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Tempeh flour from chickpea (*Cicer arietinum* L.) nutritional and physicochemical properties

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Abstract

The effects of solid state fermentation (SSF) on physicochemical and nutritional properties of chickpea flour were studied. Fermented (tempeh) flour showed higher particle size index, gelatinization temperature, dispersability and resistant starch content, and lower gelatinization enthalpy and water solubility than unfermented flour. SSF increased the content of the essential amino acids (EAA) Ile, total sulphur (Met + Cys), total aromatic (Phe + Tyr), and Thr in 37, 41, 107, and 39 g kg^{-1} protein, respectively; Trp content decreased 8 g kg^{-1} protein. Total sulphur (EAA score = 0.87) was limiting in unfermented flour and Trp (0.93) in tempeh flour. SSP improved the *in vitro* and true protein digestibility (72.2–83.2% and 83.7–88.8%, respectively), protein efficiency ratio (PER, 1.59–2.31), cPER (1.54–2.21), and corrected protein digestibility (0.73–0.89). Chickpea tempeh flour may be considered for the fortification of widely consumed legume-based food products.

Keywords: Chickpea; Tempeh; Nutritional quality

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legumes in the world; it is a staple food crop in some tropical and subtropical countries. This crop is extensively cultivated in the Northwest of México, being a good source of proteins (180–290 g kg^{-1} of sample, DM) and essential amino acids such as Lys, Leu, Ile, and Trp; however, chickpea proteins are deficient in total sulphur-containing (Met + Cys) essential amino acids (Reyes-Moreno, Cuevas-Rodríguez, Milán-Carrillo, Cárdenas-Valenzuela, & Barrón-Hoyos, 2004). Furthermore, chickpea has several undesirable attributes, such as long cooking time, protease inhibitors, phytates and phenolic compounds, which must be decreased or eliminated for

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the effective utilization of this legume (Milán-Carrillo, Reyes-Moreno, Armienta-Rodelo, Carabez-Trejo, & Mora-Escobedo, 2000). Solid state fermentation (SSF) represents a technological alternative for processing a great variety of legumes and/or cereals to improve their nutritional quality and to obtain edible products with palatable sensory characteristics. Tempeh is a nutritious oriental fermented food produced by SSF of soybeans. Several other substrates have been used to prepare tempeh, e.g. common beans, chickpeas for animal consumption, rapeseed, lupine, home bean, ground nut, wheat, corn/soybean (Cuevas-Rodríguez, Milán-Carrillo, Mora-Escobedo, Cárdenas-Valenzuela, & Reyes-Moreno, 2004; Hachmeister & Fung, 1993; Sharma & Khetarpaul, 1997). In general, SSF can be performed with *Rhizopus* sp. fungi; an important function of the fungus during fermentation is the synthesis of enzymes, which hydrolyze some of the substrate constituents and contribute to the development of desirable texture, flavour and aroma of the product. Enzymatic hydrolysis may also decrease or eliminate antinutritional factors; consequently, the nutritional quality of the fermented food may be improved (Hachmeister & Fung, 1993). The potential of using SSF to improve the nutritional value of cereals and/or legumes has been evaluated (Egounlety, Aworth, Akingbala, Houben, & Nago, 2002; Mugula & Lyimo, 2000). However, there is still a need for more information related to the impact of SSF on the nutritional quality of chickpea. Therefore, the objective of this investigation was to evaluate the effects of SSF on physicochemical and nutritional properties of chickpea flour.

2. Materials and methods

2.1. Materials

Chickpea (*C. arietinum* L., cv Blanco Sinaloa 92) was cultivated at the Culiacán Valley Experimental Station of the National Research Institute for Forestry, Agriculture and Livestock (INIFAP), Sinaloa, México. Grains were harvested, cleaned and stored at 4 °C in tightly sealed containers until used. The *Rhizopus oligosporus* strain was obtained from the Laboratory of Microbiology, National School of Biological Sciences, National Polytechnic Institute (Mexico, DF).

