	2.10^{Ba}	3.64^{Ba}	2.90^{Aa}	2.41^{Aa}	4.00^{Ba}	2.69^{Aa}	3.77^{Aa}	3.73^{Ba}	2.36^{ABa}	2.10^{Aa}	3.79^{Ba}
	8	2.11^{Aab}	1.76^{Aa}	2.57^{ABabc}	2.51^{Aabc}	2.10^{Aab}	1.86^{Aab}	2.95^{Aabc}	2.28^{Aab}	2.39^{Aab}	3.71^{Cc}	3.05^{ABbc}	2.62^{Aabc}
	12	1.87^{Aab}	1.55^{Aa}	1.87^{Aab}	2.03^{Aabc}	3.17^{Abc}	3.26^{ABc}	2.51^{Aabc}	2.21^{Aabc}	3.01^{ABbc}	2.93^{BCbc}	2.90^{ABbc}	2.18^{Aabc}
C20:4 n-6	0	1.80^{Aa}	2.34^{Babc}	2.35^{Aabc}	2.36^{Aabc}	2.49^{Abc}	1.95^{Aab}	2.13^{Aabc}	2.41^{Abc}	2.16^{ABabc}	2.28^{ABabc}	2.15^{Aabc}	2.72^{Ac}
	4	2.40^{Aa}	2.14^{Ba}	2.33^{Aa}	2.67^{Aa}	2.49^{Aa}	2.44^{Ba}	2.04^{Aa}	2.32^{Aa}	2.41^{Ba}	2.66^{Ba}	2.39^{Aa}	2.35^{Aa}
	8	2.00^{Aa}	2.08^{ABa}	2.26^{Aab}	2.17^{Aab}	2.19^{Aab}	2.26^{ABab}	2.26^{Aab}	2.30^{Aab}	1.98^{Aa}	2.22^{Aab}	2.39^{Aab}	2.70^{Ab}
	12	2.24^{Aa}	1.84^{Aa}	1.90^{Aa}	2.16^{Aa}	2.27^{Aa}	2.29^{ABa}	2.06^{Aa}	2.05^{Aa}	2.03^{Aa}	1.95^{Aa}	2.16^{Aa}	2.10^{Aa}
C20:5 n-3	0	0.25	0.31	0.27	0.29	0.23	0.28	0.25	0.25	0.26	0.33	0.36	0.25
	4	0.27	0.33	0.27	0.29	0.26	0.29	0.30	0.26	0.28	0.25	0.29	0.31
	8	0.26	0.33	0.26	0.26	0.26	0.26	0.19	0.25	0.23	0.27	0.27	0.25
	12	0.23	0.28	0.24	0.23	0.25	0.23	0.23	0.22	0.23	0.23	0.20	0.23
C22.5 n-3	0	0.64^{Aa}	0.77^{Aab}	0.83^{Bb}	0.85^{Ab}	0.86^{Ab}	0.71^{Aab}	0.75^{Aab}	0.80^{Aab}	0.72^{Aab}	0.80^{Bab}	0.75^{Aab}	0.88^{Ab}
	4	0.75^{Ba}	0.70^{Aa}	0.79^{Ba}	0.88^{Aa}	0.78^{Aa}	0.80^{Aa}	0.67^{Aa}	0.80^{Aa}	0.77^{Aa}	0.83^{Ba}	0.78^{Aa}	0.79^{Aa}
	8	0.65^{Aa}	0.70^{Aab}	0.77^{Bab}	0.69^{Aab}	0.74^{Aab}	0.77^{Aab}	0.74^{Aab}	0.72^{Aab}	0.68^{Aab}	0.76^{ABab}	0.77^{Aab}	0.83^{Ab}
	12	0.73^{ABa}	0.70^{Aa}	0.64^{Aa}	0.75^{Aa}	0.75^{Aa}	0.75^{Aa}	0.65^{Aa}	0.69^{Aa}	0.69^{Aa}	0.66^{Aa}	0.70^{Aa}	0.69^{Aa}

Table 5.9 (Continued) Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 °C.

Fatty acid	Time (d)	Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C22:6 n-3	0	0.33^{Aa}	0.46^{Aabc}	0.50^{Aabc}	0.55^{Abc}	0.65^{Ac}	0.42^{Aab}	0.52^{Abc}	0.50^{Aabc}	0.49^{Aabc}	0.54^{Abc}	0.49^{Aabc}	0.64^{Bc}
	4	0.46^{Aa}	0.41^{Aa}	0.41^{Aa}	0.53^{Aa}	0.57^{Aa}	0.51^{Aa}	0.44^{Aa}	0.57^{Aa}	0.45^{Aa}	0.53^{Aa}	0.57^{Aa}	0.43^{Aa}
	8	0.46^{Aa}	0.42^{Aa}	0.53^{Aa}	0.56^{Aa}	0.48^{Aa}	0.54^{Aa}	0.49^{Aa}	0.51^{Aa}	0.45^{Aa}	0.40^{Aa}	0.55^{Aa}	0.52^{ABa}
	12	0.63^{Ba}	0.57^{Ba}	0.51^{Aa}	0.49^{Aa}	0.49^{Aa}	0.39^{Aa}	0.42^{Aa}	0.44^{Aa}	0.44^{Aa}	0.42^{Aa}	0.44^{Aa}	0.50^{ABa}
∑ SFA	0	27.00^{Aab}	24.80^{Cab}	27.26^{ABab}	23.50^{Aab}	24.25^{ABab}	26.44^{Aab}	21.98^{Aab}	22.33^{Aab}	25.03^{Aab}	19.38^{Aa}	32.44^{Bb}	22.04^{Aab}
	4	23.95^{Aa}	22.05^{Ba}	30.13^{Ba}	25.48^{Aa}	21.45^{ABa}	31.28^{Aa}	24.47^{Aa}	29.16^{Aa}	29.04^{Aa}	20.64^{Aa}	19.67^{Aa}	28.61^{Ba}
	8	20.14^{Aab}	19.01^{Aa}	22.65^{ABab}	23.06^{Aab}	20.21^{Aab}	19.81^{Aab}	24.49^{Aab}	22.56^{Aab}	21.86^{Aab}	31.60^{Bc}	25.20^{ABb}	21.99^{Aab}
	12	17.82^{Aa}	18.50^{Aab}	19.60^{Aabc}	20.50^{Aabc}	25.61^{Bbc}	26.49^{Ac}	22.64^{Aabc}	21.90^{Aabc}	26.16^{Ac}	26.40^{Bc}	24.45^{ABabc}	20.87^{Aabc}
∑ MUFA	0	37.94^{Aab}	34.51^{Dab}	39.85^{ABab}	31.30^{Aab}	33.52^{ABab}	38.90^{Aab}	29.70^{Aab}	31.98^{Aab}	35.04^{Aab}	24.61^{Aa}	47.26^{Bb}	28.69^{Aab}
	4	32.59^{Aa}	28.70^{Ca}	44.07^{Ba}	35.59^{Aa}	30.56^{ABa}	45.45^{Aa}	34.40^{Aa}	42.52^{Aa}	41.75^{Aa}	28.35^{Aa}	25.51^{Aa}	41.73^{B}
	8	27.30^{Aa}	24.60^{Ba}	29.95^{ABa}	31.74^{Aa}	26.82^{Aa}	24.73^{Aa}	34.16^{Aab}	28.82^{Aa}	30.02^{Aa}	45.24^{Bb}	33.78^{ABa}	29.63^{Aa}
	12	23.10^{Aab}	21.70^{Aa}	26.01^{Aabc}	26.71^{Aabc}	36.11^{Bbc}	38.37^{Ac}	31.17^{Aabc}	30.74^{Aabc}	36.72^{Aabc}	37.16^{ABbc}	33.78^{ABabc}	28.89^{Aabc}
∑ PUFA	0	26.40^{Aab}	25.63^{Cab}	28.55^{Bab}	24.42^{Aab}	28.58^{Aab}	25.14^{ABab}	22.42^{Aab}	24.41^{Aab}	25.24^{ABab}	19.46^{Aa}	35.81^{Ab}	24.43^{Aab}
	4	24.66^{Aa}	20.73^{Ba}	30.46^{Ba}	26.93^{Aa}	23.20^{Aa}	32.67^{Ba}	23.66^{Aa}	32.00^{Aa}	31.50^{Ba}	22.90^{ABa}	21.29^{Aa}	31.35^{Ba}
	8	20.62^{Aab}	18.15^{ABa}	24.21^{ABabc}	23.29^{Aabc}	20.73^{Aab}	19.66^{Aab}	27.04^{Abc}	22.34^{Aab}	22.04^{Aab}	31.27^{Cc}	27.43^{Abc}	24.82^{ABabc}
	12	19.48^{Aabc}	16.61^{Aa}	18.88^{Aab}	20.14^{Aabc}	27.62^{Abc}	28.40^{ABc}	22.72^{Aabc}	21.49^{Aabc}	26.12^{ABbc}	25.83^{BCbc}	25.37^{Aabc}	21.54^{Aabc}
∑n-3 PUFA	0	4.40^{Aab}	4.45^{Bab}	4.91^{Bab}	4.31^{Aab}	4.89^{Aab}	4.32^{ABab}	3.90^{Aa}	4.19^{Aab}	4.32^{ABab}	3.51^{Aa}	6.09^{Ab}	4.26^{Aab}
	4	4.23^{Aa}	3.54^{Aa}	5.11^{Ba}	4.60^{Aa}	4.01^{Aa}	5.60^{Ba}	4.10^{Aa}	5.40^{Aa}	5.22^{Ba}	3.97^{Aa}	3.74^{Aa}	5.32^{Ba}
	8	3.48^{Aab}	3.21^{Aa}	$4.14A^{Babc}$	4.02^{Aabc}	3.59^{Aab}	3.44^{Aab}	4.37^{Aabc}	3.76^{Aab}	3.75^{Aab}	5.13^{Bc}	4.64^{Abc}	4.22^{Aabc}
	12	3.47^{Aab}	3.09^{Aa}	3.25^{Aa}	3.50^{Aab}	4.65^{Ab}	4.63^{ABb}	3.80^{Aab}	3.56^{Aab}	4.37^{ABab}	4.23^{Aab}	4.23^{Aab}	3.60^{Aab}
∑n-6 PUFA	0	21.99^{Aab}	21.18^{Cab}	23.64^{Bab}	20.11^{Aab}	23.68^{Aab}	20.83^{ABab}	18.52^{Aab}	20.22^{Aab}	20.93^{ABab}	15.95^{Aa}	29.72^{Ab}	20.17^{Aab}
	4	20.44^{Aa}	17.19^{Ba}	25.34^{Ba}	22.33^{Aa}	19.19^{Aa}	27.07^{Ba}	19.56^{Aa}	26.60^{Aa}	26.28^{Ba}	18.93^{ABa}	17.56^{Aa}	26.03^{Ba}
	8	17.14^{Aab}	14.95^{ABa}	20.07^{ABabc}	19.26^{Aabc}	17.14^{Aab}	16.22^{Aab}	22.66^{Abc}	18.58^{Aab}	18.30^{Aab}	26.13^{Cc}	22.79^{Abc}	20.61^{ABabc}
	12	16.02^{Aabc}	13.51^{Aa}	15.63^{Aab}	16.64^{Aabc}	22.97^{Abc}	23.77^{ABc}	18.92^{Aabc}	17.93^{Aabc}	21.75^{ABbc}	21.60^{BCbc}	21.14^{Aabc}	17.94^{Aabc}

Mean values with different small superscript letters presented within each row of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Mean values with different capital superscript letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

5.3.9.2 Fatty Acids in Grilled (HTHO) Processed Chicken Meat

Contrary to the fatty acid findings observed in LTLO samples as discussed previously, natural antioxidant application and the reheating process did not have any effect on all fatty acids in samples cooked by HTHO during the 12 days of storage time (Appendix Table D6). However, antioxidants provided a higher protection to fatty acids over the storage time compared to the non-treated samples. Storage time significantly reduced the amount of C18:3 n-3, C20:4 n-6, C22.5 n-3, C20:5 n-3, C22:6 n-3 and total of PUFAs, ∑n-3 and ∑n-6 in HTHO samples, regardless of the antioxidant and reheating process, while the highest reduction was found at day 8 of storage time (Appendix Table D6). No significant interaction ($p > 0.05$) was found between three factors for all fatty acids in HTHO samples (Table 5.10).

Table 5.10 Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 ºC.

Fatty acid	Time	Controls				Natural Antioxidant Treatments							
	(d)	CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C14:0	0	0.38	0.34	0.42	0.31	0.29	0.38	0.35	0.30	0.27	0.34	0.49	0.38
	4	0.38	0.32	0.35	0.32	0.31	0.36	0.27	0.46	0.34	0.41	0.41	0.48
	8	0.32	0.30	0.36	0.37	0.25	0.32	0.48	0.37	0.36	0.30	0.36	0.30
	12	0.36	0.25	0.37	0.39	0.35	0.35	0.29	0.40	0.37	0.39	0.32	0.30
C16:0	0	17.17	16.73	18.11	13.65	13.64	15.92	15.29	13.42	12.43	15.40	21.62	17.02
	4	17.34	15.61	15.66	13.81	14.68	15.45	12.35	19.82	15.80	18.42	17.51	21.04
	8	14.34	13.22	16.31	16.79	12.07	14.06	20.84	17.08	16.36	14.17	16.94	13.71
	12	15.39	11.70	16.89	17.39	15.38	15.41	13.66	17.69	16.35	17.39	14.42	14.47
C18:0	0	5.81	6.70	6.46	5.69	5.80	6.14	5.86	5.47	5.21	5.84	7.40	6.53
	4	6.40	6.10	5.89	5.69	5.98	6.07	5.37	7.00	5.90	6.79	6.73	7.78
	8	5.90	5.50	6.46	6.38	5.32	5.63	7.39	6.65	6.03	5.60	6.56	6.09
	12	6.39	5.07	6.53	6.41	5.83	5.70	5.96	6.38	6.06	7.03	5.93	5.74
C20:0	0	0.40	0.44	0.41	0.33	0.30	0.36	0.39	0.34	0.28	0.31	0.46	0.36
	4	0.44	0.41	0.37	0.30	0.35	0.40	0.31	0.41	0.39	0.45	0.40	0.48
	8	0.30	0.28	0.38	0.34	0.28	0.31	0.46	0.35	0.39	0.34	0.39	0.34
	12	0.37	0.25	0.40	0.39	0.35	0.36	0.31	0.43	0.38	0.40	0.37	0.32
C16:1 n-7	0	2.53	1.88	2.38	1.01	1.53	2.01	1.66	1.47	1.46	2.11	2.94	2.14
	4	2.34	2.02	2.22	1.65	1.60	1.61	1.18	2.52	2.12	1.90	1.89	2.39
	8	1.52	1.50	2.05	2.39	1.12	1.69	2.87	2.11	2.13	1.65	1.73	1.17
	12	1.29	1.05	2.09	2.20	1.98	2.01	1.47	2.26	1.93	1.76	1.55	1.63
C18:1 n-9c	0	31.44	29.14	33.43	23.29	22.57	27.01	26.91	22.85	19.96	26.48	38.81	29.09
	4	30.70	27.15	26.59	23.18	26.01	26.18	21.36	34.34	26.52	32.76	30.81	38.80
	8	23.22	21.09	27.74	28.54	20.31	22.79	38.06	28.78	28.49	24.65	29.96	22.52
	12	26.02	17.97	29.34	29.74	24.51	26.78	22.48	31.83	28.60	29.97	25.73	25.44

Table 5.10 (Continued) Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Fatty acid	Time (d)	Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C18:1 n-9t	0	2.45	2.14	2.40	1.72	1.88	2.04	2.08	1.87	1.67	1.90	2.64	2.05
	4	2.28	2.02	2.01	1.85	1.96	2.03	1.67	2.41	1.97	2.34	2.21	2.62
	8	1.96	1.71	2.09	2.11	1.70	1.83	2.62	2.21	2.21	1.88	2.13	1.72
	12	2.00	1.61	2.21	2.18	2.04	2.07	1.74	2.37	2.13	2.10	1.89	1.98
C18:2 n-6	0	18.15	16.88	21.56	15.90	14.77	16.69	17.72	15.15	13.13	15.51	22.20	17.95
	4	18.76	15.02	15.79	14.00	16.86	16.64	14.05	21.09	16.62	22.98	20.54	24.20
	8	13.96	13.25	16.47	15.99	12.22	13.82	21.86	16.29	16.89	14.28	18.55	14.02
	12	17.12	11.92	18.20	16.54	14.25	15.65	13.62	18.05	16.87	18.37	15.55	14.02
C18:3 n-3	0	2.62	2.34	3.33	2.34	2.03	2.55	2.69	2.13	1.78	2.30	3.47	2.72
	4	2.80	2.13	2.34	2.04	2.42	2.50	2.00	3.29	2.42	3.35	3.06	3.92
	8	1.85	1.83	2.44	2.40	1.59	1.98	3.43	2.31	2.44	1.96	2.65	1.93
	12	2.52	1.50	2.69	2.42	2.10	2.25	1.82	2.65	2.47	2.69	2.19	1.93
C20:4 n-6	0	1.80	2.02	2.35	2.41	2.50	2.06	2.38	2.25	2.14	2.14	2.09	2.10
	4	2.17	1.75	1.90	2.05	2.15	2.17	2.33	2.00	1.99	2.26	2.19	2.30
	8	1.61	1.85	2.09	2.14	1.98	1.99	1.95	1.85	2.22	1.96	2.13	2.07
	12	1.87	2.01	2.16	1.71	1.43	1.68	2.05	1.35	1.83	2.13	1.85	1.82
C20:5 n-3	0	0.26	0.21	0.26	0.24	0.29	0.24	0.25	0.26	0.29	0.25	0.25	0.30
	4	0.25	0.23	0.28	0.26	0.25	0.25	0.24	0.26	0.30	0.23	0.21	0.23
	8	0.20	0.26	0.27	0.25	0.21	0.23	0.20	0.24	0.23	0.22	0.20	0.25
	12	0.18	0.29	0.24	0.21	0.22	0.20	0.24	0.31	0.24	0.23	0.21	0.22
C22.5 n-3	0	0.60	0.70	0.76	0.70	0.79	0.70	0.72	0.71	0.70	0.73	0.68	0.74
	4	0.71	0.55	0.67	0.71	0.77	0.76	0.73	0.69	0.66	0.74	0.73	0.79
	8	0.56	0.65	0.71	0.74	0.69	0.67	0.63	0.62	0.66	0.60	0.69	0.69
	12	0.67	0.73	0.69	0.60	0.52	0.58	0.68	0.52	0.64	0.66	0.58	0.65

Table 5.10 (Continued) Effect of natural antioxidants on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

Fatty acid	Time (d)	Controls				Natural Antioxidant Treatments							
		CON	A.R.H	BHT	A.R.H	ROS	A.R.H	SRB	A.R.H	SFS	A.R.H	GGR	A.R.H
C22:6 n-3	0	0.39	0.43	0.56	0.52	0.50	0.40	0.45	0.42	0.51	0.47	0.47	0.46
	4	0.44	0.30	0.40	0.46	0.49	0.45	0.53	0.46	0.37	0.47	0.51	0.47
	8	0.33	0.44	0.45	0.47	0.45	0.47	0.38	0.40	0.44	0.46	0.48	0.46
	12	0.40	0.47	0.38	0.36	0.36	0.40	0.41	0.57	0.39	0.40	0.34	0.40
∑SFA	0	23.76	24.20	25.40	19.97	20.04	22.80	21.89	19.53	18.19	21.89	29.98	24.29
	4	24.56	22.44	22.26	20.12	21.32	22.27	18.28	27.68	22.44	26.07	25.05	29.78
	8	20.86	19.29	23.50	23.87	17.91	20.32	29.17	24.44	23.13	20.41	24.25	20.44
	12	22.51	17.26	24.20	24.59	21.92	21.82	20.22	24.90	23.15	25.21	21.04	20.83
∑MUFA	0	36.42	33.17	38.21	26.02	25.98	31.06	30.65	26.19	23.09	30.50	44.40	33.28
	4	35.33	31.18	30.82	26.68	29.58	29.83	24.20	39.28	30.61	37.00	34.91	43.81
	8	26.70	24.30	31.88	33.04	23.13	26.31	43.55	33.10	32.83	28.18	33.82	25.41
	12	29.31	20.63	33.64	34.12	28.54	30.86	25.69	36.46	32.66	33.83	29.17	29.05
∑PUFA	0	23.83	22.58	28.81	22.12	20.87	22.64	24.20	20.92	18.55	21.39	29.16	24.27
	4	25.13	19.98	21.37	19.51	22.94	22.77	19.89	27.80	22.37	30.01	27.24	31.91
	8	18.52	18.27	22.43	21.99	17.15	19.17	28.45	21.71	22.88	19.48	24.70	19.42
	12	22.75	16.92	24.35	21.85	18.87	20.76	18.82	23.45	22.43	24.48	20.72	19.05
∑n-3 PUFA	0	3.88	3.68	4.91	3.81	3.60	3.90	4.11	3.53	3.29	3.74	4.87	4.22
	4	4.20	3.21	3.69	3.46	3.93	3.96	3.50	4.71	3.76	4.78	4.51	5.41
	8	2.94	3.18	3.87	3.85	2.95	3.35	4.64	3.57	3.77	3.24	4.02	3.33
	12	3.76	2.99	3.99	3.60	3.19	3.43	3.15	4.05	3.74	3.98	3.32	3.21
∑n-6 PUFA	0	19.95	18.90	23.90	18.31	17.27	18.75	20.09	17.40	15.27	17.65	24.29	20.05
	4	20.93	16.77	17.68	16.05	19.01	18.81	16.38	23.09	18.62	25.23	22.72	26.50
	8	15.57	15.10	18.55	18.14	14.20	15.81	23.81	18.14	19.11	16.24	20.68	16.09
	12	18.99	13.93	20.36	18.25	15.68	17.33	15.67	19.40	18.69	20.50	17.40	15.84

5.4 Discussion

5.4.1 Effect of Natural Antioxidants on Thiobarbituric Acid-Reactive Substances (TBARS)

TBARS values in cooked meat in the current study were within the range reported by Yu et al. (2002) and higher than those in previous works (Lau et al., 2008: Min et al., 2008; Selani et al., 2011). Lau et al. (2008) found that the TBARS values in chicken meat cooked in a microwave reached up to 13.93 mg MDA/kg meat. Significantly higher TBARS values were observed in HTHO cooked meat than sous-vide cooked samples (Appendix Table D2). This could be due to the high temperature used during grilling meat samples, despite the cooking time being shorter than in sous vide cooked samples. Another explanation for this could be the cooking process have been conducted under aerobic conditions, which cooking process can disrupt the cell membranes and facilitate the lipid compounds to come into direct contact with oxygen and compounds that have the ability to catalyse meat lipid oxidation (Bragagnolo, 2009). It has been reported that the accelerating rate of lipid oxidation depends on the cooking temperature (Bax et al., 2011). Dominguez et al. (2014) reported that foal steaks, roasted at 200 ^{o}C had a higher TBARS value compared to that cooked in the microwave, grilled and fried less than 200 ^{o}C. Lanari et al. (1995) and Nute (2009) found that warmed over flavour becomes noticeable when TBARS values range between 0.6 to 2.0 mg MDA/kg beef meat. Moreover, after reheating LTLO cooked samples, the TBARS values were significantly decreased compared to those before reheating process. In contrary to this, reheating process markedly increased TBARS values of HTHO cooked samples compared to those before reheating (Appendix Table D2). These findings are in agreement with those reported by Pikul (1985), who reheated cooked chicken leg and breast meat, and

reported a significant increase in TBARS values in reheated samples. It seems that the formulation of warmed over flavour in multi-reheating cooked meat samples is depending on cooking methods. In addition, TBARS values in LTLO samples significantly increased from 4.04 to 9.76 mg MDA/kg meat over 12 days of storage time ($p < 0.001$). In HTHO cooked samples, the TBARS values were increased over 8 days and subsequent decrease to day 12 (Appendix Table D.2) could be due to the breakdown of malondialdehyde, and produced volatile compounds during the ongoing exposure to heat and oxygen (Bax et al., 2012). Non-treated samples of LTLO and HTHO were affected more by storage time than those treated with antioxidants (Figures 5.2 and 5.5). This is consistent with Naveena et al. (2013) and Selani et al. (2011) who reported that the highest increase in lipid oxidation was seen in non-treated samples over the storage time compared to those containing antioxidants. Addition of antioxidant inhibited lipid oxidation in beef cooked meat during the storage time (Colindres and Brewer 2011; Selani et al., 2011), this corresponds with our results (Figures 5.2 and 5.5). The significant effect of natural antioxidants observed on lipid oxidation in samples cooked in the current study at each interval of storage time (Figures 5.2 and 5.5), was due to the phenolic compounds in their structure that have the ability to terminate free radical reactions, thereby quenching metal and reactive oxygen species (Reische, 2002; Huang et al., 2005). Interestingly, the effectiveness of ROS was more in sous vide cooked meat by the reduction of TBARS values. Naveena et al. (2013) observed a similar finding in previous work where rosemary reduced the oxidative deterioration in both cooked buffalo and chicken meat. This finding explains/highlights that the capability of natural antioxidants to reduce the oxidation process could be more effective at low temperature and low oxygen. Accordingly, the highest reduction of TBARS values in BHT treatment after grilling (Figure 5.5) could be due to the synthetic antioxidant being more stable at the high temperatures

used. This synthetic antioxidant was originally preferred for stabilisation of fats in baked and fried products due to its stability in a wide range of pH and processing conditions (Fasseas et al., 2007; Rowe et al., 2007; Karre et al., 2013). The application of natural antioxidants was provided a greater protection against an increase of TBARS values in both before and reheated meat samples compared to the non-treated samples (Figures 5.1 and 5.5). These findings clearly found that the supplementation of natural antioxidants could reduce the warmed-over flavour attribute in reheated cooked meat under refrigeration storage time.

