Effective of different homogeneous methods on physicochemical, textural and sensory characteristics of soybean (Glycine max L.) yogurt
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ABSTRACT
The effect of ultrasonication (US), shear mixing (SM) and high pressure homogenization (HPH) on soybean (Glycine max L.) milk-produced set-type soybean yogurt was evaluated. The US treated with 280 W sample had the lowest water holding capacity value and highest syneresis value. Rheological constants were predicted by means of fitting the rheological model of non-Newton model. The soybean yogurt samples were found as a pseudoplastic fluid nature and the SM treated with 20,000 r/min sample had the highest apparent viscosity value and the US treated with 280 W had the lowest value. The HPH treatment caused a general decrease of hardness and adhesiveness in comparison to other samples. The overall acceptability scores of all the samples were above 6 on a scale of 1–10 indicating acceptability yogurts and the lower score in beany flavor for the yogurt might be partly due to the objectionable beany flavor still present in the samples.

Introduction
Soy milk is a water extract of soybean (Glycine max L.) that can provide a plentiful and inexpensive supply of protein and calories (Syah et al., 2015). It is the highest soybean-based product consumed in the world, not only because of its high nutritional value but also as an alternative to cow’s milk for lactose-intolerant individuals, those allergic to milk proteins, or those avoiding milk for other reasons (Reilly, Lanou, Barnard, Seidl, & Green, 2006) (Falade, Ogundele, Ogunshe, Fayemi, & Ocloo, 2014). However, the presence of indigestible oligosaccharides such as stachyose and raffinose and the raw bean flavor have limited the wide consumption of soy milk. To address those matters, fermentation of soybean products with lactic acid bacteria has been studied extensively, to develop more digestible and palatable foods such as fermented soybean cheese (Jianming, Yuan, Ping, Feng, & Liying, 2013; Vargas-Bello-Pérez, Fehrmann-Cartes, Íñiguez-González, Toro-Mujica, & Garnsworthy, 2015), sour milk beverage (do Amaral Santos, da Silva Libeck, & Schwan, 2014; Rathi, Upadhay, Dabur, & Goyal, 2015) and soybean yogurt (Saito et al., 2014; Ye, Ren, Wu, Wang, & Liu, 2013).

Soy milk is an oil-in-water emulsion, in which the continuous phase is formed by complex protein dispersion. To obtain this emulsion, the most conventional processing method is the heat treatment of the product after soaking and grinding soybeans with water (Cruz et al., 2007). The food industry is interested in new technologies to obtain high-quality and stability products. Ultrasonication (US) applications in milk fermentation science and technology are noteworthy. It is a promising tool for milk homogenization. US could subdivide the fat globules and clumps in milk to such a small size that they will no longer rise to the top of the milk as a distinct layer in the time before the milk is normally consumed. Improvements over conventional homogenization could lead to yogurt of superior quality (Wu, Hulbert, & Mount, 2000). In particular, US has been used to treat dairy systems to achieve endpoints, including the inactivation of enzymes and bacteria, homogenization, crystallization, degassing and extraction of various components and to alter the physical properties of gels made from...
milk and milk protein concentrate (Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010). High pressure homogenization (HPH) is a promising technique, particularly suitable for continuous production of fluid foods (Da Cruz et al., 2010) (Castañón-Rodriguez et al., 2015). HPH can significantly modify the structure and texture of whey proteins and reduce droplet size, and, thus, could improve food quality (Ramassamy, Chen, & Rattan, 2015). When liquid food passes through the high pressure valve it generates an increment of flow speed and a loss of pressure, bringing about cavitation, chisel effect, turbulence and collision of dispersed particles of liquid foods, such as fat droplets and microorganisms, which may be destroyed (Floury, Bellette, Legrand, & Desrumaux, 2004; Poliseli-Scopel, Hernández-Herrero, Guamis, & Ferragut, 2012). High shear mixing of small volumes is typically performed in the laboratory: the liquid medium is drawn axially into the dispersion head at high rotation speeds of the rotor and forced radially out the slots of the stator, producing large shear and thrust forces that provide mixing and disrupt cells (Koh et al., 2014).

Some attempts have been made to produce yogurts with increased solubility through the application of US, high shear mixing and high pressure. However, they were not compared against with each other. In the research, the effect of US, SM and HPH treatment before soybean yogurt fermentation were evaluated, and the rheological, texture profile analysis, water holding capacity, syneresis and other physicochemical properties of the finished yogurt were studied.

