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PHYSICAL CHARACTERISTICS AND SOME CHEMICAL COMPOSITION OF LEAF PROTEIN CONCENTRATES AND BAGASSE EXTRACTED FROM SACRED FIG AND GUINEA GRASS USING SIMPLE COAGULATION TECHNIQUES

***AKAEZE, N.C., OLABIMITAN, D.A. AND IMONITIE, S.O.**
Department of Animal Science, Faculty of Agriculture, University of Benin, P.M.B.1154 Benin City, Nigeria
*Corresponding author: nneamaka.akaeze@uniben.edu

Abstract
This study evaluated the yield, physical characteristics, and chemical composition of leaf protein concentrates (LPC) and bagasse from sacred fig (Ficus religiosa L.) and Guinea grass (Megathyrsus maximus Jacq.) using three processing methods: heat coagulation, alum precipitation, and acid coagulation. Fresh leaves were harvested, washed, and blended to extract juice. Leaf protein concentrates were coagulated using heat (boiling), alum (2g per 100ml juice), or sulfuric acid (adjusted to pH 3.5). The curds were filtered, sun-dried, and analyzed for proximate and mineral composition following standard procedures. Results showed that heat coagulation produced the highest LPC yield for Ficus religiosa (2.30%), while alum precipitation gave the highest yield for Guinea grass (2.08%). Acid coagulation yielded 1.50% for Ficus religiosa and 1.59% for Guinea grass. Bagasse yield was significantly higher for Ficus religiosa (61.22%) compared to Guinea grass (21.08%). Proximate analysis revealed that acid coagulation produced the highest crude protein content in both species (Ficus: 42%; Guinea grass: 49.57%), while alum precipitation yielded the highest ether extract (Ficus: 16.83%; Guinea grass: 12.43%). Heat coagulation gave the highest crude fibre (Ficus: 3.14%; Guinea grass: 2.38%) and ash content (Ficus: 6.28%; Guinea grass: 6.30%). Mineral analysis showed that acid coagulation produced the highest concentrations of calcium, magnesium, potassium, phosphorus, iron, manganese, copper, zinc, and chlorine in Ficus religiosa LPC, while heat coagulation was superior for most minerals in Guinea grass LPC. The study concludes that acid coagulation is the preferred method for maximizing crude protein and mineral content in LPC from both species, while alum precipitation optimizes fat extraction. These underutilized leaves can serve as cost-effective protein sources for fighting malnutrition in third world countries as well as improve livestock protein utilization.

Keywords: *Leaf protein concentrate, Ficus religiosa, Megathyrsus maximus, Bagasse, Coagulation methods*

Introduction

Protein deficiency remains one of the major nutritional challenges facing livestock production in developing countries, where conventional protein sources such as soybean meal, groundnut cake, and fishmeal are increasingly expensive and often compete directly with human food requirements (Akaeze *et al.*, 2015). The cost of feed accounts for approximately 50-75% of total production expenses in animal agriculture, with the protein fraction being a major contributor to this escalating cost (Nwokoro, 2015).

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Consequently, there is an urgent need to explore alternative, locally available, and cost-effective protein sources that can reduce dependence on conventional feed ingredients without compromising animal performance.

Leaf protein concentrate (LPC) represents a promising solution to this challenge. It is a concentrated form of protein extracted from the green foliage of plants and has been recognised as potentially the cheapest and most abundant source of available protein (Tripathi *et al.*, 2011). The concept of extracting protein from leaves has a long history, with early investigations by Osborne *et al.* (1920) and Chibnall and Schryver (1921), and was significantly advanced by the work of Pirie (1971, 1978), who developed practical methods for leaf protein fractionation. Leaf protein concentrate is not only rich in high-quality protein (typically containing 25-45% crude protein on a dry matter basis) but also provides substantial amounts of essential vitamins, particularly beta-carotene (provitamin A), and minerals such as iron, calcium, and magnesium (FAO/WHO, 1985a; Singh, 1984).

