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Proceeding Paper

Effects of Processing Methods on Phytochemical Compositions of Selected Plant Materials with Animal Nutrition Potentials [†]

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[†] Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: <https://iecps2021.sciforum.net/>.

Abstract: Plants contain significant amounts of bioactive compounds that have potential benefits for livestock and humans. This study evaluated the phytochemicals of some plant materials that have potential nutritional value for animals. In this study, fresh samples (six samples per plant material) of four leaves of Siam weed (*Chromolaena odorata* L.); African basil (*Ocimum gratissimum* L.); waterleaf (*Talinum triangulare* Jacq. Willd); and Mexican sunflower (*Tithonia diversifolia* Hemsl. A. Gray) were either air-dried (between 27 and 31 °C), oven-dried (at 65 °C) or freeze-dried (at –80 °C). The leaves were milled in a 1.0 mm sieve and the phytochemical contents of each leaf sample (in triplicates) were quantified. The quantified phytochemicals were flavonoids, tannins, beta carotene and xanthophylls for each of the leaves. Data were subjected to analysis of variance and significant means separated using the Duncan multiple range test. Flavonoids, tannins and xanthophylls were found to be the highest ($p < 0.05$) in most air-dried leaf samples compared to oven-dried and freeze-dried ones. Flavonoids, tannins, beta carotene and xanthophylls in the leaves showed that all leaf samples appear to have good potential for being used as natural feed additives such as egg yolk colourants in laying chicken feed. In vivo studies using birds are recommended.

Keywords: leaves; drying methods; phytochemicals; xanthophylls



Citation: Oyedeji, O.; Oluwatosin, O.; Jegede, A.; Fafiolu, A.; Samuel, I.; Folorunso, O.; Pirgozliev, V. Effects of Processing Methods on Phytochemical Compositions of Selected Plant Materials with Animal Nutrition Potentials. *Biol. Life Sci. Forum* **2021**, *12*, 92. <https://doi.org/10.3390/IECPS2021-11988>

Academic Editor: Giedrė Samuoliene

Published: 30 November 2021

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1. Introduction

Plants, especially those considered weeds, are candidates for further research. First, these unutilised plants are not in competitive demand between livestock and humans. Second, they have key phytochemicals which can serve nutritional and non-nutritional purposes for livestock and even human beings. Plant-derived products are generally considered safe as ingredients in animal diets because they are residue-free; hence, they have the potentials to be ideal feed supplements [1]. The herbs and plant extracts used as feed additives have bio-active ingredients such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins, tannins, phenolics, polyphenols, terpenoids, polypeptide, thymol, cineole, linalool, anethole, allicin, capsaicin, allylisothiocyanate and piperine [2–4]. Tannins help the plants defend against fungal and bacterial pathogens. One of the positive consequences of tannins in grazing forages is that they can reduce the potential for bloat in livestock [5]. They are among the main sources of antioxidants found in food and beverages and are found in abundance in tree bark, wood, fruit, leaves, and roots [6]. Flavonoids are a group

of plant metabolites reported to provide health benefits through cell signalling pathways and antioxidant effects. Flavonoids in food are generally responsible for colour, taste, and the prevention of fat oxidation [7].

Apart from tannins and flavonoids, plants are rich sources of other biologically active substances such as chlorophylls and carotenoids [8]. Carotenoids contain carotenes and oxy-carotenoids (xanthophyll)—lutein and zeaxanthin, which are yellow; and citranaxanthin, lycopene, and capsanthin which are red. The xanthophylls produced by higher plants are also found in green algae. Leafy vegetables which are fed to livestock fresh or processed are reported to be a good source of carotene, vitamins—ascorbic acid, riboflavin, folic acid—and minerals such as calcium, iron, and phosphorus which have several health benefits [9]. Xanthophylls are yellow pigments of the carotenoid group, and they are present in maize, lucerne, algae, tomatoes, and green leaves. They are also the non-nutritive factors of carotenoids which produce their pigments in egg yolk from poultry diets and impact egg yolk colour [10].

Drying is one of the oldest methods of food preservation [11]. However, some reports have documented nutrient losses from vegetables and other plants during drying. Therefore, it is imperative to evaluate the phytochemicals which may qualify these plant materials as feed additives after being subjected to different drying methods. The objective of this study is to investigate the effects of drying methods on the phytochemical compositions of unutilised leaves while enabling livestock producers, researchers, and feed manufacturers to make decisions as to the appropriateness of these leaves to serve as potential feed additives safe for animal consumption.

