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Original Article

Fermentation of locust bean (*Parkia biglobosa*): modulation in the anti-nutrient composition, bioactive profile, *in vitro* nutrient digestibility, functional and morphological characteristics

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Summary The present investigation was undertaken with an objective to assess the impact of fermentation temperatures (22, 30, 38 and 46 °C for 72 h) on physico-chemical, functional and structural characteristics of locust bean (*Parkia biglobosa*). Alkaline fermentation treatment caused drastic reduction in anti-nutritional factors including tannins (~65%), phytates (~60%) and saponins (~70%) owing to hydrothermal treatment and production of microbial enzymes during fermentation. Furthermore, phytochemical profile and associated antioxidant potential were enhanced due to the breakdown of cell-wall components and polyphenolic complexes, liberating phenolic acids and flavonoids available for extraction. With respect to bioactive properties and *in vitro* starch and protein digestibility, the most pronounced effect was observed after fermentation at 38 °C for 72 h. The trend was also supported by scanning electron micrographs, which revealed modulation in the macromolecular-structural arrangement. Significant and negative correlation coefficients between anti-nutritional factors and protein digestibility ($r > -0.95$, $P < 0.05$) confirmed the degradation of protein–polyphenol matrix. Exposure of hydrophobic regions due to fermentation resulted in altered functional properties including better oil absorption capacity, higher water solubility index, reduced water absorption potential, and lower emulsification and gelation properties. Principal component analysis was further employed to statistically validate the differences among variables and observations.

Keywords *Daddawa*, digestibility, functional properties, *Parkia biglobosa*, phytochemicals, scanning electron microscopy.

Introduction

Fermentation of legumes is a traditional and promising processing treatment to enhance their biofunctional performance, flavour profile, nutrient digestibility dedicated to hydrolysis of complex molecules and reduction in anti-nutritional factors (Olasupo *et al.*, 2016). Most of the native African foods serve as major sources of nutrition especially to rural dwellers. *Iru*/*daddawa*, a protein-rich condiment, is a folk culinary preparation from fermented locust bean seeds, which is mainly utilised as a flavouring ingredient to improve the meatiness of food products (Olagunju *et al.*, 2018).

Locust bean (*Parkia biglobosa*), commonly found in western African region, is a potential legume exhibiting high nutritive and medicinal properties from leaves, roots, stems and most importantly cotyledons (Adeloye & Agboola, 2020). Fresh seeds contain 20–36% protein content, 8–32% lipid content, with considerable amounts of dietary fibres (0.4–17%), minerals (1–6%) and other bioactive compounds including phenolic acids, flavonoids and vitamins (Termote *et al.*, 2020). However, due to higher content of anti-nutritional factors such as tannins, phytates and saponins, such seeds cannot be consumed in raw, uncooked or unfermented state (Balogun *et al.*, 2014). These non-nutritive compounds have potential to bind proteins and minerals making them inaccessible for body requirements (Sharma *et al.*, 2021b). Fermented legumes have been reported to constitute lower amounts of anti-nutrients, thereby not only preventing associated ill effects, but also possessing high protein and mineral digestibility (Olagunju *et al.*, 2018).

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Locust bean seeds are fermented before consumption and resultant product is a traditional household condiment in African nations, referred to as *daddawa*. Although it is commonly processed through natural contamination, available reports confirm that *Bacillus subtilis* are the major bacterial strains involved, and it is also characterised by strong ammoniacal odour due to the breakdown of amino acids during fermentation, significantly contributing to organoleptic behaviour (Gernah *et al*., 2007). Furthermore, bioactive compounds including generally increase during fermentation ascribing to cell-wall degradation enhancing their extraction and bioconversion from bound to free state (Adebo & Gabriela Medina-Meza, 2020). These phytochemicals act as free radical scavengers, metal chelators, metal-reducing agents and free radical quenchers resulting in prevention from several cancers, cardiovascular diseases and other degenerative complications (Kaur *et al*., 2019). *Daddawa* making is a common household practice in Africa, but standardisation of the process to optimise nutritive, bioactive and technological functionality remains to be done. To our best knowledge, no study has so far investigated the influence of fermentation temperature on compositional and functional behaviour of locust bean. Thereby, the present study was undertaken with the objective to evaluate the influence of different fermentation temperatures (22–46 °C) on bioactive constituents, antioxidant potential, macromolecular-structural arrangement, nutrient digestibility and functional characteristics of locust bean seeds.

Material and methods

Raw material and processing treatment

Locust bean (*P. biglobosa*) seeds were purchased from local market around Punjab Agricultural University, Ludhiana, India. These seeds commonly belong to African countries and can grow in drylands with minimal water requirement. Seeds were first rinsed thoroughly and cooked in boiling deionised water (90 min) with added potassium bicarbonate (1 g per 100 mL water) for alkaline conditions followed by overnight resting period. They were further exposed to ashes and pounded to remove the husk layers completely. Cotyledons were then packed in double-layered muslin cloth in sterilised glass jars before subjecting to natural fermentation at different temperatures (22, 30, 38 and 46 °C) for pre optimised time (72 h) in humidity control incubators. The relative humidity was maintained between 85% and 90%. Later, drying was done in tray dryer to obtained moisture content of ~8%. Untreated raw locust bean seeds (control) and fermented samples (*daddawa*) were milled using cycotec mill (Newport Scientific, Warriewood, Australia) to obtain flour and stored under refrigerator conditions during investigation.

