Yield and physicochemical properties of low fat tofu prepared using supercritical carbon dioxide treated soy flours with different fat levels

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Abstract The consumer demands for low fat foods are increasing to reduce obesity and chronic diseases. Low-fat tofu (LFT) was prepared using soy flours treated with supercritical CO₂ (SC-CO₂) at pressures of 10, 20, and 30 MPa. After SC-CO₂ treatment, the residual oil contents of the soy flours were 12.07, 8.12, and 1.64%, respectively, whereas that of the control soy flour was 18.20%. The objective of this study was to investigate the yield and quality characteristics of LFTs, compared to the control tofu. All SC-CO₂-treated LFTs had significantly higher protein and moisture contents than the control tofu. The yields (g/100 g soy flour) of SC-CO₂-treated tofu were 442.69, 507.44, and 535.47 g, respectively, at three fat levels, whereas the yield was 385.23 g in case of the control tofu. The SC-CO₂-treated LFTs had softer textural attributes due to increasing moisture contents. In addition, the LFTs obtained higher sensory scores owing to softer texture and lower beany flavor than control sample. SC-CO₂-treated soy flours showed higher solubility in soy milk, leading to higher yield, produced softer texture of

tofu, and increased nutritional value with low fat and high protein content.

Keywords Defatted soy flour · Low-fat tofu · Supercritical CO₂ · Yield · Physicochemical characteristics

Introduction

The consumer demand for foods with low fat and calorie content has been increasing to improve health and reduce the risk of chronic diseases related to obesity and other cardiovascular problems (Yang et al. 2007). Tofu is a widely consumed soy product and is a rich source of protein, fat, dietary fiber, soy saponins, and isoflavones (Anderson and Wolf 1995; Huang et al. 2003). However, the major drawbacks of traditionally prepared tofu is that it is high in fat, may have a beany or grassy flavor, and is easily perishable, making it undesirable to some consumers (Kim et al. 2007; Rekha and Vijayalakshmi 2013). The yield and characteristics of tofu depend on the type and concentration of coagulants, processing parameters, solid content of the soy milk, and chemical composition of the soybean used to form the curd (Cai and Chang 1999; Poysa and Woodrow 2002; Noh et al. 2005; Rekha and Vijayalakshmi 2013). Numerous studies on quality characteristics of tofu have been reported, however, information about the yield and characteristics of tofu at different levels of fat is limited. Low-fat tofu (LFT) containing high levels of protein could become an attractive product for health-conscious consumers. In addition, reducing fat in tofu may diminish its undesirable beany flavor and improve its shelf-life by limiting lipid oxidation.

The use of supercritical CO₂ (SC-CO₂) is an attractive method to remove oil from food sources without

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denaturing proteins and degrading thermo-labile compounds (Sihvonen et al. 1999). This method is superior to other conventional methods that use organic solvents, mechanical pressing or enzymatic extraction (Russin et al. 2011). From the viewpoint of food applications, after removal of oil by SC-CO₂ extraction, defatted food sources have better functional properties such as water solubility, water and oil absorption, swelling and gelling, and emulsifying and foaming capacity (Riaz 2006; Kang et al. 2017). Removing oil by organic solvent extraction leaves undesirable off-flavors in the defatted meals, which reduces their usefulness in food applications (Maheshwari et al. 1995).

The aim of this study was to investigate the yield and physicochemical properties of LFTs prepared using defatted soy flours (DSF) treated with SC-CO₂ under pressures of 10, 20, and 30 MPa, at which soy flours have different levels of residual fat. The quality characteristics of tofu, including proximate composition, amino acid profiles, textural profiles, color values, and sensory attributes were measured and evaluated. In addition to LFTs, full-fat control tofu was also prepared using non-defatted control soy flour (CSF), and its quality characteristics were compared with those of LFTs.

Materials and methods

Materials

Soy flour and food-grade coagulant MgCl₂ (Soylove, Korea) were purchased from a local market in Jinju, Korea, and stored at 4 °C in air-tight glass containers until use. The chemicals used in the present study were purchased from Sigma Aldrich (Sigma-Aldrich Corp., St. Louis, Mo., USA), and were of high-purity analytical grade.