5.4.2 Effect of Natural Antioxidants on Phospholipid Content

The content of phospholipids in the current study was within the range of those reported by Pikul et al., (1985) and Alasnier et al. (2000), whilst being higher than those published by Soyer et al. (2010) who found that phospholipid content in chicken breast meat ranged from 12-23 g/100 g fat in, while similar to those reported by Pikul et al. (1984), who showed that the amount of phospholipid content in chicken breast meat is 70 g/100 g fat.

Phospholipids are in the majority of food products containing high levels of polyunsaturated fatty acids and are considered more susceptible to oxidative deterioration (Ma et al., 2007). This can occur by thermal degradation (Jayasena et al., 2013) and have phospholipids been linked to the development of warmed-over flavours and rancidity flavour in reheated meats (Igene and Pearson, 1979; Igene et al., 1980; Roldan et al., 2014). A significant reduction of phospholipid was found in LTLO compared to the HTHO cooking methods (Appendix Table D2). An increase in the phospholipid degradation in LTLO samples may be attributed to the duration of cooking. The increased hydrolysis of phospholipids as a result of cooking time was reported previously by Wang et al. (2011), who indicated that hydrolysis of

phospholipids was greater in meat roasted at 90 ^{o}C for 1 hr than in boiled meat for 30 min. Accordingly, it could be expected that a lower cooking temperature and time leads to greater degradation of phospholipids in chicken meat. Furthermore, reheating process significantly caused a reduction of phospholipid content in both LTLO and HTHO samples compared to those before subjecting to reheating process ($p < 0.001$) (Appendix Table D2). The decreased content of phospholipid in meat after reheating might be related to its decomposition and formation of a complex with protein or carbohydrate (Takagi and Yoshida, 1999). Hence, this degradation of phospholipid could lead to producing more warmed over flavour in reheated cooked meat as being highly linked with phospholipid content. Moreover, the storage time had an effect on the phospholipid content of LTLO samples. It was found that the phospholipid content in cooked meat samples significantly decreased ($p < 0.001$) throughout the storage time, the highest reduction was found on day 8 of storage time with no significant changes at the end of the storage time (Appendix Table D2). A significant effect of storage time was seen in the phospholipid content, indicating that the storage time resulted in reducing the phospholipid content which could be attributed to the autoxidation of polyunsaturated fatty acids present in its structure. A similar finding reported by Alasnier et al. (2000) reported that phospholipid content in breast and thigh meat decreased during the storage time. Soyer et al. (2010) found a similar reduction of phospholipid content in frozen chicken breast and thigh meat for 6 months. These results suggest that phospholipid content in meat is unstable under storage conditions, which could be attributed to the formation of warmed over flavour. Non-treated samples were found to have a higher reduction of phospholipid at each interval of storage time than antioxidant treatments (Figures 5.8 and 5.9). The results of the current experiment indicated that phospholipids could be protected by adding antioxidants to chicken meat before cooking. Hence,

supplementation with either natural or synthetic antioxidants impeded the degradation of phospholipid content in all LTLO and HTHO samples compared to the non-treated samples (Figures 5.8, 5.9 and Appendix Table D2). Treated samples with natural antioxidants provided a marked protection against a reduction of phospholipids compared to the non-treated samples, while similar to BHT treatment. As mentioned in Chapter 5, the underpinning mechanism by antioxidants in protecting phospholipids from thermal decomposition is not well known. This mechanism could be similar to the protective mechanism as it applies to individual fatty acids since phospholipids are considered to have elevated levels of unsaturated fatty acids. Furthermore, natural antioxidants significantly reduced the degradation of phospholipid content in both before and after reheating LTLO samples (Figure 5.7), indicating that natural antioxidants can protect the phospholipid from degradation at different conditions, which could have reduced the warmed-over flavour.

5.4.3 Effect of Natural Antioxidants on Conjugated Dienes (CDs)

The presence of conjugated dienes (CDs) in meat indicating that polyunsaturated fatty acids have two double bonds in their structure underwent an oxidation process (Feiner, 2006; Estevez et al., 2009). Conjugated diene values in cooked meat in the current study ranged from 22.36 to 68.72 µmol/g fat, which was lower than those reported in previous works (Lee et al., 2011; Hwang et al., 2013). Lee et al. (2011) found that the CD values in cooked pork meat ranged from 430 to 699 µmol/g. Hwang et al. (2013) reported that the CD values of fried chicken nuggets ranged from 505 to 966 µmol/g. This difference could be attributed to the cooking method was used and type of meat. Cooking methods had a negative impact on the oxidative stability of the chicken meat, which resulted in increased CD values. As can be seen, HTHO cooked meat had the highest formation of CDs (Appendix Table D2). This

could be due to a higher temperature, which can disrupt the cell membrane and facilitate the uptake of oxygen. Rapid formation of CDs in meat has been established as a response to the cooking methods (Weber et al., 2008; Dai et al., 2014). The higher values of CD suggest that the unsaturated fatty acids and phospholipids were unstable during the process. This was most likely due to the decomposition of polyunsaturated fatty acids after cooking and the formation of the high content of conjugated dienes. Furthermore, the CD values in LTLO samples were not significantly affected by reheating process ($p = 0.137$), while CD values in HTHO samples were significantly reduced ($p < 0.001$) after reheating process (Appendix Table D2). Hence, the formation of CDs decreased after reheating which could be due to the breakdown of conjugated dienes to secondary lipid oxidation products. Moreover, the refrigeration time significantly affected the CD values, which increased progressively over 12 days of storage time (Appendix Table D.2). Similar an increase of CD values was found in cooked ground pork stored at ^{o}C for 14 days (Juntachote et al., 2006). Furthermore, the application of antioxidants significantly reduced the formation of CD in both LTLO and HTHO samples, while ROS and BHT were found to have the lowest values of the CD (Appendix Table D.2). The lower CD values observed in cooked meat treated with antioxidants could be associated with phenolic compounds, which could stabilize the polyunsaturated fatty acids from decomposition as demonstrated by the lowest formation of secondary lipid oxidation products (TBARS) in antioxidant treatments. The CD values in untreated samples increased with storage time increased, while treated samples with natural antioxidants were provided a greater protection against an increase of CD values in both LTLO and HTHO (Figures 5.11 and 5.14). These findings are in agreement with those found by Lee et al. (2010), who found that cooked pork supplemented with different kimchi ethanolic extracts had lower CD values compared to the non-treated samples. Similar

findings were observed by Choe et al. (2011) who found that cooked ground pork treated with different concentrations of lotus, barley leaf powder and BHT had lower values of CD over storage time. Furthermore, natural antioxidant treatments worked similar to BHT in all samples either cooked by LTLO and HTHO methods. This could be due to various phenolic compounds present in their structure. Treated samples either before and after reheating process were found to have the lowest lipid oxidation compared to the non-treated control. The CD values were significantly higher in ROS, SFS and BHT samples before reheating process compared to those after reheating (Figure 5.13). These findings observed that application of natural antioxidants can inhibit the autoxidation of lipids and reduce the development of warmed-over flavour in the meat.

5.4.4 Effect of Natural Antioxidants on Conjugated Trienes (CTs)

Conjugated trienes are a by-product of secondary lipid oxidation products produced from oxidation of fatty acids containing three or more double bonds in their structure. The presence of conjugated trienes in meat is indicative of advanced lipid oxidation (Wrolstad et al., 2005). There was a significant difference ($p \leq 0.05$) between LTLO and HTHO cooking methods with HTHO cooked samples having higher CT values (Appendix Table D3). Similar findings are reported by Poiana (2012), who found that higher temperatures (193 ^{o}C) caused the greatest accumulation of CT in sunflower oil. This could be related to the cooking process that can decompose polyunsaturated fatty acids and lead to an increase in the CTs content. Furthermore, the CT in LTLO samples was not affected significantly by reheating process, while the CT values of HTHO were significantly affected by reheating process ($p < 0.001$). Hence, HTHO samples after reheating significantly decreased compared to those before reheating (Appendix Table D3). Considering the lowest accumulation of CTs was

formed in reheated LTLO cooked samples, it seems that reheating cooked meat had no effect on the CTs. The significant effect of reheating on CT values in HTHO particularly in non-treated samples could be related oxidation more polyunsaturated fatty acids by reheating temperature that could lead to producing significant changes in conjugated trienes. Moreover, storage time significantly increased the CT values in LTLO samples over 4 days, found no effect at day 8 and 12. In contrast, the CT values in HTHO samples were significantly decreased ($p < 0.001$) with increasing storage time (Appendix Table D3). These findings are in agreement with Abreu et al. (2011), who pointed out that the level of CTs increased over storage time in blue shark meat stored at -20 ^{o}C for 12 months. Their results also found that antioxidant treatments caused a lower accumulation of conjugated trienes compared to the non-treated control. Similar findings were observed in a recent study, samples containing natural antioxidants had a lower accumulation of CTs (Appendix Table D3). The oxidative stability of cooked meat treated with antioxidants based on the changes of CTs was consistent with those reported by Poiana (2012) who found that either oil supplemented with grape seeds or synthetic antioxidants (BHT) and heated in the microwave or convection oven had a lower form of conjugated trienes. The effectiveness of natural antioxidants and the synthetic antioxidant BHT to inhibit the formation of CTs in LTLO samples were BHT > SRB > GGR > SFS > ROS > non-treated samples, respectively (Appendix Table D3). However, no significant difference was observed among natural antioxidant treatments (with exception SRB) cooked by LTLO and non-treated samples, while significant reduction of CT values were found in BHT treatment compared to non-treated samples. This could be related to cooking meat under vacuum conditions that could not lead to producing significant changes in conjugated trienes. In contrast, the higher inhibition of CTs in HTHO samples was found in ROS followed by BHT, SFS, GGR, SRB

and non-treated samples (Appendix Table D3). No significant difference was found between the BHT and natural antioxidant treatments due to the ability of natural antioxidants to perform similarly with respect to reducing the CT production. Natural antioxidants were provided a greater protection against an increase of CT values in HTHO samples over time, while the highest reduction of CT by natural antioxidants was found at day 12 (Figure 5.19). These results are consistent with those reported by Al-Dalain et al. (2011) who observed that ginger, rosemary and fernel performed similarly to the synthetic antioxidant (BHT) in reducing CT formation in sunflower oil over the storage time. Moreover, the application of natural antioxidants significantly reduced the formation of CTs in LTLO samples before reheating process, while a significant inhibition of CT values was found in both before and after reheating HTHO samples (Figures 5.16 and 5.18). These results have suggested that ROS, SRB, SFS and GGR have ability to stabilise the lipid oxidation products in chicken meat.

5.4.5 Effect of Natural Antioxidants on Texture (Shear Force)

The range of shear force in the current study was within the normal range reported by Rimini et al. (2014) who observed that shear force of cooked breast meat ranged from 14.02 to 15.40 N, respectively. Meat samples cooked by the HTHO method had a higher shear force value than those cooked by LTLO (Appendix Table D3). Increased shear force value in grilled meat could be attributed to an increase in the cooking loss, which could be the reason why the HTHO samples had a higher shear force. Another explanation for this could be because the cooking process was conducted under high temperature and high oxygen. Bao and Ertbjerg (2015) suggested that meat toughness was more likely attributed to protein when meat protein undergoes the oxidation by reactive oxygen species and generate cross-links in

structural proteins. Murphy and Marks (2000) found that shear force strongly correlated with cooking temperature when shown that shear force of cooked breast meat increased with increasing cooking temperatures. Furthermore, reheating process and application of antioxidants were not affected the shear force value of both LTLO and HTHO cooked meat (Appendix Table D3). However, samples treated with antioxidants had a lower shear force compared to the non-treated samples. These findings are inconsistent with those reported by Naveena et al. (2004), who found that antioxidant treatments had significantly lower shear force than the non-treated samples. These differences between this study and ours may be due to the types of antioxidants, antioxidant dose or dipping time used which could decrease the shear force. Naveena and Mendiratta (2001) found that shear force of cooked breast treated with 1 % of ginger extract did not significantly differ from the non-treated samples while samples treated with both 3 and 5 % were found to have a significantly lower shear force than the non-treated samples. This finding indicated that shear force of chicken could be enhanced by increasing application dose of natural antioxidants. In addition, during the storage time, the shear force of cooked breast meat decreased at day 12 of storage time compared to day 0 (Appendix Table D3). Reduction of shear force in the cooked pork muscle stored at 4 ^{o}C during a 14-day storage time has also been reported (Bao and Ertbjerg, 2015). In another study by Ferrentino and Spilimbergo (2016), they found that the hardness of cooked ham decreased with storage time. This could be attributed to the deterioration of meat quality during the storage time, which can be induced by bacteria.

5.4.6 Effect of Natural Antioxidants on pH.

pH is considered a good indicator of meat stability, which is more related to the chemical reaction that generate meat deterioration (Hwang et al., 2012). The pH values of cooked

meat were within the normal range reported in previous studies carried out by Sampaio et al. (2012) who found that the pH values of cooked breast chicken meat ranged from 5.7 to 6.40, respectively. However, it was slightly higher than those published in a study conducted by Naveena et al. (2013). The pH value of LTLO cooked samples higher than HTHO samples (Appendix Table D3). This could be due to the fact that cooking meat at a lower temperature and longer time caused the rapid increase of pH. It seems that a longer cooking time and lower temperature affect the pH more than a higher temperature and shorter time. These results were consistent with those reported by Oz and Zikirova (2015) who found that beef meat cooked at < 100 ^{o}C over a long-time had higher pH values than those cooked at < 100 ^{o}C for a shorter time. In another study, a decrease in pH of cooked meat has been observed by Nithyalakshmi and Preetha, (2015), who pointed out that meat cooked at 60 ^{o}C had lower pH values than those cooked at 40 ^{o}C. Reheating meat did not cause marked changes in pH values, which it seems that reheating had no effect on pH values. A marked increase was observed in the pH value of LTLO and HTHO cooked breast meat with increasing storage time (Appendix Table D3). This could be due to the denaturation of protein and amino acid by bacteria (Choe et al., 2011). An increased pH in cooked meat with storage time has also been reported by Choe et al. (2011) and Talab (2014). Furthermore, samples supplemented with natural antioxidants had a lower pH value compared to the non-treated samples (Appendix Table D3). ROS treatment was found to have a lower pH value at each interval of storage time (Figure 5.21). This could be attributed to the phenolic acid present in plant extracts that caused a rapid drop in pH values. These results are similar to those reported by Lara et al. (2011), who found that cooked pork patties treated with rosemary, meliox and BHT had lower pH values compared to the non-treated samples. In

our study, there was no significant difference between pH of natural antioxidant treatments and BHT, an indication that both performed similarly.

5.4.7 Effect of Natural Antioxidants on Cooking Loss

Grilled (HTHO) samples were shown to have a higher cooking loss (Figure 5.24), most likely attributable to the higher core temperature reached in grilled samples, despite the cooking time being shorter. Several studies have also found a greater cooking loss because of the use of higher temperatures during the cooking process (Murphy and Marks, 2000; Lorenzo et al., 2015; Roldan et al., 2015). Roldan et al. (2015) found cooking loss was higher in oven roasted lamb meat than in LTLO cooked samples, with values 35.70 and 23.70 % respectively. Roldan et al. (2013) showed an increase in cooking loss in lamb meat with increasing cooking temperature and cooking duration. Accordingly, the reason for increasing water loss in chicken meat after cooking is more likely because the thermal process can denature and oxidize protein (Wang et al., 2009), thus reducing the ability of the meat protein to retain water in its structure by capillary forces (Aaslyng et al., 2003). Since samples containing ROS were found to have a lower cooking loss in both LTLO and HTHO samples (Figure 5.24), these results suggest that the addition of antioxidant extracts to meat before cooking could protect proteins from denaturation. Lara et al. (2011) observed similar findings when pork patties supplemented with natural (Nutrox and Meliox) and synthetic (BHT) antioxidants had the lowest cooking loss compared to the non-treated samples. Natural antioxidants provided similar protection as compared to BHT (Figure 5.24) which indicate that both performed similarly.

5.4.8 Effect of Natural Antioxidants on Colour

The higher L* values found in HTHO cooked meat than LTLO samples (Appendix Table D4), could be due to the higher cooking loss was observed. Pulgar et al. (2012) suggested that higher L* values in pork meat were related to losses of great amounts (16-20 %) of water during the cooking process which turns meat to a dry appearance. In the study carried out by Kralik et al. (2014) breast meat with higher L* values had lower pH values. A similar finding was observed in our study when HTHO cooked meat was found to have a higher L* value and lower pH values. Furthermore, reheating process did not have any effect on L* values of LTLO samples, while significantly increased the L* values in HTHO samples (Appendix Table D4). As mentioned before, this could be attributed to exuded great amounts of water during the reheating process which turns meat to a dry appearance. Furthermore, the lightness (L*) values of cooked breast chicken meat either by LTLO or HTHO methods tended to increase at the end of storage time (Appendix Table D4). These results are in agreement with those reported by Lara et al. (2011) who found an increase in lightness values in cooked pork with storage time. However, in the study carried out by Selani et al. (2011), they found that lightness (L*) of cooked breast meat was not affected by storage time. Moreover, LTLO cooked meat treated with natural antioxidants had lower L* values than BHT and non-treated samples over storage time, and the lowest L* values was found in ROS and GGR treatments compared to any other treatments (Figure 5.25). The lower in lightness (L*) values of chicken meat treated with ROS and GGR extracts might be caused by the application of plant extracts which presented a dark green and orange colour. These results are in agreement with those reported by Lee et al. (2010), who found ground pork meat treated with mustard leaf kimchi had a lower lightness compared to the positive control (ascorbic acid) and control.

Antioxidant supplementation was found to have a significant effect on the redness (a*) values in cooked and reheated LTLO and HTHO samples (Appendix Table D4). GGR treatment cooked by HTHO was found significantly higher ($p \leq 0.05$) than non-treated samples. These results are in agreement with those observed by Lee et al. (2010) who found an incremental reduction of redness in pork meat after applying 0.1 and 0.2 % of mustard leaf kimchi with the reduction being less in samples that contained 0.05 %. The reheating process significantly reduced the redness values compared to those before reheating (Appendix Table D4). This could be related to the oxidation of oxymyoglobin (ferrous Fe^{2+}) to metmyoglobin (ferric Fe^{3+}) which reduce the redness of meat and turns meat colour to brown (Mancini and Hunt, 2005). The results also show that the storage time significantly reduced the redness value in LTLO meat samples up to day 4 and increased thereafter over 12 days (Appendix Table D4). In the study carried out by Lara et al. (2011) found an increase of redness values in non-treated samples of cooked pork with storage time, while in samples containing antioxidants the values decreased. Pizato et al. (2014) observed that a* values were declined in cooked breast meat over storage time.

The highest b* values shown in sous vide (LTLO) (Appendix Table D4), could be due to duration of cooking meat that could denaturised more haemoproteins and produce metmyoglobin. Lorenzo et al. (2015) suggested that the cooking process could cause several changes of meat colour due to rapid oxidation of myoglobin. Higher b* values were observed in GGR treatment cooked by LTLO, followed by ROS and SRB treatments compared to the non-treated over storage time (Appendix Table D4) and Figure 5.26). This could be due to the colour present in plant extracts. Lara et al. (2011) also found discoloration of cooked pork patties with supplemental natural antioxidants such as Meliox. After reheating LTLO and HTHO cooked samples, b* value was significantly higher than samples before reheating

process ($p \leq 0.05$). As mentioned before this could attributed to denaturation of haem-proteins and increase formation of metmyoglobin by reheating process. The results also indicated that the storage time had a significant effect on the b* values in LTLO and HTHO samples which cause an increase of b* value up to day 8 and decreased thereafter at day 12 (Appendix Table D4). This increase agrees with the results of previous studies conducted by Pizato et al. (2015) who found an increase of b* values in cooked chicken breast meat during the storage time.

5.4.9 Effect of Natural Antioxidants on Fatty Acids

The fatty acid profile in meat can be affected by several mechanisms, which occur during cooking processes such as lipid oxidation and cooking loss (Dominguez et al., 2015). In the current study, the amount of individual fatty acids and total SFAs, MUFAs and PUFAs markedly decreased when meat samples cooked by HTHO could be attributed to the high temperature of grilling as compared to the LTLO method that can cause a greater oxidative degradation (Appendix Table D5 and D6). The reduction in HTHO samples was observed more in PUFAs (9.14 %) followed by MUFAs (5.25 %) and SFAs (4.90 %). This is due to higher degradation of predominant fatty acids such as C16:0, C18:1 n-9c and C18:2 n-6 in meat. These results were consistent with those observed by Saldanha and Bragagnolo (2007), who found a greater reduction of PUFAs (30-36 %), followed by MUFAs (18-20 %) and SFAs (6-8 %) in grilled hake fillet samples. The results indicate that high temperature cooking processes reduced all individual fatty acid content, which influenced all three families of fatty acids (SFAs, MUFAs and PUFAs) in different proportions. Several studies have demonstrated the effect of cooking methods on the fatty acid composition of meat (Weber et al., 2008; Alfaia et al., 2010; Dominguez et al., 2015). Alfaia et al. (2010) found

a reduction of both SFAs and PUFAs in beef meat cooked by the grilling method compared to those cooked by microwave and boiling methods but increased MUFAs. In contrast, a study conducted by Weber et al. (2008) showed that different cooking methods did not have any significant effect on the fatty acids of fish fillets. The reduction of PUFAs content was mainly due to oxidation degradation of polyunsaturated fatty acids, mainly C18:2 n-6 and C18:3 n-3 into primary and secondary oxidation products. Interestingly, a higher reduction of fatty acids in grilled samples could be associated with an increase in primary and secondary lipid oxidation products in the current study. The reduction of ∑n-3 and ∑n-6 PUFAs was found to be more in HTHO samples than in LTLO samples. However, the maximum losses found in n-3, and n-6 PUFA were 10.24 and 8.92 %. This finding was supported by Erickson (2002) who reported that n-3 PUFAs oxidized more rapidly than n-6 PUFAs, due to the effect of location of methylene-interrupted double bonds on the rate of oxidation. Mielnik et al. (2006) found that degradation of n-6 and n-9 could result in the production of more heptanal, while propanal originates from the oxidation of ∑n-3 PUFAs during the storage time.

In addition, reheating process significantly affected the content of C20:4 n-6 and C20:5 n-3 in LTLO samples which they increased significantly in samples after reheating process (Appendix Table D5 and D6). Moreover, the content of fatty acids gradually declined throughout the storage time in chicken samples (Appendix Table D5 and D6) which is likely to be due to susceptible lipids being attacked by free radicals. The decrease of all individual and sum of SFAs, MUFAs and PUFAs proportion after 12 days of refrigeration time is consistent with the findings of earlier study conducted by Sampaio et al. (2012), they found that all individual and sum of SFAs, MUFAs, and PUFAs proportions in cooked breast and thigh chicken meat decreased after 4 days of storage at 4 ^{o}C. Similar findings were also

shown by Mariutti et al. (2011) who found a reduction in the amount of SFAs, MUFAs, PUFAs, ∑n-3 and ∑n-6 PUFAs in grilled chicken patties stored at -18 ºC for 90 days.

Antioxidants play a significant role in retarding and delaying the lipid oxidation in meat and meat products (Velasco and Williams 2011). The high stability of MUFAs, PUFAs, ∑n-3 and ∑n-6 PUFAs in the natural antioxidant treatments (GGR, ROS and SFS) of LTLO cooking meat samples (Appendix Table D5). This could be attributed to the phenolic compounds in these antioxidant extracts, which have an ability to scavenge free radicals, inhibit lipid peroxidation and are capable of high ferric reducing power (Chan et al., 2011). Several studies have found that the stability of fatty acid composition in cooked meat containing natural antioxidants increased (Trindade et al., 2010; Mariutti et al., 2011; Sampaio et al., 2012). In the study conducted by Mariutti et al. (2011), fatty acid composition of grilled chicken meat samples containing sage and garlic extracts remained stable during the storage time compared to the non-treated samples. In the other study, rosemary either alone or mixed either with synthetic (BHT/BHA) antioxidants or oregano extract reduced the hydrolysis of most fatty acids in beef burgers after being subjected to different irradiation doses and stored for 90 days (Trindade et al., 2010). The results in our study showed that the natural antioxidants performed better than BHT (Appendix Table D5).

5.5 Conclusions

In summary, lipid oxidation occurred more in samples cooked by HTHO compared to those cooked by LTLO method as the highest amount of lipid oxidation products such as TBARS, CDs, and CTs has shown. Of all samples analysed, HTHO samples showed the highest decomposition of fatty acids, particularly, unsaturated fatty acids. LTLO cooked meat samples were found to have a higher value of pH and higher degradation of phospholipids.

Furthermore, the reheating process was found to have a significant effect on lipid oxidation products in both HTHO and LTLO cooked meat samples. Cooked meat samples without antioxidants had lower stability against oxidation deterioration over time under storage conditions. Supplementation of chicken breast meat with natural and synthetic antioxidants before the cooking process provided the greatest protection compared to the non-treated samples with regards to lipid stability of chicken breast meat stored under refrigeration temperature, especially in LTLO samples. ROS effectively inhibited lipid oxidation in cooked meat samples as indicated by the lowest phospholipid degradation and formation of TBARS, CDs and CTs. The results also indicate that natural antioxidants provided a greater protection against lipid oxidation compared to non-treated samples and similar to BHT. These findings confirmed that the application of ROS, SRB, SFS and GGR have the ability to slow down the lipid oxidation products in cooked and reheated chicken meat.

Chapter 6.

General Discussion and Recommendations for Future Studies

This study focused on the efficacy of a small range of natural antioxidant extracts with regards to chicken meat quality and to compare their effectiveness to a commonly used synthetic antioxidant.

