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RESEARCH ARTICLE
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The Nutritive Values of Pawpaw Leaf Meal as a Potential Alternative Feed Source under Different Processing Methods

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ABSTRACT

The current study evaluated the nutritional value of pawpaw leaf meal processed differently by air-drying, sun-drying, oven-drying, and fermentation. The leaf meals were subjected to proximate, fiber fractions, phytochemicals, and functional properties analyses. Data were analyzed using the SPSS statistical package for the separation of means. The Air-dried pawpaw leaf meal (PLAD) was significantly different with higher moisture content (7.3%), crude protein (28.2%), and ether extract (8.08%) while the sun-dried pawpaw leaf meal (PLSD) crude fiber (6.48%) was higher. The flavonoid level was higher in PLSD (66.70mg/g) likewise the alkaloid level (28.40%), but the phenolic value was higher in PLAD (743.76mg/g). Water absorption capacity was significant in PLSD (67.98%) but PLAD had a higher oil absorption capacity (19.36%), while PLF bulk density was higher (0.58g/mL). There was no significant difference (P>0.05) in the acid detergent fiber, however, PLF had the highest value (29.66%), likewise, for Neutral detergent fiber (29.66%). For hemicellulose, PLOD (1.66%) was significantly different (P<0.05) from the other treatment, with the lowest value. Cellulose was significantly different (P<0.05) across treatments with PLF (22.0%) having the highest value, while the lignin for PLOD had the lowest value (1.66%). The results for proximate, fiber fraction, and phytochemical evaluation have shown the benefit of the different processing methods, hence, PLM is recommended as a potential feed ingredient for livestock.

Keywords: Proximate analysis; Functional properties; Pawpaw leaf; Potential feed ingredients; Phytochemical evaluation.

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INTRODUCTION

Papaya is a green tree with silky stems that resemble palms. Its fruit is one of the most nutritious foods available all year round, with high levels of magnesium, potassium, vitamin E, vitamin C, vitamin A, vitamin B (pantothenic acid), fiber, folate, and folate (Adeoye et al., 2024). Papaya leaves are low in calories and packed with vitamins and numerous minerals in substantial amounts (Sharma et al., 2022). One of the main obstacles to raising livestock in developing nations is the increasing cost of main feeding ingredients, their scarcity and limited availability and the competition between humans and animals for these ingredients. Due to this fact, the profitability that goes to the farmer is critically reduced since feed costs well over 70% of the entire cost of production (Agama-Acevedo et al., 2016; Hamid et al., 2022). It has impacted livestock production profitability, resulting in a decreased number of farmers (Olasunkanmi et al., 2021). It is necessary to mitigate this problematic situation by taking advantage of the potential of unconventional feed ingredients like leaf meals such as pawpaw leaf, which are highly nutritious but wasted (Ebenebe et al., 2011), as there is limited knowledge regarding their nutritional characteristics (Etim et al., 2018). Therefore, these non-traditional feed sources need to be processed to eliminate antinutritional elements, reduce the amount of fiber, and identify their proximate composition and functional characteristics, which may

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have an impact on livestock acceptance, palatability, and usage (Alabi et al., 2023). Most herbaceous plants are known to contain some bioactive compounds that are useful for the health of animals, such as vitamins, phenolic acids, flavonoids, tannins, and saponins (Okunlola et al., 2023). Their functional properties can benefit animals' health; this has become obvious post-COVID, focusing on improving the immune system against such occurrences in humans and livestock (Alabi et al., 2023).This study compared and evaluated the nutritional value of pawpaw leaf using various physical processing techniques, including air-drying, sun-drying, oven-drying, and fermentation, to enable the use of pawpaw leaf as a potential substitute ingredient in livestock feed.

MATERIALS & METHODS

Experimental Location

This study was conducted at the Project Research Laboratory I of Landmark University in Kwara state, Nigeria. Kwara State is located between 4°35′ east longitude and latitudes 8° to 32° north of the Greenwich Meridian (Elemile et al., 2019) with an average annual rainfall of 101.45mm (3.99") of precipitation and 148.38 rainy days (40.65% of the time) annually. The average relative humidity for the duration of the experiment was 50%.

Pawpaw Leaf Collection, Preparation and Processing

Healthy pawpaw leaves were harvested from varieties of pawpaw trees in Omu-Aran, Irepodun LGA, Kwara State. The collected pawpaw leaves were subjected to different processing techniques in triplicates.

