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Fermentation performance and nutritional assessment of physically processed lentil and green pea flour

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Abstract

BACKGROUND

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RESULTS

Total phenolic contents were significantly ($p<0.05$) reduced for modified and fermented substrates compared to non-fermented controls. Trypsin inhibitory activity (TIA) was reduced significantly for all substrates except for unsonicated soybean and lentil fermented with *L. plantarum* and *P. acidilactici*. When physical processing was done, there was a decrease in TIA for all the substrate. Phytic acid content decreased for physically modified soybean and lentil but not significantly for green pea. Even though there was a decrease in ANFs, there was no significant change in in vitro protein digestibility for all substrates except for unsonicated *L. plantarum* fermented soybean flour and precooked *L. plantarum* fermented lentil. Similarly, there was change in amino acid content when physically modified and fermented.

CONCLUSION

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Keywords

Fermentation, high-power sonication, anti-nutritional factors (ANFs), legume proteins, protein digestibility

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Fermentation performance and nutritional assessment of physically processed lentil and green pea flour

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1. Introduction

Legumes are plants in the Leguminosae family that includes beans, peas, lentils, chickpea, and soybean, and are grown worldwide. Around 73 million metric tons (MMT) of pulses are produced globally with dry beans (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), lentil (*Len culinaris*), and dry pea (*Pisum sativum*) accounting for about 52 MMT [(1)]. There has been growing interest in the use of whole pulse, pulse flour, protein, starch, dietary fibers, and bioactive compounds for food and non-food applications. Cheaper pulse proteins and their derivatives can be substituted for animal-based protein and other essential nutritional components [(2)]. Even though pulses have higher amount of proteins, dietary fiber, minerals, and vitamins in them, their use in food products is still not prevalent for the presence of off-flavor and several anti-nutritional factors (ANFs), for example, tannins, trypsin inhibitors, phytic acid, and flatulence causing compounds, mainly, stachyose, raffinose, and verbascose. Phytic acid chelates essential dietary minerals, protein, and starch, which then reduces their bioavailability in human. Tannins and trypsin inhibitors inhibit the digestive enzymes, thus reducing the digestion and absorption of dietary proteins and carbohydrates [(3)].

Physical and biochemical processing favorably modify some physicochemical attributes of plant-based food ingredients, including pulses. Many traditional processes such as soaking at elevated temperatures, dehulling, boiling, germination, autoclaving, and microwave-assisted cooking are reported to impact the nutritional composition and anti-nutritional factors in pulses, for example, mung beans, white kidney beans, and cowpea [(4)]. Thermal treatment at high temperatures has the potential to improve and enrich the nutritional quality of legumes. Physical

treatments for example, roasting (180ºC for 20 min), microwaving (850 W for 3 min), and boiling in water (30 and 60 min) were compared for yellow soybeans and green cotyledons; trypsin inhibitors were reported to reduce by minimum 50% for all physical treatments, and phytic acid reduced by 28% and 31% when boiled for 30 and 60 min, respectively [5]. Hefnawy [6] utilized pressure cooking (121ºC for 35 min) and boiling (100ºC for 90 min) to reduce phytic acid (36-41%) tannins (29-36%), and trypsin inhibitors (93-94%) in lentils. ANFs like trypsin inhibitors, phytic acid, phenolics, and tannins are sensitive to heat and are reduced during processing. When exposed to thermal treatments, e.g., boiling or pressure cooking, ANFs in chickpea, dry beans, faba beans, dry peas, and lentils were reduced increasing their digestibility and enhancing the nutritional profile [1]

Fermentation is another simple and low-cost bioprocessing technology that has been used to enhance nutritional and quality aspects of food ingredients, reduce undesirable compounds and enrich with essential amino acids and vitamins [7]. Controlled fermentation with specific microorganism is preferred to enhance the nutritional profile, texture, color, appearance, flavor, shelf life, and protein digestibility of ingredients, including pulses [8]. Natural fermentation, on the other hand, relies on fermentation by naturally occurring microbes, which can be more than one, and possibly affecting uniformity in quality of ingredients and food products.

Fermentation of legumes by microbes e.g., bacteria, yeast, and fungi has been reported in the literature that results in enhanced nutritional profile. Fermentation of chickpea with *Rhizopus oligosporous* for 72 h increased the protein content by 21.7% [9]. Coda et al [10] showed that fermentation of faba bean flour with *Lactobacillus plantarum* reduced ANFs such as trypsin inhibitors and tannins by more than 40%. Similarly, fermentation of mucuna bean by *B. subtilis* reduced trypsin inhibitors and phenolic compounds [11]. Fermentation caused a 72% reduction in

mucuna bean total oligosaccharides like stachyose, raffinose, and verbascose that cause flatulence. These reductions may be attributed to the secretion of hydrolytic enzymes like α-galactosidase that is capable of hydrolyzing oligosaccharides and polysaccharides [3]. Fermentation is an important processing technology for food ingredients because it improves sensory qualities, reduces pathogenic microorganisms, and enhance functional and health beneficial effects of food [12]. Fermentation makes hydrolysis of proteins easier and improves protein digestibility. The pH is reduced during fermentation which plays a role in enhancing proteolytic activities and protein breakdown into smaller peptides that can be easily digested [13]. These bioactive peptides provides health benefits such as antihypertensive, antioxidant, and ACE (angiotensin I-converting enzymes) inhibitory activity [14,15]. Additionally, fermentation of leguminous substrate can bring in probiotic/ prebiotic benefits in foods. Prebiotic rich lentils ingredients consisting of raffinose family oligosaccharides, sugar alcohols, resistant starch, and fructo-oligosaccharides showed improved insulin sensitivity in men with metabolic syndrome, displaced pathogen from rumen and gastrointestinal tract, and enhanced viability of *lactobacilli* and *Bifidobacteria* [16]. Similarly, prebiotic benefits of fermented cowpea and black bean was due to the production of short-chain fatty acids suggesting improved intestinal health [17].

There has been limited research on the modification of ANFs in pulse flours through physical processes like heat treatment or high-power sonication followed by fermentation. High-power sonication (HPS) is a relatively newer application in food processing industry and is mostly used for its disruption of cell matrices at higher intensities that could potentially render fermentation of substrates effective and more beneficial. When HPS (low frequency 16-100 kHz and 10-1000 W/cm^2 power intensity) is applied to the aqueous medium, cavitation bubbles are formed and collapse leading to extreme temperatures that produce high shear and turbulence in localized

cavitation zones [18]. Cavitations disintegrate substrate cellular matrices and facilitates the solvent extraction of constituents like protein, and sugar from plant cells; the use of high-power sonication increased the sugar release of defatted soy flakes by 50% compared to untreated flakes [19], which can be utilized in fermentation by microbes to modify and improve substrate characteristics. Thus, the specific objectives of this study were to 1) compare fermentation performance for *L. plantarum* and *P. acidilactici* in physically modified (precooked or sonicated) lentil and pea flours, and 2) evaluate the impact of these processes (physical modification and fermentation) on the nutrition and anti-nutrition constituents of the flours. Even though legume flours were fermented with probiotic bacteria, study on probiotic and prebiotic effects of resulting flours *per se* was not the focus of this reporting.

2. Material and methods

2.1 Flours and reagents

Green pea seeds, lentil seeds, precooked lentil, and pea flours were provided by Dr. Donna Winham, Iowa State University (Ames, IA). Soy flour (80-90 PDI) was obtained from Archer Daniels Midland Company (Decatur, IL). De Man, Rogosa and Sharpe (MRS) media, ferric chloride hexahydrate, pancreatin, gallic acid, sulfosalicylic acid, polyvinyl-polypirrolidone (PVPP), and Folin Ciocalteu reagents were purchased from Fisher Scientific (Waltham, MA, USA). Benzoyl-DL,-arginine-p-nitoanalide hydrochloric (BAPA), and trypsin porcine pancreas were purchased from VWR (Chicago, IL). All the chemicals used were of analytical grade.

2.2 Preparation of initial substrates

Peas and lentils were processed into flours at North Dakota State University (Fargo, ND) in Dr. Clifford Hall's research laboratory as follows. First, whole pulses were soaked in water (10-parts water 1-part pulse) overnight at 25ºC. Second, pulses were drained over a 40-mesh sieve (Gilson

Inc., Lewis OH), with any material passing through the screen discarded. Then, pulses were placed on perforated baking pans in single layers (approximately 0.45 kg per tray). Heat treatment-149ºC for 18 min (lentil) or 33 min (peas) - was carried out in a Baxter OV300G Mini Rotating Rack Convection Oven (Baxter Manufacturing Co., Orting WA). The pulses were stirred at five-minute intervals until the end of their heating times. The precooked pulses were then milled with a roller mill; flour particle size distribution for lentil showed that 3.4% was retained on 80 mesh sieves, and 28.5% retained on 100 mesh sieves with 61.1% passing through. Similarly, for pea flour, 5.1% was retained on 80 mesh sieves, and 61.6% retained on 100 mesh sieves with 33.3% passing through. These processed pulses are labeled as 'precooked' pulse flours in this report.

Another set of whole peas and lentils were milled at Center for Crops Utilization Research Pilot Plant facility (Iowa State University, Ames, IA, USA) in a Witt corrugated roller mill (Witt Corrugating Inc., Wichita, KS), first by passing the beans through 0.03" gap rollers with 1/8" corrugation followed by passing through 0.02" gap rollers with 1/16" corrugation. Green pea and lentil were then ground by using a Nutri mill (Pleasant Hill Grain, NE, USA) operated at the 'fine' setting, that resulted in final particle size average $D_{(0.5)}$ of 190 and 163 µm, respectively. These pulse flours obtained are labeled as 'uncooked or raw' in this report. Each flour slurry (1:8 w/v substrate: water) of raw green peas, lentils, and soybean was sonicated for 2 and 4 min at 100% amplitude (power density~ 2.5 W/mL) using a 2.2 kW sonicator (Branson 2000 Series, Branson Ultrasonics Corporation, Danbury, CT, USA). For precooked substrates, the slurry was maintained at 1:8 w/v substrate: water and autoclaved. The schematic diagram of the process, sampling, and analytics is shown in Figure 1.