Anti-nutritional factors

Anti-nutritional factors in locust bean seeds were evaluated in terms of tannins, phytates and saponins. Folin-Denis method for estimation of tannins was undertaken according to procedure followed by Sharma *et al*. (2017) and reported as mg tannic acid per g dry flour. Furthermore, phytates were extracted in 0.5 M nitric acid was estimation was done using method outlined by Davies *et al*. (1998) and reported as mg phytic acid per g dry flour. Lipid-free samples were then taken to extract saponins in methanol and estimation was done according to the method of Fenwick & Oakenfull (1983) in terms of mg saponins per g dry flour.

Phytochemical composition and antioxidant potential

Total phenolic content (TPC) and total flavonoid content (TFC) were estimated by methods suggested by Sharma *et al*. (2021a). Extraction of bioactive compounds was done in 80% methanol (0.1% acidified) using two rounds of reflux for 120 min each. To estimate TPC, 0.5 mL extract was added with 5 mL folin–ciocalteu reagent (10 times freshly diluted) followed by 4 mL of 7.5% sodium bicarbonate solution. Absorbance was read after the incubation period of 120 min at 765 nm. Results were reported in terms of mg gallic acid equivalent (GAE) per 100 g dry locust bean flour. For TFC, 2 mL extract was added with 0.1 mL of potassium acetate (1 M) and 0.01 mL of aluminium chloride solution (10%) followed by 2.8 mL distilled water before incubation of 30 min at 25 °C. Absorbance was read at 415 nm, and results were reported as mg quercetin equivalent (QE) per 100 g dry flour.

Antioxidant potential of extracts was determined in terms of DPPH·(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity (RSA), metal chelation (Fe^{+2}) and ABTS·+ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) RSA. DPPH·RSA was estimated as per cent reduction in the absorbance of reaction mixture (0.1 mL extract in 3.9 mL 2 mM DPPH solution) after 30 min of incubation as suggested by Sharma *et al*. (2021a). Metal chelation was done using ferrous chloride–ferrozine method described by Sharma *et al*. (2012) and reported in terms of per cent Fe^{+2} chelation. To estimate ABTS·+ RSA, 1 mL extract was added to 5 mL ABTS·+ dye, and reduction in colour was observed at 734 nm. The results were expressed as μM trolox equivalent (TE) per gram of dry flour (Yilmaz *et al*., 2015).

In vitro starch and protein digestibility

In vitro starch digestibility (IVSD) was determined using method suggested by Sharma *et al*. (2021a).

Briefly, 100 mg each of flour sample, and α-amylase were added to 20 mL phosphate buffer (pH – 6.9) followed by incubation of 120 min at 37 °C in shaking water bath. Later, 1 mL of filtered extract was added with 2 mL dinitro-salicylic acid, and contents were heated in boiling water bath for 10 min. After cooling the contents to room temperature, volume was made to 50 mL with distilled water and absorbance was read at 540 nm against enzyme blank. Results were expressed as g maltose released per 100 g of sample. *In vitro* protein digestibility (IVPD) was estimated using method adopted by Sharma *et al.* (2021a) exposing the flour samples to pepsin (2 h, pH – 1.9 at 37 °C) and pancreatin (2 h, pH – 8.0 at 37 °C) digestions. Digested contents were then centrifuged, and supernatant was discarded. Residual solids were tested for protein content using Kjeldahl method and % difference in protein content before and after digestion was taken as % IVPD.

Functional properties

Two grams of flour was added to 20 mL deionised water followed by continuous shaking for 30 min before centrifugation at 4900 ***g*** for 15 min to determine water absorption capacity (WAC). The supernatant was taken in pre-weighed petri plate to be kept for drying at 100 °C until moisture free. Gel pellets were weighed, and ratio of weight gel pellet to flour sample was reported as g g^{-1} water absorption capacity (WAC). Solid residue after drying of supernatant was weighed to calculate % water solubility index (WSI) (Singh *et al.*, 2019). Similarly, oil absorption capacity was estimated by taking 2.0 g flour added with 20 mL refined soybean oil subjected to continuous shaking for 30 min. The tubes were then centrifuged at 4900 ***g*** for 15 min and excess oil was discarded. Ratio of weight of gel pellet to flour sample was taken as g g^{-1} OAC. Swelling power (SP) was estimated using similar method of WAC except the samples were heated in boiling water bath during shaking for 30 min, then centrifuged, and ratio of weight of swollen gel to flour was taken as g g^{-1} SP (Singh *et al.*, 2017a). Gel consistency (GC) was estimated by method suggested by Sharma *et al.* (2019). 0.2 g flour was added to 0.2 mL ethanol, and 3 mL acetic acid before heating the suspension for 10 min in boiling water bath. Contents were then cooled and laid on levelled surface to measure the gel migration in millimetres. Emulsion activity (EA) and emulsion stability (ES) were determined by method of Singh *et al.* (2017a). Flour sample (2.0 g) was added to 20 mL deionised water, and 20 mL refined soybean oil and continuous shaking was done for 30 min before centrifugation at 4900 ***g*** for 15 min. EA was noted in terms of % of emulsion layer to whole layer. Tubes were then heated in boiling water bath for 30 min and cooled and centrifugation was repeated to observe ES in terms of % of emulsion layer after heating to total emulsion layer before heating.