2.2. Manufacture of chickpea tempeh flour

Tempeh flour was prepared using the procedure described by Reyes-Moreno et al. (2004). Chickpea seeds were soaked at 25 °C for 16 h in four volumes of a 0.9 M acetic acid solution (pH 3.1). Seeds were then drained and their seed coats removed manually. The cotyledons were then cooked at 90 °C for 30 min, cooled at 25 °C, inoculated with a suspension of *R. oligosporus* (1×10^9 spores/l), and packed in perforated polyethylene bags (15 × 15 cm). SSF was carried at 34.9 °C for 51.3 h. The resulting chickpea tempeh was dried at 52 °C for 12 h, cooled at room temperature (25 °C) and milled (UD, Cyclone Sample Mill, UD Corp., Boulder, CO, USA) to pass through an 80-US mesh (0.180 mm) screen. Chickpea tempeh flour was kept at 4 °C in tightly sealed containers until used.

2.3. Proximate composition

The following AOAC methods (1990) were used to determine proximate composition: drying at 105 °C for 24 h for moisture (method 925.098); incineration at 550 °C for ash (method 923.03); defatting in a Soxhlet apparatus with 2:1 (v/v) chloroform/methanol for lipids (method 920.39C with minor modifications); and microK-jeldahl for protein ($N \times 6.25$) (method 960.52). Carbohydrate content was estimated by difference.

2.4. Total colour difference (ΔE)

The surface colour of the samples was measured using a Minolta colour difference meter Model CR-210 (Minolta LTD, Osaka, Japan). The parameters L (0 = black, 100 = white), a (+ value = red, − value = green) and b (+ value = yellow, − value = blue) were recorded. The L, a and b values of a white standard tile used as reference were 97.63, 0.78 and −2.85, respectively. ΔE was calculated as $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where $\Delta L = L_{\text{std}} - L_{\text{sample}}$, $\Delta a = a_{\text{std}} - a_{\text{sample}}$ and $\Delta b = b_{\text{std}} - b_{\text{sample}}$.

2.5. Particle size index (PSI)

Flour samples (100 g) were placed in a series of US standard sieves (WS Tyler Inc., Mentor, OH, USA) with the following sizes: no. 40 = 420 μm; no. 60 = 318 μm, no. 80 = 180 μm, no. 100 = 150 μm. Sieves were shaken with a Ro-Tap machine (WS Tyler Inc., Meter, Mentor, OH, USA) for 10 min. The material retained on the sieves was expressed as percent over. To complete the particle size index of flours, the following formula was applied $\text{PSI} = \sum a_i b_i$; where a_i = percent over on sieve i, and b_i = coefficient relative to sieve i. The b_i values for sieves number 40, 60 and 80 were 0.4, 0.6 and 0.8, respectively. Over from the sieve no. 100 and from the pan were added and an overall $b_i = 1.0$ was assumed.

2.6. Bulk density (ρ_A)

The ground samples were placed in a known volume stainless cylinder until topped at 25 °C. The device was topped five times and the flour density obtained dividing the sample mass by the cylinder volume.

2.7. Water activity (A_W)

This parameter was determined in 5 g flour samples, tempered at 25 °C, using a Hygrometer Aqua Lab Model

CX-2 (Decagon Devices Inc., Pullman, WA, USA), which was calibrated with a saturated potassium chloride solution ($A_W = 0.841$ at 25 °C). Readings were taken after leaving the sample for 1 h to attain headspace equilibrium.

2.8. Differential scanning calorimeter (DSC)

Thermal analysis was performed using a differential scanning calorimeter DSC (TA Instruments Model 2010, New Castle, DE, USA) previously calibrated with indium. Powder samples (2 mg, DM) were weighed directly into DSC aluminium pans, and after addition of deionised water (20 μl), pans were sealed and allowed to equilibrate for 1 h. The heating rate was 10 °C/min, from 30 to 120 °C. An empty pan was used as reference for all measurements. The parameters evaluated were: ΔH (enthalpy of crystal fusion), and T_o (onset temperature of gelatinization).

2.9. Total starch (TS)

Total starch was measured as described by Goñi, García-Alonso, and Saura-Calixto (1997). Fifty milligram samples were dispersed in 6 ml of 2 M KOH and vigorously shaken at room temperature for 30 min. After addition of 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 μl of amyloglucosidase (Sigma A-9913), the samples were incubated for 45 min at 60 °C in a shaking water bath. Starch was measured as glucose with Peridochrom Glucose GOD-PAP (Ref. 676543, Boehringer). The conversion factor from glucose to starch was 0.9.