2.3 Microorganisms and fermentation

The bacterial strains *Lactobacillus plantarum* and *Pediococcus acidilactici* were provided by Lallemand Animal Nutrition-North America (Milwaukee, WI, USA); even though the strains are probiotic in nature [20] it was not our objective to evaluate probiotic impact of fermented ingredients. The microbes were stated to have a viable count of 2.5 x 10^{11} CFU per gram of dry product. Substrate slurries (precooked, sonicated, or control) were prepared with 1:8 w/v ratio (substrate: water), adjusted to pH 6.5, inoculated with *Lactobacillus plantarum* and *Pediococcus acidilactici* at 10^8 CFU/mL and fermented in shake flasks for 72 h at 37°C and 200 rpm. As suggested by the manufacturer, the powder bacterial strains were added directly into the pH-controlled slurry; each fermentation slurry was added 0.5 g of powder in 250 mL of pH-controlled water (pH-6.5) resulting in 5 x 10^8 CFU/ mL of microbes at the time of inoculation. The microbial cell growth and pH were measured at 6, 12, 24, 48, and 72 h. The microbial viable count was calculated by the serial dilution plate count method in a biosafety cabinet [21]. The microbial growth was compared based on the specific growth rate (SGR) parameter, μ, calculated by plotting the logarithm of cell count during the exponential phase against the time. The resulting plot was fitted with a linear equation (Eq. 1). The slope of this line is the specific growth rate of a microorganism, μ.

$$\text{Ln}(X) = \mu t + \text{Ln}(X_0) \quad (1)$$

where X is number of cells at a given time t during the log phase, X_0 is the initial number of cells at the beginning of the exponential phase. Doubling times for microbial growth (t_d) were calculated by dividing 0.693 by μ. All the fermentations were performed in duplicate and average values plotted/ analyzed.

2.4 Evaluation of substrates and modified flours

2.4.1 Proximate analyses

The proximate analyses of all legume samples (uncooked, precooked and fermented) was carried out using standard methods in the Plant Polymer Research Unit Lab (USDA-ARS, Peoria, IL). All the legume samples were dried flours. Moisture, crude protein (Dumas combustion % N × 6.25), crude oil, and crude fiber contents were analyzed according to AOCS standard methods Ba 2a-38, Ba 4e-93, Am 5-04, and Ba 6-05, respectively (AOCS, 1997)[22]. Ash contents were analyzed according to AOAC method 942.05 and carbohydrate content was calculated by difference (100 − % sum of other components).

2.4.2 Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay with slight modification [23]. Flour samples (0.5 g) were extracted with 7.5 mL 1% HCl in methanol for 2 h and centrifuged at 2000 x *g* and 25°C for 10 min. The supernatant extract (0.2 mL) was mixed with 0.6 mL of distilled water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1: 1 v/v reagent: distilled water). One milliliter of saturated sodium carbonate solution (8% w/v in water) was added after 5 min and the volume was made up to 3 mL with distilled water. They were stored in dark for 30 min and absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV 160). The phenolic content was calculated as gallic acid equivalents mg GAE g^{-1} of dry flour. All assay determinations were carried out in duplicate.

2.4.3 Trypsin inhibitor assay

Trypsin inhibitor assay (TIA) was carried out using a colorimetric assay with a UV-visible spectrophotometer (Shimadzu UV 160) with slight modification [24, 25]. Briefly, 0.25 g of raw/ fermented sample was placed in a 50-mL centrifuge tube and 25 mL of 0.01 M NaOH was added. Tubes were then vortexed for 1 min and stirred on a mechanical stirrer at 500 rpm for 3 h. The mixture was centrifuged (Thermo Sorvall legend XT, Thermo Fisher Scientific, MA, USA)

at 14000 x *g* for 10 min at 4°C. One mL of supernatant was used for TIA assay where 2 mL of BAPA and 0.5 mL of trypsin were also added and mixed. The reaction was stopped by adding 1 mL of acetic acid after 10 min. The absorbance of the reaction mix was measured at 410 nm in a spectrophotometer (Shimadzu UV 160). One trypsin inhibitory unit (TIU) was equivalent to an increase of 0.01 absorbance unit at 410 nm per 10 mL of reaction mixture compared to the blank sample that had a trypsin solution added after acetic acid. TIA was defined as the number of trypsin units inhibited per mg of dry flours.

2.4.4 In vitro protein digestibility of modified substrates

The in vitro protein digestibility (IVPD) was evaluated based on a method described by Akeson & Stahmann, (1964) [26] , with modifications (Almeida et a., 2015) [27]. Briefly, 0.25 g of each raw/ fermented flour or 250 mL of deionized water (for the blank) was suspended in 15 mL of 0.1 N HCl containing 1.5 mg/mL pepsin and incubated for 3 h at 37ºC in a water bath. The pepsin hydrolysis was neutralized with the addition of 7.5 mL of 0.5 N of NaOH. Then, the pancreatic digestion was started with the addition of 10 mL of 0.2 mol/L phosphate buffer (pH 8.0), containing 10 mg of pancreatin with 1 mL of 0.005 mol/L sodium azide and incubated at 37ºC overnight. After the pancreatic digestion, 1 mL of 10 g/100 mL of trichloroacetic acid (TCA) was added, followed by centrifugation at 503 x *g* for 20 min. The supernatant was collected, and the total protein content was estimated by BCA (Bicinchoninic acid) assay. The IVPD values were calculated according to the equation:

$$\% \text{ Digestibility} = (N_s - N_b)/Ns * 100$$

Where, N_s and N_b represent the nitrogen content in supernatants of the sample and the blank, respectively.

2.4.5 Phytic acid determination

Phytic acid was determined using the method of Gao et al. (2007) [28]. Samples of 500 mg fermented modified flours were mixed with 10 mL of 2.4% HCl, mixed for 16 h, and then centrifuged at 2000 x *g* and 10ºC for 20 min. The supernatants were transferred to 14 mL Falcon tubes containing 1 g NaCl, shaken at 350 rpm for 20 min to dissolve the salt, and were settled at 4°C for 60 min. The mixtures were centrifuged at 2000 x *g* and 10ºC for 20 min, and clear NaCl treated supernatants were collected for color development. This treatment precipitated matrix components that could interfere with the colorimetric reaction. The clear supernatant (1 mL) was diluted 25-fold by mixing with 24 mL of distilled water. Three milliliters of this diluted sample were combined with 1 mL of modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid), vortexed, and centrifuged at 2000 x *g* at 10°C for 10 min. A series of calibration standards containing 0, 0.224, 0.448, 0.896, and 1.12 µg/mL PA-P (phytic acid phosphorous) were prepared from phytic acid dodeca-sodium salt hydrate the phosphorous content of which was determined as 20.11%. The absorbance of color reaction products for both samples and standards were read at 500 nm and phytic acid was expressed as g kg^{-1} of flour.

2.4.6 Amino acid composition

Analyses of amino acids were performed using the EZ:Faast™ kit (Phenomenex, Torrance, CA, USA) [29]. Around 200 mg of each protein sample (control and modified) were taken, added to 100 µL of 0.2 mM norvaline internal standard, and dried in a Speed Vac concentrator (Savant SVC-100H, Farmingdale, NY, USA) overnight in pyrolyzed tubes. Acid hydrolysis was performed in the Pico-Tag workstation (Waters Corporation, Milford, MA, USA) following the protocols [30]. Twenty-five microliters of EDTA (1 µL of 20 % EDTA to 9 µL of water) were added to the hydrolyzed sample in the tube. Four hundred microliters of extraction buffer (water: chloroform: methanol; 3:5:12 v/v) were added to each of the tubes and supernatant was

transferred to GC-MS vial. The extraction was done one more time with 400 µL of extraction buffer and extracts were pooled. Three hundred fifty microliters of chloroform and 450 µL of distilled water were added; the mixture was vortexed and then allowed to settle until clear separation was seen. The upper water-methanol phase, which contains the amino acids, was transferred to a new tube and used in EZ: Faast^{TM} extraction kit. The amino acids were analyzed by following the protocols described in EZ: Faast^{TM} user's manual. The amino acid composition was determined by GC/MS (GC-MS Agilent 5937, Palo Alto, CA, USA) using internal standard Norvaline.

2.4.7 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein solutions extracted from the physically processed and fermented substrates were subjected to SDS-PAGE with slight modification [31]. Two types of gel, 13% resolution gel (Acryl-bisacrylamide) at the bottom, and 4% percent stacking gel at the top were prepared. The protein concentration of 1.5 mg/mL was prepared in sample buffer (15.1 g/L Tris, 300 g/L urea, 2 g/L SDS, 20 mL/L glycerol, and 0.1 g/L bromophenol blue) and incubated at 80°C for 5 min. The protein standard (6,500 – 66,000 Da, Product number M3913-SigmaMarker™) and physically processed/ fermented samples were loaded onto gel at equal volume (15 µL) and electrophoresed at a constant voltage of 200V for 50 min using standard SDS buffer (25mM Tris, 191 mM glycine and 1 g SDS per L). The gels were stained with Coomassie blue for 1 h and de-stained with methanol: acetic acid: deionized water in ratio 10:2:8 until the gels were clear and transparent.

