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Original Research Article

Effect of Fermentation Time on Proximate Composition, Phytochemical and Functional Properties of *Delonix regia* Seeds

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ABSTRACT

This study looked at how the length of fermentation influenced the proximate, phytochemical, and functional characteristics of *Delonix regia* seeds. For each treatment, 600g of the drained-soaked seeds were used. the treatments were in triplicate in a completely randomized design. The seeds were naturally fermented in polythene bags for different lengths of time in a dark place. There were five treatments, denoted T1, T2, T3, T4, and T5, with corresponding fermentation durations of 0 days, 1 day, 2 days, 3 days, and 4 days. The results showed that T5 had a higher crude fat content at 20.83, while T1, the unfermented control, had the lowest at 10.37. The crude fibre content in T1 showed a high value of 9.58, whereas T2 and T5 gave the highest and lowest values among the fermented treatments at 9.30 and 7.05, respectively. T1 at 39.99 alkaloid content was higher but lower in T5 at 36.01. T2 saponin at 9.92 was higher but lower in T5 (7.40). T1 had a better water absorbing capacity (38.88), while T4 had a better oil absorbing capacity (19.60). The bulk density and specific gravity in the treatments were not significantly different. The best value for Paste clarity was at T4 (85.03).
The study showed that the phytochemical composition of the *Delonix regia* seeds dropped with increased fermentation time, and the proximate composition of the seeds improved, signifying that fermentation enhances the seeds' nutritional properties. To optimize its nutritional potential, it is recommended to ferment the seeds for at least three days.

Keywords: *Delonix regia* seeds, Fermentation, Proximate, Phytochemical, Nutritive value

Introduction

Plant materials have the potential to be enhanced through fermentation to boost their nutritional value and bioactive components for use as livestock feed. To optimize the potential of plant seeds as feed ingredients, the proximate, phytochemical, and functional characteristics must be comprehended with regard to the effects of fermentation.[1] Previous research on plant fermentation has yielded encouraging results In terms of increasing nutritional quality and bioactivity. Studies on the fermentation of soybean meal and maize, for example, have shown better protein content, greater digestibility, and lowered anti-nutritional factors (ANFs).[1, 2] *Delonix regia* seeds and other plant materials have of recent, been evaluated for use in animal feed.[3] On the potential of *Delonix regia* as livestock feed, only a limited study has been done.[4] To bridge this knowledge deficit, this study evaluated how fermentation influences the proximate, phytochemical, and functional properties of *Delonix regia* seeds.

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The fermentation process employed for the *Delonix regia* seeds was designed to have their nutritional content as well as functional properties optimized, thus making them potentially valuable livestock feed. As a way to break down complex substances, improve nutrient availability, and lower anti-nutritional factors, fermentation produces an anaerobic environment that promotes the growth of beneficial microbes.[1] The functional components of feed ingredients are known to be enhanced by fermentation, making them more appealing to animals.[3, 5]
The Flame Tree or Royal Poinciana, also called *Delonix regia*, has been used medicinally for centuries in many different cultures. According to preliminary investigations, it may offer therapeutic benefits in the form of antioxidant, anti-inflammatory, and anti-microbial characteristics.[6] These curative properties of the plant may be due to the biologically active ingredients found in it, such as flavonoids, tannins, and alkaloids.[4, 6, 7] Functional feed properties are those that boost the health and productivity of animals while also addressing basic nutritional needs. Functional feed properties have qualities that benefit the health and productivity of animals while also serving their basic nutrient requirements. Due to their enhanced nutrient availability, lowered anti-nutritional factors, and increasing biological activity, fermented plant materials tend to fall into this group.[8] *Delonix regia* seeds that have been subjected to fermentation could be utilized as a functional feed element in livestock nutrition, improving the health and production of the animals. The isolation and detection of phytochemicals from *Delonix regia* seeds, according to Abulude *et al*.[6] may provide knowledge about their probable pharmacological properties. The body's defences may be modulated, gastrointestinal health could be enhanced, and oxidative stress minimized by these bioactive chemicals, among other advantageous effects on the health of animals. This study examined how the duration of fermentation influences the proximate

composition, phytochemical profile, and functional properties of *Delonix regia* seeds as a potential alternative functional feed ingredient.

Materials and Method

Collection and processing of Delonix regia seeds

The seeds of *Delonix regia* were gathered between January and February 2022. It was identified and confirmed in the Crop and Soil Laboratory, College of Agricultural Sciences with voucher number LU/CASLAB/CRP/2022/V/018.

