Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

https://doi.org/10.17113/ftb.59.04.21.7319 original scientific paper

Antioxidant Potential, Antinutrients, Mineral Composition and FTIR Spectra of Legumes Fermented with *Rhizopus oligosporus*

Running Title: Fermentation of Legumes with *Rhizopus oligosporus*

Barinderjeet Singh Toor, Amarjeet Kaur*, Param Pal Sahota and Jaspreet Kaur

Punjab Agricultural University, Ferozepur Road, Ludhiana, Punjab-141004, India

Received: 10 May 2021
Accepted: 12 October 2021

SUMMARY

Research background. Legumes are superior sources of macro- and micronutrients which can be further enhanced by fermentation. This can assist in addressing the food security concerns. The present study aims to determine the effect of fermentation by *Rhizopus oligosporus* on nutritional and antinutritional composition of some commonly consumed legumes.

Experimental approach. Chickpea (*kabuli* and *desi*), pigeon pea, and soybean were fermented with *Rhizopus oligosporus* (at 34 °C for 52 h), dried at 45 ºC for 16-18 h and milled. Unfermented and fermented flours were evaluated for antioxidant potential, phenolic composition, antinutrients, mineral composition and FTIR spectra.

Results and conclusions. Fermentation significantly ($p<0.05$) enhanced the total phenolic and flavonoid contents, and antioxidant properties (radical scavenging activity, reducing power, ferric reducing antioxidant power and metal chelation) of chickpea *kabuli* and *desi*, and soybean. Although fermented pigeon pea exhibited excellent antioxidant properties, the effect of fermentation on such properties was either minimal or insignificant. Additionally, specific phenolics were quantified using HPLC which showed higher concentration of certain compounds such as chlorogenic acid, *p*-

*Corresponding author:
Mobile: +919888466677
E-mail: amarjit@pau.edu

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

hydroxybenzoic acid, gallic acid and vanillic acid in fermented legumes. Phytic acid in all the fermented legumes reduced ($p<0.05$), however trypsin inhibition increased ($p<0.05$). In chickpea *kabuli* and *desi,* and pigeon pea, saponins increased ($p<0.05$) while they decreased in soybean. Tannins enhanced ($p<0.05$) in chickpea *desi*, pigeon pea and soybean and reduced ($p<0.05$) in chickpea *kabuli*. Furthermore, fermentation enhanced the content as well as estimated bioavailability of minerals. FTIR spectrum of unfermented and fermented legumes showed the presence of several functional groups and modifications in the molecular structure after fermentation.

Novelty and scientific contribution. To our knowledge, this is the first study wherein legume (chickpea *kabuli* and *desi*, pigeon pea and soybean) fermentation by *Rhizopus oligosporus* has been assessed for nutritional and antinutritional profile, and FTIR spectra, which concluded that the treatment resulted in an optimal balance of nutrients and antinutrients. The process was established as a potential tool and thus can be proposed in the development of legume based novel functional foods which might help in tackling the concerns of nutritional security.

Key words: *Rhizopus oligosporus*, legumes, antioxidant, phenolics, antinutrients, minerals

INTRODUCTION

Growing population combined with climatic changes poses a huge threat to food and nutrition securities. In such a scenario, plant foods particularly legumes are playing an important role ascribed to their rich nutritional composition containing quality proteins, essential minerals and amino acids as well as carbohydrates (*1,2*). Several epidemiological studies have concluded that the regular consumption of legumes prevents the body from various health conditions like coronary heart disease, diabetes and colon cancer (*3*). Such beneficial effects are mediated by the presence of phenolics, bioactive peptides, amino acids and vitamins (*4*). However, legumes contain certain antinutrients including phytic acid, trypsin inhibitor, saponins and tannins which reduce the bioavailability of nutrients through complexation (*5*).

Several innovative strategies have been developed to mitigate the negative effects of antinutrients and to enhance the functionality of legumes. Fermentation is a potential technique which has been found to be effective against antinutrients as well as contributes to the organoleptic properties and shelf life extension (*5*). Enzymes (protease, tannase, amylase) secreted during fermentation hydrolyze complex molecular structures to simpler forms which results in enhanced digestibility and bioavailability of nutrients including minerals (calcium and phosphorus) and limiting

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

amino acids. Moreover, fermentation has the ability to mobilize the bound phenolics to improve the overall phenolic content and related antioxidant properties (*3,6*).

Rhizopus oligosporus, a filamentous fungus utilized in the present study, belongs to the family Mucoraceae and is generally recognized as safe (GRAS). *R. oligosporus* has been demonstrated as detoxifying agent against food toxins, such as Ochratoxin A. Moreover, *R. oligosporus* resists the proliferation of pathogens, for instance, *Staphylococcus aureus,* through the formation of antibiotics (*7,8*). Since long, *R. oligosporus* has been utilized for the fermentation of soybean to produce tempeh, a traditional Indonesian soy product. Recently, substrates like chickpeas, mung beans and kidney beans have been utilized for the preparation of tempeh (*9,10*). The aim of this study was to elucidate the effect of fermentation on the antioxidants, antinutrients, mineral composition as well as FTIR spectra of chickpea (*kabuli* and *desi*), pigeon pea and soybean.

MATERIALS AND METHODS

Raw materials

Chickpea *kabuli* (CPK) – L 552, chickpea *desi* (CPD) – PBG 7, pigeon pea (PP) – AL 882 and soybean (SB) – SL 958 were procured from Punjab Agricultural University, Ludhiana, India. *R. oligosporus* MTCC 556 was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

Fermentation

For fermentation, seeds were soaked overnight in water at 25 °C followed by cooking (CPK, CPD and PP for 30 min; SB for 1 h). Cooked seeds were inoculated with *R. oligosporus* suspension (1×10^9 spores/kg) and incubated for 52 h at 34±1 °C. The cooking time and fermentation process were optimized by preliminary trials. Fermented seeds were dried in a tray dryer (Model Td-12, Narang Scientific Works, DL, India) at 45±1 °C for 16–18 h and milled to flour (60 BSS mesh) using domestic mill (Saffron Home Appliances, Ahmedabad, GJ, India). Legumes were soaked, cooked, and dried using defined conditions and milled to obtain unfermented flour (60 BSS mesh). Fermented and unfermented legumes samples were kept at 4 °C in airtight pouches till analysis (*11*).

Antioxidant properties

Extract preparation

One gram of each sample was refluxed twice with 20 mL of aqueous methanol (80 % methanol acidified with 0.1 % HCl) for 2 h. The extracts were pooled and centrifuged at 1600 × *g* for 10 min

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

(Model T-8BL, Laby Instruments Industry, Ambala, HR, India). The final volume was made up to 50 mL with aqueous methanol (sample concentration–20 mg/mL). All the extracts were stored in amber glass bottles at 4 °C until analysis.

Total phenolic content (TPC)

The method reported by Singleton *et al.* (*12*) was utilized for the determination of TPC. 0.5 mL of extract was taken in test tubes and diluted to 1 mL with aqueous methanol. 5 mL of freshly prepared Folin-Ciocalteu reagent (10 % v/v) was added followed by the addition of 4 mL saturated Na_2CO_3 solution. After incubation for 2 h, the absorbance was measured at 765 nm (Model Spectro-4, Laby Instruments Industry, Ambala, HR, India) against reagent blank. Results were expressed as milligrams gallic acid equivalents per gram of dry sample (*w*(TPC as GAE)/(mg/g)).

Total Flavonoid content (TFC)

TFC was measured according to the method given by Dini *et al.* (*13*) with certain modifications. 0.5 mL of extract and 2 mL of distilled water were mixed in test tubes followed by the addition of 0.15 mL of 5 % $NaNO_2$ aqueous solution. Tubes were incubated for 5 min and thereafter 0.15 mL of 10 % $AlCl_3$ aqueous solution was added. After 6 min, 1 mL of NaOH (1 M) was added into reaction mixture followed by the immediate addition of 1.2 mL of water. Final reaction mixture was thoroughly mixed, and absorbance was measured at 510 nm against reagent blank. Quercetin was used as standard to express the results as milligrams quercetin equivalents per gram of dry sample (*w*(TFC as QE)/(mg/g)).

Radical scavenging activities ($DPPH^•$ and $ABTS^{+•}$)

$DPPH^•$ (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity was evaluated using the procedure given by Brand-Williams *et al.* (*14*) with some modifications. In test tubes, 0.1 mL of extract was mixed with 3.9 mL of freshly prepared DPPH solution (0.2 mM) followed by incubation for 30 min in dark. Absorbance was measured at 515 nm. Absolute methanol and DPPH solution were used as blank and control respectively. The standard curve of Trolox was plotted as a function of the percentage of DPPH radical scavenging activity. Results were expressed in terms of micromoles Trolox equivalents per gram of dry sample (TE/(μmol/g)).

