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Nutritional and Antinutritional Changes in Date Seeds During 96-Hour Fermentation: Implications for Poultry Feed

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Abstract

Date palm (Phoenix dactylifera L.) seeds are an agro-industrial waste with potential as alternative feed ingredient, but their utilization is limited by high fibre and antinutritional factors. This study evaluated the effect of fermentation duration (0, 24, 48, 72 and 96 hours) on the vitamin, antinutrient, mineral, proximate and fibre composition of date seeds. Samples were fermented naturally and analyzed using standard AOAC methods with three replicates per treatment. Data were subjected to one-way ANOVA (df 4,10) and means separated at $p<0.05$. Results showed fermentation significantly ($p<0.05$) improved vitamin content with optimal performance at 48 hours. Vitamin A increased by 26.56% from 169.52 µg/100g (control) to 214.55 µg/100g at 48 hrs, riboflavin increased by 75.91% from 1.91 to 3.36 mg/100g, and folate peaked at 725.51 mg/100g at 72 hrs. Antinutrient composition was markedly altered. Trypsin inhibitor was reduced by 99.01% from 6.10 TIU/mg to 0.06 TIU/mg at 72 hrs, saponin reduced by 35.71% from 11.48 to 7.38 mg/100g, and phytate reduced by 24.63% at 24 hrs. However, haemagglutinin increased by 71.59% at 72 hrs (3.45 to 5.92 mg/100g) and oxalate increased to 10.09 mg/100g, indicating need for additional heat treatment. Mineral analysis revealed phosphorus increased by 32.33% at 48 hrs (597.96 to 791.30 mg), potassium peaked at 916.17 mg at 96 hrs, and copper increased by 99.41% at 72 hrs. Proximate analysis showed fat decreased from 4.13% to 2.40% at 96 hrs while crude fibre increased from 2.66% to 22.79%. NDF increased from 67.61% to 85.67% at 96 hrs. It was concluded that 48 hours fermentation is optimal for date seed utilization as it gives best balance of increased vitamins (A and B2), improved mineral bioavailability (P), reduced trypsin inhibitor and saponin, with moderate fibre. Fermentation beyond 72 hrs increases haemagglutinin and fibre concentration.

Keywords: Date seed, fermentation duration, antinutrients, proximate composition, vitamin profile, trypsin inhibitor

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INTRODUCTION

Date palm (Phoenix dactylifera L.) is one of the oldest cultivated fruit crops in arid and semi-arid regions, with world production exceeding 9 million tonnes annually (FAO, 2022). The fruit processing industry generates substantial quantities of by-products, particularly seeds, which constitute 10-15% of the fruit weight and are estimated at over 800,000 tonnes globally per year (Al-Farsi & Lee, 2008; Heuzé et al., 2015). These seeds are largely discarded as waste or used as low-value animal feed, despite being recognized as a rich source of dietary fibre (73.1 g/100 g), phenolics (3942 mg/100 g), antioxidants, carbohydrates, fat (5-13%), protein (5-11%) and minerals such as potassium, magnesium, calcium and phosphorus (Al-Farsi et al., 2007; Bouhlali et al., 2017; Niazi et al., 2017). Recent studies have demonstrated that date seeds possess antioxidant, anti-inflammatory and antimicrobial activities due to bioactive phytochemicals including alkaloids, flavonoids, terpenoids and tannins, suggesting their potential for valorization as functional feed ingredients (El-Far et al., 2021; Taleb et al., 2023).

Despite their nutritional potential, the utilization of date seeds in monogastric nutrition, particularly poultry, is constrained by several nutritional limitations. Date seeds contain high and variable fibre fractions with crude fibre 16-51% dry matter, neutral detergent fibre 58-90%, acid detergent fibre 41-46% and lignin 4-18%, with high degree of lignification that reduces digestibility (Heuzé et al., 2015; Boudechiche et al., 2009). In addition, like other agro-industrial by-products, they contain antinutritional factors such as trypsin inhibitors, phytate, saponin, oxalate, haemagglutinin, tannin and cyanide, which interfere with nutrient digestion, mineral bioavailability and protein utilization (Shaba et al., 2015; Al-Harrasi et al., 2014). Phytate chelates divalent minerals such as phosphorus, calcium, zinc and iron, while trypsin inhibitors impair protein digestion and saponins affect growth and feed intake in broilers (Akande et al., 2010). The presence of these antinutrients necessitates appropriate processing to improve nutrient bioavailability and reduce deleterious effects.

