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Understanding the Relationship between Wheat Grain Traits and Ethanol Yield and Predicting Grain Ethanol Yield

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Table of contents

Table of contents 2

Abstract 7

Acknowledgment 8

Publications arising from this study 10

List of figures 11

List of tables 13

List of abbreviations 14

1. Introduction 16

Schematic Representation of the Thesis 18

2. Literature Review 19

2.1 Biofuels Definition and history 19

2.2 Liquid biofuels 21

2.2.1 Biodiesel 21

2.2.2 Bioethanol 21

2.2.2.1 Sugar feedstocks 22

2.2.2.2 Starch feedstock 22

2.2.2.3 Cellulose feedstocks 24

2.3 Opportunity and risks of biofuels 25

2.3.1 Renewable Resource 25

2.3.2 Environmental consequence 25

2.3.3 Energy security 27

2.3.4 New future for Rural Communities 27

2.3.5 Food prices 28
2.4 Feedstock quality 28
2.4.1 Starch 29
2.4.2 Starch measurement 32
2.4.3 Starch conversion efficiency 35
2.5 Factors causing feedstock quality variability 36
2.5.1 Varieties 36
2.5.2 Environment 36
2.5.3 Management 37
2.6 Predicting ethanol yield 37
2.6.1 Amount of Starch and free sugars 37
2.6.2 Grain N/protein 38
2.6.3 Hot water extract 39
2.6.4 Specific weight 39
2.6.5 Thousand grain weight (TGW) 40
2.6.6 Grain size and shape 41
2.6.7 Extract turbidity 41
2.6.8 Grain hardness 42
2.6.9 Grain vitreosity 42
2.6.10 Combining different traits to predict ethanol yield 43
3. Aim and Objectives of the project 45
3.1 Aim 45
3.2 Objectives 45

3.3 Null hypothesis 46
4. General Material and Methods 46
4.1 Moisture content 47
4.2 Starch concentration 47
4.3 Nitrogen concentration 49
4.4 Image analysis 49
4.5 Specific weight 50
4.6 Grain density 50
4.7 Packing efficiency 51
4.8 Grain weight and 1000 grain weight 52
4.9 Ethanol yield 54
5. Economic importance of wheat grain ethanol yield variability 56
5.1 Summary 56
5.2 Introduction 56
5.3 Cost of production 58
5.3.1 Feedstock cost 58
5.3.2 Energy Cost 60
5.3.3 Investment cost 62
5.4 Revenue 63
5.4.1 Bioethanol 63
5.4.2 Distillers dried grains with solubles 64
5.4.3 Carbon dioxide 65
5.4.4 Others 65

5.5 Economic analysis 66
5.5.1 Supply, demand and price relationship between fuel energy sources 66
5.5.2 Cost of production 67
5.5.3 Sensitivity of cost of production to changes in variables 71
5.6 Conclusion 77
6. Analysis of grain characteristics and ethanol yield of RL varieties 78
6.1 Introduction 78
6.2 Objective 78
6.3 Grain samples 78
6.4 Grain Analysis 79
6.5 Statistical analysis 79
6.6 Results 80
6.6.1 Grain chemical composition 80
6.6.2 Grain weight 85
6.6.3 Grain density 87
6.6.4 Grain dimensions 90
6.6.5 Hardness index (HI) 90
6.6.6 Combining different parameters 90
6.7 Discussion 91
7. Field experiment 97
7.1 Introduction and objectives 97
7.2 Materials and Methods 97
7.2.1 Experimental design 100

7.2.2 Crop husbandry 101
7.2.3 Specific weight 102
7.2.4 Predicted ethanol yield (PEY) 102
7.2.5 Non-starch polysaccharide 103
7.2.6 Statistical analysis 103
7.2.7 Weather data 104
7.3 Results 105
7.3.1 Analysis of variance and regression analysis 105
7.3.2 Major chemical components of the grain 112
7.3.3 Density of the grain 115
7.3.4 Grain weight 119
7.3.5 Grain shape and size 121
7.3.6 Hardness Index 123
7.4 Discussion 124
7.4.3 Variety 128
7.4.4 Year 128
7.4.5 N fertilizer 129
7.4.6 Prediction using multiple traits 129
8 General Discussion 130
9 Recommendations for future work 135
References 137
Appendices 160

Abstract

Environmental, political and security issues are pushing the world towards a search for alternative fuel. Suitability of wheat varieties varies for different end uses. To increase process efficiency and to reduce cost of production, the right quality wheat has to be used.

An economic analysis was conducted in order to quantify the significance of feedstock quality in the economics of bioethanol production. Ethanol yield (EY) variation within 84 samples was used to determine its effect on the cost of production by using cost of production data obtained from the literature and leaving all other variables constant. This analysis indicated that the best quality wheat among these samples could save up to three million pounds per year for a company with a capacity of 100000 tonnes of wheat per year.

The other part of the study was to identify quality criteria which can be used at the refinery intake. Two independent experiments were conducted. i) Based on Recommended List (RL) samples comprising 14 varieties grown for two years at 11 sites. ii) Based on a field experiment conducted for two years using different agronomic practices in order to get a range of grain quality. EY, starch, nitrogen and non-starch polysaccharide (NSP) concentrations, thousand grain weight (TGW), specific weight, grain density, packing efficiency and the grain size and shape were measured in both experiments. Regression analysis was used to establish the relationship between the grain traits and EY. Both experiments revealed that nitrogen is the single best indicator of grain EY. TGW and specific weight are the second and third best indicators of EY respectively. Grain density and length are the poorest indicators of EY. Multiple linear regression result indicated that a model built with the combination of nitrogen and TGW can give the best prediction of EY. Adding variety and site to the model will increase the prediction potential.

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Publications arising from this study

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Awole, K.D., Kettlewell, P.S., Hare, M.C., Agu, R.C., Brosnan, J.M. and Bringhurst, T.A. 2012. Effect of environment and variety on the relationships of wheat grain physical and chemical characteristics with ethanol yield. *J Sci. Food & Agri,* **92:** 577-584

Awole, K.D., Kettlewell, P.S., Hare, M.C., Agu, R.C., Brosnan, J.M., and Bringhurst, T.A. 2008. Prediction of Alcohol yield from wheat grain. *Aspects of Applied Biology **90**, Biomass and Energy Crops III*, pp. 161-164

Awole, K.D., Kettlewell, P.S., Hare, M.C., Agu, R.C., Brosnan, J.M., and Bringhurst, T.A *Selecting Best Wheat for Bio-refining.* @ International annual meetings of the American Society of Agronomy, Crop Science Society of America and Soil Science Society of America. Long Beach, California, USA. Oct 31 – Nov 4, 2010

Awole, K.D., Kettlewell, P.S., Hare, M.C., Agu, R.C., Brosnan, J.M., and Bringhurst, T.A *Does Specific Weight Predict Alcohol Yield? Abstracts of the Society of Chemical Industry/Royal Society of Chemistry conference on Wheat for Biofuels, Bioenergy and High Value Bioproducts, 29 April 2008, Jealott's Hill, Bracknell UK.*

Awole, K.D., Kettlewell, P.S., Parsons, S.T. and Hare, M.C. 2009. Bioethanol economics, the importance of grain quality. Technical note on http://www.openfields.org.uk/Library/content/Detail.aspx?ctID=ZWVhNzBlY2QtZWJjNi00YWZiLWE1MTAtNWExOTFiMjJjOWU1&rID=ODI2&sID=MQ==&bckToL=VHJ1ZQ==&qcf=&ph=VHJ1ZQ==

List of figures

Figure 1 One of the scanned images of the grain used for grain size determination ... 49

Figure 2 Multivolume pycnometer 1305, Micromeritics Instument Corporation, USA, used for grain density measurement ... 51

Figure 3 Single kernel Characterisation System machine associated with its own data processing computer used for HI. ... 53

Figure 4 The trend in UK feed wheat, milling wheat and distilling wheat consumption from 1994 to 2007. [Data source: HGCA-AHDB)] ... 60

Figure 5 The annual average crude oil price in U.S. Dollars per barrel from 1994-2007 [Data source: EIA (05 Dec 2008)] ... 67

Figure 6 The relationship of the cost of bioethanol production in p/litre with bioethanol yield litre/t of wheat based on the ethanol yield of 84 samples of wheat from HGCA-AHDB RL trials harvested in 2003 and 2004 (Samples detailed in Chapter Six) ... 74

Figure 7 The relationship between the cost of bioethanol production in p/l and DDGS price £/t ... 76

Figure 8 The relationship between the cost of bioethanol production in p/l and wheat price £/t ... 76

Figure 9 Relationship between ethanol yield and grain nitrogen a) with common model for all samples, b) with separate model fitted for each year and c) with separate model fitted for each site ... 84

Figure 10 Relationship between ethanol yield and TGW A) With common model fitted for all samples, B) With separate model fitted for each variety and C) With separate model fitted for each year. ... 87

Figure 11 The relationship between A) ethanol yield and specific weight common model for all samples B) ethanol yield and specific weight, separate models fitted for each site C) ethanol yield and specific weight, separate models fitted for each year... 89

Figure 12 Shaded and unshaded plots of the field experiment at Harper Adams University. Date taken 16/7/2008 at GS71 ... 99

Figure 13 Polythene covered and uncovered plots of the field experiment after the polythene cover was removed. Date taken 26/08/2008 at GS91 101

Figure 14 The relationship between a) PEY and starch concentration b) starch and N concentration (both years data combined) line represents best fit simple linear regression equation the values of which are indicated on Table 7.8. 113

Figure 15 The relationship between PEY and grain nitrogen concentration (a) single model for all data from both years (b) separate lines fitted for each variety based on all data from both years 114

Figure 16 The relationship between PEY and NSP separate parallel lines fitted for shaded plots (*) and not shaded plots (▲), data is from both years combined. 115

Figure 17 The relationship between PEY and specific weight (a) two parallel lines fitted for shaded and not shaded plots (b) two separate non parallel lines fitted for the two varieties (c) when specific weight is below 73 kg/hl (d) when specific weight is above 73 kg/hl. 118

Figure 18 The relationship between PEY and TGW (a) separate model fitted for each variety and (b) separate model for shaded and not shaded plots 120

Figure 19 The relationship between PEY and (a) grain surface area, both years data combined (b) grain width, only second year data (c) grain length to width ratio, only first year data 123

Figure 20. The relationship between PEY yield and hardness index, both years' data combined 124

List of tables

Table 1 Thermal and electrical energy required at different stages of bioethanol production 61

Table 2 Estimated cost of production of wheat grain bioethanol from different literature 69

Table 3 Breakdown of bioethanol production costs adapted from Bullard *et al.* (2003) 72

Table 4 Data of 2003 and 2004 summer weather in England and Scotland 79

Table 5 Result of regression analysis of ethanol yield (l/tonne) with different grain parameters. 82

Table 6 Treatment details of the field experiment in year 2007/08 98

Table 7 Treatment details of the field experiment in year 2008/09 100

Table 8 Rate and stage of nitrogen fertilizer application for the two years field experiments in kg ha^{-1} 102

Table 9 2008 and 2009 summer weather data of Newport, Shropshire, UK 105

Table 10 Summary of the analysis of variance of both years data combined for variables that the two years data could be combined based on the two-way F-test. 105

Table 11 Summary of analysis of variance of the first year experiment for variables that the two years data could not be combined 106

Table 12 Analysis of the variance of the second year data for variables that the two years data could not be combined 106

Table 13 Regression result of PEY and N with each of the grain traits both years' data combined. 108

Table 14 Regression result of PEY and N with each of the other grain traits only for the second year data 110

Table 15 Regression result of PEY and N with each of the other grain traits only for the first year data 111

Table 16 Comparison of grain ethanol yield prediction models from different authors 133

List of abbreviations

AOAC	Association of Analytical Chemists
AX	Arabinoxylans
BERR	Business, Enterprise and Regulatory Reform
BP	British Petroleum
BTG	Biomass Technology Group
CAST	Council for Agricultural Science and Technology
CERC	Crop and Environment Research Centre
COM	Commission of the European Communities
DDGS	Distillers Dried Grain with Solubles
Defra	Department for Environment, Food and Rural Affairs
DfT	Department for Transport
DTI	Department of Trade Industry
EPC	European Parliament and Council
ETBE	Ethyl Tertiary Butyl Ether
EU	European Union
EUBIA	European Biomass Industry Association
EY	Ethanol yield
FAO	Food and Agriculture Organization
FOB	Free On Board
GC	Gas Chromatography
GCC	Green Car Congress
GHG	Greenhouse Gas
GLC	Gas Liquid Chromatography
GOPOD	Glucose Oxidase-peroxidase
GS	Growth Stage

HGCA-AHDB	Home-Grown Cereals Authority–Agriculture and Horticulture Development Board
HI	Hardness Index
HPLC	High Performance Liquid Cromatography
IEA	International Energy Agency
IOB	Institute of Brewing
KT	kilo tonnes
L	Grain Length
L:W	Length to Width ratio
NIR	Near Infrared Reflectance
NSP	Non-Starch Polysaccharide
NNFCC	National Non Food Crops Centre
PE	Packing Efficiency
PEY	Predicted Ethanol Yield
R&D	Research and Development
RL	Recommended List
RTFO	Renewable Transport Fuel Obligation
SKCS	Single Kernel Characterization System
Sp wt	Specific Weight
SWRI	Scotch Whisky Research Institute
TGW	Thousand Grain Weight
UN	United Nations
UK	United Kingdom
USA	United States of America
USD	United States Dollar

1. Introduction

Bioenergy is energy obtained from plants mainly in the form of carbohydrate which has been captured from the sun through photosynthesis (CAST, 2004). The annual productivity of plants on earth is 144-180 billion tonnes of dry organic matter and the energy content of this dry matter is 200 times the annual energy consumption of the world (Lewis, 1983). Therefore plants have great potential as an alternative renewable source of energy. With a much faster rate of consumption of fossil fuels than its rate of formation, the need for alternative renewable energy is unquestionable. The transport sector is almost solely dependent on petroleum based fuels thus attention has been given to biofuels as an alternative (MalÇa and Freire, 2006). Besides the need for renewable energy sources, the main driving forces towards biofuels are: high oil price, concern to reduce Greenhouse Gas (GHG) emissions, government initiative to reduce reliance on imported fuels.

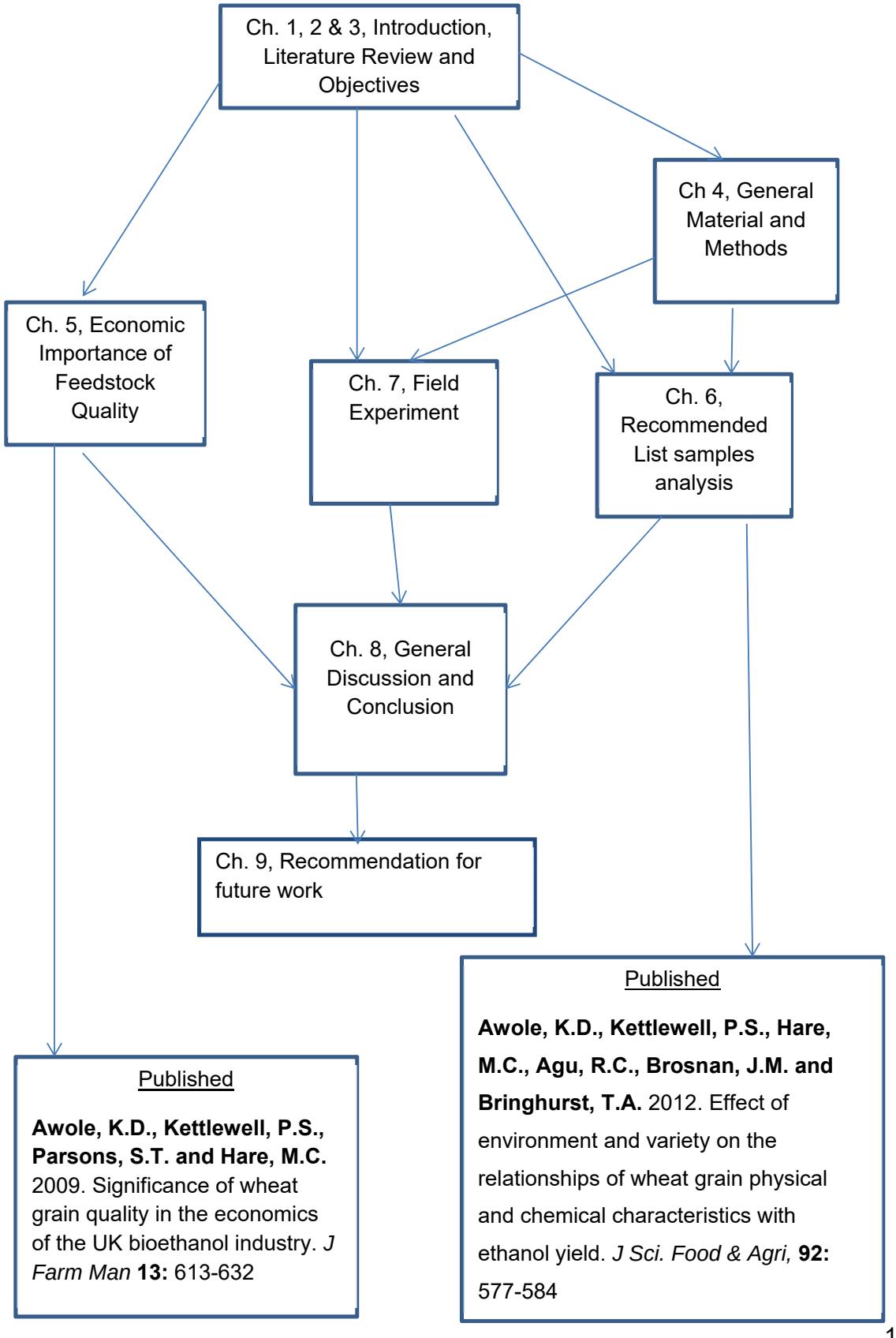
Following the UK government target of replacing 2.5% transport fuels by renewable as of 2008; the market for biofuels is rising (HGCA-AHDB, 2007a). Wheat is expected to be a principal feedstock for bioethanol production in the UK (Smith *et al*., 2006). Wheat is a relatively new feedstock for bioethanol as compared to sugar cane and maize. Thus the technology for wheat bioethanol is still developing and most of the information is required from other bioethanol feedstocks and from the potable ethanol industry and needs to be tailored for wheat bioethanol.

The potable alcohol industry in the UK uses about 0.7 million tonnes of wheat annually for production of whisky, gin and vodka spirits. Although the potable alcohol industry is restricted from using commercial enzymes, the production process of potable alcohol and bioethanol is basically similar. Both processes involve milling of the grain and mixing the flour with water and cooking. Cooking of the slurry gelatinizes the starch. Starch is

degraded to glucose by adding enzyme. The sugar is then fermented into ethanol by yeast and ethanol is distilled from the fermented mixture. This study makes full use of the information available from the potable alcohol industry.

The biofuel market is global and therefore cost competitiveness is crucial. Moreover energy efficiency is a greater concern for the biofuel industry than for the potable alcohol industry so as to increase the greenhouse gas savings and net energy outcome as compared to petrol. In order to maximize productivity as well as processing efficiency quality of the feedstock is vital. Wheat samples vary in their ethanol yielding potential. Poor quality wheat besides yielding very low ethanol takes more energy during processing (Bringhurst *et al*., 2003). So far there is no standard grain quality test for wheat bioethanol. The potable alcohol industry follows relatively simple quality criteria based on specific weight and grain nitrogen concentration. Previous studies showed that these quality criteria do not predict grain ethanol yield precisely (Taylor *et al*., 1993; Swanston *et al*., 2007). Given the importance of grain quality to the industry, a rapid and accurate test of prediction of grain ethanol yield must be available. This study has attempted to indicate the economic importance of feedstock quality to the industry and gives recommendation on what quality criteria should be used at the bio-refinery intake.

Schematic Representation of the Thesis



2. Literature Review

2.1 Biofuels Definition and history

Biofuels can be defined as fuels derived from biomass; these include the basic bioenergy resources such as wood; liquid fuels such as ethanol and methanol which are derived from bioconversion of carbohydrate as well as biodiesel processed from plant oils; gaseous forms such as biogas (IEA, 2005). This review focuses on liquid biofuels which can be used as a substitute for transport fuels. Biofuels and mineral fuels have similar physical characteristics but the way they come into existence is different (Hammond *et al*., 2007).

Humans were entirely dependent on biomass for all energy needs until the discovery of mineral fuels in the 17th century. Before that time, there was a similar energy crisis; not lack of oil but of wood (Lewis, 1983). Fossil fuels achieved such a long lasting dominant position as sources of energy mainly for two reasons; ease of use and low cost (Anon, 1983). The price of fossil fuel is no longer attractive. Currently the fossil fuel price has hit the highest in history (113 USD/bbl of crude oil at the time of writing; 1 bbl = 159 l)). Moreover the world again found itself in another energy crisis caused by rapidly increasing demand for fuels but declining supply (Hammond *et al*., 2007). Consequently there is a high demand for alternative sources of energy and biofuels are the unique option so far for the transport sector (COM, 2006).

Using bioethanol for automobiles started in the beginning of the 20th century when Henry Ford designed the Model T so as to use bioethanol produced by American farmers; similarly, using biodiesel as an engine fuel started late in the 19th century when Rudolf Diesel produced the first engine which ran on biodiesel (MalÇa and Freire, 2006). After World War II, access to cheap and surplus fossil fuel supply displaced biofuels (Anon, 1983).

In the current generation, Brazil became a model in producing and using biofuel for transportation. At least since the early 1970s until the mid-1990s, Brazil was the only country known for production and consumption of biofuels (Mol, 2007). The biofuel program in Brazil was driven by the world energy crisis of the 1970's as a means to reduce reliance on gasoline imports (Weiss, 1990). Surplus land, favourable climate and cheap sugarcane production have been in favour of the program. Brazil was the world's largest producer of bioethanol until 2006 (Mol, 2007). The USA started production of bioethanol in 1979 (Wheals *et al*., 1999); maize being the main feedstock due to accessibility and low price (Bothast and Schlicher, 2005). The USA, the biggest oil consumer in the world, is the largest producer of bioethanol since 2006 while the European Union (EU), mainly Germany, is leading world biodiesel production and consumption (Mol, 2007).

Ninety per cent of world bioethanol production is from Brazil and USA alone while the EU's contribution is only 2.8% of the world production (BP, 2006). World biofuel production is increasing tremendously. Ethanol production grew more than double between 2000 and 2005 while biodiesel production quadrupled in the same year range (Caldwell, 2007). Bioethanol is the most widely used type of biofuel in the world mainly because of higher production by volume in Brazil and America (Mol, 2007). In total, both bioethanol and biodiesel contribute only about 1% of world's liquid transport fuel (Mastny, 2006).

A number of countries have made a big commitment for substitution of conventional fossil fuels by biofuel. Among these Brazil, United States, China, Colombia, India, the Philippines, Thailand and Sweden are some of them (Mastny, 2006). Countries which are not engaged yet in biofuel production have at least planned to get engaged (Mol, 2007). The UK produced 9000 tonnes of biodiesel in the year 2004 (Hammond *et al*., 2007) and the production rate is increasing. When it comes to bioethanol, the UK has

been dependent on imports (BERR, 2007). Production of bioethanol and biobutanol in the UK began in September 2007 (British sugar, undated).

2.2 Liquid biofuels

Biofuels are enjoying unprecedented growth in production and consumption in many countries. There are several renewable fuels that could replace or can be blended with conventional fuels but bioethanol and biodiesel, commonly referred as first generation biofuels, are the most economically attractive for the time being (BERR, 2007; Peters and Thielmann, 2008). All existing engines allow a biofuel blend of up to 5% with conventional fossil fuels but with some modification to the engine, biofuel can be used as neat (100%) fuel (Hammond *et al*., 2007).

2.2.1 Biodiesel

Biodiesel is an engine fuel processed from plant oils or animal fats which have been synthesized by direct transesterification (Johnston and Holloway, 2006). Currently the two main sources of biodiesel are oil rich vegetables and waste fat and oils (Hammond *et al*., 2007).

2.2.2 Bioethanol

Ethanol is a flammable, colourless compound produced by fermentation of sugar by yeast and subsequent separation from the aqueous solution by distillation. The normal distillation process can give a 95% ethanol yield but further treatment can give 100% ethanol (Smith *et al*., 2006). Ethanol can probably replace petrol, but it can also be used as blend of ethanol/hydrocarbon (Kheshgi *et al*., 2000). Bioethanol can also be used in the production of Ethyl Tertiary Butyl Ether (bioETBE), through the chemical reaction of bioethanol with isobutylene-A which is a by-product of the petroleum refining process (MalÇa and Freire, 2006).

Feedstocks of bioethanol can be categorized into sugar, starch and cellulose. The choice of feedstock is determined by technical and economic considerations as cost of production is highly correlated with costs of feedstock (Turley *et al*., 2004).

2.2.2.1 Sugar feedstocks

This group consists of biological feedstocks that contain appreciable amount of sugar that can be converted into ethanol. Some of the highest potential crops of this group are sugar cane and sugar beet (MalÇa and Freire, 2006). So far sugarcane is the most superior feedstock for bioethanol production because of very high sugar content (Xavier, 2007). Due to climatic restrictions sugar cane cannot be a potential feedstock for bioethanol production in the UK.

Most likely sugar beet is the main sugar feedstock for bioethanol production in the UK. A study conducted to assess the viability of bioethanol production from sugar beet and wheat in the EU suggests that sugar beet is a better feedstock than wheat due to higher ethanol yield per hectare and high energy efficiency of sugar beet ethanol (Anon, 2006). Containing low dry matter content and only 16% sugar, sugar beet is a bulky crop for transportation and storage (Smith *et al*., 2006). Moreover the storage condition should be kept below 10°C in order to avoid loss of sugar through respiration (Smith *et al*., 2006). The other problem of sugar beet is that the bioethanol production is likely to be seasonal being confined in four months in the autumn/winter period (Turley *et al*., 2004). The British Sugar Biofuel Plant at Wissington produces bioethanol using the surplus production of sugar beet from Norfolk.

2.2.2.2 Starch feedstock

Cereals synthesise a high amount of starch and store it in the grain. Although cereal grains are known for their food and feed value, the projected high demand of renewable fuels requires use of such starch-rich grains as a feedstock for bioethanol production. One of the good features of cereal grains is that they can be stored for a relatively long

period of time because of their high dry matter content. Cereals are relatively short rotation annual crops and thus allow flexible production depending on demand (Nonhebel, 2002). In addition to these factors the fact that ability to use existing agricultural machinery and technology of cereal production, would make them easy to use as feedstock for biofuel production (Jørgensen *et al.*, 2007).

Other than cereals, tuber crops such as potato and cassava which have relatively high starch concentration can also be used as feedstock for bioethanol production. Potatoes have a very low dry matter content (75% water) and only 12-21% starch (Senn and Pieper, 2000). Potatoes, due to their bulky nature, are expensive to transport and store. In order to use potatoes for year round production long term storage is unavoidable but this is associated with degradation of the starch (Smith *et al*., 2006). Six months storage of potatoes could cause 8% loss in starch and the loss could be up to 16% with 8 months storage (Senn and Pieper, 2000). Some countries such as Finland have considered production of bioethanol from waste potatoes (Liimatainen *et al*., 2005)

Many factors, mainly climatic and economic factors determine the choice of feedstock at any given location. Maize is the major feedstock for bioethanol production in the United States with very little wheat and sorghum (Wheals *et al*., 1999). Brazil uses sugar cane for bioethanol production (Xavier, 2007). Germany and Poland use rye for bioethanol production extensively whereas a significant amount of triticale is being used in Sweden (Senn and Pieper, 2000). Wheat is also considered as an energy crop and some European countries are using wheat for bioethanol production (Poitrat, 1999)

According to Turley *et al*. (2004) potato and wheat are the two main starch bearing crops which can be used for bioethanol production in the UK. Although both crops give similar ethanol yield per unit area (Anon, 2006), wheat is a preferred feedstock for its cost and long term storability (Turley *et al*., 2004). Apart from the British Sugar bioethanol plant at

Wissington, all the planned UK bioethanol plants use wheat as a feedstock for bioethanol production (Smith *et al*., 2006).

