ISSN 1330-9862 *original scientific paper*
(FTB-2546)

Physicochemical, Textural and Sensory Characteristics of Probiotic Soy Yogurt Prepared from Germinated Soybean

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Received: March 23, 2010
Accepted: April 25, 2010

Summary

Soymilk prepared from germinated soybean [*Glycine max* (L.) Merrill] with different hypocotyl lengths was fermented at 42 °C for approx. 4 h to produce soy yogurt (sogurt) with the combined probiotics of *Lactobacillus helveticus* B02, *Streptococcus thermophilus* IFFI 6038 and *Lactobacillus bulgaricus* AS1.1482. The physicochemical, textural and sensory characteristics of the fermented products were subsequently analyzed. Results showed that sogurt prepared from germinated soybean with the length of hypocotyls of 3 cm displayed lower pH and higher titratable acidity, and appeared to be more acceptable by the trained panel than sogurts prepared from soybean with hypocotyl length of 0 and 6 cm ($p<0.05$). Texture profile analysis demonstrated that the hardness of sogurts significantly decreased (from 26.71 to 16.89 g), while the adhesiveness significantly increased (from –71.77 to –31.94 g·s) as hypocotyl length increased ($p<0.05$). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profile of soymilk demonstrated that the α' and α subunits of β-conglycinin (7S) and the acidic chains of glycinin (11S) were gradually degraded, which may be partly responsible for the decline of water holding capacity and the amelioration of textural properties of the germinated soy yogurt. The total concentration of free amino acids obtained by high-performance liquid chromatography (HPLC) was 515.78 μg/mL in the soymilk from ungerminated soybean (S_0), while it reached 847.44 and 880.75 μg/mL from soybean with 3-cm (S_3) and 6-cm (S_6) hypocotyls, respectively. Some of the increased levels of free amino acids may account for the improvement of flavour and reproduction of lactic acid bacteria. It could be concluded that, with appropriate germination, the physicochemical and textural properties as well as sensory characteristics of sogurt could be improved significantly.

Key words: germinated soybean, soy yogurt, probiotic, storage protein, free amino acids, physicochemical properties, textural profile analysis

Introduction

Being one of the most important cash oil crops, soybean [*Glycine max* (L.) Merrill] is widely planted. It is rich in nutritive elements like proteins, unsaturated fatty acids, lecithins, isoflavones, mineral substances, free amino acids and polypeptides (*1*). Soymilk-based yogurt, namely, soy yogurt or sogurt, is produced by the fermentation of soymilk using lactic acid bacteria. Because of its beany flavour, insufficient acidity, hard and coarse texture (*2*), sogurt is not widely accepted by consumers. Great efforts have been made by several researchers to improve the flavour and textural properties of sogurt, for instance by calcium fortification (*3*), microware treatment (*4*), ultra high pressure homogenization (*5*), the addition of mango pulp (*6*) or inulin with raffinose and glucose (*7*).

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Special issue: Probiotics, Prebiotics and Synbiotics

Several endogenous endopeptidases (*8–10*) are found to be active in soybean seedling, some of which are responsible for the hydrolyzation of soybean storage protein subunits. Consequently, the formation of three-dimensional network structure of soybean protein gel may be affected (*11*) and the textural characteristics of the fermented soymilk product could therefore be meliorated. Meanwhile, the endogenous aminopeptidase (*12*) and carboxypeptidases (*13*) that catalyze the removal of amino acids from the amino or carboxylic terminal of a polypeptide chain are also found in germinated soybean. During soymilk fermentation, the cleaved free amino acids could accelerate the reproduction of probiotics and be changed into aroma compounds that improve the flavour of sogurt.