Fermentation has emerged as a cost-effective, low-input bioprocessing technique for improving the nutritional quality of non-conventional feed resources. It is a solid-state microbial process involving natural or inoculated populations of Bacillus, Lactobacillus, Saccharomyces and Aspergillus species that produce extracellular enzymes including proteases, phytases, cellulases and lipases (Ogunyemi et al., 2017; Adegbehingbe et al., 2014). These enzymes degrade complex polysaccharides and antinutrients, hydrolyse phytate to release bound phosphorus, and break down trypsin inhibitors and saponins (Anaemene & Fadupin, 2020; Ojokoh et al., 2015). Previous studies reported that fermentation decreased crude fat, improved protein content, reduced tannin and phytate by up to 90%, and increased calcium and mineral bioavailability in quality protein maize, pigeon pea and bush mango seeds (Anaemene & Fadupin, 2020; Nwosu et al., 2021; Ayoade et al., 2018). Furthermore, microbial activity during fermentation can synthesize B-vitamins, increase vitamin A through precursor conversion, and enhance antioxidant capacity (Ayoade et al., 2018; Dewi et al., 2019). However, the extent of nutrient enhancement and antinutrient reduction is dependent on fermentation duration, substrate, microbial consortium and environmental conditions, with prolonged fermentation sometimes causing concentration of fibre and undesirable increase in certain antinutrients due to dry matter loss (Fadupin et al., 2022).

Although the effect of fermentation on proximate composition of date fruits has been reported, information on the impact of fermentation duration on vitamin, mineral, antinutrient, proximate and detergent fibre fractions of date seeds is limited. Most studies have focused on date flesh or short-term fermentation without systematic evaluation of time-course changes. Understanding the optimal fermentation period is critical to maximize vitamin synthesis, phosphorus release and trypsin inhibitor reduction while minimizing fibre concentration and haemagglutinin increase. Therefore, the objective of this study was to evaluate the effect of fermentation duration (0, 24, 48, 72 and 96 hours) on vitamin, antinutrient, mineral, proximate and fibre composition of date seeds with a view to determining the optimal fermentation time for its use as alternative feed ingredient in poultry diets.

MATERIALS AND METHODS

Study Area

The study was conducted at the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, Lafia, Nasarawa State, Nigeria. Lafia is located on latitude 08°29'48" N and longitude 08°30'58" E, with an altitude of 278m above sea level. It lies in the Southern Guinea Savannah agro-ecological zone of Nigeria with mean annual rainfall of 1,200 - 1,400mm and average temperature of 28-35°C (NIMET, 2022). The laboratory analysis was carried out in the Nutrition and Biochemistry Laboratories, Faculty of Agriculture, NSUK.

Collection and Identification of Date Palm Seeds

Date palm fruits (Phoenix dactylifera L.) was purchased from Lafia Modern Market and Shinge Market, Lafia. The seeds (pits) was manually extracted from the pulp, washed thoroughly with clean tap water to remove adhering flesh and sugar. The seeds were authenticated at the Herbarium Unit, Department of Plant Science and Biotechnology, Faculty of Agriculture, Nasarawa State University, Keffi.

Sample Preparation

The washed seeds were sun-dried for 72 hours to reduce moisture content to about 10-12% and to prevent mould growth (Ameen *et al*., 2019). The dried seeds were cleaned to remove foreign materials, stones and damaged seeds. A portion of the raw seeds were retained as control (0 hour, unfermented). The remaining seeds were milled using a hammer mill fitted with a 2.0mm sieve to obtain Date Palm Seed Meal (DPSM) for easier fermentation, the seeds were milled coarsely before fermentation to increase surface area.

