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Research Paper

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NUTRITIONAL, PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF READY-TO-USE CHICKPEA AND SOYBEAN FLOUR

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Proteins are very crucial part of the human diet as they play a vital role in maintaining many physiological functions and human immune system. In the present study two major plant protein sources viz. chickpea and soybean grain were processed by hydrothermal treatment followed by mechanical process to prepare Ready-to-Use (R-T-U) flour and their nutritional, physico-chemical and functional properties were studied. Protein content of R-T-U flour from chickpea and soybean was 22.9% and 46.3% respectively whereas fat content increased by 16.7% and 36.8% respectively during processing. Ash content of chickpea and soybean decreased during processing by 16.6% and 18.7% respectively. The protein and starch digestibility of chickpea and soybean increased significantly during processing. The results from physico-chemical properties such as gel consistency, sediment volume and SEM microgram and also from functional properties such as, pasting profile, flow curve and DSC thermal properties indicated the presence of pre-gelatinized materials in R-T-U flours. Results will be useful for utilization of convenience Ready-to-Use processed chickpea and soybean flour for development of protein rich food formulations.

Keywords: Chickpea, Soybean, DSC, Protein digestibility, Starch digestibility, Flow curve

INTRODUCTION

Legumes are recognized as the best source of vegetable protein (Molina *et al.*, 2002). Legumes, including chickpea and soybeans are important crops in the world because of their nutritional quality. They are rich sources of complex carbohydrates, protein, vitamins and minerals (Costa *et al.*, 2006). Legumes possess numerous health beneficial principles, e.g., lower glycemic index for people with diabetes (Goni and Valentin-Gamazo, 2003), increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their high dietary fiber content (Chillo *et al.*, 2008).

Chickpea (*Cicer arietinum*) is an important legume withrich sourceof protein, folate, and dietary fibre. Besides

chickpea, soybean (*Glycine max*) also has a high protein content of 40%. Soybean has been reported to have potential cholesterol lowering effect. It is suggested that this effect might be due to components such as isoflavone or a peptidepeptide sequence that alters the intestinal absorption of cholesterol and bile acids. Soybeans are also reported to possess anti-diabetic and anti-carcinogenic properties (Christina, 1999). Hypocholesterolemic characteristics of legumes could be attributed to the nature of their carbohydrates, proteins and unsaturated fats. However an involvement of dietary fibre in lowering the blood cholesterol level has also been reported (Krichevsky and Story, 1974).

Addition of legume proteins to cereal based products could be an ideal option for both increasing the quantity and quality of proteins in the diet. Moreover, legume

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proteins are rich in lysine and deficient in sulphur containing essential amino acids, whereas cereal proteins are deficient in lysine, but have adequate amounts of sulphur amino acids. Therefore, the combination of cereals with legume proteins would provide better, overall essential amino acid balanced diet, helping to overcome the protein calorie malnutrition problem.

Chickpea and soybean flour in native (uncooked) form contain several anti-nutritional factors (R-galactosides, trypsin inhibitors, tannins, hemagglutinin, saponins, lectins, phytic acid, urease, etc.) which hinder their protein and carbohydrate digestibility, nutrients absorption and bioavailability. These anti-nutritional factors can be eliminated or reduced by proper cooking/hydrothermal treatments (Nestares *et al.*, 1993; Vidal *et al.*, 1994; and Urbano *et al.*, 1995).

These two legumes need longer soaking time and sufficient heat treatment (Veny and Kiran, 2012) for cooking before consumption. Hence, it is useful to develop readyto-use cooked legumes flour which can be conveniently incorporated to any ready-to-use mixes such as soup, unleavened pancake, roti, chapatti, dosa, etc. But the information on nutritional and physico-chemical properties of pre-gelatinized ready-to-use flour from chickpea and soybean is scanty. Hence, the present investigation was carried out to develop ready-to-use chickpea and soybean flourand to evaluate their nutritional, physico-chemical and functional properties.