Defatting soy flour by SC-CO₂ treatment

A laboratory-scale SC-CO₂ unit (Ilshin Autoclave Co., Daejeon, Korea) was used to remove oil from soy flour. A schematic of the SC-CO₂ unit is shown in our previous work (Kang et al. 2017). Liquid CO₂ (purity ≥ 99.99%) contained in a siphoned cylinder was cooled to 4 °C using a system chiller before loading the sample. Soy flour (500 g) was loaded into the extraction vessel, and the head of the vessel unit was tightly sealed. Oil extraction was performed by pumping CO₂ through the vessel at a flow rate of 30–50 g/min, at a constant temperature of 40 °C for 3 h. DSF samples with different levels of fat were produced under operating pressures of 10, 20, and 30 MPa, and denoted as DSF-10, DSF-20, and DSF-30, respectively. After extraction, the vessel was gradually

depressurized. All samples were stored in a refrigerator at 4 °C in air-tight glass containers until analysis.

Total soluble solid contents of soy milk

Total soluble solid (TSS) contents of the samples were determined gravimetrically using a slightly modified method reported by Amid and Mirhosseini (2012). A flour sample (2 g) was dispersed in 100 mL of distilled water and blended (D-500 homogenizer, Wiggen Hauser, Berlin, Germany) at 10,000 rpm for 1 min. The resulting slurry was heated to 90 ± 1 °C in a water bath (BS-21, Jeio Tech., Seoul, Korea) accompanied by shaking at 185 rpm for 5, 10, 20, 30, and 60 min. After heating, the slurry was allowed to cool at room temperature for 30 min and then centrifuged at 3000 rpm for 20 min. A 25-mL aliquot of the supernatant was carefully pipetted and transferred to pre-weighed aluminum weighing dishes and dried in a laboratory convection oven (JSOF-150, JS Research Inc. Korea) at 105 °C for 3 h. The TSS content was calculated using Eq. (1), and the results were plotted as a function of time.

$$\text{TSS(g/100 g soy flour)} = 100 \times (W_1 \times D_f)/W_2 \quad (1)$$

where W_1 is the mass of solid in 25-mL supernatant, W_2 is the total sample mass, and D_f is the dilution factor (100/25).

Preparation of tofu

Tofu was prepared following a modified method proposed by Kim et al. (2007). First, 100 g of soy flour was blended with 1700 mL of tap water in a laboratory-scale automatic filtration system blender (SoyLove, IOM-201, Ronic Co. Ltd., South Korea) at 90 °C for 60 min. The resulting soy milk slurry was placed in a 2000-mL glass beaker and allowed to cool to 80 °C. Immediately, 7 g MgCl₂ was added for coagulation and the slurry was then allowed to stand for 15 min. The coagulum was transferred to a muslin cloth placed in a perforated formation frame for dewatering, and the coagulum was then pressed sequentially with air press gauge readings of 1 kg/cm² for 10 min, 2 kg/cm² for 15 min, and 3 kg/cm² for 20 min. After pressing, the cloth was removed, and the tofu was drained at room temperature for 15 min. The tofu samples were labeled as control, LFT-10, LFT-20, and LFT-30 prepared using CSF, DSF-10, DSF-20, and DSF-30, respectively. All tofu samples were stored in a refrigerator at 4 °C for subsequent analysis.

Tofu yield and proximate composition of soy flours and tofu

Tofu yield was obtained in terms of the weight of fresh tofu prepared from 100 g of soy flour and calculated using Eq. (2).

$$\text{Tofu yield (g/100 g soy flour)} = 100 \times W_1/W_2 \quad (2)$$

where W_1 denotes the weight of fresh tofu, and W_2 denotes the weight of soy flour. The proximate compositions of CSF, DSF, control tofu, and all LFT samples, including moisture, ash, lipid, carbohydrate, and protein contents ($N \times 6.25$), were determined by AOAC (2000) methods.

Amino acid analysis

Amino acid analysis was conducted following a modified method of Wang et al. (2008). Samples weighing 0.1 g were placed in heat-proof screw-cap test tubes and dissolved in 5 mL of 6 N HCl. The tubes were flushed with N_2 gas for 1 min and then sealed immediately. The samples were subsequently incubated in a laboratory oven at 110 °C for 24 h. After the reaction was complete, the supernatant was evaporated using a vacuum rotary evaporator (HS-2005S, Jisico Co., Ltd., Korea), and the concentrate was dissolved in 3 mL of sodium citrate buffer (pH 2.2). The resulting solution was filtered with 0.45- μ m PTFE filters (Sigma-Aldrich Corp., St. Louis, Mo., USA) and analyzed using an auto amino acid analyzer (Biochrom 30, Biochrom, Sweden) at 570 nm.