Fermentation Procedure and Experimental Treatments

Fermentation was carried out using natural anaerobic solid-state fermentation as described by Akinfemi (2010) and Oboh *et al*. (2003).

Procedure:

50kg of milled date palm seeds was divided into 5 equal portions of 10kg each.

Each portion will be placed in airtight plastic buckets.

Clean borehole water was added at a ratio of 1:1.5 (w/v) (1kg meal to 1.5 litres water) and thoroughly mixed.

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The buckets was tightly covered with lids and black polythene bags to ensure anaerobic condition and kept at room temperature (28-32°C) to ferment naturally by indigenous microflora.

Fermentation durations will constitute the treatments:

T1: 0 hour (Unfermented - Control)

T2: 24 hours fermentation

T3: 48 hours fermentation

T4: 72 hours fermentation

T5: 96 hours fermentation

At the end of each fermentation period, the fermented samples were oven-dried at 60°C for 48 hours to stop further microbial activity and to maintain constant weight (Kana *et al*., 2017). The dried samples were re-milled and stored in airtight labelled containers for chemical analysis. Each treatment was prepared in triplicates.

Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD). There are five treatments (fermentation durations) with three replicates per treatment, giving a total of 15 experimental units.

Model: $Yij = \mu + Fi + eij$

Where Yij = observation, μ = general mean, Fi = effect of the fermentation duration, eij = experimental error.

Chemical Analysis

Proximate Composition

Proximate composition was determined according to AOAC (2012):

Dry Matter (DM): By oven drying at 105°C to constant weight.

Crude Protein (CP): By Kjeldahl method (N x 6.25).

Crude Fibre (CF): By acid-base digestion method.

Ether Extract (EE): By Soxhlet extraction method using petroleum ether.

Ash: By incineration in muffle furnace at 550°C for 3 hours.

Nitrogen Free Extract (NFE): Calculated by difference: NFE = 100 - (CP + CF + EE + Ash + Moisture).

Fibre Fraction Analysis (Van Soest Method)

Fibre fractions will be determined according to Van Soest et al. (1991):

Neutral Detergent Fibre (NDF): Using neutral detergent solution.

Acid Detergent Fibre (ADF): Using acid detergent solution.

Acid Detergent Lignin (ADL): By treating ADF residue with 72% sulphuric acid.

Calculations:

Hemicellulose = NDF - ADF

Cellulose = ADF - ADL

Anti-nutritional Factors

Tannin: Determined by Folin-Denis method as described by Makkar and Goodchild (1996).

Phytate: Determined by the method of Haug and Lantzsch (1983) using ferric chloride precipitation.

Oxalate: Determined by titration method of Day and Underwood (1986) using potassium permanganate.

Saponin: Determined by the method of Brunner (1984) using gravimetric method.

Alkaloid: Determined by the method of Harborne (1973).

Hydrogen Cyanide (HCN): Determined by alkaline picrate method.

Trypsin Inhibitor Activity (TIA): Determined by Kakade *et al*. (1974) method.

All analyses were carried out in triplicates.

Materials and Equipment

Materials: Date palm seeds, distilled water, reagents for proximate analysis (sulphuric acid, sodium hydroxide, petroleum ether, boric acid, catalysts), reagents for fibre analysis (neutral detergent, acid detergent, cetyl trimethyl ammonium bromide), reagents for anti-nutrient analysis (Folin-Denis reagent, tannic acid standard, ferric chloride etc).

Equipment: Hammer mill, electronic weighing balance (0.01g sensitivity), hot air oven, muffle furnace, Soxhlet apparatus, Kjeldahl digestion and distillation unit, desiccator, water bath, pH meter, airtight fermentation buckets, spectrophotometer.

Statistical Analysis

All data generated were subjected to One-way Analysis of Variance (ANOVA) using SPSS statistical package (Version 27.0). Significant differences among treatment means will be separated using Duncan's Multiple Range Test (DMRT) as described by Duncan (1955) at 5% level of significance ($p<0.05$).

