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Solid State Bioconversion for Producing Functional Flours from Whole Quality Protein Maize and Common Beans with Enhanced Nutritional Value, Antioxidant and Antihypertensive Potential

Abstract

The aim was to develop functional flours with enhanced nutritional value, antioxidant and antihypertensive potential from quality protein maize and common beans throughout solid state bioconversion (SSB) process. The SSB processes were performed at 35°C/84h (maize) and 38°C/100h (beans) using a *rhizopus oligosporus* strain. The bioprocessed maize and bean (bioprocessed bean cotyledons blended with seed coats) samples were dried, cooled, and milled to obtain bioprocessed whole quality protein maize flour (BQPMF) and bioprocessed common bean flour (BCBF), respectively. SSB process increased the protein content (26.8 - 33.5%), soluble, insoluble and total dietary fiber contents (132-196%, 15-24%, and 40-43%, respectively), resistant starch (130-133%), calculated protein efficiency ratio (C-PER; 33-49 %), phenolic content (69.5-127.02%), antioxidant capacity (AoxA) (38-75%) and angiotensin converting enzyme–inhibitory (ACE-I) activity of quality protein maize and common beans. The mixture prepared from 60% BQPMF+40% BCBF had, in dry basis, 19.78% proteins, 24.65% total dietary fiber, 3.92% resistant starch, and C-PER 2.24, antioxidant activity 199.24 µmol Trolox equivalents/g sample, and IC_{50} (antihypertensive potential) 25.12 µg peptide/mL suspension. The SSB bioprocess resulted in an effective strategy to improve nutritional value, phenolic content, antioxidant activity, and antihypertensive potential of quality protein maize and common beans. The flours developed in this work (BQPMF, BCBF and its mixture) can be considered functional foods of high nutritional value and with potential for prevention and control of degenerative diseases such as hypertension and those derived from oxidative stress.

Keywords: Solid state bioconversion; Quality protein maize; Common beans; Functional flour; Nutritional value; Antioxidant activity; Antihypertensive potential

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Introduction

Maize (*Zea mays* L) is one of the world´s most important cereal crops and provides about 50% of the proteins and calories in the diet of people from developing countries. The maize proteins are deficient in the essential amino acids lysine and tryptophan. On the other hand, there are genotypes also called "quality protein maize" (QPM) which possess lysine and tryptophan levels higher than normal maize [1]. Phytochemicals such as phenolic compounds, amongst others have also been reported on several maize genotypes [2]. Maize has a higher antioxidant activity when compared to wheat, oat, and rice [3]. Maize phenolics are powerful antioxidants through radical scavenging, and thus have potential in the development of nutraceuticals rich in antioxidants [4]. Principal phenolic compounds found in whole maize grains are flavonoids and phenolic acids (Figure 1). These compounds are present as soluble free and conjugated or insoluble bound forms in whole maize kernels [2]. Most phenolic acids in cereals are found in a bound form as conjugate with sugars, fatty acids or proteins [3]. Also, bioactive peptides (Table 1) with antihypertensive activity have been obtained by enzymatic hydrolysis from proteins of maize grains [5].

Common bean (*Phaseolus vulgaris* L) plays an important role in the diet of Latin-American people, providing proteins, essential fatty acids, complex carbohydrates, vitamins and minerals [6]. Common beans are rich in antioxidants which, among other components, provides flavonoids and phenolic acids (Figure 2) [7]; likewise, this legume is a source for producing bioactive peptides (Table 2) obtained by enzymatic hydrolysis which confer antioxidant activity, antimutagenic effects [6] and an antihypertensive potential by inhibiting angiotensin converting enzyme [8,9]. In order to obtain maximum health benefits of whole grains, such as maize and beans, it is desirable to prepare mixtures from them; the additive and synergistic effects of biologically active components present in each whole grain may be responsible for the health benefits as the reduced risk of chronic diseases. Recent evidence suggests that the complex mixture of bioactive compounds in whole foods may be more healthful than individual isolated components [3]. On the other hand, technologic alternatives such as the solid state bioconversion (SSB) can be used efficiently for increasing the phenolic content and antioxidant potential of grains [4].

