J Korean Soc Appl Biol Chem (2015) 58(6):945–954
DOI 10.1007/s13765-015-0123-x

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Quality characteristics of fermented cheese analogs produced using roasted soy flour and different fermentation times with rice straw

Sook-Young Lee[1] · Dae-Yoon Cho[1] · Min-Kyoung Lee[1]

Received: 27 August 2015 / Accepted: 16 September 2015 / Published online: 21 October 2015

Abstract To improve the nutritional and physiological functionality of cheese analogs, fermented cheese analogs treated with Flavourzyme® were developed and the effects of roasting soy flour and fermentation time (1, 2, and 3 days) with rice straw on their quality characteristics, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoflavone content, and antioxidant activity were examined. Heat coagulation was the highest for the unroasted and roasted soy flour groups fermented for 1 day, with the unroasted group having higher values than the roasted group, regardless of fermentation time. The daidzein and genistein contents were the highest in the soy flour and roasted soy flour groups fermented for 2 days and 1 day, respectively. The antioxidant activity and heat extension were the highest for the soy flour group and roasted soy flour group fermented for 1 day, respectively. In the SDS-PAGE patterns, protein subunits of P26 in roasted soy flour groups were consistently decomposed by increasing the fermentation time. The yield and pH in both groups decreased as fermentation time increased. The adhesiveness of the soy flour group was highly increased by fermentation, and the cohesiveness, springiness, and appearance of the roasted soy flour group were improved by fermentation. The optimum fermentation time for the fermented cheese analogs was 1 day, as higher yields, heat coagulation, heat extension, lightness, dE, cohesiveness, and springiness were obtained. Between the two groups fermented for 1 day, the soy flour group showed better heat coagulation, genistein content, antioxidant activity, cohesiveness, springiness, and adhesiveness; however, the roasted soy flour group showed better yield and heat extension.

Keywords Antioxidant activity · Fermented cheese analog · Isoflavone · Roasted soy flour · Sensory characteristics · Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Abbreviations

FRSC	Fermented roasted soybean cheese
FSC	Fermented soybean cheese
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Introduction

Soybeans have a protein content of approximately 40 % and protein functionalities, such as solubility, emulsification, gel formation, and water holding capacity. Therefore, they have been used for the development of various protein food products (Lee et al. 2008; Lee and Lee 2009). The domestic and international demand for soybeans is increasing as they are recognized as a vegetarian, healthy, high-protein food. Additionally, healthy components of soybeans, such as isoflavone, anthocyanin, lecithin, and Bowman-Birk protease inhibitor, have been reported (Catalano et al. 2007; Park et al. 2008).

Cheonggukjang, Korean fermented soybean paste fermented by *Bacillus subtilis* is a traditional food made from soybean-containing functional substances, and several studies have been conducted on it (Choi and Ji 1989; Shon

✉ Sook-Young Lee
syklee@cau.ac.kr

[1] Division of Food Science and Technology, College of Biotechnology and Natural Resource, Chung-Ang University, Anseong-Si 456-756, Republic of Korea

et al. 2001). However, *Cheonggukjang* consumption is on the decline, especially among young people and children, due to the unpleasant odor that results when it is fermented or cooked, which is derived from butyric or valeric acid. Volatile substances, such as tetramethyl pyrazine and ammonia, contribute to the odor of *Cheonggukjang* and various studies have been conducted to attempt to mask the smell by adding subsidiary materials or improving the fermentation procedure (Ko et al. 1999; Shon et al. 2002). The majority of studies on traditional *Cheonggukjang* or food products using *Cheonggukjang* powder have focused on fermentation conditions and manufacturing procedures (An et al. 2008; Lee and Lyu 2008). However, few studies have been conducted on the rice straw fermentation technique. Studies on cheese analogs have investigated the effects of replacing milk protein with soybean protein as a cost-effective alternative (Eymery and Pangborn 1988; Bachmann 2001), and the effects of enzyme treatment on the functional properties of soy protein (Kamata et al. 1992; Lee et al. 1992; Kindstedt 1993; Cho et al. 2015).

To improve protein, nutritional, and physiological functionalities, in this study, fermented cheese analogs were developed by fermenting the proteolyzed cheese analog developed in the previous study (Cho et al. 2015) with rice straw. Raw soy flour can be used to manufacture small amounts of cheese analogs; however, it is easier to produce bulk amounts of cheese analogs for industry using roasted soy flour. Therefore, fermented cheese analogs containing proteolyzed (roasted) soy flour were prepared by fermentation with rice straw, and the effect of fermentation time was examined. The analogs were evaluated by their physicochemical properties, such as SDS-electrophoresis, heat coagulation, yield, heat extension, isoflavone content, hydrogen-donating activity, texture, and sensory characteristics.

Materials and methods

Materials

Soybean (Hwanggumkong) was purchased from Kong saranghoe in the Ill-juk area (Korea) and roasted soy flour was prepared using a mill after roasting soybean at 160 °C for 10 min to improve the milling process. Flavourzyme® (500 MG, protease from *Aspergillus oryzae*), industrial protease produced by Novozyme Co. (Denmark), soymilk residue provided by Samyouk Foods Co., and rice straw from Seoil-farm (Korea) were used. The other food materials were purchased as specified in the article of Cho et al. (2015). Reagents for the DPPH radical scavenging activity, isoflavone content, and protein marker were purchased from Sigma-Aldrich Co. (USA) and Elpis-biotech (Korea) and stored at −20 °C until use. Most of the other reagents used were of G.R (guaranteed reagent) or E.P. (extra pure) grade.

