biology and life sciences forum

MDPI

Proceeding paper

Effects of Processing Methods on Phytochemical Compositions of Selected Plants Materials with Animal Nutrition Potentials †

Oluwatobi Oyedeji [1,2,*], Oluseyi Oluwatosin [1], Adebayo Jegede [1], Adeboye Fafiolu [1], Isaac Samuel [3], Olusegun Folorunso [1] and Vasil Pirgozliev [4]

1 Centre of Excellence in Agricultural Development and Sustainable Enviornment, Federal University of Agriculture, PMB 2240, Abeokuta, Nigeria; oduguwa2002@yahoo.com (O.O.); jegedeav@funaab.edu.ng (A.J.); fafioluao@unaab.edu.ng (A.F.); folorunsoo@funaab.edu.ng (O.F.)
2 Lakeland College 5707 College Drive, Vermilion, AB T9X 1K5, Canada
3 Department of Agricultural Science Education, Federal College of Education (Technical), PMB 0100, Bichi, Kano State, Nigeria; isaacsamuel07@gmail.com
4 The National Institute of Poultry Husbandry, Harper Adams University, Newport TF10 8NB, UK; vpirgozliev@harper-adams.ac.uk
* Correspondence: oluoye2103@gmail.com

† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021.

Abstract: Plants contain significant amounts of bioactive compounds that have potential benefits to livestock and humans. This study evaluated the phytochemicals of some plant materials that have potential animal nutrition values. In this study, fresh samples (6 samples per each) of four leaves: 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 phytochemical contents of each leaf sample (in triplicates) were quantified. Phytochemicals quantified were flavonoids, tannins, beta carotene, and xanthophylls for each of the leaves. Data were subjected to Analysis of Variance and significant means separated using 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 potentials for being used as natural feed additives such as egg yolk colourants in laying chicken feeds. In-vivo studies using birds were recommended.

Keywords: Leaves; Drying methods; Phytochemicals; Xanthophylls

Citation: Oyedeji, O.; Oluwatosin, O.; Jegede, A.; Fafiolu, A.; Samuel, I.; Folorunso, O.; Pirgozliev d, V. Effects of Processing Methods on Phytochemical Compositions of Selected Plants Materials with Animal Nutrition Potentials. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Giedre Samuoliene

Published: 30 November 2021

1. Introduction

Plants especially those considered as 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 in livestock and even human beings. Plant-derived products are generally considered safe as ingredients in animal diet because they are residue-free, hence their 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 one of the main sources of antioxidants found in food and beverages and are found in abundance in the tree bark, wood, fruit, leaves, and roots [6]. Flavonoids are a group of plant metabolites reported to provide health benefits through cell signaling pathways and antioxidant effects. Flavonoids in food are generally responsible for colour, taste, and prevention of fat oxidation [7].

Apart from tannins and flavonoids, plants are rich sources of other biologically active substances like chlorophylls and carotenoids [8]. Carotenoids contain carotenes and oxycarotenoids (xanthophyll)—Lutein and zeaxanthin, which are yellow; 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—Minerals like 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 colours on egg yolks [10].

Drying has been one of the oldest methods of food preservation [11]. However, some reports have documented the losses of nutrients 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 for 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 the four leaves sourced for this study, *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 about ten days at 27–31 °C till they were dried and crispy to touch. The oven-dried leaves were placed in a large clean forced draught oven at 65 °C (Fisher Scientific, Model 655F) in labelled envelopes till the leaves were dry and of constant weights 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) at −80 °C till the leaves were freeze-dried, on 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 done 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 Ceocalteu 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 Analysis of Variance (ANOVA) using STAR 2.0.1 analytical package [15] as stipulated by [16], and means were separated with Duncan's Multiple Range Test at $p < 0.05$.

3. Results

The phytochemical values of experimental leaf samples as influenced by drying methods are shown in Table 1. The effects of 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, whereas 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 ($p > 0.05$).

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 least ($p < 0.05$). 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 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 drying method), showed that there were no significant differences across drying methods ($p > 0.05$).

Table 1. Phytochemical Values of Leaf Samples as Influences by Drying Methods.

	Flavonoids (%)					**Tannins (%)**			
	AD	**OD**	**FD**	**SEM**		**AD**	**OD**	**FD**	**SEM**
LS 1	1.88 ^{a}	1.51 ^{b}	1.70 ^{ab}	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,c} 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*.