RESULTS AND DISCUSSION

Vitamin Composition

The effect of fermentation duration on vitamin composition of date seed is presented in Table 1. Vitamin A (RE) content was significantly higher in fermented samples compared to the unfermented control (169.52 ± 5.70 µg/100g). The highest value was recorded at 48 hrs (214.55 ± 7.29 µg/100g), representing a 26.56% increase over control, while 24 hrs (208.13 ± 6.39 µg/100g) was statistically similar to 48 hrs. This increase in Vitamin A and provitamin A carotenoids during short-term fermentation has been attributed to microbial synthesis and improved extractability from plant matrix due to enzymatic breakdown of cell walls (Adebo et al., 2022; Food Safety Institute, 2023).

Thiamine (Vitamin B1) showed a progressive increase with fermentation duration. The control had 4.65 ± 0.25 mg/100g, while 96 hrs recorded the highest value of 7.35 ± 0.45 mg/100g, a 58.06% increase. Riboflavin (Vitamin B2) was significantly higher at 48 hrs (3.36 ± 0.11 mg/100g) compared to control (1.91 ± 0.05 mg/100g), representing 75.91% increase, but declined significantly at 72 hrs (1.50 ± 0.01 mg/100g). The enhancement of B-group vitamins during fermentation is well documented and is due to biosynthesis by lactic acid bacteria (LAB) and Bacillus spp. as

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metabolic by-products (LeBlanc et al., 2011; Capozzi et al., 2012). LeBlanc et al. (2011) reported that LAB such as Lactococcus lactis and Lactobacillus fermentum can synthesize riboflavin and thiamine, while certain strains may consume these vitamins during prolonged fermentation, explaining the decline at 72 hrs.

Niacin was highest at 24 hrs (2.34 ± 0.08 mg/100g), significantly greater than control (2.07 ± 0.01 mg/100g), but lowest at 72 hrs (1.12 ± 0.04 mg/100g). Folate (Vitamin B9) increased significantly from 638.91 ± 35.71 mg/100g in control to 725.51 ± 45.33 mg/100g at 72 hrs, which was the highest value observed. This is consistent with reports that LAB, particularly Lactococcus, Streptococcus, and Bifidobacterium genera, are capable of folate production during fermentation (LeBlanc et al., 2011; Food Safety Institute, 2023).

Vitamin C showed a distinct pattern, decreasing to the lowest value at 48 hrs (6.79 ± 0.19 mg/100g) from 10.88 ± 0.24 mg/100g in control, then increasing to the highest value at 72 hrs (16.34 ± 0.17 mg/100g). This surge at 72 hrs may be due to oxidative stress response of fermenting microorganisms, which produce ascorbic acid precursors to defend against stress (Solem et al., 2024).

Table 1. Mean composition of vitamin composition of date seed as affected by fermentation duration

Fermentation Duration	Vitamin A (RE) (µg/100g)	Thiamine (B1) (mg/100g)	Riboflavin (B2) (mg/100g)	Niacin (mg/100g)	Folate (B9) (mg/100g)	Vitamin C (mg/100g)
Control	169.52 ± 5.70^{c}	4.65 ± 0.25^{c}	1.91 ± 0.05^{d}	2.07 ± 0.01^{b}	638.91 ± 35.71^{b}	10.88 ± 0.24^{b}
24hrs	208.13 ± 6.39^{a}	4.98 ± 0.29^{c}	2.92 ± 0.11^{b}	2.34 ± 0.08^{a}	697.76 ± 9.86^{a}	8.34 ± 0.17^{d}
48hrs	214.55 ± 7.29^{a}	5.00 ± 0.21^{c}	3.36 ± 0.11^{a}	2.15 ± 0.08^{b}	721.59 ± 30.17^{a}	6.79 ± 0.19^{c}
72hrs	195.92 ± 4.07^{b}	5.60 ± 0.15^{b}	1.50 ± 0.01^{e}	1.12 ± 0.04^{d}	725.51 ± 45.33^{a}	16.34 ± 0.17^{a}
96hrs	192.04 ± 8.37^{b}	7.35 ± 0.45^{a}	2.24 ± 0.09^{c}	1.71 ± 0.12^{c}	603.13 ± 12.17^{b}	10.15 ± 0.63^{c}