The nutritional value of leaf protein concentrate has been demonstrated in various livestock species. Studies have shown successful incorporation of LPC into poultry diets (Ameenuddin *et al.*, 1983), fish feeds (Ngugi *et al.*, 2017), and calf rations (Odwongo and Mugerwa, 1980). Furthermore, research on rabbits has indicated that pawpaw leaf protein concentrate can replace up to 60% of soybean meal without negative effects on growth performance (Agbonghae and Nwokoro, 2019). These findings highlight the potential of LPC as a viable protein-vitamin-mineral supplement that can help mitigate the high cost of feed production for livestock farmers.

However, the widespread adoption of LPC faces several challenges. These include the presence of anti-nutritional factors such as phytate, tannins, and oxalates in some leaf materials (Telek *et al.*, 1983), the loss of heat-labile vitamins like vitamin C during processing (Kennedy, 1993), and the dark green colour that may affect consumer acceptance. Additionally, the efficiency of LPC extraction is highly dependent on the processing method employed, with common methods including heat coagulation, acid coagulation, and alum precipitation (Sayyed, 2011). Each method has distinct advantages and limitations, and the optimal method may vary depending on the plant species being processed.

The choice of plant material is critical for successful LPC production. Two plant species that are abundantly available in tropical regions and have potential as LPC sources are sacred fig (*Ficus religiosa* L.) and Guinea grass (*Megathyrsus maximus* Jacq.). *Ficus religiosa*, commonly known as the sacred fig or peepal tree, is a large deciduous tree belonging to the family Moraceae. It is widely distributed across tropical and subtropical regions and has significant medicinal value, with various parts of the plant used in traditional medicine for treating ailments such as asthma, diabetes, and gastrointestinal disorders (Ashwani *et al.*, 2018). The leaves of *Ficus religiosa* are known to contain various bioactive compounds including flavonoids, tannins, and saponins, but their potential as a protein source for animal feed has not been systematically explored.

Guinea grass (*Megathyrsus maximus* Jacq., formerly *Panicum maximum*) is a tall perennial grass belonging to the family Poaceae. It is

native to Africa but has been widely introduced across tropical regions as a pasture forage crop (Clayton *et al.*, 1974). Guinea grass is known for its high biomass production, palatability to livestock, and adaptability to various environmental conditions. It grows in densely tufted clumps with erect stems that can reach over 2 meters in height, and its leaves are long, narrow, and finely tipped (Alves and Xavier, 1986). While Guinea grass is primarily used as a forage for ruminants, its potential as a source of leaf protein concentrate for non-ruminant livestock and monogastric animals has received limited attention.

In the process of LPC extraction, the juice is separated from the fibrous residue known as bagasse. This by-product is not waste but a valuable source of indigestible fibre that can promote gut health, caecotrophy, and appetite stimulation in rabbits and other monogastric animals (Cheeke, 1987; Halls, 2010). Bagasse can also serve as bedding material for poultry and ruminant pens, or can be ensiled and used as animal feed during dry seasons when forage is scarce (Pirie, 1987). Thus, leaf fractionation offers a zero-waste approach to producing high-value protein supplements and functional fibrous feed ingredients.

The primary objective of this study was to determine the yield, physical characteristics, and chemical composition (proximate and mineral) of leaf protein concentrates and bagasse obtained from sacred fig (*Ficus religiosa*) and Guinea grass (*M. maximus*) leaves using three different processing methods: heat coagulation, alum precipitation, and acid coagulation. The findings will help identify the most effective processing method for each plant species and contribute to the development of sustainable, low-cost protein sources for livestock feed in tropical regions.

Materials and Methods

The experiment was carried out in the main Laboratory of the Faculty of Agriculture, University of Benin, Ugbowo Campus, Benin City, Nigeria. The Campus is located between Latitude 6º20'1.32" N of the Equator and Longitude 5º36'0.53" E of the Greenwich Meridian in the forest zone with an average temperature of 27.6°C (NAA, 2014; Google Earth, 2021). Fresh, mature leaves of sacred fig (*Ficus religiosa*) and Guinea grass (*M. maximus*) were harvested early in the morning and taken immediately to the laboratory for processing to prevent wilting. The leaves were thoroughly washed to remove dirt and sand particles. For each plant species and processing method, triplicate samples of 300g were used. Preliminary trials were conducted to standardize the procedures and water volumes.