Preparation of fermented cheese analogs

The fermented cheese analogs were prepared by fermenting proteolyzed cheese analogs with rice straw for different times (0, 1, 2, and 3 days). For the proteolyzed cheese analogs, (roasted) soy flour was treated with 0.3 % Flavourzyme® (enzyme content/protein content of (roasted) soy flour, w/w) for 25 min. For the detailed methods of enzyme treatment and the procedure for the manufacture of proteolyzed cheese analogs, refer to the article by Cho et al. (2015). Table 1 shows the ingredients for the manufacture of the fermented cheese analogs containing proteolyzed (roasted) soy flour. Fermentation with rice straw was performed using an NUC Household *Cheonggukjang* machine (NY-8024F, Korea), as previously described by Oh and Eom (2008) with the following modification: a layer of 1 % rice straw was placed in the *Cheonggukjang* machine and a thin layer of proteolyzed cheese analog was spread on top of the rice straw, followed by another layer of 1 % rice straw.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS gel electrophoresis was performed by the slightly modified method of Webber and Osborn (1969) for investigating protein degradation within the different fermentation times. Protein samples (1 %) were mixed with sample buffer (tris–HCl of pH 6.8, 10 % SDS, bromophenol blue, β-mercaptoethanol, and 50 % glycerol) in a ratio of 1:1, and heated at 100 °C for 2 min. The protein

Table 1 Ingredients of the fermented cheese analogs containing proteolyzed soy flour and proteolyzed roasted soy flour

Ingredients*	*g* (%)
Proteolyzed (roasted) soy flour (unfermented or fermented)	150.00 (24.3)
Distilled water	301.40 (47.6)
Soymilk residue	100.00 (15.8)
Na-caseinate	33.00 (5.2)
Soybean oil	33.00 (5.2)
Sodium chloride	5.74 (0.9)
Sodium citrate	1.25 (0.2)
Sodium pyrophosphate	1.00 (0.2)
Lactic acid	4.00 (0.6)
Total	629.39 (100.0)

* Refer to the article of Cho et al. (2015)

standard and 20 µL of each sample were loaded into 10-well 5–12 % gradient tris–HCl gels and run for approximately 2 h at 100 V.

Heat coagulation

The heat coagulation of the fermented cheese analogs was determined by the slightly modified method of Kramer and Kwee (1977). A protein solution (2 %, pH 6.8) was dispersed at 36,947×*g* for 5 min using an SMT high-flex disperser (HG92, SMT company, USA) and then agitated for 10 min. Then 1 mL of each sample was taken, while stirring for protein determination by the Biuret method using a spectrophotometer (4001/4, Spectronic Instruments Inc., USA) (A). Subsequently, each sample (10 mL) was taken and heated in a water bath at 100 °C for 20 min, cooled to room temperature, and then centrifuged at 2000×*g* for 20 min. The protein content of 1 mL of supernatant was determined by the Biuret method (B). Heat coagulation (%) was calculated as [{absorbance before heating (A) − absorbance after heating (B)}/absorbance before heating (A)] × 100.

Isoflavone analysis

Extraction and determination of isoflavone were conducted according to the method of Wang et al. (1990), where 0.5 g sample in 2 mL of 1 M HCl was heated at 100 °C in a water bath for 20 min, after which 8 mL of acetonitrile was stirred in, and kept stationary for 2 h. The supernatant was filtered (Whatman No. 42), the filtrate was collected after filtering through a 0.2 µm Teflon syringe filter, and the optical density was measured by a spectrophotometer at 260 nm (the maximum absorption wavelength of isoflavone). The conditions for the separation analysis of isoflavone are shown in Table 2.

Table 2 HPLC conditions used for the analysis of isoflavone in fermented cheese analogs containing proteolyzed (roasted) soy flour

Instrument	Gilson 350 system
Column	Higgins Analytical, Inc.; C_{18} reversed-phase column (4 × 250 mm; particle size, 5 µm)
Injection volume	20 µL
Flow rate	1.0 mL/min
Detector	117UV (PDA) detector
Mobile phase	Solvent A: acetonitrile, Solvent B: phosphate buffer
	0 min 10 % A, 0–15 min 10–25 % A,
	15–30 min 25–40 % A, 30–40 min 40–50 % A
	Re-equilibrate for 20 min at 10 % A

DPPH radical scavenging

DPPH radical scavenging was determined by the slightly modified methods of Blois (1958) and Shimada (Shimada et al. 1992). To each sample (0.2 g/mL) melted in methanol, 4 times the volume of 2 × 10^{-4} M DPPH was added and mixed by a vortex mixer. The mixture was then kept in the dark for 30 min at room temperature. Tocopherol was used as a control. The optical density of the sample in a 96-well plate was measured at 510 nm to obtain the DPPH radical scavenging (%) as follows:

[(absorbance of control group−absorbance of fermented group)/absorbance of control group]× 100.

Yield

The % yield of the sample was obtained by measuring the weight before and after preparation:

[weight of fermented cheese analog (g)/weight of total ingredients (g)] × 100.

pH

An equal amount of distilled water was added to 10 g sample, homogenized at 37,000×*g* for 10 s using an SMT high-flex disperser (HG92, SMT company, USA), and the pH was measured using a pH meter (430 CORING, NY14831, USA).

Acidity

An equal amount of distilled water was added to 10 g sample, homogenized at 37,000×*g* for 10 s, and titrated with 0.1 N NaOH. The acidity was calculated after adding 0.5 mL of 0.1 % phenolphthalein as [(quantity of 0.1 N NaOH (mL) × F × 0.009)/quantity of sample (mL)] × 100.

Moisture content

Moisture content was measured with a Moisture Analyzer (LJ16, Mettler, Swiss); for this analysis, a 2 g sample was spread out evenly on a plate and dried for 30 min at 110 °C.