The method reported by Yilmaz-Ersan *et al.* (*15*) was used for the determination of $ABTS^{+•}$ (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation) scavenging activity. 20 mM ABTS and 2.45 mM $K_2S_2O_8$ solutions were mixed in the ratio of 1:1 (v/v) and kept in the dark for 12–16 h at

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

ambient conditions for producing stock solution. The working solution was prepared by diluting this stock solution with ethanol to an absorbance of 0.7±0.02 at 734 nm. For analysis, 0.1 mL of extract, 3.9 mL of ethanol and 1 mL of freshly prepared working solution were mixed. After 6 min, absorbance was recorded at 734 nm against ethanol. The standard curve of Trolox was plotted as a function of the percentage of antioxidant activity. Results were expressed as micromoles Trolox equivalents per gram of dry sample (TE/(μmol/g)).

Reducing power

Reducing power was measured according to the procedure reported by Oyaizu (*16*). 1 mL of the methanolic extract, 2.5 mL of 1 % potassium ferricyanide and 2.5 mL of phosphate buffer (0.2 M, pH 6.6) were mixed thoroughly and incubated for 30 min at 50 °C. Thereafter, 2.5 mL of 10 % trichloroacetic acid was added into reaction mixture followed by centrifugation at 1600 × *g* for 10 min. 2.5 mL of supernatant was diluted with 2.5 mL of distilled water and mixed with 0.5 mL of 0.1 % $FeCl_3$ solution for recording the absorbance at 700 nm against prepared reagent blank. A standard curve was prepared using L-ascorbic acid and the results were expressed as milligrams ascorbic acid equivalents per gram of dry sample (AAE/(mg/g)).

Ferric reducing antioxidant power (FRAP)

FRAP was determined as per the method given by Tomasina *et al.* (*17*). 0.1 mL of extract was mixed with 2.4 mL of freshly prepared FRAP reagent followed by the incubation for 5 min at 37 °C. The absorbance was recorded at 620 nm against reagent blank. Here, FRAP reagent was prepared by mixing 20 mM $FeCl_3$ solution, acetate buffer (300 mM, pH 3.6), and 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in the ratio of 1:10:1. Trolox was utilized as standard and the results were recorded as micromoles Trolox equivalents per gram of dry sample (TE/(μmol/g)).

Metal (Fe^{2+}) chelating activity

Fe^{2+} chelating activity was performed according to Chew *et al.* (*18*) with certain modifications. The method involved the dilution of 20 μL of extract with 5 mL of distilled water followed by the addition of 50 μL of $FeSO_4.7H_2O$ (0.30 mM). After 7 min, 75 μL of ferrozine solution (0.80 mM) was added and the mixture was allowed to stand for 15 min. Absorbance was recorded at 562 nm against aqueous methanol. The following equation was used for the calculation of percentage chelating activity:

$$\%\ \text{chelating activity} = (1-A_{\text{sample}}/A_{\text{control}}) \cdot 100 \qquad /1/$$

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

where, A_{sample} and $A_{control}$ are the absorbances recorded for sample and control, respectively. Aqueous methanol was replaced with extract in the control.

Antioxidant potency composite index (APCI)

An equal weight was assigned to all the antioxidant assays (DPPH, ABTS, FRAP, reducing power, metal chelating activity) and an index value of 100 was assigned to the best score for each test. Thereafter, index scores were calculated for other samples within the test using the following equation:

Antioxidant index score = (sample score/best score) · 100 /2/

APCI was calculated by taking the average of all five tests (*19*).

Phenolic composition

As per the method described by Xiao *et al.* (*10*), samples were extracted with 80 % methanol (1:40) at 50 °C for 4 h followed by cooling to room temperature. After centrifuging the extracts at 15,000 × *g* (Model BS-SP-70BL, Biogenix System, DL, India) for 15 min, the solvent was evaporated under reduced pressure and the residue obtained was dissolved in HPLC grade methanol (80 %). The final solution was filtered through syringe filter (0.45 µm PVDF membrane) and subjected to HPLC system (Agilent Technologies, Wilmington, DE, USA) equipped with a reverse-phased ZORBAX Eclipse XDB-Phenyl column (4.6 × 250 mm, 5 µm particle size, Agilent Technologies, Wilmington, DE, USA) with the gradient elution solution A containing 80 % acetonitrile, 2 % acetic acid, 18 % water and solution B containing 9 % acetonitrile, 2 % acetic acid, 90 % water. The other parameters were adjusted as follows: solvent flow rate, 1 mL/min; column oven temperature, 25 °C; injection volume, 20 µL. Detection was performed at 276 nm for gallic acid, syringic acid and cinnamic acid, at 325 nm for chlorogenic acid, caffeic acid and ferulic acid, and at 257 nm for *p*-hydroxybenzoic acid and vanillic acid. Results were expressed as mass fraction per 100 g of sample (*w*/(mg/100g)).

Antinutrients

Phytic acid was analysed based on the method of Haug *et al.* (*20*). Samples (1 gm) were extracted with 0.2 N HCl at room temperature. After extraction, 0.5 mL of extract and 1 mL of ferric solution (0.2 g $NH_4Fe(SO_4)_2.12H_2O$ in 100 mL of 2 N HCl diluted to 1000 mL with distilled water) in stoppered test tubes were heated in boiling water bath for 30 min keeping the tubes well stoppered for the first 5 min. After heating, the tubes were cooled for 15 min in ice water and allowed to adjust to room temperature. Thereafter, 2 mL of 2,2'-bipyridine solution (10 g 2,2'-bipyridine and 10 mL

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

thioglycolic acid dissolved in distilled water and diluted to 1000 mL) was added following by thorough mixing. After 30 min, absorbance was recorded at 519 nm. Sodium phytate was utilized to obtain the standard curve and the results were calculated as milligrams phytate per gram of dry sample (*w*(phytate)/(mg/g)).

Saponins were extracted using the method of Fenwick *et al.* (*21*). Defatted samples (1 gm) with methanol were kept at room temperature for 24 h. After centrifuging the contents at 1600 × *g*, the supernatant was collected and final volume was made to 10 mL with methanol. For determination, Baccou *et al.* (*22*) method was used wherein 1 mL of extract was kept in a boiling water bath to remove the methanol. After cooling, 2 mL of ethyl acetate was added followed by the addition of 1 mL of reagent (0.5 mL anisaldehyde mixed with 99.5 mL ethyl acetate) and 1 mL of concentrated sulphuric acid. The contents were thoroughly mixed and allowed to stand for 10 min at room temperature. Absorbance was recorded at 430 nm against reagent blank and the results were expressed as milligrams saponin per gram of dry sample (*w*(saponin)/(mg/g)).

Tannins were determined using the modified method of AOAC (*23*). For extraction, 1 gm of sample was refluxed with 40 mL of 10 % methanol for 2 h. The contents were centrifuged at 1600 × *g* and the final volume was made to 50 mL with methanol. For determination, 1 mL of extract was mixed with 7.5 mL of distilled water in test tube followed by addition of 0.5 mL Folin-Denis reagent. Thereafter, 1 mL of saturated Na_2CO_3 was added and the contents were thoroughly mixed. After 30 min, absorbance was measured at 760 nm against reagent blank. The results were expressed as milligrams tannic acid equivalents per gram of dry sample (*w*(tannic acid)/(mg/g)).

The isolation and estimation of trypsin inhibitor was performed using the methods of Hajela *et al.* (*24*) and Nath *et al.* (*25*) with slight modifications. 1 g of the sample was soaked overnight in 10 mL of 0.01 M phosphate buffer (pH 7.5) containing 0.1 M NaCl. The contents were centrifuged at 4850 × *g* (Model T-8BL, Laby Instruments Industry, Ambala, HR, India) for 30 min. The supernatant was heated at 80 °C for 20 min and centrifuged again at 4850 × *g* for 30 min. Bovine pancreatic trypsin was used as the enzyme source and the substrate was N-benzoyl-DL-arginine-p-nitroanilide (BApNA). To 100 µL of the extract, 100 µL of the enzymatic solution was added followed by addition of 200 µL of 0.01 M Tris-HCl buffer (pH 7.5) containing 0.02 M $CaCl_2$. The contents were incubated at 37 °C for 10 min. 2 mL of the substrate solution (1 mM solution of N-benzoyl-DL-arginine-p-nitroanilide (BApNA) in dimethyl sulfoxide (DMSO) (9 mg:2 mL) and made to required volume using 0.01 M Tris-HCl buffer (pH 7.5)) was added and the contents were incubated again at 37 °C for 10 min. The reaction was terminated by the addition of 400 µL of 30 % acetic acid. Blank was prepared by adding acetic acid before the substrate. Control was prepared by adding 0.01 M Tris-HCl buffer

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

(pH 7.5) containing 0.02 M $CaCl_2$ instead of the extract. Protease inhibition activities were measured by recording the optical densities of test and control reaction mixtures at 410 nm. Comparison of the absorbances of test and control mixtures determined the trypsin units inhibited. Decline in optical density (OD) monitored at 410 nm as compared to control of 0.01 OD units/min was considered to be one unit inhibited per mL of the reaction medium. Results were expressed as trypsin inhibitor activity (TIA) per milligram of dry sample (Trypsin inhibitor activity/(U/mg)).