Probiotics are live microorganisms which can exert health benefits on the host when the administered amount reaches a considerable level. The health-enhancing effects of probiotic-fermented soy foods like hypocholesterolemia, antihypertension, improvement of immunity, alleviation of lactose intolerance, reduction of ovarian cancer and cardiovascular disease risks (*14,15*) have been well studied. Recently, *Lactobacillus helveticus*, a lactic acid producing rod-shaped bacterium, has been reported to be able to modulate the production of interleukin-8 by human intestinal epithelial cells (IEC) *in vitro* (*15*) and convert free amino acids into volatile aroma compounds (mostly benzaldehyde, dimethyl disulphide and 2-methyl propanol) (*16*).

There are a few researches on sogurts produced by the fermentation of germinated soybean extract, which were found to contain reduced levels of flatulence-causing oligosaccharides, and were enriched with soy phytoalexins, active isoflavone (*17*) and γ-aminobutyric acid (*18*). Nevertheless, no research on the textural properties of sogurt containing probiotics prepared from germinated soybean extract has been conducted yet. The aim of the present study is to evaluate the germination effect on the characteristics of soymilk and sogurt.

Materials and Methods

Materials

Soybeans [*Glycine max* (L.) Merrill] were purchased from the local supermarket (Guangzhou, PR China). Skimmed milk powder, with protein content of ζ=32.7 %, was purchased from Fonterra Co, Ltd. (Tauranga, New Zealand). The other reagents were analytically pure.

Microorganisms and media

Lactobacillus bulgaricus AS1.1482 and *Streptococcus thermophilus* IFFI 6038 were purchased from Microbiological Culture Collection Center (Guangdong, PR China). *Lactobacillus helveticus* B02 was obtained from Chr. Hansen Co, Ltd. (Tianjin, PR China). Stock cultures were incubated in an ordinary incubator at 37 °C for 24 h in MRS broth medium (sterilized in an autoclave at 121 °C for 20 min) and then kept at 4 °C until further use. The subcultures were obtained by inoculating stock cultures (ψ=5 %) into reconstituted skimmed milk (RSM) (ζ=12 %) and incubating at 37 °C for 10–14 h. The RSM was sterilized in an autoclave at 115 °C for 10 min.

Preparation of soymilk

Soybean germination test was carried out according to an earlier research by Feng *et al.* (*17*) with some modifications. The selected soybean seeds were surface-sterilized with 75 % ethanol for 1 min and then rinsed six times to wash away the ethanol. The surface-sterilized seeds were then soaked in sterile double distilled water at a ratio of 1:4 (*m/V*) for 14 h at 25 °C. The soaked beans were placed on a sterilized double-layer container (27×34 cm) lined with 4 sterile cheesecloths moistened with 300 mL of sterile double distilled water, and covered with 2 sterile cheesecloths with the same treatment. Then the beans were germinated in an incubator at 80 % relative humidity for 3–4 days at 25 °C in the dark until the length of hypocotyls reached 0.5, 1, 2, 3, 4, 5, 6 and 7 cm. After the seed coats were discarded, soybeans were homogenized with double distilled water at a ratio of 1:10 (*m/V*) for 3 min at 85 °C using a HR2004 blender (Philips, Guangzhou, PR China). The resulting slurries were filtered through a 180-mesh filter to yield soymilk. For the purposes of this work, the soymilk prepared from the soaked beans without germination was labelled S_0, while the other two samples that were used (soymilk from the soybeans with 3- and 6-cm hypocotyls) were labeled S_3 and S_6, respectively.

Protein content, free amino acid (FAA) and SDS-PAGE determination of soymilk

Protein content of soymilk was analyzed by Kjeldahl method (*19*). Free amino acid (FAA) evaluation was carried out by using HPLC analysis. The soymilk was centrifuged (10 000×*g*, 15 min, 4 °C; Hitachi CR 22G, Tokyo, Japan) and then the supernatant was filtered through 0.45-μm filter membrane. HPLC analysis was carried out with a Waters HPLC system fitted with a Pico·Tag® column (Waters Corp., Milford, MA, USA). For the analysis of FAAs, the mobile phase constituted of 20 mM CH_3COONa buffer (pH=7.2), triethylamine (ψ=0.018 %) and tetrahydrofuran (ψ=0.3 %) (solvent A), and mixed solvent of 20 mM CH_3COONa buffer (pH=7.2), acetonitrile and methanol with a ratio of ψ=1:2:2 (solvent B). The quantitative data for FAAs were obtained by comparison to known standards.