Heat extension

The heat extension of the fermented cheese analogs was determined by the slightly modified method of Chang (1976). Each sample was placed on a Petri dish (20 mm × 20 mm × 5 mm), heated at 220 °C in an oven for 5 min, kept at room temperature for 30 min, and subsequently assessed for extension by the increase in diameter.

Hunter color value

The sample was placed in a hexahedron-shaped (40 mm × 40 mm × 10 mm) container and lightness (L), redness (a), and yellowness (b) were measured using a color difference meter (Ultra PRO, Hunter Lab., USA).

Textural properties

Samples were cut into 20 mm × 20 mm × 20 mm cubes and their hardness, cohesiveness, springiness, adhesiveness, and chewiness were measured using a texture analyzer (Stable Micro Systems, TA. XT Express, England).

Sensory evaluation

Thirty undergraduate and graduate students in the department of Food and Nutrition at Chung-Ang University were provided with samples for the sensory evaluation of the cheese analogs fermented with rice straw. A 5-point scale method was used to evaluate appearance, color, flavor, mouthfeel, and overall quality (score of 1: very poor, to 5: very good).

Statistical analysis

All experiments were performed at least 3 times. The SAS software (SAS® 9.1, SAS Institute Inc., USA) was used for the analysis of variance. Significant differences among samples were determined by Duncan's multiple range test ($p < 0.05$) and the t test.

Results and discussion

SDS-PAGE

The SDS-PAGE patterns of the fermented cheese analogs made with proteolyzed (roasted) soy flour and roasted soy flour for different fermentation times are shown in Fig. 1. In the SDS-PAGE patterns, the protein subunits of P54 (one of 7S globulins) and acidic subunit of 11S globulins in the sample containing soy flour, and those of P26 and the acidic subunit of the 11S globulins in the sample containing roasted soy flour were consistently decomposed by increasing the fermentation time.

This was because the proteins were decomposed by fermentation with rice straw of *Bacillus* bacterium and mold. However, the bands of the basic subunit of 11S, low molecular weight, became darker in both samples. According to a report by Park et al. (2008), the molecular weight of protein subunits in *Cheonggukjang* was reported to be less than 33 kDa, which was similar to the sample containing roasted soy flour.

Heat coagulation

The heat coagulation of the fermented cheese analogs made with proteolyzed (roasted) soy flour by different fermentation times is shown in Table 3. Both groups fermented for 1 day showed the highest heat coagulations of 89.13 and 71.56 %. However, the soy flour group fermented for 3 days and the unfermented roasted soy flour group showed the lowest values of 74.70 and 49.72 %, respectively ($p < 0.001$).

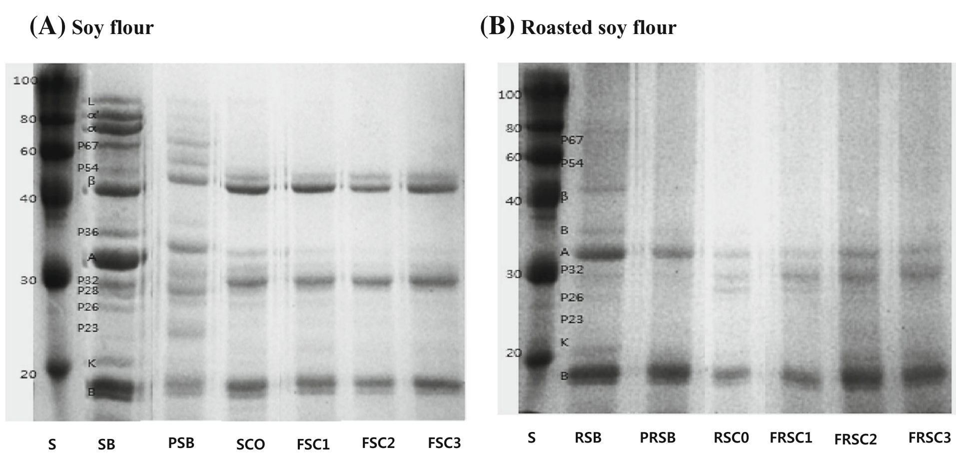

Fig. 1 Changes in SDS-PAGE patterns of fermented cheese analogs containing proteolyzed soy flour (**A**) and proteolyzed roasted soy flour (**B**) fermented with rice straw for different time periods. *L* Lipoxygenase(7S), *α'* α'-subunit of 7S globulin, *α* α-subunit of 7S globulin, *β* β-subunit of 7S globulin, *A* acidic subunit of 11S globulin, *B* basic subunit of 11S globulin, *P36* basic subunit dimer of 11S globulin, *K* Kunitz trypsin inhibitor (2S), *P32, P28, P26, and P23* 11S globulin fraction, *S* standard, *(R)SB* (roasted) soy flour, *P(R)SB* proteolyzed (roasted) soy flour treated with Flavourzyme®, unfermented [(R)SCO] and fermented cheese analogs with rice straw for 1[F(R)SC1], 2[F(R)SC2], and 3[F(R)SC3] days containing (roasted) soy flour treated with Flavourzyme®

Table 3 Changes in heat coagulation (%) of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different time periods

	Fermentation time (days)				*F*-value
	0	1	2	3	
FSC	81.71 ± 0.02[b]	89.13 ± 0.02[a]	76.84 ± 0.07[c]	74.70 ± 0.17[d]	9140.75***
FRSC	49.72 ± 0.28[d]	71.56 ± 0.13[a]	63.42 ± 0.09[b]	58.41 ± 0.31[c]	3951.53***
T-value	161.00**	189.72***	169.20***	66.31***	

** $p < 0.01$, *** $p < 0.001$

[a–d] Means with different letters in a row are significantly different by Duncan's multiple range test ($p < 0.05$)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