Mineral composition and bioavailability

Each sample was digested with solution of distilled nitric acid (3 parts) and perchloric acid (1 part) for 45 min at 90–95 °C. Digested solution was diluted with distilled water followed by filtration. Final solution was analyzed using ICP-OES (Thermo Fisher Scientific iCAP 6300) (*26*).

Phytate:mineral molar ratios were determined before and after fermentation for estimation of mineral bioavailability. The molecular weight of phytate was considered as 660 g/mol for calculation of Phy:Ca, Phy:Zn, and Phy:Fe, molar ratios (*27*).

FTIR analysis

FTIR spectrum of samples were obtained using Thermo Nicolet 6700 FTIR spectrometer, operating in the middle IR region (500–4000 $\tilde{v}$/cm^{-1}), with attenuated total refection (ATR) mode.

Statistics

All the analyses were carried out in triplicates and the results were expressed as mean ± standard deviation. To compare within changes before and after fermentation, data sets were subjected to paired t test at $p<0.05$ significance level on SPSS 18.0 statistical software (SPSS Inc.) (*28*). Pearson's correlation coefficient was performed for correlation analysis.

RESULTS AND DISCUSSION

Effect of fermentation on the antioxidant properties

Total phenolic and flavonoid content

Phenolics are ordinally correlated with the antioxidant properties due to their electron donating ability. **Table 1** shows the TPC and TFC of unfermented and fermented legumes. Fermentation significantly ($p<0.05$) enhanced the TPC of all the legumes. The highest TPC was exhibited by fermented soybean (FSB), 482 % higher than unfermented soybean (USB). Fermented chickpea *kabuli* (FCPK) and fermented chickpea *desi* (FCPD) were found to contain 52 % and 23 % higher

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

TPC than unfermented chickpea *kabuli* (UCPK) and unfermented chickpea *desi* (UCPD), respectively. The lowest but significant ($p<0.05$) improvement was observed in PP, where fermented pigeon pea (FPP) contained 12 % higher TPC than unfermented pigeon pea (UPP). The results are consistent with the findings of Xiao *et al.* (*10*) and Lee *et al.* (*29*) who have reported 80 and 39 % higher TPC in *Cordyceps militaris* SN-18 fermented chickpea and *Bacillus subtilis* BCRC 14715 fermented pigeon pea, respectively. Phenolics exist as soluble free esters, and to a major extent, as insoluble esters complexed with plant cell walls, proteins and polysaccharides (*30*). As explained by Juan *et al.* (*31*) and Vong *et al.* (*32*), the enzymes; *β*-glucosidases and *β*-glucuronidases, produced by *R. oligosporus* during fermentation of legumes could hydrolyze these complexed phenolics to soluble forms which is reflected as enhanced phenolic content.

Flavonoids are also correlated with antioxidant properties and their consumption reduces the risk of various diseases *i.e.* heart disease and cancer. As shown in **Table 1**, TFC of CPK, CPD and SB significantly ($p<0.05$) increased by 86, 60 and 90 % respectively, after fermentation while insignificant difference was found between TFC of FPP and UPP. In similar context, Juan *et al.* (*31*) and Lee *et al.* (*29*) reported 31 and 120 % higher TFC in soybean and pigeon pea fermented with *Bacillus subtilis* BCRC 14715 and attributed such enhancement to the activity of microbial enzymes which released bound flavonoids from the substrate. Adetuyi *et al.* (*33*) have reported fermentation as effective approach in enhancing the flavonoids of okra seeds. It was suggested that, during fermentation the rise in matrix acidity led to liberation of bound flavonoids to available forms. However, the studies conducted on cow pea seeds and pistachio hulls displayed contradictory results and it had been explained that with the passage of fermentation time, the microorganisms start utilizing available flavonoids which reduce their concentration (*4,34*). Thus, in the present study, the insignificant decrease in the TFC of PP might be due to the utilization of flavonoids by microorganism for their growth.

Radical scavenging activities (DPPH$^•$ and ABTS$^{+•}$)

DPPH$^•$ and ABTS$^{+•}$ are free radicals which are reduced and stabilized in the presence of electron donating compounds. Such phenomenon was used for the measurement of antiradical capacity of samples. Results obtained for DPPH$^•$ and ABTS$^{+•}$ scavenging activities have been demonstrated in the **Figs. 1a** and **1b**, respectively. A significant difference ($p<0.05$) was observed between the scavenging activities of fermented and unfermented samples of CPK, CPD and SB. In terms of DPPH$^•$ and ABTS$^{+•}$ inhibition, the highest increment was recorded in SB *i.e.* 76 % and 370 %, respectively,

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

followed by CPK (61 % and 45 %) and CPD (46 % and 29 %). PP was found to contain excellent radical scavenging activities however the effect of fermentation on such properties was insignificant.

The ability of fermentation to enhance the radical scavenging activities has been reported by various investigators. For instance, Lee *et al*. (*29*) and Juan *et al*. (*31*) observed higher scavenging of radicals in fermented pigeon pea and soybean. Similarly, Xiao *et al*. (*10*) reported 48 and 38 % higher $DPPH^{•}$ and $ABTS^{+•}$ scavenging activities in *Cordyceps militaris* SN-18 fermented chickpea. In another study, Xiao *et al*. (*35*) observed 44 and 362 % higher $DPPH^{•}$ and $ABTS^{+•}$ scavenging activities in *Cordyceps militaris* SN-18 fermented mung beans. These investigations have attributed better scavenging abilities in fermented legumes to the enrichment in phenolic and flavonoid content during fermentation. Thus, the enhanced TPC and TFC in FCPK, FCPD and FSB might be the reason behind their better radical scavenging activities. The insignificant impact on the TFC of PP was also highlighted on the radical scavenging activities upon its fermentation. In addition, Xiao *et al*. (*10*) demonstrated that the significant enhancement in the inhibition of DPPH and ABTS radicals in fermented chickpeas was ascribed to the enzymatic conversion of phenolic glycosides to more antiradical aglycones. This might also be the reason behind the enhanced radical scavenging activities in the FCPK, FCPD and especially FSB which is the excellent source of these compounds.

Reducing power

Reducing power of unfermented and fermented legumes has been demonstrated in the **Fig. 1c**. Fermentation significantly ($p<0.05$) enhanced the reducing ability of all the legumes with SB showing the highest increment of 210 % followed by CPK (51 %), CPD (61 %) and PP (9 %). In similar studies, Xiao *et al*. (*10*) and Xiao *et al*. (*30*) have reported 2–3 fold higher reducing power in *Cordyceps militaris* SN-18 fermented chickpea and oats and correlated it with the reductone enhancing ability of fermentation. Xiao *et al*. (*30*) explained that, these reductones have the ability to stabilize free radicals and cease toxic radical reactions. In addition, Xiao *et al*. (*35*) ascribed strong reducing ability of fermented mung beans to enhanced phenolics. Thus, it seemed that the enhanced phenolic and flavonoid content in FCPK, FCPD, FPP and FSB is responsible for their strong reducing power.

Ferric reducing antioxidant power (FRAP)

FRAP reflected the antioxidant potential of food matrixes owing to their ability to reduce the complex from TPTZ-Fe^{3+} to TPTZ-Fe^{2+}. **Fig. 1d** demonstrates that the fermentation significantly ($p<0.05$) increased the FRAP of all the legumes. SB achieved highest enhancement (163 %) followed by CPK (46 %), CPD (43 %) and PP (19 %), respectively. This is consistent with the finding of Razak

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

et al. (*36*) who have reported higher FRAP values in *R. oligosporus* and *Monascus purpureus* fermented cereals and attributed it to enhanced phenolic contents during fermentation. Similarly, Xiao *et al.* (*35*) demonstrated a strong correlation between the FRAP and phenolics of fermented mung beans. It was explained that, the phenolic compounds enriched during fermentation were responsible for higher reduction of TPTZ-Fe^{3+} to TPTZ-Fe^{2+} which exhibited as enhanced FRAP. Conclusively, the increment in the FRAP of CPK, CPD, PP and SB after fermentation with *R. oligosporus* was ascribed to the enrichment of the phenolics as observed in present study.

Metal chelating activity

Fe^{2+} and Cu^{2+} are the major metal ions, which initiate the radical chain reactions and catalyze the generation of toxic radicals. Chelating agents can complex the transition metal ions to stabilize them and prevent the body from oxidative damage (*37*). As demonstrated in **Fig. 1e**, fermentation produced significant ($p<0.05$) improvement in the metal chelating activity of all legumes with the highest elevation of 85 % in CPK followed by PP (45 %), SB (44 %) and CPD (37 %). The results are in agreement with the findings of Li *et al.* (*38*) who reported 329 % higher metal chelating activity in whole soybean flour fermented with *Lactobacillus casei*. It was suggested that, the increment in the phenolic content during fermentation was responsible for strong chelating abilities. Similarly, Xiao *et al.* (*35*) demonstrated the positive impact of fermentation on the chelating activity of legumes. However, the inconsistency between the chelating abilities and corresponding phenolic contents was reported. Similar was observed in present study, for instance, PP showed the second highest enhanced metal chelating activity after fermentation whereas lowest improved TPC was recorded in same extract. It seems that certain other compounds (such as tocopherols or ascorbic acid) might contribute to the metal chelation (*35*).