The *Delonix regia* seeds utilized in this study were from the Landmark University campus in Omu-Aran, Kwara State Nigeria, with coordinates: 43FH+Q9J, 251103, located on latitude 8° 9′N and longitude 5° 61′E, 564 meters above sea level. Mature pods were picked and sun-dried before being dehulled. The seeds were then steeped in water for 12 hours. Each treatment was allotted 600g from the stock of 3000g of soaked and drained seed that was initially measured with each treatment reproduced three times. The seeds were then placed in a polythene bag and stored in a dark location for varied periods to undergo natural fermentation. After fermentation, the seeds were air-dried and pulverized into a uniform size with a hammer mill. Finally, to maintain the integrity of the treated seeds during laboratory analysis, they were securely stored and labelled in an airtight container.

Proximate analysis

This was done in triplicate on the seeds for ash, crude fibre, moisture, and fat using the AOAC method.[9].

Phytochemical screening

Alkaloid

The determination was as described by Oluba *et al*.[8] To 0.5g of the sample, the extract was added 20 mL of 20% acetic acid in a 100 mL beaker and covered to stand for 4 hours. It was then filtered and placed in the water bath (Clifton, Nickel-Electro LTD, Weston-S-Mare Somerset, England) to reduce the weight to ¼. Concentrated ammonium hydroxide was added dropwise until precipitation was completed. It was then collected by filtration (Whatman Ashless Filter paper No 41, 125mm, Cat No 1441 125, GE Healthcare UK Limited, Amersham Place Little Chalfont, Buckinghamshire, HP7 9NA, UK) and weighed (PA512, Ohaus Corp. Pine Brook, NJ USA) (eqn 1)

$$\%Alkaloid = \frac{w2-w1}{w0}\ x\ 100 \text{ -------- } (eqn\ 1)$$

Where: W1 = mass of empty filter paper; W2 = mass of filter paper + residue, w0= weight of sample

Saponin

The saponin level was determined as described by Elekofehinti *et al.* [10] 1 g of the sample was weighed into 20 mL of 20% ethanol while stirring and heated at 50°C over a water bath (Clifton, Nickel-Electro LTD, Weston-S-Mare Somerset, England) for 4 hours. A second extraction using the same procedure was carried out on the filtrate obtained after filtering the extraction yield. The whole filtrate was then heated in a water bath to 85°C. After carefully pouring the concentrated solution into a 250mL separating funnel, 2.5mL of diethyl ether was added, and the mix was thoroughly agitated to homogenize it. After giving the mix enough time to settle, the aqueous layer was slowly separated by filtration. After the addition of 10 cm^3 of 5% sodium chloride solution, 60 cm^3 of n-butanol, and 60 cm^3 of the aqueous layer, it was allowed to settle, the sodium chloride layer was carefully removed, the remaining layer was additionally concentrated in a water bath, placed in a crucible, and dried in a hot air oven until a stable weight was obtained (TSL DHG-9053, Genlab Instrument Co., England). The saponin content was calculated as a percentage using the formula (eqn 2)

$$\%saponin = \frac{w2-w1}{w0}\ x\ 100 \text{ ------- } (eqn\ 2)$$

Where: W1 = weight of sample; W2 = Dried weight of sample, w0= weight of sample

Flavonoid

This was carried out by the technique of Zhang *et al*. [11]. From the extract 5mg of was taken then 5 mL of 10% aluminium chloride in methanol was added. A drop of acetic acid was also added, then diluted with 5ml methanol. Absorbance was measured using (Jenway model 6705, Bibby Scientific Limited, Stone, Staffordshire, ST15 OSA, UK) at 415nm after 40 minutes. Rutin was prepared and run for the standard curve.

Functional properties

Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)

Following the steps outlined by Lawal and Adebowale [12], water absorption capacity (WAC) and oil absorption capacity (OAC) evaluations were conducted. In a 10 mL centrifuge (80-2B, Jiangsu Zhengji Import & Export Co., LTD, China) tube with a given weight, for WAC and OAC, 5 mL each of distilled water and soybean oil were infused with 5g from the seed flour. After fully mixing all the parts, the mixture was left off for 30 minutes. It was then subjected to centrifugation for 30 minutes at ambient temperature and 3000 rpm. The supernatant was carefully emptied after centrifugation, and any remaining water or oil in the upper phase was extracted by making the sample drain at a 45° angle for 10 minutes. The tube containing the remaining material was weighed again to calculate the amount of water or oil retained per gram of the sample as in equation 3.

$$WAC/OAC = \frac{\alpha-\beta}{\beta} \text{ ----------} eqn\ 3$$

Where α = fresh weight of Sample

β = the dried weight of the sample

Paste clarity

The method put forward by Bhandari and Singhal [13] was used to evaluate paste clarity. Each 1% aqueous solution of the sample was heated for an hour in a water bath at 85°C with steady stirring. the resulting slurry was then cooled to room temperature. Then, using a UV-visible spectrophotometer (Jenway model 6705, Bibby Scientific Limited, Stone, Staffordshire, ST15 OSA, UK), the transmittance (T) was measured at 640 nm, and the paste clarity was expressed as a percentage of transmittance (%T).