According to the Department for Environment Food and Rural Affairs (Defra) wheat covered about 42.2% of the total arable land of the UK in 2006. The UK projected petroleum demand by the year 2010 is about 25 million tonnes (Anon, 2005). Assuming equal share of bioethanol and biodiesel in order to fulfil Renewable Transport Fuel Obligation (RTFO) 5% demand, 1.25 million tonnes of bioethanol will be required in 2010. One tonne of wheat yields about 0.29 tonnes of bioethanol (Smith *et al*., 2006). From the year of 2010 onwards, in order to realize the 5% RTFO demand, approximately 4 million tonnes of wheat will be required annually. Currently the UK has about 2 million tonnes of wheat surplus production annually (HGCA-AHDB, 2007b) which will cover most of the demand for biofuels but some imports of wheat could be unavoidable to fulfil the total demand.

2.2.2.3 Cellulose feedstocks

Cellulose is the most ubiquitous and major product of photosynthesis but it is poorly utilized by human beings as a food (Lewis, 1983). Therefore cellulose is also one of the potential sources of sugar for ethanol production (MalÇa and Freire, 2006). The most likely sources of cellulose for commercial-scale production of bioethanol are maize stover and straw from cereals such as wheat and rice with an annual potential of 200 million dry tonnes of feedstock (Hettenhaus, 2006). Biofuels produced from such biomass are generally referred as second generation biofuels and have an advantage of using the whole crop as opposed to first generation biofuels which can be produced from only a certain part of the crop eg. cereal grains (Peters and Thielmann, 2008).

The ability to produce bioethanol from cellulosic materials is expected to boost biofuel production. The innovation of this technology diversifies the feedstocks to be used; these

include non-food crops and agricultural, municipal and forestry wastes (Worldwatch Institute, 2007) and expected to reduce the conflict with food production.

2.3 Opportunity and risks of biofuels

2.3.1 Renewable Resource

The report of World Resources Institute (2005) showed that fossil fuel, which covered about 80% of global energy usage by 2001, is declining at an alarming rate. With the current rate of consumption, all known petroleum reserves can be depleted in less than 50 years (Sheehan, *et al.,* 1998). Biofuels are renewable and the best option to preserve our non-renewable resources. Distillers Dried Grains with Solubles (DDGS), the co-product of bioethanol production can also be used as a renewable source of energy.

2.3.2 Environmental consequence

According to HM Government (2006), climate change is becoming a serious problem and mitigation is necessary to avoid the worst social, economic and environmental consequences. Combustion of fossil fuels emits CO_2 to the atmosphere. The UK transport sector is responsible for about 30% of the UK's CO_2 emissions (Hammond *et al*., 2008). As CO_2 is a greenhouse gas it is a threat to the climate. Blending biofuels not only reduces CO_2 but also other pollutants such as sulphur particulates, hydrocarbons and carbon monoxide (Mastny, 2006; Mol, 2007).

One of the great benefits of biofuels is that they are an environmentally sustainable alternative to fossil fuels which are known to cause a great burden on the environment (Mastny, 2006). The CO_2 released by biofuels was fixed by photosynthesis during growth of the feedstock, thus biofuels are considered to be climate neutral (Reijnders and Huijbregts, 2007). Biofuels have the potential to significantly reduce greenhouse gas emissions. In addition to carbon emission during fuel use, other factors such as carbon stock and nitrous oxide emissions from soils can be important when considering the overall greenhouse gas effects. The production process of the feedstock has a great

impact on greenhouse gas saving (Gover *et al.,* 1996). Many authors have studied life cycle analysis of biofuel production. According to most of these authors biofuels can significantly reduce greenhouse gas emissions relative to fossil fuels but the range of benefit would be dependent on how the feedstock is grown (Elsayed *et al*., 2003; Punter *et al*., 2004; Billins *et al*., 2005; Farrell *et al*., 2006). Unlike the above authors, Patzek (2004) concluded that the benefit of biofuels in greenhouse gas saving is not significant. Searchinger *et al.,* (2008) also argue that if land use change is taken into account, bioethanol could increase greenhouse gas emission instead of saving. But studies conducted in the UK on biofuel production from wheat grain showed that, CO_2 emission can be reduced significantly (Punter *et al*., 2004; Woods *et al*., 2005). Growing wheat for bioethanol could be more environmental friendly as biofuel wheat requires less nitrogen than conventional production (Turley *et al*., 2004). However, the Gallagher review warns that greenhouse gas saving from biofuels can only be achieved if land use change is avoided (Gallagher, 2008).

There has been a considerable debate about the energy balance of biofuels, that is the amount of energy used (particularly fossil fuel energy) for the production of feedstock and processing and the amount of energy gained from biofuels (Mastny, 2006). Chambers *et al*. (1979) concluded that the net energy balance is negative. Since then there was much improvement to the technology of production which increased the energy efficiency and therefore all current biofuels have a positive energy balance (MalÇa and Freire, 2006).

In order to realize and maximize the environmental benefits of biofuels caution must be taken during production of feedstock. Production of biofuels on ecologically fragile lands could accelerate soil degradation and depletion of aquifers (Mastny, 2006). In addition, expansion of biofuel crops at the expense of the world's forest is a danger to the environment (Worldwatch Institute, 2007). The expansion of soybean farms in the

Amazon Basin and palm oil plantations in South East Asia, are examples of this (Worldwatch Institute, 2007).

Development of technologies, for the use of grasses and trees for biofuel production could also facilitate environmental protection as such perennial crops could protect land which has been degraded by grazing (Mastny, 2006).

2.3.3 Energy security

One of the main reasons to push for biofuels is for energy security. The fossil fuel resource is centralized in some geographical locations and the global economy is highly vulnerable to oil price which is controlled by only a few countries (Mol, 2007; Nass *et al*., 2007). For countries which do not produce their own oil, it is undesirable to rely on foreign countries for their energy needs especially – but not only - in reference to national security (Anon, 2006). Seventy five percent of the EU's oil supply was from imports in 2000 and the import is expected to rise to 85% by 2020 and the transport sector is 98% dependent on petroleum (EPC, 2003). Due to the increasing mobility of people and goods, it is reported that the need for transport fuel is increasing (MalÇa and Freire, 2006). Home produced biofuels are expected to reduce reliance on imported petroleum.

2.3.4 New future for Rural Communities

The other interesting future of biofuels is that, biofuels are compatible with the interests of farmers and rural development (Anon, 2006). Biofuels add value for crops, increase farm income and strengthen the rural economy (FAO, 2005; Mastny, 2006). Many of the major oil importing countries have a crisis in their rural areas caused by over-production of agricultural commodities, low prices, land being taken out of production (set aside) and consequently low income for the farmers (Mol, 2007). The same is true for the UK which has surplus wheat production and set aside lands. The decline in agricultural prices which lasted for decades has been reversed due to the growing use of biofuels (Gallagher, 2008). Biofuels have good potential to create rural jobs thus expected to

bring promising future for the European Union as well as the world (Anon, 2006). The capacity of growing energy crops besides fibre and food crops could change agriculture much better than any development since the green revolution (Mastny, 2006).

2.3.5 Food prices

The increase in production and globalization of biofuels brought a sharp debate on the impact of biofuels on the two most notable vulnerable groups: the poor in developing countries and small farmers. The strongest criticism biofuel is receiving is the potential impact of large scale biofuel production on food supplies, food prices and food scarcity (Mol, 2007). The increasing demand for biofuels is pushing the raw materials price too high. The inconvenience is that any crop used for energy is taken out of the food chain, in a world where many people are starving. Moreover biofuel production requires a vast area of land which could lead to competition with food production (Peters and Thielmann, 2008). The UN agency in charge of alleviating world hunger complained that food prices have risen by 40% since June 2007, weakening the capacity of the organization to respond to world hunger. However, others argue that seeking food security by pushing food prices ever lower could hurt more people than it helps (Worldwatch Institute, 2007).

2.4 Feedstock quality

Feedstock quality is a very important factor in the bioethanol industry as it affects not only the amount of ethanol produced but also the processing efficiency and the quality and quantity of co-products (Smith *et al*., 2006). The amount of ethanol attainable from a given amount of wheat grain depends on the amount of starch and fermentable sugar available in the grain and its conversion efficiency. Starch accounts for about two thirds of the grain. Other components of the grain include NSP, nitrogen, lipids, minerals and ash (Gooding and Davis, 1997).

2.4.1 Starch

In order to use wheat efficiently for biofuel production, a good understanding of the structure and properties of wheat starch and its impact on processing of the grain into ethanol is vital. The wheat reserve, starch is primarily stored in starchy endosperm being embedded in a protein matrix (Bringhurst *et al*., 2003). The wheat endosperm starch has bimodal granule size composition. The larger sized granules are named as A granules while the smaller size granules are called B granules (Morrison, 1989).

The temperature required to efficiently process the conversion of wheat to ethanol is determined by gelatinization temperature. The size and configuration of starch granules affect gelatinization temperature (Morrison, 1989). The starch in small granules is compact and thus requires a higher gelatinization temperature as compared to the large granules where the starch is relatively easily accessible (Bringhurst *et al*., 2003). According to Bathgate *et al*. (1973) the small granules cannot be gelatinized at normal mashing temperature (63 -75°C). In order to utilize the raw material efficiently, the small granules have to be gelatinized.

The major chemical components of the wheat starch granules are two polysaccharides, amylose and amylopectin which are formed by glucose polymerization (Graybosch, 1998). Amylose is often described as a linear polymer composed of α – (1, 4) - glucan, though recent reports showed that wheat amylose has some branches (Cura, *et al*., 1995). Amylose accounts for 15-37% of the total starch (Bringhurst, *et al*., 2003). So far all UK wheat varieties have a similar amylose content of about 28% (Smith, *et al*., 2006). Amylopectin is a much larger molecule and highly branched. The structure is formed from a large number of relatively short α-(1,4) linked chains linked to α-(1-6) linked branches which forms the bulk of the starch (Thomas and Atwell, 1999). Although both amylose and amylopectin are formed from D glucopyranose molecules, the differences in the structure of the two polymers cause significant difference in the functional properties of the polymers and starch (Thomas and Atwel, 1999). One of the functional

differences is in gel formation, amylose has high potential to form a gel after the starch is cooked while gel formation in amylopectin is either delayed or prevented. Likewise retrogradation (the process of recrystallization or gel formation of gelatinized starch) of the starch after cooking happens rapidly in amylose whereas it is very slow in amylopectin (Gibson *et al*., 1997).

The structural and chemical configuration of these two polymers contributes to the property of enzyme digestibility of the starch. Amylase enzymes, ‘’starch splitting enzymes’’ are used to hydrolyse starch polymers (Thomas and Atwel, 1999). Amylases such as α- and β-amylase cannot breakdown the (1-6) glycoside links, therefore amylopectin will be converted to α- and β-limit dextrins and fermentable sugars by these enzymes. Later α (1-6) links in α- and β-limit dextrins breakdown by limit dextrinase (Bringhurst *et al*., 2003).

Starch with high amylose content has a higher viscosity at a given temperature. Such starch needs very high energy to gelatinize and disperse into solution. The gelatinization temperature of high amylose starch is above 100°C which is by far higher than the gelatinization temperature of standard wheat starches, (60-70°C) (Smith, *et al*., 2006). Therefore high amylose starch could be uneconomic for biofuel production due to its high energy requirement. On the other hand high amylopectin starch (waxy starch) tends to have a higher swelling power at a certain temperature than other standard wheat starch. It could also be readily mixed and disperse into the solution. It also has no problem of retrograding during cooling as compared to high amylose starch (Hayakawa *et al*., 1997). Thus high amylopectin starch appears good for biofuel production.

Soft wheat varieties tend to have slightly higher amounts of amylose as compared to hard wheat varieties (Raeker *et al*., 1998; Capouchova and Maresova, 2003). Wheats with reduced amylose content usually referred as partial waxy wheat, whereas wheat varieties which are almost free from amylose are called waxy wheat (Graybosch, 1998).

These naturally occurring waxy mutants have the same amount of amylopectin as the wild type but lack in amylose (Hayakawa *et al*., 1997). The waxy wheat types are unsuitable for cultivation and are narrowly adapted (Yasui *et al*., 1997). US, Japan and Australia are growing partial waxy wheat whereas in the UK, some breeders are reported to be developing such waxy wheat (Smith *et al*., 2006).

Starch granules also contain proteins, lipids, moisture and ash (minerals and salts) in very small quantities (Thomas and Atwell, 1999) and this is known to have significant effect on starch processing (Bringhurst *et al*., 2003). This is because the amount of these residues is directly related to the process required to isolate the starch from the residues (Thomas and Atwell, 1999). These minor residues of protein and lipid also affect the gelatinization of the starch (Thomas and Atwell, 1999). Palmer, (1989) suggested that the amount of lipids in starch can decrease the amylolytic breakdown of the starch. Lipid in the granule is also an important factor determining propensity of starch to retrograde after cooking (Swinkells, 1985). Presence of a specific starch granule protein is also known to have an association with hardness of wheat grain endosperm (Greenwell and Schofield, 1986).

The relative distribution of large and small granules (Bringhurst *et al*., 2003) and the proportion of amylose and amylopectin (Gibson *et al*., 1997; Bringhurst *et al*., 2003; Thomas and Atwel, 1999) in starch determine its physical and chemical properties and its suitability for particular end-uses. Therefore these parameters are important in determining the processing efficiency and ethanol yield of wheat grain. Although these characteristics of the starch are important for efficient ethanol production they were not measured in this project because Smith *et al.* (2006) reported that all UK wheats currently have similar amylose content. Brosnan *et al.* (1998) also indicted that for UK wheats, the total amount of A and B granules are more important than the relative amount of the either granules.

2.4.2 Starch measurement

Starch is quantitatively the most important component of cereal grains due to its use in many food and industrial products. In biofuel production from wheat grain, it is the major component of the grain that provides fermentable sugar, thus, a premium could be paid for high grain starch concentration in the biofuel market (Kindred *et al*., 2008). Bioethanol industries in Sweden pay a premium for high starch concentration. Starch can be analysed in several ways including infrared techniques and various enzyme colorimetry methods (Bernetti *et al*., 1990; Rose *et al*., 1991; Chatel *et al*., 1997). Methods of analysis usually affect the outcome of assay accuracy, reproducibility, susceptibility to interference, sample size, complexity of analysis, cost and speed of the process (Grant *et al*., 2003). Moreover starch is terribly difficult to measure, different methods giving substantially different results for the same sample (Smith *et al*., 2006). Thus users must be aware of these characteristics and prioritize the different methods when considering quantitative starch analysis.

Measurement of starch involves two major steps: dissolution and hydrolysis of starch into sugar and measurement of the sugar concentration (Chow and Landhäusser, 2004). The first step is gelatinization and dissolution of the starch granules which can be achieved either thermally or chemically or by both (McCleary *et al*., 2006). Most of the starch determination methods are broadly categorized into acid hydrolysis and enzyme procedures (Anon, 1987).

Starch can be solubilised and hydrolysed by dilute mineral acids. Among these, perchloric acid hydrolysis (MacRae *et al*., 1974; Rose *et al*., 1991) is known to be problematic because of instability of perchloric acid (Chow and Landhäusser, 2004); and sulphuric acid hydrolysis is regarded as the simplest and fastest method (Chow and Landhäusser, 2004). Acid hydrolysis methods are relatively straightforward given standardization of temperature and time of hydrolysis (Smith *et al*., 2006). Acid hydrolysis

also facilitates complete release of the starch granules from the protein matrix by breaking down the endosperm tissue (Smith *et al*., 2006).

Acid hydrolysis of starch has had widespread use in the past. Nowadays it is largely replaced by enzymic processes. This is mainly because of its limitations such as: the need for corrosion resistant materials, high colour and salt ash content (after neutralisation), requirement of more energy for heating, relatively hard to control the reaction (Martin, 2004). There is also a risk of loss of sugar during hydrolysis (ca 10-20% depending upon time and concentration of the acid) (Smith *et al*., 2006). A comparison study between acid hydrolysis and enzymatic hydrolysis of starch by Chow and Landhäusser (2004) revealed that acid hydrolysis results in an overestimation of the starch content because of breakdown of structural carbohydrates. Moreover acid hydrolysis is more applicable to pure starch than cereal flours and it is less suitable for samples containing relatively low starch (McCleary *et al*., 1994).

Currently an enzymic procedure is widely used due to the availability of high purity enzymes in commercial starch analysis kits. Enzymic procedures vary in the pre-treatment steps (Karkalis, 1985). A very common enzymic procedure, which is widely used for starch analysis, is the Association of Analytical Chemists (AOAC) Method No. 996.11 (Megazyme ®). This method is reliable, reproducible and gives quantitative measurement of starch in samples including those containing high resistant starch (McCleary *et al*., 1997). The advantage of this procedure is, there is no loss of sugar during hydrolysis as opposed to an acid hydrolysis method (Smith *et al*., 2006) and the enzymes are precisely selective to starch avoiding the interference of NSP such as cellulose and hemicelluloses (Chow and Landhäusser, 2004). A potential source of error in enzyme procedures is insufficient gelatinization and dispersion which could lead to underestimation of the exact starch content (Smith *et al*., 2006). The disadvantages of this procedure are, it is laborious, expensive and it works well only with an experienced person.

Once the starch is converted into glucose either by acid or enzymic procedure the next step is to quantify the amount of glucose produced. The analytical procedures at this step include polarimetry (Kindred *et al*., 2008); colorimetry (McCleary *et al*., 1997; Grant *et al*., 2003; McCleary *et al*., 2006); gas-liquid chromatography (GLC) (Mitchell *et al*., 1982); gas chromatography (GC) (Carlsson *et al*., 1992) and high performance liquid chromatography (HPLC) (Choi and Mathews, 1996; Caseterline *et al*., 1999; Orak, 2006).

Polarimetric methods are used to measure the glucose content after the starch has been solubilised and hydrolysed into glucose by boiling it with HCl (Smith *et al*., 2006). In this method substances which could interfere with quantification will be removed by filtration and the concentration of glucose is determined by measuring the angle of polarization of optical rotation (Senn and Pieper, 2000). Although this method is inexpensive and simple (Smith *et al*., 2006), it has limitations in that the free sugars available in the wheat flour (Lineback and Rasper, 1998) could interfere with the result. The method also has potential error in measuring starch from samples which have high non-starch polysaccharides (NSP), because the NSP could breakdown into simple sugars during the acid hydrolysis and these simple sugars will interfere with quantification of glucose (Smith *et al*., 2006).

Glucose produced by enzyme hydrolysis can be quantified colorimetrically. A typical example of this method is AOAC 996.11. In this procedure glucose is measured spectrophotometrically at 510 nm by reaction with a glucose oxidase-peroxidase (GOPOD) reagent.

The glucose can also be measured by employing different instrumental procedures such as GC, GLC and HPLC but GLC requires lengthy derivatization steps making the technique relatively expensive and complex whereas HPLC requires a very specialized HPLC system with skilled personnel (Smith *et al*., 2006). Therefore these procedures are

less applicable when measuring the sugar content is the only interest (Chow and Landhäusser, 2004).

2.4.3 Starch conversion efficiency

The primary goal of ethanol industry is to produce as much ethanol as possible per unit of the raw material processed. Therefore the efficiency of conversion of starch into ethanol is the major determinant of distillery efficiency (Bringhurst *et al*., 2003). Quality of feedstock determines the amount of starch concentration as well as its convertibility into ethanol. The extent of conversion of starch into ethanol is largely determined by the quality of the starch itself (proportion of A and B granules and ratio of amylose to amylopectin). Other grain components such as NSP may also inhibit the rate of conversion of starch into ethanol (Smith *et al*., 2006). The fineness of milling of the wheat flour can also affect the accessibility of starch to digestion by the enzyme. Kelsall and Lynos (2003) reported that ethanol yield can be 5-10% higher in a finely ground mill than a coarser ground mill. However, Ensus, the commercial bioethanol company, cracks the wheat grain into four pieces instead of fine milling thus high starch extraction can be achieved with high heating and enzyme efficiency. Starch can also be degraded *in vitro* by the action of endogenous α amylases. Samples with high endogenous α amylase or low Hagberg falling number may give less ethanol yield due to loss of starch (Smith *et al*., 2006). The overall processing efficiency of the bioethanol industry is expected to be better than that of the potable alcohol industry due to the possibility of using chemicals and enzymes in the bioethanol industry.

Viscosity is one of the major causes of distillery inefficiency. It reduces the throughput and increases the energy requirement (Ingledew *et al*., 1999). NSP is the cause of the viscosity of the slurry (Weightman *et al*., 2008). There are enzymes which solubilise non-starch polysaccharides therefore the viscosity problem can be solved with cost in the bioethanol industry (Smith *et al*., 2006). According to Smith *et al*. (2006) samples which

give high ethanol yield generally have low viscosity. Therefore choosing high ethanol yielding samples perhaps can address the viscosity problem.

2.5 Factors causing feedstock quality variability

2.5.1 Varieties

Varieties vary in their suitability for a particular end use. Experience of the potable alcohol industry indicated that varieties show variability in ethanol yield and ease of processing (Bringhurst *et al*., 2003). For instance, the variety Riband has been the most favoured variety for distilling due to its higher ethanol yield per tonne and ease of processing (Brosnan, 2001). Nowadays varieties such as Glasgow are looking better because of higher ethanol yield per ha than Riband (HGCA-AHDB, 2007a). However, the reason behind variation in ethanol yielding potential of varieties is not well understood (Riffkin *et al*., 1990). Until recently there has not been any breeding effort specific to ethanol yield perhaps this is mainly due to limited knowhow of the genetic factors contributing to ethanol yield and lack of screening tests applicable for a large population at the early stage of a breeding programme (Swanston *et al.,* 2005). Currently there are only a few varieties which are readily acceptable by the potable alcohol industry (Swanston *et al*., 2005). The potable alcohol industry avoids use of hard wheat varieties and varieties with 1BL/1RS rye translocation but these varieties could also be good for bioethanol industry due to the use of chemicals and enzymes (Smith *et al*., 2006).

2.5.2 Environment

Variation in feedstock quality due to environment refers to variation caused by site/location and season variability. The environment in the UK is generally good for wheat growth as the UK wheat yield is among the highest in the world (Sylvester-Bradley *et al*., 2005). Moreover wheat from northern UK usually has a higher grain starch concentration (HGCA-AHDB, 2007a). Year is also known to cause variability in grain ethanol yield. A field study conducted for three years showed that ethanol yield has been

generally higher in year 2005 than 2003 and 2004 (Smith *et al*., 2006). This can be attributed to the weather difference between years. The variation between sites is generally more than between varieties (Smith *et al*., 2006). Variation between sites could be due to soil type, management type, disease and weather.

2.5.3 Management

The most influential management factor in growing wheat for ethanol yield is the amount and time of application of nitrogen fertilizer which is known to affect the ethanol yield through grain protein concentration (Swanston *et al*., 2005). It is likely that ethanol yield will be favoured from reduced and early application of nitrogen fertilizer (HGCA-AHDB, 2007a). Kindred *et al*. (2008) reported that applied N affects grain nitrogen concentration and therefore ethanol yield regardless of the varieties. But the study had been conducted on only two varieties therefore further research with a wider range of varieties is required to understand the relative response of varieties to applied N and the rate of change of grain nitrogen concentration and ethanol yield. All good management practices should remain as it has been for good quality feed wheat. Any poor management practices could lower the ethanol yield as any stresses which reduce photosynthesis reduce the starch concentration of the grain.

2.6 Predicting ethanol yield

Predicting the potential ethanol yield of the grain not only improves the productivity of the industry but also increases energy saving and greenhouse gas reduction. Some authors have studied some grain parameters with the aim of predicting ethanol yield as reviewed below. There is a need for a much fuller investigation of variation in ethanol yield in order to develop a robust prediction tool for ethanol processing yield.

2.6.1 Amount of Starch and free sugars

The most logical way of increasing ethanol yield is by improving the starch and sugar concentration of the grain. But according to Riffkin *et al*. (1990) high ethanol yield is not

necessarily associated with high starch concentration. The quality of the starch such as the amylose to amylopectin ratio and the ratio of small to large granules are also important (Riffkin *et al*., 1990; Brosnan *et al*., 1998).

Together with the starch, the free sugars (Lineback and Rasper, 1998) and the NSP available in the grain could also release some sugar during mashing and fermentation and improve the ethanol yield (Kindred *et al*., 2008). However, until the start of this PhD project there was no study concerning the effect of NSP and free sugars variation in ethanol processing yield.

Although starch concentration is not significantly correlated with ethanol yield in the study of Swanston *et al*. (2007); starch concentration showed a significant positive relationship with ethanol yield, explaining 37% of the variation in ethanol yield in the study of Kindred *et al*. (2008). This controversy could arise from the methodology used for starch analysis; megazyme total starch assay kit (Swanston *et al*., 2007) compared with acid hydrolysis (Kindred *et al*., 2008) and could also be because of the different varieties used in the two studies as starch concentration is significantly affected by genotype. The other reason could be because of the difference in design of the two experiments; replicated field experiment in case of Kindred *et al.* (2006) and bulk samples collected from each sites in the other one.

2.6.2 Grain N/protein

There is a strong negative relationship between grain nitrogen and starch concentration (Kindred *et al*., 2008). Therefore measuring grain nitrogen could be an indirect indication of potential ethanol yield (Dupont and Altenbach, 2003). Grain nitrogen can be measured easily, accurately and precisely by using Kjeldahl or the Dumas method (Smith *et al*., 2006). Grain protein concentration can be calculated from grain nitrogen concentration using a conversion factor of 5.7 (Jones, 1931).

Ethanol yield and grain nitrogen concentration are highly negatively correlated (Kindred *et al*., 2008; Swanston *et al*., 2005; 2007 Taylor and Roscrow, 1990). Grain nitrogen concentration explained almost 70% of the variation in ethanol yield (r^2=0.68) but most of the variation arose from site differences (Swanston *et al*., 2007). Whereas in the study of Kindred *et al*. (2008) grain nitrogen concentration explained only 64% of the variation in ethanol yield when a common regression equation was used for both varieties and fitting separate lines improved the percentage to 71%.

Swanston *et al*. (2005) indicated that the relationship between ethanol yield and grain nitrogen changes at lower concentrations of grain protein and differ between varieties. Moreover there is a variation in ethanol yield between samples with the same grain nitrogen concentration, this indicates that grain nitrogen alone is not effective to predict ethanol yield between varieties at given sites (Swanston *et al*., 2007).

2.6.3 Hot water extract

Hot water extract values can be determined after fine grind milling by scaling down the Institute of Brewing (IOB) recommended method of analysis (1997). Hot water extract in conjunction with grain nitrogen give a rapid means of predicting ethanol yield as the measured ethanol yield showed good association with predicted ethanol yield (Swanston *et al*., 2005). In the study of Swanston *et al*. (2007) hot water extract was highly affected by genotype and environment and its potential to predict ethanol yield was poor for the best variety Glasgow and the poorest variety Deben. Moreover as this method requires laboratory work it may not be appropriate to use at the refinery intake.

2.6.4 Specific weight

Specific weight is a measure of the bulk density of the grain, i.e. the weight of grain that can be contained in a unit volume packed in a standard way. Specific weight is measured by a specific cylinder called chondrometer. Distillers use grain specific weight as one quality criterion for distilling wheat. Specific weight has an association with extracted flour

yield (Marshall *et al*., 1986) and has a high influence on grain transport and storage costs (Brooker and Bakke, 1992; Gooding and Davies, 1997) but the relationship between ethanol yield and specific weight is very weak (Taylor and Roscrow, 1990; Taylor *et al*., 1993). However it is possible that samples with very low specific weight (< 70 kg/hl) could result in poor ethanol yield (Smith *et al*., 2006).