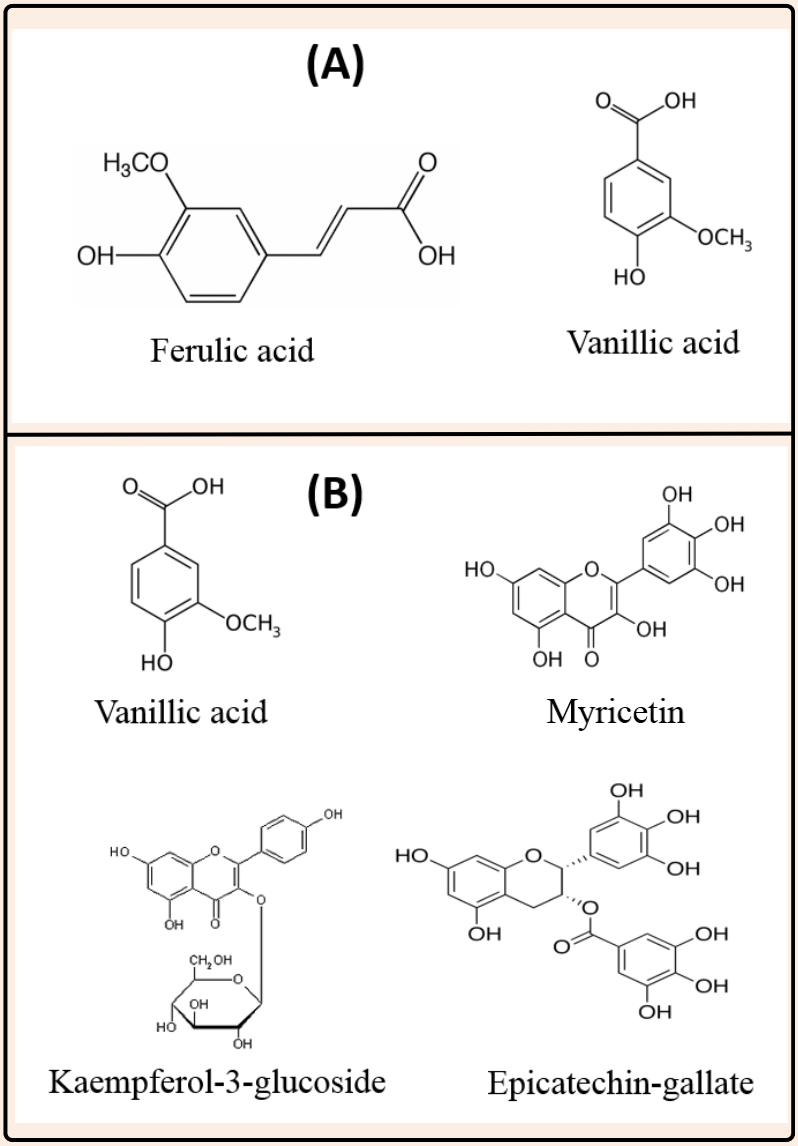

Figure 1: Phenolic compounds with antioxidant activity predominant in

a) White maize
b) Common beans

Table 1: Bioactive peptides with angiotensin converting enzyme–Inhibitory (ACE-I) activity obtained by enzymatic hydrolysis from proteins of maize and common beans.

Grain	Peptide	Protein Source	Reference
Maize	LRP; LSP; LQP	α-zein	[5]
Common Bean	PVNNPQIH	Phaseolin	[9]

Holker & Lenz [10] defined the solid state bioconversion (SSB) as the microbial bioprocessing of a solid food substrate that acts as a physical support and source of nutrients in the presence of low free liquid. It is a traditional technology used in Asia to improve the nutritional quality and palatable characteristics of cereals and legumes. Tempeh is a nutritious oriental bioprocessed food produced by SSB of soybeans with *Rhizopus oligosporus*. Several other substrates have been used to prepare tempeh, e.g. common beans, chickpeas, rice, oat, lupine, home bean, ground nut, wheat, corn/soybean [4,11-13]. The SSB would increase the phenolic, isoflavones content, and antioxidant activity of fungal processed cereals and legumes which will enhance the potential health-relevant functionality [14]. The objective of this research was to develop functional flours with enhanced nutritional value, and antioxidant and antihypertensive potential from quality protein maize and common beans throughout solid state bioconversion (SSB) process.

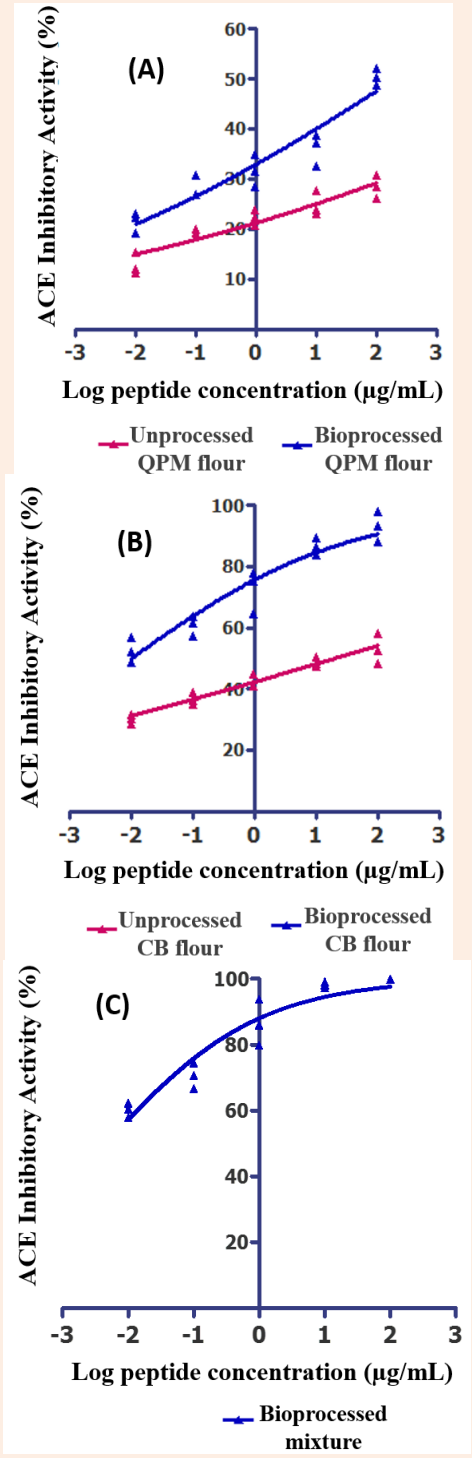

Figure 2: ACE-inhibitory activity of bioprocessed QPM and common bean (CB) flours treated with pancreatin.

a) Unprocessed and Bioprocessed QPM flours;

b) Unprocessed and Bioprocessed CB flours;

c) Bioprocessed mixture (60% Bioprocessed QPM flour + 40% Bioprocessed CB flour).