Colour profile

Tristimulus colour values (L^*, a^* and b^*) were determined using CIE system Hunter Lab Colorimeter (MiniScan XE plus, Reston, USA) where L^* denotes brightness (0 – dark black to 100 – bright white), a^* measures red ($-a$) to green ($+a$) and b^* indicates yellow ($-b$) to blue ($+b$) Sharma *et al.* (2021a).

Macromolecular-structural arrangement – scanning electron microscopy (SEM)

Modulation in the macromolecular arrangement during fermentation was studied with the help of scanning electron micrographs obtained from SEM, JSM 6100, JEOL (Akishima, Tokyo, Japan). Moisture-free samples were exposed to image capturing at varying magnifications by placing them onto carbon sample holders under vacuum condition, which eliminated the need of metallisation of flour prior to analysis. Accelerating voltage of the working equipment was fixed at 1 kV for all samples.

Statistical analysis

Independent experiments were done in triplicates, and all calculations were done at dry weight basis. Results were expressed as mean ± standard deviation ($n = 3$). One-way analysis of variance (ANOVA) was employed as statistical model to evaluate the difference among samples at $P < 0.05$ followed by post hoc Tukey's test to confirm the difference among variables. Pearson's correlation matrix (at $P < 0.05$) was used to estimate the association between different observations. Principal component analysis (PCA) was employed to validate the differences among observations and variables. XLSTAT.2021.1 was used to conduct statistical analysis.

Results and discussion

Anti-nutritional factors

Anti-nutritional factors are non-nutritive biochemical compounds naturally present in plant materials as defence mechanism which when consumed in excess may cause allergic responses and also inhibit the absorption of proteins and minerals; however, recent studies illustrated that limited consumption of these compounds may have beneficial effects in terms of antioxidant activity and lower risk of other lifestyle

diseases (Mohan *et al*., 2016). Untreated locust bean seeds exhibited 2.81 mg g^{-1} tannin content which significantly ($P < 0.05$) reduced to 0.98 mg g^{-1} after 72 h of fermentation at 38 °C; however, upsurge was seen at 46 °C and similar trend was observed for phytate and saponin content which declined from 12.48 to 4.95 mg g^{-1} and 6.80 to 1.86 mg g^{-1}, respectively, after fermentation at 38 °C as shown in Fig. 1. The lower reduction in anti-nutrients content with further increment in fermentation temperature to 46 °C was suggestive that 38 °C was the optimum for fermenting microorganisms to cause maximum degradation. The diminution in the anti-nutritional compounds could be attributed to two major factors; firstly, the hydrothermal treatment to seeds prior to fermentation breaking protein–polyphenol complex, resulting in higher leaching of water-soluble compounds in steep water such as tannins (Villacrés *et al*., 2020). Secondly, production of microbial enzymes such as tannase, phytase and β-glucosidase during fermentation could degrade the respective compounds (Khan *et al*., 2018). Furthermore, Mohapatra *et al*. (2019) also documented ~35–58% reduction in different anti-nutritional factors after combined effect of hydrothermal and fermentation treatments. Lower saponin content in fermented samples was predominantly due to disturbed stability of aglycones, lowering their water solubility and toxicity (Lai *et al*., 2013). Additionally, fermentation also results in the activation of inherent enzymes which have potential to degrade the anti-nutritional compounds as suggested by Olukomaiya *et al*. (2020) who stated lower phytate content in fermented lupin flour.

Phytochemical composition and antioxidant potential

Phytochemicals are biologically active compounds, which do not possess any nutritional properties but influence human health by preventing against several degenerative diseases. Polyphenols comprising phenolic acids and flavonoids are the major classes present in plant materials. TPC and TFC of untreated locust bean seeds were 222.19 mg GAE per 100 g and 53.94 mg QE per 100 g, respectively which varied significantly ($P < 0.05$) with fermentation treatments at different temperatures as presented in Table 1. Many of these compounds are heat sensitive and water soluble, due to which there was significant reduction due to hydrothermal treatment and later their content increased during fermentation, however, depending upon processing temperature. These compounds were