Three processing methods were employed as described by Pirie (1987), Sayyed (2011), and modified by Nwokoro (2015). For heat coagulation, the leaves were blended with water, and the slurry was pressed through a sieve cloth (muslin cloth) to separate the juice from the fibrous bagasse. The juice was then heated to boiling, and the curd (LPC) that formed was separated from the whey using Whatman filter paper and sun-dried. The temperature and time taken for curd formation were recorded. For alum precipitation, after juice extraction, crushed alum (2g per 100ml of juice) was added with stirring. The resulting curd was filtered through Whatman filter paper and sun-dried. For acid coagulation, the juice was adjusted to pH 3.5 using 5N H2SO4 added with stirring, and the coagulated curd was filtered and sun-

dried. The bagasse from each method was also sun-dried. Precautions taken included immediate processing after harvest, use of mature leaves free from physical injury, thorough washing of equipment, and sun-drying rather than oven-drying to preserve nutrients.

The LPC and bagasse samples were analyzed in triplicate for proximate composition (moisture, ash, crude protein, crude fibre, ether extract) using AOAC (2010) methods. Moisture content was determined by oven-drying at 100°C for 24 hours. Crude protein was calculated as percent nitrogen (determined by the Kjeldahl method) multiplied by 6.25. Crude fibre was determined by sequential acid and alkali digestion. Ash content was determined by incineration at 550°C for 6 hours in a muffle furnace. Ether extract was determined by petroleum ether extraction using a Soxhlet apparatus. Nitrogen-free extract (NFE) was calculated by difference: NFE = 100 - (%CP + %EE + %CF + %Ash).

Mineral analysis was performed on the LPC samples after dry-ashing 1g of sample at 550°C in a muffle furnace and dissolving in deionized water to standard volume. Sodium and potassium were determined by flame photometry. Phosphorus was determined by the vanadomolybdate method. Magnesium, calcium, manganese, iron, copper, and zinc were determined using an atomic absorption spectrophotometer. Chlorine was determined by titration. All analyses followed AOAC (2010) procedures.

Data collected from the study were subjected to analysis of variance using GenStat 12th edition for Windows at 5% level of probability. Means with significant differences were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results

The results showing the percentage yield of the percentage extraction yield of the LPC and bagasse of sacred fig and guinea grass using the three different extraction methods is presented on table 1. It reveals significant differences in the yield for the three different methods across the two test plants, with heat coagulation (2.30%) and alum coagulation (2.08%) recording the highest yield.

Table 1: Percentage Yield of Leaf Protein Concentrate (LPC) and Bagasse from Sacred Fig and Guinea Grass Using Three Processing Methods

Plant Species	Processing Method	LPC Yield (%)	Bagasse Yield (%)
Ficus religiosa	Heat Coagulation	2.30^{b}	61.22^{c}
Ficus religiosa	Alum Precipitation	1.41^{a}	-
Ficus religiosa	Acid Coagulation	1.50^{a}	-
Guinea Grass	Heat Coagulation	1.84^{b}	21.08^{a}
Guinea Grass	Alum Precipitation	2.08^{c}	-
Guinea Grass	Acid Coagulation	1.59a	-
	SEM	0.08-0.16	-

Means with different superscripts within a column and plant group differ significantly ($p<0.05$).
SEM = Standard Error of Mean

Table 2: Physical Characteristics of Whole Leaf, LPC, Bagasse, and Whey of Sacred Fig and Guinea Grass Before Drying (Fresh)

Plant Species	Character	Leaf	LPC	Bagasse	Whey
Ficus religiosa	Colour	Green	Dark green (Alum)	Green	Brownish transparent (Alum)
			Light green (Heat)		Brownish turbid (Heat)
			Dark green (Acid)		Brownish transparent (Acid)
	Texture	Smooth	Smooth	Fibrous	-
	State	Solid	Semi-solid	Solid	Liquid
Guinea Grass	Colour	Green	Dark green (Alum)	Green	Brownish turbid (Acid)
			Light green (Heat)		Brownish transparent (Alum)
			Dark green (Acid)		Brownish turbid (Heat)
	Texture	Smooth	Smooth	Fibrous	-
	State	Solid	Semi-solid	Solid	Liquid