Table 2: Chemical composition and nutritional properties of quality protein maize and common bean flours[1].

Property	Unprocessed	BQPMF[2]	Unprocessed	BCBF[3]	(FAO, 2013)[4]
	QPM flour		CB flour		
Chemical composition *(%,dw)*					
Protein	10.3±0.14^{d}	13.75±0.21^{c}	22.96±0.37^{b}	29.12±0.23^{a}	
Lipids	6.1±0.09^{a}	3.49±0.13^{b}	2.54±0.04^{c}	1.71±0.03^{d}	
Total Dietary Fibre	12.63±0.49^{d}	18.04±0.97^{c}	25.78±0.22^{b}	36.10±0.27^{a}	
Soluble	1.89±0.29^{c}	5.67±0.12^{b}	5.74±0.09^{b}	11.32±0.17^{a}	
Insoluble	10.74±0.08^{d}	12.37±0.23^{c}	20.04±0.19^{b}	24.78±0.21^{a}	
Resistant Starch	1.19±0.02^{d}	2.77±0.07^{b}	2.41±0.08^{c}	5.55±0.09^{a}	
Nutritional					
EAA[5]					
His	3.19±0.02^{b}	4.01±0.03^{a}	2.41±0.02^{d}	2.59±0.02^{c}	1.6
Ile	2.61±0.07^{c}	3.06±0.03^{b}	3.09±0.04^{b}	3.33±0.03^{a}	3
Leu	8.27±0.08^{b}	9.52±0.05^{a}	7.21±0.07^{c}	7.18±0.06^{c}	6.1
Lys	4.25±0.10^{d}	5.68±0.06^{c}	6.52±0.03^{a}	6.31±0.05^{b}	4.8
Met+Cys	5.70±0.09^{b}	6.21±0.05^{a}	2.08±0.03^{d}	2.51±0.04^{c}	2.3
Phe+Tyr	6.98±0.04^{d}	9.58±0.08^{a}	8.55±0.06^{c}	9.39±0.03^{b}	4.1
Thr	3.47±0.02^{c}	4.28±0.03^{a}	3.52±0.04^{c}	3.78±0.02^{b}	2.5
Trp	0.83±0.01^{d}	0.97±0.02^{c}	1.35±0.02^{a}	1.29±0.01^{b}	0.66
Val	6.01±0.04^{a}	4.55±0.03^{b}	3.53±0.02^{d}	3.69±0.04^{c}	4
Total	41.31	47.86	38.26	40.07	29.06
EAA chemical score	0.88	1	0.9	1	
Limiting EAA	Lys	---	Met+Cys	---	
IVPD[6]	78.37±0.12^{c}	83.60±0.17^{b}	72.2±0.11^{d}	88.2±0.10^{a}	
C-PER[7]	1.58±0.04^{c}	2.10±0.02^{b}	1.62±0.03^{c}	2.41±0.07^{a}	

[1]Values are mean +SD, a-d Means with the same letter in the same row are not significantly different (Duncan, $p<0.05$)

[2]BQPMF= Bioprocessed quality protein maize flour

[3]BCBF= Bioprocessed common bean flour

[4]Requirements of amino acids for older child, adolescent, and adult (3 years and older) according FAO (2013)

[5]EAA=Essential amino acid (g/100g protein)

[6]IVPD= *In vitro* protein digestibility (%)

[7]C-PER=Calculated protein efficiency ratio.

Materials and Methods

Source of food materials

The common beans (*Phaseolus vulgaris* L) var Nayarit black and quality protein maize (Zea mays L) var V537C were cultivated at the Culiacan Valley Experimental Station of the National Research Institute for Forestry, Agriculture and Livestock, Sinaloa, México. Grains were harvested, cleaned and stored at 4°C in tightly sealed containers until used. The *Rhizopus oligosporus* NRRL 2710 strain was obtained from American Type Culture Collection, Manassas, USA.