After carefully rinsing the fresh *Carica papaya* leaves in distilled water, they were sliced into smaller pieces using a sharp, sterile knife. They were then kept at room temperature before being divided into batches for the different processing methods. The pawpaw leaves were sun-dried, oven-dried, air-dried for 2-3 days. The fermented process was carried out by placing sliced leaves in glass jars and filled with water. The lid was tightly covered to ensure a good anaerobic environment for 3 days, after which the water was drained and the leaves oven-dried at 40ºC for 48 hours for proper drying. The sample was then blended into powder in an electric blender (Warring MS-100). The samples were sealed in containers and stored with clear labels before use.

Proximate Analysis of Pawpaw Leaves

Pawpaw leaves were analyzed for their crude protein content, moisture content, ether extract, crude fiber and ash content. The proximate constituent of the leaves was assessed at the Animal Science Laboratory of Landmark University using the Association of Official Analytical Chemist Methods (AOAC, 2012).

(Quantitative analysis) Phytochemical Screening

Total Flavonoid Level

A modified method outlined by Alabi et al. (2023) was used to determine the total flavonoid content of both extracts. The sample was prepared by mixing 0.5mL of appropriately diluted material, 0.5mL of methanol, 50% $AlCl_3$, 50% potassium acetate, and 1.4mL of water, this mixture was allowed to sit at room temperature for half an hour. The total flavonoid content was then calculated by measuring the absorbance of the reaction mixture at 415nm.

Determination of Total Phenol Content

The total amount of phenol was ascertained using the Oluba et al. (2021) method. To neutralize the extracts, 2.0mL of 7.5% sodium carbonate was used, it was then oxidized with 2.5mL of 10% Folin-Ciocalteau's reagent (v/v). A UV/V Spectrophotometer (model L7) was used to measure the absorbance of the sample at 765nm after it had been incubated for 40 minutes at 45°C. The concentration of total phenol was then measured as gallic acid equivalent.

Determination of Alkaloid

The method of Ojinnaka et al. (2017) was slightly modified to ascertain the alkaloid content of the pawpaw leaf sample material. A 250mL conical flask was filled with 200 millilitres of ethanol and 10% acetic acid after five grams of the sample had been weighed in it. The mixture was covered with foil and left to stand at room temperature for 2 hours. After that, the mixture was immersed in a water bath heated to 50°C and left to evaporate until it had been reduced to one-quarter of its initial amount. Droplets of concentrated ammonia solution were added and allowed to settle down. Whatman filter paper No. 42 was to filter the precipitate, and the mixture was oven-dried at 45 degrees Celsius to a constant weight.

Functional Properties of Pawpaw Leaves

Bulk Density

Using the technique of Alabi et al. (2023), a measuring cylinder was placed on a tarred balance and the weight was recorded. Then some quantity of leaf meal was weighed into the cylinder and tapped until a stable volume of the powdered sample was obtained. The volume was then recorded. After which the content in the cylinder was placed on the balance to get the weight of the sample.

Water Absorption Capacity

The method adopted by Alabi et al. (2023) was used to determine WAC. A well-blended powdered sample of 1.00g was weighed into the beaker and recorded. Then 10mL of water was added and stirred vigorously for 5 minutes, the content of the beaker was then transferred into weighted centrifuge tubes (recorded the weight of the centrifuge tubes as W_1). The samples were centrifuged at 3500rpm for 30 minutes, then the supernatant was decanted and adhering water inside the tube was removed. Then the weight of the residue and tube was recorded.

Oil Absorption Capacity

For OAC, the Alabi et al. (2023) method was employed. A well-blended powdered sample weighing 1.00g was placed in the beaker. Soybean oil (10mL) was added to the sample and stirred vigorously for 5min. The content of the beaker was transferred into the weighted centrifuge tubes. It was then centrifuged at 3500rpm for 30min. The supernatant was decanted to remove any adhering oil inside the tube, and the weight of the sediment and tube was recorded.

Data Analysis

Results were subjected to statistical analysis using the IBM SPSS statistics 22 package, and Duncan's multiple range test was used to differentiate the means at a significance value of 0.05.