Textural profile analysis of tofu

Textural profile analysis (TPA) of the tofu samples was conducted using a Stable Micro Systems texture analyzer (TA-XT Express, Surrey, England). Before analysis, fresh tofu samples were cut into pieces measuring 2 cm in length, 2 cm in width, and 1 cm in height, and equilibrated to room temperature for 30 min. These tofu samples were compressed twice to 35% deformation using a cylindrical plunger (D: 25 mm). Force-time deformation curves were obtained at a crosshead speed of 180 mm/min with a 5.0 g trigger force. The textural parameters of hardness, cohesiveness, adhesiveness, springiness, gumminess were measured for each tofu sample. Five samples were analyzed for each treatment.

Color values

The color values of the tofu samples were measured using a colorimeter (Minolta CR-310, Japan). A standard white plate with lightness (L^*) = 96.10 ± 0.10 , redness (a^*) = 1.38 ± 0.04 , and yellowness (b^*) = 1.33 ± 0.01

was used for calibration. The color values were expressed in Hunter L^* (0.00 = dark black, 100.00 = white), a^* (+ value = red, – value = green), and b^* (+ value = yellow, – value = blue) for each sample. The polar co-ordinate chroma, (C^*), and the total color difference (TCD*) values were calculated using Eqs. 3 and 4, respectively, proposed by Minolta (1994). The TCD* was considered between the SC-CO₂-treated samples and the control sample (indicated by index 0 in Eq. 4).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$TCD^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (4)$$

Sensory evaluation

All tofu samples were subjected to sensory evaluation following the modified methods proposed by Kim et al. (2007) and Li et al. (2017). The panel consisted of 20 semi-trained panelists who participated in previous related projects to evaluate the sensory attributes of tofu under natural light in a room. Tofu samples were cut into cubes (2 cm $\times$ 2 cm $\times$ 2 cm), assigned random code numbers and arranged on a glass plate, and then served to the panelists randomly. Drinking water was also presented to the panelists along with tofu samples for rinsing the mouth between tasting two different samples. The attributes selected for defining the quality of tofu were mouth-feel, firmness, flavor, and overall acceptability. The results were recorded on a 5-point scale (1 = dislike, 2 = neither like nor dislike, 3 = like slightly, 4 = like moderately, and 5 = like very much) for each attribute.

Statistical analysis

All the determinations were carried out three times and data were presented as mean values of three replicates $\pm$ standard deviation. The data were analyzed using SAS® (version 9.1; SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed, and Duncan's multiple range tests were used to determine the difference of means, with $p < 0.05$ representing statistical significance.

Results and discussion

Total soluble solid contents of soy milk

The total soluble solids (TSS) content of soy milk can impact the yield, textural attributes, and other quality characteristics of tofu (Rekha and Vijayalakshmi 2013). Figure 1 shows the TSS contents of soy milk prepared with

Fig. 1 As a function of time, total soluble solid (TSS) contents (%) of control soy flour (CSF) and SC-CO₂-treated defatted soy flour (DSF). Control: non-defatted soy flour; DSF-10, DSF-20 and DSF-30: defatted soy flours treated with SC-CO₂ extraction process under the pressures of 10, 20, and 30 MPa, respectively

CSF and SC-CO₂-treated soy flours heated at 90 °C as a function of time. The TSS contents of all soy milk samples increased rapidly during first 30 min of heating, and increased slightly after longer heating times. The TSS contents of soy flour samples treated with SC-CO₂ increased with increasing extraction pressures. After heating for 1 h, all soy milk samples prepared with SC-CO₂-treated soy flour had higher TSS contents with values of 42.66 g/100 g, 44.34 g/100 g, and 48.55 g/100 g for DSF-10, DSF-20, and DSF-30, respectively, compared with the TSS content of 38.77 g/100 g for soy milk prepared with CSF. The increase in the solubility of soy flours treated with SC-CO₂ may be due to increased exposure of hydrophilic groups owing to lipid removal. The higher lipid content of soy flour and other oil seed meals lead to increased hydrophobic interactions and interferes with hydrophilic characteristics, thus retarding solubility in aqueous solutions (Heywood et al. 2002; Moure et al. 2006). Konak et al. (2014) demonstrated that SC-CO₂ extraction removes non-polar lipids from oat flours, leading to remarkably higher soluble protein contents of 200 mg/g compared with the soluble protein contents of 80 mg/g in case of untreated oat flour. In a previous study (Kang et al. 2017), we showed that the SC-CO₂ extraction process can remove oil from soy flour without protein denaturation, and that SC-CO₂-treated soy flour showed superior functional properties in terms of solubility, water and oil absorption capacity, and foaming and emulsification properties than the control and hexane-treated soy flours. Sun et al. (2008) demonstrated that SC-CO₂-treated canola meal had significantly higher solubility and water-binding capacity than untreated and hexane-extracted canola meals.