Bulk density

The bulk density was obtained using the method described by Ojinnaka *et al.* [14] Weigh 50g of the seed flour in a 100 mL measuring cylinder, the cylinder's bottom was tapped ten times against the palm, and the final volume was reported in grams per millilitre (g/ml).

Specific gravity (SG) was obtained using a technique used by Omede *et al.* [24] A comparison of a substance's density to a benchmark value (the density of water) gives its specific gravity. For fermented Delonix regia seeds, SG is given as a ratio of the bulk density (BD) of the established mass of the sample under test to the density of water. To calculate the BD of the fermented Delonix regia seeds, the same approach used for estimating BD was employed. The Delonix regia seeds' SG was determined using this BD value as in equation 4.

$$SG = \frac{BD}{WD(1.0\ g/cm3)} \text{ ---------} eqn\ 4$$

Where BD= bulk density of fermented Delonix regia

WD= water's density (1.0 g/cm3).

Swelling Power(SP)

Applying the approach of Oluba *et al*[8] A 0.5g quantity of the powdered sample was mixed with 10 mL of distilled water to measure the swelling power (SP). In a graduated 100 mL cylinder, the seed flour and water were swirled and left to stand at room temperature for 18 hours. The swelling power is expressed as a percentage using the formula in inEquation 5

$$SP = \frac{x}{y}x\ 100 \text{ ...eqn 5}$$

where x = volume occupied by seed flour(mL), y= seed flour dry weight (g)

Experimental Treatments

Five treatments which were T1, T2, T3, T4 and T5 were fermented differently for 0 days, 1 day, 2 days, 3 days and 4 days respectively. Each treatment for this study was replicated three times in a Completely Randomized Design (CRD).

Statistical analysis

A one-way analysis of variance (ANOVA) was performed on all of the study's data. Statistical Package for the Social Sciences (SPSS) version

25.0 processes were used to separate the means using Duncan's multiple range tests with a 5% level of significance.

Results and Discussion

The result of proximate composition for the different treatments of *D. regia* is shown in Table 1. The crude protein (CP) was significantly different (P<0.05) with T5 having a higher value of 21.67% compared to others. Likewise, the ether extract (EE) for T5 at 20.83 was higher than in other treatments. Ash in T1 was better at 2.56% compared to other treatments, while crude fibre (CF) level was better with T5 at 7.05%. Moisture content was not significantly different (P<0.05) across the treatments.

Table 2 shows the values of phytochemical constituents of *Delonix regia*. T5 had lower values for the three parameters of alkaloid 36.01), saponin (7.40) and flavonoid (5.14) respectively compared to other treatments.

Table 3 shows the effect of different fermentation times on water absorption capacity, oil absorption capacity, bulk density, specific gravity, swelling index, paste clarity, water absorption capacity (WAC), oil absorption capacity (OAC), bulk density (BD), specific gravity (SG), swelling power (SP), and paste clarity (PC) for sample treatments T1, T2, T3, and T4, respectively. The result shows that fermentation time has a significant effect on WAC (p<0.05). T1 had the highest WAC, while others had lower values. The result showed that OAC was significantly affected by fermentation time (p<0.05). OAC for T4 had the highest OAC among the treatments. The result showed that both bulk density and specific gravity were significantly affected by fermentation time (p<0.05). T1 had the lowest bulk density and specific gravity, while T4 had the highest values. Fermentation time did not significantly affect the swelling index (p<0.05). The paste clarity was also significantly impacted (p<0.05). by fermentation time, however, T1 had the lowest paste clarity, while T4 had the highest value.

The fermentation duration significantly affected the proximate composition of *Delonix regia* seed. While the CP content in T5 (21.67%) was significant, it was within the range reported by Atanda *et al.* [15] but more than that reported by Olufayo and Falola [16]. Regardless of the fermentation period, *Delonix regia* seed showed to be a significant protein source for animal feed, particularly in poultry production. The EE values were higher than those reported by Olufayo and Falola[16] and Atanda *et al*. [15] indicating that the lipid content was higher. Longer fermentation times resulted in lower CF values, with T5 having a much higher CF value (7.05%) than Alagbe [17] findings. The ash concentration was not significantly impacted by fermentation; however, it was lower than that reported by Zuffo *et al*. [18]. Longer fermentation time lowered the level of saponins, alkaloids, and flavonoids in *Delonix regia* seed, based on the phytochemical investigation carried out. However, even though these values were lower than those presented by Atanda *et al.* [15], the presence of saponins in *Delonix regia* seed suggests possible chemo-preventive advantages, which is consistent with earlier studies by Lewu *et al.* [19] and Jukanti *et al*.[20] Because of the low saponin concentration, it is safe to use in large amounts in animal feed.[15] Fermentation also produced minimal quantities of antinutrients, ensuring that appropriate limits for animal intake were met.