Poor specific weight can arise from two different conditions. Specific weight is highly determined by the weather conditions during both grain growth and grain ripening (Atkinson *et al*., 2005). Solar radiation is crucial for good grain growth. Often there is a positive relationship between well filled grain and high specific weight (Bayles, 1977). Lack of solar radiation during the grain filling period could result in poorly filled grain and low specific weight. Such seed possibly has low starch and hence low ethanol yield. The other mechanism is specific weight can be reduced due to weathering of the grain. Rain during grain ripening and grain growth could reduce specific weight (Bracken and Bailey, 1928; Swanson, 1943). This is mainly because wetting and subsequent drying of the grain causes weathering of the grain leading to increase in the volume of the grain without significant loss in weight (Atkinson *et al*., 2005). Such losses are less likely to affect grain starch content and consequently ethanol yield. Therefore further work is required to clarify the role of specific weight in ethanol yield by considering different growth conditions.

2.6.5 Thousand grain weight (TGW)

TGW could be a good predictor of ethanol yield. There is a significant positive relationship between ethanol yield and TGW (Swanston *et al*., 2005; 2007; Taylor and Roscrow, 1990). But this trait tends to be variety specific as some varieties could combine high ethanol yield with low TGW eg. Claire (Swanston *et al*., 2005; 2007). According to Kindred *et al*. (2008) TGW is not a predictor of ethanol yield in the varieties considered in that study, confirming variety specificity of the trait.

2.6.6 Grain size and shape

Plumpness of the grain can be expressed by length: width ratio (L:W) of the grain and this showed a relationship with ethanol yield (Taylor and Roscrow, 1990; Swanston *et al*., 2005, Swanston *et al*., 2007). There is a significant negative relationship between ethanol yield and L:W ratio (Swanston *et al*., 2007). But in the study of Kindred *et al*. (2008) L:W ratio had no close association with ethanol yield.

2.6.7 Extract turbidity

Endosperm texture and ease of access to the starch granules could have a possible influence on ethanol yield (Swanston *et al*., 2007). According to the endosperm texture grains can be categorized into; mealy, known with loose packing of protein and starch granules and steely endosperm, compact packing of the starch granules (Chandra *et al*., 1999). Koliatsou and Palmer (2003) reported that starch can be easily released from a mealy endosperm than steely in barley varieties. This is due to the limitation of steely endosperm for water uptake and thus passage of hydrolyzing enzymes (Chandra *et al*., 1999). Mealy and steely grains can be separated visually but the method is subjective and requires dehusking the grain (Koliastsou and Palmer, 2003). Koliastsou and Palmer (2003) measured turbidity of some barley varieties from a suspension of finely milled flour in ethanol by turbidometer and found that mealy varieties had high turbidity and release starch more readily than steely varieties.

Swanston *et al*. (2005, 2007) studied whether turbidity can be used as a predictor of ethanol yield from wheat grain. Turbidity was not significantly correlated with ethanol yield (Swanston *et al*., 2007). The variety Consort showed high turbidity with high ethanol yield but turbidity was not a good predictor of ethanol yield in varieties such as Wizard (Swanston *et al*., 2005).

2.6.8 Grain hardness

Potable alcohol distillers prefer soft wheat varieties (Bringhurst *et al*., 2003; Kindred *et al*., 2008; Taylor and Roscrow, 1990). This is mainly because hard wheat varieties have been associated with higher nitrogen concentration, and some processing problems such as accessibility of the starch for hydrolysis (Smith *et al*., 2006). However, Taylor *et al*. (1993) showed that grain hardness has no effect on ethanol processing yield. Moreover the hard wheat variety Option showed more conversion of starch into ethanol than the soft wheat variety Riband (Kindred *et al*., 2008). Hard wheat varieties may take more energy to mill, but the effect on the overall cost of production is perhaps insignificant (Smith *et al*., 2006).

Although hard wheat varieties are not chosen by grain distillers the biofuel industry may use the hard wheat varieties. However, there is not enough information on whether hard wheat varieties could be potential varieties for bioethanol production. The comparison study of Option (hard endosperm) and Riband (soft endosperm) varieties in ethanol yield revealed that there is no significant difference in ethanol yield per ha between the two varieties (Kindred *et al*., 2008). But the study considered only one hard wheat variety therefore a wide range of hard wheat varieties should be tested to confirm their potential.

2.6.9 Grain vitreosity

Not only the grain texture but also the other physical characteristics of the grain such as vitreosity (Dexter and Edwards, 1998) may affect ethanol yield as they affect the grains processing characteristics especially the rate of starch release from the endosperm matrix (Kindred *et al*., 2008). Vitreosity is glassy characteristics of the grain. Grain vitreosity can be measured quickly and non-destructively (Wheaton and Muller, 2000). This may make it a good criterion for grain processors and/or breeders interested in high ethanol yield (Kindred *et al*., 2008).

Grain vitreosity is genetically linked with the 1BL/1RS translocation from rye (Weightman *et al*., 2008). Kindred *et al*. (2008) studied the effect of vitreosity on ethanol yield but neither of the two varieties studied has 1BL/1RS translocation and did not show much difference. Varieties with the 1BL/1RS translocation are known to have another processing problem, high viscosity, due to this, the grain distillers classed such varieties undesirable like hard wheat varieties (HGCA-AHDB, 2007a). However, a variety such as Ambrosia has the 1BL/1RS translocation but not high residue viscosity whereas a variety such as Kipling (with 1BL/1RS) gives high viscosity and poor ethanol yield (HGCA-AHDB, 2007a). Varieties with 1BL/1RS translocation and hard wheat varieties might be desirable for biofuel but have to be tested using ethanol production method typical for biofuel industry which is with the aid of commercial enzymes.

2.6.10 Combining different traits to predict ethanol yield

Combining hot water extract value with predicted fermentability (fermentability can be measured from the hot water extract as described by Dolan *et al*. (1981)) and percent nitrogen concentration gave a promising prediction of ethanol yield. The predicted ethanol yield with this equation had significant correlation with actual ethanol yield (r = 0.78) (Swanston *et al*., 2005). Application of this equation to predict ethanol yield in different samples showed a good relationship with actual ethanol yield (R^2 = 0.704) but the predicted values were invariably low (Swanston *et al*., 2007). Swanston *et al*. (2007) developed another equation with hot water extract and nitrogen concentration to predict ethanol yield and the regression explained 74% of the variation but tended to overestimate Deben and underestimate Glasgow.

According to Swanston *et al*. (2007) ethanol yield can be predicted from three parameters; grain N concentration, TGW and L:W ratio. This method explained 75% of the variation but it overestimated some varieties such as Deben which has large grain but low ethanol yield and underestimated varieties such as Glasgow which combine small grain with high ethanol yield. Removing these two varieties improved the

relationship between the predicted and measured ethanol yield by explaining 84% of the variation (Swanston *et al*., 2007). But this equation explained only 66.3% of the variation in ethanol yield in other samples (Kindred *et al*., 2008).

In the study of Kindred *et al*. (2008) using starch concentration alone as predictor explained only 37% of the variation whereas nitrogen concentration alone explained 71.4 (applied to each variety) but combining starch and nitrogen explained only 69.7% of the variation. However, in contrast to Swanston *et al*. (2007) including TGW and L:W ratio did not show any improvement in predicting ethanol yield.

Although combining different traits showed much improvement in predicting ethanol yield, some of the reasons for variation in ethanol yield remained unexplained.

3. Aim and Objectives of the project

As discussed in the literature review, there are few studies conducted to understand the relationships between wheat grain traits and ethanol yield. These studies showed some important relationships. However, there are some controversies between their results. Moreover, these studies are either focused on few grain traits or have limitation in sample coverage. For instance, Swanston *et al.* (2005) studied the relationships between a few grain quality parameters and ethanol yield in four varieties and their component mixtures; focusing on the impact of variety mixture on grain quality and ethanol yield. Swanston *et al.* (2007) investigated the relationship between grain quality parameters and ethanol yield on a few varieties grown for one year at four sites. Kindred *et al.* (2008) also studied the relationship between grain quality and ethanol yield between two varieties (hard and soft wheat varieties) focusing on the effect of nitrogen fertiliser rate and suitability of hard wheat variety for bioethanol production. There are a few more studies conducted earlier with the same aim for the potable alcohol industry (Taylor *et al.,* 1993 and Taylor and Roscrow, 1990). However, the varieties covered in these studies are out of date. None of the above studies have examined grain quality parameters as potential tests of ethanol yield over all three of the main sources of variability: varieties, sites and years. In order to develop effective test which predict potential ethanol yield of wheat grain, a wide range of samples covering several representative sites and the latest varieties grown for more than one year must be studied.

3.1 Aim

To enhance bioethanol production efficiency by using best quality feedstock

3.2 Objectives

- To examine the relationships between ethanol yield and a wide range of grain physical and chemical characteristics

- To develop a better prediction of grain ethanol yield

3.3 Null hypothesis

- There is no relationship between grain traits and ethanol yield
- It is not possible to develop a grain ethanol predicting tool based on grain traits

4. General Material and Methods

The general methodologies used throughout this project are described below. Any methods specific to one experiment are described in the respective chapter.

4.1 Moisture content

Moisture content of the samples was determined by weighing before and after oven drying for 2 hours at 130°C in accordance with the standard method of ISO R712. The moisture content data were used to adjust other traits such as starch content and TGW to a dry weight basis.

4.2 Starch concentration

The starch concentration of the grain was measured using the Megazyme (Megazyme International Ltd, Co. Wicklow, Ireland) total starch assay procedure, AOAC method 996.11 as described by McCleary *et al*. (1994). For this analysis, the grain samples were milled using a Retsch ZM 100 centrifugal mill (Retsch GmbH, Haan, Germany) fitted with a 0.5 mm screen. A buffer solution was prepared from 11.55 g of MOPS sodium salt (Sigma Aldrich, Gillingham, UK) and 900 ml of distilled water and adjusted to a pH of 7. 0.20 g of sodium azide and 0.74 g of Calcium chloride dehydrate (Sigma Aldrich, Gillingham, UK) was added to this solution. The solution was adjusted to 1 l using distilled water and stored at 4°C until needed. Approximately 100 mg of wheat flour sample (weighed accurately) in a 16 mm X 120 mm round-bottomed glass test tube was wetted by 0.2 ml of 80% (v/v) aqueous ethanol and stirred with a vortex mixer to aid dispersion. Then 3 ml of thermostable α-amylase in MOPS buffer was added and again the tube was stirred vigorously and incubated in a boiling water bath for 6 min (after the second min the tube was stirred to prevent the possibility of some of the sample expelling from the tube when the ethanol is evaporating) after the incubation, the contents of the tube was stirred vigorously until it made a homogenous mixture. Then the tube was placed in a water bath at 50°C and 4 ml sodium acetate buffer followed by 0.1 ml amyloglucosidase added and stirred and then incubated for 30 min at 50°C. The solution was transferred to a 100 ml volumetric flask. Then filled with distilled water to 100 ml and mixed well. 10 ml aliquot of the solution was centrifuged at 3000 rpm for 10 min then 0.1 ml of this solution was incubated for 20 min at 50°C with 3 ml of GOPOD reagent. Finally the

concentration of the colour developed by the sample was measured by absorbance reading at 510 nm against a reagent blank in a spectrophotometer (DU® 640, Beckman). Wheat and maize starch with known concentration were used as a standard with all the samples to ensure the methodology and effectiveness of the enzymes. The starch concentration was calculated from the absorbance reading using the formula given with the kit.

Starch% w/w = ∆A x F x 1000 x 1/1000 x 100/W x 162/180

= ∆A x F/W X90

Where:

∆A = Absorbance (reaction) read against the reagent blank

F = 100 (µg of glucose)/ absorbance for 100 µg of glucose (conversion from absorbance to µg)

1000 = volume correction (0.1 ml taken from 100 ml).

1/1000 = conversion from µg to mg

100/W = Factor to express “starch” as a percentage of flour weight.

W = the weight in milligrams (“as is” basis) of the flour analysed

162/180 = Adjustments from free glucose to anhydro glucose (as occurs in starch)

Starch % w/w (dry wt. Basis) = starch % w/w (as is) x 100/ (100 – moisture content % w/w)

Since starch measurement is complicated and requires experience, preliminary work was done to experience the method and to ensure repeatability of results. After repeated analysis with high and low starch concentration samples, the main experimental samples were analysed. Duplicate analysis was conducted on each sample and an average was

taken. When the difference between two measurements was more than 5%, a third measurement was done and the outlier was discarded. Wheat and maize starches of known concentration were used as a standard with all the samples.

4.3 Nitrogen concentration

The grain nitrogen concentration data of the samples were obtained from Scotch Whisky Research Institute (SWRI) which was determined by Near Infrared Reflectance (NIR) using an Infratec Model 1251 Food and Feed Analyser (Foss Instruments Ltd) at SWRI. Crude protein concentration was calculated as N X 5.7 (Jones, 1931).

4.4 Image analysis

A minimum of 200 grains from each sample were scanned by a flat-bed scanner (Scan Express, Mustek, Taiwan). Images of the grain were obtained as top view of the ventral side of the grain (Figure 1). Grain size parameters such as length, width, perimeter and area of the grain were measured from the scanned image using Image-Pro® Plus software version 4.5 (Media Cybernetics Inc.). Other secondary data such as length:width ratio and roundness of the grain (calculated from ratio of perimeter squared to area) were calculated from the primary data.

Figure 1 One of the scanned images of the grain used for grain size determination

4.5 Specific weight

The amount of samples available did not allow determination of specific weight by a chondrometer. Therefore, specific weight was measured by weighing a sample of grain that exactly filled a 100 ml cylinder as used by Swanston *et al.* (2005). The grain was poured into the cylinder from approximately the same distance. Specific weight data was obtained from the mean of three measurements on each sample.

4.6 Grain density

The gas pycnometer was used to measure the density of whole grain (Figure 2). This instrument operates on Archimedes principle of gas displacement to measure volume. Although other gases such as nitrogen can be used, helium was used in this experiment. Helium gives the best accuracy as it follows the gas law most precisely (Fang and Campbell, 2000). This pycnometer allows sample cups of several sizes (5, 35 and 150 cm^3). In this analysis a cup size of 35 cm^3 was used. The pycnometer measures the volume of numerous grains precisely but its accuracy does not allow it to measure volume of a single grain.

A weighed wheat grain sample which fills a 35 cm^3 cup was placed in the sample chamber and the expansion valve was closed. Then the fill valve was opened and the sample chamber was charged to a relative pressure *p1* and the pressure was recorded at this stage. *P2* was also recorded after the expansion valve was opened and the original gas in the sample chamber was released to fill both the sample chamber and the expansion chamber. Using *p1* and *p2* the volume of the sample was calculated as follows:

$$Vs = Vc - Ve/(p1/p2-1)$$

Where:

Vs = the volume of the sample

Vc = the volume of the empty, closed sample chamber

Ve = the volume of the expansion chamber.

Vc and Ve are determined by calibration.

Knowing the mass of the sample m, the density (d) is determined by:

d=m/Vs

Density measurement was done three times for each sample and an average was taken.

Figure 2 Multivolume pycnometer 1305, Micromeritics Instument Corporation, USA, used for grain density measurement

4.7 Packing efficiency

Packing efficiency, the ratio of specific weight to grain density, was calculated as defined by Pushman and Bingham (1975).

4.8 Grain weight and 1000 grain weight

Some seeds of known weight were counted by using a digital seed analyser to determine thousand grain weight. In order to study the single grain weight distribution in the lot, individual grain weight of some 300 grains from each sample were weighed using the Single Kernel Characterization System (SKCS 4100, Perten Instruments) (Figure 3) at Rothamsted Research Institute.

Figure 3 Single kernel Characterisation System machine associated with its own data processing computer used for HI.

SKCS measures diameter, weight, moisture content, and hardness index of each grain. The machine rejects some seeds based on its own criteria based on size. The rejected seeds are broken seeds and seeds exceptionally too small or too big. For this experiment a minimum of 215 seeds were measured from each sample.

4.9 Ethanol yield

The ethanol yield data of these samples were obtained from SWRI. Ethanol yield was assessed in a laboratory by reflecting commercial practice of potable alcohol processing as described by Brosnan *et al*. (1999). Wheat grain samples were milled by Buller Miag dick mill fitted with 2 mm screen. Thirty grams of each sample was mixed with 21 ml water (45°C) in a stainless steel beaker and put in a water bath at 45°C. At this point, 25 µl of α-amylase (Temayl 120L, Novozymes Ltd, Nottingham UK) was added to the solution and then mixed. The beakers were then covered and the temperature of the bath was raised to 85°C. The solution was then kept at this temperature for 30 min. The slurry was then transferred to an autoclave and cooked for 15 min at 142°C. The slurry was again transferred to 85°C and another 25 µL α-amylase was added and left for 30 min. After this, the beakers were then transferred to 65°C water bath and 50 ml of hot water (65°C) added to each beaker. Barley malt grist was added to each beaker at a rate of 20% of the dry weight of the wheat sample used. Then the beakers were left in 65°C water bath for 1 h. The beakers were then cooled by placing them in an ice bath for 20 min. The beaker content was then transferred to 250 ml volumetric flask and 1 g of type M distillers yeast (KerryBioscience Ltd, Tralee, Ireland). Distilled water was added to each flask until the weight of each sample reached 250 g. Flasks were then left in 30°C water bath for 68 h for the fermentation. The flasks were then removed; water was added again to compensate the loss of weight due to CO_2 during fermentation. Two millilitres of antifoam was added to each flask and the flasks were heated by Bunsen burner for the distillation. The evaporated ethanol was then collected and density of the ethanol was

measured using Paar 5000 density meter and expressed in ethanol yield litres per tonne of wheat processed.

5. Economic importance of wheat grain ethanol yield variability

5.1 Summary

The purpose of this chapter is to quantify the economic impact of variation in ethanol yield from wheat grain used as the feedstock in the emerging UK bioethanol industry. Analysis of ethanol yield of 84 Recommended List samples showed that the annual cost saving from best quality wheat compared with poor quality wheat could exceed £3.7 million per *annum* or £41 per tonne of wheat processed in a plant with a capacity of 100k tonnes of wheat per year.

5.2 Introduction

Bioethanol production is not new technology: it has been used when the world oil price has been high and when there has been restricted oil supply; for example, during the first and second world wars (Batchelor *et al*., 1994). In recent decades Brazil has become a model in producing biofuel. At least from the early 1970s until the mid-1990s, Brazil was the only country known for production of bioethanol (Mol, 2007). The recent escalation of oil price and increased interest in environmental issues has caused a resurgence of ethanol production. More and more countries are considering biofuel as a means of attaining greater energy security in addition to addressing environmental problems (Peters and Thielmann, 2008). Although the UK is less reliant on imported fuel than countries such as the USA, the environmental benefits of biofuels should not be overlooked (Robinson, 2002). The UK is committed to the Kyoto agreement to reduce greenhouse gas emissions, and in the UK an energy White Paper seeks reduction of carbon dioxide emissions by 60% before 2050 (DTI, 2003). The transport sector is responsible for about 30% of the UK's CO_2 emissions (Smith *et al*., 2006).

Biofuel production in the UK had been exclusively biodiesel until the start of bioethanol production in 2007 by British Sugar (GCC, 2007). Ensus has also begun producing bioethanol in 2010 refining about 400 million litres of bioethanol per year from locally grown wheat (Hazzledine *et al*., 2011). A similar amount of bioethanol is expected from Vivergo Fuels, another plant, later in 2012 (Vivergo, 2012). Another seven wheat grain bioethanol plants are planned with a total production capacity of about 1.6 million tonnes of bioethanol per year (NNFCC, 2008). The current bioethanol production in the UK has been promoted by government policy (blending quota), tax incentives and relatively high crude oil prices.

The economic feasibility of bioethanol in the UK has been investigated by several authors (e.g. Batchelor *et al*., 1994; Bullard *et al*., 2003; Turley *et al*., 2004). The results indicated that the production cost of bioethanol is generally higher than that of petrol. Studies conducted in China on cassava bioethanol and in the US on maize bioethanol suggested that the two most important factors in the economics of bioethanol production are petrol price and feedstock costs (Keim and Venkatasubramanian, 1989; Hu *et al*., 2004). When petrol is expensive and/or feedstock cost is cheap, bioethanol would be more viable (Anon, 1988). However, the relationship between these two is not very clear: while some suggest there is a strong correlation, others claim the relationship is only by chance.

The bioethanol market is global and highly competitive; so end-product price is exogenous to firms and encouraging the success of bioethanol production in the UK should focus on minimizing the cost of production (Smith *et al*., 2006). Bullard *et al*. (2003) suggested that the cost of production of bioethanol can be reduced, given significant investment in research and development (R&D). R&D is required to increase the ethanol yield of wheat and to improve the processing efficiency. It is important to note that increases in ethanol yield could result from purchases of the wheat feedstock. The UK potable alcohol distilleries have a Recommended List of varieties of wheat for distilling quality. Studies have shown that there is still high variation between and within these varieties in ethanol yielding potential (Kindred *et al*., 2008; Swanston *et al*., 2005;

2007). Several grain characteristics such as starch and nitrogen concentration TGW, grain length to width ratio have an association with ethanol yield and can be used as indicators of high ethanol yielding wheat (Awole *et al.,* 2008).

Poor quality wheat results in lower strength ethanol, and as a result it not only yields less ethanol but takes more energy to do so (Bringhurst *et al*., 2003). Moreover, poor quality wheat gives a larger proportion of residual material which again takes more energy for cooling and drying.

The main purpose of this analysis is to examine significance of the feedstock quality variability in the overall economics of wheat grain bioethanol production, so as to determine the need for clear feedstock quality criteria. Although the quality of wheat has an impact on the amount of ethanol produced and on energy-cost-saving, this analysis is limited to the effect on variability in ethanol yield. It is difficult to demonstrate the variation in energy consumption, since the energy data obtained from laboratory analysis is not representative of the larger scale industrial production of bioethanol.

5.3 Cost of production

Economic analysis requires detailed understanding of the ways in which costs are built, starting from production and proceeding through to marketing. The overall cost of bioethanol production and transportation, including the blending cost, should be comparable not only with petrol prices but also with the 'free on board' (fob) price of bioethanol imported from other countries. The major costs of bioethanol production can be categorized into three main parts as indicated below.

5.3.1 Feedstock cost

Feedstock is any biomass resource destined for conversion to energy or biofuel. Apart from the British sugar plant at Wissington which produces bioethanol from sugar beet,

all the other planned bioethanol plants in the UK use wheat as a feedstock. Therefore feedstock in this paper refers to wheat. Feedstock cost is the price that bioethanol producers must pay for the wheat delivered to their factory gate. Feedstock accounts for between 55-70% of the total cost of bioethanol production (Anon, 1983; Schultze *et al*., 2005), and therefore feedstock cost is an important factor in the economics of the industry.

The traditional markets for UK wheat are for animal feed and for milling - of which the majority is for bread and biscuit making (Hollins *et al*., 2006) - and also for brewing and distilling (Smith *et al*., 2006). Milling wheat and feed wheat have distinct properties: the main distinctions being grain protein concentration and potential grain yield per hectare. Feed wheat has been preferred by the potable alcohol industry and this will be the same for the biofuel industry because of its lower protein concentration and lower market price than that of milling wheat.

The price of feedstock is highly volatile depending on market conditions, expectations concerning future harvests and world stocks. In recent years the primary commodity price has been rising tremendously. World prices of wheat and maize increased by 136 and 31 percent respectively between March 2007 and April 2008 (Pfuderer and Castillo, 2008). There has, however, been a substantial decrease in feed wheat price after May 2008. Bioethanol production is very sensitive to wheat price (Batchelor *et al*., 1994). The price of feedstock (feed wheat) can be affected by the supply and demand for human consumption (milling wheat), supply and demand for livestock feed (feed wheat and other cereal feeds) and the supply and demand for alcoholic beverages, but it is more sensitive to supply than demand. Moreover the feedstock costs of bioethanol producers depend on the size of the order they are able to place and the contractual agreements made (DfT, 2006a). The historical data show that the demand for milling, feed and distilling wheat has been increasing during the past 15 years. The most notable one in the UK is feed wheat consumption (Figure 4). This indicates the potential competition for wheat in the near future which could raise the feedstock price.

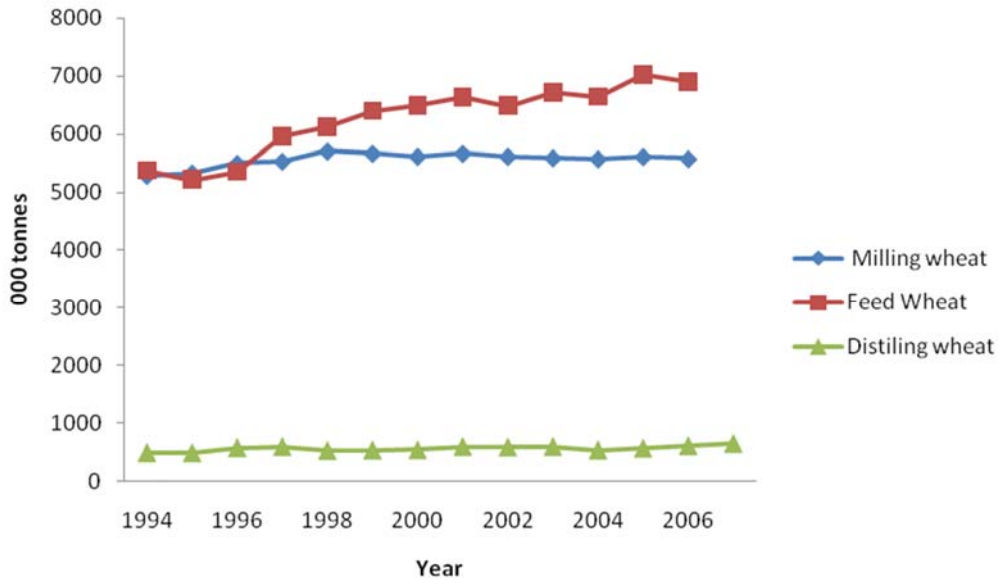


Figure 4 The trend in UK feed wheat, milling wheat and distilling wheat consumption from 1994 to 2007. [Data source: HGCA-AHDB)]

5.3.2 Energy Cost

Energy used in the production process accounts for the second largest cost, next to feedstock cost, in the economics of bioethanol production. Schulze *et al*. (2005) studied the economics of bioethanol production from four feedstocks: sugar cane; wheat; maize and sugar beet (in Brazil, central Europe, US and Germany respectively). They concluded that the energy costs account for 10-16 percent of the total cost of bioethanol production depending on location and feedstock type. According to their study, the energy costs of bioethanol production from wheat in central Europe are approximately 10 percent of the total cost.

The major processes of bioethanol production from wheat are milling, cooking, fermentation and distillation. The energy requirement of each sub-processes of wheat-based bioethanol production is indicated in Table 1 using estimates from two sources.

Table 1 Thermal and electrical energy required at different stages of bioethanol production

Process	Thermal energy use % of total Meredith, (2003)	Electrical energy use % of total Meredith, (2003)	Thermal energy use % of total Schulze, (2005)
Grain recovery and milling	0	1	Not included
Cooking and liquefaction	4-6	0	10
Fermentation	1	0	30
Distillation and dehydration	43-48	0	20
Evaporation and drying of DDGS	31-36	3-4	40
Utilities	4-6	4-5	Not included
Building	1	0	Not included
Sub totals	91	9	100

The most intensive energy-demanding steps are those which involve heating; these are cooking, distilling and drying the residue to produce DDGS. Reducing thermal energy required at these stages will have a significant effect on reducing the cost of energy and reducing greenhouse gases. Good plant design involving integration of heat-consuming steps such as distillation and dehydration could reduce the energy cost (Schulze *et al*., 2005). A number of commercial simulators such as Aspen Plus (Brien and Craig, 1996: Wooley *et al*., 1999; Krishnan *et al*., 2000) and Super Pro Designer (Kwiatkowski, 2006) have been used to optimize the processing efficiency and associated costs.

In addition to the processing efficiency, the quality of feedstock used has a substantial impact on energy saving. Both good quality and poor quality wheat require the same

amount of energy during cooking, but poor quality wheat yields lower strength ethanol and high residual matter. Consequently, poor quality wheat requires more energy for each litre of ethanol produced during dehydration of the ethanol and drying of DDGS (Bringhurst *et al*., 2003). Smith *et al*. (2006) estimated that the value of high quality grain in UK bioethanol production could be millions of pounds a year. Therefore, both quality of the feedstock used and processing efficiency are very important in the economics of bioethanol production through energy saving.