Bioprocessing of quality protein maize and common bean

The bioprocessed quality protein maize (BQPMF) and common bean (BCBF) were prepared according Cuevas-Rodríguez et al. [1] and Reyes-Bastidas et al. [12], respectively, with modifications. Preliminary studies were realized to determinate the optimal fermentation temperature and time [15]. The solid state bioconversion (SSB) processes of quality protein maize and common bean were optimized to obtain bioprocessed maize and bean flours with high antioxidant activity, phenolic content and protein content. Kernels from quality protein maize (QPM) were placed in a domestic blender at low velocity to obtain fragments that passed through a 3-US mesh (6.73mm) screen. Fragmented whole QPM and common bean seeds, respectively, were soaked (25°C/8 h) in acetic acid solution (pH=3.0). Common bean seed coats were removed manually, dried and milled. Soaking, as well as fragmenting and dehulling steps of QPM and beans, respectively, were used as precondition for mold and enzyme penetration, and for proper mycelial growth. Soaked fragmented maize and bean cotyledons were cooked (90°C/30 min) in acidified distilled water (pH=3.0), drained, cooled (25°C/3 h), inoculated with a *R. oligosporus* NRRL 2710 suspension (1x106 spores/mL), and packed in perforated polyethylene bags (15x15 cm). The SSB for fragmented maize and bean cotyledons was performed at 35°C/84 h and 38°C/100 h, respectively. The resulting bioprocessed samples were dried (50°C/8 h), cooled (25°C), and milled. The bioprocessed quality protein maize flour (BQPMF) was packed and kept at 4°C. The bioprocessed bean cotyledons flour was blended with its milled seed coats, packed, and stored at 4°C.

Macronutrients analysis

The official AOAC [16] methods 925.09B, 920.39C and 960.52 were used to determine moisture, lipids and protein (Nx6.25) contents, respectively. Soluble and insoluble dietary fiber (SDF/IDF) were measured according to the AOAC [16] enzymatic-gravimetric method for total dietary fiber (method 985.29), using the total dietary fiber assay kit from Sigma-Aldrich (TDF 100A). Resistant starch (RS) was determined using a Megazyme Resistant Starch kit. The enzymatic assay was conducted according to a laboratory protocol based on AACC [17] method 32-40.01.

In vitro protein digestibility (IVPD)

The IVPD was determined according to Hsu et al. [18]. A multi-enzyme solution was used. This solution consisted of 8mg of pancreatic trypsin type IX (15600U/mg, Sigma T-0303), 15.5mg of bovine pancreatic chymotrypsin type II (83.9 U/mg, Sigma C-4129), 6.5mg of porcine intestinal peptidase grade III (102 U/g, Sigma P-7500) and 5 mL of distilled water. Five milliliter aliquots of the multi-enzyme solution were added to 50 mL of aqueous protein suspension (6.25 g of protein/L, pH 8.0), with stirring at 37°C in a water bath. The rapid pH drop was recorded automatically over a 10 min period using a pH meter. IVPD was calculated from the equation

$$IVPD = 210.46 - 18.10\ X$$

when X = pH after 10 min. All measurements were made by triplicate.

Essential amino acid analysis

Essential amino acid content was determined using the method described by Lopez-Cervantes et al. [19] with some modifications. Flour samples (50 mg) were weighed in a tube with a screw cap, and then 10 mL of 6 M HCl was added. The solutions were incubated at 110°C for 24 hours. The resulting solution was vacuum-filtered through Whatman no. 41 paper. The filtrate was diluted with ultra-pure water (Milli-Q) with the purpose of obtaining a final concentration of approximately 0.1 mg/mL. Finally, 300 µL of this solution was added to vials, in triplicate, dried in a vacuum oven at 60 °C overnight, and derivatized with 300 µL of 9-fluorenymethyl-chloroformate (FMOC). In the chromatographic analysis an aliquot (20 µL) was analyzed using an analytical scale (4.6 mm x 250 mm) SGE Hypersil ODS C18 column (SGE, Dandenong, Australia) kept at 38°C and connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector LC 5100. The mobile phases used were as follows: (A) 30 mM ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol/water; (B) 15:85 (v/v) methanol/water; and (C) 90:10 (v/v) acetonitrile/water. Fluorescence detection was at 270 nm and 316 for excitation and emission, respectively. A calibration curve was constructed using a mix of standard amino acids.

Samples (25 mg) were mixed with 3 mL of 4.2 M NaOH and incubated in sealed tubes (N2 atmosphere) at 120°C for 4 h. After hydrolysis, the sample was adjusted to pH 9, washed with borate buffer (pH 9), vacuum filtered and then diluted to 50 mL with borate buffer. After centrifugation, the supernatant was filtered (0.45µm) and then a 20 µL aliquot was analyzed as described above. Tryptophan was detected at 280 nm with an ultraviolet detector.

Chemical score (CS)

The most limiting amino acid in the sample was identified, for which the content of each of the essential amino acids (EAA) was compared with that recommended for FAO (3 years and older) [20]. The chemical score was calculated as follows:

$$CS = (\text{Content of the most limiting EAA} / \text{REAAR}) \times 100$$

Where CS is the chemical score; EAA is the essential amino acid and REAAR is the recommended essential amino acid requirement. All determinations were made by triplicate.

Calculated protein efficiency ratio (C-PER)

The C-PER was calculated using the procedure of the AOAC [16]. This procedure was based on the IVPD and the essential amino acids (EAA) composition of the optimized mixture.

Extraction of free phenolic

One gram of dry ground sample was shaking in 10 mL of chilled ethanol–water (80:20, v/v) in a tube rotator at 50 rpm for 10 min. Then, the supernatant was recovered by centrifugation (3000xg, 10 min) (Sorvall RC5C, Sorvall Instruments, Dupont, Wilmington, DE, USA). The extracts were concentrated to 2 mL at 45°C using a vacuum evaporator (Savant SC250 DDA Speed Vac Plus centrifugal, Holbrook, NY, USA) and stored at -20°C until use. All extractions were made by quadruplicate.