4. Discussion

Flavonoids are one of the largest groups of plant pigments [17]. They give plants yellow, orange and red colours [18,19] stated that up to 5% of flavonoid supplementation in poultry diets could improve meat colour in terms of lightness. 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 [20]. Tannins have often been considered as an anti-nutritional factor in any feedstuff [21] asserted that sometimes, tannin had a noticeable positive impact on nitrogen balance. In monogastric diets, it has been reported that low dietary concentrations of tannins had shown potential as a feed additive and consequently showed improved health status, nutrition as well as animal performance [22]. 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 of the egg yolk [21]. The authors further stated that there were increased monounsaturated fatty acids of egg yolk.

There had been an increase in the usage of pigments of plant origin, especially carotenoids (xanthophyll) in many countries. These substances are main sources of natural colourants because of their many advantages in terms of safety to human health, strong biological activity, and greater bioavailability [23]. Leafy vegetables are a richer source of beta carotene than other crops [24]. Beta carotene in most leaf samples was found to be similar except for *T. triangulare* where the beta carotene value of oven-dried leaf was the highest. The reason for this is not clear but it appears that beta carotene in the leaves was more available after quantification because of further reduction in moisture level because of the temperature of 60 °C as compared to air drying (~31 °C) and freeze-drying (−80 °C). Water leaf is said to be rich in beta carotene [25]. Since the amount of beta carotene was higher in the leafy vegetables, the content of beta carotene present in the samples depended on the processing method used. The xanthophyll contents of air-dried leaf samples were among the highest except 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 [26,27].

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. Also, 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.

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

Conflicts of Interest: The authors declare no conflict of interest

References

1. Li, H.N.; Zhao, P.Y.; Yan, L.; Hossain, M.M.; Kang, J.; Kim, I.H. Dietary phytoncide supplementation improved growth performance and meat quality of finishing pigs. *Asian Australas. J. Anim. Sci.* **2016**, *9*, 1314–1321.
2. Grashorn, M.A. Use of phytobiotics in broiler nutrition—an alternative to in feed antibiotics? *J. Anim. Feed Sci.* **2010**, *19*, 338–347.
3. Kiczorowska, B.; Klebaniuk, R.; Bąkowski, M.; Al-Yasiry, A.R.M. Culinary herbs—nutritive value and content of minerals. *J. Elem.* **2015**, *20*, 599–608.
4. Al-Yasiry, A.R.M.; Kiczorowska, B. Frankincense—therapeutic properties. *Postepy Hig. I Med. Dosw.* **2016**, *70*, 380–391.
5. Taiz, L.; Zeiger, E. *Plant Physiology*, 3rd ed; Sinauer Associates Incoroprated: Sunderland, UK, 2002; pp. 555–557.
6. Kumari, M.; Jain, S. Tannins : An Antinutrient with Positive Effect to Manage Diabetes. *Res. J. Recent Sci.* **2012**, *1*, 1–8.
7. Yao, L.H.; Jiang, Y.M.; Shi, J.; Tomás-Barberán, F.A., Datta, N., Singanusong, R.; Chen, S.S. Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.* **2004**, *59*, 113–122.
8. Komes, D.; Belščak-Cvitanović, A.; Horžić, D.; Marković, K.; Ganić, K.K. Characeterisation of pigments and antioxidants properties of three medicinal plants dried under different drying conditions. 11th International Congress Engineering and Food "Food Process Engineering in a Changing World", Athens Greece, 22–26 May 2011. Available online: https://pdfs.semanticscholar.org/1cba/de98e1a711b0ae7cf3bd57d20a6e4a0bc805.pdf (accessed on).
9. Oluwalana, I.B.; Ayo, J.A.; Idowu, M.A.; Malomo, S.A. Effect of drying methods on the physicochemical properties of waterleaf (Talinum triangulare). *Int. J. Biol. Chem. Sci.* **2011**, *5*, 880–889.
10. Chowdhury, S.D.; Hassin, B.M.; Das, S.C.; Rashid, M.H.; Ferdaus, A.J.M. Evaluation of marigold flower and orange skin as sources of xanthophyll pigment for the improvement of egg yolk color. *J. Poult. Sci.* **2008**, *45*, 265–272.
11. Foline, O.; Emenike, A.F.; Eunice, B.I. Comparative analysis of the nutritional composition of three different drying methods of *Moringa oleifera* leaves. *Int. J. Appl. Agric. Res.* **2011**, *6*, 131–138.