Tofu yield and proximate composition of soy flours and tofu

From the commercial viewpoint, tofu yield is an important quality factor, particularly for tofu processors. Tofu yield depends on several factors such as processing parameters, solid contents of soy milk, types and concentration of coagulants, protein condition, and soybean composition (Cai and Chang 1999; Poysa and Woodrow 2002; Mujoo et al. 2003; Noh et al. 2005; Rekha and Vijayalakshmi 2013). In our study, tofu yields prepared with different fat levels in soy flour treated by SC-CO₂ at different pressures were measured. Tofu yield was found to increase markedly with decreasing fat levels in soy flours, and the values are listed in Table 1. LFTs prepared with SC-CO₂-treated soy flours had significantly higher yield (g/100 g soy flour) with values of 442.69, 507.44, and 535.47 g for LFT-10, LFT-20, and LFT-30, respectively, compared with 385.23 g yield of control tofu prepared with untreated CSF. Therefore, SC-CO₂-treated soy flours produced about 1.4 times higher tofu yield compared to control soy flour. The higher yield of tofu prepared with SC-CO₂-treated soy flour may be related to the increased TSS content in soy milk. The TSS content is positively correlated with protein concentration, because the latter provides a greater number of polar groups for binding water (Zayas 1997). Li et al. (2017) reported that the protein particles in soy milk hold water molecules to form a constant network structure, thereby increasing tofu yield. The solid contents of soy milk and protein contents of soy seeds are positively correlated, and seed oil is negatively correlated with tofu yield (Poysa and Woodrow 2002). Low-fat soy flour subjected to SC-CO₂ treatment can produce higher tofu yields owing to increased TSS contents and protein concentration in soy milk. However, hexane-defatted soy flour may not produce tofu with high yield and good texture. In a previous study

Table 1 Tofu yields, and proximate compositions of control and SC-CO₂-treated soy flours and their respective tofu samples

Components	Soy flours			Soy tofu				
	Control ¹	DSF-10	DSF-20	DSF-30	Control ²	LFT-10	LFT-20	LFT-30
Tofu yield (g/100 g soy flour)	–	–	–	–	385.23 ± 0.23 ^d	442.69 ± 0.40 ^c	507.44 ± 0.49 ^b	535.47 ± 0.03 ^a
Moisture (%)	7.59 ± 1.01 ^a	7.02 ± 0.17 ^a	2.12 ± 0.34 ^b	1.88 ± 0.12 ^b	77.67 ± 1.26 ^c	78.79 ± 0.87 ^c	82.17 ± 0.29 ^b	85.83 ± 0.76 ^a
Protein (%)	35.07 ± 1.44 ^d	38.32 ± 1.01 ^c	43.98 ± 1.08 ^b	49.38 ± 1.18 ^a	10.88 ± 0.45 ^c	11.93 ± 0.31 ^b	12.12 ± 0.21 ^b	12.99 ± 0.35 ^a
Crude fat (%)	18.20 ± 0.78 ^a	12.07 ± 0.51 ^b	8.12 ± 0.55 ^c	1.64 ± 0.13 ^d	5.03 ± 0.34 ^a	1.89 ± 0.03 ^b	1.01 ± 0.21 ^c	0.12 ± 0.01 ^d
Ash (%)	4.63 ± 0.19 ^b	4.97 ± 0.20 ^b	6.51 ± 0.15 ^a	6.38 ± 0.82 ^a	0.90 ± 0.34 ^a	1.01 ± 0.18 ^a	1.13 ± 0.23 ^a	1.04 ± 0.29 ^a