Antioxidant Potency Composite Index (APCI) of unfermented and fermented legumes

Since five methods with varying principles have been employed for the determination of antioxidant activities, the estimation of APCI values (**Table 2**) from the indices of the different assays is necessary. The antioxidant potency of all the legumes enhanced with fermentation. FPP was found to possess highest antioxidant potency (99) and even UPP exhibited superior potency (89) thereby showing the excellent antioxidant potential of this legume. In FCPK, FSB and FCPD, the antioxidant potency lies in the range of 42–54 which is approximately 2 fold higher than UCPK, USB and UCPD.

Effect of fermentation on the phenolic composition

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Eight phenolic compounds were quantified using HPLC to elucidate the effect of fermentation (**Table 3**). The contents of *p*-hydroxybenzoic acid and chlorogenic acid in CPK, CPD and SB increased with fermentation while in PP, *p*-hydroxybenzoic acid increased but chlorogenic acid was not detected even upon its fermentation. Also, higher quantities of caffeic acid in FCPK and FPP, ferulic acid in FCPK and FSB, gallic acid in FCPD and FPP, syringic acid in FCPD, FPP and FSB and, vanillic acid in FCPK and FCPD were observed. However, contents of few compounds reduced with fermentation, for instance, cinnamic and syringic acids in CPK, caffeic, cinnamic and ferulic acids in CPD, cinnamic and ferulic acids in PP while reducing trend was not observed for any compound in SB. Xiao *et al.* (*10*) and Saharan *et al.* (*39*) have demonstrated the ability of fermentation to enhance the concentration of phenolic compounds and corresponding antioxidant properties. Xiao *et al.* (*30*) reported the higher contents of gallic acid, ferulic acid, chlorogenic acid, vanillin, caffeic acid and *p*-hydroxybenzoic acid in fermented oats. This was attributed to the activity of microbial enzmyes which liberated the complexed phenolic compounds. Further, the strong antioxidant activities in fermented oats was correlated to enhanced concentration of these phenolic compounds. Xiao *et al.* (*10*) discussed that the higher content of chlorogenic acid in fermented chickpeas was the major contributor to the strong antioxidant activities owing to its antioxidant potency. Conclusively, the higher antioxidant activities in FCPK, FCPD, FPP and FSB could be attributed to the enhanced concentration of specific phenolic compounds especially chlorogenic acid.

Effect of fermentation on the antinutrients

Phytic acid content of all legumes significantly ($p<0.05$) reduced after fermentation (**Fig. 2a**). SB showed the highest reduction of 55 % followed by CPK (27 %), PP (24 %) and CPD (24 %). Vong *et al.* (*32*) have demonstrated the ability of *R. oligosporus* fermentation to reduce the phytic acid in okara. Similarly, Ranjan *et al.* (*40*) reported 3.2 % lower phytate content in rice bran after fermentation with *R. oryzae*. These investigations have stated that, during moderate acidic environment of fermentation, the activity of endogenous phytase enzymes increased which hydrolyze the phytic acid present in the substrate. Thus, the lowered phytic acid recorded in FCPK, FCPD, FPP and FSB could be due to the enhanced activity of phytases during fermentation. Since, phytic acid can chelate divalent ions such as Fe^{2+}, Mg^{2+}, Zn^{2+}, and Ca^{2+} and make them unavailable (*40*), fermentation could be an effective approach to degrade phytic acid and liberate bound nutrients.

Saponins in FCPK, FCPD and FPP were found to be 40, 16 and 28 %, respectively, higher ($p<0.05$) than UCPK, UCPD and UPP (**Fig. 2b**). Xiao *et al.* (*10*) reported a similar result that saponins in chickpeas increased by 3 fold after fermentation with *Cordyceps militaris* SN-18 and attributed it to

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

the synthesis of saponins as defense mechanism during fermentation. Conversely, the saponin content of SB significantly (p<0.05) reduced (12 %) with fermentation. This is in agreement with the findings of Rui *et al.* (*41*), who stated that reduced saponin content in soybean fermented with *Lactobacillus* plantarum B1-6 was due to *β*-glucosidase produced during fermentation which hydrolyzed the saponin molecules to sapogenins and sugar side chains and reduced their solubility. Saponins are usually reported as antinutrients due to their hemolytic and membranolytic activities (*41*). However, they are also considered as bioactive components owing to their antioxidant properties (*10*).

Fermentation caused 8, 10 and 43 % increase (p<0.05) in the tannins of CPD, PP and SB respectively, while in CPK, the values reduced (p<0.05) by 16 % (**Fig. 2c**). In a similar investigation, Osman (*42*) observed 350 % higher tannin content in fermented pearl millet. This was attributed to the enzymatic hydrolysis of condensed tannins. Conversely, Egounlety *et al.* (*43*) and James *et al.* (*44*) have reported reducing effect of fermentation on the tannin content of legumes. This could be due to degradation of tannins by polyphenol oxidase and/or tannase enzymes produced during fermentation (*44*).

Trypsin inhibitor activity in CPK, CPD, PP and SB increased by 18, 46, 25 and 11 % respectively, after fermentation with *R. oligosporus* (**Fig. 2d**). This is in agreement with the findings of Egounlety *et al.* (*43*) who reported increased trypsin inhibitor activity in *R. oligosporus* fermented soybean. Prinyawiwatkul *et al.* (*45*) stated that 35 % higher trypsin inhibitor activity in *R. microsporus* fermented cowpea was due to fungal protease enzymes which released the active trypsin inhibitors from heat-resistant, inactive bound form. During fermentation, the activity of fungal protease exposes the amino acid residues implying the solubilization of heat denatured trypsin inhibitors which complex with trypsin. However, complete inactivation of these released trypsin inhibitors can be achieved by adequately cooking the flour before consumption (*45*). Contrarily, some investigations have reported reduced level of trypsin inhibitors after fermentation attributing it to the activity of hydrolytic enzymes produced during fermentation (*40,41*)

Correlation analysis

Pearson's correlation coefficients among bioactive constituents, antioxidant activities and antinutrients of unfermented and fermented legumes was determined (**Table 4**). A strong positive correlation (p<0.05) was found between TFC and different antioxidant activities such as $DPPH^•$ (r=0.884), $ABTS^{+•}$ (r=0.867), reducing power (r=0.950), FRAP (r=0.860) and metal chelating activity (r=0.761). In comparison to TFC, TPC were moderately correlated with these antioxidant activities.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

The positive correlation between TPC, TFC and antioxidant activities demonstrated the contribution of phenolics in antioxidant potential of legumes. Besides, DPPH and $ABTS^{+\bullet}$ demonstrated a strong correlation (r=0.963, p<0.01). Except metal chelation, other antioxidant activities had strong correlation with each other. Antinutrients (phytic acid, saponins and tannins) are also considered as bioactive constituents however they were found to be poorly correlated with antioxidant activities in present study.

Effect of fermentation on the mineral composition and bioavailability

Nine minerals (P, Mg, Na, K, Cu, Mn, Ca, Fe, Zn) were quantified using ICP-OES. Fermentation significantly (p<0.05) enhanced the concentration of minerals in all the legumes **(Table 5**), which has also been observed by other investigators. For instance, Ali *et al.* (*46*) reported the higher contents of macro and micro minerals in soybean fermented with *Bacillus subtilis*. Asres *et al.* (*27*) discussed that, the higher mineral contents in fermented legumes was ascribed to the activity of microbial enzymes which released the minerals from chelated complex compounds. Dhull *et al.* (*47*) discussed that phytates are majorly responsible for impaired bioavailability of minerals and attributed enhanced concentration of minerals (Ca, Na, K, Cu, Fe, Zn) in *Aspergillus awamori* fermented lentil to degradation of phytates during fermentation.

Furthermore, the bioavailability of specific minerals *i.e.* Ca, Fe, and Zn, was assessed by calculating the phytate:mineral molar ratios (**Table 5**). In FCPK and FSB, the molar ratio of phytate to calcium was below the critical value (0.24) depicting good bioavailability of calcium. Similarly, the phyate:iron molar ratio was < 1 in FPP and FSB which indicates good bioavailability of iron. However, the phytate:calcium molar ratio in FCPD and FPP and phytate:iron molar ratio in FCPK and FCPD were above critical values which is an indicative of impaired mineral bioavailability. All the unfermented and fermented legumes had superior bioavailability of zinc as their phytate:zinc molar ratio was < 15. Overall, the molar ratios of phytate to minerals decreased with fermentation which signifies the improved bioavailability of minerals in fermented legumes. This is in agreement with the findings of Asres *et al.* (*27*) who have reported enhanced mineral bioavailability in fermented teff and wheat and attributed it to reduced levels of phytic acid after fermentation.