2.10. Resistant starch (RS)

Resistant starch was measured using the method described by Saura-Calixto, Goñi, Bravo, and Mañas (1993), which determines RS from insoluble dietary fibre. The procedure consists of an enzymatic hydrolysis of starch with a heat stable alpha amylase (Sigma No. 4-3306, St. Louis, MO, USA), followed by degradation with a protease (Sigma No. P-5380) and a final hydrolysis with amyloglucosidase (Sigma A-9913) to yield glucose. The insoluble dietary fibre was obtained after several steps of rinsing and centrifugation. The RS was extracted from the insoluble residue with 2 M KOH and retreated with amyloglucosidase oxidase/peroxidase (GOD-POD). RS was calculated as glucose (mg) × 0.9 (conversion factor due to starch hydrolysis).

2.11. Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were assessed as described by Anderson, Conway, Pfeifer, and Griffin (1969). Each flour sample (2.5 g) was mixed with 30 ml of distilled water in a tared 60 ml centrifuge tube. The slurry was stirred with a glass rod for 1 min at room temperature and centrifuged at $3000 \times g$ for 10 min. The supernatant was then poured carefully into a tared evaporating dish. The WAI was calculated from the weight of the remaining gel and expressed in gram of solids/gram of original solids. The WSI (gram of solids/gram of original solids) was calculated from the weight of dry solids recovered by evaporating the supernatant overnight at 110 °C.

2.12. Dispersability

It was determined according to Mora-Escobedo, Paredes-López, and Gutiérrez-López (1994). One gram of flour sample was suspended in a graduated conic tube with 10 ml of distilled water and agitated at 1000 rpm for 5 min.

2.13. pH

The pH of flour samples was recorded using a pH meter. Each flour sample (10 g) was suspended in 100 ml of boiling distilled water. After cooling, the slurry was shaken (1500 rpm, 25 °C, 20 min) using an orbital shaker (Cole Parmer Model 21704-10, Cole Parmer International, Vernon Hills, IL, USA).

2.14. In vitro protein digestibility (IVPD)

The method proposed by Hsu, Vavak, Satterlee, and Miller (1977) was used to determine IVPD. A multienzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III (Sigma Chemical Co., St. Louis, MO, USA), was used. Chickpea flours and distilled water were used to prepare 50 ml of an aqueous protein suspension (6.25 g protein/l) with pH adjusted to 8.0, while stirring in a water bath at 37 °C. The multi-enzyme solution was maintained in an ice bath. Five millilitres aliquots of the multi-enzyme solution were added with stirring to the protein suspension at 37 °C. The rapid pH drop was recorded automatically over a 10 min period using a pH meter. IVPD was calculated from the equation $\mathrm{IVPD} = 210.46 - 18.10X$, where $X =$ pH after 10 min.

2.15. Amino acid analysis

Five to ten milligrams of each sample was placed in 2 ml ampoules containing internal standard (norleucine) and 0.4 ml of 6 M HCl. The ampoules were evacuated, sealed, and placed in an oven at 110 °C for 24 h. After hydrolysis, a 20 μl aliquot of the hydrolisate was withdrawn, dried, hydrated, re-dried, and subjected to derivatization. Samples for cysteine determination were first oxidized with performic acid at 25 °C for 18 h. Performic acid was removed with the aid of an evaporative centrifuge and the samples hydrolyzed as described above. The tryptophan content was determined in a separate analysis. The samples were hydrolyzed in polypropylene tubes with a 4.2 M KOH

solution containing 10 g of thiodiglycol at 110 °C for 18 h. After hydrolysis, KOH was neutralized with 2 M perchloric acid. The supernatant was removed, adjusted to pH 3 with diluted acetic acid and a 50 μl aliquot was used for derivatization. Quantification was achieved using a Pierce Standard H amino acid calibration mixture that was supplemented with tryptophan. The amino acid analysis was performed using the Pico-Tag system (Waters, Milford, MA, USA). After hydrolysis, aliquots were dried, mixed with 10 μl of ethanol:water:triethylamine (2:2:1), dried again and reacted with 20 μl phenylisothiocyanate reagent (ethanol:water:triethylamine:phenylisothiocyanate, 7:1:1:1) at 25 °C for 20 min (Cohen & Strydom, 1988). Excess reagent was removed with the aid of a vacuum pump. Derivatized samples were dissolved in 0.1 ml of 0.14 M sodium acetate (pH 6.4). A 10 μl aliquot was injected onto the column. Tryptophan was analyzed with a Waters C18 reversed-phase column (3.9 × 150 mm) (Waters, Milford, MA, USA) using the conditions described by Buzzigoli et al. (1990). This column was used to achieve complete resolution of tryptophan and ornithine. Ornithine was produced by alkaline hydrolysis of arginine. Analysis of the other amino acids was carried out using a Waters C18 column (3.9 × 150 mm) with gradient conditions described elsewhere (Bindlingmeyer, Cohen, & Tarvin, 1984).