2.4.8 Statistical analyses

The experimental design was a randomized complete block design (RCBD) with two replications. Statistical analyses were performed using the JMP® statistical methods (100 SAS

Campus Drive, Cary, NC). Two-way analysis of variance (ANOVA) and Tukey tests was performed to assess the effect of physical modification/fermentation. Treatment means were compared within each substrate. Results having different superscript letters within each substrate group show a significant difference ($p < 0.05$). Graphs were prepared using GraphPad Prism software (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1 Microorganism growth performance on modified substrates

The viable microbial population and change in pH during fermentation of modified substrates by *L. plantarum* and *P. acidilactici* are presented in Figures 2 and 3, respectively. The exponential growth for *L. plantarum* for all the precooked and raw substrates was observed between 6 and 24 h, except for precooked green pea flour for which it was 6-48 h. Similarly, the exponential growth of *L. plantarum* for sonicated substrates was observed between 6 and 48 h (Figure 2A). The pH was adjusted initially to 6.5 before fermentation as it was an optimal pH for microbial growth. The pH decreased significantly during the first 24 h fermentation for *L. plantarum* as the microbial population was the highest during this time (Figure 2B). After 24 h, the pH slightly increased to a pH range of 4.2- 4.8. For *P. acidilactici*, the exponential growth was observed between 6 and 24 h for all physically processed flours (Figure 3A). The pH decreased significantly during the first 24 h fermentation for *P. acidilactici*, as the microbial population was the highest during this time (Figure 3B). As the microorganism used were facultative hetero-fermentative, there is a production of lactic acid as well as acetic acid which reduces the pH.

The specific growth rates (μ), and population doubling times ($t_d = 0.693/\mu$) for *L. plantarum* and *P. acidilactici* for physically modified substrates are presented in Table 2. *L. plantarum* had the highest growth rates on 2- and 4-min sonicated soybean flours at 0.95 ± 0.03 h^{-1} and $0.76 \pm$

0.02 h^{-1} respectively, compared to unsonicated flour, followed by precooked green pea, precooked lentil, unsonicated lentil, and unsonicated green pea. Similarly, *P. acidilactici* had the highest growth rate of 0.78 ± 0.05 h^{-1}, 0.77 ± 0.03 h^{-1}, 0.77±0.03 h^{-1}, and 0.76±0.20 h^{-1} for 2 min sonicated lentil, precooked lentil, 4 min, and 2 min sonicated soybean flour, followed by 4 min sonicated lentil, respectively. Compared to *L. plantarum, P. acidilactici* had a lower population doubling time for most of the substrate, resulting in the highest growth rate.

3.2 Impact of fermentation on modified flours

3.2.1 Proximate composition of modified flours

The proximate composition of the initial substrate before fermentation is given in Table 1. Uncooked and precooked green peas had similar amounts of protein, ash, carbohydrates, moisture, and fat. Proteins and carbohydrates in physically modified/ fermented soybean were in the range of 570-580 g $kg^{-1}$and 310-330 g kg^{-1} dry flour, respectively, which are comparable to those reported by (Byanju et al., 2020) [32]. Proteins and carbohydrates in physically modified and fermented green peas were in the range of 210-260 g kg^{-1} and 650-700 g kg^{-1}, respectively, and are in close agreement with Millar et al. (2019) [33]. Similarly, physically modified and fermented lentil and precooked lentil had 260-290 g kg^{-1} protein and around 620-660 g kg^{-1} carbohydrate contents. The composition of lentil is comparable to the report by Han & Baik, (2008) [34]. When these pulses were sonicated and fermented, there were no significant changes in proximate content, except for the ash content of soybean flour.

3.2.2 In vitro protein digestibility

In vitro protein digestibility (IVPD) of physically processed fermented substrates is presented in Figure 4. The protein digestibility of physically processed substrates fermented with *L. plantarum* and *P. acidilactici* was generally above 85%. Also, the IVPD was higher for

precooked lentil and green pea flours, compared to raw counterparts, possibly due to high temperature which causes denaturation of proteins as well as inactivation of enzyme inhibitors and other anti-nutritional factors [35]. Similarly, the IVPD of raw green pea (89.12%), lentil (91.87%), and soybean (96.72%) from our research were higher than reported i.e. 82.60, 79, and 71.80%, respectively [36], [37], which might be due to different processing conditions and cultivar. It could also be due to the autoclaving (121ºC, 30 min) before fermentation. Autoclaving potentially leads to a decrease in anti-nutritional factors and exposes protein to greater denaturation and enzymatic hydrolysis [38]. The highest protein digestibility was seen in soybean, irrespective of the sonication and fermentation conditions. There was an increase in digestibility when unsonicated soybean flour was fermented with *L. plantarum* and *P. acidilactici.* When flours were sonicated for 2 min and 4 min, there was no significant change in protein digestibility of soybean flour. Also, there were no significant changes in protein digestibility for green pea and lentil when sonicated and fermented with *L. plantarum* and *P. acidilactici*. For green pea, there was a significant increase in IVPD when substrates were precooked and fermented (Figure 4, middle). Similarly, IVPD improved for lentil when it was precooked (Figure 4, bottom). Ogodo et al. (2018) [13] reported an increase in IVPD of soybean meal fermented with lactic acid bacteria (LAB) consortium from 85% to 93.5% which was due to the pH reduction, thus enhancing proteolytic enzyme activity and breaking proteins into small peptides.

3.2.3 Total phenolic content

The total phenolic contents for raw, modified, and fermented soybean, green pea, and lentil are given in Table 3. The highest phenolic content was observed in raw soybean flour (4.6 ± 0.22 mg GAE g^{-1}). After soybean flours were sonicated (2 and 4 min) and fermented with *L. plantarum*,

and *P. acidilactici*, there was a significant decrease in phenolic contents. Georgetti et al. (2009) [39] and Juan & Chou. (2010) [40] have reported the total phenolic contents of 15.4 mg GAE g^{-1} and 15.94 mg GAE g^{-1} for soybean flour, which is higher than what we obtained. The reduced phenolic content we observed is likely due to the thermal treatment before fermentation. Xu & Chang, (2008) [41] reported total phenolic content of pressure boiled (15 psi, 15 min) green pea (0.66 mg GAE g^{-1}) that is lower than our values (Table 3). Torino et al. (2013) [14] also reported the higher total phenolic content for lentil (32 mg GAE g^{-1}) compared to our results, i.e. 1.9 mg GAE g^{-1} . Green pea and lentil flours when sonicated and fermented also showed a significant decrease in phenolic contents irrespective of microorganisms used. The precooked green pea and lentil also showed the same decreasing trend when fermented. Chi & Cho. (2016) [42] also reported the decrease in total phenolic contents when soybean meal was fermented with *L. plantarum* and *L. acidophilus*, which was reportedly due to lower pH activity. The decrease in phenolic compounds during fermentation might also be due to lower pH (acidic environment), which results in abstraction of hydride and rearrangement of the structure of phenolic compounds [43], hence, unable to be detected by Folin-Ciocalteu reagents. This loss of phenolic compounds can be attributed to the chemical transformation, formation of protein-phenolic complex, and decomposition during thermal treatments [44].

3.2.4 Trypsin inhibitor activity

Trypsin inhibitors are proteins and are considered ANFs, as they hinder pancreatic protease activity and absorption of dietary proteins. TIA, expressed as the trypsin unit inhibited in a dry sample, was lower for most of the physically processed fermented soybean, green pea, and lentil flour (Figure 5). For unsonicated soybean, fermentation by *L. plantarum* did not significantly reduce TIA; on the other hand, TIA was reduced significantly by 49.8% and 52.7% when

sonicated for 2 min and 4 min, respectively, and fermented by *L. plantarum*. Similarly, fermentation alone of unsonicated soybean by *P. acidilactici* did not reduce TIA significantly ($p > 0.05$) but reduced the value by 34.5% and 46.7% when sonicated for 2 min and 4 min, respectively.

For unsonicated green peas, fermentation with *L. plantarum* significantly reduced TIA by 47.3% compared to the raw sample, while sonication pretreatment for 2- and 4-min. reduced TIA by 48.1 and 48.9% when fermented by *L. plantarum*. Similarly, fermentation of unsonicated green pea by *P. acidilactici* reduced TIA significantly by 46.9%, and the combination with sonication for 2 min and 4 min reduced TIA further by 48.9% and 46.9%, respectively. The reduction of TIA for precooked green pea was 78% when compared to its raw counterpart. Çabuk et al. [(25)] also reported the decrease in TIA when pea protein concentrate was fermented by *L. plantarum*. This reduction of TIA was due to heat treatment as well as fermentation, which degrades or modifies trypsin inhibitors resulting in losing its activity to bind to trypsin [(45)]. The highest reduction, 83%, was seen in precooked green pea followed by fermentation with *L. plantarum* or *P. acidilactici*.

For unsonicated lentil, fermentation with *L. plantarum* and *P. acidilactici* did not significantly reduce the TIA. When lentil was sonicated for 2 min or 4 min, and then fermented by *L. plantarum*, the TIA was reduced significantly by 21.9 and 24.4%, respectively, compared to raw lentil. Also, TIA was reduced by 21.4 and 27.6% when sonicated for 2 and 4 min and fermented by *P. acidilactici*. Precooked lentil followed by fermentation using *L. plantarum* and *P. acidilactici* showed the highest reductions, i.e. 80.6 and 91.6%, respectively. Physical processing and subsequent fermentation by these probiotic microbes reduced the trypsin inhibitor activity and enhanced the nutritional profiles of these substrates.

3.2.5 Phytic acid

Table 4 shows the phytic acid (PA) content of raw, physically processed, and fermented substrates. The phytic acid content of soybean flour was the highest among all substrates, particularly with raw flour at 0.41 g kg^{-1}. Ojokoh & Yimin. (2011) [46] and Shi et al. (2018) [47] reported phytic acid content of around 2.75 g kg^{-1} to 22.9 g kg^{-1} for soybean meal, which is higher than that obtained in our results (Table 4). Compared to 2 min sonication, 4 min sonication significantly reduced the phytic acid in soybean flour fermented by *L. plantarum* and *P. acidilactici* (by 42 and 41%, respectively). During fermentation, phytases are produced, which catalyzes the conversion of phytate to inorganic orthophosphate, thus reducing the phytic acid content as was observed during physical processing and fermentation of soybean flour [48].