5.3.3 Investment cost

The third most important cost in the economics of bioethanol production is the capital cost, accounting for 9-14% of the total cost of production (Schulze *et al*., 2005). When taking this cost into consideration, it is important to note that there are economies of scale. Batchelor *et al.* (1994) demonstrated that capital cost per litre is sensitive to scale of production as larger scales could significantly reduce the capital cost per litre of ethanol produced. It has been suggested that grants or soft loans could potentially reduce the production costs of bioethanol. Batchelor *et al*. (1994) suggested that a grant funding of about 40% of the capital cost can reduce the production cost by 3.7 p/litre making it cost only 35.1 p/litre for a plant size of 78000 tonnes wheat *per annum*. The experience of Brazil and USA is worth mentioning in respect to government support towards development of biofuel production. In both countries the boost in bioethanol production has only been achieved after billions of dollars of subsidies. USA alone invested about US$11 billion to subsidise bioethanol production prior to 2001 (WorldWatch Institute, 2007).

The capital cost depends on the asset life of the production plant and the rate of return expected by the investors. Most of the economic analyses of wheat-based bioethanol production consider a 15% return rate on capital costs (Batchelor *et al*., 1994; Bullard *et al*., 2003). This figure is higher than normal energy investment analysis but reflects the

rate of financial and technical risks involved in the establishment of such huge plants for a new product (Turley and Ceddia, 2003). Owing to the uncertainty of the life expectancy of any duty concession for bioethanol, it is difficult to account for the long-term depreciation of capital asset. It is unlikely that the government could guarantee a duty concession over a period of 5 years (Turley and Ceddia, 2003). Thus investors would require a higher rate of return and this makes the cost of production of bioethanol more expensive.

5.4 Revenue

Although bioethanol is the main product of the process and expected to yield returns, other co-products can be used to offset the production costs. The potential sources of revenue are discussed below.

5.4.1 Bioethanol

Bioethanol can be used as a petrol additive or it can also substitute for petrol (Walker, 2005). Bioethanol acts as an oxygenate, increasing the oxygen content of the fuel which enhances combustion, consequently resulting in increased efficiency and reduced emissions (Turley *et al*., 2004). Bioethanol represented a negligible amount (0.39%) of the total petrol usage in the UK up to 2006 (BERR, 2007). Tesco has been selling petrol of up to 5% blend at over 185 forecourts in the UK when it has been profitable to do so (Tesco, 2006). Eighty-five percent ethanol blends (E85) for flexible fuel cars have also been sold in the UK by Morrisons (Morrisons, 2006). In total, eight million litres of bioethanol, imported predominantly from Brazil, were sold in March 2006 in the UK (DfT, 2006b). Predictions show that the UK road transport demand will increase by 60% from 1990 to 2025 (NNFCC, 2008). This, together with RTFO demands of 5% biofuel blending quota by 2010 imposes a very high demand for bioethanol in the UK.

Although the price of bioethanol has to follow that of petrol, the current production costs of bioethanol are generally more expensive than its counterpart. Consequently most countries have supporting policies in the form of blending quota and tax exemption or

reductions to encourage use of bioethanol. The UK government has had a 20 p/litre of duty reduction for bioethanol since 2005.

5.4.2 Distillers dried grains with solubles

DDGS is an essential by-product of cereal bioethanol production. Taking out the starch from the grain through fermentation concentrates all the remaining nutrients of the grain by a factor of three, making DDGS rich in the three most expensive nutrients added to livestock diets: fat, protein and minerals (Jacques, 2003). Thus DDGS is a high value feedstuff for dairy cattle, beef cattle, pigs, poultry and aquaculture (EUBIA, undated).

Although DDGS can be used for other purposes as well, the current market for DDGS is in the feed sector. In addition to supplementing the local livestock cereal feed, DDGS will also play a considerable role in reducing or avoiding soya bean meal import to the UK. As the exact inclusion rate of DDGS in the animal feedstuff is not known, it is difficult to assess the market size of DDGS (Smith *et al*., 2006). The UK imported about 1.8 million tonnes of soybean meal in 2007. DDGS would compete with soya-bean meal in the feedstuff market if it contained high quality digestible protein. Currently the common use of DDGS in the feed is a substitute for alternative protein sources like soya-bean meal but DDGS can also replace energy sources in livestock feeding stuffs such as cereals (Jacques, 2003).

Even though DDGS is merely a by-product of bioethanol production it makes a substantial contribution to the economic viability of the industry (Tucker *et al*., 2004). According to Batchelor *et al*. (1994) the cost of production of bioethanol is sensitive to DDGS price. A study conducted in the USA showed that an income obtained from maize DDGS returns 40-45% of the cost of maize from which it is produced (Keim and Venkatasubramanian, 1989).

The simultaneous co-production of DDGS in millions of tonnes each year will affect the supply and consequently the price. In addition to the supply and demand there are many factors which could possibly affect the market price of DDGS and hence bioethanol

production. Among these factors, the nutrient content of the DDGS is very important (Cooper, 2007). Therefore, producing high quality DDGS is vital for commercially successful bioethanol production. The other factor which could determine the DDGS price is the price of the other feeds which DDGS is expected to replace. Soya-bean meal price as a protein source or cereal feed price such as maize, wheat and barley as an energy source, could compete with DDGS prices. Historically the price of maize DDGS in the United States generally follows that of maize grain (Cooper, 2007).

5.4.3 Carbon dioxide

Carbon dioxide (CO_2) is one of the major by-products of bioethanol production. Approximately 280 kg CO_2 per tonne of wheat is produced through yeast respiration (Smith *et al*., 2006). CO_2 can be collected and sold as an additional income source. The possible markets for CO_2 are: in the carbonated drinks industry; for greenhouses to enhance productivity; in refrigeration and packaging industries; or in fire extinguishers (Senn and Piepr, 2000). However the collection of CO_2 is very expensive, therefore it may not be economical to collect CO_2 with the current limited market (Smith *et al*., 2006). Although the North British Distillery is the only distillery in the country which collects, purifies and sells CO_2, the CO_2 market in the UK is currently saturated (Brian Watts, Manager of the North British Distillery Company Ltd, personal communication). All recent economic studies exclude CO_2 income due to lack of markets. However it is possible that in the future markets could be created for CO_2.

5.4.4 Others

In order to reduce the cost of production of bioethanol and obtain a higher economic benefit, the ethanol industry should generate value added co-products. The co-products could establish markets in the feed, food and fertilizer industry (Pass and Lambart, 2003). Home-grown cereals authority (HGCA-AHDB, 2007c) has been looking into one of the possible co-products, extraction of arabinoxylans (AX) from wheat bran. Arabinoxylan

can be used in food and pharmaceutical industries. The research revealed that arabinoxylan could be produced economically in an integrated biorefinery and can enhance the economics of bioethanol production if there is a market for arabinoxylans (Weightman *et al.,* 2009)..

A novel wheat-based biorefinery which could replace the current dry-milling process of wheat has been proposed by Arifeen *et al*. (2007a&b). This process allows the extraction of different components of the grain (bran, gluten, and starch) for different end-uses (Arifeen *et al*., 2007a&b). Such processing could produce value added co-products such as gluten (Arifeen *et al*., 2007a&b). Despite much research to diversify the co-products of bioethanol production, currently there is no commercially viable co-product apart from DDGS.

5.5 Economic analysis

5.5.1 Supply, demand and price relationship between fuel energy sources

The historical crude oil price data has shown very high escalation in recent years (Figure 5). Such a huge rise in crude oil prices has created a good opportunity for bioethanol, as demonstrated by the tremendous increase in world bioethanol production. Biofuels in general have seen a threefold increase in the past two decades (Pfuderer and Castillo, 2008). A high crude oil price has a dual impact on bioethanol production. While high oil price makes bioethanol more competitive in the market, it also increases the cost of production of bioethanol due to high energy costs for feedstock production and processing.

In theory, the supply of petrol will affect demand for bioethanol in the market. However, for the time being this relationship is set to be relatively constant as bioethanol has constant demand created by a mandatory blending quota in the UK. The demand for biofuels is likely to remain the compulsory blending quota unless bioethanol becomes cheaper than petrol in the market.

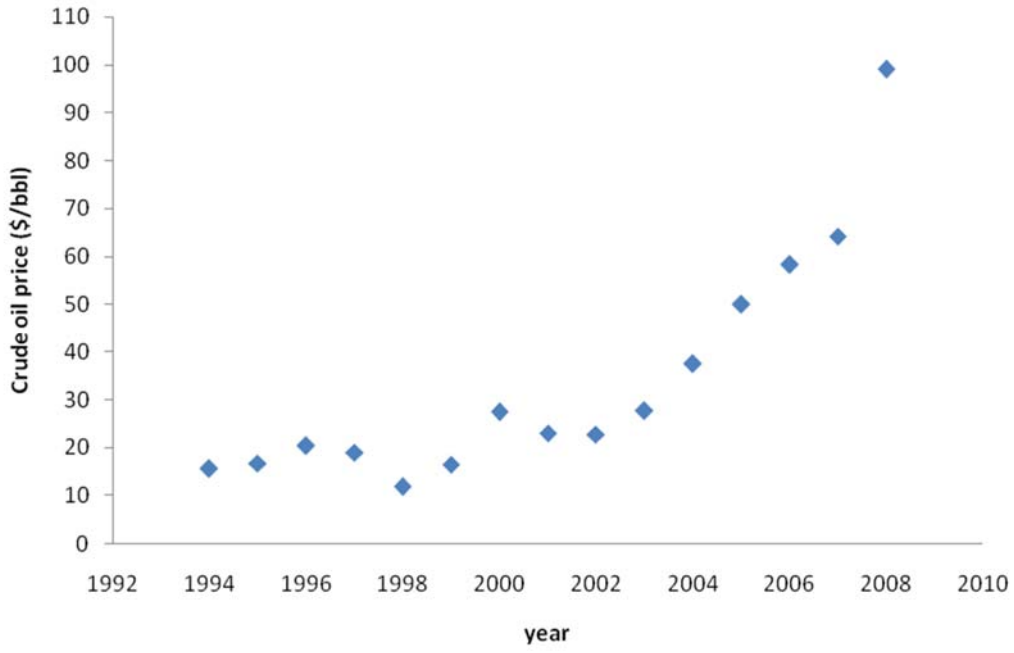


Figure 5 The annual average crude oil price in U.S. Dollars per barrel from 1994-2007 [Data source: EIA (05 Dec 2008)]

The price of feedstock is the major determining factor for the economics of bioethanol production. As wheat has many other established uses, the price of wheat for bioethanol will be affected by demand and supply for these other uses. The most common way of studying such demand and supply interaction is through historical data. However, wheat bioethanol production and the market are relatively new phenomena, and therefore the market for both the feedstock as well as the bioethanol itself is not well developed and the information needed to obtain a clear understanding of the scenario is not currently available.

5.5.2 Cost of production

Several authors have looked into the economics of bioethanol production and estimated the cost of production p/litre of ethanol (Table 2). The studies were conducted in different

years and conditions, thus showing considerable variation. The study by Batchelor *et al*. (1994) is based on a pilot plant which produces bioethanol from wheat in Sweden. All the expenses and revenues were determined from the operating company's actual data. This figure could be more reliable than other estimates as it is based on real operations rather than assumptions. Turley *et al*. (2004) and Walker *et al*. (2005) determined the cost of production based on the 1994 data of Batchelor *et al.* but adjusted to the current price of wheat and DDGS of the study year. Bullard *et al*. (2003) followed a different approach for determining the cost of production. The feedstock cost was determined as the cost required to grow wheat instead of the market price of wheat. The capital cost was considered based on different published sources of EU and North America together with consultations with several UK bioethanol project developers. The study by BTG (2004) is less relevant to UK conditions as it is entirely based on other countries' situations.

Despite the slight variation in the estimated cost of production by different authors, all concluded that bioethanol production is much more expensive than its counterpart, petrol production. The Department for Transport (DfT, 2006a) has studied the size of government concessions required to make bioethanol competitive with petrol using different scenarios of bioethanol production. It was suggested that a duty cut of 26-29 p/litre is required to incentivise bioethanol production from wheat (DfT, 2006a).

The cost of producing ethanol from wheat, updated from Bullard *et al*. (2003) for the 2011 average feed wheat price (£171/tonne) and recent DDGS price (£197/tonne) is about 50 p/litre. This is by replacing £65/tonne and £60/tonne of wheat and DDGS prices respectively. In this calculation the possible change in energy cost and capital cost over time is not considered. With the current duty reduction of 20 p/litre, wheat bioethanol costs appear cheaper than petrol prices (90 p/litre including tax at the time of writing). However, the energy content of bioethanol is not the same as petrol, therefore a litre of petrol can only be replaced by 1.49 litres of bioethanol (Peters and Thielmann, 2008).

This will still make bioethanol expensive when compared with petrol, and a 20 p duty concession is not enough to compensate.

Table 2 Estimated cost of production of wheat grain bioethanol from different literature

Authors	Estimated cost of production p/litre	Country	Condition of the study
Batchelor *et al*. (1994)	38.8	United Kingdom	The costings are based on a quote from Chematur engineering AB of Karlskoga, Sweden 78 000 t of wheat/annum Wheat price £115/t, DDGS price £110/t EY = 384.6 litre/t

Bullard *et al*. (2003)	31.94	United Kingdom	EY = 355 litre/t 100000 t/year £66/t of wheat
Turley *et al*. (2004)	28.6	United Kingdom	Based on Batchelor *et al*. (1994) and Warren *et al*. (1994) and adjusted to current feed wheat and DDGS (soya meal price)
BTG (2004) as published on EUBIA website	41	EU-25	Bioethanol production costs in the EU-25, Bulgaria and Romania Wheat price €140/t = £97.4 (currency rate at: 1/12/04)
Walker *et al*. (2005)	30	United Kingdom	Adapted from Batchelor *et al*. 1994 study for 2005 feed wheat and DDGS prices

US maize ethanol can be competitive with petrol, while Brazilian sugar cane ethanol is sometimes even cheaper than petrol (Mastny, 2006). Availability of cheap bioethanol on the international market could be a constraint for the UK bioethanol industry (Walker *et al*., 2005). Brazilian bioethanol can be produced for as low as 10 p/litre (Turley and Ceddia, 2003). Mastny (2006) reported that international trade in biofuels is currently limited because many countries have set tariffs on biofuels in order to protect domestic industries and also to ensure domestic subsidies are not spent to support other countries' industries. This is likely to change because of mandatory blending quotas, as domestic industries cannot supply full demand. Countries such as Brazil which produce ethanol in excess of local demand have the potential to influence the international market significantly.

One of the main reasons to encourage bioethanol or biofuels in general is to reduce greenhouse gas emissions. Although there has been a considerable debate on whether biofuels can reduce GHG emissions, there is a majority consensus that biofuels do reduce GHG compared with petrol, but the quantity of emission saving depends on location, type of feedstock, how the crop is grown, and the fate of by-products (Smith *et al*., 2006). Therefore, the cost effectiveness of biofuels as a means to reduce greenhouse gas emissions must also be considered. A number of authors have investigated the cost benefit analysis of bioethanol especially in regard to emission savings. IEA (2004) reported that bioethanol does save greenhouse gas emissions, but it may not be a cost-effective way of reducing emissions. The CO_2 abatement costs (CO_2 abatement cost is the additional cost of production of biofuels than fossil fuels which should be compensated by reduction in CO_2 emission) of bioethanol from different feedstock in different countries were compared (Deconti, 2008). The result indicated that apart from the Brazilian sugar cane bioethanol, the CO_2 abatement costs of all other sources of bioethanol are significantly higher than the shadow price of CO_2 (shadow price of CO_2 is an estimate of the damage costs of one additional tonne of carbon emitted into the atmosphere). Among the bioethanol feedstocks compared, wheat has the highest CO_2 abatement cost. In addition to many other reasons such as high biomass production and favourable climate, the lowest cost of production as well as the lowest CO_2 abatement cost of the Brazilian sugar cane bioethanol can be explained by long term experience and the R&D invested to improve the efficiency and productivity of the overall process. More research is justified in order to reduce the cost of production and greenhouse gas emissions, so as to make bioethanol competitive with petrol.

5.5.3 Sensitivity of cost of production to changes in variables

For the purpose of demonstrating the economic benefit of feedstock quality, the estimation made by Bullard *et al.* (2003) was used. This study estimated the cost of production of bioethanol in the UK and showed the total annual fixed cost for producing

bioethanol from a plant with a processing capacity of 100,000 tonnes of wheat per year (Table 3). According to Bullard *et al.* (2003) the fixed costs include: the capital cost and processing costs required assuming a discount rate of 15% and an economic plant lifetime of 15 years. The total income obtained from DDGS sales is also considered as fixed income and deducted from the total cost of production (i.e. as by-product credit). In their study the feedstock price was considered as the variable cost.

Table 3 Breakdown of bioethanol production costs adapted from Bullard *et al.* (2003)

Processing capacity	Annual cost (£)		
	100000 t	200000 t	300000 t
Feedstock cost	17,100,000	27,200,000	40,800,000
Conversion cost (Capital)	3,017,500	4,529,800	5,793,600
Conversion cost (processing)			
Energy	1,898,020	3,497,277	5,087,748
Staff	715,824	1,318,973	1,918,808
Maintenance	271,146	499,611	726,821
Admin. & general over heads	271,146	499,611	726,821
Working capital interest	677,864	1,249,028	1,817,053
Total annual cost	20,451,500	38,794,301	56,870,581
By-product income	-6,008,500	10,370,000	15,555,000
Net cost	15,266,500	28,424,300	41,315,850

Batchelor *et al*. (1994) demonstrated the responsiveness of the cost of bioethanol production to changes in wheat price, DDGS price and capital cost while every other cost is held constant. This present paper followed the same procedure to show the effect of variability in ethanol yield on the production cost of bioethanol.

The cost of production was calculated by leaving every other cost constant and by allowing the ethanol yield to vary. Instead of the constant ethanol yield data (355 litre/t) used in the Bullard *et al.* (2003) study, laboratory ethanol yield data of 84 samples from

14 varieties grown at 11 sites were used. These samples were part of HGCA-AHDB Recommended List samples collected from two successive harvest years (2003 and 2004). The ethanol yield data of these samples were assessed in a laboratory by reflecting commercial practice of alcohol processing as described by Brosnan *et al*. (1999) and was obtained from the SWRI. The samples showed variation in ethanol yield up to 50 litre/t of wheat.

The cost of production of a litre of bioethanol is calculated using: the ethanol yield data from these 84 samples, capital and processing costs obtained from Bullard *et al.* (2003) study and the current feed wheat and DDGS price £171/t and £197/t respectively (average price of year 2011).

Cost of production = total annual cost / bioethanol produced per year

Total annual cost = feedstock cost + conversion cost – by-product credit

Data sources

- Feedstock cost = average feed wheat price of 2011 (From HGCA-AHDB)

  Although so far there is no bioethanol production from wheat grain in the UK, we know from the experience of the potable alcohol industry that there is no price differential being applied for distilling quality.
- Conversion cost = capital, operation & maintenance cost from Bullard *et al*., (2003)
- By-product credit = DDGS sale income (average price of 2011, Farmers weekly)
- Bioethanol yield = variable for this analysis (laboratory measured ethanol yield)

The range of cost of production p/litre obtained from this calculation is indicated in Figure 6. The best quality wheat and the poorest quality wheat shows great difference in the

amount of ethanol produced per year and cost of production in pence per litre. Only 89,460 tonne of best wheat produces as much ethanol as 100000 t of poor quality wheat therefore it saves £1,802,372 from feedstock price (10540 t * £171/t). The amount saved from reduced cost of production of quality wheat is about £1,891,225 per year (40740 kl * 4.6 p/l). This implies, in total, if the plant uses the best quality wheat to produce 40.7 million litres of ethanol per year it would save about £3.7 million a year. This means it earns £41 saving for each tonne of wheat processed.

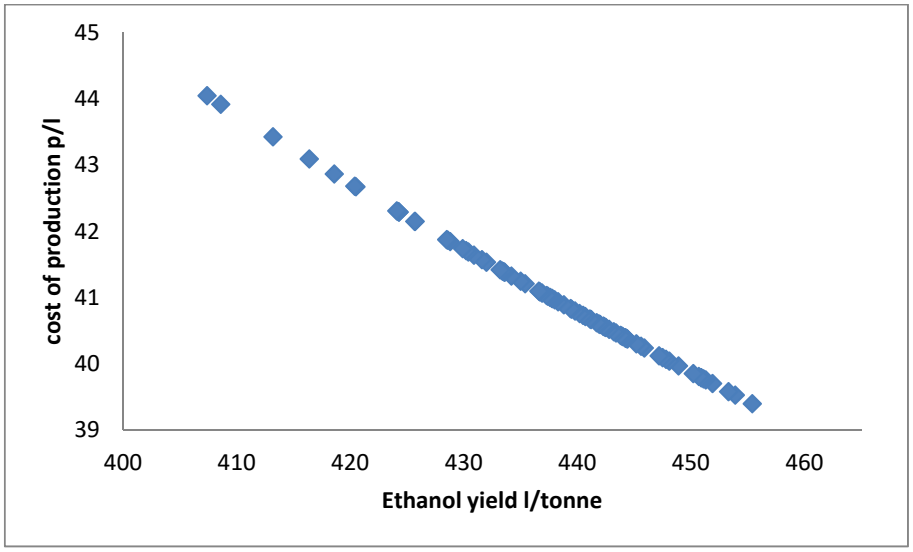


Figure 6 The relationship of the cost of bioethanol production in p/litre with bioethanol yield litre/t of wheat based on the ethanol yield of 84 samples of wheat from HGCA-AHDB RL trials harvested in 2003 and 2004 (Samples detailed in Chapter Six)

At higher plant capacity the saving would reduce slightly. For example a plant with a processing capacity of 200000 t and 300000 t wheat will save £7.1 million and £10 million per year or £35.8 and £35.3 per tonne of wheat processed respectively. For plants such as Ensus, with a capacity of 1 million tonnes of wheat, the benefit would be very large although, the exact figure cannot be shown here as the author could not find cost estimation data for such a large plant size.

This analysis did not include the possible energy saving due to quality of feedstock. A consideration of the cost reduction from energy saving is expected to boost this result substantially. This implies that a quality criterion such as described in the introduction, or a tool which predicts the potential ethanol yielding capacity of wheat and discriminates poor ethanol yielding and good ethanol yielding wheat, will save the company millions of pounds per year.

The sensitivity of cost of production to wheat price and DDGS price was also calculated using the relatively recent estimate of cost of production (Table 3) in order to check if the relationship described by Batchelor *et al*. (1994) remained the same. For example a £20 increase in price of DDGS reduces the cost of bioethanol production by 1.5 p/litre; this result closely agrees with the 2 p suggested by Batchelor *et al.* (1994) for the same DDGS price change (Figure 7). Similarly, £20 increases in wheat price increases the cost of production by 5 p/litre (Figure 8), which is the same as suggested by Batchelor *et al*. (1994). The data confirms the conclusion of Batchelor *et al.,* (1994) that bioethanol cost of production is very sensitive to the wheat price. Although the DDGS price also plays a significant role in the bioethanol cost of production, its effect is not comparable with wheat price.

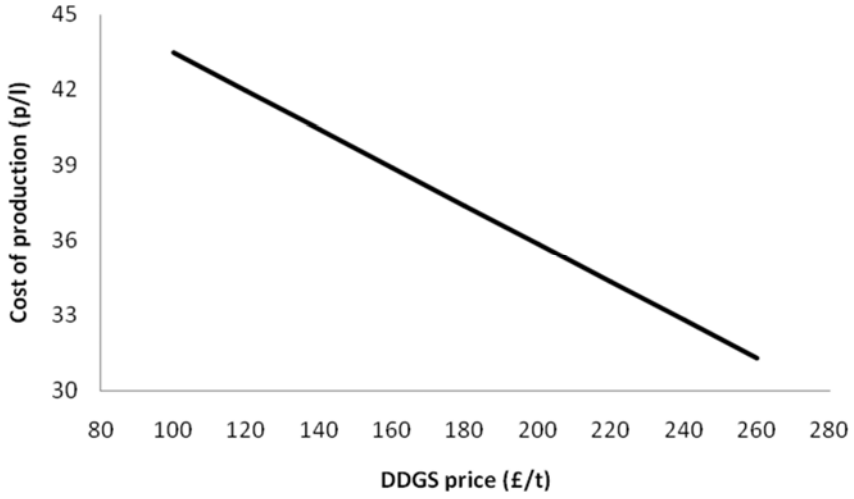


Figure 7 The relationship between the cost of bioethanol production in p/l and DDGS price £/t

Figure 8 The relationship between the cost of bioethanol production in p/l and wheat price £/t

5.6 Conclusion

In current circumstances, bioethanol is not competitive with petrol even allowing for government concessions. To improve this situation, every possible measure will have to be taken in order to make bioethanol perform better. Emphasis should be on the two major costs of the process: feedstock cost and energy cost. Better quality wheat being purchased for intake at the biorefinery will play a vital role in reducing the overall cost of bioethanol production, hence improving profitability and business sustainability. The potential ethanol yield can be maximised by producing good distilling wheat together with efficient processing to extract the available ethanol yield from the grain. More fundamental research will be needed to enhance the potential ethanol yield of wheat.

6. Analysis of grain characteristics and ethanol yield of RL varieties

6.1 Introduction

Laboratory ethanol yield determination can give accurate results but this is laborious, expensive and time consuming and therefore is not convenient to be used as a purchasing criterion at the distillery intake. A prediction tool based on the grain features such as physical and biochemical characteristics is required at the distillery intake. As indicated in the literature review, some grain features showed a promising relationship with ethanol yield but most of the results showed a discrepancy between studies. And the previous studies were focussed on understanding the relationship between these features and ethanol yield and did not cover a wide range of samples. In order to develop a reliable prediction tool a wide range of samples should be covered and the relationship between these traits and ethanol yield should be well understood. Therefore this study has covered 84 samples comprising of 14 varieties grown at 11 sites in two years. However, not all varieties were grown at all sites and in both years. (see section 6.3).

6.2 Objective

- To understand the relationship between grain physical and biochemical features with ethanol yield
- To predict grain potential ethanol yield based on grain physical and biochemical features

6.3 Grain samples

Wheat grain samples, which were grown as part of the national variety testing (HGCA-AHDB Recommended List), have been provided by the SWRI. The samples were collected from two successive harvest years (2003 and 2004) in the UK. These two years demonstrated contrasting weather conditions; 2003 was a hot and dry summer whereas

2004 was cold and wet summer (Table 4). These two years were selected to provide typical examples covering a range of grain quality.

The 2003 samples consisted of RL varieties: Claire, Consort, Dart, Deben, Dickson, Istabraq, Nijinsky, Riband, Robigus, Steadfast and Wizard. These were grown at seven sites: North Invergordon, Scottish Agronomy, SAC – Whitsomehill, SAC – Cauldshiel, NIAB –Lancs, NIAB – Norfolk and NIAB - Borders. Whereas samples of 2004 consisted of RL varieties: Ambrosia, Claire, Consort, CPBT W96, Dart, Deben, Dickson, Glasgow, Istabraq, Nijinsky, Riband and Robigus grown at four sites: Moray, NIAB, Advanta and Aberdeenshire.

Table 4 Data of 2003 and 2004 summer weather in England and Scotland

	England and Wales						Scotland					
	temperature (C °)		Rainfall (mm)		Sunshine (h)		Temperature (C °)		Rainfall (mm)		Sunshine (h)	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
Jun	15.5	15.0	67.2	58.5	209	198	13.0	12.1	75.4	130.9	164	119
July	17.0	15.4	75.6	68.5	172	166	14.8	12.8	80.1	75.8	138	132
Aug.	17.7	17.1	17.6	148.9	207	174	14.4	14.5	45.9	188.1	182	147

6.4 Grain Analysis

Grain traits measured for this study were TGW, specific weight, density, packing efficiency, starch and nitrogen concentration and image analysis. All the methodologies are described in chapter 3.