2.16. Protein quality evaluation

Protein quality evaluation of the reference and chickpea flours was performed on 40 growing male Wistar rats, weighing 45 ± 5 g at the beginning of the study. Each protein diet was tested on eight animals randomly allocated in individual cages (Eggum, 1973). The cages were housed in a room at 20 ± 1 °C and 55% relative humidity, under 12 h light/12 h dark cycles. Diets had the following composition: 10 g of protein, 9 g of fat, 2 g of vitamin mix, 5 g of mineral mix, 5 g of cellulose and corn starch to complete 100 g. Corn oil was used as the fat source. The vitamin and mineral mixes were AIN-93-VX and AIN-936-MX, and were obtained from Harland Tekland Laboratory Animal Diets (Madison, WI, USA). Sodium caseinate was used as reference. Another group of 16 animals were fed with a protein-free diet for assessment of endogenous nitrogen. Food and deionised water were given *ad libitum*. Rats were fed with test diets containing 10% protein for 3 days as preliminary acclimation period and 28 days for determination of protein efficiency ratio (PER). Feed intake was recorded every other day. Weight gain was recorded weekly. Net protein retention (NPR) was measured for an 8 days period during test days 18–26. Feed and fecal nitrogen contents were analyzed by microKjeldahl (method 960.52) (AOAC, 1990). Apparent digestibility (AD, %), PER and true digestibility (TD, %) were determined according to Eggum (1973). TD was corrected for endogenous excretion of nitrogen. The following equations were used:

$$\mathrm{PER} = \mathrm{WG}/\mathrm{PC}$$

$$\mathrm{NPR} = (\mathrm{WG} + \mathrm{WLPFG})/\mathrm{PC}$$

$$\mathrm{AD}\ (\%) = 100(\mathrm{TNintake}^{18\text{–}26\ \mathrm{d}} - \mathrm{TNfecal}^{18\text{–}26\ \mathrm{d}})/\mathrm{TNintake}^{18\text{–}26\ \mathrm{d}}$$

$$\mathrm{TD}\ (\%) = 100(\mathrm{TNintake}^{18\text{–}26\ \mathrm{d}} - \mathrm{TNfecal}^{18\text{–}26\ \mathrm{d}} - \mathrm{TNfecal\ protein\ free\ diet}^{18\text{–}26\ \mathrm{d}})/\mathrm{TNintake}^{18\text{–}26\ \mathrm{d}}$$

where WG = weight gain (g), PC = protein consumed (g), WLPFG = weight loss of protein free group (g) and TN = total nitrogen(g).

2.17. Calculated protein efficiency ratio (C-PER)

C-PER was calculated according to Satterlee, Marshall, and Tension (1979) and summarized by the AACC (2000). This procedure is based on using the IVPD and the EAAs composition of the different flour samples (untreated chickpea flour, chickpea tempeh flour).

2.18. Protein digestibility corrected amino acid score (PDCAAS)

The PDCAAS has been adopted as a current concept in protein quality evaluation since it is more relevant to human requirements (Sarwar & McDonough, 1990). The PDCAAS method was conducted in two steps. The first involved the determination of the TD (%) of casein and chickpea flour diets. In the second step, the amino acid content was used to calculate the chemical score of the protein in the diets. PDCAAS was calculated according to the following equation: PDCAAS = (TD)(lowest AA score).

2.19. Statistical analysis

Results were analyzed with Design Expert Software (version 6.04, STAT-EASE Inc., MN, USA) using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test comparisons among means. Significance was defined at $p \leqslant 0.05$.