Extraction of bound phenolic

The residues (pellets) from free phenolic extraction were hydrolyzed for 30 and 60 min with 10 mL of 2 M NaOH at 95°C and 25°C, respectively, in a shaking water bath at 60 rpm. The hydrolysate was neutralized with HCl before removing lipid with hexane. The final solution was extracted five times with 10 mL of ethyl acetate and the pool was evaporated to dryness. Bound phenolic compounds were reconstituted in 2 mL of 50% methanol and stored at -20°C until use. All extractions were made by quadruplicate.

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed essentially as described by Ou et al. [21] to determinate hydrophilic antioxidant activity of free and bound phenolic extracts. Extracts were evaluated against a standard of Trolox with Fluorescein as a probe. Briefly, AAPH [2,2-azobis (2-amidinopropane) dihydrochloride] (0.414g) was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM and made fresh daily. A fluorescein stock solution (4 x 10-3 mM) was made in 75 mM phosphate buffer (pH 7.4) and stored wrapped in foil at 5°C. Immediately prior to use, the stock solution was diluted 1:1000 with 75 mM phosphate buffer (pH 7.4). The diluted sodium fluorescein solution was made fresh daily. In regards to the plate usage, the exterior wells were not used for experimental determinations. These wells were filled with 300 µL of water, while the interior wells were used for experimental determinations. To all experimental wells, 150 µL of working sodium fluorescein solution was added. In addition blank wells received 25 µL of 75 mM phosphate buffer (pH 7.4), while standards received 25 µL of Trolox® dilution, and samples received 25 µL of sample. The plate was then allowed to equilibrate by incubating for a minimum of 30 minutes in a Microplate Reader (SynergyTM HT Multi-Detection, BioTek, Inc., Winooski, VT, USA) at 37°C. Reactions were initiated by the addition of 25 µL of AAPH solution using the microplate reader's injector for a final reaction volume of 200 µL. The fluorescence was then monitored kinetically with data taken every two minutes. Data was expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (dw) sample. All measurements were made by triplicate.

Total phenolic content (TPC)

The concentration of phenolic compounds in free and bound extracts was determined using spectrophotometric method [22]. The reaction mixture was prepared by mixing 20 µL of ethanolic or methanolic solution of free and bound extracts, respectively, with 180 µL 2M Folin-Ciocalteu (Sigma Chemical Co., St Louis, MO, USA) reagent, and 50 µL 7% $NaHCO_3$. Blank was concomitantly prepared, containing 20 µL of ethanol or methanol, 180 µL 2M Folin-Ciocalteu, and 50 µL 7% $NaHCO_3$. The samples were thereafter incubated at room temperature for 90 min. absorbance was measured at 750 nm using a Synergy Microplate Reader (Synergy™ HT Multi-Detection, BioTek, Inc., Winooski, VT). A calibration curve was prepared using gallic acid (Sigma Chemical Co., St Louis, MO, USA) as standard and total phenolics were expressed as milligrams of Gallic acid equivalents (mg GAE)/100 g dry weight sample.

Enzyme hydrolysis

The ground samples were hydrolyzed with pancreatin enzyme (a mixture of digestive enzymes) so its action simulated gastrointestinal digestion according to Humiski & Aluko [23]. The sample was mixed with deionized water to prepare 10% (w/v) solutions, then temperature and pH were adjusted at 39°C and 8.0, respectively. Hydrolysis reaction time was fixed at 2 h. After the hydrolysis, the slurries were adjusted at pH 4.0 with 2 mol/L HCl and were kept in a water bath at 95°C for 10 min to inactivate the enzyme, after that, they were centrifuged (10,000g/30 min/25°C); the supernatant containing the hydrolysates was recovered and preserved at -20°C. Degree of hydrolysis (DH) was determined with the pH-stat method. This method relies on the base consumption needed to maintain constant pH during the hydrolysis, which is then associated with the DH.

Angiotensin converting enzyme – inhibitory (ACE-I) activity

The antihypertensive potential of the hydrolysates was determined by its Angiotensin Converting Enzyme-Inhibitory (ACE-I) activity according to Miguel et al. [24] with modifications. ACE hydrolyses hippuryl-histidyl-leucine (HHL) to generate hippuric acid and the peptide His-Leu. The reaction mixture, consisting of the substrate (HHL) and hydrolysate sample was prepared in ACE buffer (50 mol/L sodium borate, containing 0.5 mol/L sodium chloride, pH 8.3) and was pre-incubated at 37°C for 5 min, after that, ACE was added to a final concentration of 2.5 mU/mL [one unit of enzyme will produce 1.0 µmol of hippuric acid from Hippuryl-His-Leu per min in 50 mM HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 300 mM NaCl at pH 8.3 at 37 °C]. Different dilutions of each mixture hydrolysate were added and incubated in the before mentioned reaction mixture for 30 min at 37°C; after that, the reactions were stopped by addition of 150 µL of 1 mol/L HCl solution, followed by addition of 1 mL of ethyl acetate, to extract hipuric acid, and mixed by vortex for 1 min. The mixture was centrifuged (14,000g/25°C/10 min), 750 µL of the organic phase was collected and transferred into a test

tube and evaporated. The residue was dissolved in 600 µL water and hippuric acid concentration was determined at 228 nm using water as a blank. In order to determinate the IC50 [defined as the concentration of peptide needed to inhibit 50% the activity of Angiotensin Converting Enzyme (ACE)] of sample; data was adjusted to a non-linear regression model using Hill's equation [GraphPad Software].