MATERIALS AND METHODS

Chickpea dhal and whole soybeans were procured from local retail store, cleaned and used for study. All chemicals used were of analytical grade.

Sample Preparation

Chickpea native flour (CPN): Chickpea dhal was powdered using hammer mill (PREMIUM micro pulveriser, PREMIUM PULMAN Pvt. Lmtd., Ahmedabad, India) to get flour passing through 250micron aperture sieve.

Processed Chickpea Flour (CPP)

Dhal was soaked overnight; washed and steamed for 40 min. Steamed dhal was dried partially and flaked using flaking machine (Aktiebolaget, Kvarnmaskiner, Malmo, Sweden). Flaked dhal was further dried and powdered using hammer millto get flour passing through 250 micron aperture sieve. Thus, flour obtained is called processed chickpea flour.

Soybean Native Flour (SBN)

Whole soybean was milled using versatile dhal mill to get dhal splits. Dhal was powdered using hammer mill to get flour passing through 250micronaperture sieve.

Processed Soybean Flour (SBP)

Soybean dhal was soaked overnight, washed and steamed for 40 min. Steamed dhal was dried partially and flaked using flaking machine. Flaked dhal was further dried and powdered using hammer mill to get flour passing through 250 micron aperture sieve. Thus, flour obtained is called processed soybean flour.

Proximate Composition

Protein, fat, ash and moisture content were determined by standard AACC methods (2000).

Physical Properties

Physical properties *viz.* loose and pack bulk density, angle of repose, coefficient of friction on metal and glass plate were determined by following the procedure of Ghasemi *et al.* (2008). Colours of flours were determined using Konica Minolta CM-5 spectrophotometer colour measurement system. Colour readings were expressed by Hunter values for L*, a*, b* and ΔE^* .

Total Amylose Content

Defatted flour (100 mg, dry weight basis) taken in a 100 ml conical flask was wetted with distilled alcohol, to which 10 ml of 1 N NaOH was added gently, stoppered, and left overnight. Subsequently, it was boiled vigorously using boiling water bath for 10 min with gentle mixing intermittently, cooled to room temperature, and the volume was made up to 100 ml with distilled water. To an aliquot of the sample (5 ml), about 50 ml water, 1 ml acetic acid (1 N) and 2 ml of iodine solution (0.2%) were added, and the volume was made up to 100 ml. The blue colour developed was read at 630 nm against iodine blank (Sowbhaghya and Bhattacharya, 1979)

Sediment Volume

The defatted flour (2 g, dry weight basis) was taken in a 50 ml glass stoppered measuring cylinder and 40 ml of 0.05 N HCl was added, mixed thoroughly and one or two drops of amyl alcohol were added to prevent frothing. The contents were left at ambient conditions without disturbing and the sediment volume was noted after 4 h (Bhattacharya and Ali, 1976).



Gel Consistency

100 mg (dry weight basis) of flour was transferred to test tubes of equal length and diameter. To each of the test tube, 0.2 ml of ethanol containing 0.025% thymol blue was added and dispersed in 2 ml of 0.2 M KOH. Tubes were then heated in a vigorously boiling water bath for 8 min and cooled at room temperature for 5 min, followed by cooling in ice water bath for 20 min. Tubes were then laid on the graph sheet vertically undisturbed and distance travelled by gel was then noted after one hour (Unnikrishanan and Bhattacharya, 1988).

Water Absorption Capacity (WAC)

Ten millilitre of water was added to 1 g of each sample, the suspension was then stirred using a magnetic stirrer for 5 min. The suspension was then centrifuged at 5000 rpm for 30 min. The density of water was assumed to be 1 g/ml. The water absorbed was calculated as the difference between the initial water added and the volume of the supernatant obtained after centrifugation (Sathe *et al.*, 1982).

Oil Absorption Capacity (WAC)

The oil (refined soybean oil with density of 0.92 g/ml) and flour (1 g in 10 ml oil) were mixed using a magnetic stirrer for 5 min and then centrifuged at 5000rpm for 30 min. The oil absorbed was calculated as the difference between the initial oil added and the volume of the supernatant obtained after centrifugation (Sathe *et al.*, 1982).