FTIR spectra of unfermented and fermented legumes

FTIR analysis was performed to identify the changes induced by fermentation in the structure of legumes using the vibrational pattern of various functional groups present. As shown in the figure (**Fig. 3**), identical compounds were present in unfermented and fermented legumes which is exhibited

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

by similar patterns of absorption bands. After fermentation, the band shifted to lower wavelengths for all the legumes along with reduction in the intensities. The amide I band (1600–1700 cm^{-1}) provides information on the protein secondary structures wherein *β*-sheet structure, random coil, α-helix structure and *β*-turn structures are observed in the ranges 1615–1638, 1638–1645, 1645–1662, and 1662–1682 cm^{-1} respectively (*48*). The changes in the carbohydrate zone (750–1200 cm^{-1} fingerprint region) and amide I zone after fermentation can be attributed to partial protein and starch depolymerization. An intense band around 3300 cm^{-1} was observed for both unfermented and fermented legumes which may be due to the stretching vibration of O-H, mainly in phenolic groups (*49*). Aromatic groups are inferred by the band around 1407 cm^{-1} (C-C stretch) and the peak near 798 cm^{-1}. Slight modification was recorded near these bands in fermented legumes since fermentation was responsible for changes in these functional groups especially aromatic and phenolic groups which include antinutritional factors, such as phytic acid, saponins and tannins.

CONCLUSIONS

Fermentation of some commonly consumed legumes; CPK, CPD, PP and SB with R*. oligosporus* showed significant changes in the nutritional and antinutritional composition. The total phenolic and flavonoid contents and antioxidant activities increased, and the phenolic composition improved upon fermentation. A strong positive correlation was observed between the flavonoid content and different antioxidant properties. Further, the phytic acid content of legumes reduced with fermentation which consequently enhanced the concentration and bioavailability of the minerals. However, tannins, saponins and trypsin inhibitors increased in most of the legumes. FTIR analysis displayed the changes in the functional groups of the legumes after fermentation. From this study, it was concluded that legume fermentation led to superior phenolic composition, enhanced antioxidant properties and higher mineral bioavailability. Thus, fermented legumes can be incorporated as functional ingredients in the development of healthy diets possessing the ability to counter the lifestyle-related oxidative stress, chronic disorders and micronutrient (mineral) deficiencies.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are thankful to the DST for providing facilities to carry out research under PURSE project – Phase II “Addressing food security through nationally enriched improved cultivars and technologies

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

for swasth Bharat". The authors also gratefully acknowledge Centre for Food Research & Analysis (CFRA), NIFTEM, Sonipat, HR, India, for phenolic acid quantification by HPLC.

AUTHORS' CONTRIBUTION

Barinderjeet Singh Toor carried out processing, experimental work, interpretation of results and manuscript writing. Amarjeet Kaur participated in designing of work, interpretation of results, manuscript editing. Param Pal Sahota contributed to designing and interpretation of results. Jaspreet Kaur was involved in interpretation of results and manuscript editing.

ORCID ID

B.S. Toor https://orcid.org/0000-0002-2381-2744

A. Kaur https://orcid.org/0000-0002-3721-3582

P.P. Sahota https://orcid.org/0000-0002-3214-336X

J. Kaur https://orcid.org/0000-0003-1866-5214

REFERENCES

1. Jeong D, Han JA, Liu Q, Chung HJ. Effect of processing, storage, and modification on in vitro starch digestion characteristics of food legumes: A review. Food Hydrocoll. 2019;90:367-76. https://doi.org/10.1016/j.foodhyd.2018.12.039
2. Zafar TA, Aldughpassi A, Al-Mussallam A, Al-Othman A. Microstructure of whole wheat versus white flour and wheat-chickpea flour blends and dough: impact on the glycemic response of pan bread. Int J Food Sci. 2020. https://doi.org/10.1155/2020/8834960
3. De Pasquale I, Pontonio E, Gobbetti M, Rizzello CG. Nutritional and functional effects of the lactic acid bacteria fermentation on gelatinized legume flours. Int J Food Microbiol. 2020;316:108426. https://doi.org/10.1016/j.ijfoodmicro.2019.108426
4. Yadav N, Kaur D, Malaviya R, Singh M, Fatima M, Singh L. Effect of thermal and non-thermal processing on antioxidant potential of cowpea seeds. Int J Food Prop. 2018;21(1):437-51. https://doi.org/10.1080/10942912.2018.1431659
5. Maleki S, Razavi SH. Pulses' germination and fermentation: Two bioprocessing against hypertension by releasing ACE inhibitory peptides. Critic Rev Food Sci Nutr. 2020:1-8. https://doi.org/10.1080/10408398.2020.1789551

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

6. Chinma CE, Azeez SO, Sulayman HT, Alhassan K, Alozie SN, Gbadamosi HD, *et al*. Evaluation of fermented African yam bean flour composition and influence of substitution levels on properties of wheat bread. J Food Sci. 2020;85(12):4281-9.
https://doi.org/10.1111/1750-3841.15527
7. Cantabrana I, Perise R, Hernández I. Uses of *Rhizopus oryzae* in the kitchen. Int J Gastron Food Sci. 2015;2(2):103-11.
https://doi.org/10.1016/j.ijgfs.2015.01.001
8. Ibarruri J, Cebrián M, Hernández I. Solid state fermentation of brewer's spent grain using Rhizopus sp. to enhance nutritional value. Waste Biomass Valorization. 2019;10(12):3687-700.
https://doi.org/10.1007/s12649-019-00654-5
9. Collados A, Conversa V, Fombellida M, Rozas S, Kim JH, Arboleya JC, *et al*. Applying food enzymes in the kitchen. Int J Gastron Food Sci. 2020:100212.
https://doi.org/10.1016/j.ijgfs.2020.100212
10. Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, *et al*. Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with *Cordyceps militaris* SN-18. J Funct Foods. 2014;10:210-22.
https://doi.org/10.1016/j.jff.2014.06.008
11. Reyes-Moreno C, Cuevas-Rodríguez EO, Milán-Carrillo J, Cárdenas-Valenzuela OG, Barrón-Hoyos J. Solid state fermentation process for producing chickpea (*Cicer arietinum* L) tempeh flour. Physicochemical and nutritional characteristics of the product. J Sci Food Agric. 2004;84(3):271-8.
https://doi.org/10.1002/jsfa.1637
12. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method Enzymol. 1999;299:152-78.
https://doi.org/10.1016/S0076-6879(99)99017-1
13. Dini I, Tenore GC, Dini A. Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter Chenopodium quinoa seeds. LWT-Food Sci Technol. 2010;43(3):447-51.
https://doi.org/10.1016/j.lwt.2009.09.010

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

14. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28(1):25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

15. Yilmaz-Ersan L, Ozcan T, Akpinar-Bayizit A, Sahin S. Comparison of antioxidant capacity of cow and ewe milk kefirs. J Dairy Sci. 2018;101(5):3788-98. https://doi.org/10.3168/jds.2017-13871

16. Oyaizu M. Studies on products of browning reactions, antioxidant activities of products of browning reaction prepared from glucose amine. Jap J Nutr.1986;44(6):307-15. https://doi.org/10.5264/eiyogakuzashi.44.307

17. Tomasina F, Carabio C, Celano L, Thomson L. Analysis of two methods to evaluate antioxidants. Biochem Mol Biol Educ. 2012;40(4):266-70. https://doi.org/10.1002/bmb.20617

18. Chew YL, Goh JK, Lim YY. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem. 2009;116(1):13-8. https://doi.org/10.1016/j.foodchem.2009.01.091

19. Todorovic V, Redovnikovic IR, Todorovic Z, Jankovic G, Dodevska M, Sobajic S. Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. J Food Compos Anal. 2015;41:137-43. https://doi.org/10.1016/j.jfca.2015.01.018

20. Haug W, Lantzsch HJ. Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric. 1983;34(12):1423-6. https://doi.org/10.1002/jsfa.2740341217

21. Fenwick DE, Oakenfull D. Saponin content of food plants and some prepared foods. J Sci Food Agric. 1983;34(2):186-91. https://doi.org/10.1002/jsfa.2740340212

22. Baccou JC, Lambert F, Sauvaire Y. Spectrophotometric method for the determination of total steroidal sapogenin. Analyst. 1977;102(1215):458-65. https://doi.org/10.1039/an9770200458

23. AOAC Official Method 952.03, In Section 26.1.37, 8th, AOAC International, Gaithersburg, 2005.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

24. Hajela N, Pande AH, Sharma S, Rao DN, Hajela K. Studies on a doubleheaded protease inhibitor from *Phaseolus mungo*. J Plant Biochem Biotechnol. 1999;8(1):57-60. https://doi.org/10.1007/bf03263059

25. Nath AK, Chadha K, Sharma P, Deepika R. Purification and characterization of a novel inhibitor from *Poinciana pulcherrima* seeds with activity towards pest digestive enzymes. Funct Plant Sci Biotechnol. 2012;6(1):75-81.