Table 3: Physical Characteristics of Whole Leaf, Bagasse, and LPC of Sacred Fig and Guinea Grass After Sun Drying (30-35°C)

Plant Species	Character	Leaf	Bagasse	LPC
Ficus religiosa	Colour	Brown	Light green	Dark green (Alum)
				Brownish green (Heat)
				Dark green (Acid)
	Texture	Coarse	Fibrous	Smooth
	State	Solid	Solid	Solid
Guinea Grass	Colour	Brownish green	Green	Dark green (Alum)
				Light green (Heat)
				Dark green (Acid)
	Texture	Smooth	Fibrous	Smooth
	State	Solid	Solid	Solid

Table 4 presents the results of the proximate composition of the LPC and bagasse of sacred fig and guinea grass processed using 3 different methods. It reveals that the crude protein content of the leaf protein concentrate was highest for acid coagulation method for both leaves and lowest for heat coagulation for both leaves. These differences were significantly different. This sequence in difference was however not observed for the crude fibre content of the two leaves when examined across the three processing methods, as heat coagulated LPC recorded the highest crude fibre content for both leaves.

Table 4: Proximate Composition of Leaf Protein Concentrate and Bagasse from Sacred Fig and Guinea Grass Using Three Processing Methods

Plant Species	Method	% Moisture	% Ash	% EE	% CP	% CF	% NFE
Ficus religiosa	Acid Coagulation	5.12a	8.04c	15.31b	42.00c	1.74a	27.71a
Ficus religiosa	Alum Precipitation	6.30b	5.32a	16.83c	35.00b	1.52a	35.06b
Ficus religiosa	Heat Coagulation	5.20a	6.28b	9.31a	33.00a	3.14b	42.38c
	SEM	0.10	0.2	0.2	0.48	0.1	0.38
Ficus religiosa Bagasse	-	10.23	4.81	4.21	21.58	8.7	50.47
Guinea Grass	Acid Coagulation	5.21a	3.99a	10.30b	49.57c	2.37	28.55a
Guinea Grass	Alum Precipitation	6.30b	3.52a	12.43c	47.83b	2.06	27.86a
Guinea Grass	Heat Coagulation	7.36c	6.30b	6.58a	36.75a	2.38	40.80b
	SEM	0.15	0.26	0.2	0.47	0.11	0.52
Guinea Grass Bagasse	-	10.78	5.23	4.31	15.75	28.55	35.37

Means with different superscripts within a column for each plant differ significantly ($p<0.05$).
Means without superscripts are not significantly different. EE = Ether Extract, CP = Crude Protein, CF = Crude Fibre, NFE = Nitrogen Free Extract, SEM = Standard Error of Mean.

Table 5: Mineral Composition of Leaf Protein Concentrate from Sacred Fig and Guinea Grass

Plant Species	Method	Ca	Mg	K	Na	P	Fe	Mn	Cu	Zn	Cl
		mg/kg									
Ficus religiosa	Acid Coagulation	1849c	1789c	3893c	9.60c	1222c	457.7c	196.9c	45.6c	322.2c	3.61b
Ficus religiosa	Alum Precipitation	409a	390a	859a	2.30a	276.6a	95.8a	43.87a	9.79a	70.73a	0.84a
Ficus religiosa	Heat Coagulation	1656b	1566b	3470b	8.50b	1084b	394.2b	173.33b	40.0b	283.37b	3.51b
	SEM	10.13	23	30	0.16	5.83	3.79	1.4	0.39	0.84	0.22
Guinea Grass	Acid Coagulation	677.7b	513.3b	1379b	3.57b	448.0b	164.1b	70.99b	16.25b	116.7b	1.48b
Guinea Grass	Alum Precipitation	304.3a	296.0a	588a	1.57a	206.7a	68.0a	30.57a	6.83a	49.3a	0.67a
Guinea Grass	Heat Coagulation	771.7c	704.0c	1590c	3.78b	529.0c	187.5c	81.60c	18.63c	134.4c	1.72c
	SEM	5.87	1.64	3.7	0.09	3.83	1.9	0.73	0.16	1.23	0.05