RESULTS

The proximate composition for pawpaw leaf air-dried (PLAD), pawpaw leaf sun-dried (PLSD), pawpaw leaf oven-dried (PLOD) and pawpaw leaf fermented (PLF) is shown in Table 1. The ether extract was significantly different ($P<0.05$) with PLOD (8.08%) having a higher value of ether extract from the other treatments and PLAD having the lowest ether extract value (4.50%). From the results, the crude protein level was not significantly different. The PLAD had a higher crude protein of 28.22%, while the PLSD had the lowest crude protein level of 24.87%. The moisture was not significantly different ($P>0.05$), but the PLOD had the highest moisture content level of 7.33%, while the PLF had the lowest moisture content of 3.67%. The ash content was not significantly different ($P>0.05$), with the PLF (2.35%) having the highest ash content and the PLSD having the lowest ash content (1.85). For crude fiber, all the treatments were significantly different ($P<0.05$) with the PLAD having the highest amount of crude fiber (6.48%), while the PLF was lower (3.98). The NFE of the PLF was significantly different ($P<0.05$) from the other treatments with a higher value of 56.59% and the PLOD having the lowest NFE (51.53%). The dry matter was not significantly different ($P>0.05$) across the various treatments, with the PLF having the highest value of 96.33% and the PLOD having the lowest dry matter value of 92.66%.

The qualitative phytochemical analysis of papaya leaves is shown in Table 2. The parameters evaluated are alkaloids, flavonoids, phenols, steroids, glycosides, total phenol, triterpenes, saponins, and tannins. The screening results showed that alkaloids, tannins, saponins, flavonoids, steroids, glycoside, and total phenol were detected in all the treatments. The screening results showed that the triterpene was absent in all the treatments.

Table 3 shows the amounts of alkaloids, flavonoids, tannins, and T. phenols in PLAD, PLSD, PLOD, and PLF. The alkaloid, flavonoid and phenolic values in the PLAD, PLSD, PLOD and PLF were significantly different ($P<0.05$). PLAD (743.76mg/g) phenolic acid value was the highest, while PLOD (466.56mg/g) had the least value. PLSD (28.40%) showed the highest alkaloid value, with PLOD (21.55%) having the lowest value. PLF (66.70mg/g) had the highest flavonoid level, with PLOD (33.70mg/g) having the lowest value.

The functional properties of papaya leaf samples exposed to different processing techniques are shown in Table 4 for the oil absorption capacity, bulk density, and water absorption capacity of papaya leaf extracts. A substantial variation was observed in the oil absorption capacity of the various treatments of the papaya leaves; it was higher in PLAD (19.36%), intermediate in PLF (17.12%) and PLSD (16.84%), and lowest in PLOD (13.98%). Water absorption capacity in PLSD has the highest water absorption capacity (67.98%) with PLAD having the lowest water absorption capacity (54.35%). The bulk density for PLOD (0.55g/mL) and PLF (0.58g/mL) showed higher and relative values.

Table 1: Proximate composition (%) of papaya leaves processed differently

Treatment	CP	EE	M	ASH	CF	NFFE	DM
PLAD	28.22±0.17a	4.50±0.14b	6.67±0.44	2.23±0.05ab	6.48±0.04a	51.90±0.18a	93.33±0.44
PLSD	24.87±0.07c	5.42±0.08b	6.67±0.16	1.85±0.21b	5.86±0.41ab	55.33±0.68a	93.33±0.16
PLOD	25.83±0.05b	8.08±0.54a	7.33±0.16	1.96±0.00b	5.27±0.21b	51.53±0.46b	92.66±0.16
PLF	26.00±0.07b	7.41±0.08a	3.67±0.16	2.35±0.05a	3.98±0.26c	56.59±0.24b	96.33±0.16
p-value	0.080	0.010	0.160	0.004	0.105	0.059	0.160

Values (mean±SE) in a column bearing different alphabets differ significantly ($P<0.05$). CP: crude protein, EE: Ether extract, CF: crude fiber, M: moisture, NFE: nitrogen-free extract, DM: dry matter. PLAD: pawpaw leaf air-dried sample PLSD: pawpaw leaf sun-dried sample PLOD: pawpaw leaf oven-dried sample PLF: pawpaw leaf fermented sample.

Table 2: Qualitative phytochemical screening of papaya leaves

Treatment	Alkaloid	Tannin	Saponin	Flavonoid	Steroid	Triterpenes	Glycosides	T. Phenol
PLAD	+++	++	+++	++	+++	-	+++	++
PLSD	+++	+++	+++	++	+++	-	+++	++
PLOD	+++	+++	+++	++	+++	-	+++	++
PLF	+++	+++	+++	++	+++	-	+++	++

Denotations represent the level of availability of the phytochemical in the treated leaves: not present = -, mild = +, moderate = ++, abundance = +++

Table 3: Phytochemical quantitative screening of papaya leaves

Treatment	Total Phenol (mg/g)	Alkaloid (%)	Flavonoid (mg/g)
PLAD	743.76±0.00a	26.23±0.00	39.00±0.00
PLSD	496.15±0.003c	28.40±0.02	41.70±0.02
PLOD	466.56±0.01d	21.55±0.02	33.70±0.02
PLF	533.99±0.03b	25.21±0.61	66.70±0.00
p-value	0.001	N/S	N/S

Values (mean±SE) in a column bearing different alphabets differ significantly ($P<0.05$).