Isoflavone analysis

The isoflavone content of the fermented cheese analogs made with proteolyzed (roasted) soy flour by different fermentation times is shown in Table 4. The contents of daidzein and genistein, glycosides of isoflavone, in the unfermented soy flour group were the highest (42.92 and 52.03 mg/100 g), while those in the soy flour group fermented for 3 days were the lowest (3.98 and 5.72 mg/100 g, $p < 0.001$). The contents of daidzein and genistein, non-glycosides, in the soy flour group fermented for 2 days (33.47 and 61.49 mg/100 g), and the roasted soy flour group fermented for 1 day (30.57 and 49.54 mg/100 g), were the highest. The rapid variation (decrease or increase) of glycoside and non-glycoside content within 1 day of fermentation was believed to be influenced by the microorganisms in rice straw. Glycosides were converted to non-glycosides by β-glucosidase in soybean, by enzymes from a rice straw strain, or by thermal hydrolysis during processing (Coward et al. 1998; Chung and Kim 2001).

According to the report of Oh and Eom (2008), most bacteria and mold were grown within 12 and 24 h fermentation with rice straw, respectively. The activities of protease and β-amylase rapidly increased within 24 and 12 h, respectively. In addition, Wu et al. (2004) reported that the isoflavone content of tofu manufactured with the addition of enzymes extracted from *Aspergillus oryzae* and *Koji* could be increased compared to that of common tofu.

Compared to the contents of daidzein and genistein, non-glycosides, in soybean cheese, which were reported as 11.24 and 20.08 mg/100 g, respectively (Haytowiyz et al. 1999), those of the fermented soybean cheese developed in this study were 33.47 and 61.49 mg/100 g, respectively, approximately three times higher. Therefore, fermentation with rice straw was thought to be effective in converting

Table 4 Changes in isoflavone content (mg/100 g) of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different time periods

Isoflavone		Fermentation time (days)				*F*-value
		0	1	2	3	
Daidzein	FSC	42.92 ± 0.36[a]	8.36 ± 0.08[b]	4.24 ± 0.02[c]	3.98 ± 0.01[d]	137.612***
	FRSC	33.18 ± 0.40[a]	8.18 ± 0.02[b]	8.23 ± 0.03[b]	8.27 ± 0.03[b]	7945.55***
T-value		32.01***	3.16 NS	−181.24***	−216.93***	
Genistein	FSC	52.03 ± 0.40[a]	10.16 ± 0.19[b]	5.74 ± 0.05[c]	5.72 ± 0.04[c]	18210.8***
	FRSC	46.58 ± 0.24[a]	5.79 ± 0.05[d]	6.21 ± 0.01[c]	6.64 ± 0.03[b]	69709.5***
T-value		16.60***	31.62**	−13.55**	−26.50***	
Daidzein	FSC	6.99 ± 0.01[c]	31.45 ± 0.63[b]	33.47 ± 0.03[a]	31.04 ± 0.62[b]	2531.99***
	FRSC	6.04 ± 0.08[d]	30.57 ± 0.10[a]	29.45 ± 0.09[b]	26.71 ± 0.12[c]	46266.3***
T-value		17.43**	1.96 NS	58.81***	9.66*	
Genistein	FSC	24.92 ± 0.18[d]	55.22 ± 0.20[c]	61.49 ± 1.65[a]	57.60 ± 0.66[b]	717.99***
	FRSC	21.30 ± 0.40[c]	49.54 ± 1.46[a]	44.87 ± 0.47[b]	43.63 ± 0.31[b]	736.13***
T-value		11.78**	5.46*	13.68**	27.23***	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *NS* not significant

[a–d] Means with different letters in a row are significantly different by Duncan's multiple range test ($p < 0.05$)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

Table 5 Changes in hydrogen-donating activity (%) of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different time periods

	Fermentation time (days)				*F*-value
	0	1	2	3	
FSC	81.64 ± 0.70[b]	85.43 ± 0.41[a]	80.11 ± 1.25[b]	74.78 ± 2.10[c]	27.64***
FRSC	75.10 ± 0.32[c]	79.64 ± 1.14[b]	84.12 ± 0.83[a]	73.47 ± 0.93[c]	61.76***
T-value	11.98***	6.78*	−3.78*	0.81[NS]	

* $p < 0.05$, *** $p < 0.001$, *NS* not significant

[a–d] Means with different letters in a column are significantly different by different by Duncan's multiple range test ($p < 0.05$)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

the glycosides of isoflavone to non-glycosides, and the development of various types of functional fermented soybean food products would be possible by this route.

Antioxidant activity

The antioxidant activities of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times are shown in Table 5. The antioxidant activity of the soy flour group fermented for 1 day was the highest at 85.43 %, while that of the roasted flour group fermented for 3 days was the lowest at 73.47 % ($p < 0.05$). In the soy flour groups, antioxidant activity increased during the first day of fermentation and then decreased, while that in the roasted soy flour groups increased until 2 days of fermentation and then decreased. These results are similar to those for the isoflavone content, because antioxidants such as tocopherol, isoflavone, and saponin in soybean are produced by fermentation (Yee et al. 1980; Santiago et al. 1992; Park and Ryu 1996; Kim et al. 2007).

pH

The pH of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times is shown in Table 6. The pH of the unfermented soy flour group was the highest at pH 4.94, and that of the roasted soy flour group fermented for 3 days was the lowest at pH 3.95 ($p < 0.001$). In both groups, pH decreased with fermentation due to the formation of organic acids. The pH in

Table 6 Changes in the pH, acidity, moisture content, yield, and heat extension of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different time periods