The changes of subunits of soybean protein during germination were determined by using SDS–PAGE according to a modified method of Laemmli (*20*) using 12.5 % separating gel and 3 % stacking gel. The gel was stained with 0.1 % (*m/V*) Coomassie Brilliant Blue R-250 in methanol (ψ=45 %) and acetic acid (ψ=10 %), and destained overnight in a solution containing methanol (ψ=30 %) and acetic acid (ψ=45 %). The protein fractions were identified by using recombinant molecular mass standard mixture (TaKaRa Biotechnology Co, Ltd, Dalian, PR China).

Reconstituted skimmed milk yogurt (RSMY) and soy yogurt preparation

The soymilk was mixed with reconstituted skimmed milk (ζ=12 %) at a ratio of 7:3 (by volume). Both the

RSM and the soymilk mixed with RSM were supplemented with 8 % (*m/V*) sucrose and then sterilized at 100 °C for 20 min. After being cooled to 42 °C, all of the sterilized media were inoculated with the 5 % mixed starter culture of *Lactobacillus bulgaricus* AS1.1482, *Streptococcus thermophilus* IFFI 6038 and *Lactobacillus helveticus* B02, at a volume ratio of ψ=1.5:1.5:2, and incubated at 42 °C for approx. 4 h. Both the RSMY and soy yogurt were removed from the incubator and refrigerated at 4 °C for further analysis.

Acidity, water holding capacity (WHC) and colour measurement of RSMY and soy yogurt

The pH of the samples was measured by using a pH meter (S20, Mettler, Küsnacht, Switzerland). The percentage of lactic acid was used as a representative of titratable acidity according to the formula:

$$w(\text{lactic acid})/\% = [(\text{volume of 0.1 M NaOH})\times \times(\text{normality of NaOH})\times 9]/\text{sample mass titrated in g} \qquad /1/$$

Samples were titrated with 0.1 M sodium hydroxide until the final pH of the solution was 8.3. The amount of titrant was then calculated to determine the acidity of the sample (*21*).

WHC of the samples was determined by a method reported earlier (*22*) with slight modifications. Inoculated soymilk-RSM solution and RSM (20 g) were incubated in polypropylene centrifuge tubes (radius 32 mm, height 115 mm) at 42 °C until the end of fermentation. After 24 h of cold storage at 4 °C, the stored samples were centrifuged (500×*g*, 10 min, 20 °C; Hitachi CR 22G, Tokyo, Japan) and the expelled whey was weighed. The following formula was used to calculate WHC:

$$w(\text{WHC})/\% = (1-m_1/m_2)\times 100 \qquad /2/$$

where m_1 is the mass of whey after centrifugation and m_2 is the mass of RSMY or soy yogurt.

Colour attributes of the samples were determined with a chroma meter CR-400/410 (Minolta, Tokyo, Japan), which gave L^*, a^* and b^* values directly. Total colour difference was calculated by the formula:

$$\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \qquad /3/$$

while the RSMY was used as reference.

Texture profile analysis of RSMY and soy yogurt

Texture profile analysis (TPA) test was performed by using a TA XT2 texture analyzer (Stable Micro Systems Ltd, Godalming, Surrey, UK). An SMS P/0.5 probe was used to measure the TPA of the samples at room temperature, which was done in 3 repetitions. During the pretest, compression and relaxation of a sample, the speed of the probe was 1.0 mm/s, while the speed of obtaining the data was 200 pps. The thickness of samples was set at 5 cm and 30 % of the original depth was compressed during the first stage.