2. Materials and Methods

2.1. Preparation and Processing of Experimental Leaves

The experimental leaves used for this study were sourced from various locations within Ogun and Oyo States, southwest Nigeria, in April and May during the rainy season when the leaves tend to be more abundant. The study materials were collected irrespective of their ages to represent the phytochemical concentrations of all plant materials used for this study. The leaves sourced were Siam weed (*Chromolaena odorata* L.), African basil (*Ocimum gratissimum* L.), Waterleaf (*Talinum triangulare* (Jacq.) Willd), and Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray). The choice of some of these experimental leaves was based on their usage as feed additives in previous studies and their bioavailability. The leaves of the plants were de-stalked and washed thoroughly with tap water and then with distilled water to remove any dirt.

All four leaves sourced for this study, namely *C. odorata*, *O. gratissimum*, *T. triangulare*, and *T. diversifolia* were either air-dried, oven-dried, or freeze-dried. The fresh leaves intended for air-drying were spread inside a ventilated room for approximately ten days at 27–31 °C until they were dried and crispy to the touch. The oven-dried leaves were placed in a large clean forced draught oven at 65 °C (Fisher Scientific, Model 655F, Waltham, MA, USA) in labelled envelopes until the leaves were dry and of constant weight on an average of three days for each leaf. The freeze-dried leaves were placed inside a lyophiliser (Labconco Free Zone Freeze Dry System, Model 7752021, Kansas City, MO, USA) at –80 °C until the leaves were freeze-dried, for an average of three days for each leaf. The dried leaves were milled to pass through a 1.0 mm sieve and stored in well-labelled sample bottles.

2.2. Chemical Analysis of Experimental Leaves

Chemical analysis was performed for each leaf sample (6 samples, each analysed in triplicates). Phytochemicals were quantified to know their concentrations in each of the leaves. Phytochemical screenings were carried out for tannins based on the Folin–Ciocalteu method and flavonoids according to protocols by [12,13]. The factors that impact colours including beta carotene and xanthophylls were analysed according to [14].

2.3. Data Analysis

The data collected on proximate and phytochemical analyses of the leaves were subjected to an analysis of variance (ANOVA) using the STAR 2.0.1 (International Rice Research Institute, Los Baños, Philippines) analytical package as stipulated by [15], and means were separated with Duncan's multiple range test at $p < 0.05$.

3. Results

The phytochemical values of the experimental leaf samples—as influenced by drying methods—are shown in Table 1. The effects of the drying methods on the flavonoids of LS 1 and 3 were significant. The air-dried LS 1 value (1.88%) was the highest ($p < 0.05$) when compared to other drying methods, including oven-dried LS 1 (1.51%). Conversely, oven-dried LS 3 had the highest ($p < 0.05$) value (2.51%) when compared to air-dried (1.18%) and freeze-dried (1.20%), which themselves are similar ($p > 0.05$).

Table 1. Phytochemical values of leaf samples as influenced by drying methods.

	Flavonoids (%)				Tannins (%)				
	AD	OD	FD	SEM	AD	OD	FD	SEM	
LS 1	1.88 ^a	1.51 ^b	1.70 ^{a,b}	0.13	LS 1	0.33 ^a	0.31 ^b	0.35 ^a	0.01
LS 2	1.17	1.44	1.63	0.09	LS 2	0.40 ^a	0.36 ^a	0.36 ^b	0.01
LS 3	1.18 ^b	2.51 ^a	1.20 ^b	0.22	LS 3	0.14	0.16	0.15	0.01
LS 4	1.81	1.83	1.82	0.02	LS 4	0.26	0.26	0.26	0.01
	Beta Carotene (mg/100 g)				Xanthophyll (mg/100 g)				
	AD	OD	FD	SEM	AD	OD	FD	SEM	
LS 1	8.42	8.23	8.23	0.01	LS 1	11.85 ^a	10.09 ^b	9.96 ^b	0.40
LS 2	6.58	6.53	6.67	0.01	LS 2	9.17	9.52	9.45	0.10
LS 3	2.06 ^b	11.01 ^a	2.08 ^b	0.02	LS 3	2.92 ^b	20.11 ^a	2.86 ^b	0.15
LS 4	4.66	4.65	4.69	0.01	LS 4	14.74	14.04	14.91	0.35

^{a,b} Means on the same row with different superscripts are significantly ($p < 0.05$) different. LS—leaf sample; AD—air-dried; OD—oven-dried; FD—freeze-dried. LS 1—*Chromolaena odorata*; LS 2—*Ocimum gratissimum*; LS 3—*Talinum triangulare*; LS 4—*Tithonia diversifolia*.