3. Results and discussion

3.1. Physicochemical properties of chickpea flours

The results of some physicochemical properties of unfermented and tempeh chickpea flours are shown in Table 1. Chickpea tempeh flour had higher ($p \leqslant 0.05$) ΔE and lower ($p \leqslant 0.05$) Hunter "L" than untreated raw chickpea flour. Soaking and cooking produced a significant increase in the "L" value of chickpea, meaning a lighter colour (data not shown), but fermentation resulted in a slightly darker colour, probably due to the influence of mycelia colour and the drying step. Despite the fermented flour had a higher ΔE than the unfermented sample, the colour of chickpea tempeh flour looked acceptable, although sensory

Table 1
Physicochemical properties of chickpea flours

Property[a]	Chickpea flour[b]	
	Unfermented	Tempeh
Colour		
Hunter "*L*" value	91.6 ± 0.38^{a}	86.3 ± 0.11^{b}
ΔE	16.7 ± 0.65^{b}	20.3 ± 0.72^{a}
PSI (%)	63.4 ± 0.64^{b}	74.5 ± 0.22^{a}
Bulk density (kg l^{-1})	0.41 ± 0.01^{a}	0.45 ± 0.01^{a}
A_W	0.42 ± 0.01^{a}	0.46 ± 0.01^{a}
T_g (°C)	66.1 ± 0.27^{b}	70.5 ± 0.25^{a}
ΔH (J g^{-1})	3.5 ± 0.07^{a}	0.9 ± 0.06^{b}
Total starch (g kg^{-1})	492 ± 11^{a}	484 ± 10^{b}
Resistant starch (g kg^{-1})	19 ± 1.1^{b}	76 ± 2.1^{a}
pH	6.3 ± 0.07^{a}	5.9 ± 0.04^{b}
WAI (kg gel kg^{-1} solids, DM)	2.2 ± 0.04^{b}	4.2 ± 0.06^{a}
WSI (kg solids kg^{-1} original solids)	28.3 ± 0.70^{a}	11.3 ± 0.58^{b}
Dispersability (%)	24.6 ± 0.78^{b}	66.5 ± 0.98^{a}

[a] ΔE = total color diference; PSI = particle size index; A_W = water activity; T_g = gelatinization temperature; ΔH = gelatinization enthalpy; WAI = water absorption index; WSI = water solubility index.
[b] Means were separated by rows using Duncan's multiple range test. Means with same letter are not significantly different at $p \leqslant 0.05$.

studies were not conducted. The PSI is a measurement of flour finesse where higher values mean smaller flour particles. The PSI value of tempeh flour (74.5%) was significantly higher than that of the unfermented flour (63.4%). Bulk density and water activity were similar for both samples. The values for water activity (0.42–0.46) were in a range where the growth of microorganisms, as well as enzymatic and chemical reactions occur slowly, meaning a long shelf life.

Gelatinization temperature and enthalpy were different ($p \leqslant 0.05$) between unfermented and tempeh chickpea flours (Table 1). Gelatinization temperature was higher while gelatinization enthalpy was lower in tempeh flour with respect to the unfermented flour. The lower gelatinization enthalpy value in the tempeh flour may be the result of more severe processing conditions, since it has been shown that a drastic thermal treatment produces starch gelatinization with a higher degree of disorganization. This suggests starch becomes more gelatinized in tempeh flour than in untreated chickpea flour.

Total starch (TS) values were slightly higher in untreated chickpea flour than in tempeh flour (49.2 *vs.* 48.4 g/100 g of dry flour). This may be a consequence of partial removal of non-starch constituents during the SSF process. Meares, Bogracheva, Hill, and Hedley (2004) reported TS values of 45.2 and 42.1 g/100 g of dry flour for untreated desi and kabuli chickpea flours, respectively, which are similar to the results obtained in this study. With respect to resistant starch (RS), tempeh flour showed higher ($p \leqslant 0.05$) values than unfermented chickpea flour (7.6 *vs.* 1.9 g/100 g dry flour). This result can be explained based on the heat treatments that the grain suffers during the SSF process. These treatments may have promoted the interaction between starch and other components (proteins, lipids or itself), making it less accessible to enzyme hydrolysis (Saura-Calixto et al., 1993). Kutos, Golob, Kac, and Plestenkak (2003) studied the effect of different thermal processing conditions on RS content of common bean (*Phaselous vulgaris* L.); they found values of RS almost twice higher in cooked samples than in uncooked samples. During the thermal processing of starch rich foods, RS is formed due to amylose retrogradation. In recent years, resistant starches have been introduced as functional ingredients important to human nutrition. The physiological importance of RS has been investigated in relation to the reduction of the glycemic and insulinemic response to a food, as well as hypocholesterolemic and protective effects against colorectal cancer (Asp, Van Amelsvoort, & Hautvast, 1996).