Sensory evaluation

Sensory properties of samples were evaluated by a trained panel consisting of 8 assessors at a mean age of 25±3. RSMY and different kinds of soy yogurt samples were served at 7 to 10 °C in plastic cups and coded with three-digit numbers. Order of presentation of the samples was randomized. A test form comprising four sensory attributes, namely, appearance, flavour, texture and overall acceptability, was given to each assessor. A standard 9-point scale was used for evaluation of sensory characteristics of samples, in which 1 was equal to the worst and 9 was equal to the best.

Statistical analysis

Data were expressed as mean±standard deviation (SD) from three independent parallel experiments. The analysis of variance was performed by ANOVA and significant differences among the means of samples were analyzed by Duncan's test with a 95 % confidence level.

Results and Discussions

Acidity, water holding capacity and colour analysis of RSMY and soy yogurt

Results presented in Table 1 demonstrate the positive effect of germination on some physicochemical characteristics of sogurt. With the fermentation of probiotic soymilk for approx. 4 h at 42 °C, all of the samples had a pH value below 4.2 accompanied with titratable acidity over 0.75 %. In comparison with RSMY, an appreciable decrease in pH was noted in sogurt after the fermentation of probiotics. The pH value of S_3 yogurt

Table 1. Changes of some physicochemical properties in RSMY and soy yogurt

Sample	pH	w(TA)/%	w(WHC)/%	Colour			
				L^*	a^*	b^*	ΔE^*ab
RSMY	$(4.13\pm0.01)^c$	$(0.86\pm0.00)^c$	$(85.07\pm0.01)^a$	$(77.42\pm0.15)^c$	$(-4.21\pm0.06)^a$	$(9.60\pm0.03)^c$	
S_0 yogurt	$(3.94\pm0.01)^b$	$(0.76\pm0.01)^a$	$(92.35\pm0.02)^c$	$(77.92\pm0.37)^d$	$(-3.67\pm0.03)^b$	$(9.07\pm0.03)^a$	0.86
S_3 yogurt	$(3.90\pm0.02)^a$	$(0.80\pm0.01)^b$	$(90.31\pm0.03)^{bc}$	$(76.16\pm0.03)^b$	$(-3.07\pm0.02)^c$	$(9.32\pm0.02)^b$	1.71
S_6 yogurt	$(3.92\pm0.01)^{ab}$	$(0.76\pm0.01)^a$	$(87.54\pm0.01)^{ab}$	$(75.06\pm0.21)^a$	$(-3.12\pm0.02)^c$	$(9.63\pm0.03)^c$	2.60

Values are mean±S.D., N=3; mean values in the same column with different letters in the superscript are significantly different ($p<0.05$); RSMY=reconstituted skimmed milk yogurt; S_0, S_3 and S_6 indicate the length of soybean hypocotyls; TA=titratable acidity; WHC=water holding capacity; L^* value represents lightness and darkness with a range from black (0) to white (100), a^* value represents the green-red spectrum with a range from green (–100) to red (+100), while b^* value represents blue-yellow spectrum with a range from blue (–100) to yellow (+100). Total colour difference ΔE^*ab was determined for different sogurts by using the control RSMY as the reference produced in the present study was 3.90, which is slightly lower than of the ungerminated soybean yogurt (3.94). The highest titratable acidity (0.80 % lactic acid) was also observed in S_3 yogurt, while a lower titratable acidity of about 0.76 % was obtained in both S_0 and S_6 yogurt. Previous studies have shown that fermentation of soymilk with probiotics incubated at 40–45 °C for a few hours decreases the pH value to 3.9–4.3 (*23*) and increases total titratable acidity for 0.64–0.97 % (*7,24*). All of the samples produced in the present study were in accordance with the previous researches.

WHC of a protein gel is an important parameter in yogurt manufacturing. After 24 h of refrigeration, the w(WHC) of S_0 yogurt was (92.35±0.02) and of S_3 yogurt was (90.31±0.03), and was found to be slightly higher than that of RSMY, which was (85.07±0.01) % and of S_6 yogurt, which was (87.54±0.01) % (Table 1). Lower WHC or whey separation is partly due to the unstable gel network of yogurts (*7*), in which the weak colloidal linkage of protein micelles cannot entrap water within its three-dimensional network.