In-Vitro Starch Digestibility

*In-Vit*ro Starch Digestibility (IVSD) was determined according to Holm *et al.* (1985).100 mg (starch equivalent) of defatted flour was taken in 100 ml conical flask.15 ml of distilled water, 0.1 ml of termamyl (alpha amylase) was added to it and cooked in boiling water bath for 30 min. 15 ml of 0.2 M glycine HCl buffer of pH 2 containing 10 mg of porcine stomach pepsin was added followed by the incubation at 37 °C for 2 h. 15 ml of 0.05 M phosphate buffer of pH 6.8 containing 15 mg of porcine pancreatin was added and the reaction mixture was then incubated at 37 °C for 2 h. Further, 15 ml of 0.05 M acetate buffer of pH 4.8 containing 20 mg of amyloglucosidase was added and incubated at 55 °C for 2 h. Total volume was made up to 100 ml. The amount of glucose released was estimated using DNS method (Miller, 1959).

In-Vitro Protein Digestibility

In-Vitro Protein Digestibility (IVPD) was determined according to Akeson and Stahmann (1964). To 100 mg

(protein equivalent) of flour, 50 ml of 0.1 N HCl was added. It was followed by addition of 12.5 mg of pepsin. It was incubated at 37 °C for 3 h. Subsequently, 25 ml of 0.05 M phosphate buffer with 6mg of pancreatin was added and was incubation at 37 °C for 24 h. Volume of the digested sample was made up to 100 ml. Protein digestibility values were obtained by Lowry's method (Lowry *et al.*, 1951).

Pasting Characteristics

Pasting characteristics were determined using Brabender viscoamylograph (Brabender GMBH & C0. Kulturstr, Germany) following standard test method according to Louis *et al.* (2009). 12% slurry (14% moisture basis) was heated from 30° to 92 °C at a rate of 7.5 °C/min and held at 92 °C for 5 min and then cooled to 50 °C at the same rate and held for 1 min at 50 °C. Measuring range used was 300 cmg at a speed of 250 rpm. The pasting indices measured were Peak Viscosity (PV), Hot Paste Viscosity (HPV), Cold Paste Viscosity (CPV), breakdown (BD) viscosity (PV-HPV); total setback (SB); (CPV-HPV). All viscosity parameters were expressed in Brabender Units (BU).

Viscosity Measurement

The viscosity was measured using plate-plate measuring system PP75 (Dia: 75 mm, gap 1 mm) (Modular compact rheometer MCR35, Anton Parr, Austria) at ambient temperature $(25 \pm 1 \,^{\circ}\text{C})$ using 28% slurry concentration for chickpea and soybean flours. The measurements were recorded at 50 data points at increasing shear rate range of 0 to 100 S⁻¹. Shear rate versus shear stress graph (viscosity curve) was plotted. As the systems were non-Newtonian in nature, the indicated viscosities are to be taken as apparent viscosities (Guha and Ali, 2011). Also, influence of temperature (10 to 40 °C) on flour slurry viscosities was studied using the same slurry concentration. Temperature versus viscosity graph was plotted.

Thermal Properties

Thermal properties of native and processed chickpea and soybean flour were assessed using Differential Scanning Calorimetry (DSC). 3 mg of flour samples were weighed into a stainless steel DSC pan, and 9ìL of distilled water was added. The mixture was hermetically sealed and equilibrated at room temperature for at least 24 h prior to heating from 25 to 180 °C ramping at 10 °C/min and a hermetically sealed empty pan was used as a reference. Nitrogen was used as a purging gas. Star^e software (ver. 9.20, Mettler Toledo) was used for the analysis of the thermograms. The onset



temperature (To), peak temperature (Tp), conclusion temperature (Tc) and transition enthalpy (Δ H) were determined.