¹Control, untreated full-fat soy flour; DSF-10, 20 and 30, defatted soy flours treated with SC-CO₂ at the pressures of 10, 20, and 30 MPa²Control, tofu prepared with control soy flour; LFT-10, 20 and 30, Low-fat tofu prepared using DSF-10, DSF-20, and DSF-30, respectively^{a–d}Different letters superscript indicate in the same row separately for soy flours and soy tofu that means are significantly different ($p < 0.05$) by Duncan's test

(Kang et al. 2017), we showed that soy flours treated with hexane had decreased fat content, but the extent of protein denaturation increased, leading to poor solubility and water absorption capacity, whereas SC-CO₂ treatment removes oil without protein denaturation and offers superior solubility and water absorption capacity. This may indicate that the SC-CO₂-treated soy flour may produce a higher tofu yield compared with control and hexane-treated soy flour. A study by Tsai et al. (1981) showed that tofu prepared from soybean flakes defatted by hexane had lower yield than that prepared with full-fat soybean.

Table 1 presents the proximate compositions of all samples of soy flour and tofu used in this study, and lists the levels of major macronutrients and moisture content. After SC-CO₂ extraction of soy flour under different pressures, the residual oil contents were 12.07% for DSF-10, 8.12% for DSF-20, and 1.64% for DSF-30, whereas the oil content was 18.20% for CSF. In addition to removal of oil, the SC-CO₂ treatment also reduced the moisture content in soy flour. Such decrease in moisture content was profound with increasing extraction pressure. The moisture contents were 7.59, 7.02, 2.12, and 1.88% for CSF, DSF-10, DSF-20, and DSF-30, respectively. The protein concentration in both soy flour and soy tofu, as well as the water-binding capacity of tofu, increased with decreasing fat levels in soy flour. All SC-CO₂-treated soy flours and their respective LFTs had significantly higher ($p < 0.05$) protein contents of 49.38 and 12.99%, 43.98 and 12.12%, and 38.32 and 11.93% for DSF-30 and LFT-30, DSF-20 and LFT-20, and DSF-10 and LFT-10, respectively, than the protein contents of 34.92 and 10.88% for CSF and control tofu, respectively. In addition, LFT-30 and LFT-20 had significantly higher moisture contents of 85.83 and 82.17%, respectively, compared with the moisture contents of 78.79 and 77.67% in the LFT-10 and control tofu, respectively. Because of the removal of fat, SC-CO₂-treated soy flours and their respective LFTs had greater protein concentrations than those of the CSF and control tofu. In addition, high protein and amino acid contents in SC-CO₂-treated LFTs may enhance water-binding capacity by exposing their hydrophilic characteristics. Many researchers (Zayas 1997; Riaz 2006; Kang et al. 2017) have shown that the defatting of soy flour increased the protein, carbohydrate, and ash contents because the level of fat was reduced. In addition, defatting by SC-CO₂ extraction did not degrade the nutritional quality of oil seeds, whereas defatting by hexane denatured the proteins in the seeds (Stahl et al. 1980). Sparks et al. (2006) reported that defatted rice bran treated by SC-CO₂ extraction had higher protein content compared with that in control and organic solvent treated rice bran.

Table 2 Amino acids composition of control and all low-fat tofu prepared with SC-CO₂-treated soy flours (unit: mg/100 g)