Natural antioxidants are considered to have various biological functions such as antioxidant, anti-inflammatory, anti-cancer and antibacterial properties (Manthey and Grohmann, 2001; Chan et al., 2011; Berdahl and McKeague, 2015). In light of the broader reasons for the application of antioxidants the more recent focus of research has been on the use of natural antioxidants. This is mainly because consumers' preference, and place increasing demands on foods containing natural antioxidants instead of synthetic ones because of the perceived health benefits (Fasseas et al., 2007), improved nutritional value and the enhanced quality of meat (Velasco et al., 2011). A further reason for the move towards natural antioxidants is to avoid reporting carcinogenic side effects associated with long-term consumption of some synthetic antioxidants (Altmann et al., 1986; Van, 1986; Gharavi, et al., 2007). The plants from which the extracts were obtained in the current study are part of the staple diet in many cultures and are often consumed at levels significantly higher than proposed in this study (Charles, 2013; Apak et al., 2011).

This project used rosemary (ROS), small red bean (SRB), sunflower seeds (SFS), and ginger (GGR) as the raw materials from which ethanolic extracts were obtained. The natural antioxidant extracts were characterised and standardised in their use in various types of chicken meat (described in Chapter 2). Firstly, the *in-vitro* influence of the natural antioxidants on the extracted lipid fractions from the chicken meat was evaluated (described

in Chapter 2), before the same natural antioxidants were applied to chicken meat (described in Chapter 4). Ultimately, the impact of the application of natural antioxidants on the warmed-over characteristics in cooked chicken meat was evaluated (described in Chapter 5).

6.1 Lipid Oxidation in Chicken Breast Meat.

Lipid and phospholipid oxidation occurred naturally and by a thermal process in chicken meat over storage time. A lower level of TBARS was found in fresh control meat which indicated that a slight amount of lipid oxidation had occurred (0.40 mg MDA/kg meat) (see Chapter 4). These results are consistent with those found by Selani et al. (2011). Thermal processes markedly increased the lipid oxidation in chicken meat. Hence, TBARS values were found to be higher in fat samples stored at elevated temperatures. This was also observed in grilled samples, sous vide cooked, and freshly cooked meat (Chapter 2, 4 and 5). The higher secondary lipid oxidation product observed could be attributed to the cooking temperature that facilitates the lipid compounds to react with oxygen and compounds that have the ability to catalyse meat lipid oxidation (Bragagnolo, 2009), which goes some way to explain the high levels of TBARS in grilled meat samples. Several researchers have demonstrated that the incidence of warmed-over flavour coincided with the development of thiobarbituric acid reactive substances (TBARS) (Lanari et al., 1995; Byrne et al., 2001; Nute, 2009). Moreover, freshly cooked samples were also induced the oxidation of phospholipids and fatty acids particularly unsaturated fatty acids. Oxidation of both phospholipids and unsaturated fatty acids increased the formation of primary (CD) and secondary lipid oxidation products (CT) (see chapter 4).

Measuring fatty acid content is an important factor in evaluating the nutritional quality of meat and its products (Mariutti, Nogueira, & Bragagnolo, 2011). Thermal processing can potentially cause hydrolysis of fatty acids in meat (Cortinas et al., 2004; Alfaia et al., 2010). In Chapter 2 and 4, cooking processes caused a significant decrease ($p \leq 0.05$) in all individual fatty acids and total SFAs, MUFAs and PUFAs in all meat samples. These findings suggest that the majority of SFAs, MUFAs and PUFAs in meat remains relatively unstable after the cooking process. The possible explanation for this is more likely that the thermal process that used could induced the lipid oxidation. Unsaturated fatty acids were more susceptible to oxidation than saturated fatty acids as evidenced by higher losses shown in the USFA. Therefore, greater degradation of USFA could be attributed to lipid oxidation in cooked meat. Similar results were reported by Cortinas et al. (2004), who demonstrated that the cooking of chicken thigh meat caused a reduction of all individuals and total SFAs, MUFAs and PUFAs compared to the raw meat. Moreover, after chicken meat samples were subjected to different cooking methods (LTLO and HTHO methods), as reported in Chapter 5, HTHO samples had a greater reduction in PUFA (9.14 %) followed by MUFA (5.25 %) and SFA (4.90 %) compared to the LTLO methods. This fact is due to higher degradation of C16:0, C18:1 n-9c and C18:2 n-6 in meat. These results are similar to those reported by Saldanha and Bragagnolo (2007), who found a high reduction of PUFA (30-36 %), followed by MUFA (18-20 %) and SFA (6-8 %) in grilled hake fillet samples. These results indicate that cooking at high temperature reduced levels of all individual fatty acids, which influenced all three families of fatty acids SFAs, MUFAs and PUFAs in different proportions. Sensitivity to oxidation processes mainly depends on the composition of lipids. Lipids containing high levels of unsaturated fatty acids are considered more prone to oxidation compared to fats high in saturated fatty acids (Min et al., 2008). Thus, a free radical

can attack quickly, react with unsaturated fatty acid double bonds, and produce warmed-over flavour (Huang, et al., 2013). It was previously observed that formation of primary and secondary lipid oxidation in either fresh or cooked meat could be minimized by antioxidant supplementation (Naveena et al., 2008; Naveena et al., 2013).

6.2. The Effect of Supplementary Natural Antioxidants on Lipid Oxidation Products in Chicken Meat

Firstly, supplementation of natural antioxidants to chicken fat or meat prior to exposing thermal process was evaluated (Chapter 2). The important goal of this particular experiment to find out the optimum level of antioxidant can inhibit or delay the process of lipid oxidation and to measure natural antioxidant capacity under standardised accelerated storage conditions (i.e. Schaal oven test). For practical purposes, it has been suggested that accelerated storage is considered a very useful tool to identify and characterise a new antioxidant and determine its activity. Hence, the application of natural antioxidants was noticed significantly reduced the formation of lipid oxidation products as indicated by decreasing TBARS, CDs and CTs values compared to samples without added antioxidants (control) (Chapter 2). However, these products were found in lower levels in samples treated with the synthetic antioxidants (BHT), the effect of natural antioxidants was comparable with that of (BHT). This is probably associated with efficiency of compounds and the chemical structure of natural extracts. Zilic et al. (2010) reported that natural sources which contain several compounds that exert anti-oxidative functions tend to retard lipid oxidation. These compounds are mainly phenolic compounds such as the chlorogenic, caffeic, ferulic and rosmarinic acids (Velasco and Williams, 2011). The phenolic compounds can retard oxidation by scavenging free-radicals, quenching singlet oxygen and chelating metals (Velasco and Williams, 2011; Karre et al., 2013).

On the contrary, the application of natural antioxidants particularly ROS extract to meat prior to the cooking process awarded a greater protection against oxidative rancidity in meat was freshly cooked, and cooked meat by LTLO and stored for 12 days. In raw meat without subjecting to any cooking method, SFS extract followed by SRB extract imposed a marked protection against oxidative rancidity by reducing TBARS, CD and CT formation. However, natural antioxidants performed significantly to decrease the lipid oxidation products in samples stored under accelerated storage condition and cooked by HTHO, their effect was slightly less than BHT. It seems that natural antioxidants in some instances performed similar to or higher than synthetic antioxidants. The highest inhibition of lipid oxidation was seen in fat treated with BHT under Schaal oven temperature and in grilled samples, which could be attributed to synthetic antioxidant stability in a wide range of pH and processing conditions (Fasseas et al., 2007; Rowe et al., 2007; Karre et al., 2013). Fluctuation effect of antioxidants observed in this study could be attributed to the active compounds in the extract. Although the application was carried out based on the total phenolic compounds, there were variations in their ability to protect lipids from autoxidation. In addition, antioxidant supplementation protected fatty acids and phospholipids from degradation and reduced the formation of TBARS, conjugated dienes and conjugated trienes.

6.3. Effect of Antioxidant on pH, Shear Force, Cooking Loss and Colour of Chicken Meat

The impact of antioxidant supplementation on chicken meat pH, colour, texture and cooking loss were evaluated in Chapters 4 and 5. The results showed that antioxidants did not have any significant effect on shear force of chicken meat freshly cooked, while in HTHO samples, a significant effect of natural antioxidants on shear force was shown ($p = 0.022$). Hence, the effectiveness of natural antioxidants and the synthetic antioxidant BHT to

enhance the meat texture (shear force) in HTHO samples was ROS > BHT > non-treated > GGR >SFS > SRD, respectively. Our findings are in agreement with those reported by Naveena and Mendirratta (2004), who found that marinating buffalo meat chunks in the GGR extract at 0, 3, 5 and 7 % v/w for 2 days at 4 ^{o}C significantly decreased shear force values. In another study conducted by Naveena and Mendirratta (2001), the addition of GGR at 1, 3 and 5% v/w to chicken breast meat reduced the shear force value. However, the sample containing the highest concentration was found to have a lower value. Further work is required to determine whether dipping time would have any effect on shear force.

The application of antioxidants significantly affected the pH values in raw and cooked chicken meat by both sous vide and grilled methods, as reported in Chapters 4 and 5. Meat samples supplemented with antioxidants and cooked by sous vide and grilled methods were found to have lower pH values than non-treated samples. This could be attributed to the phenolic acid present in plant extracts that caused a rapid drop in pH values and cooking temperature. A similar reduction of pH in cooked meat treated with antioxidants has been shown by Lara et al. (2011), who found that cooked pork patties treated with ROS, meliox and BHT had lower pH values compared to the non-treated samples. This finding is in agreement with those reported by Selani et al. (2011), who found that the antioxidant (i.e. Grape peel and seed extract, sodium erythorbate, citric acid and sugar, BHT) did not have any effect on pH values in cooked chicken meat over 9 days of storage time.

Because colour is a primary sensory attribute, it is often used to evaluate meat quality. Losses due to consumer discrimination against meat with surface discoloration, either in the home or at retail, result in losses of what may be edible meat. Aside from the packaging another effective methods to prolong shelf life is the addition of antioxidants to meat and its products. The use of antioxidants to preserve meat quality has been well documented. In the current

study, the effect of antioxidants was significantly observed on redness (a*) values in raw and cooked meat. The natural antioxidant treatments were found to have a lower oxidation of pigments than non-treated samples and BHT. This results in the notion that the addition of antioxidants could protect meat from discolouration.

Samples treated with ROS, SRB, SFS and GGR were found to have a lower cooking loss compared to the non-treated samples as reported in Chapter 4. However, statistical was not significant. In contrast, samples treated with ROS extract and cooked by LTLO and HTHO had the lowest cooking loss compared with non-treated samples, but similar to the BHT. These results suggested that the addition of antioxidant extracts to meat before cooking could protect proteins from denaturation. Lara et al. (2011) observed similar findings who showed pork patties supplemented with natural (Nutrox and Meliox) and synthetic (BHT) antioxidant had a lowest cooking loss compared to the non-treated samples .

6.4 Assessment of Natural and Synthetic Antioxidant Activity

Previous research was carried out to measure natural antioxidant activities based on the secondary lipid oxidation product such as aldehydes (MDA) (Jayathilakan, et al., 2007). In the current study, calculation of antioxidant activity of plant extracts has been conducted using the following equation:

AOA (%) = (V1-V2/V1) x 100.

Where AOA is antioxidant activity, V1 is the TBARS value of the CON samples where V2 is the TBARS value of the treated samples.

The important goal in this particular section to find out the activity of ROS, SRB, SFS and GGR applied to various types of chicken meat to inhibit the lipid oxidation products compared to the synthetic antioxidant. The activity of natural antioxidants against an

increase in lipid oxidation in raw meat was ranged from 18 to 30 %. Among natural antioxidants, SFS extract had the highest inhibition of lipid oxidation of 30 %, while the inhibition of lipid oxidation in samples treated with BHT was reached to 22 %. In meat samples, freshly cooked and LTLO samples, ROS was found to have a higher antioxidant activity of 62 and 67 %, respectively for freshly cooked and LTLO samples compared to the BHT (56-54%). The inhibition of lipid oxidation products in treated samples stored under accelerated storage conditions was ranged from 31 to 51% and in samples cooked by HTHO methods was ranged from 27 to 37% as compared to the synthetic antioxidants, the antioxidant activity of BHT was found to be the highest of 71% in samples stored under accelerated storage conditions and (60%) in HTHO (grilled) samples. As mentioned before, this could be attributed to synthetic antioxidant stability in a wide range of pH and processing conditions (Fasseas et al., 2007; Rowe et al., 2007; Karre et al., 2013). Jayathilakan et al. (2007) tested the antioxidant activity of natural (cloves, cinnamon) and synthetic antioxidants (ascorbic acid, tart-butyl hydroquinone, butylated hydroxyanisole (BHA), and propyl gallate (PG) in different species of meat such as mutton, beef and pork and reported that tart-butyl hydroquinone had the highest antioxidant activity in three species of meat followed by cloves, ascorbic acid, BHA, PG and cinnamon, respectively.

6.5 General Conclusions

The results obtained from this study support the research hypothesis: that the application of natural antioxidants to raw and cooked meat post-slaughter can improve meat quality, reduce lipid oxidation, discolouration of meat and limit the degree of warmed-over flavour in chicken meat as measured by instrumental analyses. The principle outcomes obtained from this study could be summarised as:

- Thermal processing of chicken fat and meat induced lipid oxidation and increased the degradation of both fatty acids and phospholipids
- The effect of natural antioxidants was similar to the synthetic antioxidant BHT
- Antioxidant supplementation protected fatty acids and phospholipids from degradation and reduced the formation of primary oxidation products (conjugated dienes) and secondary oxidation products (MDA, 1, 3-propanedial and conjugated trienes) in chicken meat. In raw and freshly cooked meat, natural antioxidants were found to have lower primary and secondary lipid oxidation products, whilst in fat samples and grilled samples, BHT was found to have less lipid oxidation products.
- Natural antioxidant reduced the warmed-over flavour that occurred in cooked meat and reheated during the storage time.
- The colour of meat was more stable in samples containing natural antioxidants compared to those without antioxidants added.
- The percentage of cooking and drip loss was reduced in samples treated with antioxidants. Treated samples were found to have lower pH values of meat, but antioxidants did not have any effect on the shear force.

6.6 Recommendations for further work

6.6.1 Further work is required to evaluate whether the application of natural antioxidants has any adverse, neutral, or beneficial effects on the sensory attributes such as grassy, cardboard-like, rancid flavour and odour, stale, and painty of raw and cooked chicken meat during various processing applications, and during extended storage times.

6.6.2 All antioxidants reduced the formation of lipid oxidation products, while further study is needed to isolate and identify the antioxidant compounds from natural sources (ROS, SRB, SFS and GGR), and evaluate the effect of each antioxidant compound on lipid oxidation products in chicken meat.

6.6.3 Antioxidants had a positive effect on the lipid oxidation in chicken meat, while it is important to apply these antioxidant extracts to other white meat such as turkey meat. The penetration of total phenolic was investigated in the outer layer and the core of chicken fillets, but it would be interesting to determine lipid oxidation products in the outer layer and the core to get a better understanding the process of lipid oxidation and the effect of phenolic compounds on lipid oxidation throughout the muscle.

6.6.4 Further work is also required to determine whether the application of natural antioxidants has any effect on meat spoilage microorganisms.

References

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference*, 14, 277–288.

Abedi, E., & Sahari, M. A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science & Nutrition*, 2(5), 443–63.

Abedi, E., & Sahari, M. A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition*, 2(5), 443–63.

Abou-Arab, E., & Abu-Salem, F. M. (2010). Effect of natural antioxidants on the stability of ostrich meat during storage. *Grasas Y Aceites*, 61, 102–108.

Ahmed, S. T., Islam, M. M., Bostami, A.B.M. R., Mun, H. S., & Kim, Y. J. (2015). Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum L.*) by-products. *Food Chemistry*, 188, 481-488.

Ahn, D. U., & Kim, S. M. (1998). Prooxidant effects of ferrous iron, hemoglobin, and ferritin in oil emulsion and cooked-meat homogenates are different from those in raw-meat homogenates. *Poultry Scien*ce, 77, 348–355.

Ahn, D. U., Nam, K. C., Du, M., & Jo, C. (2001). Volatile production in irradiated normal, pale soft exudative (PSE) and dark firm dry (DFD) pork under different packaging and storage conditions. *Meat Science*, 57 (4), 419–426

Ahn, J., Grün, I. U., & Fernando, L. N. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science*, 67(4), 1364–1369.

Ahn, J., Grun, I. U., & Mustapha, A. (2007). Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology*, 24, 7–14.

Aidani, E., Aghamohammadi, B., Akbarian, M., Morshedi, A., Hadidi, M., Ghasemkhani, N., & Akbarian, A. (2014). Effect of chilling, freezing and thawing on meat quality: a review. *International Journal of Biosciences*, 5 (4), 159-169.

Akowuah, G., Ismail, Z., Norhayati, I., & Sadikun, A. (2005). The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. *Food Chemistry*, 93(2), 311–317.

Alarcon-Rojo, A. D. (2010). Marination, cooking, and curing applications. In Guerrero-Legarreta, I., & Y. H. Hui (Eds.), *Handbook of poultry science and technology* (pp. 89-100). New Jersey, U.S: Hoboken.

Alasnier, C., Meynier, A.,Viau, M., & Gandemer, G. (2000). Hydrolytic and oxidative changes in the lipids of chicken breast and thigh muscle during refrigeration storage. *Journal of Food Science*, 65(1), 9-14.

Al-dalain, S. Y. A., & Al-fraihat, A. H. (2011). Effect of aromatic plant essential oils on oxidative stability of sunflower oil during heating and storage. *Pakistan Journal of Nutrition*, 10(9), 864–870.

Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A. S. H., Fontes, C. M. G. A., & Prates, J. A. M. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, 84, 769–777.

Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A. S. H., Fontes, C. M. G., Prates, J. M. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, 84(4), 769–77.

Ali, M., Ullah, I., Ahmad, S., Khan, H., & Akbar, H. (2009). Effect of commercial kebab frying on physico-chemical parameters of the tallow. *Pakistan Journal of Nutrition*, 8, 891–895.

Allen, C. D., Fletcher, D. L., Northcutt, J. K., & Russell, S. M. (1998). The relationship of broiler breast color to meat quality and shelf-life. *Poultry Science*, 77, 361–366.

Altmann, H.-J., Grunow, W., Mohr, U., Richter-Reichhelm, H. B., & Wester, P. W. (1986). Effects of BHA and related phenols on the forestomach of rats. *Food and Chemical Toxicology*, 24, 1183-1188.

Amakura, Y., Yoshimura, M., Yamakami, S., & Yoshida, T. (2013). Isolation of phenolic constituents and characterization of antioxidant markers from sunflower (*Helianthus annuus*) seed extract. *Phytochemistry Letters*, 6, 302-305.

Andersen, H. J., & Skibsted, L.H. (1992). Kinetics and mechanism of thermal oxidation and photooxidation of nitrosylmyoglobin in aqueous solution. *Journal of Agricultural and Food Chemistry*, 40(10), 741–1750.

Anderson, R, L., & Davis, S. (1982). An organic phosphorus assay which avoids the use of hazardous perchloric acid. *Clinica chimica acta*, 121, 111-116.

Andrés, S., Morán, L., Aldai, N., Tejido, M. L., Prieto, N., Bodas, R., & Giráldez, F. J. (2014). Effects of linseed and quercetin added to the diet of fattening lambs on the fatty acid profile and lipid antioxidant status of meat samples. *Meat Science*, 97(2), 156–63.

Antolovich M., Prenzler P. D., Patsalides E., & McDonald S, R. K. (2002). Methods for testing antioxidant activity. *The Analyst*, 127(1), 183–198.

Anwar, F., & Przybylski, R. (2012). Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Scientiarum Polonorum, Technologia Alimentaria*, 11, 293–302.

Apak, R., Gorinstein, S., Böhm, V., Schaich, K. M., Özyürek, M., & Güçlü, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC technical report). *Pure and Applied Chemistry*, 85(5), 957–998. 07-15

Baker, I. A., Alkass, J. E., & Saleh, H. H. (2012). Antioxidant and antimicrobial effects of rosemary and ginger extract added to Karadi yearling lamb patties. *Research Opinions in Animal and Veterinary Science*, 2(2), 109-114.

Baker, I. A., Alkass, J. E., & Saleh, H. H. (2013). Reduction of oxidative rancidity and microbial activities of the karadi lamb patties in freezing storage using natural antioxidant extracts of rosemary and ginger. *International Journal of Agriculture and Food Research*, 2(1), 31-42.

Banerjee, R., Verma, A. K., Das, A. K., Rajkumar, V., Shewalkar, A. A & Narkhede, H. P. (2012). Antioxidant effects of broccoli powder extract in goat meat nuggets. *Meat Science*, 91, 179-184.

Bao, Y., & Ertbjerg, P. (2015). Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork. *Meat Science*, 110, 174–179.

Basappa Maheswarappa, N., Subbaiah, V., Muthupalani, M., Yamagani, P. K., Mohan, K., Keshapaga, U. R., Kalappurakkal, R. C. (2014). Antioxidant activity of carnosic acid and rosmarinic acid in raw and cooked ground chicken patties. *Journal of the Science of Food and Agriculture*, 94(2), 273–279.

Bax, M., Aubry, L., Ferreira, C., Daudin, J., Gatellier, P., Remond, D., & Sante-Lhoutellier, V. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: Investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry*, 60, 2569-2576.

Bedawey, A. A. El, Mansour, E. H., Zaky, M. S., & Hassan, A. A. (2010). Characteristics of Antioxidant Isolated from Some Plant Sources. *Food and Nutrition Sciences*, 01(01), 5–12.

Belitz H, D., Grosch, W., & Schieberle P. (2009). Food Chemistry (4th revised and extended ed.), Berlin and Heidelberg: Springer-Verlag.

Belury, M. A. (2002). Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annual Review of Nutrition*, 22, 505–531.

Berdahl, D.R., and McKeague, J (2015). Rosemary and sage extracts as antioxidants for food preservation. In Shahidi, F. (Eds), *Handbook of Antioxidants for Food Preservation* (pp. 177-217). Cambridge, England: Woodhead Publishing.

Betti, M., Perez, T. I., Zuidhof, M. J., & Renema, R. A. (2009). Processing, products and food safety. Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes. *Poultry Science*, 88, 1740–1754.

Bhale, S. D., Xu, Z., Prinyawiwatkul, W., King, J. M., & Godber, J. S. (2007). Oregano and rosemary extracts inhibit oxidation of long-chain n-3 fatty acids in menhaden oil. *Journal of Food Science*, 72(9), C504–8.

Blake, S. (2010). Understanding Dietary Fats and Oils: A Scientific Guide to their Health Effects. Life Long Press.

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agriculture and Food Chemistry*, 42 (9), 1931–1937.

Botsoglou, N.A., P. Florou-Paneri, P., Christaki, E., Fletouris D.J., & Spais, A.B. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science*, 43, 223- 230.

Bragagnolo, N. (2009). Cholesterol and cholesterol oxides in meat and meat products. In Nollet, L.L, & Toldr, F.(Eds.), *Handbook of muscle foods analysis* (pp. 187-219). Boca Raton, US: CRC Press.

Buaneow, C., Usawakesmanee, W., Siripongvutikorn, S., & Tongraung, C. (2008). Effect of pH and ATP on lipid oxidation in unwashed and washed seabass mince (*Lates calcarifer*) mediated by haemoglobin. *Songklanakarin Journal of Science and Technology*, 30, 19–23.

Buege, J.A., & Aust, S.D. (1978). Microsomal lipid peroxidation methods. *Enzymology*. 52, 302 - 310.

Byrne, D., Bredie, W. L., Bak, L., Bertelsen, G., Martens, H., & Martens, M. (2001). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science*, 59(3), 229–249.

Byrne, D., Bredie, W. L., Mottram, D., & Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. *Meat Science*, 61(2), 127–139.

Campo, M.M., Nute, G.R., Hughes, S.I., Enser, M., Wood, J.D., & Richardson, R.I., (2006). Flavour perception of oxidation in beef. *Meat Science*, 72(2), 303-311).

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology: An International Journal Published for the *British Industrial Biological Research Association*, 51, 15–25.

Carpenter, R., O'Grady, M. N., O'Callaghan, Y. C., O'Brien, N. M., & Kerry, J. P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science*, 76(4), 604–10.

Carr, M. A. (2007). Shelf Life of Fresh and Frozen Pork. In Nollet, L. M. L. (Eds.), *Handbook of meat, poultry and seafood qualities* (pp. 417-429). Ames, U.S: Blackwell Publishing.

Chamul, R. S. (2007). Quality Measurements in Beef. In Nollet, L. M. L. (Eds.), *Handbook of meat, poultry and seafood qualities* (pp. 341-357). Ames, U.S: Blackwell Publishing.

Chan, E., Lim, Y., & Wong, S. (2011). Antioxidant properties of ginger leaves: An overview. *Free Radicals and Antioxidants*, 1(1), 6–16.

Charles, D. J. (2013). Antioxidant Properties of Spices, Herbs and Other Sources. New York, U.S: Springer Science Business Media.

Che Man, Y. B., & Tan, C. P. (1999). Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized palm olein during deep-fat frying of potato chips. *Journal of the American Oil Chemists' Society*, 76 (3), 331-339.

Che Man, Y. B., & Tan, C. P. (1999). Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized palm olein during deep-fat frying of potato chips. *Journal of the American Oil Chemists' Society,* 76 (3), 331-339.

Chen, C., Pearson, A.M. and Gray, J.I. (1992). Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. Food Chemistry, 43(3), 177-183.

Chen, J. H., & Ho, C. T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry*, 45, 2374–2378.

Chen, X., Jo, C., Lee, J. I., & Ahn, D. U. (1999). Lipid oxidation, volatiles and color changes of irradiated pork patties as affected by antioxidants. *Journal of Food Science*, 64, 16–19.

Choe, J.-H., Jang, A., Lee, E. S., Choi, J. H., Choi, Y. S., Han, D. J., & Kim, C. J. (2011). Oxidative and color stability of cooked ground pork containing lotus leaf (*Nelumbo nucifera*) and barley leaf (*Hordeum vulgare*) powder during refrigerated storage. *Meat Science*, 87, 12–8.