The phytic acid content of raw green pea has been reported in the range of 0.54 g kg^{-1} to 0.85 g kg^{-1} [33,49]. The lower PA content compared to the literature was due to the autoclaving process, as PA is heat-labile and forms insoluble complexes. For uncooked and precooked green pea flour, there was only a minor reduction in PA content when physically processed and fermented. For lentil, phytic acid content was reported in the range of 8.6-17.1 g kg^{-1} for various cultivar [49]. Phytic acid content was reduced greatly in unsonicated lentil, from 0.07 g kg^{-1} (raw) to ~0 g kg^{-1} and 0.03 g kg^{-1}, when fermented by *L. plantarum* and *P. acidilactici*, respectively, compared with that of the sonicated then fermented samples. Also, 2 min sonication and fermentation were optimal for reducing phytic acid in lentil. For precooked and then fermented lentil, both microorganisms were able to reduce the phytic acid contents significantly.
The lower values for fermented samples could be attributed to all the substrates being autoclaved for 30 min at 121ºC prior to fermentation and PA assays. Avanza et al. (2013) [50] and Khattab et al. (2009) [3] reported that phytic acid is heat-labile and it forms insoluble complexes between

phytate and other components like calcium and magnesium, thus decreasing the phytic acid content. Sonication followed by fermentation was effective in reducing the phytic acid content for soybean flour.

3.2.6 Amino acid composition of fermented flours

Amino acid (AA) composition of physically processed fermented flours of soybean, green pea, and lentil are presented in Tables 5, 6, and 7 respectively. Phenylalanine (Phe), leucine (Leu), and isoleucine (Ile) were the predominant essential amino acids (EAA) in soybean flour (control) (Table 5), whereas, proline (Pro), alanine (Ala), aspartic acid (Asp), and glutamic acid (Glu) were the major non-essential amino acids (NEAA). *L. plantarum* fermentation of soybean flour led to increases in Leu, Asp, and Pro, but decreased lysine (Lys), Ala, Glu, glycine (Gly), serine (Ser), and tyrosine (Tyr). Fermentation generally increased AA contents, especially when *P. acidilactici* was used. Similarly, when 4 min sonicated soybean flour was fermented by *L. plantarum*, AA contents increased; however, NEAAs and EAAs both improved when fermented by *P. acidilactici*. All the AAs in soybean meal were reported to increase when fermented by *Bacillus natto* [46] which was also the case for our studies when soybean flours were fermented, presumably due to the hydrolysis of proteins into shorter peptides and eventually to AA.

For green peas (Table 6), the most dominant AA was Phe, Asp, Glu, and Leu, while lower AA concentrations were observed for Lys, Thr, Ala, and Gly. Methionine (Met), cysteine (Cys), histidine (His), Ser, and Tyr were not detected in physically modified and fermented green pea [51], [3]. Compared with unfermented (control) green peas, precooked as well as fermented green peas showed lower EAA and NEAA contents, which might be due to the non-enzymatic browning reactions as well as heat pretreatment [47]. When green pea flours were fermented by *L. plantarum*, all AAs decreased. When 2 min sonicated green pea flour was fermented by *L.*

plantarum, Ile, and Val increased while other AAs decreased or remain unchanged. When 4 min sonicated green pea was fermented by *L. plantarum*, Pro increased while the rest remained unchanged. When green peas were fermented by *P. acidilactici*, most of the AA increased and any reductions were not to a drastic extent as noted with *L. plantarum*. Sonicating green peas for 2 and 4 min and then being fermented by *P. acidilactici* decreased all the AA. Precooking reduced the AA content in green peas. Fermentation with *L. plantarum* as well as *P. acidilactici* did not have notable benefits based on decreased AA contents. Threonine was most detrimentally affected as its amount decreased with sonication and fermentation, which may be due to the threonine aldolase enzyme that converts threonine into acetaldehyde that gives fermented aroma [(52)].

For lentil control (Table 7), Leu, Pro, Phe, and Ile were the dominant AAs, which to some extent, aligns with what was reported by Boye et al., (2010) [(53)]. Cys, Met, Ser, Tyr were not detected in unfermented as well as physically modified and fermented lentils. All the AA tended to decrease when lentil flour was fermented by *L. plantarum*. When 2 min sonicated lentil flour was fermented by *L. plantarum*, all the AA decreased except for Val and Gly. Similar results were noted for 4 min sonicated and *L. plantarum* fermented lentil flour, except for aspartic and glutamic acids which increased. Two-minute sonication and fermentation with *P. acidilactici* was beneficial for lentil flours when compared to 4 min as most of the AA increased. Pre-cooking also reduced the AA contents compared with uncooked lentils. Fermentation of precooked lentil by *L. plantarum* and *P. acidilactici* improved the contents of AAs relative to values obtained from precooked controls alone.

The increase in AA content may be due to the degradation of complex protein by bacteria or microbial metabolism during fermentation which releases peptides and amino acids [(12,54)].

Aminopeptidases are reported to be produced by LAB strains during fermentation [55]. The release of AA from proteins relies heavily on the action of aminopeptidases that are responsible for the cleavage of AA from the N-terminus of peptides to liberate free AA. Serine was reduced or even not detected in soybean, green pea, and lentil which might be due to the action of serine dehydratase responsible for the deamination of serine into ammonia and pyruvate and ultimately to organic acids [56]. In general, AA is generated during fermentation through biodegradation pathways involving extracellular proteolysis of proteins by proteolytic enzymes or intracellular biosynthetic pathways involving biosynthesis from AA precursors. Therefore, fermentation of these substrates using *L. plantarum* and *P. acidilactici* may have followed such a pathway that may explain the increase or reduction in AA content [56].

3.2.7 SDS-PAGE

Figure 6 presents the electrophoretic pattern of protein subunits obtained from green pea, lentil, and soybean. In unmodified (raw) substrates (lanes A1, B1, and C1), and precooked substrate (lane A6 and B6), there were high molecular weight (MW) bands as well as higher intensity (darker in color) at MW>36 kDa. Similarly, physical processing and then fermentation by *L. plantarum* and *P. acidilactici* led to considerable protein modification in all substrates, as indicated by the reduced band intensity (lighter in color) (green pea: A2-A5, A7-A8, lentil: B2-B5, B7-B8, soybean: C2-C5). This is possibly due to extensive proteolytic activity on the protein during fermentation, as was reported by Di Stefano et al. (2019) [57]; green lentils and yellow pea fermented with *L. plantarum* had decreased subunit band intensity due to proteolytic hydrolysis of proteins resulting in fractions with MW < 10 kDa. Kiers et al. (2000) [58] also fermented soybean with *Bacillus subtilis* and reported that the protein bands virtually disappeared after

fermentation. Such alteration in protein subunits could be expected to result in varying degree of functional characteristics in legume flours/ ingredients that require further research.

4. Conclusions

Both modified and unmodified soybean flour, green pea flour, and lentil flour supported the growth of *L. plantarum* and *P. acidilactici*. The fermentation of this physically processed legume and pulse flours influenced the non-nutritive compounds. The phytic acid contents were significantly reduced for soybean flour when sonicated. Also, phytic acid was generally reduced with the fermentation of physically modified lentil. Similarly, trypsin inhibitors were also reduced for most of the physically processed and fermented substrates. Total phenolic content was reduced significantly when physically processed substrates were fermented. Physical modification along with fermentation did not affect the protein digestibility for nearly all the substrates. Only the precooked green peas improved protein digestibility from physical modification and fermentation, which is beneficial to green pea utilization. This study demonstrated the impact of physical modification such as sonication/precooking on the fermentation performance of some pulse-based ingredients, leading to reduction in many anti-nutritional compounds and enhanced nutritional quality.

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Table 1 Proximate composition of plant substrate before (control) and after physical modification