6.5 Statistical analysis

Statistical analysis was conducted by using Genstat Version 10 (Lawes Agricultural Trust, Rothamsted). Correlation analysis was conducted between all traits in order to

assess the relationship between different traits. As there was no replication of samples collected at the sites, it is not possible to conduct analysis of variance which would permit the quantification of the genetic and environmental effects on these traits and ethanol yield. Regression analysis was conducted for ethanol yield with each of the other traits. Three separate steps were followed during the regression analysis. i) Fitting a common line for all the data ii) fitting separate lines with common slope for different years, sites and varieties iii) fitting separate lines with different slope. The significance of the improvements from step i to ii and ii to iii were determined. When the improvement is significant separate lines were fitted for each year, site or variety, whichever showed significant improvement. Stepwise multiple linear regression analysis was also conducted to select the best combination of traits to predict ethanol yield. For stepwise multiple linear regression, $P<0.05$ was used as critical value for new traits to enter into the model. Higher R^2 value was considered a criterion for the order of entrance into the model.

6.6 Results

A summary of most of the regression analyses is given in Table 5; only significant relationships are presented. The unbalanced representation of sites and varieties within the two years prevents direct comparison of specific sites and varieties. However the results give a clear indication of the general effect of site, year and variety on ethanol yield and its relationships with the grain characteristics. Particular caution must be used in interpreting year differences, since none of the sites were identical for the two years, although they represented similar climatic regions.

6.6.1 Grain chemical composition

There is a significant overall positive relationship between ethanol yield and starch concentration. Starch concentration alone explains 16% of the variation in ethanol yield.

According to this function, ethanol yield increases by 1.75 litres for every 10 kg increase in starch concentration. Fitting separate models for each variety gave no significant improvement in the variability accounted for, neither did the year. However, fitting separate models for each site improved the explained variance by 55% but the overall relationship between ethanol yield and starch concentration became non-significant when site was included in the model. This indicated that the overall relationship between ethanol yield and starch concentration was derived from site differences in both variables.

Table 5 Result of regression analysis of ethanol yield (l/tonne) with different grain parameters.

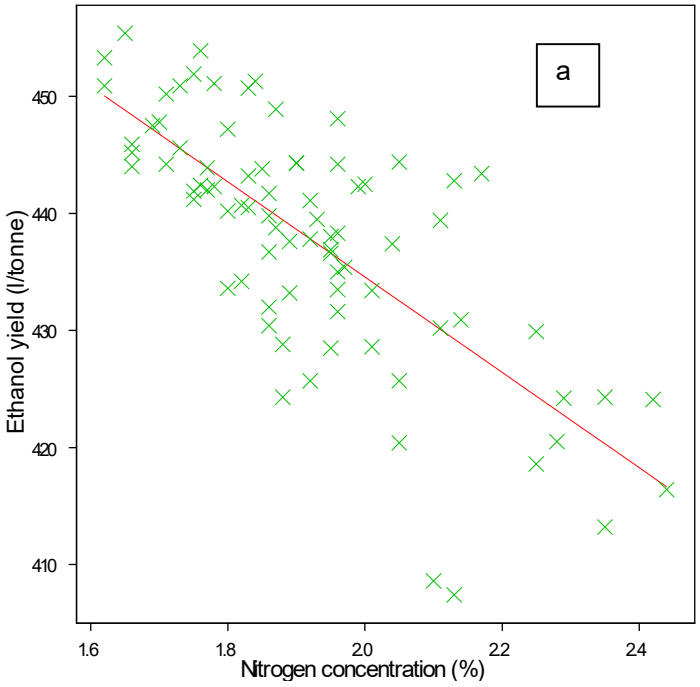
Grain parameters	Overall R^2	Overall p	Overall Standard error	Parameter estimate value	Parameter estimate p
Starch	15.6	<0.001	9.52	1.752	<0.001
Nitrogen	53.1	<0.001	7.15	-40.7	<0.001
Site included *	76.6	<0.001	5.05	-28.91	<0.001
Year included *	58.9	<0.001	6.69	-46.1	<0.001
Specific weight[a]	33.7	<0.001	8.44	66.2	0.006
Site included *	74.3	<0.001	5.25	1.439	<0.001
Year included **	33.5	0.027	8.45	1.938	<0.003
Density	4.4	0.032	10.1	-88.3	0.032
Site included *	73.9	<0.001	5.30	-106.5	0.002
Packing efficiency [a]	39.9	<0.001	8.04	3.948	<0.001
Site included *	76.1	<0.001	5.06	1.782	<0.001
Variety included *	47.3	0.048	7.52	5.794	<0.001
Area	24.2	<0.001	9.03	3.083	<0.001
Variety included *	33.8	0.044	8.43	4.430	<0.001
Year included *	27.9	0.027	8.61	3.547	<0.001
Width	21.3	<0.001	9.20	20.54	<0.001
Year included **	27.5	0.019	8.83	14.36	0.011
Length: width	9.6	0.003	9.86	-20.27	0.003
Diameter	31.7	<0.001	8.54	39.40	<0.001
Variety included *	45.8	0.006	7.61	56.46	<0.001
Year included *	35.9	0.014	8,27	43.73	<0.001
TGW	45.6	<0.001	7.65	1.618	<0.001
Variety included *	58.2	0.002	6.70	2.061	<0.001
Year included *	49.1	0.012	7.39	1.739	<0.001
Upper quartile	40.2	<0.001	8.02	1.057	<0.001
Variety included *	53.5	0.003	7.07	1.401	<0.001
Year included *	45.3	0.005	7.67	1.184	<0.001
Lower quartile	48.9	<0.001	7.41	1.452	<0.001
Variety included *	61.8	0.001	6.41	1.808	<0.001
Year included *	51.9	0.016	7.19	1.538	<0.001
Median	45.2	<0.001	7.68	1.200	<0.001
Variety included *	58.0	0.002	6.72	1.535	<0.001
Year included *	48.9	0.010	7.41	1.295	<0.001

* Separate model with common slope but different intercept

** Separate model with different slope and different intercept

[a] a curve fitted for the regression instead of straight line

Nitrogen concentration showed a strong negative correlation with ethanol yield accounting for 53% of the variation (Figure 9a). Ethanol yield decreased by 7.14 litres for every one percent increase in protein concentration. Fitting separate models for varieties gave no significant improvement to the variance accounted for. However, fitting separate models with a common slope for years improved the explained variance by 6% (Figure 9b). The statistics justified fitting separate models with a common slope, but different intercepts for each site, which accounted for 76% of the variation in ethanol yield (Figure 9c).



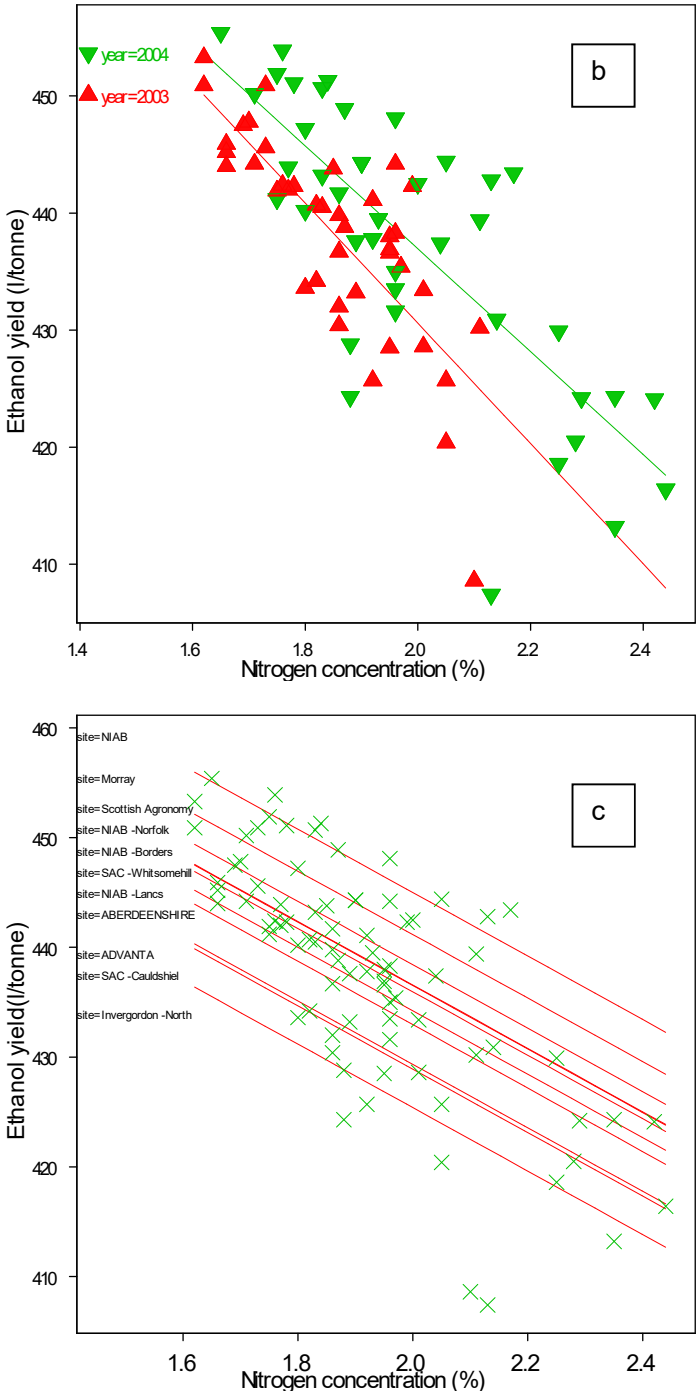


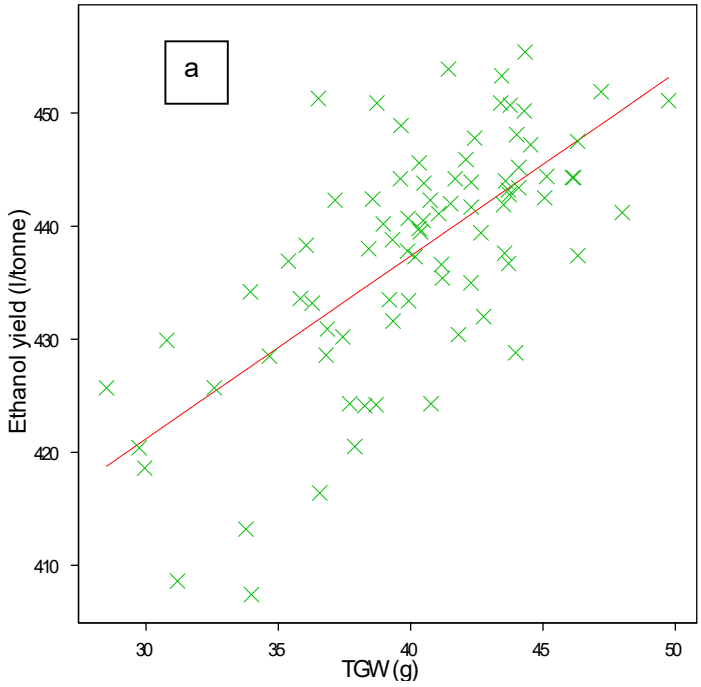
Figure 9 Relationship between ethanol yield and grain nitrogen a) with common model for all samples, b) with separate model fitted for each year and c) with separate model fitted for each site

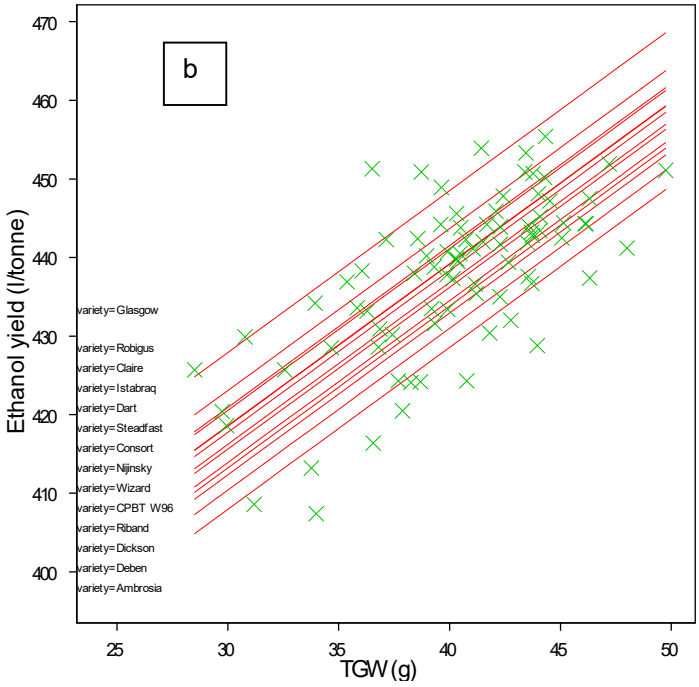
Starch concentration was also plotted against the nitrogen concentration data of all the samples. The relationship between starch and nitrogen concentration was significant ($p<0.05$) but the R^2 for this relationship was very low (4.5%).

6.6.2 Grain weight

TGW also showed a fairly good positive relationship with ethanol yield. TGW alone explained 46% of the variation in ethanol yield (Figure 10a). Unlike the chemical composition of the grain, TGW was affected by all factors: site, variety and year. The statistics justified fitting separate models with common slope for each variety and year: models with different slopes have no statistical significance. Site, variety and year improved the explained variance by 25% ($p<0.001$), 13% ($p< 0.001$), 4% ($p< 0.05$) respectively (Figure 10b – c). Although site did improve the explained variance, the overall relationship between TGW and ethanol yield was not significant in this model so there was no justification for fitting separate models for each site in this case.

The distribution of grain weight within the sample lot was also studied. Upper quartile, lower quartile, range, minimum, maximum and median was also plotted against ethanol yield. Among these, lower quartile, upper quartile and median showed fairly good relationships with ethanol yield (Table 5.). However, none of these predict grain ethanol yield better than TGW.


a
Ethanol yield (l/tonne)
450
440
430
420
410
30
35
40
45
50
TGW (g)


b
Ethanol yield (l/tonne)
470
460
450
440
430
420
410
400
variety=Glasgow
variety=Robigus
variety=Claire
variety=Istabraq
variety=Dart
variety=Steadfast
variety=Consort
variety=Nijinsky
variety=Wizard
variety=CPBT W96
variety=Riband
variety=Dickson
variety=Deben
variety=Ambrosia
25
30
35
40
45
50
TGW (g)

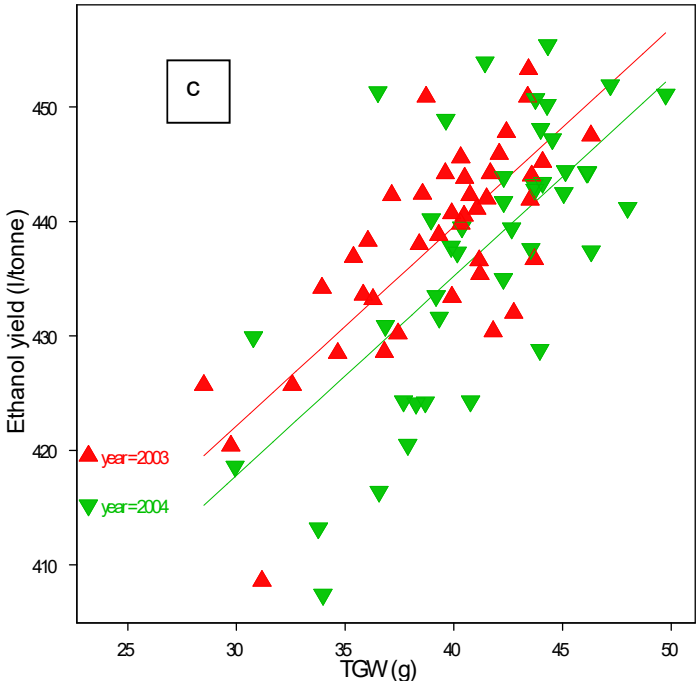
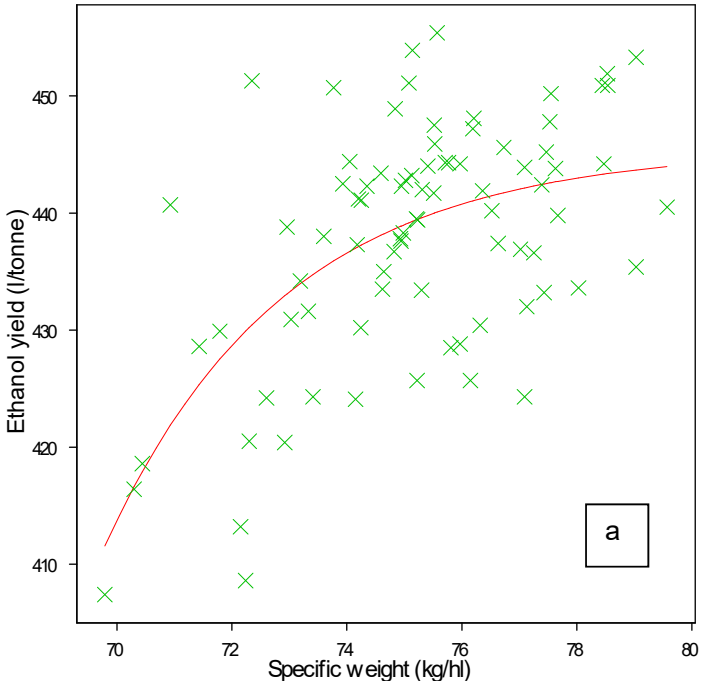
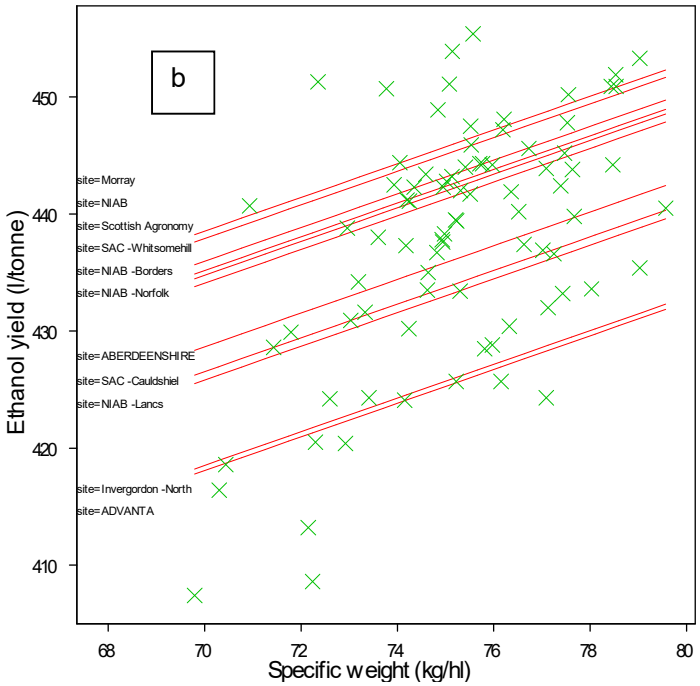


Figure 10 Relationship between ethanol yield and TGW A) With common model fitted for all samples, B) With separate model fitted for each variety and C) With separate model fitted for each year.

6.6.3 Grain density

Specific weight is bulk density of the grain, including air spaces, whereas grain density is the density of the grain alone and both were analysed in this experiment. Specific weight also had a positive relationship with ethanol yield. As opposed to the above other grain parameters, the relationship between specific weight and ethanol yield was better described by a curve rather than a line (Figure 11a). This model explained 34% of the variation in ethanol yield. Moreover, including site in the model improved the explained variance by 41%. Therefore separate parallel lines were fitted for each individual site (Figure 11b). There was no need for separate models for varieties as the change in explained variance was not significant. However, the statistics justified fitting separate lines with different slopes and intercepts for each of the years (Figure 11c).


Ethanol yield (l/tonne)
450
440
430
420
410
70
72
74
76
78
80
Specific weight (kg/hl)
a


b
site=Morray
site=NIAB
site=Scottish Agronomy
site=SAC -Whitsomehill
site=NIAB -Borders
site=NIAB -Norfolk
site=ABERDEENSHIRE
site=SAC -Cauldshiel
site=NIAB -Lancs
site=Invergordon -North
site=ADVANTA
Ethanol yield (l/tonne)
450
440
430
420
410
68
70
72
74
76
78
80
Specific weight (kg/hl)

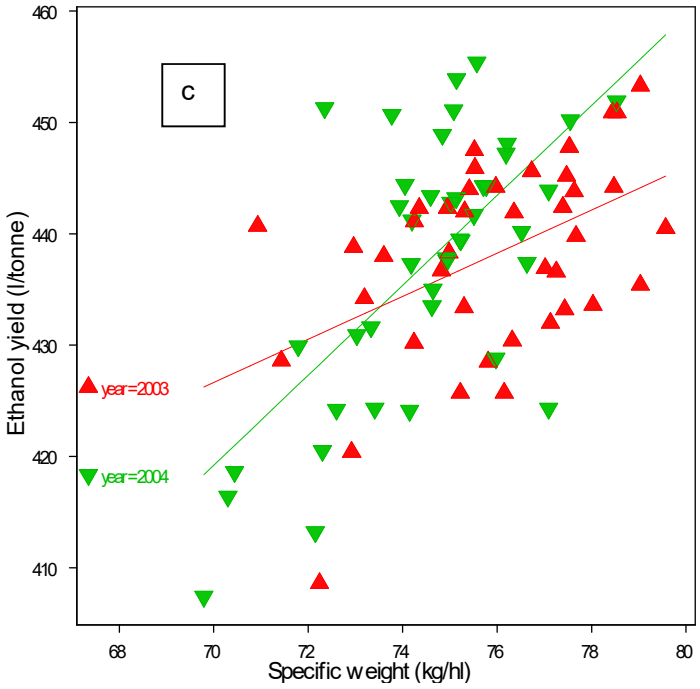


Figure 11 The relationship between A) ethanol yield and specific weight common model for all samples B) ethanol yield and specific weight, separate models fitted for each site C) ethanol yield and specific weight, separate models fitted for each year.

The relationship between grain density and ethanol yield was less significant ($p<0.034$) explaining only 4% of the variation in ethanol yield. Although fitting separate models for varieties and years had no significance; site, like all the above grain parameters, had a significant effect on the explained variance (74%).

Packing efficiency showed a better relationship with ethanol yield than did specific weight and density. It explained 45% of the variation in ethanol yield. As for specific weight, a curve described the relationship better than a straight line. Fitting separate models for sites improved the explained variance by 31%, but there was no need to fit separate models for year and variety as the change in the explained variance were not significant.

6.6.4 Grain dimensions

The explained variances of the relationships between ethanol yield and diameter, width, L:W, area, perimeter and roundness were 32%, 21%, 10%, 24%, 10% and 11% respectively (all highly significant, $p<0.001$). However, there was no significant relationship between ethanol yield and length. Including site in these models improved the explained variance to 70% for all of them, but the relationship within site became insignificant in these models. Including variety in these models gives a significant improvement only for diameter and area: 14% ($p<0.01$) and 10% ($p<0.05$) respectively. When year was included in the model significant improvement was obtained for diameter, width and area by 4% ($p<0.05$). The regression lines had a common slope and different intercepts for diameter and area but different slopes and different intercepts for width (Table 5).

6.6.5 Hardness index (HI)

Although HI showed significant regression with ethanol yield, the explained variance is very low to allow prediction of ethanol yield from HI (R^2=5.2). Including year and variety in the model has no significant effect on the explained variance. Like all other parameters including site has a very large effect on the explained variance (R^2=71.6; $p<0.001$).

6.6.6 Combining different parameters

In this study the highest explained variance was found by combining nitrogen and TGW and including site and variety in the model. Nitrogen and TGW alone explained 67% ($p<0.001$) of the variation in ethanol yield. But including variety improved the model to 74% ($p<0.001$). When site was also added to this model, the explained variance increased to 82% ($p<0.001$).

Without including site and variety in the model, variation in ethanol yield was best explained by a model composed of nitrogen, length, packing efficiency, perimeter and starch concentration of the grain (73%, $p<0.001$). The equation for this model is:

PEY = 295.5 - 5.047 N + -14.57 L + 1.519 PE + 9.07 P +0.840 S

Where:

PEY = Predicted ethanol yield (l/tonne)

N = Grain nitrogen concentration (g/100g dry matter)

L = Length (mm)

PE = packing efficiency

P = perimeter (mm)

S = Grain starch concentration (g/100g dry matter)

6.7 Discussion

It is the starch in the grain which will be fermented and converted into ethanol yield. Therefore, theoretically, measuring starch concentration should indicate the potential ethanol yield. In this study, although starch concentration was significantly correlated with ethanol yield, it explained only a very low proportion of the variation (16%). This was 11% less than that found by Kindred *et al*. (2008). On the other hand Swanston *et al*. (2007) reported that there is no correlation between ethanol yield and starch concentration of the grain. Contrary to these, Smith *et al*. (2006) reported a very good relationship between starch concentration and ethanol yield (78%). Kindred *et al*. (2008) suggested that the poor correlation could arise from the difficulty of measuring starch precisely. However data obtained from both the most commonly used starch measuring methodologies (acidic methodology – by Kindred *et al*. (2008); enzymic methodology – by Swanston *et al*. (2007) and this study) showed poor correlations. Therefore the relationship between ethanol yield and starch concentration is not reliable and starch concentration cannot be a good predictor of ethanol yield. The reason for this needs further investigation. Starch measuring methodologies may not be efficient enough to detect small variations between samples thus fail to show a precise correlation with ethanol yield. It may

also possibly be due to quality of starch or other characteristics of the grain that interfere with the starch conversion to ethanol e.g. nitrogen or NSP.

Grain nitrogen concentration showed an inverse relationship with ethanol yield confirming previous findings of Swanston *et al*. (2005 & 2007); Kindred *et al*. (2008) and Riffkin *et al*. (1990). In the present study nitrogen concentration explained about half of the variation in ethanol yield. This is 15% and 11% less than that has been found by Swanston *et al*. (2007) and Kindred *et al*. (2008) respectively. This is probably due to the wider range of samples (site, year and variety) covered in the current study. Unlike the report of Swanston *et al.* (2005) and Kindred *et al.* (2008), the relationship between ethanol yield and nitrogen concentration was not affected by variety. However, the relationship was highly affected by site. There is substantial difference in ethanol yield between sites at a given nitrogen concentration. This agrees with the finding of Swanston *et al* (2007). The study also showed that ethanol yield can slightly vary across years at a given nitrogen concentration.

Specific weight is one of the most frequently used measures for grain quality and usually is referred to as a crude measure of grain shrivelling (Gooding and Davis, 1997). According to Fenwick (1990) high specific weight is an indication of clean, plump, well filled and healthy grain. Shrivelled grain has more bran/endosperm ratio and as a result has less flour yield and starch concentration. Previous studies by Taylor and Roscrow (1993) and Taylor *et al*. (1990) showed that there is no correlation between ethanol yield and specific weight. Nevertheless the potable alcohol industry still considers specific weight as one of the quality criteria for alcohol yield. The result from this study showed that, when considering a common model for all samples from different sites and varieties, specific weight is a fair predictor of ethanol yield (33%) even better than starch concentration. Low specific weight can be used as an assessment for potential ethanol yield but at high specific weight the relationship is not linear. This relationship was not affected by variety but by the site. The significant interaction found in the regression of specific weight and ethanol yield between years can be attributed to the

weather difference during the two years. Specific weight is highly determined by the weather conditions during both grain growth and grain ripening (Atkinson *et al.,* 2005). Specific weight can be reduced either due to weathering or due to poor grain filling or both (Bayles, 1997 and Swanson 1943). Specific weight affected by grain filling could show a better relationship with ethanol yield than specific weight differences caused by grain weathering; as weathering is less likely to affect grain starch concentration as much as grain filling.