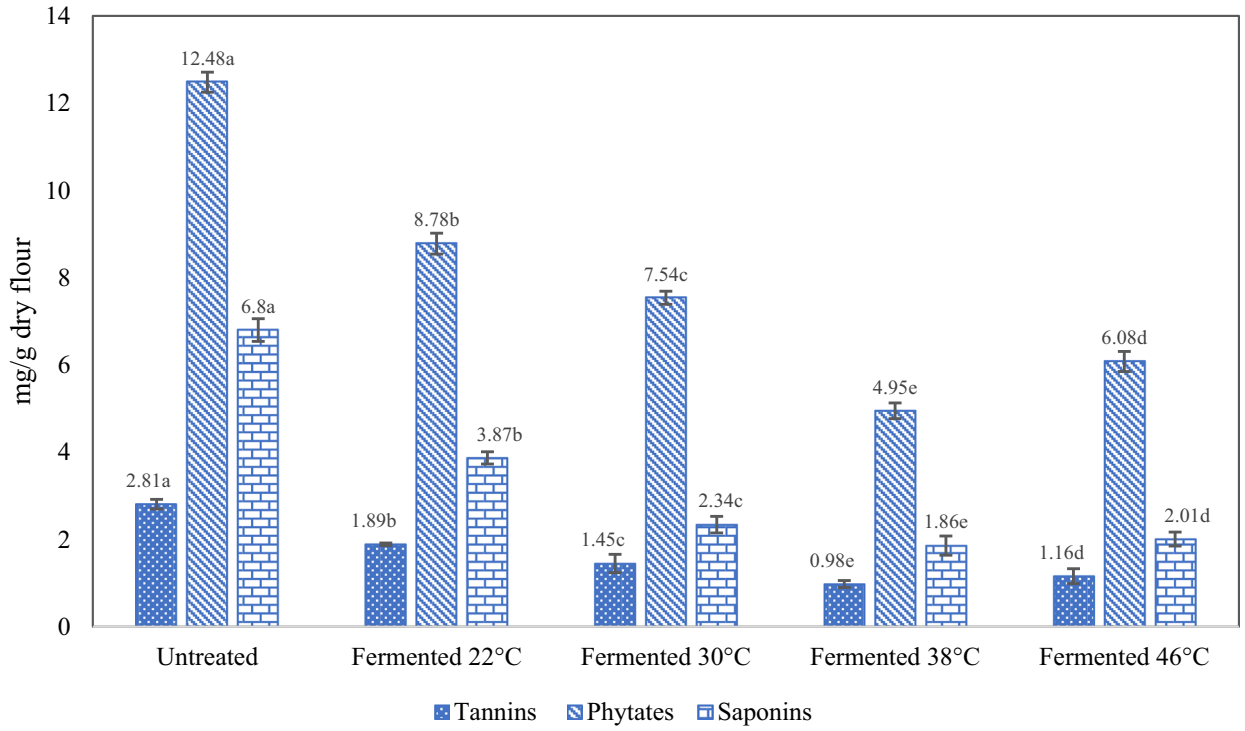

Figure 1 Effect of fermentation temperatures on anti-nutritional factors of Locust bean. Results are expressed as mean ± standard deviation ($n = 3$); Values with different alphabets (a–e) represent significant difference at $P < 0.05$.

Table 1 Impact of fermentation temperature on phytochemical compounds and associated antioxidant potential of Locust bean

	Phytochemical constituents		Antioxidant potential		
Treatments	**Total phenolic content (mg GAE per 100 g)**	**Total flavonoid content (mg QE per 100 g)**	**DPPH· RSA (%)**	**ABTS·+ RSA (μmol TE per g)**	**Metal chelation (%)**
Untreated	222.19 ± 2.85d	53.94 ± 1.09e	16.38 ± 0.20e	96.89 ± 1.06c	24.11 ± 0.12d
Fermented 22 °C	189.37 ± 1.98e	132.36 ± 1.14d	20.46 ± 0.26d	74.38 ± 0.98d	21.53 ± 0.18e
Fermented 30 °C	376.83 ± 2.47c	235.86 ± 1.36c	27.36 ± 0.38c	92.57 ± 0.78c	26.99 ± 0.11c
Fermented 38 °C	644.81 ± 3.45a	331.53 ± 1.84a	39.26 ± 0.89a	142.84 ± 0.93a	38.80 ± 0.09a
Fermented 46 °C	525.47 ± 3.29b	297.65 ± 2.43b	33.57 ± 0.92b	117.93 ± 1.03b	31.57 ± 0.16b

Results are expressed as mean ± standard deviation ($n = 3$); Values with different alphabets (a–e) in a column are significantly different at $P < 0.05$. GAE, gallic acid equivalent; QE, quercetin equivalent; TE, Trolox equivalent

highest when locust bean seeds were fermented at 38 °C (3-fold increase in TPC and 6-fold increase in TFC) followed by 46 °C. The lowest TPC at 22 °C treatment could be ascribed to the fact that growth of fermenting microorganisms was not supported at that temperature. The results are consistent to the findings of the Saharan *et al.* (2020) who observed similar increase in the bioactive constituents of the legumes during fermentation treatment. The authors further argued that the activity of enzymes such as xylanase, amylases, proteases and glycosidase enhanced during fermentation, which degraded protein molecules, and consequently, bound polyphenols were released free and higher extraction was achieved. Also, breakdown of higher polyphenolic compounds due to enzymatic action could have resulted in higher quantification of bioactive compounds. Similar results were also reported by Sandhu & Punia (2017) stating that enhanced β-glucosidase activity degraded phenolic compounds to yield higher content to total flavonoids.