Discussion

Yield of Leaf Protein Concentrate and Bagasse

The yield of leaf protein concentrate was significantly influenced by both plant species and processing method. For *Ficus religiosa*, heat coagulation produced the highest LPC yield (2.30^{b}%), which was significantly greater than yields from alum precipitation (1.41a%) and acid coagulation (1.50a%). This finding is not consistent with reports by Akaeze *et al.* (2015) for rubber leaf protein concentrate (5.6%) and Isuzu (2014) for pumpkin leaf protein concentrate (4.19%), although the yields obtained in this study were lower. The lower yield from *Ficus religiosa* may be attributed to the inherent nature of the plant material, including the presence of tannins and other phenolic compounds that can bind proteins and reduce extractability (Telek *et al.*, 1983).

For Guinea grass, alum precipitation produced the highest LPC yield (2.08^{c}%), followed by heat coagulation (1.84^{b}%) and acid coagulation (1.59^{a}%). The yield from alum precipitation was lower than the 2.93% reported by Sayyed (2011) for *Raphanus sativus* using the same method, again reflecting species-specific differences in protein content and extractability. The higher yield from alum precipitation compared to other methods may be due to the ability of alum to effectively coagulate proteins across a broader pH range, as well as its capacity to form insoluble complexes with proteins.

The bagasse yield from *Ficus religiosa* (61.22c%) was significantly higher than that from Guinea grass (21.08a%), indicating that sacred fig leaves have a much higher fibre content. This is consistent with the woody nature of *Ficus religiosa* compared to the herbaceous Guinea grass. The bagasse yield from Guinea grass was lower than the 23.62% recorded by Agbonghae (2016) for pawpaw leaf bagasse, suggesting variations in leaf morphology and fibre composition across species.

Proximate Composition

The crude protein content of LPC varied considerably between species and methods. Acid coagulation produced the highest crude protein content in both species: 42.00c% for *Ficus religiosa* and 49.57c% for Guinea grass. These values are notably higher than those reported for *Amaranthus hybridus* (34.8%) by Adeyeye and Omolayo (2011) and for rubber leaf protein concentrate (32.64%) by Akaeze *et al.* (2015). The high protein content in Guinea grass LPC from acid coagulation (49.57^{c}%) falls slightly short of that reported for *Telfaira occidentalis* LPC (54.80%) by Sodamade *et al.* (2013). The superiority of acid coagulation for protein extraction may be explained by the fact that adjusting the pH to 3.5, near the isoelectric point of many leaf proteins (particularly rubisco, which has a pI of 5.3-5.9), minimizes protein solubility and promotes maximum precipitation (Komatsu *et al.*, 1996; Nelson and Surzycki, 1976).

The ether extract (fat) content was highest with alum precipitation for both species (Ficus: 16.83c%; Guinea grass: 12.43c%). These values are substantially higher than those reported for *Amaranthus hybridus* (9.6%) and *Telfaira occidentalis* (10.7%) by Adeyeye and Omolayo (2011). The high fat content in alum-precipitated LPC may be due to the ability of alum to co-precipitate lipoproteins and membrane lipids along with the protein fraction. Acid coagulation also produced relatively high fat content (Ficus: 15.31b%; Guinea grass: 10.30b%),

while heat coagulation gave the lowest fat values (Ficus: 9.31a%; Guinea grass: 6.58a%), possibly due to thermal degradation or melting and separation of lipids during heating.

Crude fibre content was low in all LPC samples (1.52-3.14%), confirming that the extraction process effectively separates protein from the fibrous matrix. This is a key advantage of LPC over unprocessed leaf meal, as high fibre content can reduce nutrient digestibility and feed intake in monogastric animals (Pirie, 1978). The bagasse, however, contained high levels of crude fibre (Ficus: 8.70%; Guinea grass: 28.55%), making it suitable as a fibrous feed for ruminants or as a bedding material.

Ash content, which reflects mineral richness, was highest in acid-coagulated *Ficus religiosa* LPC (8.04c%) and heat-coagulated Guinea grass LPC (6.30b%). These values are lower than those reported for *Amaranthus hybridus* (17.2%) by Adeyeye and Omolayo (2011) but still represent substantial mineral contributions. The negative relationship between ash and crude protein content observed by Cheeke *et al.* (1981) was also evident in this study, where samples with higher ash tended to have lower protein content.