		Fermentation time (days)				*F*-value
		0	1	2	3	
pH	FSC	4.80 ± 0.02[a]	4.06 ± 0.01[b]	3.97 ± 0.10[b]	3.95 ± 0.01[b]	161.73***
	FRSC	4.94 ± 0.01[a]	4.46 ± 0.08[b]	4.42 ± 0.06[b]	4.31 ± 0.02[b]	46.29***
T-value		−10.67**	−6.93*	−5.77*	−22.05**	
Acidity	FSC	1.20 ± 0.08[c]	2.11 ± 0.03[b]	2.46 ± 0.03[a]	2.49 ± 0.02[a]	350.15***
	FRSC	1.12 ± 0.01[c]	1.95 ± 0.02[b]	1.96 ± 0.01[b]	2.01 ± 0.02[a]	1924.61***
T-value		1.32[NS]	6.24*	25.39***	23.33***	
Moisture content (%)	FSC	32.03 ± 2.06[a]	29.03 ± 3.59[ab]	23.99 ± 1.52[bc]	22.11 ± 0.11[c]	8.13*
	FRSC	17.28 ± 1.81[c]	25.35 ± 1.13[b]	25.40 ± 1.14[b]	38.91 ± 0.81[a]	96.28***
T-value		7.61*	1.38 [NS]	−1.05 [NS]	−28.98**	
Yield (%)	FSC	87.46 ± 2.95[a]	68.79 ± 0.78[b]	55.68 ± 0.27[c]	51.67 ± 1.38[d]	5762.07***
	FRSC	81.93 ± 2.11[a]	72.83 ± 1.19[b]	63.74 ± 0.45[c]	57.34 ± 0.40[d]	234.51***
T-value		3.61*	−4.80*	−25.15**	−17.73**	
Heat extension (Cm)	FSC	2.13 ± 0.05	2.03 ± 0.05	2.10 ± 0.08	2.07 ± 0.05	1.00[NS]
	FRSC	2.07 ± 0.05[a]	2.17 ± 0.05[a]	1.94 ± 0.02[b]	2.05 ± 0.04[a]	10.67**
T-value		1.41 [NS]	−2.83*	2.61[NS]	0.38[NS]	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *NS* not significant

[a–d] Means with different letters in a row are significantly different by Duncan's multiple range test (p < 0.05)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

the roasted soy flour group after fermentation was higher and nearer to isoelectric point than that of the soy flour group, regardless of fermentation time.

Acidity

The acidity of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times is shown in Table 6. The acidity of the soy flour group fermented for 3 days was the highest at 2.49 %, and the acidity of the unfermented roasted soy flour group was the lowest at 1.12 % ($p < 0.001$). In both groups, acidity increased as fermentation time increased, and the acidity of the soy flour group was higher than that of the roasted soy flour group, regardless of fermentation time. Acidity generally increased as fermentation time increased, because of the release of carboxylic acid by protease and lactic acid produced by bacteria, such as *Lactobacillus* spp., during fermentation with rice straw (Alvaro et al. 1999).

Moisture content

The moisture content of the fermented cheese analogs prepared using proteolyzed (roasted) soy flour with different fermentation times is shown in Table 6. The moisture content of the roasted soy flour fermented for 3 days was the highest at 38.91 %, and that of the unfermented roasted soy flour was the lowest at 17.28 % ($p < 0.001$). The moisture content of the soy flour group decreased as the fermentation time increased, whereas that of the roasted soy flour group increased. The moisture content of the soy flour group decreased because more hydrophobic residues were exposed by microbial proteolysis during fermentation. The moisture content of the roasted soy flour group showed a large increase because of more hydrophilic residues such as free carboxylic acids, and free amino groups, as the denatured protein could be more easily degraded by microbial proteases during fermentation.

Yield

The yields of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times are shown in Table 6. The yield of the unfermented soy flour group was the highest, 87.46 %, and that of the soy flour group fermented for 3 days was the lowest at 51.67 % ($p < 0.001$). The yields of the roasted and unroasted soy flour groups decreased as the fermentation time increased. The yield of the soy flour sample, nearer to the isoelectric point of soy protein (pH 4.6) at which more curds were formed, was much higher than that of the roasted soy flour sample before fermentation. The lower yield of the roasted soy flour sample was due to the fact that the roasting decreased the water holding capacity, one of protein functionality, as the hydrophobic residues inside the protein molecule are exposed to the outside the protein by thermal denaturation.

The yield of the soy flour group showed a greater decrease after fermentation because the pH was further from the isoelectric point and the degradation of the acidic subunit of 11S globulin was greater than that for the roasted soy flour group (Fig. 1). The yield of the roasted soy flour group decreased as the fermentation time increased, although the moisture content showed an increase, because the roasting decreased the emulsion capacity by excessive thermal decomposition and the 7S globulin of roasted soy flour was degraded as shown by the SDS-PAGE patterns (Fig. 1). It has been reported that the emulsion capacity decreased by excessive proteolysis and it has been suggested that there was an optimum mean molecular size of proteins for obtaining the best emulsification (Quaglia and Orban 1990; Lee et al. 1995).

Heat extension

The heat extension of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times is shown in Table 6. The heat extension of the roasted soy flour group increased during the first day of fermentation and then decreased. Mahoney et al. (1982) showed that the heat extension of cheese increased because of the decrease in the size of the protein molecules by Flavourzyme® treatment. The heat extension of the roasted soy flour group increased significantly and then decreased because of the difference between soy flour and roasted soy flour resulting from Flavourzyme® treatment and fermentation. The cell size of the roasted soy flour was relatively small, as shown in Fig. 1.