Materials and methods

Materials

Soybean used in this study was provided by Yihai Kerry (Shanghai, China). The commercial yogurt starter cultures containing Lactobacillus bulgaricus and Streptococcus thermophilus were purchased from Chuanxiu (Chuanxiu technology limited, Beijing, China).

Soybean milk elaboration

About 500 g of dry, mature, whole soybeans were washed and soaked in distilled water containing sodium bicarbonate (0.8 %) at 25°C for 12 h. After decanting the water, the soaked soybeans were mixed with 8 times their weight of distilled water for 3 min and comminuted to get soy milk in a lab-type soy milk machine (Soypower, MJ820, NY, U.S.A.). The resulting suspension was filtered through three layers of cheesecloth. The total solids, fat and protein content of unstandardized soybean milk were 7.80%, 0.81%, 3.13%. Then 4% sucrose was added to the native solution.

US treatments were carried out using an ultrasonic processor (THC-28, Tianhua, Jining, China) operating at a constant frequency of 24 kHz. The processor was fitted with an ultrasonic probe which had a 22 mm diameter tip. Soy milk samples (about 700 mL) were transferred to a 1000 mL beaker fitted with a recirculating water jacket and equilibrated for 5 min at a temperature of 45°C. Based on preliminary experiments, treatment time was 5 min with a continuous application of ultrasound at a power output of 280 W (US-280). Shear mixing was accomplished using a rotor-stator homogenizer (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at speed 20,000 r/min for 2 min (SM-20). HPH treatments were performed in a homogenize machine (Ah-basic, ATS Engineering Inc, Brampton, Canada) at 100MPa (HPH-100).

The soybean without homogeneous processing for yogurt making was used the control. These concentrations used in this study were selected based on our preliminary tests. Then the samples were all treated by heating (90°C, 10 min). After processing, the sample was cooled and stored at 4°C for a maximum of 6 h prior to use.

Soybean yogurt

The commercial cultures (Chuanxiu technology limited, Beijing, China) containing L. bulgaricus and S. thermophilus were thawed and diluted at 0.1% (v/v) in sterile peptone water just before inoculation. The culture was inoculated at 0.03% (v/v) in aliquots of the processed soybean milk samples (100 mL) and incubated for 12 h at 30°C. Then the samples were held at 4°C for 24 h.

Texture evaluation

Texture profile analysis was conducted using the methods of Bedani et al. (Bedani, Vieira, Rossi, & Saad, 2014). Texture test of samples were performed at 4°C in a TA-TX2 texture analyzer (Stable Micro System, Surrey, UK) equipped with a 25 mm cylindrical probe were used to perform a puncture test. The cylinder compressed the sample to a depth of 15 mm in a double compression cycle, using a crosshead speed 1 mm s⁻¹. From the measurement of load (N) as a function of time (s) the following attributes were analyzed: hardness, cohesion energy, springiness, chewiness and gumminess.

Rheological analysis

Prior to measurements, the samples were stirred to mimic typical consumer behavior before soybean yogurt consumption. Prestirring is a common preparatory procedure prior to viscometry testing of yogurt, as it is simple and does not induce substantial loss of structure. The rheological properties were analyzed according to the method described by Rezaei et al. (Rezaei, Khomeiri, Aalami, & Kashaninejad, 2014) using a R/S plus rotational rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a fixed outer cylinder and rotating measuring bob. The radius of rotating cylinder was 12.50 mm, the length of the cylinder and the gap width was 50.00 mm and 2.00 mm, respectively. Rheological curves were obtained after 5 min stabilization at 8 ± 1°C. T Flow curves were performed upward and downward at shear rates ranging from 10 to 290 s⁻¹. Both delay time and integration time were set at 5 s. Experiment data were fitted to the non-Newton model (Equation (1)) in order to determine the consistency (k) and the flow behavior indexes (n). The measurements were performed in triplicate.

\[ \sigma = k \gamma^n \]  

where \( \sigma \) is shear stress (Pa) while \( n \) is flow behavior index, \( k \) is consistency coefficient (Pa s^n) and \( \gamma \) is the shear rate (1/s).