Colour measurement shows the slight difference of colour between different kinds of sogurts. With the growth of soybean sprouts, L^* values of sogurts decreased while a^* and b^* values increased, demonstrating that sogurts made from germinated soybeans were darker, more greenish and yellowish. In comparison with RSMY, total colour differences of sogurts were increased with the process of germination (Table 1).

Texture profile analysis of RSMY and SY

Texture profile analysis imitates the conditions in the mouth by compressing a product twice (*25,26*). Results in Table 2 demonstrate that, except for the cohesiveness, germination significantly improved the textural characteristic of sogurts, furthermore, the positive effect increased with the course of germination. Hence, all of the textural properties of S_6 yogurt were similar to those of the control RSMY.

As a critical parameter for evaluation of textural characteristics of food, hardness is used to estimate the maximum force of the first compression. The highest hardness was measured in S_0 yogurt (26.71±1.35) g, while the lowest one was observed in RSMY and S_6 yogurt (approx. 16 g). Textural and rheological properties of coagulated yogurt products are, to a great extent, determined by their internal structure. It is generally accepted that soybean glycinin is a hexamer consisting of five kinds of subunits, each subunit containing acidic and basic chains linked by a disulphide bond (*27*). Consequently, the tight and rigid molecular structure finally results in a firm and brittle protein gel. The microstructure of RSMY consists of a three-dimensional network of casein particles, containing spherical molecules of different sizes, some of which can barely form a chain structure (*28*). As a result, loose network architecture with low hardness was formed (Table 2).

Cohesiveness of sogurts maintained the same level during soybean seedling growth, indicating that germination did not diminish the strength of internal bonds of all samples. With the unchanged levels of cohesiveness, multiplication of hardness and cohesiveness, namely gumminess, also decreased. Adhesiveness is commonly calculated as the field surface of a negative peak. After germination, the values of adhesiveness increased approx. by 31.93 (S_3 yogurt) and 55.49 % (S_6 yogurt), demonstrating that more force was needed to remove the material adhered to the mouth during eating sogurts produced from germinated soybean. The springiness of S_6 yogurt (0.99±0.01) was slightly higher than of the others, indicating that it returned more easily to its original shape after the deforming force was removed.

Sensory evaluation of RSMY and soy yogurt

Sensory evaluation of RSMY and soy yogurt is presented in Table 3. Compared to S_0 yogurt, the sensory characteristics of soy yogurt obtained from 3-cm long hypocotyls markedly improved. Nevertheless, the appearance, flavour and overall acceptability were negatively affected by the subsequent growth of hypocotyls. The lowest appearance score was obtained in S_6 yogurt, where dark yellow colour, surface cracks and whey separation were observed. With slightly lower whey separation, the RSMY also got a low score in appearance. Off-flavours, beany, grassy and the lack of characteristic flavour have been described as the main flavour problems in soy yogurts (*2*). After the fermentation of RSM and soymilk with RSM, both the RSMY and soy yogurt had a strong characteristic dairy and soy aroma. However, a slightly beany flavour remained in S_0 yogurt and an obvious soybean sprout and astringent flavour appeared in the S_6 one, which reduced the flavour scores of these soy yogurts remarkably. The higher flavour score in S_3 yogurt may be attributed to the lowest beany flavour after germination. Due to the relatively high gel hardness and grainy sensation, S_0 yogurt got the lowest texture score. On the whole, having the highest overall acceptability score of 7.44±0.91 on a nine-point scale, the S_3 yogurt appeared to be more acceptable to the trained assessors than others. It can be concluded that, with appropriate germination,