Specific weight is the product of the grain's density and its packing efficiency. As a result of strong correlations between grain density and grain nitrogen concentration (Riffkin *et al.,* 1990) and of nitrogen concentration with ethanol yield an inverse relationship was expected between grain density and ethanol yield. However, the grain density measured in this study showed no significant correlation with ethanol yield. Pushman and Bingham (1975) also reported that there is no correlation between grain density and flour yield. Thus, the poor relationship between ethanol yield and specific weight is due to a poor correlation between grain density and ethanol yield. The packing efficiency which is the factor of grain shape and grain surface texture seems more related to ethanol yield and showed a better explained variance than specific weight. The relationship of ethanol yield with grain density or with packing efficiency has not been studied before.

TGW varies with growing condition and maturity and more importantly between varieties and it is considered as a good indicator of grain size and mostly used in handling and processing of grains (Sablani and Ramaswamy, 2003). With the expectation that large, well filled, dense grain will possess more endosperm compared with bran, TGW has been used as an indicator of flour yield (Gooding and Davis, 1997). TGW has shown a good correlation with ethanol yield in our study confirming the findings of Taylor and Roscrow (1993) and Swanston *et al*. (2005 and 2007). TGW was highly influenced by genotypic and environmental factors. 2003 samples had higher ethanol yield than 2004 samples at a given TGW. This was probably because the longer sunshine hours of 2003 favoured grain filling and starch concentration. The result also

indicated that varieties should be considered separately when TGW is taken as an indicator of ethanol yield. Varieties Glasgow and Ambrosia have the highest and lowest ethanol yield of the varieties tested at a given TGW. Other studies have also shown that Glasgow is one of the best candidates in terms of its ethanol yield as well as grain yield per hectare (HGCA-AHDB, 2008 and Swanston *et al.,* 2007). Ambrosia has the 1BL/1RS translocation (HGCA-AHDB, 2008). Varieties with the 1BL/1RS translocation are suggested to have processing problems by increasing viscosity of the slurry (Kindred *et al.,* 2008). This might be the reason for the poor performance of Ambrosia from the rest of the varieties at a comparable TGW. But the site effect was different. Although site did improve the explained variance, TGW became non-significant when site was included in the model. This implies that TGW was not a good indicator of ethanol yield within samples from the same site. Davis-Knight and Weightman (2008) also suggested that TGW may not be an indicator of ethanol yield when agronomic factors rather than genetic factors affect the weight of the grain.

Grains which vary in shape and size possibly have a different bran to endosperm ratio, and thus vary in starch concentration and consequently in ethanol yield. Taylor and Roscrow (1993) and Swanston *et al*. (2005 and 2007) reported a good correlation between length to width ratio and ethanol yield. Similarly in the current study, grain diameter appeared to be a good indicator of ethanol yield even better than L:W ratio. However, grain diameter showed a strong correlation with TGW and thus these two parameters can be used alternatively but not together.

The HI of these samples showed a very poor correlation with ethanol yield. This agrees with the report of Taylor *et al*. (1993). However the varieties included in this study are mainly soft wheat varieties. Including hard wheat varieties, to give a much wider range of HI is required in order to draw a clearer conclusion on the relationship between ethanol yield and grain hardness.

It is not clear why starch was a poorer indicator of ethanol yield than grain nitrogen concentration, but due to the ease and cheapness of determining grain nitrogen concentration compared with starch concentration, the better predictive potential of nitrogen concentration is more useful. Grain nitrogen can be determined by NIR more accurately than starch which allows fast determination of many samples (Smith *et al.,* 2006). The other interesting fact behind the relationship between grain nitrogen concentration and ethanol yield is that grain nitrogen concentration can be manipulated by nitrogen fertiliser application (Kindred *et al.,* 2008). Less nitrogen application not only gives higher ethanol yield per tonne through its effect on grain nitrogen but also reduces greenhouse gas emission associated with nitrogen fertilizer production and application. About 50% of greenhouse gas emission associated with wheat production is from nitrogen fertilizer (Simmonds, 1995). Grain nitrogen concentration also has an inverse relationship with grain yield per hectare (Weightman *et al.,* 2008). This implies that high grain yielding wheat is also higher ethanol yielding. Kindred *et al*. (2008) suggested that the prediction potential of grain nitrogen can be explained by its inverse relationship with grain starch concentration. However, in this study, there was no correlation between grain nitrogen and starch concentration confirming the finding of Weightman *et al*. (2008). In the study of Kindred *et al*. (2008) a multiple linear regression based on grain starch concentration and nitrogen concentration explained 69.7% of the variance but in this study a regression based on these two parameters explained only 54.8%, not much more than nitrogen alone (53%).

In general, the results indicated that variation in ethanol yield and the relationships between grain parameters and ethanol yield was more controlled by the environment than variety. Grain shape and size parameters such as TGW and diameter were the only parameters affected by variety. All the varieties gave fairly good ethanol yield regardless of site and year. The highest ethanol yield (455 l/tonne) was achieved from variety Consort and the least ethanol yield obtained was from Variety Deben (407 l/tonne). However, among the variety means, Istabraq was the highest (444 l/tonne) and CPBT W96 was the least (435 l/tonne). The relatively low varietal difference observed here could be because these varieties are already on the national

recommended variety list for distilling quality and have thus been selected for relatively good distilling quality. When comparing with other cereals, the best ethanol yield obtained from this study is less than what can be obtained from maize (475 l/tonne), sorghum (475 l/tonne) and millet (462 l/tonne) (Agu *et al.,* 2006). However, the least ethanol yield obtained from this study was better than what can be achieved from other cereals such as rye (364 l/tonne) and oats (317 l/tonne) (El Bassam, 2010). Comparable ethanol yield was also obtained from triticale by Davis-Knight and Weightman (2008).

Some sites are especially good for producing high ethanol yielding wheat, but to understand this, further study would be needed to relate the environmental characteristics of site to ethanol yield. None of the single parameters studied showed a very good relationship with ethanol yield therefore combining different parameters would be useful. To this effect, the current quality criteria of the potable alcohol industry, specific weight and grain nitrogen is compared with the result of this analysis. An important finding of this study is that assessing TGW as grain quality, together with grain nitrogen, gives a better prediction of ethanol yield than specific weight and grain nitrogen. It appears that TGW should replace the norm of measuring specific weight for distilling quality. In addition, if site and variety could be considered during purchasing they could help to predict ethanol yield more precisely. A combination of many more parameters such as starch, length, perimeter and packing efficiency could improve the prediction power. However, from the point of view of time, cost and effort required to measure these parameters, it may not be worth and or feasible including them.

7. Field experiment

7.1 Introduction and objectives

The study which is reported in the previous chapter was conducted on a wide range of samples consisting of several varieties grown on various sites in two consecutive years. That experiment showed some promising relationships between some grain features and ethanol yield. Some of the grain features gave consistent results with previous findings and others did not. Moreover, there has been huge variation in ethanol yield between sites which could make the relationship between these traits and ethanol yield vague.

This chapter describes a study where, a field experiment was conducted on one location to grow a wide range of grain quality by deliberately manipulating the crop husbandry and environment. The aim of this study was to grow seeds with a range of grain quality from very poor to the best quality, to study the relationships between grain quality traits and grain ethanol yield and to develop a model, which predicts grain potential ethanol yield. The two experiments (RL samples and field experiment) have the same aim but use a different approach. However, the field experiment data analysis was modified based on the result of the RL samples, for eg, NSP was included on the field experiment study and traits such as density, packing efficiency, upper quartile, lower quartile (single grain distribution within a lot) were not measured during the field experiment specially the second year.

7.2 Materials and Methods

The summer weather is known to be the major determinant of grain quality (Kettlewell *et al*., 2003). Brocklehurst *et al.* (1978) showed that shading reduces grain filling and results in low grain weight. Shading, representing very cloudy weather, affects chemical composition and size of the grain. According to Atkinson *et al*., (2005) subsequent dry and wet weather after grain filling causes weathering of the grain which changes volume and shape of the grain but not weight of the grain. Therefore shade and irrigation treatments have been introduced in this study to simulate good and bad summer weather conditions. The shade treatment was applied

during the grain filling period Zadoks *et al.,* (1974) growth stage system (GS) 69 to 83) so as to affect the starch accumulation in the grain (Figure 12). Overhead irrigation treatment was also applied during grain ripening starting from GS87 and this was expected to cause grain weathering which is common in wet summer conditions. The treatments also comprise covering of some plots with polythene to keep rain off during grain ripening (Figure 13). This polythene treatment is expected to give the best grain quality as the starch accumulation was not affected by shade nor it is exposed to weathering from irrigation and rain. Some of the plots were also shaded as well as irrigated to obtain poorly filled and weathered grain. The control treatment was also included with no shading, polythene cover or irrigation. In order to study the genotypic effect on all these variations, two soft wheat varieties were used (Table 6). Varieties Glasgow and Deben are known in the potable alcohol industry for their high and poor alcohol yielding potential respectively (HGCA-AHDB, 2008).

Table 6 Treatment details of the field experiment in year 2007/08

Treatment number	Treatment description
1	Glasgow untreated
2	Glasgow covered with clear polythene from GS 87
3	Glasgow shaded from GS 69 to GS 83
4	Glasgow overhead irrigated from GS 87 for 2 weeks
5	Glasgow shaded and irrigated
6	Deben untreated
7	Deben covered with clear polythene from GS 87
8	Deben shaded from GS 69 to GS 83
9	Deben overhead irrigated from GS 87 for 2 weeks
10	Deben shaded and irrigated

Figure 12 Shaded and unshaded plots of the field experiment at Harper Adams University. Date taken 16/7/2008 at GS71

The field experiment was repeated in 2008/9 with some amendments. During the second year everything was carried out the same but two different rates of nitrogen fertilizer were applied as factorial design to add to the crop husbandry factors creating variation of the grain characteristics (Table 7). As the first year resulted in a wide gap of grain quality between shaded and unshaded treatments, the shading time was also narrowed down during the second year (from the Zadoks growth stage GS 71 to GS 77).

Table 7 Treatment details of the field experiment in year 2008/09

Main plot	Sub plot	Main plot treatment description
1	N1*	Glasgow untreated
	N2**	
2	N1	Glasgow covered with clear polythene from GS 87
	N2	
3	N1	Glasgow shaded from GS71 to GS 77
	N2	
4	N1	Glasgow overhead irrigated from GS 87 for 2
	N2	weeks
5	N1	Glasgow shaded and irrigated
	N2	
6	N1	Deben untreated
	N2	
7	N1	Deben covered with clear polythene from GS 87
	N2	
8	N1	Deben shaded from GS71 to GS 77
	N2	
9	N1	Deben overhead irrigated from GS 87 for 2 weeks
	N2	
10	N1	Deben shaded and irrigated
	N2	

N1* recommended rate N2** reduced rate

7.2.1 Experimental design

The field experiment was conducted at Harper Adams University College, Shropshire UK in 2007/08 and 2008/09. In 2007/08 the experiment was conducted on Birds Nest field. The site had grown grass the previous year. The second year experiment was conducted on the adjacent Horse Foxhole field which also had grass in the previous year. The soil type was sandy clay loam. The trial consisted of a randomized factorial design comprising seven blocks

and ten treatments in the first year and 20 treatments in the second year. The main plot size was 12 x 1.8 m and the sub-plot size was 6 x 1.8 m.

Figure 13 Polythene covered and uncovered plots of the field experiment after the polythene cover was removed. Date taken 26/08/2008 at GS91

7.2.2 Crop husbandry

Planting was conducted in October each year using a seed rate of 300 seeds m^{-2}. The trial was sprayed with herbicide, insecticide and fungicide following the standard practice, to keep the crop free from weeds, pest and diseases. Soil nitrogen content was analysed at Eurofins Laboratory, Wolverhampton, UK, from a soil sample taken in March each year before nitrogen fertilizer application. Based on the available nitrogen in the soil, nitrogen fertilizer was adjusted to a total of 180 kg ha^{-1} soil available plus applied nitrogen (Table 8).

Table 8 Rate and stage of nitrogen fertilizer application for the two years field experiments in kg ha^{-1}

Year 2008		Year 2009	
Growth stage	Rate	Growth stage	Rate
GS 25	42.4	GS 29	40.5
GS 32	42.5	GS 31	45
GS 37	40	GS 39	N1* = 41.5, N2** = 20.75
Total applied	124.9	N1 = 127, N2 = 106.25	
Soil available	55.1	53	

N1* recommended rate N2** reduced rate

Harvesting was conducted using a combine harvester (Nurserymaster, Wintersteiger, Ried, Austria). One kg of subsample was taken from each plot for grain quality and ethanol yield analysis. The analysis conducted on this grain were, density and packing efficiency (only first year) specific weight, thousand grain weight, grain starch, nitrogen and NSP concentration, grain size (width, length, perimeter, surface area, length to width ratio grain roundness), HI and predicted ethanol yield. The methodology for all of these except that of specific weight, NSP and predicted ethanol yield were described in chapter three.

7.2.3 Specific weight

Specific weight was measured using a chondrometer. A chondrometer measures bulk density of the grain expressed as kg/hl.

7.2.4 Predicted ethanol yield (PEY)

Due to resource constraints, it was not possible to conduct laboratory ethanol yield measurement on these samples. Watson (2010) compared the laboratory ethanol yield and the NIR predicted ethanol yield of samples of the same variety grown in the same year in the adjacent field to this experiment. His results indicated that the NIR method can give a good prediction of the actual ethanol yield (Watson*, et al.,* 2010). Therefore the NIR prediction was used for this analysis, Ethanol yield of each sample was predicted using NIR at the SWRI. The NIR machine was calibrated to measure ethanol yield referenced by the laboratory

ethanol distilling ‘wheat cook’ method using 933 samples collected all over the UK for seven consecutive years (Sylvester-Bradley *et al.,* 2010). The machine emits near infra-red light on the samples and the amount of light absorbed by the samples is proportional to ethanol yield (Smith *et al.,* 2006). The machine takes about a kilogram of whole grain wheat and produces an expected ethanol yield based on average of ten subsamples. This method has been used within the Scottish grain distilleries for commercial purposes (Agu R., Personal communication).

7.2.5 Non-starch polysaccharide

Non-starch polysaccharide was measured at the laboratory of Englyst Carbohydrates Ltd Southampton, UK using Englyst Fiberzym kit, Measurement of dietary fibre as NSP by colorimetry. This method determines total, soluble and insoluble dietary fibre as NSP. The procedure involves removal of starch by enzymic digestion and hydrolysis and measurement of NSP. The method is described in detail by Englyst *et al*. (1994).

7.2.6 Statistical analysis

Statistical analysis was performed using Genstat Discovery edition 4. Firstly, a two-way F test was conducted to check the variance of the two years experiments. The analysis indicted that the residual mean squares of the two experiments were significantly different for thousand grain weight (F=0.03), specific weight, width, grain roundness and length to width ratio at F<0.001 while there is no significant difference for PEY, HI, nitrogen, NSP, starch, grain length, grain perimeter and grain surface area. Therefore the two years data was combined only for those traits which did not show significant differences in variance on the two-way F test. Summary of the two years results are detailed in Appendix 1 and 2.

Analysis variance was applied in order to check the effect of variety, agronomic treatments and nitrogen fertilizer rate.

Pearson's correlations and regression analysis were used to study the relationships between PEY and the measured grain traits. Multiple linear regression was also used to predict EY based on the grain traits. Year, variety, fertilizer rate and shade effect (shaded versus not shaded) were used as a group to understand their impact and identify the best fit model. For each relationship between PEY and a grain trait, initially a common model based on all the data was tested. Next, each group was added one by one to check if they could improve the explained variance. Finally, a common model or separate model with common slope but different intercept or separate models with different slopes were fitted for each relationships based on the changes in the explained variance. Similar regression analysis was also conducted for grain nitrogen concentration with the other grain traits in order to check if they are better or worse than their relationship with PEY.

7.2.7 Weather data

Weather data was obtained from Harper Adams University College's weather station. The data shows that 2009 summer had higher rainfall and less solar radiation than 2008; however the maximum and minimum temperatures were almost the same in both years (Table 9). The maximum and minimum temperatures for both years are almost the same as the long term average of England from 1981-2010 which is 19.6°C and 10.2°C maximum and minimum respectively. However, the average rainfall recorded for the same period in England (58.3 mm) is much less than recorded in 2008 and 2009 at Harper Adams weather station.

Table 9 2008 and 2009 summer weather data of Newport, Shropshire, UK

	2008				2009			
Month	Max temp (ºC)	Min temp (ºC)	Rainfall (mm)	Solar energy (MJ)	max temp (ºC)	min temp (ºC)	Rainfall (mm)	Solar energy (MJ)
May	18.2	9.1	47.5	509.2	17.5	7.2	50.2	233.5
June	19.2	9.0	35.2	532.3	20.4	9.7	92.2	163.2
July	21.4	12.0	94.4	297.8	21.0	11.5	110.6	204.2
August	20.6	12.8	83.6	199.2	21.7	11.8	37.8	88.1
total	79.4	42.9	260.7	1538.5	80.6	40.2	290.8	689
Mean	19.9	10.7	65.2	384.6	20.1	10.0	72.7	172.2

7.3 Results

7.3.1 Analysis of variance and regression analysis

Analysis of variance was used to check the effectiveness of the treatments in creating variation between samples. Summary of the results are presented in Tables 10, 11 and 12.

Table 10 Summary of the analysis of variance of both years data combined for variables that the two years data could be combined based on the two-way F-test.

Variety	PEY l/tonne	HI	N %	NSP %	Starch %	Length mm	Perimeter mm	Area mm
Glasgow	431.2	15.13	2.093	8.852	66.10	5.7416	13.757	11.514
Deben	428.3	37.84	2.086	9.085	64.40	6.0500	14.430	12.450
F	NS	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment								
con	440.1^{B}	17.13^{AB}	1.923^{A}	8.971	67.83^{A}	6.0922^{B}	14.629^{A}	13.128^{A}
poly	432.0^{B}	19.52^{B}	2.038^{A}	9.246	67.46^{A}	6.0891^{B}	14.582^{A}	12.841^{B}
sha	418.5^{A}	40.47^{C}	2.285^{B}	9.292	61.54^{B}	5.6300^{A}	13.298^{B}	10.281^{C}
irr	437.7^{B}	16.49^{A}	1.944^{A}	8.997	68.03^{A}	6.0762^{B}	14.678^{A}	13.302^{A}
Irr & sha	420.5^{A}	38.82^{C}	2.256^{B}	9.447	61.39^{B}	5.5913^{A}	13.281^{B}	10.360^{C}
F	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001
Year								
2007/2008	428.7	30.94	2.091	9.085	63.60	6.1069	14.309	11.598
2008/2009	430.3	24.26	2.089	9.243	66.07	5.7902	13.986	12.174
F	NS	<0.001	NS	NS	<0.001	<0.001	<0.001	<0.001

Con= untreated, poly= polythene covered, sha= shaded, irr= overhead irrigation, irr & sha= shaded and overhead irrigation applied

Table 11 Summary of analysis of variance of the first year experiment for variables that the two years data could not be combined

Variety	Density (g/c^3)	Packing efficiency	Specific weight (Kg/hl)	TGW (g)	Length to width ratio	Grain roundness	Width (mm)
Glasgow	1.383	46.44	72.55	36.66	2.33	17.79	2.58
Deben	1.385	45.57	70.73	42.19	2.32	17.83	2.72
F	NS	0.012	<0.001	<0.001	NS	NS	<0.001
Treatment							
con	1.359^{A}	49.22^{C}	74.95^{B}	48.12^{A}	2.18^{AB}	17.02^{A}	2.88^{C}
poly	1.403^{B}	47.05^{B}	78.75^{C}	46.68^{A}	2.23^{B}	17.62^{B}	2.82^{B}
sha	1.405^{B}	42.27^{A}	65.56^{A}	27.44^{B}	2.51^{C}	18.82^{C}	2.31^{A}
irr	1.354^{A}	49.51^{C}	74.25^{B}	47.81^{A}	2.16^{A}	16.93^{A}	2.90^{C}
Irr and sha	1.399^{B}	41.97^{A}	64.66^{A}	27.06^{B}	2.51^{C}	18.71^{C}	2.33^{A}
F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Con= untreated, poly= polythene covered, sha= shaded, irr= overhead irrigation, irr & sha= shaded and overhead irrigation applied

Table 12 Analysis of the variance of the second year data for variables that the two years data could not be combined

Variety	TGW (g)	Specific weight (kg/hl)	Width (mm)	Grain roundness	Length to width ratio
Glasgow	37.64	74.15	2.8286	15.956	2.00
Deben	39.95	70.45	2.8666	16.317	2.07
F	<0.001	<0.001	0.001	<0.001	<0.001
Treatment					
con	45.55^{A}	74.63^{A}	2.9918^{AB}	15.962^{A}	2.004^{A}
poly	44.87^{A}	74.81^{A}	2.9521^{B}	16.064^{AB}	2.030^{A}
sha	29.64^{B}	69.83^{B}	2.5990^{C}	16.512^{B}	2.124^{B}
irr	44.75^{A}	73.17^{A}	3.0415^{A}	15.854^{A}	1.965^{C}
Irr and sha	29.18^{B}	69.06^{B}	2.6537^{C}	16.289^{B}	2.062^{D}
F	<0.001	<0.001	<0.001	<0.001	<0.001
N Fertilizer					
Reduced	38.31	72.18	2.8384	16.154	2.0409
Full rate	39.29	72.41	2.8568	16.119	2.0003
F	NS	NS	NS	NS	NS

Con= untreated, poly= polythene covered, sha= shaded, irr= overhead irrigation, irr & sha= shaded and overhead irrigation applied

There was significant difference between the two varieties in everything except the PEY and nitrogen concentration in both years and in density, L:W ratio and grain roundness during the first year (Table 10-12). Deben is significantly higher than Glasgow in all grain size and weight measurements. Deben has also significantly higher NSP and HI. Glasgow has significantly higher starch concentration, specific weight and packing efficiency (Table 10 -12).

The agronomic treatments did not cause any significant difference in NSP. In the case of PEY, nitrogen, starch, grain length, grain perimeter, TGW, and specific weight (only second year for specific weight) the shading and irrigation treatments created significant difference only between shaded and un-shaded plots. However the shading and irrigation treatments caused more significant variations in HI, specific weight, packing efficiency, grain surface area, grain roundness, grain width and length to width ratio (Table 10 - 12).

The nitrogen fertilizer rate did not cause significant difference in almost all the traits including PEY. There was also significant difference between the two years in most of the traits. Since the treatments were introduced only to create variation between samples, the interaction effects were not studied (Table 10 - 12).

The regression results are also summarized in Table 13 to 15 below. Only major relationships are presented in graphs.

Table 13 Regression result of PEY and N with each of the grain traits both years' data combined.

	PEY (l/tonne)					N (%)				
				Parameter estimate					Parameter estimate	
Grain trait	Overall R^2	Overall *P*	Overall standard deviation	value	*p*	Overall R^2	Overall *p*	Overall standard deviation	*value*	*p*
Starch	16.3	<0.001	19.7	1.97	<0.001	16.8	<0.001	0.291	-0.0297	<0.001
Year included	28.8	<0.001	18.2	-3.815	<0.001	17.7	NS	0.290	0.0807	NS
Shade included	20.8	0.05	19.2	2.386	0.005	26.0	<0.001	0.275	-0.0402	<0.001
Variety included	15.9	NS	19.7	-0.52	NS	17.3	NS	0.290	0.0621	NS
Nitrogen	88.4	<0.001	7.31	-63.23	<0.001					
Variety included	88.9	0.001	7.15	3.227	<0.001					
Shade included	88.6	0.019	7.23	2.72	0.019					
Year included	88.4	NS	7.31	1.31	NS					
NSP	1.0	NS	21.4	-2.44	NS	2.2	0.018	0.316	0.0495	0.018
Variety included	0.6	NS	21.5	1.34	NS	1.7	NS	0.317	0.282	NS
Shade included	14.8	<0.001	19.9	-16.69	<0.001	21.5	<0.001	0.283	-0.2910	<0.001
Year included	0.7	NS	21.5	1.96	NS	1.7	NS	0.317	-0.0042	NS
Area	17.9	<0.001	19.5	5.97	<0.001	26.2	<0.001	0.275	-0.1072	<0.001
Variety included	22.0	<0.001	19.0	9.50	<0.001	28.2	NS	0.271	0.0178	NS
Shaded included	21.9	<0.001	19.1	12.98	<0.001	29.1	0.001	0.269	-0.1663	0.001
Year included	24.2	<0.001	18.8	-7.61	<0.001	34.6	<0.001	0.259	0.1220	<0.001
Length	5.7	<0.001	20.9	15.9	<0.001	12.9	<0.001	0.299	-0.3465	<0.001
Year included	14.4	<0.001	19.9	-40.2	<0.001	25.8	<0.001	0.275	0.751	<0.001
Variety included	9.5	0.002	20.5	9.99	0.002	15.5	0.007	0.294	-0.1260	0.007
Shade included	16.4	0.008	19.7	-30.8	0.008	22.5	0.012	0.0282	0.423	0.012
Perimeter	11.8	<0.001	20.3	9.53	<0.001	20.8	<0.001	0.2850	-0.1858	<0.001
Year included	19.4	<0.001	19.4	-15.92	<0.001	30.9	<0.001	0.266	0.2762	<0.001
Variety included	17.3	<0.001	19.6	11.51	<0.001	24.7	<0.001	0.277	-0.1455	<0.001
Shade included	14.3	0.008	20.0	-13.55	0.008	22.2	0.030	0.282	0.1569	0.030

Table 13 continued

Grain trait	PEY (l/tonne)					N (%)				
	Overall R^2	Overall *P*	Overall standard deviation	Parameter estimate		Overall R^2	Overall *p*	Overall standard deviation	Parameter estimate	
				value	*p*				*value*	*p*
HI	9.6	<0.001	20.4	-0.41	<0.001	8.8	<0.001	0.305	0.0058	<0.001
Year included	19.9	<0.001	19.2	0.959	<0.001	14.0	<0.001	0.296	-0.01043	<0.001
Variety included	13.6	0.001	19.9	-12.05	0.001	17.6	<0.001	0.290	0.2595	<0.001
Shade included	14.7	<0.001	19.8	-13.14	<0.001	21.4	0.036	0.283	0.00645	0.036

Table 14 Regression result of PEY and N with each of the other grain traits only for the second year data

	PEY (l/tonne)					N (%)				
				Parameter estimate					Parameter estimate	
Grain trait	Overall R^2	Overall *P*	Overall standard deviation	value	*p*	Overall R^2	Overall *p*	Overall standard deviation	value	p
TGW	4.9	0.005	21.0	0.591	0.005	8	<0.001	0.299	-0.0107	<0.001
Fertilizer included	4.3	NS	21.1	1.75	NS	7.5	NS	0.030	-0.0280	NS
Variety included	4.3	NS	21.1	1.61	NS	7.6	NS	0.299	-0.0340	NS
Shade included	9.2	0.007	20.5	21.88	0.007	9.4	NS	0.296	-0.2060	NS
Specific weight	1.2	NS	21.3	0.738	NS	1.9	NS	0.305	-0.0125	NS
Fertilizer included	0.6	NS	21.4	1.58	NS	1.4	NS	0.306	-0.0247	NS
Variety included	1.2	NS	21.3	-3.91	NS	5.3	0.018	0.300	0.0339	0.018
Shade included	0.5	NS	21.4	-0.99	NS	2.3	NS	0.304	0.0784	NS
Width	3.8	0.013	21.2	22.68	0.013	7.8	<0.001	0.300	-0.450	<0.001
Fertilizer included	3.2	NS	21.2	1.59	NS	7.2	NS	0.300	-0.0245	NS
Variety included	6.6	0.017	20.9	-44.2	0.017	12.9	0.002	0.291	0.788	0.002
Shade included	7.4	0.013	20.8	21.33	0.013	10.1	0.035	0.296	-0.255	0.035
Grain roundness	2.4	0.039	21.3	-9.63	0.039	3.8	0.013	0.306	0.1675	0.013
Fertilizer included	1.8	NS	21.4	1.67	NS	3.3	NS	0.307	-0.0266	NS
Variety included	15.2	<0.001	19.9	54.8	<0.001	17.7	<0.001	0.283	-0.818	<0.001
Shade included	1.7	NS	21.4	-0.21	NS	3.8	NS	0.306	0.0645	NS
Length:width ratio	2.2	0.044	21.4	-42.6	0.044	3.6	0.014	0.306	0.746	0.014
Fertilizer included	1.7	NS	21.4	1.93	NS	3.1	NS	0.307	-0.0299	NS
Variety included	9.8	<0.001	20.5	187.3	<0.001	11.7	<0.001	0.293	-2.777	<0.001
Shaded included	1.5	NS	21.4	-1.19	NS	3.9	NS	0.306	0.0758	NS