Chickpea tempeh flour showed higher ($p \leqslant 0.05$) WAI and lower ($p \leqslant 0.05$) WSI than untreated flour; partial protein denaturation and starch gelatinization occurring during the cooking step may be responsible for these changes. The dispersability was much higher in Tempeh flour than in unfermented flour.

3.2. Essential amino acid (EAA) content of chickpea tempeh flour

EAA content of untreated chickpea and tempeh flours are shown in Table 2. Unfermented and tempeh flours contained 38.23 and 40.5 g EAA/100 g protein, respectively; these values are higher than those recommended by the FAO/WHO for children 2–5 years old (33.9 g EAA/100 g of protein). When compared with FAO/WHO (1991) reference standards, proteins from unfermented chickpea showed higher values of EAA for His, Ile, Leu, Lys, total aromatic (Phe + Tyr), Thr, and Val; however, they had lower levels of total sulfur (Met + Cys) and similar content of Trp. In general, EAA content of proteins from unfermented chickpea was improved by the SSF process; the

Table 2
Essential amino acids content of chickpea flours

EAA[a]	Chickpea flour[b]		EAA requirements 2–5 years[c]
	Unfermented	Tempeh	
His	2.43^{b}	2.54^{a}	1.9
Ile	3.19^{b}	3.56^{a}	2.8
Leu	7.14^{a}	7.22^{a}	6.6
Lys	6.39^{a}	6.09^{b}	5.8
Met + Cys	2.18^{b}	259^{a}	2.5
Phe + Tyr	8.80^{b}	9.87^{a}	6.3
Thr	3.46^{b}	3.85^{a}	304
Trp	1.10^{a}	1.06^{b}	1.1
Val	3.54^{b}	3.76^{a}	3.5
Total	38.23^{b}	40.5^{a}	33.9
Limiting EAA	Met + Cys	Trp	
EAA score	0.87	0.96	

[a] g EAA kg^{-1} total protein.
[b] Means were separated by rows using Duncan's multiple range test. Means with same letter are not significantly different at $p \leqslant 0.05$.
[c] FAO/WHO (1991).

content of Ile, total sulphur (Met + Cys), total aromatic (Phe + Tyr), Thr, and Val increased significantly ($p \leqslant 0.05$) in 0.37, 0.41, 1.07, 0.39, and 0.22 g/100 g protein, respectively. However, Lys and Trp levels decreased from 6.39 to 6.19 g/100 g protein and from 1.10 to 1.06 g /100 g protein, respectively. Robinson and Kao (1977) prepared tempeh from chickpea and reported a significant increase in Met content. Paredes-López and Harry (1988) reported that the amino acids Lys and Met were released in higher amounts during fermentation. They suggested that a biochemical mechanism such as transamination might be taking place during SSF.

The EAA scores of proteins from unfermented and fermented chickpea flours were evaluated taking into account the suggested pattern of amino acid requirements for children 2–5 years old (FAO/WHO, 1991) (Table 2). Total sulphur (Met + Cys) was the first limiting EAA in proteins from untreated chickpea with an EAA score of 0.87. Therefore, the EAA score and limiting amino acids of unfermented chickpea flour were affected by the SSF process; in proteins from chickpea tempeh flour, Trp was the first limiting EAA with an EAA score of 0.93.

3.3. Nutritional properties of chickpea tempeh flour

In vitro protein digestibility (IVPD) and biological values of proteins from unfermented and fermented chickpea flours are shown in Table 3. The IVPD was improved by the SSF process; proteins from unfermented and tempeh flours had IVPD of 72.20% and 83.20%, respectively. Paredes-López and Harry (1990) reported an increase of the IVPD in common beans as a consequence of the same process. Increases in IVPD could be explained by the elimination of antinutritional factors (e.g. hydrolysis of phytic acid during fermentation) and protein denaturation during the cooking step, which results in proteins that are more vulnerable to enzyme action. True digestibility (TD) is an indicator of the amount of nitrogen/protein absorbed from a particular diet. The results of the animal studies showed that the TD of chickpea tempeh flour increased significantly ($p \leqslant 0.05$) when compared to unfermented chickpea flour. As expected, rats fed the control casein used dietary protein more efficiently when compared to counterparts fed the untreated chickpea and chickpea tempeh flours (Table 3).