Means bearing different superscript letters within the same column differ significantly ($p < 0.05$); means bearing the same superscript letter within the same column do not differ significantly ($p > 0.05$). Values are Mean ± SD of three replicates (n = 3). Superscripts are read down each column only and are not comparable across rows or between columns. One-way ANOVA (df = 4, 10) showed that fermentation duration significantly affected all properties ($p < 0.05$).

Antinutrient Composition

Table 2 shows antinutrient composition. All antinutrients were significantly ($p < 0.05$) affected by fermentation duration. Haemagglutinin content increased significantly with extended fermentation. Control had the lowest value of 3.45 ± 0.24 mg/100g, while 72 hrs and 96 hrs had significantly higher values of 5.92 ± 0.24 and 5.63 ± 0.19 mg/100g respectively. Similar increase in hemagglutinin during fermentation of Quality Protein Maize was reported by Fadupin et al. (2022), who attributed it to concentration effect due to dry matter loss. This indicates that fermentation alone may not degrade lectins, and additional heat treatment is required (Yusuf et al., 2008).

Saponin was significantly reduced. Control had the highest value of 11.48 ± 0.66 mg/100g, while the lowest values were recorded at 72 hrs (7.38 ± 0.36 mg/100g) and 48 hrs (7.57 ± 0.39 mg/100g), representing 35.71% reduction. The reduction in saponin during fermentation is due to leaching and enzymatic breakdown by microorganisms (Oghbaei & Prakash, 2016; Hamid et al., 2022).

Phytate was significantly lower at 24 hrs (0.462 ± 0.005 mg/100g) compared to control (0.613 ± 0.008 mg/100g), a 24.63% reduction, while 72 hrs had the highest value (0.661 ± 0.013 mg/100g). The reduction in phytate at short fermentation duration is attributed to production of microbial phytase enzymes that hydrolyze phytate to release inorganic phosphate and inositol (Rasaq, 2017; Shi et al., 2017). Hirabayashi et al. (1998) found that fermentation with *Aspergillus* efficiently degraded phytate, providing more available phosphorus, which explains the concomitant increase in phosphorus at 48 hrs in this study.

Oxalate did not differ significantly among control (6.91 ± 0.23 mg/100g), 24 hrs (7.04 ± 0.47 mg/100g) and 48 hrs (6.84 ± 0.22 mg/100g), but increased significantly at 72 hrs (10.09 ± 0.14 mg/100g). Fadupin et al. (2022) also reported increase in oxalate during fermentation of cereals. Tannin content was lowest at 48 hrs (13.39 ± 0.17 mg/100g) compared to control (14.72 ± 0.63 mg/100g). The reduction in tannins is attributed to polyphenol oxidase activation and tannase activity by lactobacillus during fermentation that breaks down tannin-protein complexes (Adebo et al., 2022; Gernah et al., 2022).

Trypsin inhibitor activity showed the most substantial reduction. Control had 6.10 ± 0.38^{a} TIU/mg, while values at 72 hrs (0.06 ± 0.00 TIU/mg) and 96 hrs (0.09 ± 0.00 TIU/mg) represent 99.01% and 98.52% reduction respectively. This agrees with Wang et al. (1986) who achieved 94.2% degradation of trypsin inhibitor using Bacillus subtilis, and Chi and Cho (2016) who reported effective degradation of trypsin inhibitor in soybean meal by Bacillus spp. through secretion of proteases (Feng et al., 2007; Shi et al., 2017). Probiotics in fermented feed decompose proteins including trypsin inhibitors through secretion of proteases (Shi et al., 2017).