Mineral Composition

The mineral composition of LPC showed dramatic differences between species and processing methods. For *Ficus religiosa*, acid coagulation produced the highest concentrations of almost all minerals analyzed, including calcium (1849c mg/kg), magnesium (1789c mg/kg), potassium (3893c mg/kg), phosphorus (1222c mg/kg), iron (457.7c mg/kg), manganese (196.9c mg/kg), copper (45.6c mg/kg), and zinc (322.2c mg/kg). These values are substantially higher than those reported for lucerne LPC by Siebrits *et al.* (1986), indicating that acid coagulation is particularly effective at co-precipitating minerals with the protein curd. The low pH (3.5) likely increases the solubility of mineral ions, allowing them to become trapped within the coagulated protein matrix.

For Guinea grass, however, heat coagulation produced the highest concentrations of most minerals, including calcium (771.7c mg/kg), magnesium (704.0c mg/kg), potassium (1590c mg/kg), phosphorus (529.0c mg/kg), iron (187.5c mg/kg), manganese (81.60c mg/kg), copper (18.63c mg/kg), and zinc (134.4c mg/kg). This species-specific response suggests that the chemical environment within the leaf juice, including the presence of chelating agents such as oxalates and phytates, significantly affects mineral behaviour during coagulation. *Ficus religiosa* leaves are known to contain various phenolic compounds and tannins (Ashwani *et al.*, 2018), which may bind minerals differently than the components in Guinea grass.

The high iron content in LPC from both species, particularly *Ficus religiosa* acid-coagulated LPC (457.7c mg/kg) and Guinea grass heat-coagulated LPC (187.5c mg/kg), is noteworthy. Iron deficiency is a major global health problem affecting both humans and animals, and LPC could serve as a potent, low-cost iron supplement (WHO, 1985). The calcium and phosphorus values obtained are also sufficient to contribute meaningfully to bone formation and metabolic functions (Olusanya, 2008).

Sodium content was low across all samples (1.57-9.60 mg/kg), which is beneficial as excessive sodium intake can lead to hypertension and other health

issues. The chlorine content followed similar patterns to sodium, as expected.

Conclusion

This study has successfully demonstrated that both sacred fig (*Ficus religiosa*) and Guinea grass (*Megathyrsus maximus*) leaves are viable sources of high-quality leaf protein concentrate with significant potential as alternative protein supplements in livestock feed. The choice of processing method significantly influences yield, proximate composition, and mineral profile. For maximizing crude protein content, acid coagulation is the superior method for both species, producing LPC with 42% protein from sacred fig and nearly 50% protein from Guinea grass. For maximizing fat extraction, alum precipitation is most effective. For mineral-rich LPC, acid coagulation is preferred for sacred fig, while heat coagulation is better for Guinea grass. The bagasse by-product, particularly from sacred fig (61%), is a valuable source of fibrous material for ruminant feeding or bedding. These findings support the promotion of leaf protein concentrate technology as a sustainable, low-cost strategy for improving protein nutrition in tropical livestock systems.

Recommendations

Based on the findings of this study, the following recommendations are made. Livestock farmers and feed manufacturers should adopt acid coagulation as the preferred method for producing high-protein leaf protein concentrate from both sacred fig and Guinea grass leaves, as this method consistently yielded the highest crude protein content (42-50%). For applications where fat content is important, such as in high-energy diets, alum precipitation is recommended. The production of leaf protein concentrate should be encouraged at both small-scale village level and industrial scale, as the technology is simple and requires minimal equipment. Further research should focus on in vivo feeding trials to evaluate the bioavailability of nutrients from these LPCs in various livestock species, including rabbits, poultry, pigs, and fish. Studies should also investigate pre-treatments to reduce anti-nutritional factors such as tannins in *Ficus religiosa* leaves, which may affect palatability and nutrient absorption. The bagasse should not be discarded but utilized as a fibrous feed supplement for ruminants or as bedding material. Finally, economic analysis of LPC production from these species should be conducted to determine cost-effectiveness and guide adoption by farmers.

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