Scanning Electron Microscopy (SEM)

Scanning electron microphotographs of flours were obtained using the LEO 435 VP (LEO Electron Microscopy Ltd., Cambridge, UK) at 20 kV. Samples were gold coated in a vacuum and scanned under at 2000X magnification and a representative microphotograph was taken and presented for interpretation.

Data Analysis

All the data were analysed using the Graphad Instat software. Each experiment was performed in triplicate, and the results were expressed as the mean values \pm standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by multiple comparison test (Tukey's test) at 5% level of significance. Values of $p \le 0.05$ were considered statistically significant.

RESULTS AND DI SCUSSI ON

The proximate composition analysis of native and processed legumes is presented in Table 1. The moisture content of R-T-U chickpea and soybean flour was 6.7% and 7.3%, indicating safe storage stability. The protein content of processed R-T-U flour from chickpea and soybean flour was 22.9% and 46.3% respectively. The protein content of the processed R-T-U chickpea flour decreased by 4.3% whereas it increased by 8.11% in processed soybean. This may be due to the differences in structural, morphological and functional quality of their proteins. The fat content of

both the legumes increased during processing and processed soybean showed higher increase (36.8%) than that of processed chickpea (16.7%). The hydrothermal treatment followed by mechanical degradation (roller flaking) might have increased the extractability of fat. Ash content of chickpea and soybean decreased by 16.6% and 18.7% respectively during processing. This may be due to excess water soaking and washing of the legumes.

Loose bulk density and pack bulk density values of native chickpea and soybean flour were 435 kg/m³, 587 kg/ m³ and 377 kg/m³, 466 kg/m³ respectively, and increased significantlyin both legumes after processing. Angle of repose of native and processed legumes ranged between 60° and 65° (Table 2). The coefficient of friction of legume flours on the metal plate showed higher values than on the glass plate. Higher coefficient of friction indicates poor flowability. These results are useful to design processing equipments, packing, transport and storage facilities of the products.

Colour values of chickpea and soybean flour changed significantly after processing. L* values measure lightness, a* values measure redness when positive and b* values measure yellowness when positive whereas ΔE gives deviation from the standard. There was a decrease in L* values for both processed flours compared to native, indicating darkening of the colour during processing (Table 2). This may be due to non-enzymatic browning reaction during hydrothermal treatment.

Total amylose content of native chickpea was higher (19.6%) compared to the native soybean (0.33%) (Table 3). Amylose content influences the physico-chemical properties

	CPN	СРР	SBN	SBP
Moisture Content	9.1±0.6 ^a	$6.7 {\pm} 0.52^{b}$	7.8±0.35 ^{ab}	7.3±0.8
Protein	23.96±0.8 ^a	22.91±0.6 ^a	42.8±1.0 ^b	46.3±0.
Fat	$4.8{\pm}0.2^{a}$	5.6 ± 0.4^{a}	17.1±0.6 ^b	23.4±1.
Ash	3.0±0.15 ^a	2.5 ± 0.3^{a}	6.4±0.28 ^c	5.2±0.4
Carbohydrate*	59.1±1.3 ^c	$60.4{\pm}1.0^{d}$	25.9±0.9 ^b	17.8±0.

Table 1: Proximate Composition (%) of Native and Processed Flours from Chickpea and Soybean

Note: *Carbohydrate by difference. (CPN: Chickpea native; CPP: Chickpea processed; SBN: Soybean native; SBP: Soybean Processed). Results are mean of three determinations \pm SD. Values with same letters (within rows) are not significantly different at $p \le 0.05$.