26. Özcan MM, Dursun N, Juhaimi FA. Macro-and microelement contents of some legume seeds. Environ monito assess. 2013;185(11):9295-8. https://doi.org/10.1007/s10661-013-3252-x

27. Asres DT, Nana A, Nega G. Complementary feeding and effect of spontaneous fermentation on anti-nutritional factors of selected cereal-based complementary foods. BMC Pediatr. 2018;18(1):1-9. https://doi.org/10.1186/s12887-018-1369-3

28. Alihosseini N, Moahboob SA, Farrin N, Mobasseri M, Taghizadeh A, Ostadrahimi AR. Effect of probiotic fermented milk (kefir) on serum level of insulin and homocysteine in type 2 diabetes patients. Acta Endocrinol-Buch. 2017;13(4):431-6. https://doi.org/10.4183/aeb.2017.431

29. Lee BH, Lai YS, Wu SC. Antioxidation, angiotensin converting enzyme inhibition activity, nattokinase, and antihypertension of *Bacillus subtilis* (natto)-fermented pigeon pea. J Food Drug Anal. 2015;23(4):750-7. https://doi.org/10.1016/j.jfda.2015.06.008

30. Xiao Y, Rui X, Xing G, Wu H, Li W, Chen X, et al. Solid state fermentation with *Cordyceps militaris* SN-18 enhanced antioxidant capacity and DNA damage protective effect of oats (*Avena sativa* L.). J Funct Foods. 2015;16:58-73. https://doi.org/10.1016/j.jff.2015.04.032

31. Juan MY, Chou CC. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with *Bacillus subtilis* BCRC 14715. Food Microbiol. 2010;27(5):586-91. https://doi.org/10.1016/j.fm.2009.11.002

32. Vong WC, Hua XY, Liu SQ. Solid-state fermentation with *Rhizopus oligosporus* and *Yarrowia lipolytica* improved nutritional and flavour properties of okara. LWT. 2018;90:316-22.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

https://doi.org/10.1016/j.lwt.2017.12.050

33. Adetuyi FO, Ibrahim TA. Effect of fermentation time on the phenolic, flavonoid and vitamin C contents and antioxidant activities of okra (*Abelmoschus esculentus*) seeds. Nigerian Food J. 2014;32(2):128-37.
https://doi.org/10.1016/s0189-7241(15)30128-4

34. Ehsan K, Ehsan O, Rudi H, Jaafar HZ. Solid state fermentation effects on pistachio hulls antioxidant activities. King Khalid Univ. Res. J. 2010;15:260-6.

35. Xiao Y, Zhang Q, Miao J, Rui X, Li T, Dong M. Antioxidant activity and DNA damage protection of mung beans processed by solid state fermentation with *Cordyceps militaris* SN-18. Innov Food Sci Emerg Technol. 2015;31:216-25.
https://doi.org/10.1016/j.ifset.2015.06.006

36. Razak DLA, Rashid NYA, Jamaluddin A, Sharifudin SA, Long K. Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with *Rhizopus oligosporus* and *Monascus purpureus*. Biocatal Agric Biotechnol. 2015;4(1):33-8.
https://doi.org/10.1016/j.bcab.2014.11.003

37. Zhang Z, Lv G, Pan H, Fan L, Soccol CR, Pandey A. Production of powerful antioxidant supplements via solid-state fermentation of wheat (*Triticum aestivum* Linn.) by *Cordyceps militaris*. Food Technol Biotechnol. 2012;50(1):32-9.

38. Li S, Jin Z, Hu D, Yang W, Yan Y, Nie X, *et al.* Effect of solid-state fermentation with *Lactobacillus casei* on the nutritional value, isoflavones, phenolic acids and antioxidant activity of whole soybean flour. LWT-Food Sci Technol. 2020;125:109264.
https://doi.org/10.1016/j.lwt.2020.109264

39. Saharan P, Sadh PK, Duhan S, Duhan JS. Bio-enrichment of phenolic, flavonoids content and antioxidant activity of commonly used pulses by solid-state fermentation. J Food Meas Charact. 2020;14(3):1497-1510.
https://doi.org/10.1007/s11694-020-00399-z

40. Ranjan A, Sahu NP, Deo AD, Kumar S. Solid state fermentation of de-oiled rice bran: Effect on in vitro protein digestibility, fatty acid profile and anti-nutritional factors. Food Res Int. 2019;119:1-5.
https://doi.org/10.1016/j.foodres.2019.01.054

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

41. Rui X, Wang M, Zhang Y, Chen X, Li L, Liu Y, *et al*. Optimization of soy solid-state fermentation with selected lactic acid bacteria and the effect on the anti-nutritional components. J Food Process Preserv. 2017;41(6):e13290.
https://doi.org/10.1111/jfpp.13290

42. Osman MA. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. J Saudi Soc Agric Sci. 2011;10(1):1-6.
https://doi.org/10.1016/j.jssas.2010.06.001

43. Egounlety M, Aworh OC. Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). J Food Eng. 2003;56:249-54.
https://doi.org/10.1016/s0260-8774(02)00262-5

44. James S, Nwabueze TU, Ndife J, Onwuka GI, Usman MA. Influence of fermentation and germination on some bioactive components of selected lesser legumes indigenous to Nigeria. J Agric Food Res. 2020;2:100086.
https://doi.org/10.1016/j.jafr.2020.100086

45. Prinyawiwatkul W, Eitenmiller RR, Beuchat LR, McWatters KH, Phillips RD. Cowpea flour vitamins and trypsin inhibitor affected by treatment and fermentation with *Rhizopus microsporus*. J Food Sci. 1996;61(5):1039-42.
https://doi.org/10.1111/j.1365-2621.1996.tb10928.x

46. Ali MW, Shahzad R, Bilal S, Adhikari B, Kim ID, Lee JD, *et al*. Comparison of antioxidants potential, metabolites, and nutritional profiles of Korean fermented soybean (*Cheonggukjang*) with *Bacillus subtilis* KCTC 13241. J Food Sci Technol. 2018;55(8):2871-80.
https://doi.org/10.1007/s13197-018-3202-2

47. Dhull SB, Punia S, Kidwai MK, Kaur M, Chawla P, Purewal SS, *et al*. Solid-state fermentation of lentil (*Lens culinaris* L.) with *Aspergillus awamori*: Effect on phenolic compounds, mineral content, and their bioavailability. Legum Sci. 2020;2(3):e37.
https://doi.org/10.1002/leg3.37

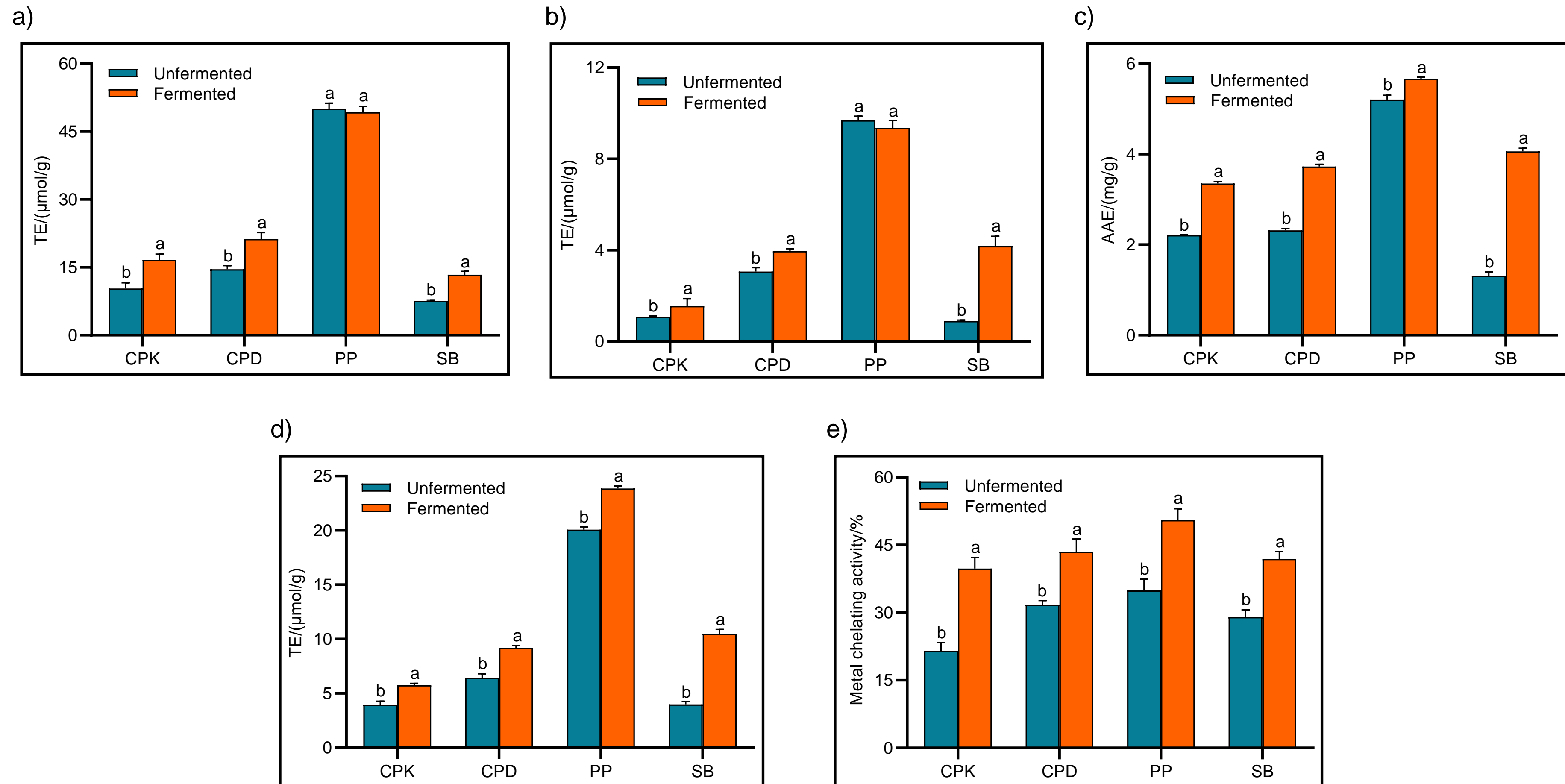
48. Hou F, Ding W, Qu W, Oladejo AO, Xiong F, Zhang W, *et al*. Alkali solution extraction of rice residue protein isolates: Influence of alkali concentration on protein functional, structural properties and lysinoalanine formation. Food Chem. 2017;218:207-15.