Substrate	Treatment	% Moisture	Protein (DB) (g kg^{-1})	Fat (DB) (g kg^{-1})	Ash (DB) (g kg^{-1})	Fiber (DB) (g kg^{-1})	Carbohydrate (DB (g kg^{-1})
Green Pea	Control	4.60 ± 0.56^{a}	225.7 ± 7.5^{abc}	4.3 ± 4.0^{a}	29.7 ± 3.0^{ab}	65.1 ± 4.3^{ab}	675.2^{a}
	LP	5.04 ± 0.21^{a}	221.5 ± 1.8^{bc}	6.5 ± 1.3^{a}	32.8 ± 2.9^{a}	64.0 ± 4.1^{ab}	675.2^{a}
	LP (2 min)	5.97 ± 0.77^{a}	219.1 ± 15.4^{bc}	2.8 ± 0.6^{a}	30.6 ± 0.2^{ab}	69.6 ± 5.6^{a}	677.9^{a}
	LP (4 min)	6.55 ± 0.28^{a}	224.4 ± 3.6^{abc}	1.4 ± 1.9^{a}	30.8 ± 0.5^{ab}	64.4 ± 3.3^{ab}	679.0^{a}
	PA	5.40 ± 0.14^{a}	218.7 ± 9.0^{ab}	5.7 ± 1.5^{a}	28.0 ± 0.5^{ab}	63.8 ± 2.6^{ab}	683.8^{a}
	PA (2 min)	4.69 ± 0.36^{a}	211.8 ± 6.6^{c}	4.1 ± 0.8^{a}	26.9 ± 1.2^{ab}	63.8 ± 4.8^{ab}	693.4^{a}
	PA (4 min)	6.80 ± 1.09^{a}	224.3 ± 5.4^{abc}	1.9 ± 0.0^{a}	24.9 ± 0.6^{b}	63.0 ± 0.0^{ab}	685.9^{a}
Precooked Green Pea	Control	6.22 ± 0.90^{a}	255.9 ± 0.4^{a}	9.9 ± 6.9^{a}	27.4 ± 0.1^{ab}	50.0 ± 8.3^{bc}	656.8^{a}
	LP	6.00 ± 0.23^{a}	236.0 ± 16.3^{abc}	6.4 ± 0.5^{a}	28.3 ± 2.1^{ab}	35.7 ± 0.4^{cd}	693.6^{a}
	PA	5.13 ± 0.33^{a}	251.7 ± 1.8^{ab}	8.5 ± 0.2^{a}	27.6 ± 0.8^{ab}	33.0 ± 1.3^{d}	679.2^{a}
Lentil	Control	3.97 ± 0.12^{a}	270.1 ± 2.2^{a}	3.7 ± 0.8^{a}	27.5 ± 0.1^{a}	41.1 ± 0.1^{a}	657.6^{a}
	LP	4.51 ± 0.28^{a}	269.3 ± 7.7^{a}	7.8 ± 1.7^{a}	28.2 ± 0.7^{a}	37.9 ± 0.0^{a}	656.8^{a}
	LP (2 min)	4.32 ± 0.45^{a}	269.2 ± 14.2^{a}	1.2 ± 3.4^{a}	30.7 ± 0.1^{a}	41.7 ± 2.1^{a}	657.2^{a}
	LP (4 min)	4.40 ± 0.22^{a}	268.4 ± 4.5^{a}	0.6 ± 0.6^{a}	29.6 ± 0.6^{a}	37.9 ± 1.9^{a}	663.5^{a}
	PA	4.36 ± 0.96^{a}	267.2 ± 9.3^{a}	5.1 ± 0.4^{a}	27.9 ± 2.6^{a}	40.6 ± 1.0^{a}	659.2^{a}
	PA (2 min)	3.49 ± 0.06^{a}	270.7 ± 9.3^{a}	0.8 ± 0.3^{a}	27.2 ± 0.7^{a}	38.6 ± 0.3^{a}	662.7^{a}
	PA (4 min)	3.48 ± 0.01^{a}	265.8 ± 6.9^{a}	2.2 ± 1.3^{a}	25.8 ± 0.2^{a}	37.4 ± 0.2^{a}	668.8^{a}
Precooked Lentil	Control	5.25 ± 0.21^{a}	291.9 ± 5.8^{a}	7.6 ± 4.3^{a}	29.4 ± 1.9^{a}	46.1 ± 4.3^{a}	625.0^{a}
	LP	3.37 ± 0.12^{a}	272.0 ± 6.5^{a}	3.3 ± 0.3^{a}	30.6 ± 0.2^{a}	38.2 ± 1.0^{a}	655.9^{a}
	PA	6.08 ± 0.29^{a}	274.5 ± 4.1^{a}	3.7 ± 0.8^{a}	28.5 ± 1.5^{a}	39.1 ± 3.6^{a}	654.2^{a}
Soy flour	Control	3.39 ± 0.22^{a}	584.8 ± 6.8^{a}	1.7 ± 2.1^{a}	65.7 ± 0.4^{d}	28.7 ± 7.2^{a}	319.1^{a}
	LP	4.45 ± 0.20^{a}	588.0 ± 5.8^{a}	4.1 ± 2.5^{a}	68.1 ± 0.0^{bc}	25.5 ± 0.1^{a}	314.3^{a}
	LP (2 min)	3.37 ± 0.17^{a}	584.7 ± 14.5^{a}	5.0 ± 3.1^{a}	70.2 ± 0.8^{a}	26.8 ± 3.4^{a}	313.3^{a}
	LP (4 min)	4.38 ± 0.04^{a}	587.0 ± 3.0^{a}	2.5 ± 1.8^{a}	68.8 ± 0.1^{ab}	26.3 ± 2.1^{a}	315.4^{a}
	PA	5.56 ± 0.80^{a}	576.0 ± 4.0^{a}	2.6 ± 2.0^{a}	67.0 ± 0.3^{cd}	24.3 ± 0.1^{a}	330.1^{a}
	PA (2 min)	3.67 ± 0.02^{a}	578.4 ± 2.2^{a}	6.3 ± 1.3^{a}	67.3 ± 0.2^{c}	31.9 ± 1.6^{a}	316.1^{a}
	PA (4 min)	3.69 ± 1.86^{a}	589.0 ± 10.0^{a}	2.8 ± 3.3^{a}	67.0 ± 0.5^{cd}	28.1 ± 3.9^{a}	313.1^{a}

Abbreviations: LP: *Lactobacillus plantarum*; PA: *Pediococcus acidilactici*; LP (2 min): Sonicated for 2 min and fermented by LP; LP (4 min): Sonicated for 4 min and fermented by LP; PA (2 min): Sonicated for 2 min and fermented by PA; PA (4 min): Sonicated for 4 min and fermented by PA

Values are mean ± standard deviations (n=2). Results having different superscript letters within each substrate are significantly different ($p < 0.05$) as determined by Tukey's test.

Table 2 Specific growth rates (μ), and doubling time (t_d) for microorganisms used to ferment physically processed substrates

Microorganism	Substrate	Sonication time (min)	μ (h^{-1})	t_d (h)
L. plantarum	Green Pea	0	0.70 ± 0.03[a]	1.00 ± 0.04
		2	0.34 ± 0.01[b]	2.07 ± 0.05
		4	0.28 ± 0.01[b]	2.46 ± 0.09
	Precooked Green Pea	0	0.72 ± 0.02[a]	0.96 ± 0.02
	Lentil	0	0.71 ± 0.03[a]	0.97 ± 0.05
		2	0.64 ± 0.03[a]	1.08 ± 0.05
		4	0.49 ± 0.04[b]	1.42 ± 0.12
	Precooked Lentil	0	0.73 ± 0.01[a]	0.95 ± 0.01
	Soybean	0	0.67 ± 0.03 [a]	1.03 ± 0.04
		2	0.95 ± 0.03[b]	0.73 ± 0.03
		4	0.76 ± 0.02[a]	0.91 ± 0.02
P. acidilactici	Green Pea	0	0.70 ± 0.01[a]	0.99 ± 0.02
		2	0.75 ± 0.00[b]	0.92 ± 0.00
		4	0.76 ± 0.00[b]	0.91 ± 0.00
	Precooked Green Pea	0	0.64 ± 0.01[c]	1.08 ± 0.02
	Lentil	0	0.71 ± 0.03[a]	0.97 ± 0.05
		2	0.78 ± 0.05[a]	0.92 ± 0.00
		4	0.76 ± 0.00[a]	0.91 ± 0.00
	Precooked Lentil	0	0.77 ± 0.03[a]	0.90 ± 0.04
	Soybean	0	0.70 ± 0.06[a]	0.99 ± 0.09
		2	0.76 ± 0.02[a]	0.91 ± 0.02
		4	0.77 ± 0.03[a]	0.91 ± 0.04

Values are mean ± standard deviations (n=2). Results having different superscript letters within each substrate are significantly different ($p < 0.05$) as determined by Tukey's test.

Table 3 Total phenolic compounds of physically processed fermented substrate

Substrates	Sonication time (min)	Microbes for fermentation	TPC (mg GAE g^{-1})
Soybean	Raw	None	4.60 ± 0.22 [a]
	0	*L. plantarum*	2.80 ± 0.14 [b]
	2		2.65 ± 0.46 [b]
	4		2.38 ± 0.54 [b]
	0	*P. acidilactici*	2.71 ± 0.31 [b]
	2		3.19 ± 0.14 [ab]
	4		2.75 ± 0.61 [b]
Green pea	Raw	*None*	1.81 ± 0.32 [a]
	0	*L. plantarum*	0.70 ± 0.12 [b]
	2		0.49 ± 0.04 [bcd]
	4		0.21 ± 0.18 [bcd]
	0	*P. acidilactici*	0.58 ± 0.11 [bc]
	2		0.10 ± 0.05 [cd]
	4		0.01 ± 0.01 [d]
Precooked Green Pea	Raw	*None*	1.75 ± 0.02 [a]
	0	*L. plantarum*	0.71 ± 0.07 [b]
	0	*P. acidilactici*	0.58 ± 0.04 [bc]
Lentil	Raw	*None*	1.90 ± 0.09 [a]
	0	*L. plantarum*	1.61 ± 0.01 [ab]
	2		1.09 ± 0.01 [bcd]
	4		0.52 ± 0.17 [d]
	0	*P. acidilactici*	1.45 ± 0.45 [abc]
	2		0.94 ± 0.10 [cd]
	4		0.59 ± 0.13 [d]
Precooked Lentil	Raw	*None*	1.69 ± 0.01 [ab]
	0	*L. plantarum*	0.66 ± 0.03 [d]
	0	*P. acidilactici*	0.66 ± 0.16 [cd]

Values are mean ± standard deviations (n=2). Results having different superscript letters within each substrate are significantly different ($p < 0.05$) as determined by Tukey's test.