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Table 2. Mean composition of antinutrient (phytochemical) composition of date seed as affected by fermentation duration

Ferm. Duration	Haemagglutinin (mg/100g)	Saponin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Tannins (mg/100g)	Trypsin Inhibitor Activity (TIU/mg)
Control	3.45 ± 0.24^{c}	11.48 ± 0.66^{a}	0.613 ± 0.008^{b}	6.91 ± 0.23^{c}	14.72 ± 0.63^{b}	6.10 ± 0.38^{a}
24hrs	3.84 ± 0.04^{b}	8.51 ± 0.30^{b}	0.462 ± 0.005^{d}	7.04 ± 0.47^{c}	15.75 ± 0.62^{a}	0.76 ± 0.04^{b}
48hrs	3.64 ± 0.08^{bc}	7.57 ± 0.39^{c}	0.484 ± 0.008^{c}	6.84 ± 0.22^{c}	13.39 ± 0.17^{d}	0.77 ± 0.03^{b}
72hrs	5.92 ± 0.24^{a}	7.38 ± 0.36^{c}	0.661 ± 0.013^{a}	10.09 ± 0.14^{a}	13.73 ± 0.21^{cd}	0.06 ± 0.00^{c}
96hrs	5.63 ± 0.19^{a}	8.01 ± 0.20^{bc}	0.493 ± 0.005^{c}	9.27 ± 0.32^{b}	14.60 ± 0.68^{bc}	0.09 ± 0.00^{c}

Means bearing different superscript letters within the same column differ significantly ($p < 0.05$); means bearing the same superscript letter within the same column do not differ significantly ($p > 0.05$). Values are Mean ± SD of three replicates (n = 3). Superscripts are read down each column only and are not comparable across rows or between columns. One-way ANOVA (df = 4, 10) showed that fermentation duration significantly affected all properties ($p < 0.05$).

Mineral Composition

Table 3 presents mineral composition. All minerals were significantly affected ($p < 0.05$). Sodium decreased progressively with fermentation, from 18.77 ± 0.78 mg in control to 13.10 ± 0.28 mg at 96 hrs, a 30.21% reduction, due to leaching during soaking and fermentation. In contrast, potassium increased significantly, with the highest values at 96 hrs (916.17 ± 6.10 mg) and 48 hrs (891.57 ± 2.32 mg) compared to control (748.22 ± 19.96 mg). The increase in mineral bioavailability during fermentation is due to reduction in antinutritional factors such as phytate and tannins that chelate minerals, and production of organic acids that improve mineral solubility (Agriculture Institute, 2024; Hirabayashi et al., 1998). Phosphorus increased significantly from 597.96 ± 13.28 mg in control to highest at 48 hrs (791.30 ± 18.21 mg), a 32.33% increase, attributed to phytate breakdown releasing bound phosphorus (Rasaq, 2017). Similarly, manganese increased significantly, with the highest value at 72 hrs (15.66 ± 0.72^{a} mg) compared to control (11.88 ± 0.19 mg). Copper was highest at 72 hrs (3.39 ± 0.15 mg) compared to control (1.70 ± 0.10 mg), representing 99.41% increase, while iron was higher at 24 hrs (5.97 ± 0.06 mg) than control (5.50 ± 0.36 mg).

Table 3. Mean composition of mineral composition of date seed as affected by fermentation duration

Ferm. Duration	Sodium (mg)	Manganese (mg)	Magnesium (mg)	Potassium (mg)	Copper (mg)	Iron (mg)	Phosphorus (mg)
Control	18.77 ± 0.78^{a}	11.88 ± 0.19^{c}	26.72 ± 0.57^{b}	748.22 ± 19.96^{c}	1.70 ± 0.10^{c}	5.50 ±0.36^{bc}	597.96 ± 13.28^{c}
24hrs	17.48 ± 0.85^{b}	15.29 ± 0.74^{a}	24.09 ± 1.13^{c}	746.30 ± 26.79^{c}	2.13 ± 0.06^{b}	5.97 ± 0.06^{a}	687.61 ± 64.98^{b}
48hrs	14.64 ± 0.14^{c}	13.82 ± 0.59^{b}	26.23 ± 0.51^{b}	891.57 ± 2.32^{a}	2.19 ± 0.14^{b}	5.11 ± 0.18^{c}	791.30 ± 18.21^{a}
72hrs	13.42 ± 0.23^{d}	15.66 ± 0.72^{a}	24.78 ± 0.65^{c}	801.31 ± 32.84^{b}	3.39 ± 0.15^{a}	4.64 ± 0.08^{d}	675.72 ± 17.24^{b}
96hrs	13.10 ± 0.28^{d}	13.30 ± 0.64^{b}	28.38 ± 0.37^{a}	916.17 ± 6.10^{a}	1.24 ± 0.03^{d}	5.67 ±0.30^{ab}	607.76 ± 27.80^{c}