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

https://doi.org/10.1016/j.foodchem.2016.09.064

49. Sivam AS, Sun-Waterhouse D, Perera CO, Waterhouse GI. Application of FT-IR and Raman spectroscopy for the study of biopolymers in breads fortified with fibre and polyphenols. Food Res Int. 2013;50(2):574-85.
https://doi.org/10.1016/j.foodres.2011.03.039

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

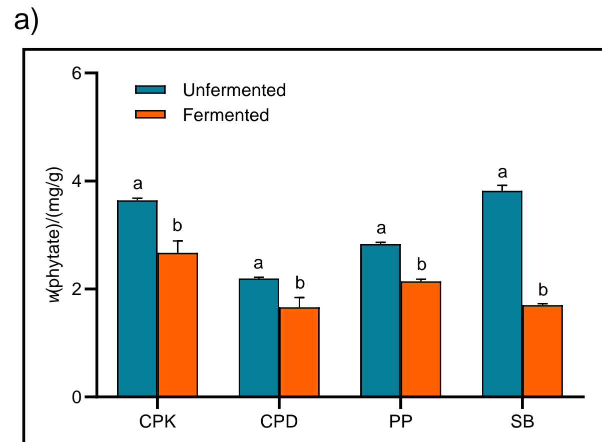


Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

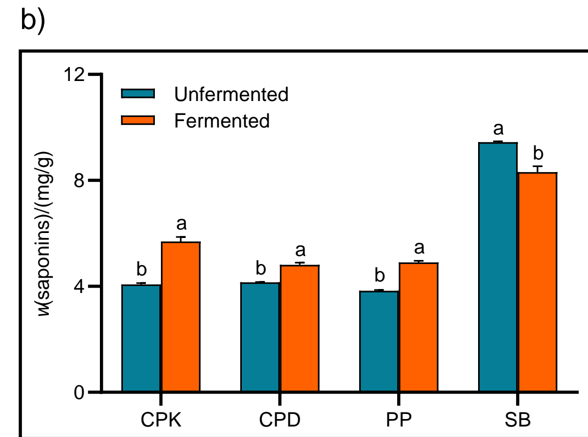
Fig. 1. DPPH radical scavenging activity (a), ABTS radical cation scavenging activity (b), Reducing power (c), Ferric reducing antioxidant power (d), and Metal chelating activity (e). Error bars represent the standard deviation of means of three replicates. Different letters (a, b) on bars indicate significant difference ($p<0.05$) between unfermented and fermented legume. CPK, chickpea *kabuli*; CPD, chickpea *desi*; PP, pigeon pea; SB, soybean

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

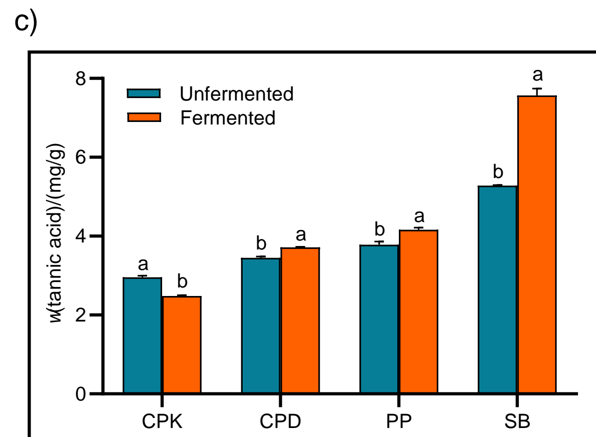
a)



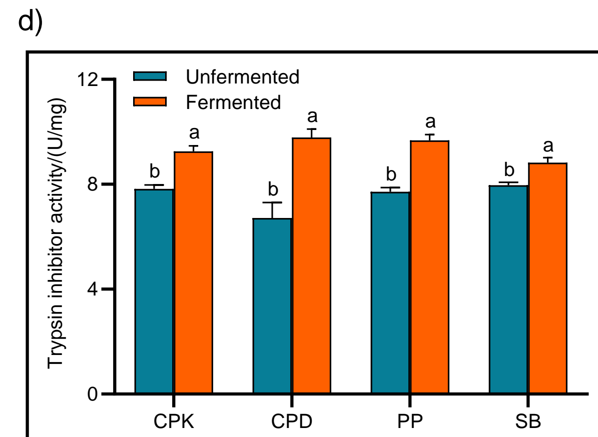
b)



c)



d)



Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Fig. 2. Phytic acid (a), Saponins (b), Tannins (c), Trypsin inhibitor activity (d). Error bars represent the standard deviation of means of three replicates. Different letters (a, b) on bars indicate significant difference ($p<0.05$) between unfermented and fermented legume. CPK, chickpea *kabuli*; CPD, chickpea *desi*; PP, pigeon pea; SB, soybean