Amino acids	Control tofu	LFT-10	LFT-20	LFT-30
Total essential amino acids	2680.89 ± 1.38 ^a	2691.06 ± 0.79 ^b	2714.89 ± 0.86 ^a	2796.2 ± 0.86 ^a
Threonine	248.37 ± 2.33 ^a	244.86 ± 2.12 ^a	243.61 ± 2.84 ^a	246.67 ± 1.90 ^a
Valine	227.11 ± 0.87 ^a	219.79 ± 1.02 ^b	222.64 ± 0.79 ^{ab}	219.54 ± 0.48 ^b
Methionine	150.62 ± 1.69 ^b	150.92 ± 1.54 ^b	151.99 ± 1.18 ^b	162.81 ± 1.29 ^a
Isoleucine	192.06 ± 0.15 ^b	194.22 ± 0.88 ^{ab}	198.28 ± 1.09 ^a	201.80 ± 1.00 ^a
Leucine	437.64 ± 2.40 ^a	442.38 ± 1.84 ^a	444.71 ± 1.23 ^a	442.69 ± 0.99 ^a
Phenylalanine	397.60 ± 3.08 ^a	394.62 ± 2.51 ^a	397.83 ± 0.21 ^a	399.29 ± 2.57 ^a
Histidine	235.23 ± 0.80 ^b	233.34 ± 1.33 ^b	239.89 ± 0.76 ^b	305.06 ± 0.94 ^a
Lysine	319.55 ± 3.59 ^b	323.95 ± 3.27 ^{ab}	327.10 ± 1.03 ^a	330.00 ± 0.77 ^a
Arginine	472.71 ± 4.28 ^a	486.98 ± 2.61 ^a	486.84 ± 2.64 ^a	488.34 ± 3.01 ^a
Total nonessential amino acids	3435.55 ± 0.82 ^d	3510.34 ± 0.64 ^c	3549.48 ± 0.49 ^b	3684.47 ± 0.59 ^a
Aspartic acid	675.86 ± 3.31 ^a	652.48 ± 2.59 ^a	656.16 ± 2.01 ^a	663.18 ± 2.09 ^a
Serine	288.09 ± 1.59 ^c	321.99 ± 0.54 ^a	302.74 ± 1.08 ^b	325.35 ± 1.37 ^a
Glutamic acid	769.16 ± 1.85 ^a	784.22 ± 1.51 ^a	793.84 ± 1.30 ^a	798.90 ± 1.01 ^a
Proline	602.33 ± 1.05 ^c	651.52 ± 1.75 ^b	671.85 ± 1.07 ^a	692.89 ± 0.85 ^a
Glycine	316.20 ± 0.59 ^a	308.30 ± 2.01 ^a	308.98 ± 1.44 ^a	309.54 ± 1.02 ^a
Alanine	339.44 ± 1.41 ^a	327.67 ± 1.29 ^a	326.23 ± 0.51 ^a	328.68 ± 1.71 ^a
Cysteine	170.24 ± 1.05 ^a	167.03 ± 1.04 ^a	168.44 ± 0.86 ^a	169.36 ± 0.57 ^a
Tyrosine	274.23 ± 1.81 ^d	297.13 ± 1.04 ^c	321.24 ± 0.55 ^b	396.57 ± 0.27 ^a
Total	6116.44 ± 1.10 ^d	6201.40 ± 0.71 ^c	6264.37 ± 0.68 ^b	6480.67 ± 0.72 ^a

Control, tofu prepared with control soy flour; LFT-10, 20 and 30, Low-fat tofu prepared with SC-CO₂-treated soy flour at the pressures of 10, 20, and 30 MPa

^{a-d}Different letters superscript indicate in the same row that means are significantly different ($p < 0.05$) by Duncan's test

Amino acid analysis

Amino acid composition may influence the functional properties such as hydration, solubility, water binding, and gelation properties of protein rich meal (Zayas 1997). Table 2 shows the amino acid compositions of the different tofu samples used in this study. A total of 17 amino acids were identified in each tofu sample. Glutamic acid was most predominant in all samples, followed by proline, aspartic acid, arginine, leucine, phenylalanine, tyrosine, lysine, alanine, glycine, and histidine, and the sulfur-containing amino acid, cysteine, was present in the smallest amount. Amino acid contents increased in LFTs with decreasing fat levels in soy flour and tofu. Including essential and non-essential amino acids, LFT-30 had the highest amino acid content of 6480.67 mg/100 g, followed by LFT-20 with 6264.37 mg/100 g, LFT-10 with 6201.40 mg/100 g, and control tofu with 6116.44 mg/100 g. Thus, the amino acid contents of all LFTs were found to be significantly higher than that of control tofu. The higher amino acid contents in LFTs could be ascribed to the reduced levels of fat in DSF and their respective

LFTs. SC-CO₂ extraction removes oil from dry-milled corn germ flour and soy flour without denaturation of protein and amino acids, and after oil removal, the protein and amino acid concentrations were significantly higher than those of the control and the hexane-treated samples (Christianson et al. 1984; Kang et al. 2017). Similarly, Park et al. (2008) showed that the total, essential, and free amino acid contents were greater in SC-CO₂-treated mackerel viscera than in the untreated samples.