Table 15 Regression result of PEY and N with each of the other grain traits only for the first year data

Grain trait	PEY (l/tonne)					N (%)				
	Overall R^2	Overall *P*	Overall standard deviation	Parameter estimate		Overall R^2	Overall *p*	Overall standard deviation	Parameter estimate	
				value	*p*				value	p
TGW	72.2	<0.001	11.4	1.725	<0.001	84.9	<0.001	0.1310	-0.02909	<0.001
Variety included	94.9	<0.001	4.87	0.889	<0.001	93.1	<0.001	0.0888	-0.01424	<0.001
Shaded included	91.5	<0.001	6.30	1.798	<0.001	94.6	<0.001	0.0784	-0.03672	<0.001
Specific weight	81.4	<0.001	9.32	3.332	<0.001	79.8	<0.001	0.152	-0.05139	<0.001
Variety included	86.2	<0.001	8.03	1.663	<0.001	88.8	<0.001	0.113	-0.02626	<0.001
Shade included	90.1	<0.001	6.82	-31.93	<0.001	91.6	<0.001	0.0979	0.5781	<0.001
Width	76.5	<0.001	10.5	66.41	<0.001	88.7	<0.001	0.113	-1.1147	<0.001
Variety included	95.6	<0.001	4.52	17.58	<0.001	93.5	<0.001	0.0860	-0.2877	<0.001
Shade included	90.2	0.010	6.77	46.7	0.010	94.8	<0.001	0.0770	-1.000	<0.001
Grain roundness	79.4	<0.001	9.82	-22.50	<0.001	77.5	<0.001	0.160	0.3464	<0.001
Variety included	82.0	0.002	9.18	7.22	0.002	77.8	NS	0.159	0.0551	NS
Shade included	90.3	<0.001	6.74	-32.17	<0.001	91.7	<0.001	0.0970	0.5695	<0.001
Length:width ratio	88.0	<0.001	7.49	-119.92	<0.001	88.1	<0.001	0.116	1.8848	<0.001
Variety included	93.0	0.004	5.70	-24.18	0.004	88.9	0.021	0.113	0.390	0.021
Shade included	90.5	<0.001	6.64	-24.35	<0.001	92.0	<0.001	0.0954	0.4598	<0.001
Density	33.6	<0.001	17.6	-489.4	<0.001	29.3	<0.001	0.283	7.25	<0.001
Variety included	42.5	0.010	16.4	-419	0.010	34.0	0.012	0.274	6.96	0.012
Shade included	89.5	<0.001	7.02	-39.66	<0.001	91.3	<0.001	0.0992	0.6467	<0.001
Packing efficiency	81.7	<0.001	9.26	5.387	<0.001	76.2	<0.001	0.164	-0.08111	<0.001
Variety included	82.1	NS	9.15	3.54	NS	78.7	0.004	0.155	0.1130	0.004
Shaded included	91.6	<0.001	6.27	-28.90	<0.001	92.3	0.038	0.0936	-0.0311	0.038

7.3.2 Major chemical components of the grain

There was a significant positive relationship between PEY and starch concentration ($p<0.001$). However it explained only 16% of the variation in ethanol yield (Figure 14a). Fitting separate lines for years has improved the explained variance by 13%. Fitting separate lines for varieties was not required as there was no improvement to the explained variance. Adding the shade effect as a group improved the explained variance by 4% ($p<0.05$). There was also significant negative relationship between starch and nitrogen concentration. (Figure 14b). As depicted in the Figure, there is distinct cluster of data which is created by the shade effect. Therefore the shaded and not shaded plots were analysed separately. The result indicated that there is no significant relationship between starch and nitrogen concentration or ethanol yield when not shaded plots were analysed alone. However there is significant relationship between starch and ethanol yield (R^2=19.3) and between starch and nitrogen concentration (R^2=17.8) when shaded plots were analysed alone.

Nitrogen concentration showed a strong negative correlation with PEY explaining 88% of the variation accounted ($p<0.001$). The equation for this model is EY=-63.23N+561.89 (Figure 15a). There was no need to fit separate lines for each year as there were no changes in the explained variance while including year to the model (Table 13). The statistics has justified fitting separate lines for each variety. Variety Glasgow has given a better ethanol yield at any nitrogen level than variety Deben (Figure 15b). Fitting separate lines for shaded and not shaded samples improved the explained variance by 0.2% which was significant at $p=0.019$.

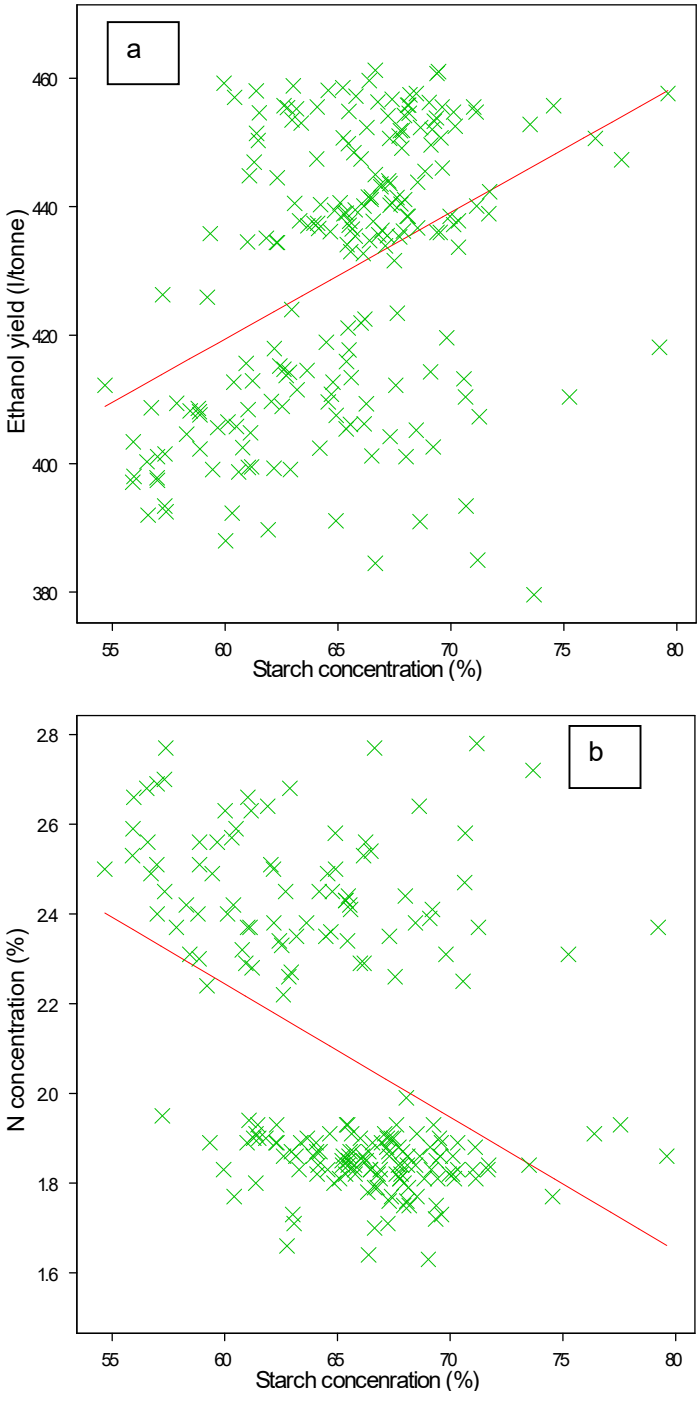


Figure 14 The relationship between a) PEY and starch concentration b) starch and N concentration (both years data combined) line represents best fit simple linear regression equation the values of which are indicated on Table 13

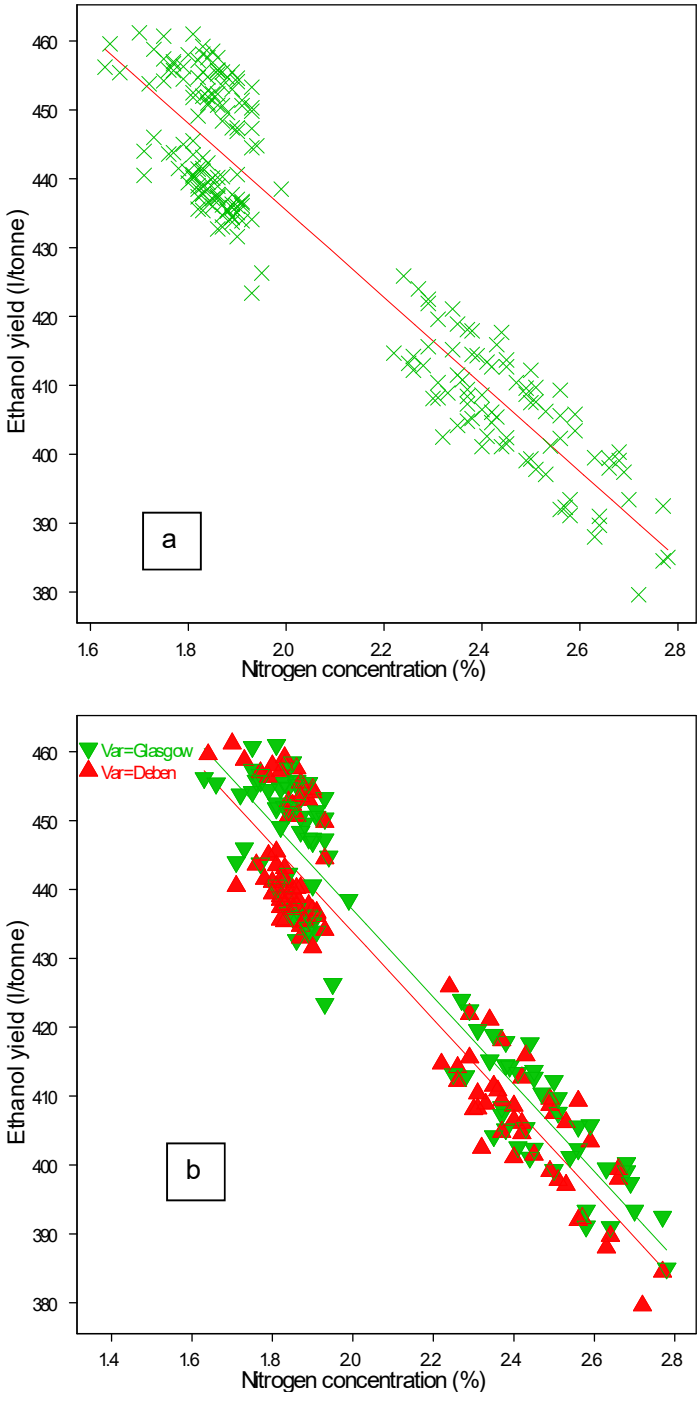


Figure 15 The relationship between PEY and grain nitrogen concentration (a) single model for all data from both years (b) separate lines fitted for each variety based on all data from both years

There was negative weak correlation between PEY and NSP. However the regression was not significant. Fitting separate model for year and variety, did not make the regression significant. However, fitting separate parallel line for shaded and not shaded plots makes the relationship significant ($p<0.001$) and explains 14.8 % of the variation in ethanol yield (Figure 16).

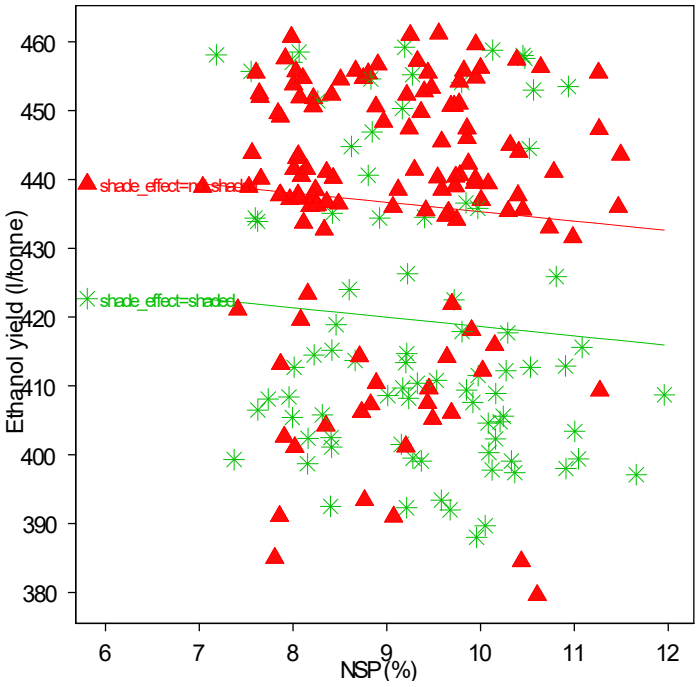


Figure 16 The relationship between PEY and NSP separate parallel lines fitted for shaded plots (*) and not shaded plots (▲), data is from both years combined.

Only the second year data was used to fit separate lines for the two fertilizer rates for the relationships between PEY and starch, PEY and nitrogen and PEY and NSP but the relationships were not significant in all the cases.

7.3.3 Density of the grain

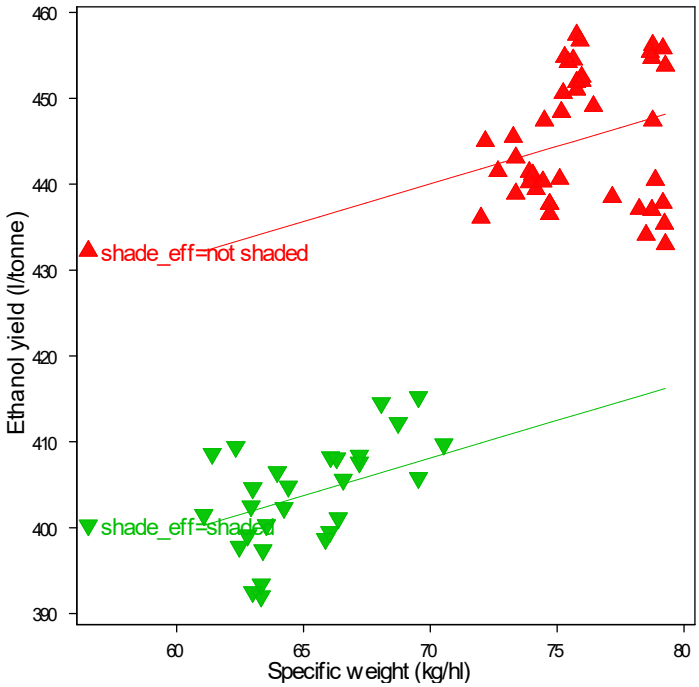
The two-way F test indicated that there is significant difference between the residual mean squares of the two years experiments specific weight data therefore the two years data were analysed separately. The first year specific weight data's minimum, maximum, average, median and standard deviation is 61.1, 79.3, 71.6, 73.9 and 5.8. Whereas the second year

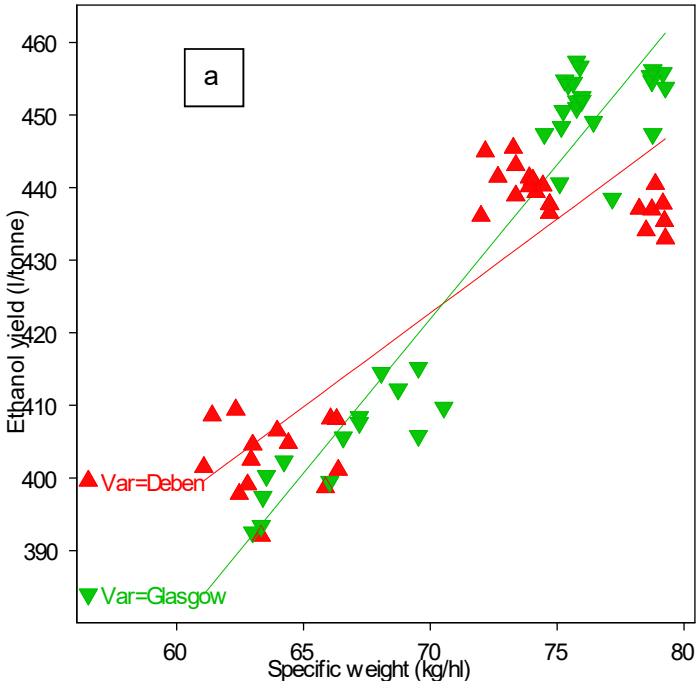
specific weight data's minimum, maximum, average, median and standard deviation is 56.8, 79.4, 72.4, 72.9, 4.0.

There was a significant positive relationship between specific weight and PEY only during the first year. During the first year, the variation in specific weight explained about 81% of the variation in PEY. Adding variety and shade effect as a group also improved the explained variance as indicated in Table 15 and Figure 17 a & b. However, during the second year this relationship was not significant. Moreover, adding variety, fertilizer rate and shade effect as a group did not make the relationship significant. The reason behind lack of relationship between specific weight and PEY during the second year is that most of the samples during the second year had a high specific weight. Therefore the higher specific weights and the smaller specific weights from both years were analysed separately.

This result showed that the relationship between specific weight and PEY is stronger at lower specific weight levels (Figure 17 c & d).

There was significant negative relationship between grain density and PEY. Density alone explained 33% of the variation in ethanol yield. The equation for this model is EY = -489.4D + 1106. Separate lines with different slopes were fitted for variety Deben and Glasgow as the explained variance showed 9% improvement than a single model for both varieties. Adding the shade effect as a group improved the variance accounted by 55%. As this data is only from one year, year and fertilizer rate were not used as a group.


460
450
440
430
420
410
400
390
Ethanol yield (l/tonne)
shade_eff=not shaded
shade_eff=shaded
60
65
70
75
80
Specific weight (kg/hl)


a
460
450
440
430
420
410
400
390
Ethanol yield (l/tonne)
Var=Deben
Var=Glasgow
60
65
70
75
80
Specific weight (kg/hl)

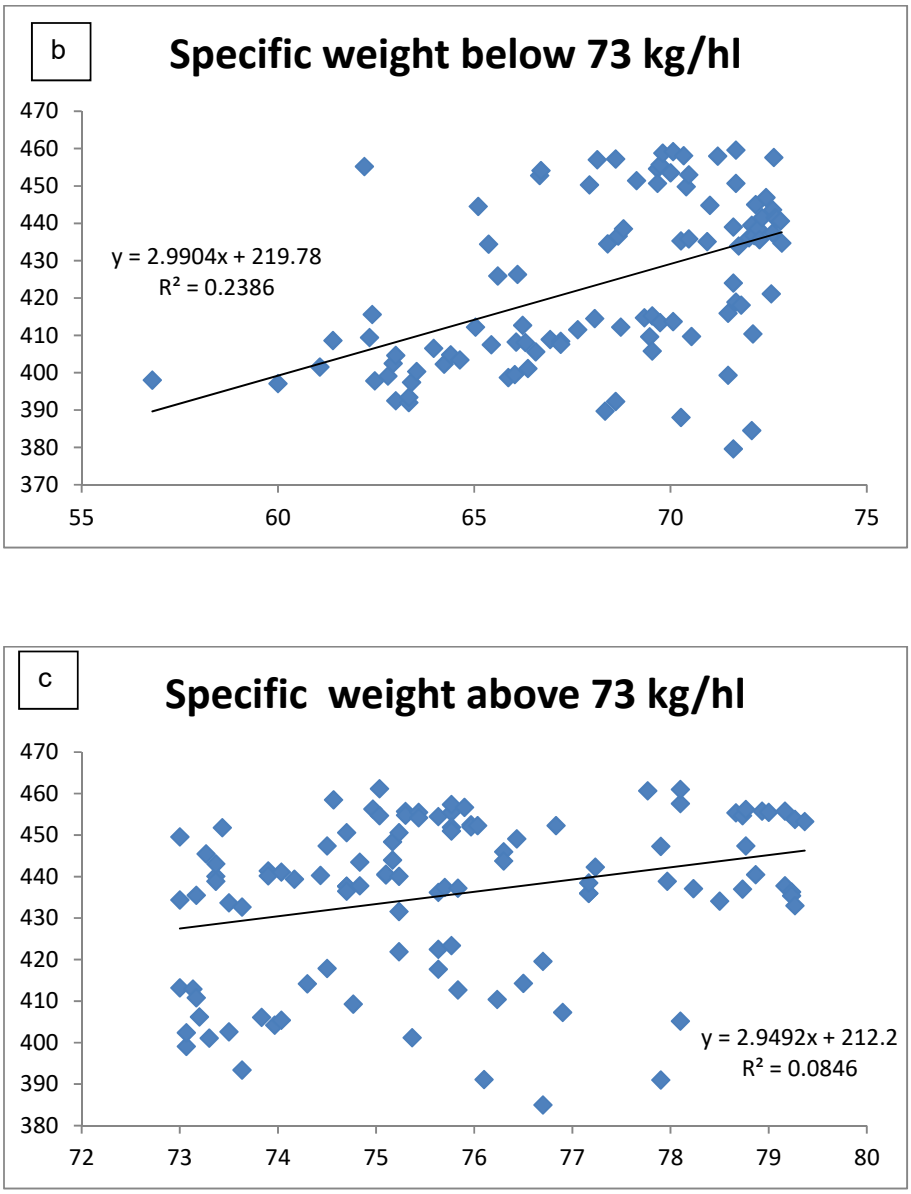


Figure 17 The relationship between PEY and specific weight (a) two parallel lines fitted for shaded and not shaded plots (b) two separate non parallel lines fitted for the two varieties (c) when specific weight is below 73 kg/hl (d) when specific weight is above 73 kg/hl.

Packing efficiency (PE) explained 82% of the variation in ethanol yield ($p<0.001$). The equation for this model is EY = 5.4PE + 180.9. Adding variety to the model has not improved the explained variance. However, adding the shade effect improved the explained variance by

10%. The explained variance of packing efficiency looks much higher than others; however the reason is that packing efficiency was measured only during the first year.

Grain density was not measured during the second year due to its relative poor relationship with ethanol yield as compared to others during the first year. Packing efficiency also was not analysed during the second year because it is the derivative of specific weight and it does not predict ethanol yield better than specific weight during the first year.

7.3.4 Grain weight

Like specific weight, the two years TGW data also cannot be combined. Both years, the relationship between TGW and ethanol yield was significant but the variations accounted due to TGW were highly different between the two years (Tables 14 and 15). During the second year adding variety and fertilizer rate as a group did not add to the explained variance. However, during the first year, variety as a group has a significant effect. The shade effect improved the explained variance during both years.

The figures shown below are from the first year. Figure 18a indicates that variety Glasgow gives better ethanol yield than Deben at any TGW levels however, the yield difference is higher at higher TGW levels. On Figure 18b the lines fitted for shaded and not shaded plots show opposite direction. This is because Deben combines higher TGW with relatively lower ethanol yield as compared to Glasgow.

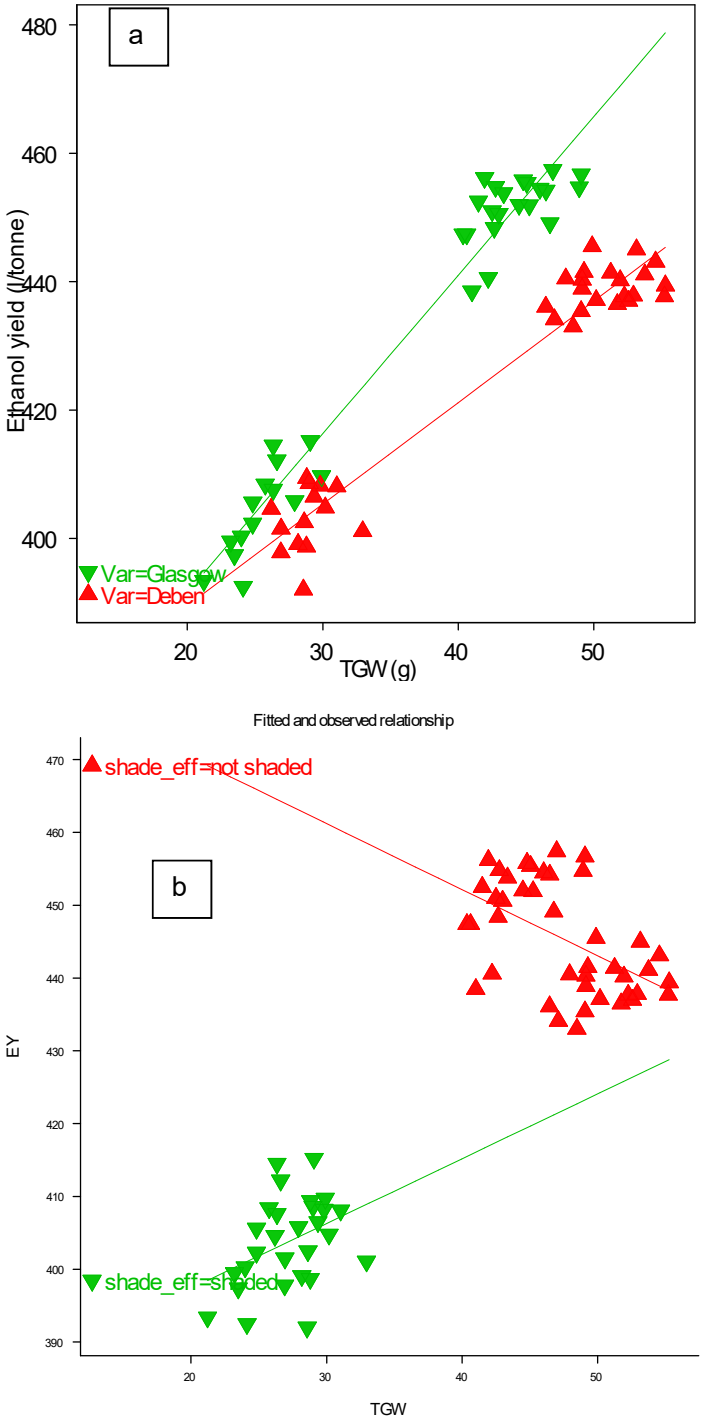


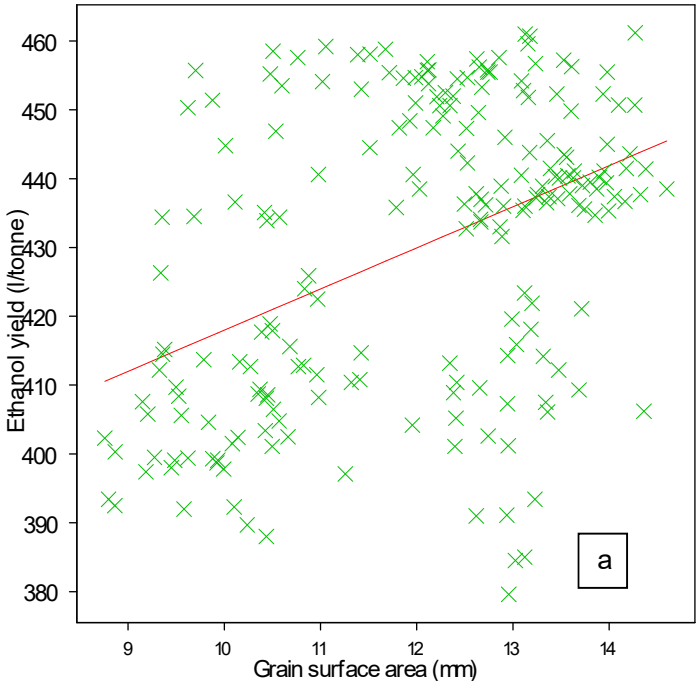
Figure 18 The relationship between PEY and TGW (a) separate model fitted for each variety and (b) separate model for shaded and not shaded plots

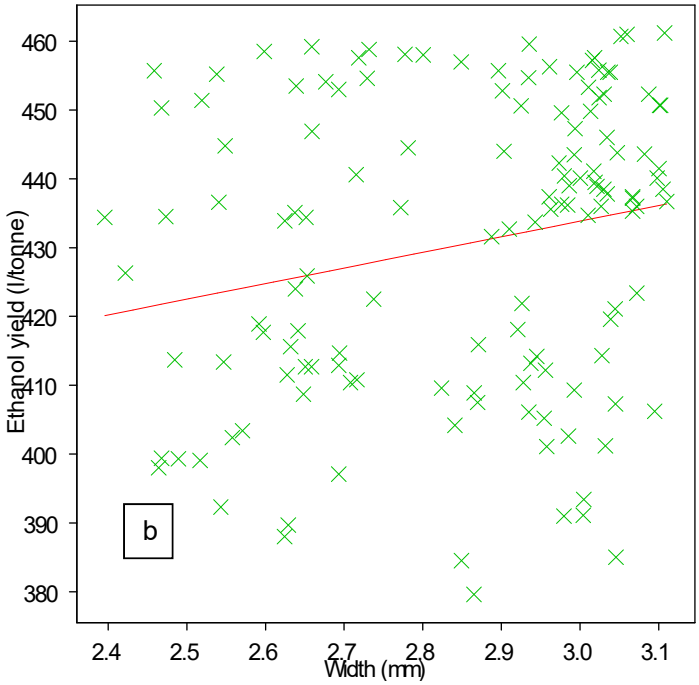
7.3.5 Grain shape and size

It was appropriate to combine the two years results of the grain size measurements obtained from image analysis such as area, perimeter and length, but width, length to width ratio (L:W) and grain roundness could not be combined. All showed significant relationship with PEY (Tables 13 - 15)The statistics justified fitting separate lines with different slopes for each year for area, perimeter and length improving the explained variance by 6%, 7% and 8%, respectively. It was required to fit separate lines with common slope for each variety for area, perimeter, length as the change in the explained variance increased by 4%, 5% and 4%, respectively which were significant at $p<0.05$. Using shade effect as a group improved the explained variance by 4%, 4% and 11%, respectively.