Table 4: Functional properties of papaya leaves

Treatment	WAC (%)	OAC (%)	BD (g/mL)
PLAD	54.35±1.63c	19.36±1.34a	0.50±0.00b
PLSD	67.98±0.98a	16.84±1.51ab	0.51±0.01b
PLOD	64.46±2.50ab	13.98±0.44b	0.55±0.00a
PLF	59.17±1.34bc	17.12±0.08ab	0.58±0.01a
p-value	0.534	0.025	0.69

Values (mean±SE) in a column bearing different alphabets differ significantly (P<0.05). OAC: oil absorption capacity BD: bulk density, WAC: water absorption capacity.

Table 5: Fiber Fractions (%) of Papaya Leaves

Treatment	NDF	ADF	HEM	CELL	LIG	SILICA
PLAD	21.83±1.96bc	20.00±1.73bc	1.83±0.60	14.66±0.33	3.66±1.20	2.00±0.00a
PLSD	26.11±0.31ab	23.00±0.57b	2.83±0.33	20.00±2.00	2.33±1.20	1.00±0.00b
PLOD	19.66±1.01c	18.00±0.57c	1.66±0.44	18.33±3.84	1.66±0.88	1.33±0.33b
PLF	29.66±1.74a	27.00±2.64a	2.66±0.44	22.00±1.73	4.00±0.57	1.00±0.00b
p-value	0.171	0.281	0.682	0.035	0.450	0.001

Values (mean±SE) in a column bearing different alphabets differ significantly (P<0.05). ADF: Acid detergent fiber, NDF: Nitrogen detergent fiber, HEM: Hemicellulose, CELL: Cellulose, SIL: Silicon, LIG: Lignin

Table 5 shows the fiber fractions of papaya leaf samples subjected to various processing techniques. There was no significant difference (P>0.05) in the acid detergent fiber (ADF), with the PLF having the highest value (29.66%), while PLSD had a value of 26.11%. Neutral detergent fiber (NDF) percentage in the various treatments is not significantly different, with PLF (29.66%) having the highest value and PLOD (19.66%) having the lowest value. In hemicellulose, PLOD (1.66%) was different from the other treatment, having the lowest value. PLSD (2.83%) has the highest value. Cellulose was significantly different (P<0.05). The PLF (22.0%) has the highest value, and the PLAD (14.66%) has the lowest value. Silica was significantly different. PLAD (2.00%) has the highest value, and PLSD (1.00%) and PLF (1.00%) have the lowest value. Lignin PLF has the highest value of (4.00%), while PLOD has the lowest value of (1.66%).

DISCUSSION

In the current study, the proximate composition of the pawpaw leaves subjected to different processing methods was significantly different (P<0.05) across the treatments as shown in Table 1. The PLF with 3.67% moisture content was better, which can be associated with the effect of fermentation on the leaf fiber (Godswill Awuchi et al., 2019). However, the ash content was moderate, with the highest being 2.35% in PLF and the lowest at 1.85% in PLSD, which could suggest the effect of fermentation on the bioavailability of minerals in the leaves, possibly through the breakdown of anti-nutritional factors (Olasunkanmi et al., 2021). The ether extract was significant in PLOD at 8.08% compared to other treatments, possibly due to the concentration effect of the processing methods on POD and PLF. It could indicate fermentation capacity to concentrate lipids and fatty esters in the leaf cell walls, which may be valuable depending on usage (Fadzilah et al., 2020). PLF crude fiber (3.98%) was the lowest, reflecting the potential of fermentation in a partial breakdown of fibrous parts, making leaf digestibility possible. It is worthy to note that when considering pawpaw leaf meal for feed formulation, monogastric animals may quickly digest crude fiber, whereas enough amounts of fiber are necessary for the intestinal tracts of ruminant animals to operate efficiently (Ifemeje et al., 2015). Proteins have vital structural and biological roles in feed, influencing its texture, flavour, and appearance (Twinomuhwezi et al., 2020). The crude protein was highest in PLAD (28.22%), while PLSD (24.87%) showed the least crude protein (Table 1), this may imply that a controlled environment could have benefited airdrying in reducing protein degradation, while microbial activities might have partly affected fermentation, and that oven heat caused slight protein denaturation (Twinomuhwezi et al., 2020). However, it shows that pawpaw leaf meal is a potential alternative protein source for feed composition. NFE in the pawpaw leaves is not significantly different across the various treatments, however, the PLF (56.59%) had the highest NFE value, suggesting that carbohydrate availability might be due to the effect of fermentation in potentially breaking down complex carbohydrates into simple and digestible forms (Olajide & Adeloye, 2018).