Textural attributes of tofu

The quality characteristics such as textural attributes are closely related to the processing parameters used in producing tofu, and include temperature, time, pH, type of coagulant, ionic strength, moisture content, solid content of soy milk, and chemical composition of soybean (Mujoo et al. 2003; Rekha and Vijayalakshmi 2013). Table 3 lists the TPA results of all SC-CO₂-treated LFTs and control tofu. Textural attributes such as hardness, springiness, and gumminess decreased proportionately with decreasing fat levels in soy flour. By contrast, the values of cohesiveness

Table 3 Textural profile and color value analysis of control, and SC-CO₂-treated low-fat tofu

Tofu	Textural attributes			Color value						
	Hardness (g s)	Cohesiveness (g mm)	Adhesiveness (g s)	Springiness (mm)	Gumminess (g)	L*	a*	b*	C*	TCD
Control	414.30 ± 4.49 ^a	0.46 ± 0.05 ^b	– 21.30 ± 15.99 ^b	0.84 ± 0.08 ^a	223.78 ± 10.71 ^a	98.20 ± 0.14 ^d	2.66 ± 0.03 ^a	16.93 ± 0.01 ^a	17.13 ± 0.04 ^a	
	303.40 ± 2.25 ^b	0.51 ± 0.06 ^{ab}	– 10.70 ± 5.37 ^b	0.83 ± 0.07 ^{ab}	163.85 ± 4.25 ^b	100.39 ± 0.18 ^c	1.93 ± 0.02 ^b	15.67 ± 0.03 ^b	15.78 ± 0.05 ^b	2.63 ± 0.23 ^c
10										
LFT-	241.20 ± 1.73 ^c	0.54 ± 0.01 ^a	– 9.23 ± 0.58 ^b	0.82 ± 0.02 ^{ab}	123.08 ± 15.35 ^c	101.70 ± 0.12 ^b	1.92 ± 0.03 ^b	15.67 ± 0.04 ^b	15.78 ± 0.07 ^b	3.79 ± 0.19 ^b
20										
LFT-	169.00 ± 17.32 ^d	0.54 ± 0.02 ^a	– 4.30 ± 3.12 ^a	0.81 ± 0.10 ^b	77.74 ± 16.42 ^d	102.85 ± 0.15 ^a	1.90 ± 0.02 ^b	15.62 ± 0.04 ^b	15.73 ± 0.06 ^b	4.89 ± 0.21 ^a
30										

Control: tofu prepared with control soy flour; LFT-10, 20 and 30: Low-fat tofu prepared with SC-CO₂-treated soy flour at the pressures of 10, 20, and 30 MPa

^{a-d}Different letters superscript indicate in the same column that means are significantly different ($p < 0.05$) by Duncan's test; standard color values: L* = 96.10 ± 0.10, a* = 1.38 ± 0.04, b* = 1.33 ± 0.01 (L*: lightness, a*: redness, b*: yellowness, C*: Chroma value, TCD: total color difference)

increased with decreasing fat content in the soy flour. Therefore, all SC-CO₂-treated LFTs were significantly softer texture than the control tofu. However, the SC-CO₂-treated tofu had significantly lower ($p < 0.05$) hardness values of 169.17, 241.20, and 303.40 g for LFT-30, LFT-20, and LFT-10, respectively, while the hardness value had 414.3 g for control. Compared with control tofu, the cohesiveness of SC-CO₂-treated tofu increased with decreasing fat level. The values of springiness and gumminess of LFTs decreased with decreasing fat level. All LFTs treated with SC-CO₂ were softer, less springy, and less gummy, probably owing to their high TSS and moisture contents. The high value of cohesiveness may be ascribed to the presence of high protein instead of fat in LFTs, which could form stronger ionic bonds with water molecules and coagulants compared to the control tofu. Generally higher levels of fat may be related to softer texture in processed food products such as emulsified sausages, fresh pork sausages, coarse ground sausages, cookies etc. (Zoulias et al. 2002; Keeton 1994). In our study, however, tofu prepared with SC-CO₂-treated soy flours produced softer texture. This may be related to increase in moisture contents of LFTs. Zayas (1997) reported that water retention and protein solubility are critical factors affecting the textural properties of soy products.