Hunter color value

The color values of the fermented cheese analogs made with proteolyzed (roasted) soy flour by different fermentation times are shown in Table 7. The lightness of the roasted soy flour was higher than that of the soy flour, regardless of fermentation time. The redness of the unfermented soy flour was the lowest at 2.89 ($p < 0.001$), and that of both groups increased during the first day of fermentation. The redness of the roasted soy flour group was higher than that of the soy flour group, and the lightness of the roasted soy flour group was lower than that of the soy flour group.

This was similar to the results of Uh et al. (2006), as lightness decreased and redness increased with an increase in heating temperature and time. The yellowness of the soy flour increased as the fermentation time increased, and that of the roasted soy flour increased during the first day of fermentation and then decreased. The yellowness of the soy flour group was higher than that of the roasted soy flour group, regardless of fermentation time. The dE of both groups decreased until 2 days of fermentation and then decreased.

Table 7 Changes in the color of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different periods of time

		Fermentation time (days)				*F*-value
		0	1	2	3	
L (lightness)	FSC	77.72 ± 0.12[a]	69.86 ± 0.30[b]	60.94 ± 0.86[c]	59.19 ± 0.31[d]	650.80***
	FRSC	59.81 ± 0.04[a]	55.04 ± 0.08[b]	51.11 ± 0.19[d]	51.82 ± 0.27[c]	842.33***
T-value		193.34***	67.62***	15.70**	25.27**	
a (redness)	FSC	2.89 ± 0.06[c]	7.52 ± 0.17[a]	6.35 ± 0.05[b]	6.34 ± 0.07[b]	646.55***
	FRSC	7.55 ± 0.21[b]	9.04 ± 0.32[a]	9.22 ± 0.13[a]	9.07 ± 0.13[a]	25.26***
T-value		−30.55***	−5.91*	−29.43**	−25.48**	
b (yellowness)	FSC	15.91 ± 0.02[c]	19.04 ± 0.06[b]	20.10 ± 0.33[a]	19.54 ± 0.44[ab]	122.28***
	FRSC	15.87 ± 0.35[b]	18.21 ± 0.40[a]	16.37 ± 0.11[b]	15.81 ± 0.09[b]	28.11***
T-value		0.17[NS]	2.88[NS]	15.33**	11.64**	
dE	FSC	79.35 ± 0.12[a]	72.77 ± 0.32[b]	64.45 ± 0.81[c]	64.93 ± 1.18[c]	225.54***
	FRSC	62.31 ± 0.08[a]	58.65 ± 0.19[b]	54.43 ± 0.16[d]	54.91 ± 0.24[c]	786.39***
T-value		170.56***	53.67***	17.18**	11.76**	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *NS* not significant

[a–d] Means with different letters in a row are significantly different by Duncan's multiple range test ($p < 0.05$)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

Textural properties

The textural properties of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times are shown in Table 8. The hardness of the soy flour groups was higher than that of the roasted soy flour groups, as the β-subunits of 7S and P54 were not decomposed in the soy flour groups, as shown in the SDS-PAGE pattern (Fig. 1). The cohesiveness of the soy flour groups decreased as the fermentation time increased, and those of the roasted soy flour groups increased until 2 days of fermentation and then decreased ($p < 0.001$). The

Table 8 Changes in the textural properties of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different periods of time

		Fermentation time (days)				*F*-value
		0	1	2	3	
Hardness (g)	FSC	2212.34 ± 30.22[d]	2433.39 ± 30.00[c]	8123.82 ± 100.53[a]	7608.95 ± 129.33[b]	2.55***
	FRSC	1385.75 ± 11.92[b]	1133.08 ± 23.74[c]	1090.56 ± 40.74[c]	1695.25 ± 16.11[a]	260.30***
T-value		−51.08***	48.07***	91.70***	64.17***	
Cohesiveness (%)	FSC	29.94 ± 1.61[a]	27.42 ± 1.02[a]	23.16 ± 0.75[b]	23.72 ± 1.13[b]	0.24***
	FRSC	16.70 ± 1.85[b]	21.81 ± 0.27[a]	22.61 ± 0.50[a]	19.07 ± 0.77[b]	14.70***
T-value		7.64**	7.49*	0.87[NS]	4.81*	
Springiness (mm)	FSC	0.39 ± 0.02[a]	0.28 ± 0.01[b]	0.20 ± 0.01[c]	0.18 ± 0.00[d]	0.59***
	FRSC	0.14 ± 0.01[d]	0.20 ± 0.00[b]	0.18 ± 0.00[c]	0.22 ± 0.01[a]	106.61***
T-value		16.01**	8.61*	4.24*	−8.01*	
Adhesive force (g/cm²)	FSC	−188.38 ± 24.66[c]	−2003.03 ± 77.74[b]	−2346.24 ± 57.43[a]	−2341.44 ± 57.35[a]	1.25***
	FRSC	−241.89 ± 26.71[a]	−89.07 ± 14.79[b]	−27.63 ± 5.33[c]	−11.51 ± 0.51[c]	75.61***
T-value		2.08[NS]	−34.20***	−56.85***	−57.46***	
Chewiness (gmm)	FSC	24.84 ± 4.35[d]	186.22 ± 13.26[c]	382.83 ± 17.9[a]	320.15 ± 18.17[b]	0.32***
	FRSC	32.90 ± 2.45[d]	48.94 ± 1.26[b]	44.62 ± 2.36[c]	69.93 ± 1.72[a]	249.82***
T-value		−2.28 [NS]	14.58**	26.49**	19.39**	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, *NS* not significant

[a–d] Means with different letters in a row are significantly different by Duncan's multiple range test ($p < 0.05$)

F(R)SC fermented cheese analogs containing (roasted) soy flour treated with Flavourzyme®

cohesiveness of the soy flour groups was higher than that of the roasted soy flour groups, regardless of fermentation time. This was related to the higher heat coagulation of the soy flour groups (Table 3).