	2	CPN	СРР	SBN	SBP
Loose Bulk Density (kg/m ³)		435±8 ^b	652±10 ^d	377±7 ^a	432±8 ^b
Pack Bulk Density (kg/m ³)		587±9 ^c	765±5 ^d	466±10 ^a	502±4 ^b
Angle of Repose (°)		61±1 ^b	65±3 ^b	65±2 ^b	60±3 ^a
	Metal Plate	$1.73 {\pm} 0.07^{b}$	1.15±0.08 ^a	$1.07{\pm}0.04^{a}$	1.19±0.08
Coefficient of friction	Glass Plate	1.42±0.06 ^b	1.07±0.04 ^a	0.93±0.03 ^a	1.07±0.05
Colour	L*	$88.13 {\pm} 0.02^{d}$	83.61±0.01 ^a	86.21±0.02 ^e	84.83±0.04
	a*	2.13±0.01 ^d	1.78±0.005 ^c	$0.08{\pm}0.00^{a}$	0.12±0.01
	b*	22.05±0.1 ^b	26.85±0.03 ^d	$23.05 \pm 0.09^{\circ}$	21.01±0.08
	dE*	24.33±0.08 ^a	30.53 ± 0.03^{d}	25.91±0.09 ^c	24.77±0.08

Note: (CPN: Chickpea native; CPP: Chickpea processed; SBN: Soybean native; SBP: Soybean Processed). Results are mean of three determinations \pm SD. Values with same letters (within rows) are not significantly different at $p \le 0.05$.

	CPN	СРР	SBN	SBP
Total Amylose Content (%)	19.6±0.25 ^b	20.9±0.3 ^c	0.33±0.01 ^a	$0.5{\pm}0.03^{a}$
Sediment Volume (ml)	7.2±0.3 ^a	9.0±0.4 ^b	11.1±0.5 ^c	13.3±0.6 ^d
Gel Consistency (mm)	87±2.0 ^a	140 ± 3^{bc}	145±1.5 ^b	$150{\pm}0.8^{b}$
WAC(g/g)	$0.8{\pm}0.02^{a}$	2.2±0.1 ^d	$1.4{\pm}0.08^{b}$	1.8±0.1 ^c
OAC (g/g)	1.1±0.03 ^a	1.1±0.01 ^a	1.3±0.02 ^b	1.3 ± 0.05^{b}
IVSD (%)	80.45±1.2 ^c	$83.3 {\pm} 0.7^{d}$	22.5 ± 0.5^{a}	25.2±0.9 ^b
IVPD (%)	$70.8{\pm}0.8^{\circ}$	73.5 ± 1.0^{d}	55.5±0.6 ^a	58.3±0.7 ^b

Note: (CPN: Chickpea native; CPP: Chickpea processed; SBN: Soybean native; SBP: Soybean Processed). Results are mean of three determinations \pm SD. Values with same letters (within rows) are not significantly different at $p \le 0.05$.

and reactivity of starch. It inhibits the swelling, especially in the presence of lipids, which can form insoluble complexes during swelling and gelatinization (Atkin *et al.*, 1998). Moreover total amylose content increased during processing, this may be due to thermal and subsequently mechanical breakdown of the starch molecule. Similar observation was reported by Guha and Ali (2006) during extrusion cooking of rice.

Higher sediment volume is an index of the presence of pre-gelatinized starch. Sediment volume and gel consistency of chickpea and soybean flour increased significantly during processing (Table 3) indicating the presence of pregelatinized starch.

Water absorption capacity of processed chickpea and processed soybean flour increased by 2.75 and 1.28 folds respectively, indicating a suitable ingredient for preparation protein rich foods such as unleavened pancake, soup mix, etc. No change in oil absorption capacity was observed for both the flours after processing, indicating their suitability for low-fat snacks food formulations (Table 3). The increased capacity of flour to absorb and retain water may help to improve binding of the structure, enhance flavour



retention, improve mouth feel and reduce moisture loss from the food materials (Prinyawiwatkul *et al.*, 1997).