Plant food, leaves, and other plant parts are natural sources of phytochemicals, which are bioactive substances. These chemicals protect other substances in concert with nutrients and dietary fiber (Ifemeje et al., 2015). Adeoye et al. (2024), reported that the potential for employing pawpaw leaves as nutrient-dense animal feed is increased by their phytochemical composition. One of such phytochemicals is alkaloids, which are toxic amines that are naturally present in plants but serve as a defence mechanism (Taylor & Hefle, 2017). However, the concentration of alkaloids was not significantly different but consistent across all treatments as demonstrated in Table 3. The presence of flavonoids in pawpaw leaf meal raises the possibility of using them as animal feed because they are non-nutritive plant chemicals with disease-preventive qualities (Nhon Hoang et al., 2023). The result of the flavonoids shows that PLF has the highest content (66.70mg/g), demonstrating the flavonoid retention capacity of fermentation. The PLAD phenolic content (743.76mg/g) was the highest, showing the effect of airdrying in conserving the leaf meal phenolic content. The PLOD at 466.56mg/g with the lowest value might have undergone heat denaturation (Table 3).

Feed ingredients functionality is a quality unrelated to their nutritional value that influences how they are used and applied and how they impact the final appearance, flavor, and texture (Ajatta et al., 2016). The 19.36% for OAC in PLAD seen in Table 4 was significantly different from the other treatments. This suggests that the preparation methods influence its oil absorption capacity, an important consideration when choosing feed for animals, as it affects the mouthfeel, flavor, texture, and yield of the product (Oluba et al., 2021; Alabi et al., 2023). According to Bedasso (2021), OAC has been associated with lipid-protein interactions involving noncovalent bonds such as hydrogen bonding, hydrophobic interactions, and electrostatic interactions, as well as the physical trapping of oil within proteins, as demonstrated in the PLAD. The PLSD value of 67.98% for WAC was higher than that of the other treatments, showing higher retention capacity, according to Godswill Awuchi et al. (2019), it is congruent with the hydrophilic groups that bind the water molecule availability and the macromolecule's ability to form a gel. The bulk density (BD) is the mass of numerous feed material particles per total volume they occupy (Godswill Awuchi et al., 2019). This implies that the bulkiness of pawpaw leaves in this study is influenced by their processing method, with the PLF (0.58g/mL) and PLOD (0.55g/mL) having a better value.

Feed digestibility can be determined by employing fiber fraction analysis. In Table 5 of this study, the acid detergent fiber (ADF) was significantly different, with the PLF having the highest value (27.00%), indicative of fermentation potential breakdown of fiber through microbial activities. The nitrogen detergent fiber (NDF) in the various treatments was significantly different, with PLF (29.66%) showing a higher value potentially due to the concentration of fibrous materials through fermentation. Hemicellulose is a class of complex carbohydrates that envelop the cellulose fibers of plant cells and other carbohydrates (Ning et al., 2017), with PLSD (2.83%) having the highest value, although relatively consistent across treatments. Cellulose, the fundamental structural element of plant cell walls, makes up approximately 33% of all vegetable matter (Singh & Kim, 2021). The cellulose content was significantly different, with the highest value of 22.00% in PLF potentially resulting from the effects of fermentation in cellulose concentration, suggesting its usefulness in ruminant feed due to a slow digestion of cellulose. More mature plants always have higher levels of lignin (Olajide, 2016), with the PLF having the highest value of 4.00%, significantly different from the other treatments and a possible demerit depending on usage. However, the PLOD showing the lowest value of 1.66%, will be suitable for consideration in diet formulation.

Conclusion

The study's results indicated that papaya leaves have the potential to be used as animal feed, regardless of the processing method, based on the evaluations of the leaves' nutritional, phytochemical, and functional properties and the purpose for usage. However, all the processes can be employed for feeding trials to determine the most effective inclusion level of pawpaw leaves for different animal species and how they interact with other feed components to optimize animal diets.

Conflict of Interest: All authors declare no conflict of interest.

Authors Contribution: OOP and AOO: Conceptualization, Writing, original draft preparation, review and editing, Project administration, Formal analysis. Funding acquisition. AES and AG: Investigation, Methodology, Formal analysis, Data gathering

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