Results and Discussion

Chemical composition and nutritional properties of BQPMF and BCBF

The solid state bioconversion (SSB) process increased ($p<0.05$) the protein content (+ 26.8 - 33.5 %) and decreased ($p<0.05$) the lipid (- 32.7- 42.8%) contents of raw maize and common beans (Table 2). Previous studies of SSB with cereals and legumes have reported a significant increase in total protein content during the fermentation [1,12,25]. Sánchez-Magaña et al. [26] reported that the increase in protein content reflects the decrease of other constituents, which might have been lost by leaching during the initial steps of SSB or might have been consumed by the fungus for its growth. Ruiz-Terán & Owens [27] observed also a significant reduction in lipids during the fermentation of soybean, due to oxidation of fatty acids released by lipases from *R. oligosporus*; these fatty acids are used by the fungus as a source of energy.

The soluble, insoluble and total dietary fiber, and resistant starch contents in both, maize and common beans, increased ($p<0.05$), in dry weight, in 132-196%, 15-24%, 40-43%, and 130-133%, respectively, after bioprocessing (Table 2). These changes may be due to leaching out some compounds during steeping and cooking steps (before fermentation) and to fungi growth which have consumed carbohydrates and fat as an energy sources and the development of a fiber-rich fungous mycelium [28]. Resistant starch (RS) is formed during thermal processing of starch-rich foods, such as maize and bean grains. Gelatinization and retrogradation of the starch are important processes that govern the formation of RS; these phenomena occur during the steps of cooking and cooling of the SSB process [25]. RS is a fraction of starch that is not digested by amylolytic enzymes in the digestive track but is fermented by the gut bacteria in the colon and produces short-chain fatty acids and other organic acids. There is an interest in consuming dietary fibre and RS because of its health benefits including reduction of the glycemic and insulinemic responds to foods, obesity, diabetes, cardiovascular disease and colon cancer [29].

The EAA content of unprocessed and bioprocessed QPM and common bean flours is shown in Table 2. In general, the EAA content of proteins from unprocessed QPM and common beans was improved by SSB process; however, in QPM the Val levels decreased by 1.46 g/100 g protein; while in common beans the Leu, Lys and Trp levels decreased ($p<0.05$) 0.03, 0.21 and 0.06 g/100 g protein, respectively, although their final contents were still higher than those of the reference standards. The most significant effect of SSB process on QPM proteins was the improvements in the Lys and Trp contents; these EAA had increments in 1.43 and 0.14 g/100 g protein, respectively. Cuevas-Rodríguez et al. [1] also reported increments in Lys and Trp during SSB of QPM. Common bean proteins contain relatively high levels of Lys, but this amino acid decreased during SSB. Paredes-López & Harry [30] reported a significant reduction in Lys and Met during fermentation due, in part, to the conversion of amino acids by the action of transaminases produced by the fungus. However, the effect on the amino acid composition depends on the substrate employed due to the fact that the fungus does not depend upon a specific amino acid for growth.

The EAA scores of proteins from unprocessed and bioprocessed QPM and common beans flours were evaluated taking into account the suggested pattern of amino acid requirements for older children, adolescents and adults (3 years and older) [20] (Table 2). In proteins from untreated QPM, Trp was the first limiting EAA, with an EAA score of 0.88. Barragan-Salgado & Serna-Saldıvar [31] reported EEA scores for proteins from unprocessed QPM of 0.72 and 0.66, respectively. Therefore, the EEA scores and limiting amino acid of raw QPM flour were affected by the SSB process; in proteins from BQPMF there was not limiting EAA with EAA score of 1.00. Total sulfur (Met+Cys) was the limiting EAA in proteins from unprocessed bean flour with an EAA score of 0.90; SSB improved the EAA balance, resulting in a score of 1.00.

The SSB processes improved the *in vitro* protein digestibility (IVPD) of the QPM and common beans from 78.4 to 83.6 % and from 72.2 % to 88.2 %, respectively (Table 2). This improvement has been also observed using chickpea [25]. The increase in protein digestibility could be explained by the increase in susceptibility of proteins to the enzymatic hydrolysis due to the elimination of antinutritional factors (e.g. phytic acid, enzymatic inhibitors, tannins, etc.) during fermentation and denaturing of proteins during the cooking step. The SSB also increased the C-PER of the samples in 33-49 % (Table 2). This increase can be due to the improvement in digestibility and EAA content of the proteins.