The springiness of the unfermented soy flour group was the highest at 0.39 mm, while that of the unfermented roasted soy flour group was the lowest at 0.14 mm ($p < 0.001$). The springiness of the soy flour group decreased as the fermentation time increased. The springiness of the soy flour groups was higher than that of the roasted soy flour groups until 2 days of fermentation, but the springiness of the roasted soy flour group was lower than that of the soy flour group at 3 days of fermentation. The adhesiveness of the unfermented soy flour group was the lowest at -188.38 g/cm^2, and that of the soy flour group fermented for 2 days was the highest at -2346.24 g/cm^2, which was not significantly different from that of the soy flour group fermented for 3 days. The adhesiveness of the soy flour groups increased as the fermentation time increased, but those of the roasted soy flour groups decreased. The adhesiveness of the soy flour groups was much higher than that of the roasted soy flour groups after fermentation.

The chewiness of the soy flour group fermented for 2 days was the highest (382.83 gmm), and those of both unfermented groups were the lowest ($p < 0.001$). The chewiness is calculated by hardness $\times$ cohesiveness $\times$ springiness. The chewiness of the soy flour groups following fermentation was greatly increased, which was similar to hardness results, while that of the roasted soy flour groups was similar to the springiness results.

Sensory evaluation

The sensory evaluation of the fermented cheese analogs made with proteolyzed (roasted) soy flour for different fermentation times is shown in Fig. 2. The color of the unfermented soy flour group was evaluated as the best, and that of the soy flour group fermented for 3 days was evaluated as the worst ($p < 0.05$); the same results were obtained for the roasted soy flour groups ($p < 0.05$). The flavor and mouthfeel of the soy flour group fermented for 2 days and the roasted soy flour group fermented for 1 day were evaluated as the best, while that of the unfermented soy flour group was evaluated as the worst ($p < 0.05$). The appearance of the roasted soy flour groups fermented for 1 and 2 days was evaluated as the best ($p < 0.05$), which might be because the cohesiveness of these groups were higher. There were no significant differences in overall quality, which could be because of the substances responsible for bean-like or *Cheonggukjang* flavor, which were removed or reduced by Flavourzyme® treatment and rice straw fermentation.

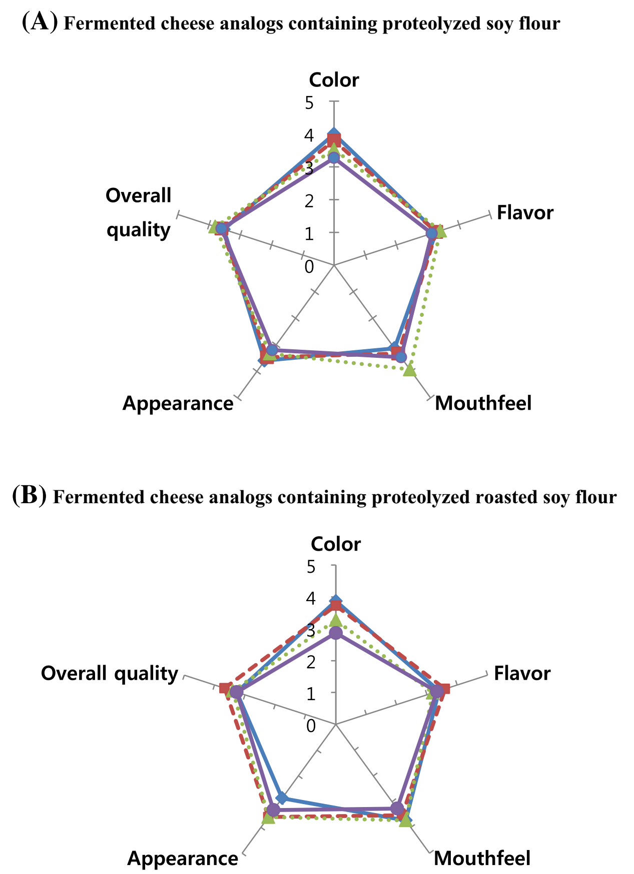

Fig. 2 Sensory characteristics of fermented cheese analogs containing proteolyzed (roasted) soy flour fermented with rice straw for different periods of time (unfermented (-◆-) and fermented cheese analogs for 1(--■--), 2(··▲··), and 3(-●-) days containing (roasted) soy flour treated with Flavourzyme®)

In conclusion, 1 day of fermentation time was the most desirable fermentation time for the production of fermented cheese analogs containing proteolyzed (roasted) soy flour, as an acceptable pH and the highest yield, heat coagulation, heat extension, lightness, dE, cohesiveness, springiness were obtained for this fermentation time. The pH of fermented cheese analogs containing proteolyzed soy flour and proteolyzed roasted soy flour were 4.06 and 4.46, respectively, which were acceptable pH values, similar to the pH of yogurt. Between the two groups that were subjected to fermentation for 1 day, the soy flour group showed better heat coagulation (89.13 %), genistein content, antioxidant activity, cohesiveness, springiness, and adhesiveness; however, the roasted soy flour group showed better yield (72.83 %) and heat extension.

Acknowledgments This research was supported by the Chung-Ang University in 2009.