Chou, S.T., Chao, W.W., & Chung, Y.C. (2002). Antioxidative activity and safety of 50% ethanolic red bean extract (*Phaseolus radiatus L. var. Aurea*). *Journal of food science*, 68, 21-25.

Colindres, P., & Brewer, M. S. (2011). Oxidative stability of cooked, frozen, reheated beef patties: Effect of antioxidants. *Journal of the Science of Food and Agriculture*, 91, 963–968.

Cortinas, L., Villaverde, C., Galobart, J., Baucells, M. D., Codony, R., & Barroeta, a. C. (2004). Fatty Acid Content in Chicken Thigh and Breast as Affected by Dietary Polyunsaturation Level. *Poultry Science*, 83, 1155-1164.

Cross, H.R., Leu, R., & Miller, M.F. (1987). Scope of warmed-over flavour and its importance to the meat industry. In St Angelo, A. J, & Bailey, M.E. (Eds.). *Warmed-over flavour of meat* (pp. 1-18). London, England: Academic Press, Inc.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313–7352.

Dai, Y., Liu, D. Y., Chen, M., Lei, Y., Zhai, Y. N., Xu, X. L., & Zhou, G. H. (2014). The effect of various postproduction heat treatment on lipid composition and oxidation in chinese nanjing salted duck. *Journal of Food Processing and Preservation*, 38(3), 837–844.

De Cordova, C. S., Locatelli, C., Assunção, L. S., Mattei, B., Mascarello, A., Winter, E., Creczynski-Pasa, T. B. (2011). Octyl and dodecyl gallates induce oxidative stress and apoptosis in a melanoma cell line. Toxicology in Vitro: *An International Journal Published in Association with BIBRA*, 25(8), 2025–34.

Decker, E.A. (2002). Antioxidant Mechanisms. In Akoh, C.C., & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (*2nd*.pp. 535-560). New York: Marcel Dekker, Inc.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, 22(3), 296–302.

Domínguez, R., Borrajo, P., & Lorenzo, J. M. (2015). The effect of cooking methods on nutritional value of foal meat. *Journal of Food Composition and Analysis*, 43, 61–67.

Domínguez, R., Gómez, M., Fonseca, S., & Lorenzo, J. M. (2014). Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science*, 97(2), 223–30.

Domínguez, R., Gómez, M., Fonseca, S., &. Lorenzo, J. M (2015). Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat. *LWT - Food Science and Technology* 58, 439-445.

El-Alim, S. S. L. A., Lugasi, A., Hóvári, J., & Dworschák, E. (1999). Culinary herbs inhibit lipid oxidation in raw and cooked minced meat patties during storage. *Journal of the Science of Food and Agriculture*, 79(2), 277–285.

El-Shourbagy, G., & El-Zahar, K. M. (2014). Oxidative stability of ghee as affected by natural antioxidants extracted from food processing wastes. *Annals of Agricultural Sciences*, 59(2), 213–220.

Eric Chan, W. C., Lim, Y. Y., & Wong, S. K. (2011). Antioxidant properties of ginger leaves: An overview. *Free Radicals and Antioxidants*, 1(1), 6–16.

Erickson, M.C. (2002). Lipid oxidation of muscle foods. In. Akoh, C.C, & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (*2nd*. pp. 383-429). New York, U.S: Marcel Dekker, Inc.

Erkan, N., Ayranci, G., & Ayranci, E. (2008). Antioxidant activities of rosemary (*Rosmarinus Officinalis L.*) extract, blackseed (*Nigella sativa L.*) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry*, 110(1), 76–82.

Estevez, M., Morcuende, D. & Ventanas, S. (2009). Determination of oxidation. In Nollet, L.L., & Toldr, F. (Eds.), *Handbook of muscle foods analysis* (pp. 221-239). Boca Raton, U.S: CRC Press.

Fan, L., & Michael Eskin, N.A. (2015). The use of antioxidants in the preservation of edible oils. In Shahidi, F. (Eds), *Handbook of Antioxidants for Food Preservation* (pp. 373-388). Cambridge, England: Woodhead Publishing.

Farhoosh, R., Khodaparast, M. H. H., Sharif, A., & Rafiee, S. A. (2012). Olive oil oxidation: Rejection points in terms of polar, conjugated diene, and carbonyl values. *Food Chemistry*, 131(4), 1385–1390.

Fasseas, M. K., Mountzouris, K. C., Tarantilis, P. a., Polissiou, M., & Zervas, G. (2008). Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, 106 (3), 1188-1194.

Faustman, C., & Cassens, R. G. (1991). The effect of cattle breed and muscle type on discoloration and various biochemical parameters in fresh beef. *Journal of Animal Science*, 69(1), 184–93.

Feddern, V., Kupski, L., Cipolatti, E. P., Giacobbo, G., Mendes, G. L., Badiale-Furlong, E., & de Souza-Soares, L. (2010). Physico-chemical composition, fractionated glycerides and fatty acid profile of chicken skin fat. *European Journal of Lipid Science and Technology*, 112 (11), 1277-1284.

Feiner, G. (2006). Meat Products Handbook; Practical Science and Technology. Abington Cambridge, England: Woodhead Publishing Limited.

Ferrentino, G., & Spilimbergo, S. (2016). A combined high-pressure carbon dioxide and high power ultrasound treatment for the microbial stabilization of cooked ham. *Journal of Food Engineering*, 174, 47-55.

Flavia, P., Zorica, V. and Delia, B. (2014). Effects of temperature and storage time on the quality of alimentary animal fats. *International Food Research Journal*, 21(4), 1507-1514.

Fletcher, D. L., Qiao, M., & Smith, D. P. (2000). The relationship of raw broiler breast meat color and pH to cooked meat color and pH. *Poultry Science*, 79:784-788.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation andpurification of total lipids from animal. *Journal of Biological Chemistry*, 1957, 226; 497-509.

Frankel, E. N. (1980). Lipid oxidation. *Progress in Lipid Research*, 19(1-2), 1–22.

Fraqueza, M. J., & Barreto, S. (2011). Gas mixtures approach to improve turkey meat shelf life under modified atmosphere packaging: the effect of carbon monoxide. *Poultry Science*, 90(9), 2076–2084.

Gao, T., Li, J., Zhang, L., Jiang, Y., Liu, Y., Zhang, X., & Zhou, G. (2015). Effect of different tumbling marination methods and time on the quality characteristics of prepared pork chops. *Food Science and Technology*, 28 (2), 260-267.

Gearhart, W.M., & Stuckey, B.N. (1955). A comparison of commercially used phenolic antioxidants in edible animal fats. Journal of the American Oil Chemists Society, 32(7), 386-390.

Gharavi, N., Haggarty, S., & S. El-Kadi, A. (2007). Chemoprotective and Carcinogenic Effects of tert-Butylhydroquinone and Its Metabolites. *Current Drug Metabolism*, 8, 1-7.

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysia young ginger (*Zingiber officinale Roscoe*). *Molecules*, 15(6), 4324–4333.

Gheisari, H. R., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2010). Sodium chloride or heme protein induced lipid oxidation in raw, minced chicken meat and beef. *Czech Journal of Food Science*, 28(5), 364-375.

Gong, Y., Parker, R. S., & Richards, M. P. (2010). Factors affecting lipid oxidation in breast and thigh muscle from chicken, turkey and duck. *Journal of Food Biochemistry*, 34, 869–885.

Gordon, M. H. (2001). The development of oxidative rancidity in foods. In Pokorny, J, Yanishlieva,N. (Eds.), *Antioxidants in food Practical applications* (pp. 7-20.) Abington Cambridge, England. Woodhead Publishing Ltd.

Goswami, M., Prabhakaran, P. P., and Tanwar, V. K. (2014) Antioxidant and antimicrobial effects of condiments paste used as nitrite replacer in chicken mince. *Veterinary World*, 7(6), 432-438.

Grady, M. N. O., Monahan, F. J., Burke, R. M., & Allen, P. (2000). The effect of oxygen level and exogenous a-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat science*, 55, 39-45.

Grossi, A., Bolumar, T., Søltoft-Jensen, J., & Orlien, V. (2014). High-pressure treatment of brine enhanced pork semitendinosus: Effect on microbial stability, drip loss, lipid and protein oxidation, and sensory properties. *Innovative Food Science & Emerging Technologies*, 22, 11–21.

Guidi, A., & Castigliego, L. (2010). Poultry meat colour. In Guerrero-Legarreta, I. (Eds.), *Handbook of poultry science and technology: Secondary processing* (*Vol .2*. pp. 359-378). New Jersey, U.S: A John Wiley & Sons, Inc.

Guzmán, E., Baeten, V., Fernández Pierna, J. A., & García-Mesa, J. A. (2011). Application of low-resolution Raman spectroscopy for the analysis of oxidized olive oil. *Food control*, 22 (12), 2036–2040.

Haile, D. M., De Smet, S., Claeys, E., & Vossen, E. (2013). Effect of light, packaging condition and dark storage durations on colour and lipid oxidative stability of cooked ham. *Journal of Food Science and Technology*, 50(2), 239–47.

Hall, C. (2001). Sources of natural antioxidants: oilseeds, nuts, cereals, legumes, animal products and microbial sources. In Pokorny, J, Yanishlieva, N. (Eds.), *Antioxidants in food practical applications* (pp. 159-198). Abington Cambridge, England: Woodhead Publishing Ltd.

Han, X., Shen, T., & Lou, H. (2007). Dietary Polyphenols and Their Biological Significance. *International Journal of Molecular Sciences*, 8(9), 950–988.

Hansen, C.L., Berg, F. van der, Ringgaard, S., Stodkilde-Jorgensen, H. & Karlsson, A.H. (2008). Diffusion of NaCl in meat studied by ^{1}H and ^{23}Na magnetic resonance imaging. *Meat Science*, 80, 851–856.

Hassan, M. A. M., & Youssef, A. M. K. (2012). Effect of Toasting and Microwaving on Gross Chemical Composition, Total phenolics, antioxidant activity and phenolic acids fractionation of white beans flour (*Phaseolus vulgaris* L.). *World Applied Sciences Journal*, 18 (6), 731-736.

Hayes, J.E. (2008). Sensory descriptors for cooked meat products. In: Nollet, L.L. & Toldr, F. (Eds.), *Handbook of processed meats and poultry analysis* (pp. 399–421). Boca Raton, U.S: CRC Press.

Hernández-Hernández, E., Ponce-Alquicira, E., Jaramillo-Flores, M. E., & Guerrero Legarreta, I. (2009). Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. *Meat Science*, 81(2), 410–7.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49 (4), 447-457.

Hosseini, S.E., & Mehr, E. (2015). The effect of meat marinating with lactic and citric acid on some physicochemical and electrophoretic pattern of beef burger. *Iranian Journal of Veterinary Medicine*, 9, 103-108.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6), 1841-1856.

Huang, L., Xiong, Y. L., Kong, B., Huang, X., & Li, J. (2013). Influence of storage temperature and duration on lipid and protein oxidation and flavour changes in frozen pork dumpling filler. *Meat Science*, 95(2), 295–301.

Huang, Y., He, Z., Li, H., Li, F., & Wu, Z. (2012). Effect of antioxidant on the fatty acid composition and lipid oxidation of intramuscular lipid in pressurized pork. *Meat Science*. 91(2), 137-141.

Huff-Lonergan, E. (2010). Chemistry and biochemistry of meat. In Toldra, F. (Eds.), *Handbook of meat processing* (pp. 5-24). Ames, U.S: Blackwell Publishing.

Huff-Lonergan, E., & Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat science,* 71, 194-204.

Humada, M. J., Sañudo, C., & Serrano, E. (2014). Chemical composition, vitamin E content, lipid oxidation, colour and cooking losses in meat from Tudanca bulls finished on semi-extensive or intensive systems and slaughtered at 12 or 14 months. *Meat Science,* 96(2PtA), 908-915.

Hwang, K. E., Choi, Y. S., Choi, S. M., Kim, H. W., Choi, J. H., Lee, M. A., & Kim, C. J. (2013). Antioxidant action of ganghwayakssuk (*Artemisia princeps Pamp*) in combination with ascorbic acid to increase the shelf life in raw and deep fried chicken nuggets. *Meat Science*, 95(3), 593–602.

Hwang, K. E., Kim, H. W., Choi, Y. S., Lee, S. Y., Yeo, E. J., Ham, Y. K., Kim, C. J. (2012). Evaluation of the antioxidant effect of ganghwayakssuk (*Artemisia princeps Pamp*) extract alone and in combination with ascorbic acid in raw chicken patties. *Poultry Science,* 92, 3244–3250.

Ibrahim, H., Abou-Arab., & Abu Salem, F. (2011). Antioxidant and antimicrobial effects of some natural plant extracts added to lamb patties during storage. *Grasas Y Aceites*, 62(2), 139–148.

Igene, J. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry*, 5(4), 263–276.

Igene, J.O., & Pearson, A.M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal of Food Science*, 44(5), 1285-1290.

Igene, J.O., Pearson, A.M., Dugan, L.R., Jr. & Price, J.F. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry*, 5, 263-276.

Iqbal, S., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry*, 100(1), 246–254.

Jääskeläinen, E., Hultman, J., Parshintsev, J., Riekkola, M.-L., & Björkroth, J. (2016). Development of spoilage bacterial community and volatile compounds in chilled beef under vacuum or high oxygen atmospheres. *International Journal of Food Microbiology*, 223, 25–32.

James, C., & James, S. J (2010). Freezing/Thawing. In Fidel Toldrá. (Eds.) *Handbook of meat processing* (pp. 105-125). Iowa, U.S: A John Wiley & Sons, Inc.

Javidipour, I., Tüfenk, R., & Baştürk, A. (2015). Effect of ascorbyl palmitate on oxidative stability of chemically interesterified cottonseed and olive oils. *Journal of Food Science and Technology*, 52(2), 876–84.

Jayasena, D.D., Ahn, D.U., Nam, K.C., & Jo, C. (2013). Factors affecting cooked chicken meat flavour: a review. *World's Poultry Science Journal*, 69(03), 515-526.

Jayathilakan, K., Sharma, G. K., Radhakrishna, K., & Bawa, S. (2007). Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chemistry*, 105(3), 908–916.

Jittrepotch, N., Ushio, H., & Ohshima, T. (2006). Oxidative stabilities of triacylglycerol and phospholipid fractions of cooked Japanese sardine meat during low temperature storage. *Food Chemistry*, 99, 360–367.

Johnson,I. T. (2001). Antioxidants and antitumour properties. In Pokorny, J., Yanishlieva , N. (Eds.), *Antioxidants in food practical applications* (pp. 100-119). Abington Cambridge, England. Woodhead Publishing Ltd.

Journi, M., Hammouda, H., Trabelsi-Ayadi, M., & Chérif, J. K. (2015). Quantitative determination and identification of phenolic compounds of three Tunisian legumes: *Vicia faba*, *Lens culinaris* and *Phaseolus vulgaris*. *Advances in Chemistry and Biochemistry Sciences* 02(03), 1–12.

Juntachote, T., Berghofer, E., Siebenhandl, S., & Bauer, F. (2006). The antioxidative properties of Holy basil and Galangal in cooked ground pork. *Meat Science*, 72(3), 446–456.

Juntachote, T., Berghofer, E., Siebenhandl, S., & Bauer, F. (2007). The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. LWT - *Food Science and Technology*, 40, 324–330.

Kahl, R. & Kappus, H. (1993). Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Zeitschrift fur Lebensmittel-untersuchung und-forschung*, 196 (4), 329-338.

Karamać, M., Kosińska, A., Estrella, I., Hernández, T., & Dueñas, M. (2012). Antioxidant activity of phenolic compounds identified in sunflower seeds. *European Food Research and Technology*, 235(2), 221–230.

Kargiotou, C., Katsanidis, E., Rhoades, J., Kontominas, M., & Koutsoumanis, K. (2011). Efficacies of soy sauce and wine base marinades for controlling spoilage of raw beef. *Food Microbiology*, 28(1), 158–63.

Karre, L., Lopez, K. & Getty, K.J. (2013). Natural antioxidants in meat and poultry products. *Meat science*, 94(2), 220-227.

Kawakatsu, M., Terao, J., & Matsushita, S. (2014). Phospholipid oxidation catalyzed by ferrous ion and ascorbic acid. *Agricultural and Biological Chemistry*, 48(5), 1275–1279.

Ke, P. J., & Woyewoda, A. D (1979). Microdetermination of thiobarbituric acid values in marine lipids by a direct spectrophotometric method with a monophasic reaction system. *Analytica chimica acta*, 106 (2), 279–284.

Kerler, J., & Grosch, W. (1997). Character impact odourants of boiled chicken: changes during refrigerated storage and reheating. *European Food Research and Technology*, 205, (3), 232–238.

Khan, M. A., & Shahidi, F. (2001). Effects of natural and synthetic antioxidants on the oxidative stability of borage and evening primrose triacylglycerols. *Food Chemistry*, 75, 431–437.

Khan, M. I., Min, J. S., Lee, S. O., Yim, D. G., Seol, K. H., Lee, M., & Jo, C. (2015). Cooking, storage, and reheating effect on the formation of cholesterol oxidation products in processed meat products. *Lipids in Health and Disease*, 14(1), 89.

King, A. J., & Bosch, N. (1990). Effect of NaCl and KCl on rancidity of dark turkey meat heated by microwave. *Journal of Food Science*, 55(6):1549-1551

Kishk, Y. F.M., & El Sheshetawy, H. E. (2010). Optimization of ginger (*Zingiber officinale*) phenolics extraction conditions and its anti-oxidants and radical scavenging activities using response surface methodology. *World Journal of Dairy Food Science*. 5 (2), 188-196.

Kosinska, A., & Karamac, M. (2006). Antioxidant capacity of roasted health-promoting products. *Polish Journal of Food and Nutrition Sciences*, 15, 193-198.

Kralik, G., Djurkin, I., Kralik, Z., Skrtic, Z., & Radisic, Z. (2014). Quality indicators of broiler breast meat in relation to colour. *Animal Science Papers and Reports*, 32(2), 173–178.

Kraybill, H.R., Dugan, L.R., Beadle, B.W., Vibrans, F.C., Swartz, V., & Rezabek, H. (1949). Butylated hydroxyanisole as an antioxidant for animal fats. *Journal of the American Oil Chemists' Society*, 26(9), 449-453.

Laack, R.L.J.M. (1994). Spoilage and preservation of muscle food. In: Kinsman, D. M., Kotula, A. W. and Breidenstein, B. C. (Eds.), *Muscle foods: meat poultry and seafood technology* (pp. 378-404). New York: Chapman and Hall, Inc.

Lanari, M.C., Schaefer, D.M., & Scheller, K.K. (1995). Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Science*, 41(3), 237–250.

Lara, M. S., Gutierrez, J. I., Timón, M., & Andrés, I. (2011). Evaluation of two natural extracts (*Rosmarinus officinalis* L. and *Melissa officinalis* L.) as antioxidants in cooked pork patties packed in MAP. *Meat Science*, 88(3), 481–488.

Lau, D. W., & King, A. J. (2003). Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *Journal of Agricultural and Food Chemistry*, 51(6), 1602–1607.

Lee, E.J., & Ahn, D.U. (2005). Quality characteristics of irradiated turkey breast rolls formulated with plum extract. *Meat Science*, 71(2), 300-305.

Lee, M. A., Choi, J. H., Choi, Y. S., Han, D. J., Kim, H. Y., Shim, S. Y., & Kim, C. J. (2010). The antioxidative properties of mustard leaf (*Brassica juncea*) kimchi extracts on refrigerated raw ground pork meat against lipid oxidation. *Meat Science*, 84, 498–504.

Lee, M. A., Choi, J. H., Choi, Y. S., Kim, H. Y., Kim, H. W., Hwang, K.-E., & Kim, C.J. (2011). Effects of kimchi ethanolic extracts on oxidative stability of refrigerated cooked pork. *Meat Science*, 89(4), 405–11.

Lemos, A. L. S. C., Nunes, D. R. M., & Viana, A. G. (1999). Optimization of the still-marinating process of chicken parts. *Meat science*, 52, 227–234.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, 91(2), 93–98.

Liang, C., Chan, L., Ding, H., So, E. C., & Lin, R. (2012). Free radical scavenging activity of 4 - (3,4-dihydroxybenzoyloxymethyl) phenyl-o-*β*-d-glucopyranoside from origanum vulgare and its protection against oxidative damage. *Journal of Agriculture and Food Chemistry*, 60, 7690-7696.

Liang, Q., Cui, J., Li, H., Liu, J., & Zhao, G. (2013). Florets of Sun fl ower (*Helianthus annuus* L.): Potential new sources of dietary fiber and phenolic acids. *Journal of Agriculture and Food Chemistry*, 61. 3435-3442.

Lin, L.Z., Harnly, J. M., Pastor-Corrales, M. S., & Luthria, D. L. (2008). The polyphenolic profiles of common bean (*Phaseolus vulgaris L.*). *Food Chemistry*, 107(1), 399–410.

Lorenzoa, J, M., Cittadinib, A., Munekatac, P. E., & Domíngueza, P. (2015). Physicochemical properties of foal meat as affected by cooking methods. *Meat science,* 108, 50–54.

Love, J (1987). Mechanism of Iron Catalysis of Lipid Oxidation in Warmed-Over Flavor of Meat. In St Angelo, A. J, & Bailey, M.E. (Eds.). *Warmed-over flavour of meat* (pp. 19-41). London, England: Academic Press, Inc.

Luis, J. C., & Johnson, C. B. (2005). Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of their in vitro antiradical activity. *Spanish Journal of Agricultural Research*, 3(1), 106–112.

Luthria, D. L., & Pastor-Corrales, M. A. (2006). Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris L.)* varieties. *Journal of Food Composition and Analysis*, 19(2-3), 205–211.

Ma, H., Ledward, D., Zamri, A, Frazier, R., & Zhou, G. (2007). Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle. *Food Chemistry*, 104(4), 1575-1579.

Maizura, M., Aminah, A. and Wan Aida, W. M. (2011). Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale)* and turmeric (*Curcuma longa*) extract. *International Food Research Journal*, 18, 529-534.

Mancini, R., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, 71, 100-121.

Mansour, E. H., & Khalil, A. H. (2000). Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry*, 69(2), 135–141.

Manthey, J. A., & Grohmann, K. (2001). Phenols in citrus peel by products. concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agriculture, Food Chemistry*, 49 (7), 3268–3273.

Maqsood, S., Abushelaibi, A., Manheem, K., & Kadim, I. T. (2015). Characterisation of the lipid and protein fraction of fresh camel meat and the associated changes during refrigerated storage. *Journal of Food Composition and Analysis*, 41, 212–220.

Mariutti, L. R. B., Nogueira, G. C., & Bragagnolo, N. (2011). Lipid and cholesterol oxidation in chicken meat are inhibited by sage but not by garlic. *Journal of Food Science*, 76, C909–15.

Martínez, M. L., Penci, M. C., Ixtaina, V., Ribotta, P. D., & Maestri, D. (2013). Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions. *LWT - Food Science and Technology*, 51(1), 44–50.

McCarthy, T., Kerry, J. P., Kerry, J. F., Lynch, P. B., & Buckley, D. J. (2001). Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Science*, 58(1), 45-52.

McKee, L. (2007). Microbiological and Sensory Properties of Fresh and Frozen Pork Products. In Nollet, L. M. L. (Eds.), *Handbook of meat, poultry and seafood qualities* (pp. 395-404). Ames, U.S: Blackwell Publishing.

Mensink, R. P and Jogchum, P. (2002). Dietary fats and coronary heart disease. In Akoh, C.C., & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (2nd pp. 383-429). New York, U.S: Marcel Dekker, Inc.

Meynier, A., Genot, C., & Gandemer, G. (1998). Volatile compounds of oxidized pork phospholipids. *Journal of the American Oil Chemists' Society,* 75(1), 1–7.

Middleton, T. F., Ferket, P. R., & Boyd, L. C. (1997). The effect of ethoxyquin on the quality of ground poultry mortality carcasses preserved by lactic acid fermentation and phosphoric acid stabilization. *Poultry Science*, 80, 1154–1163.

Mielnik, M. B., Aaby, K., & Skrede, G. (2003). Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. *Meat Science*, 65(3), 1147–1155.

Mielnik, M. B., Olsen, E., Vogt, G., Adeline, D., & Skrede, G. (2006). Grape seed extract as antioxidant in cooked, cold stored turkey meat. LWT - *Food Science and Technology*, 39(3), 191–198.

Miller, R. K. (1994). Quality characteristics. In: Kinsman, D. M., Kotula, A. W., & Breidenstein, B. C. (Eds.), *Muscle foods: meat poultry and seafood technology (*pp. 296-332). New York, U.S: Chapman and Hall, Inc.

Min, B., & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products -A review. *Food Science Biotechnology,* 14 (1), 152 – 163.

Min, B., & Ahn, D. U. (2009). Factors in various fractions of meat homogenates that affect the oxidative stability of raw chicken breast and beef loin. *Journal of Food Science*, 74 (1), C41-48.

Min, B., Cordray, J. C., & Ahn, D. U. (2011). Antioxidant effect of fractions from chicken breast and beef loin homogenates in phospholipid liposome systems. *Food Chemistry*, 128 (2), 299-307.

Min, B., Nam, K. C., Cordray, J., & Ahn, D. U. (2008). Endogenous Factors Affecting Oxidative Stability of Beef Loin, Pork Loin, and Chicken Breast and Thigh Meats. *Journal of Food Science*, 73(6), C439-C446.

Min, D. B., & Boff, J. M. (2002). Lipid oxidation of edible oil. In: Akoh, C.C., & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (*2nd*. pp. 353-381).New York, U.S: Marcel Dekker, Inc.

Mottram, D.S. (1998). The chemistry of meat flavour. In Shahidi, F. (Eds.), *Flavor of meat, meat products and seafoods* (*2nd*. pp 5-26). London, England: Blackie Academic and Professional.