Antioxidant activity is usually estimated by measuring the potential of bioactive extracts to quench/scavenge-free radicals, reactive oxygen species, chelation of metals and their reducing power. DPPH RSA, ABTS·+ RSA and % metal chelation (Fe^{+2}) of locust bean extracts enhanced in accordance with TPC and TFC as shown in Table 1. Significant ($P < 0.05$) positive correlations were observed between TPC and % DPPH·RSA ($r = 0.98$, $P < 0.05$), ABTS·+ RSA ($r = 0.93$, $P < 0.05$) and % metal chelation ($r = 0.98$, $P < 0.05$); however, TFC exhibited significant correlation only with % DPPH· RSA ($r = 0.98$, $P < 0.05$). Since three of the assays have different principles to estimate antioxidant potency, it was difficult to establish concrete relationship among them; however, their association with phytochemical constituents could be inferred from positive and significant ($P < 0.05$) correlation coefficients. Lee *et al.* (2004) also stated that polyphenols impart antioxidant activity owing to their structural arrangement, due to which they could easily bind metals to form complexes and scavenge-free radicals present in the biological systems.

In vitro starch and protein digestibility

As illustrated in Fig. 2, IVSD and IVPD increased significantly from 5.84 to 23.78 g maltose released per 100 g dry flour and 65.91 to 74.56 g hydrolysed protein per 100 g protein, respectively, with combined effect of hydrothermal treatment and fermentation. The most pronounced effect was observed at 38 °C as with further increase in fermentation, IVSD and IVPD declined significantly ($P < 0.05$). For IVPD, there was no significant difference between 30 and 46 °C fermented flours, which depicted that temperature variation in any direction from 38 °C caused reduction in IVPD making it optimum condition for protein digestibility. Macro-nutrient digestion of food ingredients indicates their biological performance as IVSD is a measure of energy contribution after starch hydrolysis to glucose while IVPD dictates the amount of protein available for physiological functionality. Gelatinised starch is prone to amylolytic attack, and thus, higher enzymatic activity caused breakdown of starch granules to smaller fragments. Dhital *et al.* (2016) also stated that the breakdown of protein and fibre matrix exposed starch granules to enzymatic action. Furthermore, the production of microbial enzymes such as tannase, phytase and glucosidases resulted in degradation of cell-wall constituents and other anti-nutritional factors, liberating the proteins from polyphenolic complexes and making them accessible to proteolytic enzymes. The aforesaid relationship was confirmed by negative correlation between IVPD and tannins ($r = -0.96$, $P < 0.05$), phytates ($r = -0.95$, $P < 0.05$) and saponins ($r = -0.97$, $P < 0.05$). Ketnawa & Ogawa (2019) also reported similar results after fermentation of soybean seeds.

Functional properties and colour profile

Functional properties of food ingredients dictate their suitability and potential in end products and are dependent upon structural and biochemical characteristics. Effect of fermentation temperature influenced

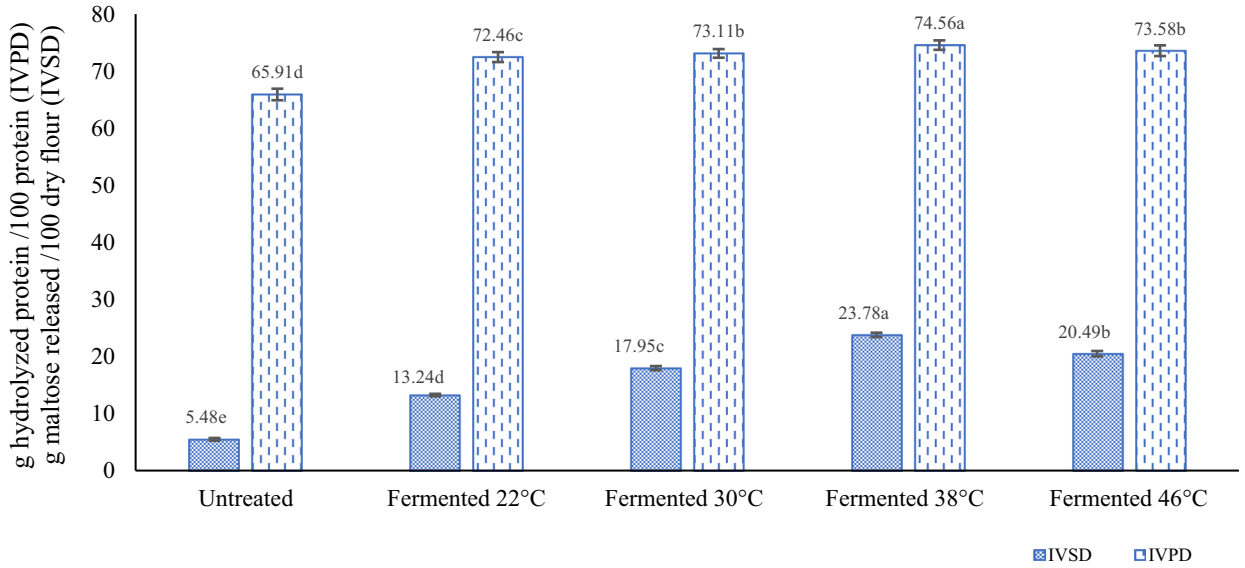

Figure 2 Effect of fermentation temperatures on in vitro starch digestibility (IVSD) and in vitro protein digestibility (IVSD) of Locust bean. Results are expressed as mean ± standard deviation (n = 3); Values with different alphabets (a–e) represent significant difference at $P < 0.05$.