Total phenolic content (TPC) and antioxidant activity (AoxA) of BQPMF and BCBF

The solid state bioconversion (SSB) increased ($p<0.05$) free, bound and total phenolic contents of both substrates, raw QPM and common beans, in 177.9-316.54%, 27.7-45.88%, and 69.5-127.02%, respectively (Table 3). Processing of the whole raw quality protein maize and common beans using SSB increased ($p<0.05$) the total hydrophilic antioxidant capacity (AoxA) in 38-75% when compared with the unprocessed materials (Table 3). It was also observed that the AoxA of free and bound phenolic compounds significantly increased ($p<0.05$) in 103-163% and 17-38%, respectively, after SSB (Table 3). The highest increments in TPC and AoxA corresponded to bioprocessed common bean flour (BCBF). Maiti & Majumdar [13] suggested that fungal β-glucosidase catalyze the release of aglycones from the bean substrate and consequently there is an increase in phenolic content. Kuan-Chen et al. [14] reported that β-glucosidase activity

of dehulled black soybean samples increased with SSB time using three different *Rhizopus strains [oligosporus* (BCRC 31996, NTU-5), *oryzae* (BCRC 30894)] and resulted in the accumulation of isoflavone aglycones. These results are in agreement with those reported by other researchers [4,13,14] who found that solid state bioconversion (SSB) would increase the TPC of fungal processed legumes and cereals which will enhance the potential health-relevant functionality, like Sánchez-Magaña et al. [26] who found that SSB process of chickpea improved *in vitro* α-amylase and α-glucosidase inhibition activities of phenolic extracts in 83 and 370%, respectively. Likewise these authors suggest that SSB is a good strategy to enhance health-linked functionality of chickpea, due to improved TPC, AoxA and content of strong natural inhibitors of enzymes associated with diabetes.

Table 3: Phenolic content and antioxidant and hypertensive potential of quality protein maize and common bean flours[1].

Property	Unprocessed QPM flour	BQPMF2	Unprocessed CB Flour	BCBF[3]
Phenolic content[4]				
Free	61.22±0.37^{c}	170.19±0.25^{b}	57.23±0.34^{d}	238.39±5.10^{a}
Bound	143.65±3.65^{c}	183.41±3.81^{b}	133.13±4.51^{d}	194.21±4.60^{a}
Total	208.38±4.19^{c}	353.11±4.31^{b}	190.67±4.27^{d}	432.85±6.30^{a}
Antioxidant activity[5]				
Free phytochemicals	36.79±1.32^{d}	74.69±1.60^{b}	38.84±1.30^{c}	102.30±2.13^{a}
Bound pytochemicals	111.25±2.23^{c}	130.40±2.18^{a}	90.64±1.63^{d}	125.03±2.54^{b}
Total	148.11±3.09^{c}	205.09±3.31^{b}	129.48±2.25^{d}	227.33±3.12^{a}
Antihypertensive activity (IC50)[6]	ND	358±11^{a}	79.22±0.21^{b}	0.0145±0.008^{c}

[1]Values are mean +SD, a-d Means with the same letter in the same row are not significantly different (Duncan, $p<0.05$)

[2]BQPMF= Bioprocessed quality protein maize flour

[3]BCBF= Bioprocessed common bean flour

[4]mg of Gallic acid equivalents (GAE)/100 g dry sample

[5]µmol Trolox equivalents (TE) / g dry sample

Maiti & Majumdar [13] reported the use of different GRAS (generally recognized as safe) filamentous fungi to enhance phenolic content and antioxidant activity of various foods crops by using SSB. Most phenolics in plants are conjugated to sugars as glycosides [32]. Salar et al. [4] reported the role of some hydrolytic enzymes (α-amylase, β-glucosidase, xylanase) in the release of bound phenolic compounds in *thamnidium* fermented maize.

Degree hydrolysis (DH) of BQPMF and BCBF

The degree hydrolysis (DH) values at 60 min were 37.5, 47.4, 45.5 and 58.4% for raw QPM, raw common beans, bioprocessed QPM flour (BQPMF), and bioprocessed common beans flour (BCBF), respectively. The SSB process improved the DH of QPM and common beans. It is possible that antinutritional factors (e.g. phytic acid, enzymatic inhibitors, tannins, etc.) still remain in raw QPM and common beans, which can result in a minor DH than the bioprocessed samples, while soaking and cooking treatments applied previously to the fermentation, can be promoted leaching and/or inactivation of antinutritional factors, increasing the DH of the samples. During fermentation step also occurs elimination of antinutritional factors caused by the action of the fungus, while during the cooking step, proteins are denatured contributing both steps to increasing the DH [33].

Angiotensin converting enzyme–Inhibitory (ACE-I) activity of BQPMF and BCBF

Non-linear fit plots of the data of ACE-I activity for bioprocessed QPM flour (BQPMF) and bioprocessed common beans flour (BCBF) are shown in Figure 2. Can be observed that BCBF had the highest ACE-I percentage (>90%). However, to determinate the potential antihypertensive of the samples, IC_{50} values were calculated. The BQPMF and BCBF had better IC_{50} than unprocessed maize (358 µg/mL vs ND) and common beans (0.0145 vs 79.22 µg/mL), respectively (Table 3). During bioprocessing by SSB of maize and common bean exists release of phenolic compounds, peptides and many other bioactive compounds capable of inhibiting ACE.

Valdez-Ortiz et al. [8] reported that common bean seeds are a valuable source of ACE inhibitors. Therefore, inhibition of ACE reduces the activity of Angiotensin II but increases Bradykinin levels, and thus can result in a lowering of blood pressure. It has been reported that many natural ACE inhibitors isolated from the hydrolysis of various grains proteins such as amaranth [34],

common beans [8] or during gastrointestinal digestion or food processing [35] can be used as pharmaceuticals and physiological functional food supplements for hypertension therapy. The results obtained in this work are encouraging, because IC_{50} values were in agreement or lower than those reported for pancreatic hydrolysates from common beans (60-319 µg / mL) [8] and pepsin-pancreatin hydrolysates from lima bean (250-692 µg/mL) [36].