Movileanu, I., Núñez De González, M. T., Hafley, B., Miller, R. K., & Keeton, J. T. (2013). Comparison of dried plum puree, rosemary extract, and bha/bht as antioxidants in irradiated ground beef patties. *International Journal of Food Science*, 360732(7), 1-7.

Muela, E., Sanudo, C., Campo, M. M., Medel, I., & Beltran, J. A. (2010). Effect of freezing method and frozen storage duration on instrumental quality of lamb throughout display. *Meat Science*, 84(4), 662–669.

Munekata, P.E.S., Calomeni, A.V., Rodrigues, C.E.C., Fávaro-Trindade, C.S., Alencar, S.M., & Trindade, M.A. (2015). Peanut skin extract reduces lipid oxidation in cooked chicken patties. *Poultry science*, 94(3):442-446.

Murphy, R. Y., & Marks, B. P. (2000). Effect of meat temperature on proteins, texture, and cook loss for ground chicken breast patties. *Poultry Science*, 79(1), 99–104.

Naczka, M., & Shahidib, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 41(5), 1523–1542.

Nam, K. C., & Ahn, D. U. (2002). Carbon monoxide-heme pigment is responsible for the pink color in irradiated raw turkey breast meat. *Meat Science*, 60(1), 25–33.

Nam, K. C., Min, B. R., Yan, H., Lee, E. J., Mendonca, A., Wesley, I., & Ahn, D. U. (2003). Effect of dietary vitamin E and irradiation on lipid oxidation, color, and volatiles of fresh and previously frozen turkey breast patties. *Meat Science*, 65, 513–521.

Nanke, K. E., Sebranek, J. G., & Olson, D. G. (1998). Color characteristics of irradiated vacuum-packaged pork, beef, and turkey. *Journal of Food Science*, 63 (6), 1001–1006.

Naveena, B. M., & Mendiratta, S. K. (2001). Tenderisation of spent hen meat using ginger extract. *British Poultry Science*, 42, 344–349.

Naveena, B. M., Mendiratta, S. K., & Anjaneyulu, A. S. R. (2004A). Tenderization of buffalo meat using plant proteases from *Cucumis trigonus Roxb* (Kachri) and *Zingiber officinale roscoe* (Ginger rhizome). *Meat Science*, 68(3), 363–9.

Naveena, B.M., & Mendiratta, S.K (2004B). The tenderization of buffalo meat using ginger extract. *Journal of Muscle Foods*, 15,235–244.

Naveena, B. M., Sen, R., Vaithiyanathan, S., Babji, Y., & Kondaiah, N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Science*, 80, 1304–1308.

Naveena, B. M., Vaithiyanathan, S., Muthukumar, M., Sen, R., Kumar, Y. P., Kiran, M., & Chandran, K. R. (2013). Relationship between the solubility, dosage and antioxidant capacity of carnosic acid in raw and cooked ground buffalo meat patties and chicken patties. *Meat Science*, 95, 195–202.

Naveena, B.M. & Mendiratta, S.K. (2001). Tenderisation of spent hen meat using ginger extract. *British poultry science*, 42, 344–349.

Naveena, B.M. & Mendiratta, S.K. (2004). The tenderization of buffalo meat using ginger extract. Journal of Muscle Foods, 15 (4), 235-244.

Nawar, W.W. (1996). Lipids. In: Fennema, O.R., Ed., *Food Chemistry*. Marcel Dekker, New York.

Nerin, C., Tovar, L., Djenane, D., Camo, J., Salafranca, J., Beltran, J. A. and Roncales, P. (2006). Stabilization of beef meat by new active packaging containing natural antioxidants. Journal of Agricultural and Food Chemistry, 54 (20), 7840-7846.

Nerín, C., Tovar, L., Djenane, D., Camo, J., Salafranca, J., Beltrán, J. A., & Roncalés, P. (2006). Stabilization of beef meat by new active packaging containing natural antioxidants. *Journal of Agriculture and Food Chemistry*, 52, 5598-5605.

Nieto, G., Diaz, P., Banon, S., & Garrido, M.D. (2010). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet. *Meat Science,* 85, 82–88.

Nile, S. H., & Park, S. W. (2015). Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. *Industrial Crops and Products*, 70, 238–244.

Nissen, L. R., Byrne, D.V., Bertelsen, G., & Skibsted, L. H. (2004). The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Science*, 68,485–495.

Nithyalakshmi, V., & Preetha, R. (2015). Effect of cooking conditions on physico-chemical and textural properties of Emu (*Dromaius novaehollandiae*) meat. *International Food Research Journal*, 22, 1924-1930.

Nunez-Gonzalez, F. A (2010). Marination, cooking, and curing: principles. In Guerrero-Legarreta, I., & Hui, Y. H) (Eds.), *Handbook of poultry science and technology* (pp. 81-89). New Jersey, U.S: Hoboken.

Nute, G.R. (2009). Sensory descriptors. In Nollet, L., & Toldra, F. (Eds.). *Handbook of Muscle Foods Analysis* (pp. 513-523). Boca Raton, U.S: Taylor & Francis Group, LLC.

O'Fallon, J. V., Busboom, J. R., Nelson, M. L., & Gaskins, C. T (2007). A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science.* 85, 1511–1521.

O'Fallon, J. V., Busboom, J. R., Nelson, M. L., & Gaskins, C. T (2007). A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of. Animal. Science,* 85, 1511–1521.

O'Keefe, S. F. (2002). Nomenclature and Classification of Lipids. In Akoh, C.C, & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (pp. 19-56). *2nd Edition.* New York: Marcel Dekker, Inc.

Offer, G. & Trinick, J. (1983). On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Science,* 8, 245–281.

Offer, G., & Trinick, J. (1983). On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Science,* 8, 245–281.

Oz, F., & Zikirov, E. (2015). The effects of sous-vide cooking method on the formation of heterocyclic aromatic amines in beef chops. *LWT - Food Science and Technology*, 64(1), 120–125.

Ozer, O., & Sariçoban, C. (2010). The effects of butylated hydroxyanisole, ascorbic acid, and α-tocopherol on some quality characteristics of mechanically deboned chicken patty during freeze storage. *Czech Journal of Food Sciences*, 28(2), 150–160.

Packer, V.G., Melo, P.S., Bergamaschi, K.B., Selani, M.M., Villanueva, N.D., de Alencar, S.M. and Contreras-Castillo, C.J. (2015). Chemical characterization, antioxidant activity and application of beetroot and guava residue extracts on the preservation of cooked chicken meat. *Journal of Food Science and Technology*, 52(11), 7409-7416.

Pearson, A. M., & Gray, J. I. (1983). Mechanism Responsible for Warmed-Over Flavor in Cooked Meat. In Waller, G. R., & Feather M. S. (Eds.), *The maillard reaction in foods and nutrition* (pp 287–300). Washington DC, U.S: American Chemical Society.

Pegg, R. B. (2005). Lipid oxidation/stability. In: Wrolstad, R.E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, J. S., Shoemaker, C. F., Smith, D., & Sporns, P., (Eds.), *Handbook of food analytical chemistry, water, proteins, enzymes, lipids, and carbohydrates*. (pp. 513-529) New Jersey, U.S: Wiley interscience.

Pereira de Abreu, D. A., Paseiro Losada, P., Maroto, J., & Cruz, J. M. (2011). Natural antioxidant active packaging film and its effect on lipid damage in frozen blue shark (*Prionace glauca*). *Innovative Food Science & Emerging Technologies*, 12(1), 50–55.

Pikul, J. (1985). Oxidation Products in Chicken Meat After Frozen Storage, Microwave and Convection Oven Cooking, Refrigerated Storage, and Reheating. *Poultry Science*, 64, 93-100.

Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1984). Relative role of phospholipids, triacylglycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *Journal of Food Science*, 49, 704–708.

Pikul, J., Leszczynskl, D. E., & Kummerow, F. A. (1985). Influence of fat content and composition on malonaldehyde concentration in chicken meat and skin. *Poultry Science*, 64, 311-317.

Pizato, S., Cortez-Vega, W. R., & Prentice, C. (2015). Quality assessment of cooked chicken breast meat at different storage temperatures. *International Food Research Journal*, 22, 143-154.

Poiana, M.-A. (2012). Enhancing oxidative stability of sunflower oil during convective and microwave heating using grape seed extract. *International Journal of Molecular Sciences*, 13, 9240-9259.

Post, R. C., & Heath, J. L. (1983). Marinating broiler parts: the use of a viscous type marinade. *Poultry Science*, 62, 977-984.

Puangsombat, K., & Smith, J. S. J. (2010). Inhibition of heterocyclic amine formation in beef patties by ethanolic extracts of rosemary. *Journal of Food Science*, 75(2), T40-47.

Pulgar, J. D. D., Gázquez, A., & Ruiz-Carrascal, J. (2012). Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science*, 90(3), 828–835.

Radha Krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., & Sukumar, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology*, 171, 32–40.

Reische, D.W., Lillard, D.A. & Eitenmiller, R.R. (2002). Antioxidants. In Akoh, C.C., & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology*. (2th pp. 507-534). New York: Marcel Dekker, Inc.

Rhee, K.S., & Ziprin, Y. A. (2001). Pro-oxidative effects of NaCl in microbial growth-controlled and uncontrolled beef and chicken. *Meat Science*, 57, 105-112.

Richards, M. P., & Hultin, H. O. (2000). Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. *Journal of Agricultural and Food Chemistry*, 48(8), 3141-3147.

Rimini, S., Petracci, M., & Smith, D. P. (2014). The use of thyme and orange essential oils blend to improve quality traits of marinated chicken meat. *Poultry Science*, 93(8), 2096–2102.

Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering*, 109(1), 98–103.

Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering*, 109(1), 98–103.

Rogers, R. W. (2007). Packaging and freezing of beef as related to sensory properties. In Nollet, L. M. L. (Eds.), *Handbook of meat, poultry and seafood qualities* (pp. 370-373). Ames, U.S: Blackwell Publishing.

Rojas, M. C. & M.S. Brewer (2007). Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. *Journal of Food Science*, 72, 282-288.

Rojas, M. C., & M.S. Brewer (2007). Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. *Journal of Food Science*, 72, 282-288.

Roldan, M., Antequera, T., Armenteros, M., & Ruiz, J. (2014). Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food chemistry*, 149, 129-136.

Roldan, M., Loebner, J., Degen, J., Henle, T., Antequera, T., & Ruiz-Carrascal, J. (2015). Advanced glycation end products, physico-chemical and sensory characteristics of cooked lamb loins affected by cooking method and addition of flavour precursors. *Food Chemistr*y, 168, 487–495.

Rowe, C., Pohlman, F., Jr, A. B., & Johnson, Z. (2007). Effect of Salt, Trisodium Phosphate, Synthetic Antioxidants, and Conjugated Linoleic Acid on Sensory and Quality Characteristics of Beef Striploins. *Arkansas,* 1995, 81-83

Ruiz, J., Muriel, E., Perez-Palacios, T., & Antequera, T. (2009). Analysis of Phospholipids in Muscle Foods. In Nollet, L.L, & Toldr, F. (Eds.), *Handbook of muscle foods analysis* (pp. 168-182). Boca Raton, U.S: CRC Press.

Rusaczonek, A., Żebrowska, M., Waszkiewicz-robak, B., & Ślusarczyk, E. (2007). Evaluation of phenolic compounds content and antioxidant capacity of herbs. *Polish Journal of Food and Nutritional Science*, 57, 483–488.

Saldanha, T., & Bragagnolo, N. (2007). Cholesterol oxidation is increased and PUFA decreased by frozen storage and grilling of atlantic hake fillets (Merluccius hubbsi). Lipids, 42, 671–678.

Sampaio, G., Saldanha, T., Soares, R., & Torres, E. (2012). Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. *Food Chemistry*, 135, 1383-1390.

Sánchez-Escalante, A., Torrescano, G., Djenane, D., Beltrán, J. A., Giménez, B., & Roncalés, P. (2011). Effect of antioxidants and lighting conditions on color and lipid stability of beef patties packaged in high-oxygen modified atmosphere. *Journal of Food*, 9(1), 49–57.

Sanders, D. H. (1969). Fluorescent dye tracing of water entry and retention in chilling of broiler chicken carcasses. *Poultry Science*, 48:2032–2037.

Sasse, A., Colindres, P., & Brewer, M. S. (2009). Effect of Natural and Synthetic Antioxidants on the Oxidative Stability of Cooked, Frozen Pork Patties. *Journal of Food Science*, 74(1), S30–S35.

Sato, K., & Hegarty, G. R. (1971). Warmed-over flavour in cooked meats. *Journal of Food Science*, 36, pp. 1098-1102.

Saucier, L., Gendron, C., & Gariépy, C. (2000). Shelf life of ground poultry meat stored under modified atmosphere. *Poultry Science*, 79(12), 1851–6.

Sebranek, J. G. (1995). Poultry and poultry products. In Jeremiah, L. E. (Eds), *Freezing effects on food quality* (pp. 85-109). New York, U.S: Marcel Dekker, Inc.

Selani, M. M., Contreras-Castillo, C. J., Shirahigue, L. D., Gallo, C. R., Plata-Oviedo, M., & Montes-Villanueva, N. D. (2011). Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. *Meat Science*, 88, 397–403.

Shah, M. A., Bosco, S. J. D., & Mir, S. A. (2014). Plant extracts as natural antioxidants in meat and meat products. *Meat Science*, 98(1), 21–33.

Shahdi & Wanasundara (2002). Extraction and analysis of lipids. In. Akoh, C.C, & Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (*2nd*. pp 151-178). New York, U.S: Marcel Dekker, Inc.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects -A review. *Journal of Functional Foods*, 18, 820–897.

Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53(20), 7749-7759.

Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53, 7749-7759.

Sharedeh, D., Gatellier, P., Astruc, T., & Daudin, J.-D. (2015). Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. *Meat Science*, 110, 24–31.

Shirin Adel P. R., & Prakash, J. (2010). Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *Journal of Medicinal Plants Research*, 4(24), 2674-2679.

Siddiq, M., Ravi, R., Harte, J. B., & Dolan, K. D. (2010). Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris L*.) flours. LWT - *Food Science and Technology*, 43(2), 232–237.

Singh, P., Sahoo, J., Chatli, M. K., & Biswas, A. K. (2014). Shelf life evaluation of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste as natural preservatives at refrigerated. *International Food Research Journal*, 21(4), 1363-1373.

Singleton, V.L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.

Singleton, V.L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.

Smith, D.P., & Northcutt, J.K. (2004). Induced red discoloration of broiler breast meat: I effect of blood, bone marrow and marination. *International Journal of Poultry Science*, 3 (4), 248-252.

Soyer, A., Özalp, B., Dalmış, Ü., & Bilgin, V. (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry*, 120, 1025-1030.

Su, L., Yin, J., Charles, D., Zhou, K., Moore, J., & Yu, L. (2007). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry*, 100 (3), 990-997.

Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating. *Food Chemistry*, 104(3), 997–1005.

Sun, Q., Senecal, A., Chinachoti, P., & Faustman, C. (2002). Effect of water activity on lipid oxidation and protein solubility in freeze-dried beef during storage. Journal of Food Science, 67(7), 2512–2516.

Sutivisedsak, N., Cheng, H. N., Willett, J. L., Lesch, W. C., Tangsrud, R. R., & Biswas, A. (2010). Microwave-assisted extraction of phenolics from bean (*Phaseolus vulgaris L*.). *Food Research International*, 43(2), 516-519.

Taghvaei, M., Jafari, S. M., Mahoonak, A. S., Nikoo, A. M., Rahmanian, N., Hajitabar, J., & Meshginfar, N. (2014). The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. *LWT - Food Science and Technology*, 56 (1), 124-130.

Takagi, S., & Yoshida, H. (1999). Microwave heating influences on fatty acid distributions of triacylglycerols and phospholipids in hypocotyl of soybeans (*glycine* max L.). *Food Chemistry*, 66, 345-351.

Talab, A. S. (2014). Effect of cooking methods and freezing storage on the quality characteristics of fish cutlets. *Advance Journal of Food Science and Technology*, 6(4), 468–479.

Tavasalkar, S. U., Mishra, H. N., & Madhavan, S. (2012). Evaluation of antioxidant efficacy of natural plant extracts against synthetic antioxidants in sunflower oil. *Open Access Scientific Reports*, 1-6.

Tavassoli, S., & Djomeh, Z. E. (2011). Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (*Rosmarinus officinalis* L.). *Global Veterinaria*, 7(4), 337–341.

Teets, S., Sundararaman, M., & Were, L. M. (2008). Electron beam irradiated almond skin powder inhibition of lipid oxidation in cooked salted ground chicken breast. *Food Chemistry*. 111, 934-941

Thiansilakul, Y., Benjakul, S., & Richards, M. P. (2011). Effect of myoglobin from Eastern little tuna muscle on lipid oxidation of washed Asian seabass mince at different pH conditions. *Journal of Food Science*, 76(2), C242-249.

Thummaluru, R. M. R., & Gurrala, S. (2016). Development and validation of HPLC-UV method for the estimation of fulvestrant in bulk drug. *Journal of Pharmacy Research*, 10(1), 21-24

Tichivangana, J. Z., & Morrissey, P. A. (1985). The influence of ph on lipid oxidation in cooked meats from several species. *Irish Journal of Food Science and Technology*, 9(2), 99-106.

Toldrá, F. (2006). Meat: chemistry and biochemistry. In Hui, Y. H. (Eds.), *Handbook of food science, technology, and engineering*. (pp. 28-1-28-14). Boca Raton: Taylor & Francis Group, LLC.

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. *Meat Science*, 70, 493-508.

Totosaus, A., Perez-Chabela, M. L., & Guerreroin, I. (2007). Color of fresh and frozen poultry. In Nollet, L. M. L. (Eds.), *Handbook of meat, poultry and seafood qualities* (pp. 455-465). Ames: Blackwell Publishing.

Trindade, R. A., Mancini-Filho, J., & Villavicencio, A. L. C. H. (2010). Natural antioxidants protecting irradiated beef burgers from lipid oxidation. *LWT - Food Science and Technology*, 43, 98–104.

Tsai, L. L., Yen, N. J., & Chou, R. G. R. (2012). Changes in Muscovy duck breast muscle marinated with ginger extract. *Food Chemistry*, 130(2), 316–320.

Tsuzuki, E., Garcia, E. de M., Murakami, A., Sakamoto, M., & Galli, J. (2003). Utilization of sunflower seed in laying hen rations. *Revista Brasileira de Ciência Avícola*, 5(3), 179–182.

Tsuzuki, W., Suzuki, Y., Yamada, S., Kano, S., Ohnishi, H., Fujimoto, T., & Horigane, A. (2014). Effect of oxygen absorber on accumulation of free fatty acids in brown rice and whole grain wheat during storage. LWT - *Food Science and Technology*, 58(1), 222–229.

Vaithiyanathan, S., Naveena, B. M., Muthukumar, M., Girish, P. S., & Kondaiah, N. (2011). Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 °C). *Meat Science*, 88 (3), 409–14.

Van Esch, G. J. (1986). Toxicology of tert-butylhydroquinone (TBHQ). *Food and Chemical Toxicology*, 24(10-11), 1063-1065.

Varakumar, S., Umesh, K. V., & Singhal, R. S. (2017). Enhanced extraction of oleoresin from ginger (*Zingiber officinale*) rhizome powder using enzyme-assisted three phase partitioning. *Food Chemistry*, 216, 27–36.

Vaudagna, S.R., Sánchez, G., Neira, M.S., Insani, E.M., Picallo, A.B., Gallinger, M.M., & Lasta, J.A. (2002). Sous vide cooked beef muscles: effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International journal of food science & technology*, 37(4), 425-441.

Velasco, V., & Williams, P. (2011). Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural research*, 71(2), 313-322.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *Journal of Agricultural and Food Chemistry*, 46 (10), 4113-4117.

Vieira, C., Diaz, M. T., Martínez, B., & García-Cachán, M. D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science*, 83(3), 398–404.

Waldroup, P. W., & Waldroup, A.L. (2005). Fatty acid effect on carcass the influence of various blends of dietary fats added to corn-soybean meal based diets on the fatty acid composition of broilers. *International Journal of Poultry Science*. 4 (3), 123-132.

Wang, D. Y., Liu, F., & Zhu, Y. Z. (2011). Changes of phospholipids in duck muscle by different heating methods. *Journal of Food Processing and Technology*, 02(05), 1-5.

Wang, R. R., Pan, X. J., & Peng, Z. (2009). Effects of heat exposure on muscle oxidation and protein functionalities of pectoralis majors in broilers. *Poultry Science*, 88, 1078–1084.

Weber, J., Bochi, V. C., Ribeiro, C. P., Victório, A. D. M., & Emanuelli, T. (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chemistry*, 106(1), 140–146.

Wilson, R. F& Rinne, R. W. (1976) Studies on lipid synthesis and degradation in developing soybean cotyledons. *Plant Physiology*, 57: 375-381.

Wood, J. D., Enser, M., Fisher, A. V, Nute, G. R., Sheard, P. R., Richardson, R. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78(4), 343-358.

Wrolstad, R. E., Durst, R. W., & Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science and Technology*, 16(9), 423–428.

Wu, X., & Beecher, G. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural*, 52, 4026-4037.

Xiong, Y., L., & Kupski, D. R. (1999 a). Monitoring phosphate marinade penetration in tumbled chicken filets using a thin-slicing, dye-tracing method. *Poultry Science*, 78(7), 1048–1052.

Xiong, Y.L. & Kupski, D.R. (1999b). Time-dependent marinade absorption and retention, cooking yield, and palatability of chicken filets marinated in various phosphate solutions. *Poultry science*, 78, 1053–1059.

Yanishlieva-Maslarova, N. V. (2001). Inhibiting oxidation. In. Pokorny, J., Yanishlieva, N. (Eds.) *Antioxidants in food Practical applications* (pp. 22-57.). Abington Cambridge, Woodhead Publishing Ltd.

Ye, F., Liang, Q., Li, H., & Zhao, G. (2015). Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthus annuus* L.). *Industrial Crops and Products*, 76, 574–581.

Yeh, H., Chuang, C., Chen, H., Wan, C., Chen, T., & Lin, L. (2014). Bioactive components analysis of two various gingers (*Zingiber officinale Roscoe*) and antioxidant effect of ginger extracts. LWT - *Food Science and Technology*, 55(1), 329–334.

Yoshida, H., Hirakawa, Y., Tomiyama, Y., Nagamizu, T., & Mizushina, Y. (2005). Fatty acid distributions of triacylglycerols and phospholipids in peanut seeds (Arachis hypogaea L.) following microwave treatment. *Journal of Food Composition and Analysis*, 18(1), 3-14.

Yu, L., Scanlin, L., Wilson, J., & Schmidt, G. (2002). Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. *Journal of Food Science*, 67(2), 582–585.

Yusop, S. M., O'Sullivan, M. G., Kerry, J. F., & Kerry, J. P. (2010). Effect of marinating time and low pH on marinade performance and sensory acceptability of poultry meat. *Meat Science*, 85(4), 657–663.

Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. (2010). Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chemistry*, 118, 656–662.

Zhang, Z. F., Zhou, T. X., & Kim, I. H. (2013). Effects of dietary olive oil on growth performance, carcass parameters, serum characteristics, and fatty acid composition of breast and drumstick meat in broilers. *Asian-Australasian Journal of Animal Sciences*, 26, 3, 416-422.

Zhou, G. H., Xu, X. L., & Liu, Y. (2010). Preservation technologies for fresh meat - A review. *Meat Science*, 86(1), 119–128.

Zhuang, H., Barth, M. M., & Hileband, D (2002). Fatty acid oxidation in plant tissues. In: Akoh, C.C., Min, D.B. (Eds.), *Food lipids: chemistry, nutrition, and biotechnology* (*2nd*. pp. 431-469). New York: Marcel Dekker, Inc.

Zilic, S.; Maksimovic-Dragisic, J.; Maksimovic, V.; Maksimovic, M.; Basic, Z.; Crevar, M.; Stankovic, G. (2010). The content of antioxidants in sunflower seed and kernel. *Helia*, 33(52), 75–84.

Zou, Y. P., & Chang Sam, K. C. (2014). Antioxidant and antiproliferative properties of extract and fractions from small red bean (*Phaseolus vulgaris* L.). *Journal of Food and Nutrition*, 1(105), 1-11.

Appendix A (Statistics Analysis for Chapter 2)

Table A 1 Lipid composition of various portions of chicken tissue.

	Chicken Tissue			
Parameters	Breast	Thigh	Adipose	Skin
Dry matter g/kg meat	251.3 ± 4.96a	270.4 ± 2.22b	534.5 ± 4.11c	826.6 ± 4.36d
Fat g/kg dry basis	66.6 ± 8.92a	271.9 ± 4.71b	777.8 ± 8.51c	971.7 ± 2.23d

Mean values with different small letters presented within each row differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A2 Effect of natural antioxidant extracts at different levels on TBARS values, Conjugated dienes, Phospholipid content and Conjugated trienes in fat from chicken portions during the accelerated storage time (ST).