The fermentation period substantially impacted the functional properties of *Delonix regia* seed. Longer durations resulted in lower WAC because of the interaction of protein and carbohydrate levels, which influence the seed's water absorption ability.[11] The inclusion of highly charged hydrophilic components, such as polar amino acid residues, aids in hydrogen bond formation, resulting in greater water trapping.[8] Because of the weak intermolecular interactions between starch molecules, the starch content also contributed to water absorption capacity [21] OAC levels increased with longer fermentation times, peaking at 19.60% in T5. The capability of the proteins in fermented Delonix regia seeds to bind with oil will make it useful in feed formulations that demand great oil absorption. This property will make the fermented seed fit for many beneficial applications in the feed sector, including the production of high-energy feed.[22] Also, when added to diets, its Oil Absorption Capacity (OAC) will improve the flavour and texture of feed formulations.[22, 23] The fermentation time increased bulk density and specific gravity an indicator of the level of material compactness.[24] Fermentation time had no significant effect on swelling power which is always influenced by the water-binding capacity of the seed flour.[14] Longer fermentation time resulted in greater paste clarity which is a measure of the transparency of a substance in a paste form.[8]

Conclusion

This study showed that the nutritional value and functional properties of *Delonix regia* seed are significantly impacted by the length of fermentation. The results highlight its potential to be an important component of functional animal feed, particularly in poultry farming. The reduced concentration of phytochemical constituents and antinutrients, along with the important functional properties, further support its safe and effective utilization. Further research should focus on optimizing fermentation conditions to maximize the beneficial attributes of *Delonix regia* seed in animal feed formulations

Table 1: Proximate constituents of the experimental treatments of *Delonix regia*

Parameter	T1	T2	T3	T4	T5
Moisture	6.37 ± 0.63	5.83 ± 0.44	6.17 ± 0.60	7.00 ± 0.76	6.33 ± 0.44
Ash	2.57 ± 0.47	3.83 ± 0.17	2.67 ± 0.44	3.22 ± 0.14	3.32 ± 0.18
Ether extract	10.37 ± 3.19	11.00 ± 0.29	14.69 ± 0.85	17.33 ± 3.19	20.83 ± 6.22
Crude Fibre	9.59 ± 0.34	9.30 ± 0.23	6.64 ± 0.25	8.03 ± 0.50	7.03 ± 0.21
Crude Protein	19.33 ± 0.70	19.73 ± 1.27	18.13 ± 1.11	21.67 ± 1.32	21.67 ± 0.34
NFE	50.31 ± 0.31	50.31 ± 0.31	51.70 ± 0.70	42.75 ± 0.75	40.82 ± 0.44

The standard error of means (SEM) is given as ± for each treatment

Table 2: Effect of soaking time on phytochemical constituents of *Delonix regia* seed

Parameter	T1	T2	T3	T4	T5
Flavonoid	6.99 ± 0.10	6.91 ± 0.09	6.73 ± 0.13	7.06 ± 0.04	7.02 ± 0.03
Saponin	9.96 ± 0.04	19.98 ± 0.04	19.93 ± 0.05	29.64 ± 0.33	10.01 ± 0.03
Alkaloid	39.99 ± 0.02	59.61 ± 0.32	39.97 ± 0.04	56.28 ± 3.15	56.26 ± 3.14

The standard error of means (SEM) is given as ± for each treatment

Table 3: Functional properties of *Delonix regia* seed with different fermentation time

Parameter	T1	T2	T3	T4	T5
WAC	38.88 ± 0.47	35.55 ± 0.67	34.93 ± 0.36	36.47 ± 0.52	35.64 ± 0.08
OAC	18.66 ± 0.20	16.30 ± 0.19	17.65 ± 0.20	18.53 ± 0.55	19.60 ± 0.15
SP	30.42 ± 0.42	21.25 ± 6.88	30.33 ± 0.33	29.58 ± 0.42	31.67 ± 0.42
SG	1.63 ± 0.01	1.67 ± 0.20	1.65 ± 0.01.	1.61 ± 0.02	1.69 ± 0.02
PC	64.07 ± 0.52	78.57 ± 0.20	83.73 ± 0.67	85.03 ± 0.27	85.00 ± 0.15

The standard error of means (SEM) is given as ± for each treatment

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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