Table 3
Nutritional properties of chickpea flours

Property[a]	Chickpea flour[b]		Casein
	Unfermented	Tempeh	
Protein digestibility (%)			
In vitro	72.20 ± 0.1^{c}	83.20 ± 0.1^{b}	90.06 ± 0.12^{a}
In vivo			
Apparent	81.10 ± 1.4^{c}	86.20 ± 1.3^{b}	90.70 ± 1.6^{a}
True	83.70 ± 1.1^{c}	88.80 ± 1.5^{b}	93.20 ± 1.2^{a}
PER	1.59 ± 0.08^{c}	2.31 ± 0.10^{b}	2.50 ± 0.05^{a}
NPR	2.65 ± 0.06^{b}	3.02 ± 0.10^{a}	3.02 ± 0.06^{a}
C-PER	1.54 ± 0.07^{c}	2.21 ± 0.08^{b}	2.48 ± 0.09^{a}
PDCAAS	0.73 ± 0.05^{c}	0.92 ± 0.02^{b}	1.11 ± 0.03^{a}

[a] PER = protein efficiency ratio; NPR = net protein retention; C-PER = calculated protein efficiency ratio; PDCAAS = protein digestibility corrected amino acid score.

[b] Means were separated by rows using Duncan's multiple range test. Means with the same letter are not significantly different $p \leqslant 0.05$.

The improved EAA patterns or scores (Table 2) and the higher nitrogen retention values observed in the chickpea tempeh flour clearly improved rat performance (Table 3). PER and NPR were improved significantly ($p \leqslant 0.05$) from 1.59 to 2.31 and from 2.65 to 3.02, respectively, as a consequence of the SSF process. The improvement of PER during fermentation can be attributed to better availability of amino acids, greater digestibility of the proteins in the substrates, and the conditions used during tempeh production. The high protein quality of chickpea tempeh flour makes this kind of products particularly attractive for countries where the high prevalence of protein-energy malnutrition is due largely to the poor nutritional quality of the diet.

C-PER's for proteins from unfermented and fermented chickpea flours were 1.54 and 2.21, respectively (Table 3); these values are higher than those reported by other researchers (Cuevas-Rodríguez et al., 2004; Faris & Takruri, 2002; Sullivan & Carpenter, 1993) for corn meal (1.1), wheat flour (0.8), soy flour (1.3), and quality protein maize (1.43). C-PER model is based on the essential amino acid profile and protein digestibility analysis.

The PDCAAS index reflects the ability of the test protein to meet the protein needs of an individual. This is a better predictor of protein quality for humans than the rat growth method, which is in many cases the only convenient *in vivo* approach (Sarwar & McDonough, 1990). FAO/WHO (1991) recommends that PDCAAS must be >0.6 to meet the amino acid needs of pre-school age children (2–5 years). It is considered that PDCAAS may replace the need for assessing the overall protein quality. SSF process increased ($p \leqslant 0.05$) the PDCAAS of untreated chickpea flour; PDCAAS of unfermented and fermented chickpea flours were 0.73 and 0.90, respectively. Both values are higher than those reported for cooked chickpeas (0.46) and chickpea dip with taninah (0.70) (Faris & Takruri, 2002). Cuevas-Rodríguez et al. (2006) reported PDCAASof 0.55 and 0.83 for unfermented and fermented quality protein maize flours, respectively.

4. Conclusions

This study showed that solid state fermentation (SSF) could be used to improve the nutritional characteristics of chickpea. In comparison to unfermented chickpea flour, tempeh flour showed higher ($p \leqslant 0.05$) particle size index, gelatinization temperature, dispersability, and resistant starch content, and a lower ($p \leqslant 0.05$) gelatinization enthalpy and water solubility index. The essential amino acids content of untreated chickpea was improved by the SSF process; the contents of Ile, total sulphur (Met + Cys),

total aromatic (Phe + Tyr), and Thr were significantly increased ($p \leqslant 0.05$). The SSF process increased ($p \leqslant 0.05$) the nutritional indicators *in vivo* protein digestibility, protein efficiency ratio, net protein retention, calculated protein efficiency ratio, and protein digestibility corrected amino acid score. Based mainly on its nutritive value, chickpea fermented flour may be considered for the fortification of widely consumed legume-based food products.

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