Tissue	**Antioxidants (AO)**							
	TBARS value (mg MDA/kg fat)							
	CON	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**
Breast	45.63d	22.26b	24.54b	26.75bc	31.37c	12.82a	2.28	<0.001
Thigh	43.70e	25.70bc	31.59cd	22.81b	33.01d	13.92a	2.22	<0.001
Adipose	63.97d	40.06b	72.02e	46.24c	45.41bc	25.15a	2.04	<0.001
Skin	33.80c	8.57a	12.86b	16.16b	15.87b	6.72a	1.38	<0.001
	Conjugated dienes (µmol/g fat)							
	CON	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**
Breast	54.95b	39.92a	38.49a	35.60a	40.52a	34.69a	2.13	<0.001
Thigh	26.57a	31.05c	28.29ab	27.98ab	29.88bc	33.92d	1.00	<0.001
Adipose	10.62a	15.76c	12.25b	12.08b	11.61b	12.34b	0.31	<0.001
Skin	18.10d	16.54c	11.86a	12.51a	15.64b	23.93e	0.31	<0.001
	Phospholipid content (g100/g fat)							
	CON	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**
Breast	55.87b	53.66b	53.22b	51.15ab	51.94ab	46.71a	2.29	0.005
Thigh	28.38	28.34	31.00	29.17	30.35	27.41	1.43	0.091
Adipose	3.70	3.61	3.50	3.45	3.86	4.07	0.27	0.158
Skin	6.11	5.36	5.69	5.95	5.43	5.50	0.40	0.333
	Conjugated trienes (µmol/g fat)							
	CON	**ROS**	**SRB**	**SFS**	**GGR**	**BHT**	**SED**	**AO**
Breast	21.76d	13.97bc	14.50bc	12.03ab	14.89c	9.58a	0.98	<0.001
Thigh	11.65	13.02	12.55	11.69	12.90	12.75	0.50	0.060
Adipose	1.77a	4.00c	2.59ab	2.81b	2.53ab	2.81b	0.35	<0.001
Skin	3.72b	4.87c	3.46ab	3.21a	5.51d	7.37e	0.12	<0.001

Mean values with different small letters presented within each row of each tissue differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A3 Effect of natural antioxidant extracts at different levels on fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken breast tissue during the accelerated storage time at 62.8 °C.

Antioxidant (AO)		C14:0	C16:0	C18:0	C20:0	C16:1 n-7	C18:1 n-9	C18:2 n-6	C18:3 n-3
CON		0.19ab	11.87bc	4.19c	0.22ab	1.35	23.13bc	12.47bc	1.49ab
ROS		0.20b	11.93bc	4.13bc	0.22ab	1.43	23.51c	12.65c	1.54ab
SRB		0.18a	11.18a	3.92a	0.21a	1.33	21.89a	11.82a	1.43a
SFS		0.20b	12.07c	4.17c	0.23b	1.46	23.87c	12.94c	1.56b
GGR		0.19ab	11.51ab	4.01ab	0.21a	1.40	22.50ab	12.16ab	1.45ab
BHT		0.18ab	11.28a	3.91a	0.21a	1.40	22.50ab	12.02ab	1.47ab
Antioxidant (AO) x Level (L)									
AO	**L**								
CON	0 mg	0.19	11.87cde	4.19c	0.22	1.35	23.13	12.47bc	1.49abc
ROS	10 mg	0.20	11.81cde	4.14bc	0.21	1.41	23.04	12.41bc	1.50abc
	20 mg	0.20	12.06de	4.12bc	0.23b	1.45	23.97bc	12.88cd	1.58bc
SRB	10 mg	0.18	11.44abcd	4.08bc	0.21	1.34	22.10	12.02ab	1.43ab
	20 mg	0.18	10.91ab	3.75a	0.21	1.32	21.68a	11.62a	1.43ab
SFS	10 mg	0.20	11.66bcde	4.09bc	0.22	1.40	22.80	12.39bc	1.48abc
	20 mg	0.20	12.48e	4.24c	0.23b	1.52	24.94c	13.48d	1.63c
GGR	10 mg	0.18	11.14abc	3.97ab	0.21	1.33	21.44a	11.66a	1.37a
	20 mg	0.21	11.87cde	4.06bc	0.22	1.47	23.56	12.65bc	1.53abc
BHT	10 mg	0.18	11.81cde	4.06bc	0.22	1.49	23.59	12.61bc	1.54abc
	20 mg	0.18	10.74a	3.75a	0.20a	1.30	21.41a	11.44a	1.39a
Antioxidant (AO) x Storage time (ST)									
AO	ST								
CON	0	0.21bcd	12.48d	4.30d	0.23ef	1.31	24.68d	13.19de	1.58de
	3	0.19abcd	12.09cd	4.27d	0.23ef	1.38	23.47bcd	12.69cde	1.53de
	7	0.16ab	11.05abc	4.00bcd	0.20abcd	1.34	21.23abc	11.54ab	1.35ab
ROS	3	0.22d	12.24d	4.22d	0.23ef	1.44	24.17d	13.03de	1.61e
	7	0.18abc	11.63bcd	4.04cd	0.21bcd	1.41	22.85bcd	12.27bc	1.47bd
SRB	3	0.21cd	12.02cd	4.14d	0.23ef	1.44	23.84cd	12.85cde	1.60e
	7	0.15a	10.34a	3.69a	0.19a	1.22	19.94a	10.79a	1.27a
SFS	3	0.21bcd	12.46d	4.27d	0.24f	1.47	24.80d	13.44e	1.64e
	7	0.20bcd	11.68bcd	4.06cd	0.22cde	1.45	22.95bcd	12.43cd	1.47bd
GGR	3	0.21cd	12.15d	4.19d	0.23ef	1.45	23.94cd	12.92cde	1.57de
	7	0.18abc	10.86ab	3.84abc	0.20abc	1.34	21.07ab	11.40a	1.33a
BHT	3	0.20bcd	11.86cd	4.07d	0.22de	1.50	23.83cd	12.68cd	1.58de
	7	0.16a	10.69a	3.74ab	0.20ab	1.29	21.17ab	11.37a	1.36abc
SED									
AO		0.008	0.19	0.05	0.007	0.07	0.50	0.24	0.04
AO x L		0.010	0.23	0.06	0.008	0.08	0.60	0.29	0.05
AO x ST		0.013	0.31	0.08	0.011	0.11	0.80	0.39	0.06
AO x L x ST		0.015	0.36	0.09	0.012	0.13	0.92	0.45	0.08
p value									
AO		0.006	<.001	<.001	0.016	0.360	<.001	<.001	0.008
AO x L		0.231	<.001	<.001	0.004	0.125	<.001	<.001	<.001
AO x ST		<.001	<.001	<.001	<.001	0.086	<.001	<.001	<.001
AO x L x ST		0.152	0.008	<.001	0.304	0.241	0.045	0.032	0.105

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A3 (continued) effect of natural antioxidant extracts at different levels on fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken breast tissue during the accelerated storage time at 62.8 ºC.

Antioxidant (AO)		C20:4 n-6	C20:5 n-3	C22:5 n-3	C22:6 n-3	∑SFA	∑MUFA	∑PUFA	∑n-3	∑n-6
CON		1.19c	0.13b	0.46b	0.28c	16.71bc	24.47ab	16.02bcd	2.35b	13.67bc
ROS		1.13bc	0.11a	0.43ab	0.27bc	16.71bc	24.93b	16.13cd	2.35b	13.78c
SRB		1.10b	0.11a	0.44b	0.25bc	15.70a	23.22a	15.15a	2.23	12.92a
SFS		1.09ab	0.11a	0.43ab	0.24ab	16.88c	25.33b	16.36d	2.33b	14.03c
GGR		1.15bc	0.11a	0.43ab	0.24abc	16.15ab	23.90ab	15.54abc	2.24	13.30ab
BHT		1.03a	0.10a	0.39a	0.22a	15.77a	23.90ab	15.23ab	2.18a	13.05a
Antioxidant (AO) x Level (L)										
AO	**L**									
CON	0 mg	1.19d	0.13c	0.46	0.28bc	16.71cd	24.47abc	16.02cde	2.35b	13.67cd
ROS	10 mg	1.17cd	0.11bc	0.44	0.28bc	16.60cd	24.45abc	15.92bcde	2.34b	13.58cd
	20 mg	1.10bc	0.10ab	0.42	0.26abc	16.82cd	25.42bc	16.34de	2.36b	13.98de
SRB	10 mg	1.22d	0.12bc	0.46	0.30c	16.16bc	23.44ab	15.54abcd	2.30b	13.23bc
	20 mg	0.99a	0.09a	0.43	0.21a	15.24ab	23.00a	14.77ab	2.16	12.60ab
SFS	10 mg	1.16cd	0.11abc	0.44	0.24abc	16.40cd	24.20abc	15.83bcde	2.28	13.55cd
	20 mg	1.03ab	0.11abc	0.41	0.23ab	17.37d	26.46c	16.89e	2.38b	14.51e
GGR	10 mg	1.21d	0.12bc	0.45	0.25abc	15.73abc	22.77a	15.06abc	2.19	12.87ab
	20 mg	1.09abc	0.11ab	0.41	0.23ab	16.56cd	25.03abc	16.02cde	2.28	13.74cd
BHT	10 mg	1.07abc	0.11ab	0.4	0.23ab	16.49cd	25.08abc	15.96cde	2.28	13.68cd
	20 mg	0.99a	0.10ab	0.38	0.21a	15.06a	22.71a	14.51a	2.08a	12.43a
Antioxidant (AO) x Storage time (ST)										
AO	**ST**									
CON	0	1.14	0.13	0.47	0.25ab	17.46de	25.99cd	16.76ef	2.44b	14.32efg
	3	1.22	0.12	0.45	0.34c	17.02cde	24.86cd	16.36def	2.45b	13.91defg
	7	1.23	0.13	0.46	0.23ab	15.65abc	22.57ab	14.94abcd	2.17ab	12.77bc
ROS	3	1.15	0.11	0.43	0.29bc	17.14de	25.61cd	16.63ef	2.45b	14.18fg
	7	1.11	0.10	0.43	0.24ab	16.27bcd	24.25bc	15.63bcde	2.25ab	13.38cd
SRB	3	1.13	0.10	0.42	0.27abc	16.82cde	25.28cd	16.37def	2.39b	13.97defg
	7	1.08	0.11	0.46	0.24ab	14.58a	21.16a	13.94a	2.07a	11.87a
SFS	3	1.14	0.11	0.43	0.24ab	17.40e	26.27d	17.00f	2.42b	14.58g
	7	1.05	0.11	0.42	0.24ab	16.37bcde	24.39c	15.72cde	2.24ab	13.48cde
GGR	3	1.16	0.12	0.44	0.23a	17.00cde	25.39cd	16.43ef	2.36b	14.08efg
	7	1.13	0.11	0.43	0.25ab	15.30ab	22.41a	14.64abc	2.26 ab	12.52b
BHT	3	1.04	0.11	0.39	0.21a	16.56abc	25.33cd	16.00def	2.29ab	13.72def
	7	1.03	0.10	0.39	0.22a	14.98a	22.46s	14.46ab	2.07a	12.39ab
SED										
AO		0.04	0.005	0.02	0.01	0.25	0.54	0.27	0.04	0.23
AO x L		0.05	0.006	0.02	0.02	0.29	0.64	0.32	0.05	0.28
AO x ST		0.06	0.008	0.03	0.02	0.40	0.86	0.43	0.06	0.37
AO x L X ST		0.07	0.009	0.04	0.02	0.46	0.99	0.49	0.07	0.43
p value										
AO		0.002	<.001	0.013	<.001	<.001	<.001	<.001	<.001	<.001
AO x L		<.001	<.001	0.23	<.001	<.001	<.001	<.001	<.001	<.001
AO x ST		0.433	0.674	0.914	<.001	<.001	<.001	<.001	<.001	<.001
AO x L X ST		0.021	<.001	0.784	0.003	0.003	0.047	0.018	0.081	0.016

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A4 Effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken thigh tissue during the accelerated storage time at 62.8 °C.

Antioxidant (AO)		C14:0	C16:0	C18:0	C20:0	C16:1 n-7	C18:1 n-9	C18:2 n-6	C18:3 n-3
CON		0.36b	17.44b	5.19b	0.33	2.45b	36.53b	19.42b	2.70b
ROS		0.35b	17.15b	5.15b	0.33	2.40b	36.07b	19.13b	2.66b
SRB		0.36b	17.15b	5.16b	0.33	2.39b	35.96b	19.09b	2.67b
SFS		0.37b	17.45b	5.22b	0.34b	2.46b	36.76b	19.55b	2.71b
GGR		0.36b	17.16b	5.13b	0.33	2.44b	36.04b	19.12b	2.67b
BHT		0.34a	16.04a	4.83a	0.31a	2.22a	33.67a	17.95a	2.47a
Antioxidant (AO) x Level (L)									
AO	**L**								
CON	0 mg	0.36	17.44cd	5.19c	0.33	2.45cd	36.53cde	19.42cd	2.70c
ROS	10 mg	0.35	17.17bcd	5.14bc	0.33	2.41bcd	36.14cde	19.16bcd	2.66bc
	20 mg	0.36	17.14bcd	5.16bc	0.34	2.40bcd	35.99bcde	19.10bcd	2.66bc
SRB	10 mg	0.37	17.55cd	5.27c	0.34	2.46cd	37.01de	19.60cd	2.74c
	20 mg	0.35	16.74abc	5.06abc	0.32	2.32abc	34.92abc	18.57abc	2.60bc
SFS	10 mg	0.38	17.82d	5.32c	0.35	2.53d	37.74e	20.00d	2.78c
	20 mg	0.36	17.08bcd	5.12bc	0.34	2.39bcd	35.78bcde	19.10bcd	2.64bc
GGR	10 mg	0.37	17.46cd	5.23c	0.34	2.51d	36.86cde	19.51cd	2.71c
	20 mg	0.35	16.86bc	5.04abc	0.32	2.36abcd	35.21abcd	18.73abc	2.62bc
BHT	10 mg	0.34	16.24ab	4.89ab	0.31	2.25ab	34.02ab	18.21ab	2.51ab
	20 mg	0.33	15.85a	4.77a	0.31	2.20a	33.31a	17.68a	2.42a
Antioxidant (AO) x Storage time (ST)									
AO	**ST**								
CON	0	0.38de	18.49f	5.47g	0.35	2.62df	39.04d	20.69e	2.90c
	3	0.35abcde	17.11abcde	5.11bcdef	0.34	2.39abcd	35.51abc	18.90abcd	2.64ab
	7	0.34ab	16.72abc	5.00bc	0.31	2.35abc	35.04abc	18.67abcd	2.56ab
ROS	3	0.34abcd	16.84abcde	5.08bcde	0.34	2.35abc	35.59abc	18.77abcd	2.60ab
	7	0.36abcde	17.47cdef	5.22cef	0.33	2.45cdef	36.54cd	19.49cde	2.72bc
SRB	3	0.35abcde	17.11bcde	5.15cdef	0.33	2.39abcde	36.10c	19.06bcd	2.67b
	7	0.36abcde	17.19cde	5.18cdef	0.33	2.39abcde	35.83bc	19.12bcd	2.68bc
SFS	3	0.35abcde	17.10bcde	5.11cde	0.34	2.41cdef	36.26c	19.22bcd	2.66b
	7	0.38e	17.80cef	5.34fg	0.35	2.50cdef	37.26cd	19.88de	2.76bc
GGR	3	0.35abcd	16.73abcd	5.02bcd	0.32	2.40bcde	35.20abc	18.66abc	2.58ab
	7	0.37bde	17.60cdef	5.24cef	0.34	2.47cdef	36.88cd	19.59cde	2.75bc
BHT	3	0.34abc	15.94a	4.77a	0.32	2.23ab	33.57a	17.78a	2.46a
	7	0.33a	16.15ab	4.90ab	0.30	2.22a	33.77ab	18.12ab	2.47a
SED									
AO		0.01	0.21	0.07	0.01	0.04	0.47	0.25	0.04
AO x L		0.01	0.25	0.08	0.01	0.05	0.56	0.30	0.05
AO x ST		0.01	0.34	0.10	0.02	0.06	0.75	0.40	0.07
AO x L x ST		0.01	0.39	0.12	0.02	0.07	0.87	0.46	0.08
p value									
AO		<.001	<.001	<.001	0.028	<.001	<.001	<.001	<.001
AO x L		0.070	0.002	0.007	0.255	0.001	<.001	<.001	0.005
AO x ST		<.001	<.001	<.001	0.188	0.003	<.001	<.001	<.001
AO x L x ST		0.073	0.182	0.412	0.456	0.403	0.331	0.369	0.071

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A4 (continued) effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken thigh tissue during the accelerated storage time at 62.8 ºC.

Antioxidant (AO)		**C20:4 n-6**	**C22:5 n-3**	**C22:6 n-3**	**∑SFA**	**∑MUFA**	**∑PUFA**	**∑n-3**	**∑n-6**
CON		0.65	0.22	0.22a	23.53b	38.98b	23.06b	3.13b	20.07b
ROS		0.69	0.22	0.30b	23.20b	38.47b	22.69b	3.17b	19.82b
SRB		0.68	0.23	0.26b	23.21b	38.35b	22.66b	3.15b	19.77b
SFS		0.67	0.22	0.24b	23.59b	39.22b	23.14b	3.17b	20.21b
GGR		0.68	0.22	0.24b	23.19b	38.47b	22.69b	3.13b	19.80b
BHT		0.64	0.22	0.25b	21.66a	35.89a	21.27a	2.93a	18.59a
Antioxidant (AO) x Level (L)									
AO	**L**								
CON	0 mg	0.65	0.22	0.22	23.53cd	38.98cd	23.06cd	3.13bc	20.07cd
ROS	10 mg	0.68	0.22	0.29	23.20bcd	38.55cd	22.72bcd	3.17bc	19.84bcd
	20 mg	0.69	0.22	0.30	23.21bcd	38.39bcd	22.66bcd	3.18bc	19.79bcd
SRB	10 mg	0.68	0.22	0.26	23.74cd	39.47cd	23.24cd	3.23c	20.28cd
	20 mg	0.68	0.23	0.25	22.68bc	37.24abc	22.08abc	3.08abc	19.25abc
SFS	10 mg	0.67	0.22	0.24	24.09d	40.27d	23.67d	3.23c	20.67d
	20 mg	0.66	0.22	0.25	23.10bcd	38.17bcd	22.62bcd	3.11bc	19.76bcd
GGR	10 mg	0.68	0.23	0.24	23.61cd	39.37cd	23.13cd	3.18bc	20.19cd
	20 mg	0.67	0.22	0.25	22.77bc	37.58abc	22.24abc	3.09abc	19.40abc
BHT	10 mg	0.65	0.22	0.26	21.98ab	36.27ab	21.59ab	2.99ab	18.86ab
	20 mg	0.64	0.21	0.24	21.35a	35.51a	20.96a	2.88a	18.32a
Antioxidant (AO) x Storage time (ST)									
AO	**ST**								
CON	0	0.69	0.22	0.22abcd	24.92fh	41.66d	24.73f	3.34f	21.38e
	3	0.61a	0.21	0.20ab	23.11bcdef	37.90abc	22.35abcde	3.04abcd	19.51abcd
	7	0.65	0.22	0.23abcde	22.56abc	37.39abc	22.10abc	3.02abc	19.32abc
ROS	3	0.66	0.21	0.25abcde	22.80bcde	37.95abc	22.24abcde	3.06abcde	19.43abcd
	7	0.71c	0.23b	0.34e	23.60cdefgh	38.99cd	23.15cdef	3.29df	20.20cde
SRB	3	0.65	0.22	0.21abc	23.16cdefg	38.49c	22.60bcde	3.09abcdef	19.71bcd
	7	0.70bc	0.23b	0.31bde	23.27cdefg	38.21bc	22.73bcde	3.22bcdef	19.82bcd
SFS	3	0.65	0.21	0.18a	23.11bcde	38.67c	22.74bcde	3.05abcde	19.86bcd
	7	0.69	0.23b	0.31fg	24.08cefgh	39.76cd	23.55cef	3.29df	20.57de
GGR	3	0.66	0.22	0.21ab	22.60abcd	37.59abc	22.12abcd	3.01ab	19.32abcd
	7	0.69	0.23	0.27cdef	23.77cdefgh	39.35cd	23.25cdef	3.25cdef	20.27cde
BHT	3	0.62ab	0.20a	0.22abcd	21.51a	35.80a	21.07a	2.89a	18.40a
	7	0.66	0.23	0.28cef	21.82ab	35.99ab	21.48ab	2.98a	18.78ab
SED									
AO		0.02	0.01	0.02	0.28	0.51	0.29	0.05	0.25
AO x L		0.02	0.01	0.02	0.34	0.60	0.35	0.06	0.30
AO x ST		0.03	0.01	0.03	0.45	0.81	0.47	0.07	0.40
AO x L x ST		0.03	0.01	0.04	0.52	0.94	0.54	0.09	0.47
p value									
AO		0.097	0.542	0.009	<.001	<.001	<.001	<.001	<.001
AO x L		0.989	0.940	0.980	0.002	<.001	<.001	0.017	<.001
AO x ST		0.003	<.001	<.001	<.001	<.001	<.001	<.001	<.001
AO x L x ST		0.821	0.670	0.420	0.193	0.340	0.337	0.031	0.396

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A5 Effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from adipose tissue during the accelerated storage time at 62.8°C.

Antioxidant (AO)		C14:0	C16:0	C18:0	C20:0	C16:1 n-7	C18:1 n-9	C18:2 n-6	C18:3 n-3
CON		0.42bc	18.67b	4.98c	0.51c	2.82c	40.59c	19.47c	3.00c
ROS		0.42c	18.75b	4.99c	0.51c	2.81c	40.52c	19.43c	2.98c
SRB		0.39ab	17.66a	4.70ab	0.47ab	2.63ab	38.23a	18.31ab	2.82ab
SFS		0.40abc	18.75b	5.00c	0.49bc	2.79c	40.46c	19.50c	2.97c
GGR		0.39abc	17.99ab	4.78bc	0.47ab	2.70bc	39.11b	18.76b	2.90bc
BHT		0.37a	17.13a	4.54a	0.46a	2.56a	37.17a	17.90a	2.75a
Antioxidant (AO) x Level (L)									
AO	**L**								
CON	0 mg	0.42bc	18.67d	4.98c	0.51d	2.82d	40.59e	19.47ef	3.00e
ROS	10 mg	0.43c	19.02d	5.06c	0.51d	2.85d	41.10e	19.75f	3.04e
	20 mg	0.41abc	18.48bcd	4.91bc	0.51d	2.78cd	39.95de	19.11def	2.92de
SRB	10 mg	0.40abc	18.16	4.85bc	0.50cd	2.73bcd	39.31cde	18.83cde	2.92de
	20 mg	0.37a	17.16	4.54ab	0.45ab	2.54ab	37.17ab	17.79ab	2.71ab
SFS	10 mg	0.41abc	18.63	4.97c	0.48bcd	2.78cd	40.33de	19.44def	2.97de
	20 mg	0.40abc	18.87d	5.03c	0.50cd	2.79cd	40.59e	19.55ef	2.98de
GGR	10 mg	0.42a	18.68cd	4.98c	0.51d	2.82d	40.56de	19.46def	3.01e
	20 mg	0.37a	17.31	4.58ab	0.43a	2.58abc	37.68bc	18.06bc	2.78bc
BHT	10 mg	0.38a	17.82	4.74bc	0.46abc	2.67abcd	38.81bcd	18.62bcd	2.86cd
	20 mg	0.37a	16.44a	4.34a	0.45ab	2.46a	35.54a	17.18a	2.64a
Antioxidant (AO) x Storage time (ST)									
AO	**ST**								
CON	0	0.44d	19.66ef	5.24e	0.53cd	2.99g	42.73d	20.55d	3.16e
	3	0.42bcd	19.07cdef	5.08cde	0.53cd	2.87defg	41.47d	19.89d	3.06e
	7	0.39abcd	17.28abc	4.62abc	0.48abc	2.61abcd	37.56bc	17.96ab	2.78bcd
ROS	3	0.40bcd	17.91bcde	4.76bcd	0.48abc	2.70bcdef	38.77c	18.61c	2.85cd
	7	0.43d	19.59f	5.22e	0.54d	2.93g	42.27d	20.25d	3.11e
SRB	3	0.35a	16.18a	4.31a	0.45ab	2.43a	35.12a	16.88a	2.60a
	7	0.42cd	19.14def	5.08cde	0.50bcd	2.83defg	41.35d	19.75d	3.03e
SFS	3	0.39abcd	17.89bcd	4.73bc	0.49abcd	2.70cdef	38.83c	18.75c	2.89d
	7	0.41bcd	19.62f	5.26e	0.49abc	2.87dfg	42.09d	20.24d	3.05e
GGR	3	0.38abc	16.71ab	4.42ab	0.46ab	2.54abc	36.33ab	17.48ab	2.73abc
	7	0.40bcd	19.27def	5.14de	0.48abc	2.87dfg	41.90d	20.04d	3.06e
BHT	3	0.37ab	16.39a	4.34a	0.45ab	2.48ab	35.56ab	17.15ab	2.67ab
	7	0.37abc	17.87bcd	4.75bcd	0.46ab	2.65abcde	38.79c	18.64c	2.83cd
SED									
AO		0.01	0.32	0.09	0.02	0.05	0.71	0.33	0.05
AO x L		0.01	0.38	0.10	0.02	0.06	0.84	0.40	0.06
AO x ST		0.02	0.51	0.14	0.03	0.08	1.13	0.53	0.08
AO x L X ST		0.02	0.58	0.16	0.03	0.09	1.31	0.62	0.09
p value									
AO		<.001	<.001	<.001	0.002	<.001	<.001	<.001	<.001
AO x L		0.002	<.001	<.001	0.004	<.001	<.001	<.001	<.001
AO x ST		<.001	<.001	<.001	0.023	<.001	<.001	<.001	<.001
AO x L x ST		0.015	<.001	<.001	0.056	<.001	<.001	<.001	<.001

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A5 (continued) effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from adipose tissue during the accelerated storage time at 62.8 ºC.