The two year data of width, grain roundness and length to width ratio were analysed separately. All the three variables showed very high R^2 during the first year and very low R^2 during the second year (Tables 14 & 15).

There was no need to fit separate lines for fertilizer rate as the changes in the explained variance were not significant in all the cases. In order to illustrate the pattern of the data in these relationships, three graphs; Figure 19a) both years combined Figure 19b) only first year data and Figure 19c) only second year data are shown below. The choice is based on the highest R^2 within the three groups.


Ethanol yield (l/tonne)
460
450
440
430
420
410
400
390
380
9
10
11
12
13
14
Grain surface area (mm)
a


Ethanol yield (l/tonne)
460
450
440
430
420
410
400
390
380
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
Width (mm)
b

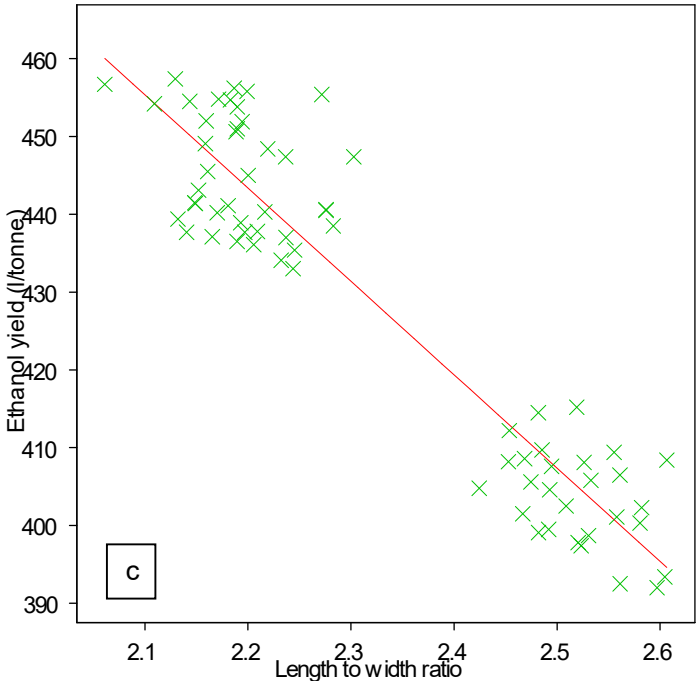


Figure 19 The relationship between PEY and (a) grain surface area, both years data combined (b) grain width, only second year data (c) grain length to width ratio, only first year data

7.3.6 Hardness Index

Hardness Index is negatively correlated with PEY. It explained about 10% of the variation accounted (Figure 20). Adding variety and year as a group improved the explained variance by 3% and 10% respectively. Adding fertilizer rate (second year) and shade effect as a group was not required as the changes in the explained variance were not significant.

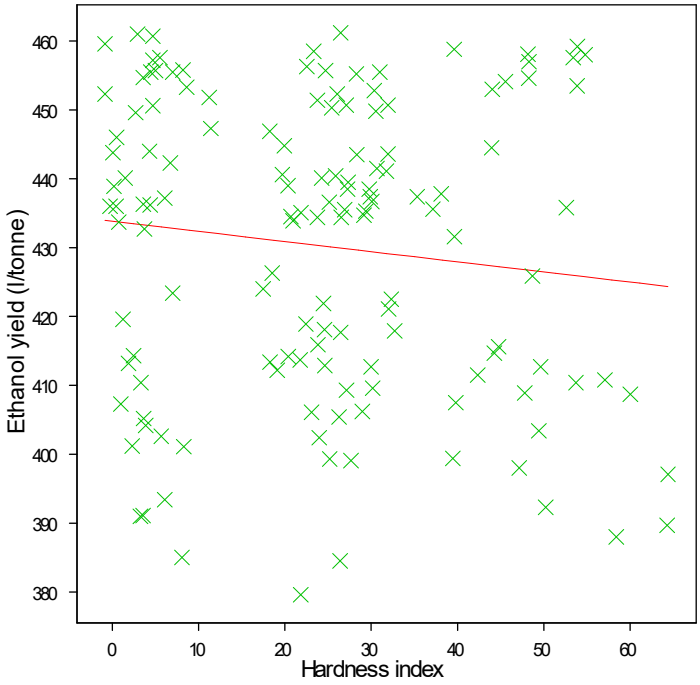


Figure 20. The relationship between PEY yield and hardness index, both years' data combined

7.4 Discussion

The three major chemical components of wheat grain are starch, protein and NSP. All three were analysed in this experiment. The result showed that starch is positively correlated with PEY whereas protein and NSP are inversely correlated with PEY. However, the relationship between PEY and NSP is not significant. As it is the starch, which is converted to ethanol, it is expected to see a strong positive relationship between starch and ethanol. However in this experiment, variation in starch concentration represented only a small portion of variation in ethanol yield. The lack of strong correlation between starch concentration and ethanol yield could be because of difficulty of measuring starch concentration precisely (Kindred *et al.,* 2008) In addition to the complication of the starch analytical methods, milling and mixing of the sample could also cause problem on the precision of the result unless proper milling and mixing is done. For this experiment, best effort was made to practice the Megazyme total starch assay method and to ensure repeatability and accuracy of results before working on

the actual samples. Quality of the starch such as proportion of large and small starch granules (Brosnan *et al.,* 1999), conversion efficiency of the starch (Agu *et al*., 2006) or it might be because of the available free sugars (Lineback and Rasper, 1988) and NSP (Kindred *et al*., 2008) contributing to the ethanol yield in addition to starch. Lacerenza *et al*. (2008) reported that starch concentration is rather important in selecting between small grain cereals for highest ethanol yield. The lack of a strong direct relationship between ethanol yield and starch concentration in wheat grain does not pose a big problem for refineries as long as there are other grain traits which could be used to predict potential ethanol yield. However, further investigation about the relationship between ethanol yield and starch concentration might be important for wheat breeders in their search for genes which are responsible for high ethanol yield.

The experiment showed that protein concentration is the single best indicator of ethanol yield of wheat grain. Similar findings were reported by Swanston *et al*. (2005), Kindred *et al*. (2008) and Agu *et al*. (2009). According to this experiment, almost 90% of the variation in wheat grain ethanol yield comes from variation in grain protein concentration. This figure could be exaggerated as the ethanol yield was assessed using the NIR method which was developed to predict ethanol yield mainly from nitrogen concentration. Interestingly, the relationship between ethanol yield and grain protein concentration was not affected by year or nitrogen fertilizer rate. This makes protein concentration a reliable test for grain ethanol yield potential. The result also showed that Glasgow gives superior ethanol yield than Deben at any nitrogen concentration level. Thus, although nitrogen concentration is important for all varieties, varietal difference is also important for ethanol yield.

There were two theories considered regarding the relationship between NSP and ethanol yield. i) it may contribute to ethanol yield through breakdown of NSP into simple sugars (Kindred *et al*., 2008) ii) It may negatively influence ethanol yield by limiting the release of starch from the endosperm matrix (Davis-Knight *et al*., 2009). Although there is a negative relationship between PEY and NSP, the variation in ethanol yield observed in this experiment

cannot be significantly explained by the variation in NSP. This result agrees with the finding of Davis-Knight *et al*. (2009). The relationship was not affected by any of the variations like, year, variety and fertilizer rate. Dornez *et al*. (2008) also reported that grain NSP levels are not affected by nitrogen fertilizer rate. There is actually minor difference in NSP between samples which might make it difficult to detect its relationship with ethanol yield. According to this result non starch polysaccharide has no use as a potential test for grain ethanol yield.

Specific weight appeared to be a better indicator of grain ethanol yield than starch concentration. Specific weight is usually associated with large well filled grains (Fenwick, 1990). That might explain its relationship with ethanol yield. However the relationship between ethanol yield and specific weight is weaker when specific weight is more than a certain limit around 73 kg/hl. This probably explains why some distilleries set 72 kg/hl specific weight as a minimum purchase criterion (Brown, 1990).

Although the combined two years data showed some relationship, the regression between ethanol and specific weight during 2008/9 was not significant. This is because most of the second year samples have specific weight higher than 70 kg/hl where the relationship with ethanol yield starts to decrease. In contrast, the samples from 2007/8 have a wide range of specific weight from very low to very high. Moreover, the variety Deben covered with polythene tends to bias the relationship as these samples have high specific weight yet low ethanol yield. It appears that in the case of Deben, even if the grain is large enough, the genetic character limits its ethanol yield.

The density and packing efficiency result reported here is only from the first year. During the second year density was not measured because the first year experiment and results from Awole *et al*. (2012) showed that density is not a better trait than specific weight. Based on the first year data, density explained only 33% of the variation in ethanol yield whereas specific weight explained 81% of the variation in ethanol yield. Since density, specific weight and

packing efficiency are associated traits and specific weight appeared to be the best in its relationship with ethanol yield, the other two traits were not required for further analysis.

The other important aspect of the grain which is worth considering as a potential test for bioethanol yield is its shape and size. The shape and size of the grain can indicate how much starch it possesses. The grain size is also important for processing efficiency as too small grains can pass through the mill and be processed as whole. This could reduce the amount of starch extracted and fermented and as a result reduce ethanol yield (Bringhurst *et al*., 2003).

Many size and shape parameters of the grain were considered in this study as a potential indicator of grain bioethanol yield. Among these TGW and width were the best of all. However, they both have very low predictive potential when compared to grain nitrogen concentration. According to Swanston *et al*. (2007), variety Deben could combine large grains with relatively low ethanol yield whereas variety Glasgow could combine small grains with relatively higher ethanol yield. This could explain why this study showed a relatively weak relationship between TGW and ethanol yield. Varieties with no contrasting size characteristics like these could give better indication of ethanol yield beyond TGW and N concentration. Importance of TGW in predicting grain ethanol yield was also reported by Swanston *et al*. (2005, 2007). Swanston *et al*. (2007) indicated that TGW and L:W ratio combined with grain protein could predict 75% of the variation in ethanol yield. In the current study the combination of protein concentration and TGW explained 89% of the variation in ethanol yield. Adding width or L:W ratio does not improve this model. However, Kindred *et al*.(2008) reported that neither TGW nor L:W ratio could help in predicting grain ethanol yield. This is because of lack of wide variation in grain size and shape among the samples covered in that specific study. Davis-Knight and Weightman (2008) reported that high TGW could be found with high grain protein which yields poor ethanol yield. This indicates that TGW could only be useful for this purpose when associated with low grain protein concentration.

Distillers prefer soft wheat varieties over hard wheat in order to avoid processing problems caused by higher viscosity worts (Brown, 1990). This study looked at whether grain hardness affects ultimate ethanol yield. The result indicated that there is a weak relationship between hardness index and ethanol yield. Kindred *et al.* (2008) also reported that hard wheat varieties can potentially give the same ethanol yield as soft wheat varieties. Bioethanol distillers can overcome the processing problem of hard wheat varieties by using commercially available enzymes, which the potable alcohol industries could not use because of the Scotch Whisky Act 1988 which prohibits the use of commercial enzyme preparations (Bringhurst *et al*., 2003).

7.4.3 Variety

Soft white or red winter wheat varieties are considered best for ethanol yield by the potable alcohol industry (Bringhurst *et al*., 2003). Varieties Glasgow and Deben are among these varieties; Glasgow being among the highest ethanol yielding varieties and Deben with low ethanol yield potential. However in this experiment, the analysis of variance showed that there is no significant difference between NIR predicted ethanol yield of Glasgow and Deben. This could be the problem of NIR method, measuring only the N effect. Although most of the relationships studied above are the same for both varieties, variety affects the two most important relationships; nitrogen concentration versus ethanol yield and the grain shape and size parameters with ethanol yield. That is at any grain nitrogen concentration and grain size and/or shape Glasgow gives more ethanol yield than Deben. This indicates that, if nitrogen concentration and TGW are set as purchase criteria for bioethanol yield, the variety potential has to be considered as well.

7.4.4 Year

There was a big difference between the two years results mostly caused by modification of the shading treatment. During the first year the shading treatment was so severe that it caused a wide gap between the results of shaded and not shaded treatments. As a result, the shading period was narrowed down during the second year which resulted in less variation in grain characteristics. All the grain shape and size parameters and starch concentration showed

stronger relationships with ethanol yield during the first year than the second year which is probably because of the extreme variation observed among the first year data. Ethanol yield of all treatments except the shaded ones have reduced from year one to two this is probably because of the reduced solar energy during the second year.

7.4.5 N fertilizer

Only two rates of nitrogen fertilizer (full recommended rate and reduced rate) were applied as a treatment on this experiment. It did not affect the relationship between the grain characteristics and ethanol yield. A wider range of nitrogen fertilizer rate trial may give different result. Daniel and Triboi (2000) reported that nitrogen fertilizer increases grain nitrogen while it has minimal effect on grain weight. That could probably explain the increase in relationship between grain nitrogen and ethanol yield and decrease in the relationship between TGW and ethanol yield from year one to year two.

7.4.6 Prediction using multiple traits

Multiple linear regression was used to obtain the best predictive tool by combining multiple traits. In the present data set, nitrogen concentration alone explains 88.4% of the variation in ethanol yield. Adding other traits did not increase the explained variance much. Adding TGW alone improves the explained variance by only 0.2% while adding both TGW and starch concentration increases the explained variance by only 0.4%. Adding other traits did not change the explained variance significantly. In another study, grain nitrogen concentration alone explained 84.8% of the variation in ethanol yield (Smith *et al*., 2006). A model composed of grain nitrogen, TGW and L:W ratio also predicted 75% of the variation in ethanol yield (Swanston *et al*., 2007). According to Kindred *et al*. (2007) maximum prediction could be achieved (71.4%) only by grain protein concentration and adding variety to the model. In all cases the majority of the accounted variance in ethanol yield comes from grain nitrogen concentration.

8 General Discussion

The UK wheat bioethanol industry is benefited from the country's higher wheat grain yield per hectare of land and quality of the grain as compared to other European countries. However the UK bioethanol has to be competitive globally not only with wheat bioethanol but also with other feedstock too. Currently 35% of bioethanol used in the UK is sourced from maize (DfT, 2012). The total biofuel usage in the country for the year 2010/11 is 3.27% of the total road transport fuel. This is still under the 3.5% target of the RTFO for the year. Therefore there is still very high demand for home grown, homemade bioethanol. One way of increasing supply is through improving the production efficiency. For efficient production the right quality feedstock has to be used. Quality of the feedstock is vital in the economics of bioethanol production (Awole *et al.,* 2009). This thesis has helped to identify what measures should be used in order to determine the right quality wheat grain for bioethanol production.

Bioethanol and potable alcohol productions are principally similar. Even though there is experience of potable alcohol production in the UK for about two decades, bioethanol production is relatively new. Although this thesis focuses on the bioethanol production, it makes full use of the available methodologies of the potable alcohol production. The major difference between the two productions is the restriction of using commercial enzymes in case of the potable alcohol. These enzymes may enhance the processing efficiency and bioethanol yield (Smith *et al.,* 2006). It may also give bioethanol industry more choice of varieties such as hard wheat varieties which are not acceptable in the potable alcohol industry. After the start of this PhD project, Davis-Knight *et al*. (2009) developed an enzyme only bioethanol production method which is typical of the bioethanol industry. The comparison of the two methodologies by Davis-Knight *et al*. (2009) indicated that there is a good agreement between the results. Therefore the result of methodology of the potable alcohol is applicable to that of bioethanol industry.

This study has attempted to examine most of the physical, chemical and physicochemical characteristics of wheat grain which might be useful for predicting grain potential ethanol yield.

The work was conducted in two different ways (i) using HGCA-AHDB Recommended List varieties grown at several sites for two years. (ii) Another study conducted for two years at one location with treatments which could yield a range of grain quality. All the traits showed some relationship with ethanol yield. However, most of the relationships were not strong enough to be able to predict grain potential ethanol yield. The second experiment was complicated by using the NIR method to get ethanol yield which might exaggerated the relationship between N and ethanol yield while probably undermining the other relationships. The choice of the varieties with contrasting nature in the relationship between grain weight and ethanol yield, which was not known at the onset of this project, also complicated the second experiment. Moreover, the treatments used in the second experiment created a cluster of data instead of continuous variation. However, the result of this experiment is also in line with the hypothesis and previous findings.

More than two thirds of the wheat grain is starch. Protein and NSP are the two major components of the grain next to starch (Englyst *et al.,* 1999, MAFF, 2000). All are expected to have direct or indirect impacts on ethanol yield. In both experiments starch showed a weak but positive relationship with ethanol yield. Interestingly it explained the same amount of variation in ethanol yield in both experiments. This weak positive relationship was also observed in another study (Kindred *et al.,* 2008). However, both experiments revealed strong negative correlation between nitrogen concentration and ethanol yield. This is also supported by many other studies (Agu *et al.,* 2009, Misailidis, 2010). The study also showed that site and genetic variations could affect the relationship between nitrogen concentration and ethanol yield. NSP was also expected to have negative or positive impact on ethanol yield but both this study and the study of Davis-Knight *et al.* (2009) showed that it has little or no impact on ethanol yield. However it is possible that it might have impact on processing efficiency. Studying the processing efficiency was beyond the scope of this project. Further study should probably focus on conversion efficiency of starch into ethanol in addition to the amount of

starch concentration. The other important thing to look into might be the other constituents of the grain such as lipids and ash which could affect ethanol yield either positively or negatively.

In both experiments, the second best indicator of grain potential ethanol yield was TGW. In addition, both experiments showed that its relationship with ethanol yield is affected by genetic and seasonal or climatic variations. Ethanol yield prediction potential of TGW reduced by half from the first experiment to the second one. This is because of the varieties used in the two studies. In the field experiment, only two varieties Glasgow and Deben were analysed. Glasgow combines small grain with higher ethanol yield while Deben combines large grains with low ethanol yield. This has affected the relationship between TGW and ethanol yield of the field experiment. It was hypothesized that not only the mean grain weight but also the grain weight distribution within the lot could affect grain ethanol yield. To investigate this, the relationship between the upper quartile, lower quartile and median grain weight and ethanol yield was studied during the first experiment. However, none of this could predict ethanol yield better than the mean grain weight and were not investigated in the second experiment. Apparently TGW does not work for all varieties for the future it might be useful to identify these varieties.

Specific weight is the most widely used quality indicator of wheat grain for many end uses. It is also the third most important factor in predicting grain potential ethanol yield. Like TGW, the variation accounted in ethanol yield due to specific weight has reduced from experiment one to two. This again, is probably due to the nature of Glasgow and Deben as explained above. The studies showed that specific weight could be used as indicator of ethanol yield especially at lower specific weight levels. Both experiments indicated that the impact of specific weight on ethanol yield varies with seasonal or climatic conditions.

Both studies have considered many size and shape parameters as a potential indicator of grain ethanol yield. Among these parameters length to width ratio and width appear more

important. Width has explained almost the same amount of variation in ethanol yield in both experiments.

There is agreement between the two experiments that best prediction of ethanol yield can be achieved by combining grain protein concentration with TGW. According to experiment one, maximum prediction could be achieved by combining TGW and protein concentration plus site and variety added to the model. While in the second experiment maximum prediction was achieved by protein concentration and variety only. Combining TGW with protein improves the variation accounted by protein only but not as much as variety does. Moreover, adding TGW on the model of protein and variety does not give significant improvement to the variation explained. This is again due to the character of Deben and Glasgow as explained above.

In conclusion, with a wide variety of samples TGW and protein concentration could give maximum prediction of ethanol yield and can be used as purchase criteria at the refinery intake. Adding variety and site could boost the prediction power

Both experiments showed that there is influence of temporal, spatial and genetic variation in the relationship between the grain traits and ethanol yield. In the first experiment the genetic effect was much less than the spatial influence. This is probably because of the fact that all those varieties were already chosen to be good distilling wheat. The model suggested above should be validated for its stability with samples collected from different sites, years and varieties. This was not done due to time constraint.

Table 16 Comparison of grain ethanol yield prediction models from different authors

Authors	Sample composition	Variables suggested	R^2

Swanston *et al.*, (2007)	Ten varieties, four sites and oneA s year	Protein, TGW and G:L ratio	75%
Kindred *et al.,* (2008)	2 varieties, one site & one year	Starch and protein	70%
Agu et al., (2009)	Ten varieties, four sites & one year	Hardness and protein	56%
Nikiforos *et al.,* (2010)	Several varieties, four sites and 2 years	Protein and CRP profile	82%
This thesis	Two experiments discussed above	Protein and TGW plus site and variety	82%

Table 16 shows comparison of results from different authors in regards to predicting grain ethanol yield. All of the models have protein in common confirming the well-established relationship between protein and ethanol yield. One of the models suggests starch in addition to protein. However, starch measurement is difficult as discussed above. The other two models suggest measurement of grain weight and or size in addition to grain protein. However, grain weight and size have varietal influence some varieties combining large grain with poor ethanol yield and others vice versa as discussed above. The remaining two models suggest measuring grain hardness in addition to protein and this seems better applicable than all others.

In terms of the explained variance, Nikiforos *et al.,* (2010) and this thesis have come with the highest and comparable results and both works are based on several sites and varieties and a couple of years . Among the two, the model suggested by Nikiforos *et al.,* (2010) which is based on grain protein and crush response profile (CRP, a measure of grain hardness which can be obtained from CKCS machine, Osborne *et al.,* 2001) seems better than the model suggested from this thesis because the model based on the protein and CRP predicts ethanol yield better than protein and TGW without adding site and variety to the model.

9 Recommendations for future work

In relation to the chemical composition of the grain, the minor components such as ash and lipid content of the grain and their effect on ethanol yield have not been studied so far. Although these components are small in comparison to starch, protein and NSP of the grain, it is possible that these components could show some relationship with ethanol yield and reduce the variations in ethanol yield unexplained by the recommended model.

Attempts made to predict grain ethanol yield based on starch concentration were largely unsuccessful. Future studies should focus on conversion efficiency of starch into ethanol in addition to starch concentration. Starch which has survived the conversion process and is present in DDGS should be measured after ethanol analysis. What affects the conversion efficiency? Whether starch quality, other components of the grain and/or the processing efficiency is also a subject of further study. Clarifying the relationship between ethanol yield and starch concentration could establish a prediction model based on starch concentration. Current laboratory starch measuring methodologies are, however, expensive, labour and time demanding to screen a large quantity of samples. More research to find a cheap and quick method which measures starch concentration would also be needed. Addressing this issue will be useful for growers, breeders and processors.

One comprehensive study can be conducted to address the issues above. This research can be conducted by collecting wheat samples of most representative varieties with similar protein concentration from across the major wheat growing sites for over a period of two years. As the relationship between protein concentration and ethanol yield is well established, the rest of the unknowns could be studied from these samples. Variety and N test trials available in major wheat growing sites of the UK can be used for this purpose. It is important to replicate the varieties across sites uniformly. This will help to measure environmental and genetic influence on the relationship between grain traits and ethanol yield. It will also enable correction factors to be calculated for varieties and sites to the model suggested from this work. It will also help to discriminate varieties for which TGW should be used or not.

Wheat grain samples collected from this research will be used to analyse starch, protein ash and lipid concentration, TGW, ethanol yield and starch remained in the DDGS. The relationship between ethanol yield and these parameters could be studied in an attempt to improve the suggested model.

The model should then be verified with samples collected from the market. It will be useful to test the applicability of the model with wheat samples grown out of the UK as it is possible that wheat grown outside the UK could be used by the refineries.

It is also important to look into two varieties with similar N and TGW, yet different ethanol yield in order to understand what genetic factor affects ethanol yield. Samples from the above experiment can be used for this study too. All grain components of these varieties should be examined including their conversion efficiency into ethanol. The null hypothesis for this experiment will be there is no conversion efficiency difference between two varieties with similar protein/starch content.

For future researches, it will be very useful to utilize the resources available from the GREEN Grain project, (Sylvester-Bradley *et al.,* 2010) in order not to duplicate efforts and costs.

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Appendices

Appendix 1. Summary of the first (2007/08) year data

	Minimum	Maximum	Median	Average	Standard deviation
PEY	392	457.4	437.4	428.7071	21.47689
N	1.63	2.77	1.89	2.085507	0.334597
starch	54.6786	70.1906	64.75315	63.59581	4.192285
TGW	21.2202	55.2773	42.569	39.42554	10.61342
Specific weight	61.0667	79.2667	73.9	71.64334	5.826579
Density	1.33393	1.44315	1.393455	1.38386	0.025799
Packing efficiency	39.0097	51.6259	46.3868	46.00505	3.609387
Perimeter	12.9491	15.4791	14.44085	14.30901	0.771243
Length	5.5975	6.55375	6.12403	6.106794	0.278469
Width	2.16085	3.05771	2.76372	2.648481	0.283463
Area	8.75825	14.3812	12.0413	11.59831	1.672078
L:W	2.06097	2.60665	2.2437	2.325117	0.167842
Grain roundness	16.224	19.3071	17.69835	17.81293	0.852126
HI	3.43533	58.8733	28.60755	30.05334	15.17823
NSP	7.53052	10.7338	9.137345	9.084607	0.914381

Appendix 2. Summary of the second (2008/09) year data

	Minimum	Maximum	Median	Average	Standard deviation
PEY	379.6	461.2	435.7	430.2671	21.45521
N	1.64	2.78	1.9	2.0885	0.31035
starch	55.9059	79.6102	66.1716	66.07398	4.343656
TGW	20.5532	62.4853	41.9029	38.79775	8.557341
Specific weight	56.8	79.3667	72.91665	72.41208	4.008628
HI	-0.84657	64.4011	24.6589	24.25644	17.05511
perimeter	12.3535	15.4826	14.0954	13.98596	0.774074
length	5.10171	6.43274	5.78436	5.789892	0.309704
width	2.39579	3.11025	2.92686	2.848373	0.200489
area	9.34009	14.599	12.62965	12.1767	1.425553
L:W	1.88218	2.22237	2.0438	2.036987	0.086338
Grain roundness	15.4083	17.0057	16.1704	16.13421	0.392974
NSP	7.03641	11.9601	9.300835	9.243271	1.110117