Table 2 Impact of fermentation temperature on functional properties and colour profile of Locust bean

Functional properties	Untreated	Fermented 22 °C	Fermented 30 °C	Fermented 38 °C	Fermented 46 °C
Water absorption capacity (g g^{-1})	3.09 ± 0.15a	2.48 ± 0.18b	2.29 ± 0.11c	2.07 ± 0.13d	2.21 ± 0.19c
Water solubility index (%)	3.11 ± 0.23e	6.28 ± 0.13d	7.73 ± 0.17c	10.26 ± 0.37a	8.98 ± 0.31b
Oil absorption capacity (g g^{-1})	0.86 ± 0.02d	0.93 ± 0.03c	0.95 ± 0.03bc	1.02 ± 0.04a	0.98 ± 0.03ab
Swelling power (g g^{-1})	4.39 ± 0.45c	4.74 ± 0.34a	4.61 ± 0.32b	4.27 ± 0.21d	4.42 ± 0.29c
Gel consistency (mm)	35.7 ± 0.2e	44.5 ± 0.4d	48.4 ± 0.2c	54.8 ± 0.3a	51.5 ± 0.4b
Emulsion activity (%)	45.42 ± 1.02a	13.82 ± 0.78d	14.98 ± 0.38cd	16.24 ± 0.23b	15.67 ± 0.29bc
Emulsion stability (%)	24.27 ± 0.77a	12.44 ± 0.42c	13.83 ± 0.48c	16.95 ± 0.57b	15.02 ± 0.37b
L^*	79.26 ± 0.37a	67.48 ± 0.49b	59.25 ± 0.58c	51.87 ± 0.33e	55.48 ± 0.41d
a^*	2.21 ± 0.12e	4.67 ± 0.11d	5.95 ± 0.14c	7.83 ± 0.59a	6.58 ± 0.13b
b^*	23.07 ± 0.34a	22.13 ± 0.58b	20.58 ± 0.48c	17.98 ± 0.18e	18.75 ± 0.45d

Results are expressed as mean ± standard deviation (n = 3); Values with different alphabets (a–e) in a raw are significantly different at $P < 0.05$.

the functional properties as a function of extent of microbial activity during fermentation. Compositional and structural analysis have suggested that fermentation at 38 °C caused most pronounced effect, and similar trend was seen in functional properties of locust bean flours as shown in Table 2. With further increase in temperature to 46 °C, the degree of fermentative changes declined as a result of unsuitability of higher temperature for microbial growth and hydrolytic activity of enzymes. Severity of fermentation resulted in loss of WAC (3.09–2.07 g g^{-1}), while WSI increased (3.11–10.26 g g^{-1}) significantly ($P < 0.05$), most probably ascribing to amylolytic hydrolysis of starch molecules resulting in the formation of lower sugars and dextrin which possess lower water holding power and further augment the release of soluble constituents (Cornejo & Rosell, 2015). Fractional unfolding of primary protein structure during heating and fermentation exposed hydrophobic regions, which could have encouraged the association between oil droplets and lipophilic protein regions, explaining the increment in OAC (0.86–1.02 g g^{-1}) after fermentation process. Swelling power of flours is a property of amylopectin units, and its degradation during fermentation to lower sugars promoted starch inter-crosslinking within the amorphous regions, and consequently, SP reduced (Singh *et al.*, 2017b). Enzymatic hydrolysis was further responsible for gel weakening of locust bean flours linearly with the extent of fermentation as indicated by increasing GC from 35.7 to 54.8 mm. This could be