Antioxidant (AO)		C20:4 n-6	C22:5 n-3	∑SFA	∑MUFA	∑PUFA	∑n-3	∑n-6
CON		0.18c	0.25	24.82c	43.41c	22.90c	3.25c	19.65c
ROS		0.18bc	0.26	24.90c	43.33c	22.85c	3.24c	19.61c
SRB		0.17ab	0.28	23.42ab	40.87ab	21.58ab	3.09	18.48ab
SFS		0.19c	0.24a	24.86c	43.25c	22.89c	3.21bc	19.68c
GGR		0.17ab	0.29	23.84bc	41.82b	22.11b	3.18	18.93b
BHT		0.16a	0.30b	22.70a	39.74a	21.11a	3.05a	18.06a
Antioxidant (AO) x Level (L)								
AO	L							
CON	0 mg	0.18bc	0.25	24.82b	43.41e	22.90ef	3.25bc	19.65ef
ROS	10 mg	0.18bc	0.30	25.25b	43.95e	23.26f	3.33c	19.93f
	20 mg	0.18bc	0.23ab	24.55b	42.72de	22.43def	3.15abc	19.28def
SRB	10 mg	0.18bc	0.25bc	24.14b	42.04cde	22.19cde	3.17abc	19.02cde
	20 mg	0.16a	0.30	22.72a	39.70ab	20.96ab	3.01a	17.95ab
SFS	10 mg	0.19c	0.18a	24.72b	43.11de	22.77def	3.14abc	19.63def
	20 mg	0.19c	0.29	25.01b	43.39de	23.01ef	3.27bc	19.74ef
GGR	10 mg	0.19c	0.27	24.81b	43.38de	22.93def	3.28bc	19.65def
	20 mg	0.16a	0.30	22.88a	40.26bc	21.30bc	3.08ab	18.22bc
BHT	10 mg	0.17ab	0.29	23.62ab	41.48bcd	21.94bcd	3.15abc	18.79bcd
	20 mg	0.15a	0.32c	21.79a	38.00a	20.29a	2.95a	17.33a
Antioxidant (AO) x Storage time (ST)								
AO	ST							
CON	0	0.19b	0.27c	26.13e	45.72d	24.17d	3.43g	20.74d
	3	0.18ab	0.25ab	25.35cde	44.34d	23.38d	3.31efg	20.07d
	7	0.17a	0.23a	22.99abc	40.17bc	21.14bc	3.01abc	18.13bc
ROS	3	0.17a	0.24ab	23.78bcd	41.46c	21.86c	3.09cd	18.77c
	7	0.19b	0.29c	26.02e	45.20d	23.83d	3.40g	20.44d
SRB	3	0.17a	0.27c	21.48a	37.55a	19.92a	2.87a	17.05a
	7	0.17a	0.28c	25.37de	44.18d	23.23d	3.31fg	19.92d
SFS	3	0.17a	0.32c	23.72bcd	41.53c	22.13c	3.21def	18.92c
	7	0.20b	0.16a	26.00e	44.96d	23.65d	3.21def	20.44d
GGR	3	0.17a	0.33c	22.18ab	38.87ab	20.71ab	3.06bc	17.65ab
	7	0.17a	0.24abc	25.50de	44.77d	23.51d	3.30fg	20.21d
BHT	3	0.16a	0.28c	21.75a	38.04ab	20.27ab	2.95ab	17.32ab
	7	0.16a	0.33c	23.65bcd	41.44c	21.95c	3.15cde	18.80c
SED								
AO		0.007	0.02	0.41	0.75	0.38	0.05	0.34
AO x L		0.008	0.02	0.49	0.89	0.45	0.06	0.40
AO x ST		0.011	0.03	0.66	1.20	0.61	0.08	0.54
AO x L x ST		0.012	0.03	0.76	1.39	0.70	0.10	0.62
p value								
AO		0.003	0.004	<.001	<.001	<.001	<.001	<.001
AO x L		0.001	<.001	<.001	<.001	<.001	<.001	<.001
AO x ST		0.012	<.001	<.001	<.001	<.001	<.001	<.001
AO x L x ST		0.054	<.001	<.001	<.001	<.001	<.001	<.001

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A6 Effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken skin tissue during the accelerated storage time at 62.8°C.

Antioxidant (AO)		**C14:0**	**C16:0**	**C18:0**	**C20:0**	**C16:1 n-7**	**C18:1 n-9**	**C18:2 n-6**	**C18:3 n-3**
CON		0.43b	19.25b	5.38b	0.44	2.77	41.07	20.33	3.05b
ROS		0.43b	19.50b	5.45b	0.43	2.81	41.70	20.71	3.13bc
SRB		0.43b	19.76b	5.52b	0.43	2.84	42.27	21.05	3.17bc
SFS		0.43b	19.66b	5.48b	0.41	2.82	41.98	20.97	3.12bc
GGR		0.44b	19.81b	5.53b	0.42	2.84	42.29	21.04	3.16c
BHT		0.39a	17.93a	4.99a	0.37a	2.56a	38.27a	19.03a	2.84a
Antioxidant (AO) x Level (L)									
AO	L								
CON	0 mg	0.43	19.25bc	5.38bc	0.44c	2.77bc	41.07bc	20.33bc	3.05bc
ROS	10 mg	0.43	19.28bc	5.39bc	0.44c	2.79bc	41.25bc	20.51bc	3.10bc
	20 mg	0.43	19.71c	5.50c	0.41abc	2.83c	42.14c	20.91c	3.17c
SRB	10 mg	0.44	19.85c	5.54c	0.42bc	2.85c	42.45c	21.14c	3.18c
	20 mg	0.43	19.66c	5.49c	0.43bc	2.82c	42.08c	20.97c	3.16c
SFS	10 mg	0.43	19.46bc	5.41bc	0.40abc	2.79bc	41.55bc	20.74bc	3.09bc
	20 mg	0.44	19.86c	5.55c	0.42bc	2.86c	42.40c	21.19c	3.15c
GGR	10 mg	0.44	19.93c	5.57c	0.44c	2.86c	42.57c	21.16c	3.18c
	20 mg	0.43	19.69c	5.49c	0.40abc	2.82c	42.00c	20.91c	3.15c
BHT	10 mg	0.40	18.47b	5.13ab	0.38ab	2.65b	39.45b	19.64b	2.94b
	20 mg	0.38	17.39a	4.84a	0.35a	2.47a	37.09a	18.41a	2.73a
Antioxidant (AO) x Storage time (ST)									
AO	ST								
CON	0	0.44b	19.72bc	5.52bc	0.43bc	2.85bc	42.09bc	20.70bcd	3.07bcd
	3	0.41b	18.71bc	5.22bc	0.43bc	2.69bc	39.92bc	19.77b	2.98bc
	7	0.43b	19.32bc	5.40bc	0.45c	2.78bc	41.19bc	20.53bcd	3.11bcd
ROS	3	0.42b	19.12bc	5.35bc	0.42bc	2.74bc	40.92bc	20.33bcd	3.08bcd
	7	0.44b	19.87c	5.55c	0.43bc	2.87c	42.48c	21.09bd	3.19cd
SRB	3	0.43b	19.85c	5.54c	0.44c	2.83c	42.50c	21.18d	3.19d
	7	0.44b	19.66bc	5.49c	0.41abc	2.84c	42.04bc	20.93bcd	3.14cd
SFS	3	0.43b	19.58bc	5.45bc	0.43bc	2.82bc	41.81bc	20.93bcd	3.15cd
	7	0.43b	19.75c	5.51c	0.39ab	2.83bc	42.14c	21.00bd	3.09bcd
GGR	3	0.44b	19.82c	5.55c	0.44c	2.84c	42.28c	21.05bd	3.18cd
	7	0.44b	19.80c	5.52c	0.40abc	2.84c	42.29c	21.02bd	3.15cd
BHT	3	0.37a	17.25a	4.80a	0.36a	2.45a	36.79a	18.26a	2.71a
	7	0.41b	18.62b	5.17b	0.38ab	2.67b	39.76b	19.79bc	2.96b
SED									
AO		0.01	0.23	0.07	0.01	0.03	0.50	0.25	0.04
AO x L		0.01	0.28	0.08	0.01	0.04	0.60	0.30	0.04
AO x ST		0.01	0.37	0.11	0.02	0.05	0.80	0.40	0.06
AO x L x ST		0.01	0.44	0.12	0.02	0.06	0.95	0.47	0.07
p value									
AO		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
AO x L		0.115	0.011	0.013	0.016	0.004	0.009	0.007	0.002
AO x ST		0.014	<.001	<.001	0.002	<.001	<.001	<.001	<.001
AO x L x ST		0.073	0.011	0.013	0.022	0.010	0.010	0.006	0.001

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table A6 Effect of natural antioxidant extracts at different levels on the fatty acid profile (g of fatty acids/100 g of fat) in fat from chicken skin tissue during the accelerated storage time at 62.8 °C.

Antioxidant (AO)		**C20:4 n-6**	**C22:6 n-3**	**∑SFA**	**∑MUFA**	**∑PUFA**	**∑n-3**	**∑n-6**
CON		0.28ab	0.21	25.49b	43.84	23.87	3.29	20.61b
ROS		0.28ab	0.21	25.80b	44.51	24.33	3.39	20.99b
SRB		0.29b	0.19	26.13b	45.10	24.70	3.40	21.34b
SFS		0.29b	0.19	25.98b	44.80	24.56	3.36	21.25b
GGR		0.28b	0.20	26.20b	45.13	24.68	3.41	21.32b
BHT		0.26a	0.21	23.68a	40.83a	22.33a	3.06a	19.29a
Antioxidant (AO) x Level (L)								
AO	**L**							
CON	0 mg	0.28	0.21bcd	25.49bc	43.84bc	23.87bc	3.29bc	20.61bc
ROS	10 mg	0.28	0.22bcd	25.55bc	44.04bc	24.10bc	3.37bc	20.79bc
	20 mg	0.28	0.19abcd	26.04c	44.97c	24.55c	3.40c	21.19c
SRB	10 mg	0.29	0.11ab	26.25c	45.31c	24.72c	3.33bc	21.43c
	20 mg	0.29	0.27d	26.01c	44.90c	24.68c	3.47c	21.25c
SFS	10 mg	0.29	0.28d	25.70bc	44.34bc	24.40c	3.42c	21.03bc
	20 mg	0.29	0.10a	26.27c	45.26c	24.73c	3.29bc	21.48c
GGR	10 mg	0.28	0.25cd	26.38c	45.43c	24.88c	3.48c	21.45c
	20 mg	0.28	0.15abc	26.02c	44.82c	24.49c	3.34bc	21.19c
BHT	10 mg	0.27	0.22bcd	24.39b	42.10b	23.06b	3.19b	19.90b
	20 mg	0.26	0.20abcd	22.97a	39.56a	21.60a	2.93a	18.67a
Antioxidant (AO) x Storage time (ST)								
AO	**ST**							
CON	0	0.27	0.22b	26.10bc	44.93bc	24.26bcd	3.39bc	20.97bcd
	3	0.26	0.18a	24.77bc	42.61bc	23.20b	3.16b	20.04b
	7	0.30b	0.22b	25.60bc	43.97bc	24.16bcd	3.33bc	20.83bcd
ROS	3	0.29	0.31c	25.31bc	43.66bc	24.00bcd	3.39bc	20.61bcd
	7	0.27	0.11a	26.29c	45.35c	24.66bd	3.39bc	21.37bd
SRB	3	0.30b	0.25b	26.27c	45.33c	24.92d	3.44c	21.48d
	7	0.28	0.14a	26.00bc	44.88c	24.48bcd	3.37bc	21.21bcd
SFS	3	0.30b	0.26b	25.89bc	44.63bc	24.64bd	3.41bc	21.23bd
	7	0.28	0.12a	26.08c	44.97c	24.49bcd	3.30bc	21.28bd
GGR	3	0.29	0.24b	26.25c	45.12c	24.76bd	3.42bc	21.34bd
	7	0.28	0.16a	26.16c	45.13c	24.61bd	3.40bc	21.29bd
BHT	3	0.26a	0.18a	22.78a	39.23a	21.41a	2.89a	18.52a
	7	0.27	0.23b	24.58b	42.43b	23.25bc	3.23bc	20.06bc
SED								
AO		0.01	0.03	0.31	0.53	0.29	0.05	0.25
AO x L		0.01	0.03	0.37	0.64	0.35	0.06	0.30
AO x ST		0.01	0.04	0.49	0.86	0.47	0.08	0.41
AO x L x ST		0.01	0.05	0.58	1.01	0.55	0.09	0.48
p value								
AO		0.006	0.941	<.001	<.001	<.001	<.001	<.001
AO x L		0.894	<.001	0.010	0.008	0.009	<.001	0.008
AO x ST		0.004	<.001	<.001	<.001	<.001	<.001	<.001
AO x L x ST		0.017	<.001	0.007	0.010	0.002	<.001	0.006

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

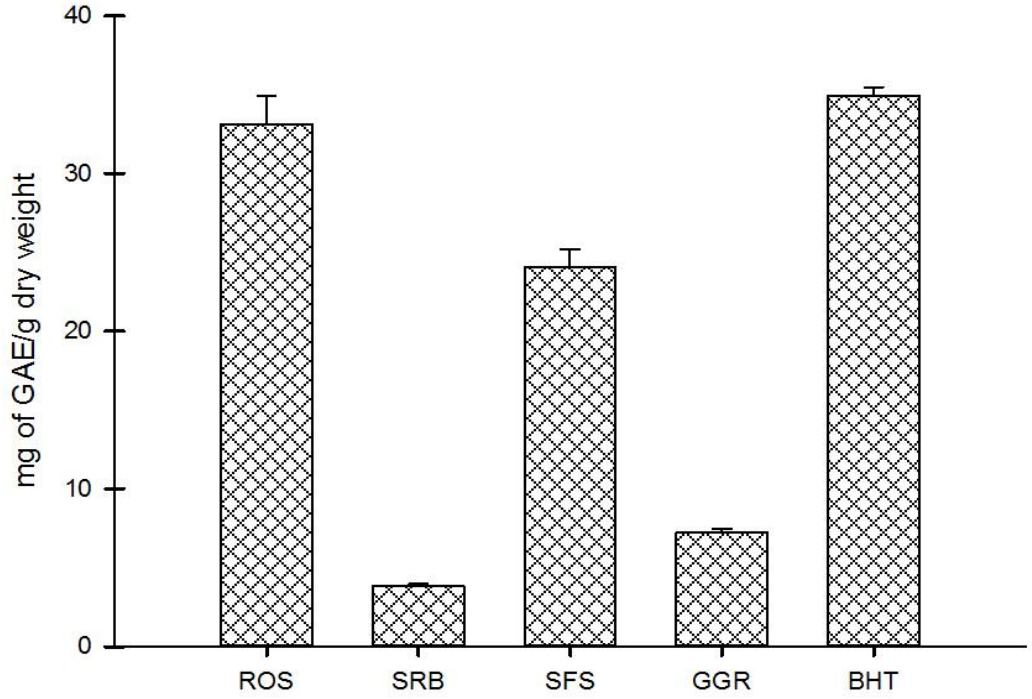

Figure A. 1 Total phenolic content (mg of GAE/g dry weight) of plant extract as the results expressed mg of GAE/g dry weight) (Means ± SED; n = 3). Rosemary, (ROS); Small Red Bean, (SRB); Sunflower Seed, (SFS); Ginger, (GGR); Butylated Hydroxytoluene, (BHT).

Appendix B (Statistics Analysis for Chapter 3)

Table B1 Effect of natural antioxidants (AO) and dipping time (DT) on moisture uptake (%) of raw and thawed chicken fillets.

Main effects	Raw Meat	Thawed Meat
Antioxidants (AO)		
CON	1.59ab	1.03
ROS	1.76ab	1.33
SRB	2.06a	1.29
SFS	1.40bc	1.22
GGR	1.02c	1.01
Dipping time (DT)		
5 min	0.43d	0.52c
20 min	1.50c	1.15b
30 min	1.93b	1.17b
60 min	2.40a	1.86a
SED		
AO	0.17	0.25
DT	0.15	0.22
AO x DT	0.35	0.50
p value		
AO	<.001	0.588
DT	<.001	<.001
AO x DT	0.019	0.286

Mean values with different small letters presented within each column of each meat (raw and thawed) differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table B2 Effect of natural antioxidants and dipping time on total phenolic content (mg GAE/100 g meat) in different layers of raw and thawed breast chicken meat.

Main effects	Raw Meat	Thawed Meat
Antioxidant (AO)		
CON	33.01c	54.33e
ROS	55.14a	98.22a
SRB	40.26b	62.09d
SFS	39.22b	73.70c
GGR	38.09b	84.54b
BHT	40.41b	76.48c
Meat Layer (ML)		
Membrane	44.05a	85.53a
Tenderloin	44.62a	77.50b
The core	34.40b	61.65c
Dipping Time (DT)		
5 min	33.32c	70.15c
20 min	47.95a	77.33b
30 min	40.78b	57.59d
60 min	42.04b	94.50a
SED		
AO	1.01	1.80
ML	1.42	2.54
DT	1.16	2.07
AO x ML	2.46	4.40
AO x DT	2.01	3.59
ML x DT	2.85	5.08
AO x ML x DT	4.93	8.80
p value		
AO	<0.001	<0.001
ML	<0.001	<0.001
DT	<0.001	<0.001
AO x ML	<0.001	<0.001
AO x DT	<0.001	<0.001
ML x DT	<0.001	<0.001
AO x ML x DT	0.035	<0.001

Mean values with different small letters presented within each column of each meat (raw and thawed) differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Appendix C (Statistics Analysis for Chapter 4)

Table C1 Proximate analysis of chicken meat

Treatment	Dry Matter g/kg meat		Fat g/kg DM	
	Raw Meat	Cooked meat	Raw Meat	Cooked meat
CON	247.90 ± 3.22a	312.40 ± 1.40cd	42.04 ± 19.86a	45.05 ± 2.63a
BHT	267.90 ± 2.49b	310.30 ± 9.74cd	46.11 ± 13.28a	44.75 ± 20.38a
ROS	257.40 ± 6.04ab	315.30 ± 2.70d	32.18 ± 15.71a	41.00 ± 0.19a
SRB	247.40 ± 6.87a	305.60 ± 4.26cd	28.38 ± 13.32a	31.33 ± 4.69a
SFS	258.50 ± 4.44ab	298.50 ± 0.23c	52.26 ± 11.43a	43.52 ± 0.99a
GGR	244.90 ± 1.90a	302.60 ± 8.63cd	27.67 ± 8.29a	36.71 ± 5.52a

The data shown are the average and standard deviation of three independent samples. Values with different small letters within each column differ significantly ($p \leq 0.05$) according to the Tukey's HSD test

Table C2 Effect of natural antioxidants application on TBARS values, Conjugated Dienes and Conjugated Trienes of raw and sous-vide processed chicken breast meat following storage at 4°C.

	TBARS value		*Conjugated Dienes*		*Conjugated Trienes*	
	Raw	Sous-vide	Raw	Sous-vide	Raw	Sous-vide
Main effects						
Antioxidant (AO)						
CON	0.50b	5.33d	44.04b	52.30	21.01b	26.96
ROS	0.39ab	2.04a	36.91a	48.87	18.37ab	24.20
SRB	0.38ab	2.89b	33.24a	47.61	16.79a	24.77
SFS	0.35a	4.20c	32.30a	50.76	15.96a	24.86
GGR	0.41ab	2.15a	32.46a	52.08	17.29a	26.49
BHT	0.39b	2.32ab	33.01a	49.43	16.63a	25.90
Average	0.40	3.16	35.33	50.18	17.67	25.53
Storage Time (ST)						
0	0.34a	1.68a	28.09a	44.50a	13.64a	21.92a
3	0.48b	4.57c	33.05b	52.29b	16.29b	25.78b
7	0.39a	3.22b	44.84c	53.74b	23.10c	28.88c
SED						
AO	0.04	0.22	1.74	1.87	1.23	0.99
ST	0.03	0.16	1.23	1.32	0.87	0.70
AO x ST	0.07	0.38	3.01	3.24	2.13	1.71
p value						
AO	0.012	<0.001	<0.001	0.104	0.003	0.056
ST	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AO x ST	0.468	<0.001	0.034	0.407	0.555	0.393

Mean values with different small letters presented within each column of each meat (raw and cooked) differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. TBARS value (mg MDA/kg meat); Conjugated Dienes (µmol/g fat); Conjugated Trienes (µmol/g fat).

Table C3 Effect of natural antioxidants application on phospholipid (g/100 g fat) and pH value of raw and sous-vide processed chicken breast meat following storage at 4°C.

	phospholipid content		pH value	
	Raw	**Sous-vide**	**Raw**	**Sous-vide**
Main effects				
Antioxidant (AO)				
CON	41.51	38.28a	5.97b	6.15
ROS	41.40	41.63d	5.80a	6.06
SRB	40.47	40.93cd	5.91ab	6.05
SFS	41.50	40.51bc	5.92ab	6.03
GGR	40.91	39.89b	5.80a	6.10
BHT	41.63	39.59b	5.84ab	6.03
Average	41.24	40.13	5.87	6.07
Storage Time (ST)				
0	43.30c	41.14b	5.85	6.10
3	41.57b	40.56b	5.86	6.05
7	38.84a	38.69a	5.91	6.06
SED				
AO	0.59	0.34	0.05	0.05
ST	0.42	0.24	0.03	0.03
AO x ST	1.03	0.58	0.09	0.08
p value				
AO	0.348	<0.001	0.004	0.155
ST	<0.001	<0.001	0.223	0.348
AO x ST	0.004	<0.001	0.725	0.920

Mean values with different small letters presented within each column of each meat (raw and cooked) differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table C4 Effect of natural antioxidants application on characteristics of chicken breast meat following storage at 4°C.

	Cooking loss	Shear force	L* value	a* value	b* value	Drip loss %
Main effects						
Antioxidant(AO)						
CON	23.42b	17.36	59.27	2.24a	6.86a	2.44b
ROS	22.45ab	16.77	60.52	2.99bc	10.12b	1.76a
SRB	22.50ab	16.17	59.87	2.00a	7.18a	1.91a
SFS	22.43ab	16.82	60.57	2.48ab	7.40a	2.09ab
GGR	21.82ab	16.76	59.85	3.23c	10.24b	2.06ab
BHT	20.51a	16.80	60.28	2.48ab	7.67a	2.08ab
Storage Time (ST)						
0	21.97ab	15.42a	61.01b	2.47	7.38a	1.16a
3	23.11b	17.82b	59.58a	2.47	8.62b	2.15b
7	21.49a	17.10b	59.60a	2.77	8.73b	2.86c
SED						
AO	0.53	0.59	0.82	0.22	0.46	0.13
ST	0.75	0.41	0.58	0.15	0.33	0.09
AO x ST	1.31	1.02	1.41	0.38	0.80	0.23
p value						
AO	0.013	0.541	0.609	<.001	<.001	<.001
ST	0.015	<.001	0.026	0.091	<.001	<.001
AO x ST	0.628	0.481	0.233	0.624	0.654	0.023

Mean values with different small letters presented within each column differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table C5 Effect of natural antioxidants application on fatty acid composition (g of fatty acid/kg DM) of raw chicken breast meat during the storage time at 4 °C.

Main effects	TFA	C14:0	C16:0	C18:0	C20:0	∑ SFA	C16:1 n-7	C18:1 n-9	∑ MUFA
Antioxidant (AO)									
CON	54.20a	0.24a	11.65	5.36a	0.24	17.49	1.46	20.77	22.23
ROS	59.80ab	0.26ab	13.29	5.59ab	0.26	19.40	1.66	22.31	23.97
SRB	63.90ab	0.28ab	14.12	5.74ab	0.29	20.43	1.76	24.64	26.40
SFS	62.80ab	0.28ab	13.63	6.03ab	0.25	20.19	1.63	23.98	25.61
GGR	67.70b	0.34b	14.86	6.33b	0.27	21.80	2.03	25.90	27.93
BHT	59.80ab	0.29ab	13.70	5.53ab	0.27	19.78	1.94	23.12	25.06
Storage Time (ST)									
0	63.80	0.29	14.32	5.87	0.29b	20.77	1.89	24.81	26.70
3	58.90	0.27	12.78	5.83	0.26ab	19.15	1.47	21.92	23.39
7	61.50	0.28	13.52	5.58	0.24a	19.62	1.88	23.63	25.51
SED									
AO	4.32	0.03	1.05	0.32	0.02	1.34	0.27	2.02	2.27
ST	3.05	0.02	0.74	0.23	0.01	0.95	0.19	1.43	1.60
AO x ST	7.48	0.05	1.82	0.55	0.03	2.33	0.47	3.50	3.92
p value									
AO	0.073	0.026	0.091	0.049	0.069	0.073	0.336	0.186	0.210
ST	0.286	0.394	0.134	0.382	<.001	0.228	0.052	0.141	0.126
AO x ST	0.109	0.112	0.094	0.071	<.001	0.076	0.235	0.204	0.218

Main effects	C18:2 n-6	C18:3 n-3	C20:4 n-6	C22:5 n-3	C20:5 n-3	C22:6 n-3	∑PUFA	∑ n-3	∑n-6
Antioxidant (AO)									
CON	11.12a	1.40	0.31a	0.25	0.88	0.57	14.52a	3.09ab	11.43a
ROS	12.59ab	1.65	0.43b	0.28	0.94	0.53	16.42ab	3.40ab	13.02ab
SRB	13.27ab	1.71	0.39ab	0.28	0.78	0.61	17.03ab	3.38ab	13.65ab
SFS	13.15ab	1.77	0.36ab	0.27	0.89	0.55	16.99ab	3.48ab	13.51ab
GGR	13.83b	1.83	0.45b	0.31	0.98	0.55	17.95b	3.66b	14.28b
BHT	11.52ab	1.48	0.40ab	0.28	0.80	0.52	14.99ab	3.07a	11.92ab
Storage Time (ST)									
0	12.65	1.62	0.39	0.27	0.81	0.56	16.29	3.26	13.03
3	12.49	1.60	0.42	0.28	0.98	0.55	16.32	3.40	12.91
7	12.60	1.69	0.36	0.28	0.84	0.56	16.34	3.37	12.96
SED									
AO	0.85	0.17	0.04	0.04	0.09	0.07	1.00	0.19	0.84
ST	0.60	0.12	0.03	0.02	0.06	0.05	0.71	0.14	0.60
AO x ST	1.47	0.30	0.07	0.06	0.15	0.12	1.74	0.33	1.46
p value									
AO	0.019	0.118	0.013	0.687	0.185	0.795	0.012	0.028	0.014
ST	0.965	0.738	0.092	0.867	0.025	0.989	0.998	0.544	0.980
AO x ST	0.115	0.398	0.074	0.899	0.326	0.389	0.068	0.038	0.096

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table C6 Effect of natural antioxidants application on fatty acid composition (g of fatty acid/kg DM) of sous vide chicken breast meat during the storage time at 4 °C.