Chemical composition and nutritional and nutraceutical properties of the bioprocessed mixture

The bioprocessed mixture (60% EQPMF+40%BCBF) contained 19.78% (dw) proteins, 2.65% (dw) lipids, 24.65% (dw) total dietary fiber, and 3.92% (dw) resistant starch (Table 4). The presence of dietary fiber and resistant starch in foods is important in health because they have been considered as functional ingredients to reduce colon cancer and battle obesity [29].

Table 4: Chemical composition, physicochemical, nutritional and nutraceutical properties of the functional flour.

Property	Functional Flour[1]	Requirements for older child, adolescent, and adult (3 years and older)[2]
Chemical Composition (% dw)		
Protein	19.78±0.17	
Lipids	2.65±0.07	
Total Dietary Fibre	24.65±0.12	
Soluble Fibre	7.67±0.10	
Insoluble Fibre	16.98±0.15	
Resistant Starch	3.92±0.06	
Nutritional		
EAA[3] (g / 100 g protein)		
His	3.41±0.03	1.6
Ile	3.14±0.03	3
Leu	8.51±0.05	6.1
Lys	5.91±0.06	4.8
Met+Cys	4.75±0.03	2.3
Phe+Tyr	9.31±0.10	4.1
Thr	3.98±0.07	2.5
Trp	1.10±0.02	0.66
Val	4.21±0.04	4
Total	44.32	33.9
EAA[3] chemical score	100	---
Limiting EAA	--	---
In vitro protein digestibility (%)	88.1±0.22	---
Calculated protein efficiency ratio	2.24±0.04	---
Nutraceutical		
Antioxidant activity[4]	199.24±2.67	---
Antihypertensive potential (IC_{50})[5]	25.12±0.53	---

[1]BQPMF= Bioprocessed quality protein maize flour; BCBF=Bioprocessed common bean flour

[2]Requirements of amino acids for older child, adolescent, and adult (3 years and older) according FAO (2013)

[3]EAA = essential aminoacids

[4]µmol Trolox equivalent / g sample (dw)

[5]µg peptide/mL suspension

The EAA content of the bioprocessed mixture was found to be higher than the requirements for older child, adolescent, and adult (3 years and older) (44.32 vs 33.9 g/100 g protein), its EAA score was 1.00, and did not present limiting EAA (Table 4). The bioprocessed mixture had *in vitro* protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER) values of 88.1% and 2.24, respectively (Table 4). Serna-Saldívar et al. [37] reported minor values of IVPD and C-PER (83.1-84.87% and 1.22-1.35, respectively) for wheat bread fortified with defatted soybean and sesame meals. Also, a similar value of IVPD (87.9%) and a minor value of C-PER (1.86) were reported by Alarcón-Valdez et al. [38] for infant food based a mixture of nixtamalized maize (26.7%) and extruded chickpea (73.3%) flours. Serna-Saldívar et al. [37] recommended the use of *in vitro* techniques due to the fact that they are fast and accurate indicators of protein digestibility and PER. They reported that although C-PER values underestimated the values obtained from rats, this *in vitro* technique predicted the same absolute differences between treatments observed in the rat bioassay.

The bioprocessed mixture showed an antioxidant activity of 199.24 µmol TE/g dry mixture and an antihypertensive potential IC_{50} value of 25.12 µg/mL suspension (Table 4). This IC_{50} value was lower than those reported for fermented products of soybean (80-360 µg/mL) [Pyo & Lee (35)] and lentils (180-200 µg/mL) [33]. The IC_{50} value of the bioprocessed mixture corresponded to an intermediate value between the IC_{50} values of bioprocessed QPM flour and bioprocessed common bean flour (0.0145 - 358 µg/mL; Table 3), while the antioxidant activity of the bioprocessed mixture was similar to the antioxidant activity value of bioprocessed QPM flour [205.09 µmol trolox equivalent/g sample (dw); Table 3] and lower than bioprocessed common bean flour [227.33 µmol trolox equivalent/g sample (dw); Table 3]. Although, the antihypertensive and antioxidant potential of bioprocessed common bean flour was better than the bioprocessed mixture, there aren´t reports in literature about the antihypertensive and antioxidant potential of mixtures of bioprocessed cereals and legumes, as the mixture of bioprocessed QPM flour + bioprocessed common beans flour. Likewise, according to Adom & Liu [3], the complex mixture of bioactive compounds in whole foods may be more healthful than individual isolated components.

Conclusion

The solid state bioconversion resulted in an effective strategy to improve nutritional value, phenolic content, antioxidant activity and antihypertensive potential of quality protein maize and common beans. The flours developed in this work, bioprocessed quality protein maize and common beans flours (BQPMF and BCBF, respectively), and its mixture (60% BQPMF+40% BCBF), can be considered functional foods of high nutritional value and with potential for the prevention and control of degenerative diseases such as hypertension and those derived from oxidative stress.

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