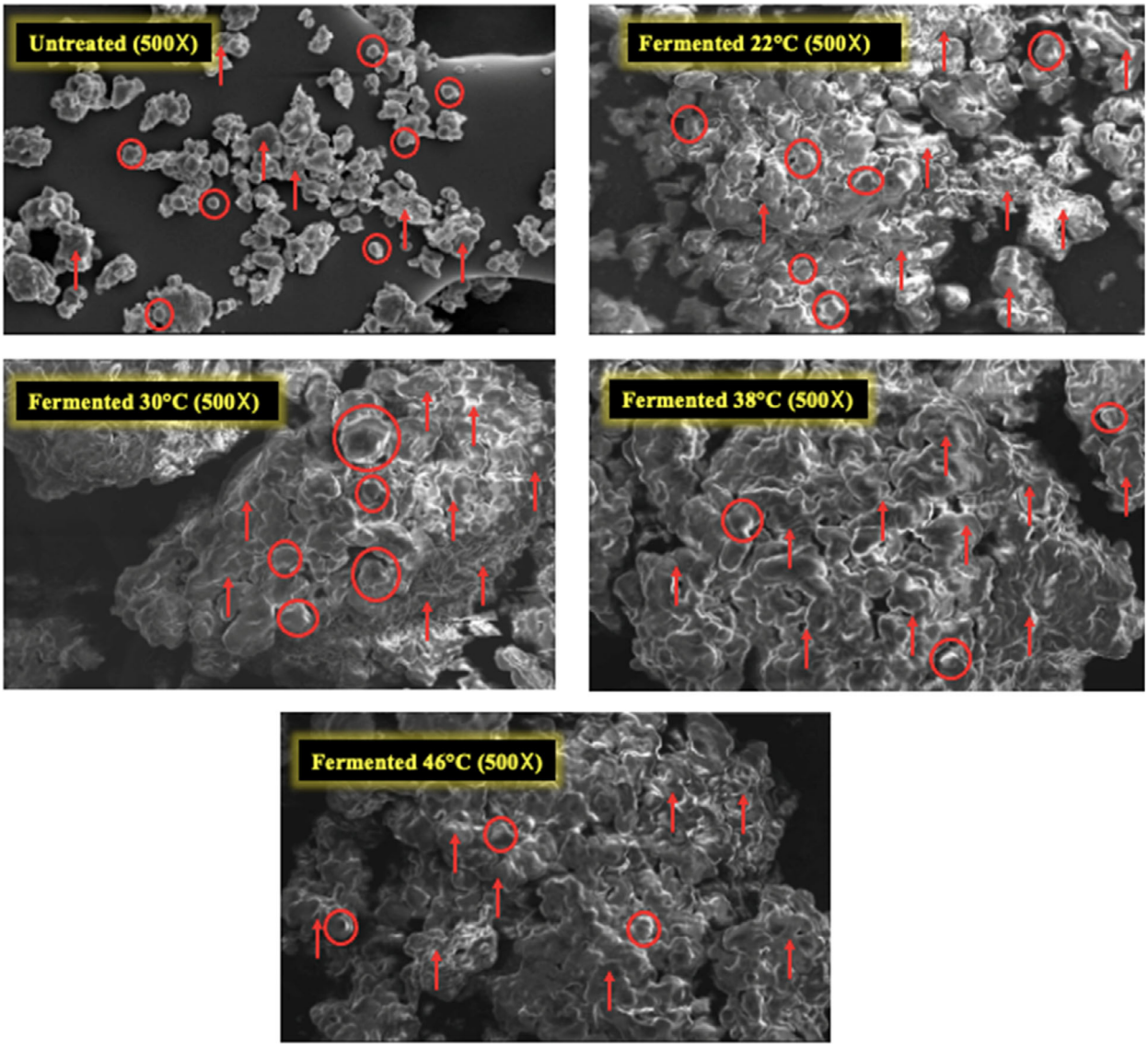

Figure 3 Scanning electron micrographs of untreated and fermented locust bean flours. Encircled molecules are locust bean starch granules and up arrows indicate starch-protein matrix (uniform thick mass in treated samples due to gelatinization).

attributed to lower viscosity of gels from fermented flours under acidic conditions, and the results were also consistent with findings of Adiandri & Hidayah (2019) who studied the functional properties of fermented sorghum flour. Loss of EA and ES after hydrothermal treatment could be ascribed to denaturation of proteins; however, severity of fermentation brought little but significant ($P < 0.05$) improvement. Abd Elmoneim *et al*. (2005) also documented that fermentation induced flexibility in proteins and exposed hydrophobic regions, which enhanced emulsification strength of treated flours. Tristimulus markers of colour profile were significantly influenced by the temperature of fermentation as shown in Table 2. L^* value declined from 79.26 (untreated flour) to 51.87 (fermented at 38 °C) suggesting darkening of the flour due to oxidative and enzymatic changes. Red to green chromaticity coordinate, that is a^* value increased from 2.21 to 4.76–7.84, while b^* was found to reduce from 23.07 to 22.13–17.98 at different processing temperatures. Variation in the colour towards red (increasing a^*) and blue (decreasing b^*) could be attributed to oxidation reactions due to higher enzymatic activity, and Maillard series of reactions including Strecker

degradation of amino acids during high temperature processing such as boiling and drying as reported by Diez-Simon *et al*. (2020).

Macromolecular-structural arrangement

Scanning electron micrographs shown in Fig. 3 were obtained for untreated and fermented locust bean flours. Raw flour exhibited complete and well-shaped starch granules, which were affected differently in response to different fermentation temperatures. The starch–protein matrix revealed that the most severe impact was caused at 38 °C. Gelatinisation of starch could be seen in all fermented samples attributing to hydrothermal treatment, and thus, the major variation among different samples was due to higher action of hydrolytic enzymes in samples fermented at 38 °C > 46 °C > 30 °C > 22 °C. Furthermore, no visible boundaries between flour particles were suggestive of enzymatic breakdown of the cell-wall constituents to produce uniform thick mass. Microbial production of amylases, proteases and lipases was the major factor to modulate the starch–protein structural arrangement (Handoyo & Morita, 2006), which significantly altered the *in vitro* digestibility of the nutrients as detailed in respective section.

Principal component analysis

Data variance from multivariate analysis was expressed by two dimensional factors F1 (82.62%) and F2 (16.87%) as shown in PCA plot (Fig. 4). The first dimension exhibited three variables including untreated locust bean flour and those fermented at 38 and 46 °C, while remaining two were distinguished in the second dimension. PCA is a statistical technique to present complex information in a simplified manner. Observations and variables which are in close proximity to each other possess positive correlation while those in opposite quadrants hold negative association with each other. Specifically,

- Locust bean seeds fermented at 22 °C were characterised by *b** and swelling power in the first quadrant.
- The second quadrant was equipped with untreated locust bean with variables including anti-nutritional factors, ES, EA, *L** and WAC.