References

Alvaro V, Javier V, Raul SV, Alfonso C, Justo P, Juan B, Franciso M (1999) Peptide characteristics of sunflower protein hydrolysates. J Am Oil Chem Soc 76(12):1455–1460

An SH, Lee SH, Park GS (2008) Quality characteristics of tofu prepared with various concentrations of commercial Cheonggukjang powder. Korean J Food Cook Sci 24(2):258–265

Bachmann HP (2001) Cheese analogues: a review. Int Dairy J 11:505–515

Blois MS (1958) Antioxidant determination by the use of a stable free radical. Nature 181:1199–1200

Catalano M, Ragona L, Molinari H, Tava A, Zetta L (2007) Anticarcinogenic Bowman Birk inhibitor isolated from snail medic seeds (Medicago scutellata): solution structure and analysis of self-association behavior. Biochemistry 42(10):2836–2846

Chang PK (1976) Partially delactosed whey used as NFDM replacement in American process cheese food offers economic advantages. Food Prod Dev 10(9):51–55

Cho DY, Lee MK, Kim EA, Lee SY (2015) Analysis of the isoflavone content, antioxidant activity, and SDS-PAGE of cheese analogs produced with different proteolysis and soymilk residue contents. J Korean Soc Appl Biol Chem 58(4):501–509

Choi SH, Ji YA (1989) Change in flavor of Chungkookjang during fermentation. Korean J Food Sci Technol 21(2):229–234

Chung HS, Kim CJ (2001) Contents of genistein and daidzein in tofu treated with enzyme, β-glucosidase Korea Soybean Digest 18(2):51–64

Coward L, Smith M, Kirk M, Bames S (1998) Chemical modification of isoflavones in soyfoods during cooking and processing. Am J Clin Nutr 68:1486–1491

Eymery O, Pangborn RM (1988) Influence of fat, citric acid and sodium chloride on texture and taste of a cheese analog. Science des Aliments 8:15–32

Haytowiyz DB, Beecher GR, Bhagwat S, Holden JM, Murphy PA (1999) Development of a database on the isoflavone content of foods. IFT Annual Meeting 106

Kamata Y, Chiba K, Yamauchi F, Yamada M (1992) Selection of commercial enzymes for soymilk-curd production by limited proteolysis with immobilized enzyme reactor. J Jap Soc Food Sci Tech 39:102–105

Kim OM, Gu YA, Jeong YJ (2007) Characteristics of mung bean powders after various hydrolysis protocols. Korean J Food Preserv 14:301–307

Kindstedt PS (1993) Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. Crit Rev Food Sci Nutr 33:167–187

Ko HS, Cho DH, Hwang SY, Kim YM (1999) The effect of quality improvement by Chungkukjang's processing methods. Korean J Food Nutr 12(1):1–6

Kramer A, Kwee WH (1977) Functional and nutritional properties of tomato protein concentrate. J Food Sci 43:207–211

Lee MK, Lee SY (2009) The quality characteristics of soy wan-jas made with different proteolytic enzyme treated textured soy proteins. J Korean Soc Appl Biol Chem 52:708–715

Lee YM, Lyu ES (2008) Physico-chemical and sensory characteristics of Cheonggukjang powder added hamburger patty. Koran J Food Cook Sci 24(6):742–747

Lee SY, Park PSW, Rhee KC (1992) Textural properties of cheese analogs containing proteolytic enzyme-modified soy protein isolates. J Am Oil Chem Soc 69(8):755–759

Lee SH, Cho YJ, Chun SS, Kim YH, Cho C (1995) Functional properties of proteolytic enzyme-modified isolated sesame meal protein. Korean J Food Sci Technol 27:708–715

Lee SY, Kim EB, Kim EJ, Lee HN, Lee MK, Oh JS, Kim SO (2008) The quality characteristics of soy cutlets using textured soy protein treated with different enzymes. Korean J Food Cult 23(4):507–513

Mahoney RR, Lazaridis HN, Rosenau JR (1982) Protein size and melt-ability in enzyme-treated, direct-acidified cheese products. J Food Sci 47:670–671

Oh HI, Eom SM (2008) Changes in microflora and enzyme activities of Cheonggukjang prepared with germinated soybeans during fermentation. Korean J Food Technol 40(1):56–62

Park EY, Ryu BH (1996) Antioxidant producing microorganism from Korean fermented food. Proceeding Kor Soc, Appl Microbiol **330**

Park IK, Yang SH, Choi YS (2008) Quality characteristics of soy ice cream prepared with fermented soybean powder base and oligosaccharide and its blood glucose lowering effect. Korean J Food Sci Technol 40:88–95

Quaglia GB, Orban E (1990) Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (Sardina pilchardus) protein hydrolysates. J Food Sci 55(6):1571–1573

Santiago LA, Hiramatsu M, Mori A (1992) Japanese soybean paste miso scavenges free radicals and inhibits lipid peroxidation. J Nutr Sci Vitaminol 38:297–302

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 40:945–948

Shon MY, Seo KI, Park SK, Cho YS, Sung NJ (2001) Some biological activities and isoflavone content of Chungkugjang prepared with black beans and Bacillus strains. J Korean Soc Food Sci Nutr 30(4):662–667

Shon MY, Kim MH, Park SK, Park JR, Sung NJ (2002) Taste components and palatability of black bean Chungkugjang added with kiwi and radish. J. Korean Soc Food Sci Nutr 31(1):39–44

Uh JH, Park M, Hsieh FH, Eun JB (2006) Physicochemical properties of roasted soybean flour in the temperature and time. Proceeding Korean J Food Preserv 196

Wang G, Kuan SS, Francis OJ, Ware GM, Carman AS (1990) A simplified HPLC method for the determination of phytoestrogens in soybean and its processed products. J Agric Food Chem 38:185–190

Webber K, Osborn M (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem 244:4406–4412

Wu ML, Chang JC, Lai YH, Cheng SL, Chiou RY (2004) Enhancement of tofu isoflavone recovery by pretreatment of soy milk with koji enzyme extract. J Agric Food Chem 52:4785–4790

Yee JJ, Shipe WF, Kinsella JE (1980) Antioxidant effects of soy protein hydrolysates on copper-catalyzed methyl linoleate oxidation. J Food Sci 45:1082–1083