Table 2. Texture profile analysis of RSMY and soy yogurt

Sample	Hardness/g	Adhesiveness/(g·s)	Springiness	Cohesiveness	Gumminess
RSMY	(16.03±0.51)^{a}	(–31.14±5.21)^{c}	(0.98±0.01)^{b}	(0.46±0.01)^{a}	(7.38±0.01)^{a}
S_0 yogurt	(26.71±1.35)^{c}	(–71.77±10.53)^{a}	(0.95±0.00)^{a}	(0.45±0.02)^{a}	(12.15±0.86)^{c}
S_3 yogurt	(21.33±0.19)^{b}	(–48.86±4.84)^{b}	(0.96±0.01)^{a}	(0.44±0.02)^{a}	(9.36±0.39)^{b}
S_6 yogurt	(16.89±0.48)^{a}	(–31.94±2.04)^{c}	(0.99±0.01)^{b}	(0.45±0.01)^{a}	(7.59±0.40)^{a}

Values are mean±S.D., N=3; mean values in the same column with different letters in the superscript are significantly different ($p<0.05$); RSMY=reconstituted skimmed milk yogurt; S_0, S_3 and S_6 indicate the length of soybean hypocotyls

Table 3. Sensory evaluation of RSMY and soy yogurt

Sample	Appearance	Flavour	Texture	Overall acceptability
RSMY	$(5.93\pm0.60)^{b}$	$(7.64\pm0.34)^{d}$	$(7.73\pm0.53)^{c}$	$(7.25\pm0.44)^{c}$
S_0 yogurt	$(6.74\pm0.53)^{c}$	$(5.41\pm0.90)^{b}$	$(5.86\pm0.60)^{a}$	$(5.89\pm0.67)^{b}$
S_3 yogurt	$(6.78\pm0.45)^{c}$	$(6.73\pm0.48)^{c}$	$(6.96\pm0.60)^{b}$	$(6.94\pm0.56)^{c}$
S_6 yogurt	$(4.74\pm0.51)^{a}$	$(4.23\pm0.86)^{a}$	$(7.10\pm0.71)^{bc}$	$(4.54\pm0.92)^{a}$

Values are mean±S.D., N=3; mean values in the same column with different letters in the superscript are significantly different ($p<0.05$); RSMY=reconstituted skimmed milk yogurt; S_0, S_3 and S_6 indicate the length of soybean hypocotyls

Table 4. Changes in the concentrations of free amino acids during the germination of soybean

Amino acid	$\gamma(S_0)/(\mu g/mL)$	$\gamma(S_3)/(\mu g/mL)$	$\gamma(S_6)/(\mu g/mL)$
Asp	13.50	72.59	70.35
Glu	51.28	24.66	14.03
Ser	11.05	61.69	64.40
Gly	9.30	15.60	18.27
His	17.22	54.06	76.96
Arg	247.49	246.36	228.12
Thr	43.42	124.90	146.18
Ala	3.69	55.65	50.84
Pro	11.93	27.56	36.42
Tyr	30.97	27.95	26.55
Val	11.58	25.40	29.17
Met	16.83	10.13	9.96
Cys	0.26	0.19	0.23
Ile	6.85	15.06	15.08
Leu	8.48	16.90	16.77
Trp	13.40	10.41	9.12
Phe	6.60	19.64	22.41
Lys	11.91	38.68	45.90
Total	515.78	847.44	880.75

Free amino acids were analyzed by HPLC as described in Materials and Methods; S_0, S_3 and S_6 indicate the length of soybean hypocotyls

the sensory characteristics of sogurt could be markedly improved.