Means bearing different superscript letters within the same column differ significantly ($p < 0.05$); means bearing the same superscript letter within the same column do not differ significantly ($p > 0.05$). Values are Mean ± SD of three replicates (n = 3). Superscripts are read down each column only and are not comparable across rows or between columns. One-way ANOVA (df = 4, 10) showed that fermentation duration significantly affected all properties ($p < 0.05$).

Proximate Composition

Table 4 shows proximate composition. Moisture, fat, fibre, ash and energy were significantly ($p < 0.05$) affected, while protein ($p = 0.24$) and carbohydrate ($p = 0.0684$) were not significantly affected. Moisture increased significantly from 9.96 ± 0.17% in control to 12.72 ± 0.09% at 96 hrs due to water absorption and metabolic water production during fermentation. Fat decreased significantly from 4.13 ± 0.13% in control to lowest at 96 hrs (2.40 ± 0.17%) and 48 hrs (2.53 ± 0.10%), due to utilization of lipids as energy source by fermenting microorganisms (Cui et al., 2021; Hamid et al., 2022).Protein content did not differ significantly, ranging from 21.97 ± 0.62^{a}% to 24.07 ± 1.82%. The lack of significant increase is contrary to reports of increased crude protein due to microbial biomass synthesis (Feng et al., 2007; Shi et al., 2017). However, the numerically higher value at 24 hrs (24.07%) suggests slight improvement. Fibre content increased significantly from 2.66 ± 0.08% in control to 22.79 ± 0.51% at 96 hrs. This increase might be due to dry matter loss, where soluble carbohydrates are consumed by microbes, concentrating fibre fraction (Gernah et al., 2022). Energy was significantly lower at 96 hrs (258.08 ± 5.44 kJ) compared to control (278.95 ± 11.43 kJ).

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Table 4. Mean composition of proximate composition of date seed as affected by fermentation duration

Fermentation Duration	Moisture (%)	Fat (%)	Protein (%)	Fibre (%)	Ash (%)	Carbohydrate (CHO) (%)	Energy (kJ)
Control	9.96 ± 0.17^{c}	4.13 ± 0.13^{a}	22.98 ± 0.88^{a}	2.66 ± 0.08^{c}	3.29 ± 0.02^{b}	38.07 ± 0.62^{a}	278.95 ± 11.43^{a}
24hrs	10.60 ± 0.53^{b}	3.52 ± 0.19^{b}	24.07 ± 1.82^{a}	21.06 ± 0.50^{b}	3.56 ± 0.10^{a}	36.77 ± 1.46^{a}	281.55 ± 2.12^{a}
48hrs	12.60 ± 0.32^{a}	2.53 ± 0.10^{d}	21.97 ± 0.62^{a}	21.00 ± 0.73^{b}	3.31 ± 0.01^{b}	38.17 ± 1.64^{a}	274.58 ± 2.80^{a}
72hrs	12.67 ± 0.28^{a}	3.19 ± 0.18^{c}	23.02 ± 0.92^{a}	21.06 ± 0.17^{b}	1.68 ± 0.13^{d}	38.55 ± 2.01^{a}	274.87 ± 8.14^{a}
96hrs	12.72 ± 0.09^{a}	2.40 ± 0.17^{d}	23.77 ± 0.90^{a}	22.79 ± 0.51^{a}	2.85 ± 0.11^{c}	34.75 ± 1.62^{a}	258.08 ± 5.44^{b}