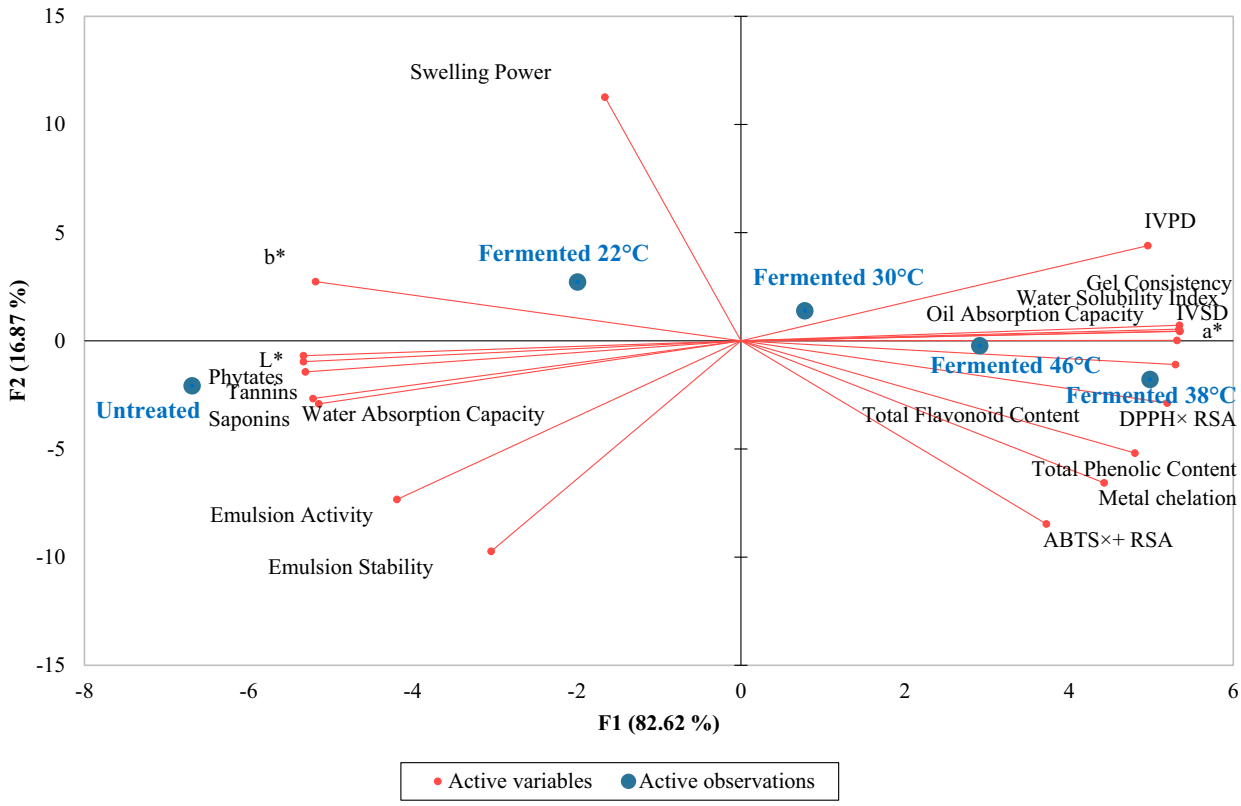

Figure 4 Principal component analysis defining the data variance by two factors F1 (82.62%) and F2 (16.87%) for association between different variables and observations.

- Fermented locust bean at 38 and 46 °C were in close proximity signifying higher correlation with variables including phytochemical constituents and antioxidant assays.
- The fourth quadrant was characterised by GC, OAC, WSI, *a**, IVSD and IVPD with fermented locust bean at 30 °C.

Conclusion

The outcomes of present investigation were suggestive that 38 °C was the optimum temperature to minimise anti-nutritional factors without adversely affecting the functional properties of locust bean. Enhanced *in vitro* starch and protein digestibility further improved the potential of locust bean for utilisation in value-added products. Major fermentation induced changes were associated with inherent hydrolytic enzymes synthesised during treatment. Reduction in anti-nutritional factors resulted in degradation of protein–polyphenol complexes making bioactive constituents available for extraction in addition to activation of endogenous hydrolytic enzymes. Disruption of starch molecules due to fragmentation into smaller units as revealed by micrographs and exposure of hydrophobic regions due to fermentation were the major reasons for modulated functionality of locust bean flour. This study can be considered as potential reference for future investigations to assess detailed arrangement of biomolecules after fermentation of locust bean.

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Conflict of interest

None.

Author contribution

Caleb Maina Yakubu: Data curation (equal); Formal analysis (equal); Methodology (equal); Writing-original draft (equal). **Rajan Sharma:** Conceptualization (equal); Formal analysis (equal); Writing-original draft (equal); Writing-review & editing (equal). **Savita Sharma:** Conceptualization (equal); Project administration (lead); Supervision (lead); Writing-review & editing (equal).

Ethical approval

Ethics approval was not required for this research.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15288.

Data availability statement

Author elect not to share data.

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