Free amino acid determination

The changes in the levels of free amino acids during soybean germination were measured by HPLC analysis. As shown in Table 4, arginine was the most abundant acid in both ungerminated and germinated soybean extracts. During the process of germination, the total contents of FAA in soymilk were increased from 515.78 up to 847.44 µg/mL (S_3 yogurt) and 880.75 µg/mL (S_6 yogurt). Meanwhile, alanine was approx. 13.7 to 15.1 times higher than the initial content and the contents of some essential amino acids (His, Thr, Val, Ile, Leu, Phe, Lys) were also shown to be increased. The increased levels of free amino acids were mainly released due to the hydrolysis of polypeptide chains of storage proteins by exopeptidases (*12,13*) after the imbibition of soybean seeds. Since the catabolism of amino acids (such as Arg, Thr and His) by lactic acid bacteria is fully understood (*29*), the combined probiotic starters are therefore capable of metabolizing the increased levels of Thr, His or other amino acids and producing more dissolved hydrogen ions as well as enough organic acids in the germinated soybean extract (Table 1). The flavour of germinated soybean yogurts is also improved as more volatile aroma compounds are converted (*16*).

SDS-PAGE analysis

Changes of storage protein subunits during soybean germination are shown in Fig. 1. The α′ and α subunits of β-conglycinin (7S) and the acidic chains of glycinin

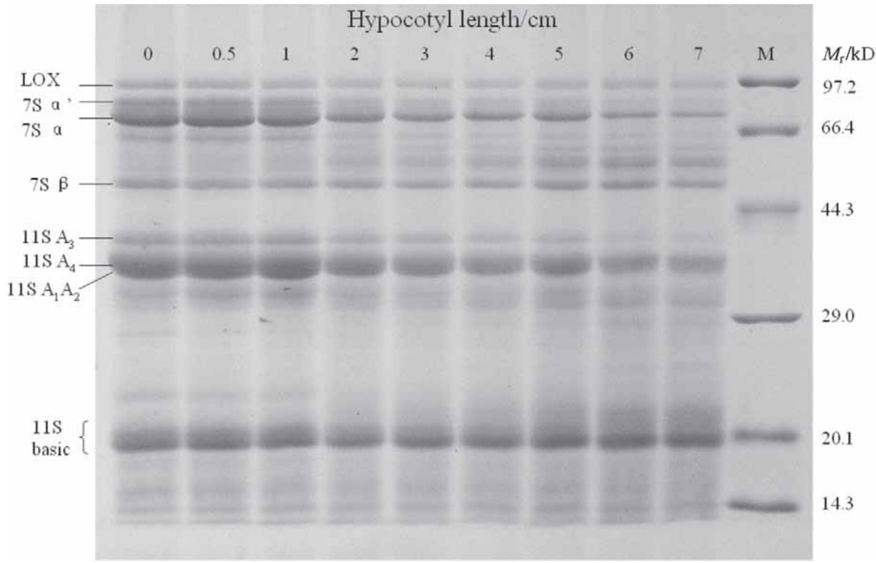

Fig. 1. SDS-PAGE gel electrophoresis of soymilk prepared from soybean with hypocotyls of different length. From left to right the lanes contain samples of soymilk as the hypocotyls grew from 0 up to 0.5, 1, 2, 3, 4, 5, 6 and 7 cm, respectively. The protein fractions were identified by using recombinant standard mixtures with different molecular masses (line M). LOX=lipoxygenase

(11S) were preferentially metabolized right after the soybean imbibition. This finding is in accordance with the studies of Sathe *et al.* (*30*) and Wilson *et al.* (*31*). Of the three subunits of β-conglycinin, the larger α′ and α subunits were rapidly degraded, generating new β-conglycinin cross-reactive polypeptides of approx. 50.0 kDa molecular mass as soybean hypocotyls reached 2–3 cm. It has been reported that several proteases like protease C1 (*8*), a novel serine protease (*9*) and a cysteine endopeptidase D3 (*10*) are responsible for the initial degradation of the α′ and α subunits of β-conglycinin. As the hypocotyls grew up to 5 cm, protein bands with molecular mass between 25.0 and 35.0 kDa appeared in the gel, indicating that the acidic chains A_3, A_4, A_2 and A_1 of glycinin were rapidly hydrolyzed to a smaller molecular mass form. This may be attributed to the protease G1, which is found to catalyze a limited specific proteolysis of acidic polypeptides of glycinin (*31*). No observable decrease in the basic chains of glycinin was shown in this gel profile.