Table 4 Phytic acid content of physically processed fermented substrates

Substrates	Sonication time (min)	Microbes for fermentation	Phytic acid content (g kg^{-1})
Soybean	Raw	None	0.41 ± 0.01 [a]
	0		0.36 ± 0.00a [ab]
	2	*L. plantarum*	0.33 ± 0.02 [b]
	4		0.29 ± 0.01 [b]
	0		0.31 ± 0.01 [b]
	2	*P. acidilactici*	0.34 ± 0.02 [b]
	4		0.29 ± 0.03 [b]
Green pea	Raw	None	0.03 ± 0.00 [a]
	0		0.02 ± 0.01 [a]
	2	*L. plantarum*	0.02 ± 0.02 [a]
	4		0.01 ± 0.01 [a]
	0		0.03 ± 0.00 [a]
	2	*P. acidilactici*	0.02 ± 0.01 [a]
	4		0.02 ± 0.01 [a]
Precooked Green Pea	Raw	None	0.03 ± 0.01 [a]
	0	*L. plantarum*	0.02 ± 0.01 [a]
	0	*P. acidilactici*	0.01 ± 0.01 [a]
Lentil	Raw	None	0.07 ± 0.02 [ab]
	0		0.01 ± 0.01 [d]
	2	*L. plantarum*	0.04 ± 0.01 [bcd]
	4		0.07 ± 0.02 [ab]
	0		0.03 ± 0.01 [bcd]
	2	*P. acidilactici*	0.03 ± 0.00 [cd]
	4		0.05 ± 0.02 [abc]
Precooked Lentil	Raw	None	0.08 ± 0.00 [a]
	0	*L. plantarum*	0.05 ± 0.01 [abcd]
	0	*P. acidilactici*	0.01 ± 0.00 [cd]

Values are mean ± standard deviations (n=2). Results having different superscript letters within each substrate are significantly different ($p < 0.05$) as determined by Tukey's test.

Table 5 Amino acid composition (μmole/mg) of physically processed fermented soybean

Treatment	Essential amino acid (μmole/mg)							Non-essential amino acid (μmole/mg)						
	Ile	Leu	Lys	Phe	Thr	Val	Met	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Control	147±4^{bcd}	219±3^{c}	31±5^{cd}	223±10^{cd}	70±3^{a}	152±9^{ab}	24±3^{b}	108±8^{cd}	102±4^{d}	101± 9^{d}	99±4^{cd}	177±6^{d}	50±2^{a}	27±2^{b}
LP	194±24^{ab}	272±2^{b}	ND	238±10^{c}	56±7^{a}	155±1^{ab}	28±5^{b}	68±0^{e}	148±0^{c}	69± 5^{e}	42±7^{e}	234±20^{bc}	ND	ND
LP-2 min	143±9^{cd}	345±19^{a}	72±11^{a}	307±8^{a}	8±1^{b}	164±5^{a}	22±7^{b}	80±2de	122±12^{cd}	168±6^{abc}	72±8de	216±10^{cd}	ND	53±7^{a}
LP-4 min	231±14^{a}	305±12^{ab}	42±9^{bcd}	333±4^{a}	70±9^{a}	98±12^{c}	26±3^{b}	156±6^{ab}	240±5^{a}	174±5^{ab}	146±7^{ab}	380±1^{a}	ND	33±1^{b}
PA	160±6^{bcd}	225±6^{c}	62±3^{ab}	230±5^{c}	64±8^{a}	163±5^{a}	32±7^{b}	123±16^{bc}	200± 17^{b}	155±8^{bc}	116±11^{bc}	246±9^{bc}	58±6^{a}	62±3^{a}
PA-2 min	114 ±10^{d}	154±19^{d}	22±6^{d}	200±1^{d}	55±6^{a}	115±19^{bc}	93±11^{a}	79±14de	197± 9^{b}	143±11^{c}	72±14de	179±21^{d}	ND	17±4^{bc}
PA-4 min	165±12^{bc}	277±0^{ab}	57±9^{abc}	266±1^{b}	60±8^{a}	111±17^{bc}	74±7^{a}	161±5^{a}	251±7^{a}	191±6^{a}	151±4^{a}	279±15^{b}	53±11^{a}	30±8^{b}

Abbreviations: LP: *Lactobacillus plantarum*; PA: *Pediococcus acidilactici*; LP-2 min: Sonicated for 2 min and fermented by LP; LP-4 min: Sonicated for 4 min and fermented by LP; PA-2 min: Sonicated for 2 min and fermented by PA; PA-4 min: Sonicated for 4 min and fermented by PA. ND: Not detected

Table 6 Amino acid composition (μmole/mg) of physically processed fermented green pea flour

Treatment	Essential amino acid (μmole/mg)							Non-essential amino acid (μmole/mg)						
	Ile	Leu	Lys	Phe	Thr	Val	Met	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Green pea														
Control	88±7^{bc}	142±15^{ab}	24±9^{b}	175±0^{ab}	37±9^{a}	88±7^{bcd}	ND	49±1^{abc}	141±16^{ab}	124±18^{a}	59±3^{ab}	135±8^{b}	ND	ND
LP	68±8^{cde}	123±11^{bc}	24±5^{b}	110±8^{c}	19±4^{b}	76±2^{bcd}	ND	48±6^{bc}	119±12^{abc}	51±12^{cd}	55±5^{abc}	97±10^{cd}	ND	ND
LP-2 min	127±13^{a}	176±7^{a}	ND	160±9^{ab}	10±1^{bc}	166±21^{a}	ND	61±6^{ab}	110±19^{bcd}	74±1^{bc}	58±1^{abc}	116±3^{bc}	ND	ND
LP-4 min	95±2^{b}	134±6^{b}	20±2^{b}	183±2^{a}	25±6^{ab}	102±14^{b}	ND	46±3^{bc}	154±5^{a}	113±7^{a}	47±6^{bc}	172±1^{a}	ND	ND
PA	83±6^{bcd}	133±4^{b}	39±2^{a}	154±13^{b}	20±6^{b}	97±9^{bc}	ND	75±17^{a}	143±9^{ab}	100±3^{b}	72±14^{a}	113±15^{c}	ND	ND
PA-2 min	62±1^{def}	107±11^{bcd}	ND	84±7^{cd}	ND	71±8^{bcd}	ND	32±5^{c}	77±3^{d}	43±8^{cd}	38±5^{bcd}	97±11^{cd}	ND	ND
PA-4 min	47±2^{ef}	87±1^{cde}	ND	62±5de	ND	49±2^{d}	ND	41±1^{bc}	78±5^{d}	69±1^{bc}	44±1^{bc}	60±3^{e}	ND	ND
Precooked green pea														
Control	42±2^{f}	79±1de	ND	55±6^{e}	ND	59±0^{cd}	ND	49±2^{abc}	79±4^{cd}	64±5^{c}	34±1^{cd}	65±8de	ND	ND
LP	37±8^{f}	70±14^{e}	ND	47±8^{e}	ND	71±10^{bcd}	ND	35±5^{c}	73±11^{d}	32±2^{d}	18±5^{d}	67±9de	ND	ND
PA	48±2^{ef}	92±7^{cde}	ND	50±1^{e}	ND	66±6^{bcd}	ND	38±4^{bc}	71±3^{d}	49±5^{cd}	39±8^{bcd}	59±5^{e}	ND	ND

Abbreviations: LP: *Lactobacillus plantarum*; PA: *Pediococcus acidilactici*; LP-2 min: Sonicated for 2 min and fermented by LP; LP-4 min: Sonicated for 4 min and fermented by LP; PA-2 min: Sonicated for 2 min and fermented by PA; PA-4 min: Sonicated for 4 min and fermented by PA. ND: Not detected

Table 7 Amino acid composition (μmole/mg) of physically processed fermented lentil

Treatment	Essential amino acid (μmole/mg)							Non-essential amino acid (μmole/mg)						
	Ile	Leu	Lys	Phe	Thr	Val	Met	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Lentil														
Control	106±1^{ab}	145±10^{ab}	ND	108±21^{a}	ND	80±8^{abc}	ND	43±8^{ab}	78±11^{abc}	30±2^{c}	47±1^{cd}	128±6^{a}	ND	ND
LP	46±10^{c}	106±7^{bcd}	ND	52±3^{bc}	ND	57±0^{cd}	ND	41±4^{ab}	47±7^{bc}	55±3^{abc}	43±3^{cd}	56±9^{c}	ND	ND
LP-2 min	84±7^{b}	143±6^{ab}	ND	52±5^{bc}	ND	111±11^{a}	ND	29±4^{b}	38±1^{c}	ND	76±4^{ab}	82±1^{bc}	ND	ND
LP-4 min	49±15^{c}	90±25^{cd}	ND	105±10^{a}	15±4^{a}	66±15^{bcd}	ND	60±10^{a}	103±8^{ab}	78±6^{a}	39±9^{cd}	75±18^{c}	ND	ND
PA	97±0^{ab}	84±8^{d}	ND	48±12^{c}	ND	54±1^{cd}	ND	38±6^{ab}	57±19^{bc}	47±8^{bc}	31±5^{d}	58±4^{c}	ND	ND
PA-2 min	92±2^{ab}	164±1^{a}	ND	104±15^{a}	ND	113±6^{a}	ND	54±4^{ab}	125±13^{a}	43±1^{bc}	85±9^{a}	110±8^{ab}	ND	ND
PA-4 min	49±2^{c}	95±11^{cd}	5±0^{b}	91±21^{abc}	22±8^{a}	50±12^{cd}	ND	50±8^{ab}	118±4^{a}	81±19^{a}	44±7^{cd}	68±3^{c}	ND	ND
Precooked lentil														
Control	46±7^{c}	76±11^{d}	14±4^{a}	85±7^{abc}	ND	44±6^{d}	ND	42±9^{ab}	103±9^{ab}	69±3^{ab}	25±5^{d}	57±6^{c}	ND	ND
LP	119±5^{a}	133±7^{abc}	ND	67±11^{abc}	ND	92±8^{ab}	ND	49±7^{ab}	47±4^{bc}	34±4^{c}	47±5^{cd}	58±3^{c}	ND	ND
PA	99±1^{ab}	133±8^{abc}	ND	99±0^{ab}	ND	102±3^{a}	ND	43±5^{ab}	117±5^{a}	68±5^{ab}	54±5^{bc}	85±11^{bc}	ND	ND