The tannin results for LS 1 revealed that the values of air-dried (0.36%) and freeze-dried (0.35%) were similar ($p > 0.05$) while the value of oven-dried (0.31%) was the smallest ($p < 0.05$). For LS 2, where 0.36% was the result for oven-dried and that of freeze dried ($p > 0.05$), the highest ($p < 0.05$) value was obtained for the LS 2 air-dried (0.40%) sample. Meanwhile, the results for LS 3 (0.14%, 0.16% and 0.15%) and LS 4 (0.26% for the drying method) showed that there were no significant differences across drying methods ($p > 0.05$).

4. Discussion

Flavonoids are one of the largest groups of plant pigments [16]. They give plants yellow, orange, and red colours, and [17,18] stated that up to 5% of flavonoid supplementation in poultry diets could improve meat colour in terms of lightness. The supplementation of flavonoids up to 60 g/kg in broiler diets was found to improve antioxidant activity in breast muscles while performance was not compromised [19]. Tannins have often been considered an anti-nutritional factor in any feedstuff; [20] asserted that tannin has at times had a noticeable positive impact on nitrogen balance. In monogastric diets, it has been reported that low dietary concentrations of tannins showed potential as a feed additive and consequently showed improved health status for nutrition as well as animal performance [21]. Tannin at 0.20% in the diets of laying hens of 50 weeks did not have any adverse effect on egg weights, shell thickness and yolk colour, but decreased the level of cholesterol in the egg yolk [20]. The authors further stated that there were increased monounsaturated fatty acids in the egg yolk.

There has been an increase in the usage of pigments of plant origin, especially carotenoids (xanthophyll), in many countries. These substances are the main sources of natural colourants because of their many advantages in terms of safety to human health, strong biological activity, and great bioavailability [22]. Leafy vegetables are a richer source of beta carotene than other crops [23]. Beta carotene in most leaf samples was found to be similar except for *T. triangulare* where the beta carotene value of the oven-dried leaf was the highest. The reason for this is not clear but it appears that beta carotene in leaves was more available after quantification because of the further reduction in moisture level because of the temperature of 60 °C as compared to air drying (~31 °C) and freeze-drying (−80 °C). Waterleaf is said to be rich in beta carotene [24]. Since the amount of beta carotene was higher in the leafy vegetables, the beta carotene contents present in the samples depended on the processing method used. The xanthophyll contents of air-dried leaf samples were among the highest except for LS 3. Heat treatment seemed to make xanthophyll available in this leaf. The reason for this remains unclear. According to these results, xanthophylls were mostly preserved in the air-dried leaves. Although it is still unknown whether leaves that are rich in xanthophylls can serve as total substitutes for synthetic egg yolk colourants, local scavenging chickens in Nigeria appear to have golden yellow yolk because they have access to all kinds of food materials including green leaves. Reports have also shown that the inclusion of leaf products in the diets of chickens could result in their eggs having increased yolk colour intensity [25,26].

5. Conclusions

Phytochemical compositions in the leaves have placed the leaves in a good position as potential feedstuffs and additives, especially in poultry birds. Feeding trials are required to confirm the results above in their egg yolks or skins if the feeds could be laced with these leaves. Additionally, it is important to consider the economic viability of each drying method as well as the availability of the leaves to be used as potential feed additives.

Author Contributions: Conceptualisation, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin) and A.J.; methodology, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin) and A.J.; software, O.O. (Oluwatobi Oyedeji) and O.O. (Olusegun Folorunso); validation, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin), A.J., A.F., I.S. and V.P.; formal analysis, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin) and A.J.; data curation, O.O. (Oluwatobi Oyedeji), A.J., I.S.; writing—original draft preparation, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin); writing—review and editing, O.O. (Oluseyi Oluwatosin) and I.S.; supervision, O.O. (Oluseyi Oluwatosin), A.J. and O.F.; project administration, O.O. (Oluwatobi Oyedeji), O.O. (Oluseyi Oluwatosin) and A.F.; funding acquisition, O.O. (Oluseyi Oluwatosin), A.J. and A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The World Bank Centre of Excellence in Agricultural Development and Sustainable Environment (CEADESE) and the Federal University of Agriculture Abeokuta under Grant Number 0023.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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