Recently, researchers have reported that gels made from soybean glycinin are harder than the β-conglycinin one (*32*). As germination continued, the molecular structure of soybean storage protein was more loose since the acidic chains of glycinin (11S) degraded gradually. After that the cross-linking of soybean protein three-dimensional network decreased, and softer, less hard S_3 and S_6 yogurt gels were formed (Table 2). Nevertheless, as hypocotyls grew to 6 cm, the relatively over-degraded subunits of soybean storage protein caused larger weakening of colloidal linkage of soy protein micelles and the formation of an unstable network with lower water holding capacity (Table 1).

Lipoxygenase (LOX) is responsible for the beany flavour of soy products formed during catalytic oxidation of polyunsaturated fatty acids containing *cis*,*cis*-1,4-pentadiene units to the corresponding conjugated hydroperoxydiene derivatives by the addition of molecular oxygen (*33*). Fig. 2 shows that LOX decreased gradually with the growth of hypocotyls and the lowest LOX activity was observed in 3-cm long hypocotyls, which was accounted for the lowest beany flavour in S_3 yogurt (Table 3).

Protein content measurement

Protein content is an important factor that affects the quality of acid coagulation of protein gel products. The protein content of soymilk increased at the initial phase of germination and then declined as the germination progressed. Right after imbibition, (2.61±0.07) g per 100 g of protein were obtained, while the maximum protein content was observed in 0.5-cm long hypocotyls ((2.91± 0.06) g per 100 g). After that, the protein content declined and remained in the range of 2.3 to 2.1 g per 100 g, and finally declined to the lowest level ((1.94±0.04) g per 100 g) as hypocotyls grew to 7 cm (Fig. 2). With the growth of hypocotyls, some physicochemical properties of sogurt products were diminished because of the low content of protein (Table 1). Consequently, the length of hypocotyls should be well considered during the production of germinated soybean yogurts.

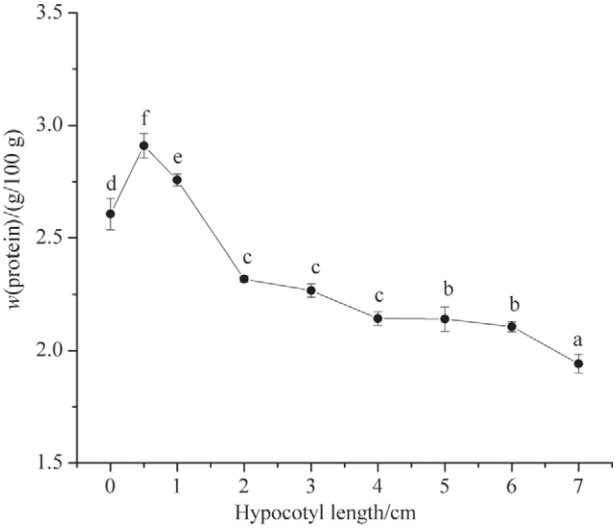

Fig. 2. Protein content of soymilk prepared from soybean with hypocotyls of different length. S_0 soymilk was prepared from the soaked beans without germination. The determinations were performed in triplicate for each sample. Different letters represent significant differences ($p<0.05$)

Conclusions

The results of the present study indicate that, after the fermentation of probiotics, all of the sogurts had a pH value ranging from 3.90 to 3.94 and titratable acidity between 0.75 and 0.80 %. With the degradation of the acidic chains of glycinin (11S) and α′ and α subunits of β-conglycinin (7S), the water holding capacity of sogurts decreased, and textural properties improved (with increasing adhesiveness, decreasing hardness and gumminess). The enhanced levels of free amino acids after germination were responsible for the conversion of aroma compounds, flavour improvement and growth of lactic acid bacteria. In addition, with appropriate germination, physicochemical and sensory properties as well as textural characteristics of germinated soybean yogurt could be significantly improved.

Acknowledgement

This project was supported by the National Natural Science Foundation of China (Grant No. 30770056).

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