Abbreviations: LP: *Lactobacillus plantarum*; PA: *Pediococcus acidilactici*; LP-2 min: Sonicated for 2 min and fermented by LP; LP-4 min: Sonicated for 4 min and fermented by LP; PA-2 min: Sonicated for 2 min and fermented by PA; PA-4 min: Sonicated for 4 min and fermented by PA. ND: Not detected

Figures

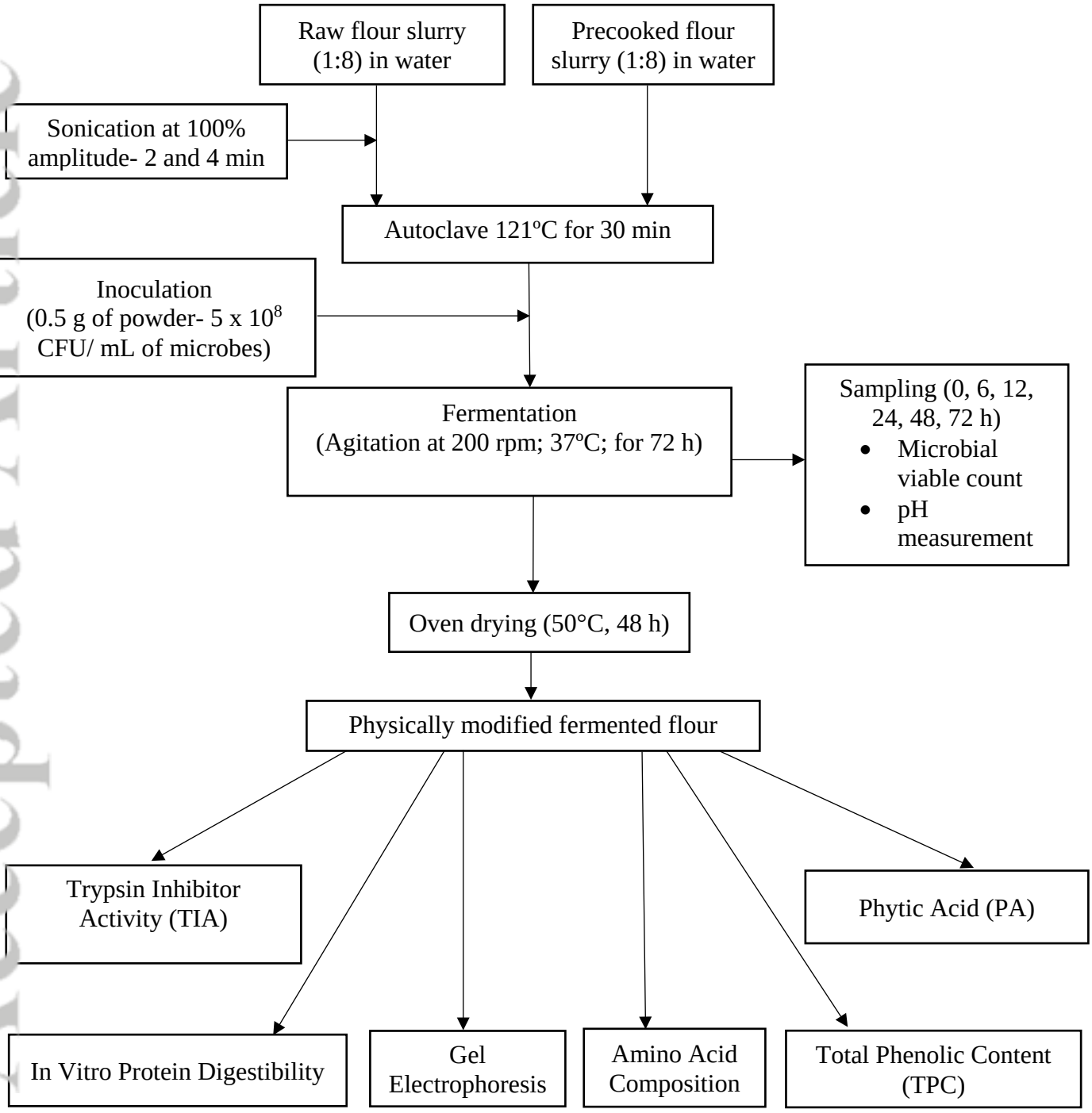


Figure 1 Conceptual framework: flour modification with two processing options and evaluation of resulting ingredients

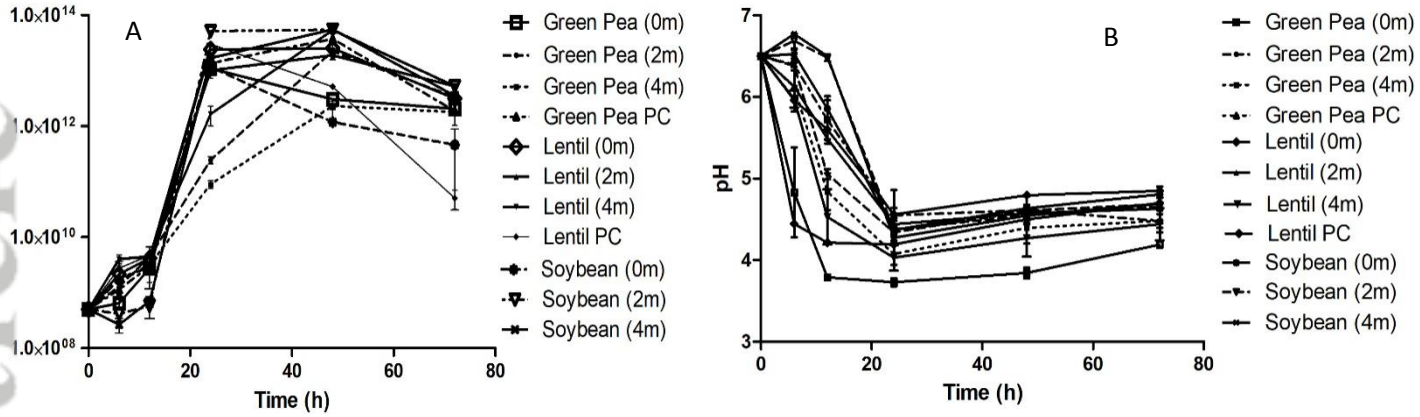


Figure 2 Microbial viable population (A) and pH (B) of *Lactobacillus plantarum* for physically modified substrates

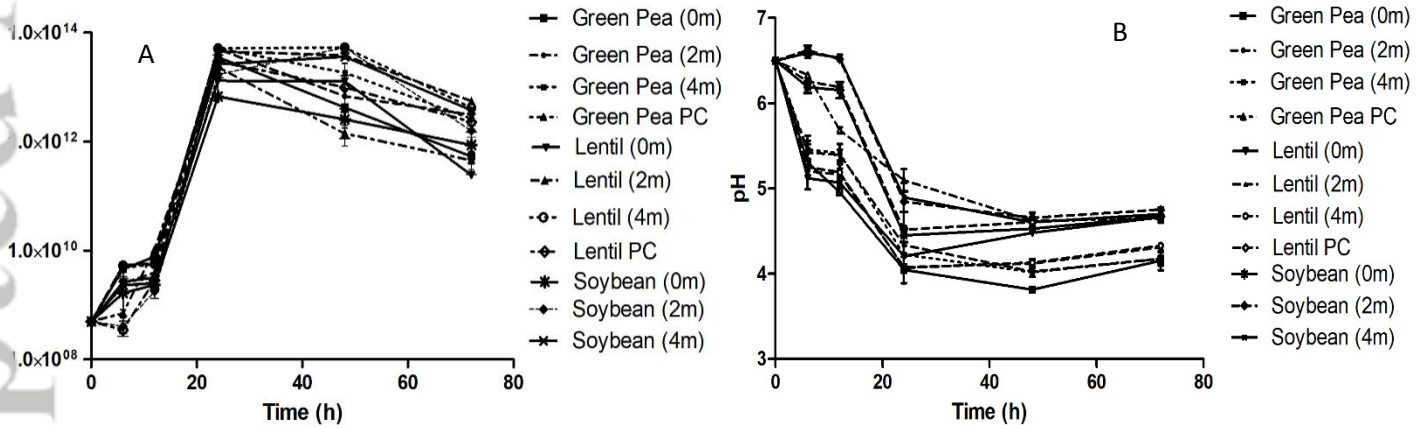


Figure 3 Microbial viable population (A) and pH (B) of *Pediococcus acidilactici* for physically modified substrates