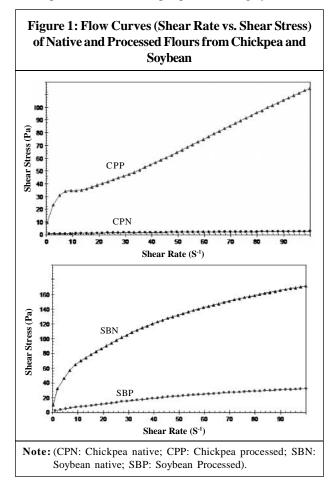
The IVSD and IVPD are the important predictors of physiological effects of a particular food. IVSD measures the susceptibility of starch to digestive enzymes and commonly used as a faster method to predict in-vivo Glycemic index (Susanna and Prabhasankar, 2013) whereas IVPD gives an indication of protein absorption. IVSD and IVPD of chickpea and soybean increased significantly (p \leq 0.05) during processing compared to their native counterparts (Table 3). Similar observations of increase in IVSD and IVPD during processing of chickpea and other legumes were also reported by other investigators (Saleh and Tarek, 2006; Veny and Kiran, 2012; and Kayembe and Rensburg, 2013). The increase in IVSD and IVPD of Readyto-Use processed legume flours is desirable as an ingredient for developing protein-rich health food formulations.

The visco-amylogram pasting profile of chickpea and soybean flour revealed that the pasting indices*i.e.* peak viscosity, hot paste viscosity and cold paste viscosity of processed chickpea decreased by 71.7%, 92.3% and 93.2% respectively compared to its native counterpart, whereas no significant changes in pasting profile were observed in soybean during processing (Table 4). This may be due to the presence of high protein content in soybean which has negligible role in increasing viscosity in slurry compared to starch. The ready-to-usepre-gelatinized legumes flour with low pasting profile is desirable for preparation of protein-rich calorie dense food formulations.

	CPN	СРР	SBN	SBP
PV	184±5 ^c	52±4 ^b	11 ± 2^a	13±1 ^a
HPV	143±6 ^b	11±3 ^a	6±1 ^a	7±1 ^a
CPV	251 ± 8^{b}	17±4 ^a	8±1 ^a	9±1 ^a
BD	41 ± 3^{b}	41±2 ^b	5±1 ^a	6±1 ^a
SB	108 ± 7^{c}	16±1 ^b	2±0.5 ^a	2±0.5
nati SBI	viscosities are in ve; CPP: Chick P: Soybean Pro rminations ± SD	pea processed ocessed). Res	; SBN: Soyb ults are mea	ean native n of thre

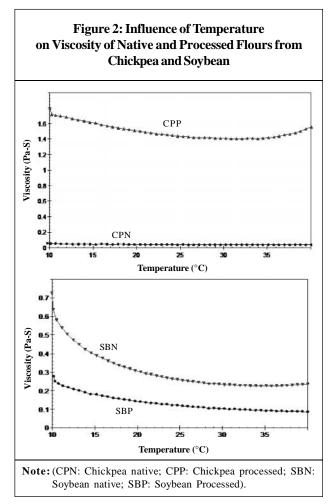
Flow behaviour of native and processed legume flours was studied by plotting the shear rate-shear stress relationship of 28% slurry at 25 °C over the shear rate range of 0 to 100S⁻¹ and are shown in Figure 1. It could be noted that an increase in shear rate increased the shear stress value. Moreover, processed chickpea flour exhibited higher shear stress than that of its native counterparts at any given shear rate. Whereas, processed soybean flour showed lower shear stress than that of its native counterpart at any given shear rate.

The viscosities of legumes slurries were measured at different temperature (10 to 40 °C) at a fixed shear rate and are presented in Figure 2. Viscosity of native and processed slurries decreased with increase in temperature. Moreover, viscosity of processed chickpea flour was higher than that of its native counterparts at any measured temperature, whereas processed soybean flour showed the opposite trend. This may be due to the less starch (carbohydrate) content of soybean, which might became unavailable due to complex formation with lipid/protein during hydrothermal



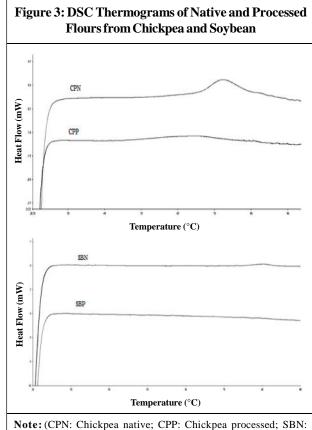
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processing. The pattern of the flow behaviour of legume flours also indicated non-Newtonian pseudoplasticbehaviour of the processed legume slurries which is an important parameter to consider for the design of the flow systems, selection of pumps, scale up and mechanization of the process.