Means bearing different superscript letters within the same column differ significantly ($p < 0.05$); means bearing the same superscript letter within the same column do not differ significantly ($p > 0.05$). Values are Mean ± SD of three replicates (n = 3). Superscripts are read down each column only and are not comparable across rows or between columns. One-way ANOVA (df = 4, 10) showed that fermentation duration significantly affected moisture, fat, fibre, ash and energy ($p < 0.05$), but not protein ($p = 0.24$) or carbohydrate ($p = 0.0684$).

Fibre Fraction Composition

Table 5 shows fibre fraction composition. All fibre fractions were significantly ($p < 0.05$) affected. Neutral detergent fibre (NDF) increased significantly from 67.61 ± 6.77% in control to highest at 96 hrs (85.67 ± 0.56%). Acid detergent fibre (ADF) increased significantly from 47.80 ± 1.48% in control to highest at 24 hrs (63.70 ± 2.54%) and 48 hrs (63.53 ± 1.94%). Cellulose increased significantly from 40.40 ± 0.77% in control to highest at 48 hrs (65.65 ± 4.26%), a 62.50% increase. Lignin increased progressively from 1.02 ± 0.09% in control to highest at 96 hrs (2.08 ± 0.13%), representing 103.9% increase. The increase in fibre fractions is consistent with the increase in crude fibre and is attributed to concentration effect and solubilization of hemicellulose (Fadupin et al., 2022).

Table 5. Mean composition of fibre fraction composition (g/kg dm) of date seed as affected by fermentation duration

Fermentation Duration	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Acid Detergent Lignin (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Control	67.61 ± 6.77^{c}	47.80 ± 1.48^{c}	7.64 ± 0.29^{c}	21.32 ± 1.05^{d}	40.40 ± 0.77^{c}	1.02 ± 0.09^{e}
24hrs	81.36 ± 3.37^{ab}	63.70 ± 2.54^{a}	8.74 ± 0.21^{a}	29.22 ± 0.69^{b}	46.24 ± 1.12^{b}	1.29 ± 0.06^{d}
48hrs	75.49 ± 3.55^{bc}	63.53 ± 1.94^{a}	8.85 ± 0.35^{a}	12.92 ± 0.64^{e}	65.65 ± 4.26^{a}	1.48 ± 0.06^{c}
72hrs	83.78 ± 5.83^{ab}	50.78 ± 2.13^{c}	7.90 ± 0.13^{bc}	37.43 ± 1.10^{a}	45.09 ± 0.64^{b}	1.80 ± 0.10^{b}
96hrs	85.67 ± 0.56^{a}	55.19 ± 2.61^{b}	8.34 ± 0.41^{ab}	24.06 ± 0.90^{c}	48.70 ± 2.64^{b}	2.08 ± 0.13^{a}

Means bearing different superscript letters within the same column differ significantly ($p < 0.05$); means bearing the same superscript letter within the same column do not differ significantly ($p > 0.05$). Values are Mean ± SD of three replicates (n = 3). Superscripts are read down each column only and are not comparable across rows or between columns. One-way ANOVA (df = 4, 10) showed that fermentation duration significantly affected all properties ($p < 0.05$).

Conclusion

Fermentation significantly improved vitamin content, with the highest vitamin A and riboflavin at 48 hrs, thiamine at 96 hrs, and folate and vitamin C at 72 hrs. The most notable antinutrient reduction was trypsin inhibitor, with 99.01% reduction at 72 hrs, and saponin with 35.71% reduction. However, haemagglutinin and oxalate increased at extended fermentation durations. Mineral bioavailability improved, particularly phosphorus at 48 hrs and potassium at 96 hrs. Based on overall nutrient enhancement and antinutrient reduction, 48 hrs fermentation is optimal for date seed as poultry feed ingredient, while 72 hrs requires heat treatment to destroy haemagglutinin.

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