12. Mahajan, R.T.; Badgujar, S.B. Phytochemical investigations of some laticiferous plants belonging to Khandesh region of Maharashtra. *Ethnobot. Leafl.* **2008**, *2008*, 151.
13. Peixoto Sobrinho, T.J.S.; Silva, C.H.T.P.; Nascimento, J.E.; Monteiro, J.M.; Albuquerque, U.P.; Amorim, E.L.C. Validation of spectrophotometric methodology for quantify flavonoid content in Bauhinia cheilantha (Bongard) Steudel. *Rev. Bras. De Ciências. Farm.* **2008**, *44*, 683–689.
14. Harborne, A.J. *Phytochemical Methods*; Chapman and Hall: London, UK, 1973
15. Statistical Tool for Agricultural Research, 2014. International Rice Research Institute. Available online: http://bbi.irri.org (accessed on).
16. Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics*; A Biometric Approach McGraw Hill International Book Company: New York, NY, USA, 1980; p. 63.
17. Brodowska, K.M. Natural Flavonoids: Classification, Potential Role, and Application of Flavonoids Analogues. *European Journal of Biol*ogical *Research* **2017**, *7*, 108–123.
18. Pikulski, M.; Brodbelt, J.S. Differentiation of Flavonoid Glycoside Isomers by Using Metal Complexation and Electrospray Ionization Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1437–1453.
19. Kamboh, A.A.; Leghari, R.A.; Khan, M.A.; Kaka, U. Flavonoids Supplementation—An Ideal Approach to Improve Quality of Poultry Products. *World Poult. Sci. J.* **2019**, *75*, 115–126.
20. Brenes, A.; Viveros, A.; Goni, I.; Centeno, C.; Saura-Calixto, F.; Arija, A. Effect of Grape Seed Extract on Growth Performance, Protein and Polyphenol Digestibilities, and Antioxidant Activity in Chickens. *Span. J. Agric. Res.* **2010**, *8*, 326–333.
21. Antongiovanni, M.; Minieri, S.; Buccioni, A.; Galigani, I. Effect of a Tannin from Chestnut Wood (Castanea sativa Miller) on Cholesterol and Fatty Acids of Eggs. http://www.wpsa.com/index.php/Wpsa-Proceedings/2015/xxii-European-Symposiumon-the-Quality-of-Poultry-Meat-and-the-Xvi-European-Symposium-on-the-Quality-Ofeggs-and-Egg-Products/2150-Effect-of-a-Tannin-from-Chestnut-Wood-Castanea-Sativamiller-on-Cholesterol-and-Fatty-Acids-of-Eggs/File (accessed on 2 May 2018).
22. Brus, M.; Dolinšek, J.; Cencič, A.; Škorjanc, D. Effect of chestnut (Castanea sativa Mill.) wood tannins and organic acids on growth performance and faecal microbiota of pigs from 23 to 127 days of age. *Bulg. J. Agric.* **2013**, *19*, 841–847.
23. Rossi, P.; Nunes, J.K.; Rutz, F.; Anciuti, M.A.; Moraes, P.V.D.; Takahashi, S.E.; Bottega, A.L.B.; Dorneles, J.M. Effect of sweet green pepper on yolk colour and performance of laying hens. *J. Appl. Poult. Res.* **2015**, *24*, 10–14.
24. Podsedek, A. Natural Antioxidants and Antioxidant Capacity of Brassica Vegetables: A Review. *Food Sci. Technol.* **2007**, *40*, 1–11.
25. Ezekwe, M.O.; Besong, S.A.; Igbokwe, P.E. Beneficial influence of purslane and waterleaf supplement on cardiovascular disease risk in humans. *Hum. Niger. Food J.* **2010**, *16*, 39.
26. Togun, V.A.; Farinu, G.O.; Ojebiyi, O.O.; Akinlade, J.A.; Popoola, O.B. Evaluation of three dietary levels of wild sunflower (*Tithonia diversifolia*, Hemsi, A. Gray) forage meal on growth and carcass measurement of male rabbits. *J. Anim. Vet. Adv.* **2006**, *5*, 791–794.
27. Aro, S.O.; Tewe, O.O.; Aletor, V.A. Potentials of Siam Weed (*Chromolaena odorata*) leaf meal as egg yolk colourant for laying hens. Livestock Research for Rural Development. **2009**, *21*, 171. Association of Official Analytical Chemists (1990). Official Methods of Analysis, (Arlington, VA, AOAC). Available online: http://www.lrrd.org/lrrd21/10/aro21171.htm (accessed on 4 March 2016).