Color value of tofu

All tofu samples prepared in this study were white or yellowish, and the results of color values of the samples are given in Table 3. The lightness or L* values of LFTs prepared using soy flours treated with SC-CO₂ at the pressures of 10, 20 and 30 MPa increased significantly with values of 100.39, 101.70, and 102.85, respectively, compared to the value of 98.20 for control tofu. The redness or a* values and yellowness or b* values of LFTs decreased significantly than control tofu. The polar co-ordinate chroma, (C*), which is an indication of how dull/vivid the product (Gonçalves et al. 2007), and the control tofu was obtained significantly higher vividness than those of LFTs. The C* values were found to be 17.13, 15.78, 15.78, and 15.73 for control, LFT-10, LFT-20, and LFT-30, respectively. The total color differences (TCD*) were 2.63, 3.79, and 4.89 in case of LFT-10, LFT-20 and LFT-30, respectively, compared with control tofu. This indicates that the color differences increased with increasing pressures of SC-CO₂ treatment for producing low-fat soy flours. The higher lightness (L*), lower redness (a*) and yellowness (b*) in SC-CO₂-treated LFTs could be due to reduced fat, and defatting process may remove the carotenoids and some other color components from soy flours.

Sensory evaluation

The tofu samples were evaluated for the quality attributes like mouth-feel, firmness, flavor, and overall acceptability, and the results are shown in Fig. 2. Higher scores of all attributes indicated a greater preference for all tofu samples used in this study. Mouth-feel was liked better in LFT-30 and LFT-20 samples as compared to LFT-10 and control. The preference scores of firmness were not significantly different for LFT-30 and control tofu, but LFT-30 had comparatively higher scores than control. Flavor scores increased markedly with the SC-CO₂ pressure used to produce DSF and the corresponding LFTs. Flavor scores were 4.25 for LFT-30, 3.65 for LFT-20, and 2.61 for LFT-10, whereas the lowest score was 2.18 for control tofu. Amongst all tofu samples, LFT-30 showed highest score of 4.57 for overall acceptability, followed by 3.47, 3.10 and 3.05 for LFT-20, control, and LFT-10, respectively. Therefore, SC-CO₂-treated LFTs (LFT-20 and LFT-30) had significantly higher scores ($p < 0.05$) for overall acceptability than the control tofu. This could be attributed

to the relatively lower beany or grassy flavor, higher moisture or juiciness, softness, and lower springiness of LFTs compared to the control. Higher juiciness in LFTs may enhance the preference of mouthfeel. Higher score for firmness in LFT-30 and control may be related to the variation of consumers' preference for soft and hard tofu. Generally, soy products including tofu may provide unpleasant flavors such as beany or grassy (Riaz 2006; Maheshwari et al. 1995). The SC-CO₂ treatment on soy flour may remove these off-flavors leading to an increased flavor preference for LFTs. Maheshwari et al. (1995) also demonstrated that SC-CO₂ extraction was effective for removing off-flavors and volatile odor compounds from soybean and corn proteins. In addition, SC-CO₂ extraction exhibits excellent initial flavor and storage stability of defatted meals because of the inactivation of peroxidase enzymes (Christianson et al. 1984). By contrast, Riaz (2006) showed that aqueous extracts prepared from hexane-defatted soy flour have undesirable off-flavors that reduce their usefulness in food applications.

Fig. 2 Sensory evaluation of control tofu and all samples of low-fat tofu prepared using SC-CO₂-treated soy flours. **a** Control tofu prepared using control soy flour; **b–d** low fat tofu (LFT) prepared using SC-CO₂-treated soy flour at pressures of 10, 20, and 30 MPa,

respectively. The values were recorded on a 5-point scale; where 1 = dislike, 2 = neither like nor dislike, 3 = like slightly, 4 = like moderately, 5 = like very much

Conclusion

The SC-CO₂ extraction process removes oil from soy flour without protein denaturation, resulting in improved nutritional quality and functional properties including TSS content. Therefore, soy flour treated with SC-CO₂ at different pressures can produce low-fat and high-protein tofu. SC-CO₂-treated tofu had good nutritional properties with significantly higher yield, softer textural and better sensory attributes. In addition, SC-CO₂ treatment may remove the beany flavor of soy flour, as tofu made from these flours had higher flavor likeability scores. In comparison with the control tofu prepared using CSF, the SC-CO₂-treated LFT may be attractive to health-conscious consumers who prefer a low-fat, low-calorie, and healthy diet.

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