A typical DSC endotherm for native chickpea and soybean flours were obtained (Figure 3). Native chickpea exhibited higher gelatinization enthalpy (4.29 J/g) than that of native soybean (0.78 J/g) (Table 5). Gelatinization enthalpy depends on a number of factors such as crystallinity, intermolecular bonding, etc. Transition temperatures (T_0 , Tp and Tc) for native chickpea and native soybean were 67.11, 71.87, 78.11 °C and 77.26, 80.13, 83.23 °C respectively (Table 5). Biliaderis (1990) and Leszkowiat *et al.* (1990) have suggested that higher transition temperature indicates more stable amorphous regions and lower degree of chain branching. The processed chickpea and processed soybean flours did not show any gelatinization endotherm when



Soybean native; SBP: Soybean Processed).

Table 5: DSC Characteristics of Native and Processed Flours from Chickpea and Soybean

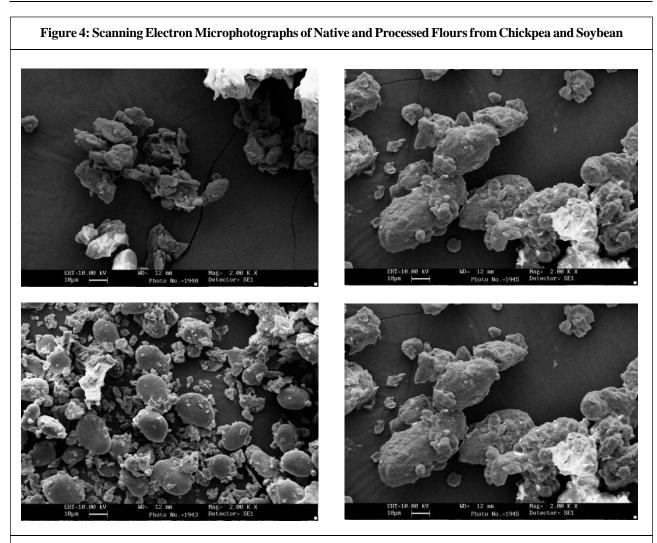
	T ₀ (°C)	T _p (°C)	T _c (°C)	$\Delta H (J / g)$
CPN	67.11	71.87	78.11	4.29
СРР	ND	ND	ND	ND
SBN	77.26	80.13	83.23	0.78
SBP	ND	ND	ND	ND

Note: ND: Not determined. (CPN: Chickpea native; CPP: Chickpea processed; SBN: Soybean native; SBP: Soybean Processed).

heated up to 100 °C, which indicated their loss of crystallinity during processing.

Scanning electron microphotographs corresponding to native and processed legume flours showed considerable differences. Native legumes' granules are spherical, round while in processed flours the granular structure entirely disappears. In processed flour, the entire granule population seems to be clustered to form an aggregated mass





Note: (CPN: Chickpea native; CPP: Chickpea processed; SBN: Soybean native; SBP: Soybean Processed).

comprising of several small granules. Further, the disruption of the granules indicated partial re-orientation of starch and protein in both the processed flours (Figure 4).

CONCLUSION

Chickpea and soybean were hydrothermally processed followed by mechanical flaking to prepare pre-cooked readyto-use flour. The physico-chemical and functional properties such as gel consistency, sediment volume, DSC thermograms and SEM microphotographsof processed legumesindicated the presence of pre-gelatinized flours. The processing also improved the *in-vitro* starch and protein digestibility of chickpea and soybean flour. The ready-to-use pre-cooked chickpea and soybean flour would provide a convenience protein source to use as an ingredient to prepare protein rich healthy food formulations.

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