Treatment	TFA	C14:0	C16:0	C18:0	C20:0	∑ SFA	C16:1 n-7	C18:1 n-9	∑ MUFA
Antioxidant (AO)									
CON	52.10	0.25	11.84	5.17	0.20ab	17.47	1.58ab	20.06	21.64
ROS	55.70	0.23	12.08	5.12	0.22ab	17.64	1.59ab	21.30	22.88
SRB	56.00	0.24	12.12	5.36	0.22ab	17.86	1.43a	21.45	22.88
SFS	58.70	0.27	12.69	5.37	0.23b	18.57	1.61ab	22.53	24.14
GGR	51.30	0.22	11.16	5.04	0.17a	16.54	1.28a	19.43	20.71
BHT	58.10	0.28	13.50	5.16	0.25b	19.15	2.24b	23.06	25.30
Storage Time (ST)									
0	59.80b	0.28b	13.32b	5.47b	0.22	19.30b	1.87	23.17b	25.04b
3	53.60ab	0.23a	11.71a	5.08a	0.22	17.24a	1.46	20.62ab	22.08ab
7	52.50a	0.23a	11.66a	5.06a	0.21	17.08a	1.54	20.13a	21.66a
SED									
AO	3.88	0.02	0.93	0.22	0.02	1.16	0.24	1.73	1.93
ST	2.74	0.01	0.66	0.16	0.01	0.82	0.17	1.22	1.36
AO x ST	6.72	0.04	1.60	0.38	0.03	2.01	0.42	3.00	3.34
p value									
AO	0.319	0.065	0.223	0.608	0.002	0.32	0.007	0.290	0.222
ST	0.025	<.001	0.024	0.019	0.358	0.017	0.051	0.038	0.036
AO x ST	0.504	0.051	0.567	0.523	<.001	0.533	0.486	0.499	0.503

	C18:2 n-6	C18:3 n-3	C20:4 n-6	C22:5 n-3	C20:5 n-3	C22:6 n-3	∑PUFA	∑n-3	∑n-6
Antioxidant (AO)									
CON	9.94a	1.31	0.35c	0.25	0.69	0.44ab	12.98a	2.70a	10.28a
ROS	11.78ab	1.54	0.27ab	0.27	0.79	0.50b	15.15ab	3.10ab	12.05ab
SRB	11.92ab	1.55	0.24ab	0.25	0.78	0.51b	15.25ab	3.09ab	12.16ab
SFS	12.52b	1.68	0.30bc	0.27	0.74	0.48ab	15.98b	3.17b	12.82b
GGR	10.91ab	1.39	0.26ab	0.25	0.79	0.49ab	14.08ab	2.92ab	11.17ab
BHT	10.66ab	1.34	0.22a	0.27	0.72	0.43a	13.63ab	2.76ab	10.88ab
Storage Time (ST)									
0	12.07	1.63b	0.27	0.27	0.75	0.47	15.46	3.12b	12.34
3	11.15	1.44ab	0.27	0.25	0.74	0.48	14.33	2.92ab	11.41
7	10.64	1.33a	0.28	0.26	0.77	0.47	13.75	2.83a	10.92
SED									
AO	0.84	0.14	0.02	0.01	0.03	0.02	0.99	0.15	0.85
ST	0.59	0.10	0.01	0.01	0.02	0.02	0.70	0.11	0.60
AO x ST	1.45	0.24	0.03	0.02	0.06	0.04	1.72	0.26	1.46
p value									
AO	0.043	0.090	<.001	0.272	0.061	0.006	0.041	0.013	0.049
ST	0.062	0.016	0.528	0.233	0.598	0.705	0.058	0.025	0.068
AO x ST	0.318	0.178	0.041	0.521	0.928	0.612	0.321	0.222	0.334

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Appendix D (Statistics Analysis for Chapter 5)

Table D1 Proximate analysis of chicken meat

Treatment	DM g/kg meat		Fat g/kg DM	
	Sous vide (LTLO)	**Grilled (HTHO)**	**Sous vide (LTLO)**	**Grilled (HTHO)**
CON	287.90 ± 9.27a	317.10 ± 10.28a	44.18 ± 4.56a	69.72 ± 20.26a
BHT	297.60 ±1.81a	319.70 ±4.23a	77.17 ± 21.23a	61.90 ± 26.27a
ROS	293.40 ± 7.16a	323.30 ±12.42a	63.88 ± 9.25a	56.36 ± 24.99a
SRB	316.40 ±7.41a	327.20 ±9.63a	53.53 ± 8.24a	43.91 ± 5.94a
SFS	300.00 ±11.65a	325.60 ±1.85a	60.88 ± 15.23a	59.21 ± 9.59a
GGR	291.20 ±39.19a	322.10 ±5.16a	55.77 ± 21.63a	71.82 ± 32.21a

The data shown are the average and standard deviation of three independent samples. Values with different small letters within each column differ significantly ($p \leq 0.05$) according to the Tukey's test.

Table D2 Effect of natural antioxidants application on TBARS (mg MDA/kg meat), phospholipid content (mg/ 100 g fat) and conjugated dienes (μmol/g fat) comparing a 'Low Temperature, Low Oxygen' (Sous Vide) and a 'High Temperature, High Oxygen' (Grilled) cooking method for raw chicken breast meat during storage at 4 ºC.

	TBAR		**phospholipid**		**conjugated dienes**	
Main effects	**Sous vide**	**grilled**	**Sous vide**	**grilled**	**Sous vide**	**grilled**
Antioxidants (AO)						
CON	10.74e	19.79d	43.23a	42.49a	30.75c	55.38d
ROS	3.57a	12.49b	44.96b	45.78b	27.08ab	37.67a
SRB	9.97d	14.42c	45.25b	44.88b	28.49bc	42.59c
SFS	6.20c	13.22bc	44.46b	46.18b	28.58bc	42.01bc
GGR	5.39b	12.50b	44.80b	45.23b	27.07ab	42.66c
BHT	4.99b	7.91a	45.02b	45.79b	25.49a	38.12ab
Average	6.81	13.39	44.62	45.06	27.91	43.07
Reheating (RH)						
BRH	7.33b	12.97	46.02b	46.55b	28.34	45.16
ARH	6.29a	13.80	43.22a	43.57a	27.48	40.98
Storage Time (ST)						
0	4.04a	7.84a	47.97c	47.70c	25.83a	47.46b
4	4.48a	8.78b	46.74b	45.57b	27.09ab	42.60a
8	8.97b	19.53d	41.96a	43.89a	29.14bc	40.04a
12	9.76c	17.40c	41.80a	43.07a	29.58c	42.19a
SED						
AO	0.24	0.30	0.41	0.52	0.99	1.47
RH	0.14	0.17	0.24	0.30	0.57	0.85
ST	0.20	0.24	0.33	0.42	0.81	1.20
AO x RH	0.34	0.42	0.58	0.73	1.40	2.08
AO x ST	0.48	0.60	0.82	1.03	1.98	2.94
RH x ST	0.28	0.35	0.47	0.60	1.15	1.70
AO x RH x ST	0.68	0.85	1.16	1.46	2.81	4.16
p value						
AO	<.001	<.001	<.001	<.001	<.001	<.001
RH	<.001	<.001	<.001	<.001	0.137	<.001
ST	<.001	<.001	<.001	<.001	<.001	<.001
AO x RH	<.001	<.001	0.016	0.120	0.130	<.001
AO x ST	<.001	<.001	<.001	0.004	0.027	<.001
RH x ST	<.001	<.001	0.274	<.001	<.001	<.001
AO x RH x ST	<.001	<.001	<.001	<.001	0.043	<.001

Mean values with different small letters presented within each column of each cooking method differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process

Table D3 Effect of natural antioxidants on conjugated trienes (µmol/g fat), pH values and shear force (N) of chicken breast meat cooked by Sous Vide and grilled cooking method during the storage time at 4 °C.

	Conjugated trienes		**pH value**		**Shear force (N)**	
Main effects	**Sous vide**	**grilled**	**Sous vide**	**grilled**	**Sous vide**	**grilled**
Antioxidants (AO)						
CON	14.83[c]	26.75c	6.37c	6.28b	12.47	13.22ab
ROS	13.69[bc]	15.60a	6.23a	6.24ab	13.12	12.76a
SRB	12.40[ab]	19.31b	6.32bc	6.22a	12.94	14.21b
SFS	13.60[abc]	18.21ab	6.37c	6.19a	11.81	14.08ab
GGR	13.33[abc]	19.07b	6.31bc	6.23ab	13.48	13.50ab
BHT	11.83[a]	16.07ab	6.30ab	6.23ab	11.97	12.93a
Average	13.28	19.17	6.32	6.23	12.63	13.45
Reheating (RH)						
BRH	13.43	20.77	6.33	6.24	12.39	13.49
ARH	13.13	17.58	6.30	6.22	12.87	13.42
Storage Time (ST)						
0	12.27a	24.73c	6.27ab	6.22a	13.76b	13.39ab
4	14.93b	22.48c	6.32b	6.23a	12.33a	12.85a
8	12.93a	13.22a	6.26a	6.19a	12.78ab	14.35b
12	12.99a	16.26b	6.42c	6.29b	11.66a	13.22a
SED						
AO	0.63	1.14	0.02	0.02	0.64	0.36
RH	0.37	0.66	0.01	0.01	0.37	0.21
ST	0.52	0.93	0.02	0.02	0.53	0.29
AO x RH	0.90	1.62	0.03	0.03	0.91	0.51
AO x ST	1.27	2.29	0.05	0.05	1.29	0.72
RH x ST	0.73	1.32	0.03	0.03	0.74	0.41
AO x RH x ST	1.79	3.23	0.07	0.06	1.82	1.01
p value						
AO	<.001	<.001	<.001	0.003	0.068	0.022
RH	0.422	<.001	0.055	0.17	0.204	0.818
ST	<.001	<.001	<.001	<.001	0.001	0.004
AO x RH	0.040	<.001	0.451	0.168	0.074	0.143
AO x ST	0.350	<.001	<.001	0.578	0.081	0.176
RH x ST	<.001	<.001	0.003	<.001	0.361	0.08
AO x RH x ST	0.004	<.001	<.001	0.149	0.619	0.372

Mean values with different small letters presented within each column of each cooking method differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process

Table D4 Effect of natural antioxidants on L, a* and b* values of chicken breast meat cooked by Sous Vide and grilled cooking method during the storage time at 4 °C.*

	Sous vide			Grilled		
Main effects	**L* value**	**a* value**	**b* value**	**L* value**	**a* value**	**b* value**
Antioxidants(AO)						
CON	80.64de	0.11d	17.84ab	84.04	-1.85a	15.89
ROS	77.23a	-1.40a	18.90c	83.97	-1.30ab	15.50
SRB	79.93cd	-0.70bc	18.48bc	84.44	-1.17ab	15.05
SFS	78.90bc	-0.16cd	17.44a	83.69	-1.58ab	15.71
GGR	78.60b	-0.81b	22.34d	84.04	-0.97b	15.29
BHT	81.05e	-0.96ab	17.31a	84.31	-1.22ab	15.08
Average	79.39	-0.65	18.72	84.08	-1.34	15.42
Reheating						
BRH	79.40	-0.59	18.50	83.62	-1.14	15.18
ARH	79.38	-0.71	18.94	84.54	-1.55	15.66
ST						
0	79.15ab	-0.78ab	18.68ab	84.29ab	-1.51	14.98a
4	79.94b	-1.02a	18.39a	83.53a	-1.29	14.90a
8	78.68a	-0.37b	19.10b	83.90ab	-1.30	15.97b
12	79.78b	-0.44b	18.72ab	84.59b	-1.29	15.81b
SED						
AO	0.38	0.20	0.28	0.44	0.26	0.35
RH	0.22	0.11	0.16	0.25	0.15	0.20
ST	0.31	0.16	0.23	0.36	0.21	0.29
AO x RH	0.54	0.28	0.40	0.62	0.37	0.49
AO x ST	0.76	0.40	0.56	0.87	0.52	0.70
RH x ST	0.44	0.23	0.33	0.51	0.30	0.40
AO x RH x ST	1.08	0.56	0.80	1.24	0.74	0.99
p value						
AO	<.001	<.001	<.001	0.606	0.017	0.105
RH	0.946	0.283	0.009	<.001	0.008	0.019
ST	<.001	<.001	0.026	0.023	0.663	<.001
AO x RH	0.597	0.346	0.893	0.956	0.036	0.614
AO x ST	0.017	0.457	0.001	0.146	0.537	0.943
RH x ST	0.435	0.786	0.221	0.271	0.656	0.778
AO x RH x ST	0.590	0.937	0.974	0.778	0.422	0.595

Mean values with different small letters presented within each column of each cooking method differ significantly ($p \leq 0.05$) according to the Tukey's HSD test.

Table D5 Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 °C.

	T.F.A	C14:0	C16:0	C18:0	C20:0	∑SFA	C16:1 n-7	C18:1 n-9c	C18:1 n-9t	∑MUFA
Antioxidants (AO)										
CON	72.00a	0.33a	14.98a	5.98a	0.37	21.66a	1.66	25.18a	1.96a	28.80a
ROS	84.50b	0.38ab	17.09	6.58	0.40	24.44	1.91	30.20b	2.20ab	34.31b
SRB	81.10ab	0.37ab	16.54	6.40	0.38	23.69	1.90	28.87ab	2.16ab	32.94
SFS	85.40b	0.40b	17.62b	6.58	0.41	25.01b	2.15	30.43b	2.27b	34.86b
GGR	84.60b	0.38ab	16.96	6.68b	0.39	24.41	1.81	29.68ab	2.17ab	33.66
BHT	81.80ab	0.37ab	16.80	6.46	0.39	24.02	1.91	29.08ab	2.16ab	33.15
Reheating										
BRH	82.60	0.38	16.82	6.48	0.39	24.06	1.89	29.33	2.15	33.38
ARH	80.50	0.36	16.51	6.42	0.39	23.68	1.89	28.48	2.16	32.53
ST										
0	85.00bc	0.39b	17.38ab	6.53	0.40ab	24.70ab	2.06b	30.13ab	2.25b	34.44bc
4	88.20c	0.40b	17.89b	6.79b	0.41b	25.49b	2.10b	31.55b	2.28b	35.93c
8	76.75ab	0.34a	15.67a	6.33	0.38ab	22.72a	1.63a	26.89a	2.04a	30.56a
12	76.30a	0.35a	15.73a	6.13a	0.37a	22.58a	1.78ab	27.05a	2.05a	30.87ab
SED										
AO	3.94	0.02	0.81	0.22	0.02	1.04	0.17	1.60	0.09	1.81
RH	2.27	0.01	0.47	0.13	0.01	0.60	0.10	0.93	0.05	1.05
ST	3.22	0.02	0.66	0.18	0.02	0.85	0.14	1.31	0.07	1.48
AO x RH	5.57	0.03	1.14	0.31	0.03	1.48	0.24	2.27	0.13	2.57
AO x ST	7.88	0.04	1.62	0.44	0.04	2.09	0.34	3.21	0.18	3.63
RH x ST	4.55	0.02	0.93	0.25	0.02	1.20	0.20	1.85	0.10	2.10
AO x RH x ST	11.14	0.06	2.29	0.62	0.05	2.95	0.48	4.54	0.25	5.13
p value										
AO	0.010	0.042	0.037	0.034	0.255	0.034	0.125	0.018	0.030	0.020
RH	0.362	0.130	0.519	0.632	0.871	0.530	0.995	0.359	0.976	0.418
ST	<.001	<.001	<.001	0.003	0.008	0.001	0.002	<.001	<.001	<.001
AO x RH	0.189	0.097	0.098	0.488	0.026	0.131	0.134	0.151	0.222	0.137
AO x ST	<.001	0.001	0.001	<.001	<.001	<.001	0.034	<.001	0.004	<.001
RH x ST	0.039	0.026	0.048	0.056	0.009	0.042	0.243	0.048	0.226	0.061
AO x RH x ST	<.001	<.001	<.001	0.001	<.001	<.001	0.034	<.001	<.001	<.001

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process

Table D5 (Continued) Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'Low Temperature, Low Oxygen' (Sous Vide) cooking method during the storage time at 4 °C.

Main effects	C18:2 n-6	C20:4 n-6	C18:3 n-3	C22.5 n-3	C20:5 n-3	C22:6 n-3	∑PUFA	∑n-3	∑n-6
Antioxidants (AO)									
CON	15.70a	2.10a	2.28a	0.28	0.70a	0.47	21.53a	3.73[a]	17.80[a]
ROS	19.06b	2.30b	2.86b	0.26	0.77b	0.51	25.75b	4.39[b]	21.36[b]
SRB	18.18ab	2.20ab	2.68ab	0.24	0.73ab	0.49	24.51ab	4.14[ab]	20.37[ab]
SFS	19.02b	2.21ab	2.85b	0.26	0.74ab	0.46	25.55b	4.31[b]	21.23[b]
GGR	19.62b	2.37b	2.95b	0.27	0.77b	0.52	26.51b	4.51[b]	21.99[b]
BHT	18.11ab	2.27ab	2.68ab	0.26	0.78b	0.51	24.61ab	4.23[ab]	20.38[ab]
Reheating									
BRH	18.69	2.20	2.80	0.26	0.73	0.49	25.17	4.28	20.89
ARH	17.88	2.28	2.63	0.27	0.76	0.49	24.32	4.16	20.16
ST									
0	19.15bc	2.26bc	2.90b	0.28b	0.78b	0.51	25.87bc	4.46b	21.41bc
4	19.82c	2.39c	3.02b	0.28b	0.78b	0.49	26.78c	4.57b	22.21c
8	17.25ab	2.23b	2.49a	0.26ab	0.74a	0.49	23.47ab	3.98a	19.49ab
12	16.90a	2.09a	2.46a	0.23a	0.70a	0.48	22.85a	3.87a	18.99a
SED									
AO	1.01	0.06	0.18	0.01	0.02	0.02	1.20	0.19	1.02
RH	0.58	0.03	0.11	0.01	0.01	0.01	0.69	0.11	0.59
ST	0.82	0.05	0.15	0.01	0.01	0.02	0.98	0.15	0.83
AO x RH	1.43	0.08	0.26	0.02	0.03	0.03	1.70	0.26	1.44
AO x ST	2.02	0.12	0.37	0.03	0.04	0.04	2.40	0.37	2.03
RH x ST	1.17	0.07	0.21	0.01	0.02	0.03	1.39	0.22	1.17
AO x RH x ST	2.85	0.17	0.52	0.04	0.05	0.06	3.39	0.53	2.87
p value									
AO	0.003	<.001	0.008	0.084	<.001	0.076	0.002	0.002	0.002
RH	0.167	0.027	0.128	0.065	0.005	0.797	0.222	0.281	0.214
ST	<.001	<.001	<.001	<.001	<.001	0.475	<.001	<.001	<.001
AO x RH	0.371	0.080	0.301	0.108	0.297	0.083	0.430	0.495	0.419
AO x ST	0.001	0.023	0.001	0.652	0.107	<.001	0.002	0.003	0.001
RH x ST	0.028	0.197	0.026	0.883	0.592	0.747	0.032	0.029	0.033
AO x RH x ST	<.001	0.002	<.001	0.127	0.012	0.006	<.001	<.001	<.001

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process

Table D6 Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

	T.FA	C14:0	C16:0	C18:0	C20:0	∑ SFA	C16: 1n-7	C18:1 n-9c	C18:1 n-9t	∑ MUFA
Antioxidants (AO)										
CON	72.50	0.33	15.19	5.98	0.36	21.86	1.77	2.02	25.84	29.63
ROS	69.90	0.33	14.58	5.81	0.34	21.05	1.69	1.95	24.52	28.16
SRB	78.80	0.36	16.27	6.26	0.38	23.27	1.94	2.12	28.33	32.39
SFS	76.30	0.35	15.79	6.06	0.37	22.56	1.88	2.03	27.18	31.09
GGR	83.20	0.38	17.09	6.60	0.39	24.46	1.93	2.16	30.15	34.23
BHT	77.60	0.36	16.08	6.19	0.37	22.99	2.00	2.07	27.73	31.80
Reheating										
BRH	76.90	0.35	15.86	6.13	0.37	22.71	1.90	2.08	27.48	31.46
ARH	75.80	0.35	15.81	6.17	0.36	22.68	1.84	2.03	27.10	30.97
ST										
0	77.50	0.35	15.87	6.08	0.36	22.66	1.93	2.07	27.58	31.58
4	80.50	0.37	16.46	6.31	0.39	23.52	1.95	2.12	28.70	32.77
8	73.70	0.34	15.49	6.12	0.35	22.30	1.83	2.01	26.35	30.19
12	73.80	0.35	15.51	6.09	0.36	22.30	1.77	2.03	26.54	30.33
SED										
AO	5.22	0.03	1.10	0.26	0.02	1.39	0.22	0.12	2.12	2.43
RH	3.01	0.02	0.63	0.15	0.01	0.80	0.13	0.07	1.22	1.40
ST	4.26	0.02	0.90	0.21	0.02	1.14	0.18	0.10	1.73	1.98
AO x RH	7.38	0.04	1.55	0.37	0.03	1.97	0.31	0.17	2.99	3.43
AO x ST	10.43	0.06	2.19	0.52	0.05	2.78	0.44	0.24	4.23	4.85
RH x ST	6.02	0.03	1.27	0.30	0.03	1.61	0.26	0.14	2.44	2.80
AO x RH x ST	14.76	0.08	3.10	0.74	0.06	3.94	0.62	0.34	5.99	6.86
p value										
AO	0.155	0.387	0.28	0.067	0.361	0.220	0.735	0.536	0.139	0.184
RH	0.716	0.951	0.939	0.828	0.504	0.975	0.646	0.472	0.754	0.726
ST	0.318	0.691	0.676	0.672	0.100	0.673	0.720	0.712	0.503	0.528
AO x RH	0.383	0.296	0.477	0.584	0.331	0.497	0.523	0.257	0.388	0.391
AO x ST	0.165	0.308	0.168	0.071	0.035	0.142	0.105	0.126	0.127	0.126
RH x ST	0.227	0.343	0.289	0.261	0.256	0.287	0.574	0.082	0.195	0.208
AO x RH x ST	0.537	0.588	0.590	0.570	0.406	0.602	0.449	0.577	0.459	0.456

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process

Table D6 (Continued) Effect of natural antioxidant application on fatty acid composition (g/kg DM) of chicken breast meat cooked by a 'High Temperature, High Oxygen' (Grilled) cooking method during the storage time at 4 °C.

	C18:2 n-6	C18:3 n-3	C20:4 n-6	C22.5 n-3	C20:5 n-3	C22:6 n-3	∑PUFA	∑n-3	∑n-6
Antioxidants (AO)									
CON	15.63	2.20	1.89	0.23	0.65	0.40	21.00	3.48	17.52
ROS	15.11	2.18	1.99	0.24	0.68	0.44	20.65	3.54	17.11
SRB	17.23	2.54	2.02	0.25	0.66	0.45	23.15	3.91	19.25
SFS	16.83	2.43	2.08	0.25	0.67	0.44	22.70	3.79	18.91
GGR	18.38	2.73	2.07	0.24	0.69	0.45	24.56	4.11	20.45
BHT	16.81	2.50	2.10	0.25	0.70	0.45	22.80	3.90	18.91
Reheating									
BRH	16.90	2.46	2.05	0.24	0.68	0.44	22.77	3.82	18.95
ARH	16.43	2.39	2.00	0.24	0.68	0.44	22.19	3.76	18.43
ST									
0	17.13	2.53	2.19c	0.26b	0.71b	0.47b	23.28b	3.96ab	19.32b
4	18.04	2.69	2.11bc	0.25ab	0.71b	0.45ab	24.24b	4.09b	20.15b
8	15.63	2.23	1.99b	0.23a	0.66a	0.44ab	21.18a	3.56a	17.62a
12	15.85	2.27	1.82a	0.23ab	0.63a	0.41a	21.20a	3.53a	17.67a
SED									
AO	1.26	0.23	0.08	0.01	0.02	0.03	1.48	0.23	1.26
RH	0.73	0.13	0.04	0.01	0.01	0.01	0.86	0.13	0.73
ST	1.03	0.19	0.06	0.01	0.02	0.02	1.21	0.18	1.03
AO x RH	1.78	0.32	0.11	0.02	0.03	0.04	2.10	0.32	1.78
AO x ST	2.51	0.46	0.15	0.03	0.05	0.05	2.96	0.45	2.52
RH x ST	1.45	0.26	0.09	0.01	0.03	0.03	1.71	0.26	1.45
AO x RH x ST	3.55	0.64	0.22	0.04	0.07	0.07	4.19	0.64	3.56
p value									
AO	0.138	0.129	0.064	0.626	0.225	0.360	0.100	0.055	0.109
RH	0.511	0.592	0.307	0.521	0.968	0.62	0.497	0.651	0.473
ST	0.068	0.048	<.001	0.025	<.001	0.047	0.026	0.004	0.036
AO x RH	0.277	0.328	0.150	0.019	0.293	0.966	0.308	0.393	0.294
AO x ST	0.207	0.249	0.026	0.393	0.013	0.067	0.195	0.204	0.190
RH x ST	0.224	0.242	0.965	0.081	0.955	0.130	0.237	0.294	0.228
AO x RH x ST	0.606	0.491	0.055	0.588	0.147	0.590	0.571	0.373	0.607

Mean values with different small letters presented within each column of each fatty acid differ significantly ($p \leq 0.05$) according to the Tukey's HSD test. BRH, before reheating process; ARH, after reheating process