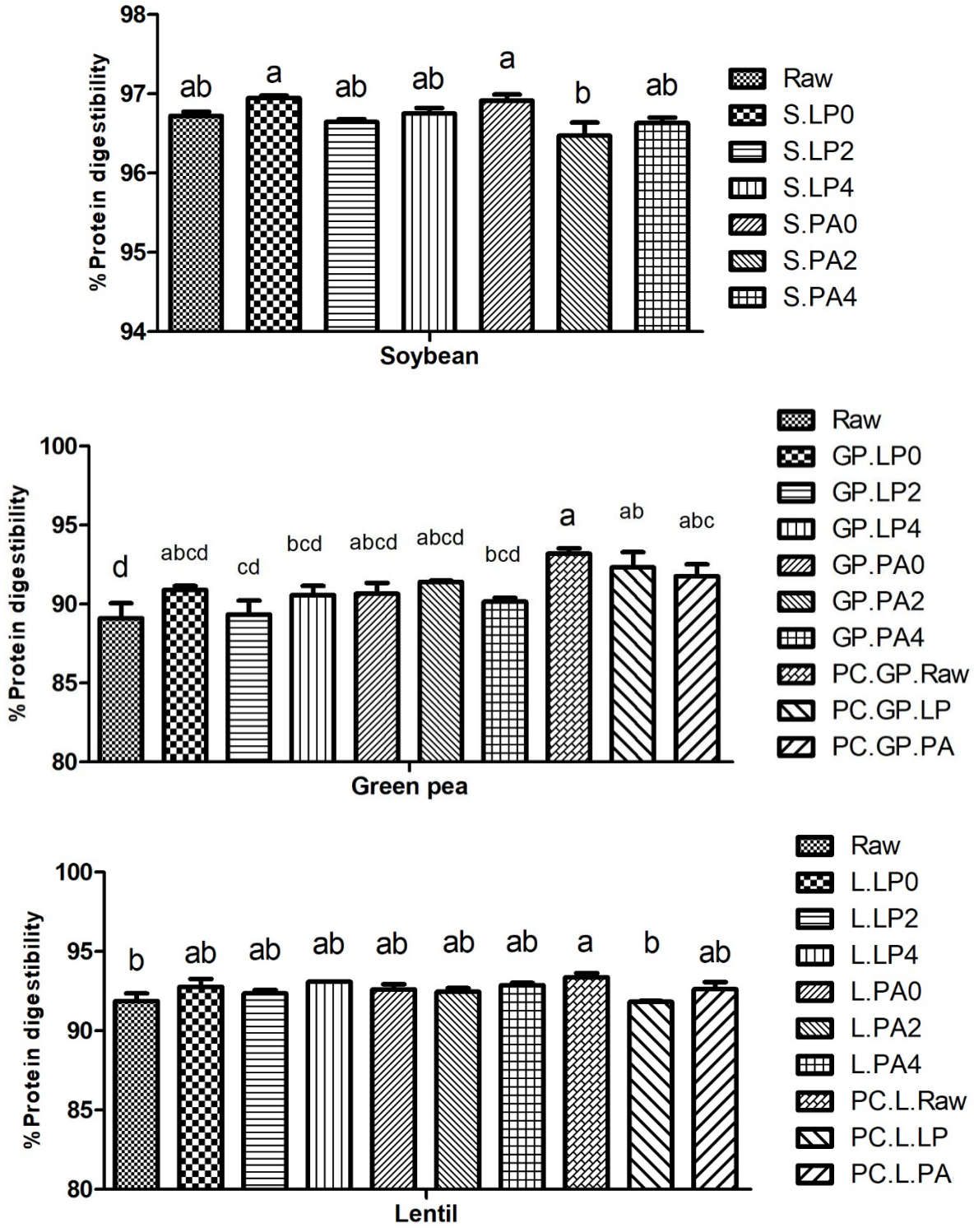


Figure 4 In vitro protein digestibility of physically modified fermented substrates: Top: Soybean; Middle: Green pea; Bottom: Lentil

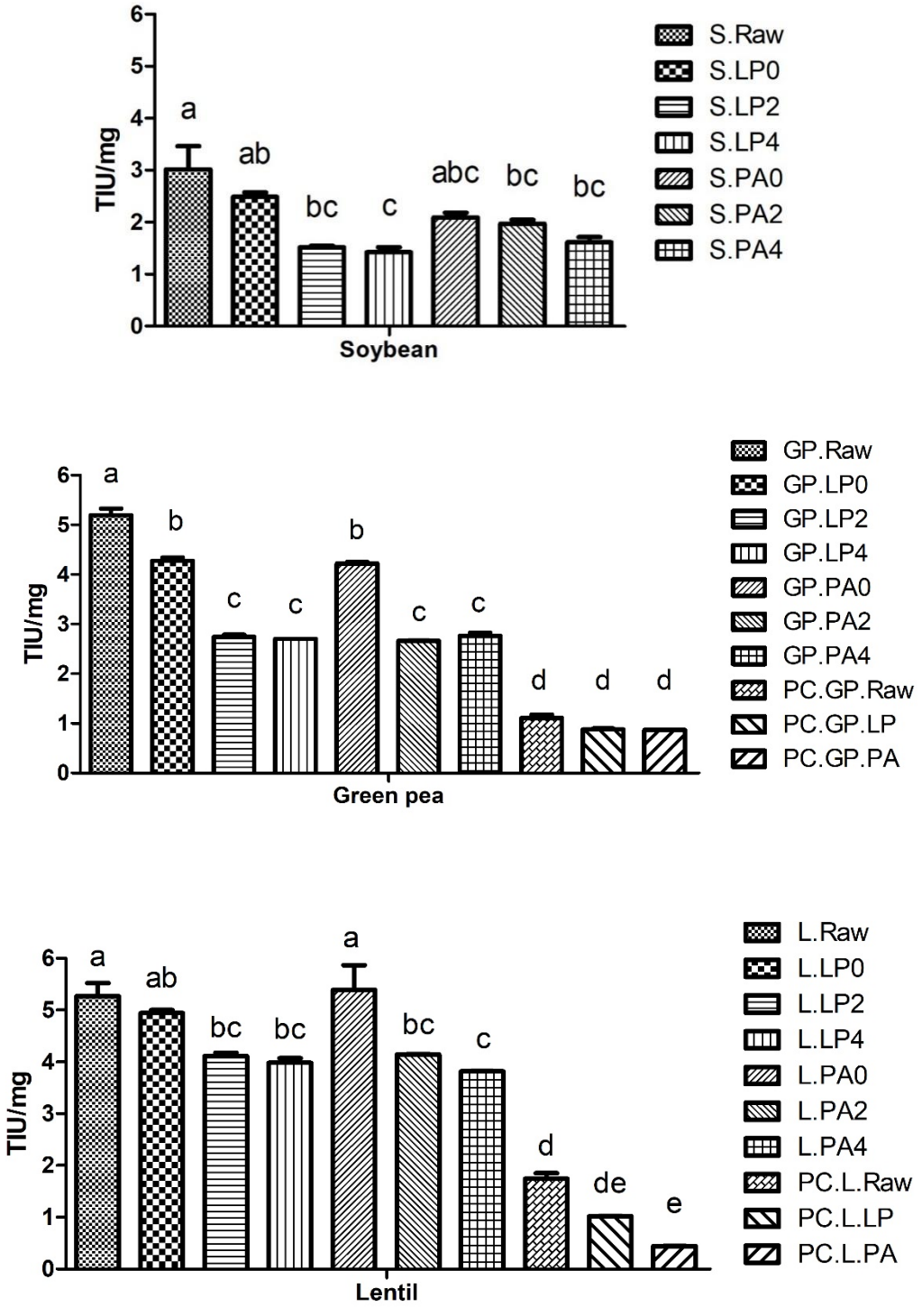
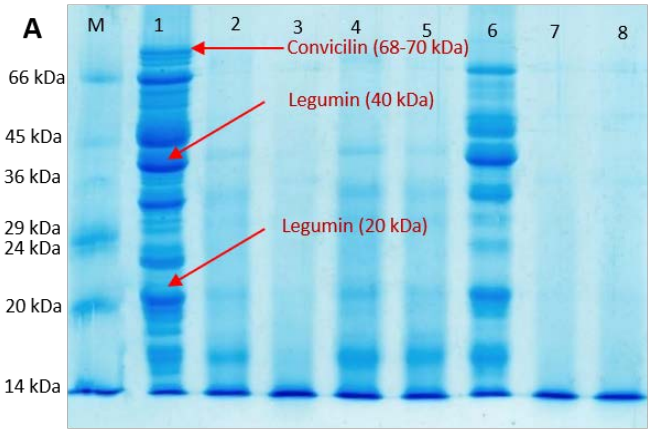
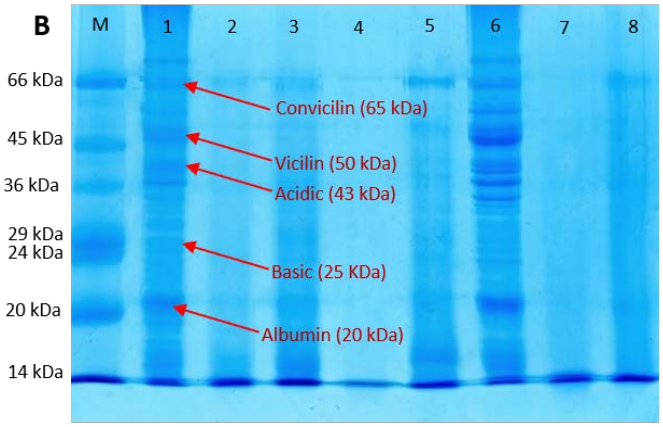


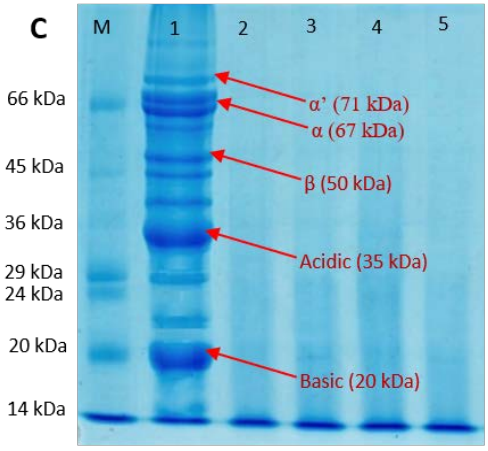
Figure 5 Trypsin inhibitory activity of physically processed fermented substrates: Top: Soybean; Middle: Green pea; Bottom: Lentil



Green pea protein: M-Marker, 1-Raw, 2-LPF, 3-LPF4, 4-PAF, 5-PAF4, 6-PC. raw, 7-PC. LP, 8-PC. PA



Lentil protein: M-Marker, 1-Raw, 2-LPF, 3-LPF4, 4-PAF, 5-PAF4, 6-PC. raw, 7-PC. LP, 8-PC. PA



Soybean protein: M-Marker, 1-Raw, 2-LPF, 3-LPF4, 4-PAF, 5-PAF4

Figure 6 Gel electrophoresis of physically processed and fermented substrate. (A) Green pea, (B) Lentil, (C) Soybean