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

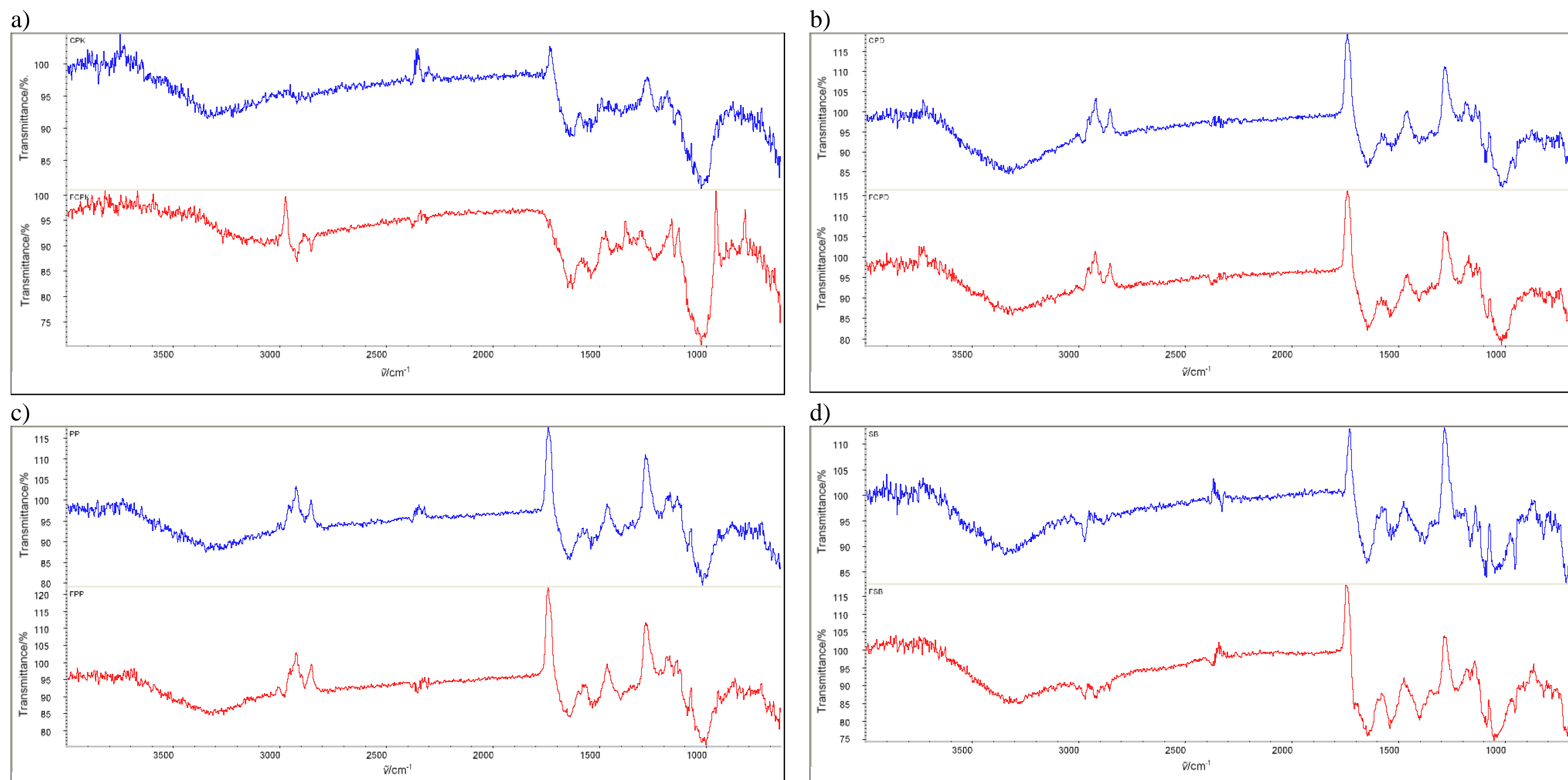


Fig. 3. FTIR analysis of unfermented ( ) and fermented ( ) legumes. Chickpea *kabuli* (a), Chickpea *desi* (b), Pigeon pea (c), Soybean (d)

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 1. Total phenolic and flavonoid content of unfermented and fermented legumes

	Chickpea *kabuli*		Chickpea *desi*		Pigeon pea		Soybean	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
w(TPC as GAE)/(mg/g)	$(1.05\pm0.02)^{b}$	$(1.61\pm0.10)^{a}$	$(1.01\pm0.03)^{b}$	$(1.24\pm0.03)^{a}$	$(3.24\pm0.04)^{b}$	$(3.64\pm0.04)^{a}$	$(0.87\pm0.06)^{b}$	$(5.07\pm0.06)^{a}$
w(TFC as QE)/(mg/g)	$(13.02\pm0.90)^{b}$	$(24.17\pm0.47)^{a}$	$(16.93\pm0.88)^{b}$	$(27.08\pm0.92)^{a}$	$(33.06\pm0.88)^{a}$	$(32.01\pm0.37)^{a}$	$(11.39\pm0.76)^{b}$	$(21.66\pm0.75)^{a}$

Mean ± standard deviation of three replicates. Means with different superscripts (a, b) depict significant difference ($p<0.05$) between unfermented and fermented legume. TPC, total phenolic content; TFC, total flavonoid content

Table 2. Antioxidant Potency Composite Index of unfermented and fermented legumes

	Chickpea *kabuli*		Chickpea *desi*		Pigeon pea		Soybean	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
DPPH index	20.68	33.30	29.18	42.55	100	98.55	15.16	26.74
ABTS index	11.04	15.99	31.64	40.90	100	96.57	9.18	43.18
RP index	39.13	59.22	40.95	65.85	91.93	100	23.15	71.73
FRAP index	16.54	24.09	26.98	38.55	84.07	100	16.70	43.91
MCA index	42.55	78.72	62.77	86.17	69.15	100	57.45	82.98
APCI	25.99	42.26	38.30	54.81	89.03	99.02	24.33	53.71

DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); RP, reducing power; FRAP, ferric reducing antioxidant power; MCA, metal chelating activity; APCI, antioxidant potency composite index

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 3. Phenolic composition of unfermented and fermented legumes

	Chickpea *kabuli*		Chickpea *desi*		Pigeon pea		Soybean	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
	w/(mg/100g)							
Caffeic acid	ND	4.0	0.7	ND	0.3	0.4	ND	ND
Cinnamic acid	10.3	4.5	27.5	ND	3.6	ND	ND	ND
Chlorogenic acid	ND	1.0	0.8	56.0	ND	ND	ND	109.9
Ferulic acid	18.8	21.1	5.7	5.4	2.2	1.1	0.8	11.3
Gallic acid	ND	ND	ND	20.6	4.7	33.7	ND	ND
Syringic acid	1.4	ND	5.3	9.5	12.8	30.1	10.1	86.7
Vanillic acid	ND	1.4	35.6	88.8	ND	ND	ND	ND
p-hydroxybenzoic acid	2.6	158.7	2.6	61.5	1.3	28.8	26.6	46.2

ND, not detected

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 4. Correlation among bioactive constituents, antioxidant activities and antinutrients of unfermented and fermented legumes

	TPC	TFC	DPPH	ABTS	RP	FRAP	MCA	Phytic acid	Saponins	Tannins
TPC	1.000	0.547	0.476	0.631	0.738*	0.657	0.578	-0.498	0.175	0.657
TFC		1.000	0.884**	0.867**	0.950**	0.860**	0.761*	-0.564	-0.425	-0.096
DPPH			1.000	0.963**	0.883**	0.960**	0.536	-0.251	-0.477	-0.145
ABTS				1.000	0.908**	0.981**	0.578	-0.392	-0.383	0.084
RP					1.000	0.923**	0.756*	-0.563	-0.359	0.080
FRAP						1.000	0.648	-0.374	-0.315	0.099
MCA							1.000	-0.774*	0.026	0.228
Phytic acid								1.000	0.157	-0.268
Saponins									1.000	0.735*
Tannins										1.000

* Significant at $p<0.05$

** Significant at $p<0.01$

TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); RP, reducing power; FRAP, ferric reducing antioxidant power; MCA, metal chelating activity

Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Table 5. Mineral composition and their bioavailability in unfermented and fermented legumes

	Chickpea *kabuli*		Chickpea *desi*		Pigeon pea		Soybean	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
	w/(mg/100 g)							
Phosphorous	$(111.27±7.99)^b$	$(278.54±9.23)^a$	$(83.03±7.69)^a$	$(86.41±6.29)^a$	$(74.84±5.49)^b$	$(115.89±8.39)^a$	$(196.21±9.94)^b$	$(299.13±10.08)^a$
Magnesium	$(98.87±4.39)^b$	$(126.35±5.36)^a$	$(67.04±5.65)^a$	$(68.51±4.85)^a$	$(45.35±3.90)^b$	$(93.79±7.67)^a$	$(76.29±5.49)^b$	$(120.42±7.66)^a$
Sodium	$(18.09±1.73)^b$	$(25.99±2.4)^a$	$(20.91±2.13)^b$	$(26.57±.2.63)^a$	$(7.63±0.68)^b$	$(16.571±1.54)^a$	$(15.23±1.43)^b$	$(26.42±2.08)^a$
Potassium	$(431.70±21.73)^b$	$(661.95±29.03)^a$	$(214.75±16.53)^a$	$(213.73±.15.75)^a$	$(757.44±20.08)^b$	$(980.99±26.42)^a$	$(335.19±11.73)^b$	$(589.61±15.20)^a$
Copper	$(0.78±0.01)^b$	$(1.53±0.08)^a$	$(0.68±0.05)^b$	$(0.93±0.09)^a$	$(2.16±0.19)^b$	$(4.70±0.53)^a$	$(0.88±0.09)^b$	$(1.89±0.11)^a$
Manganese	$(8.30±0.79)^b$	$(20.69±2.13)^a$	$(5.79±0.36)^a$	$(6.81±0.51)^a$	$(4.69±0.51)^b$	$(7.11±0.69)^a$	$(1.54±0.12)^b$	$(3.06±0.46)^a$
Calcium	$(40.40±2.99)^b$	$(81.55±4.07)^a$	$(21.22±1.98)^a$	$(21.31±2.16)^a$	$(17.09±1.93)^b$	$(44.35±3.70)^a$	$(75.03±5.60)^b$	$(117.61±7.69)^a$
Iron	$(5.06±0.45)^b$	$(6.29±0.79)^a$	$(4.66±0.32)^b$	$(5.86±0.63)^a$	$(11.94±1.07)^b$	$(21.15±1.98)^a$	$(16.30±1.03)^b$	$(31.12±2.25)^a$
Zinc	$(2.93±0.19)^b$	$(4.19±0.36)^a$	$(5.19±0.49)^a$	$(5.15±0.36)^a$	$(3.23±0.29)^b$	$(6.89±0.60)^a$	$(2.75±0.3)^b$	$(4.43±0.56)^a$
Phytate/Calcium	0.55	0.20	0.63	0.47	1.01	0.29	0.31	0.09
Phytate/Iron	6.09	3.59	3.99	2.40	2.01	0.86	1.98	0.46
Phytate/Zinc	12.32	6.31	4.19	3.19	8.69	3.08	13.76	3.80

Mean ± standard deviation of three replicates. Means with different superscripts (a, b) depict significant difference ($p<0.05$) between unfermented and fermented legume