Color measurement

Color determinations were made at 5°C by Labscan XE (Hunterlab, Reston, USA). Soybean yogurt samples (50 mL) at
5°C were stirred and placed in an aluminum cylinder (outside diameter 55 mm), with the surface optically flat before measuring, and the sensor was mounted directly on top of the cylinder to prevent ambient light noise. The values were calibrated with a standard plate (L₀ = 93.37, a₀ = −0.91 and b₀ = 0.19). L*, a* and b* were the color values of the samples. These analyses were performed in triplicate.

Water-holding capacity (WHC)

WHC was determined with a modified procedure adapted from Akalin et al. (Akalin, Unal, Dinkci, & Hayaloglu, 2012). A sample of about 20 g of each sample was centrifuged for 10 min at 3,000 g and 20°C. The whey was separated from the samples, which were then reweighed. The WHC was expressed as weight of drained whey per 100 g soybean yogurt sample.

Syneresis

Syneresis was determined by the method reported by Izadi et al. (Izadi, Nasirpour, Garoosi, & Tamjidi, 2015) with some modifications. This involved placing 30 g of unstripped sample in a funnel lined with a Whatman filter paper number 1 (Whatman International Ltd., Maidstone, England). The graduated cylinder was then held at 4°C for 5 h and the volume of liquid collected was recorded. The following formula was used to calculate syneresis:

\[
\text{Syneresis (} \% \text{)} = \frac{V_1}{V_2} \times 100.
\]

where: \( V_1 \) = volume of whey collected after drainage; \( V_2 \) = volume of soybean yogurt sample.

Sensory evaluation

Five aspects (appearance, texture, beany flavor, acidity and overall acceptability) were evaluated by 11 experienced assessors (between 20 and 30 years of age, 7 males). Participation was free for anyone who wished to take part and had mainly students and staff of the Laboratory of Dairy Science in Shanghai Jiao Tong University. As selection criteria, people who were sick, have flu or those with some kind of allergy or intolerance to milk were excluded, besides those consuming dairy foods at least weekly were included. Ten-point system was used for evaluation according to method of Isanga and Zhang (Isanga & Zhang, 2009).

Sensory evaluation scores: extremely unacceptable = 1; unacceptable-barely acceptable = 2–4; acceptable-very acceptable = 5–9; extremely acceptable = 10. The sensory cards included an ‘Observations’ section, in which the panellists were asked to indicate any defects noticed or any descriptors considered useful to better define the yellow soybean yogurt samples.

Statistical analysis

Experimental data were analyzed using SPSS software (version 19.0, Statistical Package for the Social Sciences Inc., Chicago, IL, USA). The one-way ANOVA procedure followed by Duncan’s multiple range tests was adopted to determine the significant difference (\( p < 0.05 \)) between treatment means, and the results were expressed as mean ± SD.

Results and discussion

The pH change

The pH values of the soybean yogurt were showed in Table 1. All the samples are within the target range (3.32–3.50). The HPH-100 sample had lower pH values (3.32). The HPH-100 sample had lower pH values (3.32), which may be explained by the starter which results in more lactate production and therefore lower pH value (Aryana & McGrew, 2007).

WHC and syneresis of soybean yogurt

The WHC of yogurt is an indicator of its ability to retain serum in the gel structure. The ability of a yogurt product to exhibit minimal whey separation is an important factor for its retail success, because whey separation negatively affects consumer perception (Bong & Moraru, 2014). The homogenization treatment had an effect on the WHC of soybean yogurt (Table 1). The control had the lowest WHC value (48.13%) and the US-280 sample had the highest value (49.42%). Higher WHC of the soybean yogurt samples could be related to higher solvation of the micellar system and to a more branched soybean yogurt microstructure, not easy to lose water when submitted to centrifugal forces. Meanwhile, the decreases were obtained in control and HPH-100 samples, which could arise from the looser bonds between H₂O molecules and soybean protein (Akalin et al., 2012).

As shown in Table 1, syneresis was found to be higher in US-280 sample. It may be due that syneresis in the US treatment samples are caused by an increased cross-linking among protein molecules through various interactions. The higher protein concentrations could increase the number of cross-linking in the soybean yogurt gel network, which may induce an increase in syneresis (Noh, Park, Pak, Hong, & Yun, 2005). One of the interesting findings is that higher pH values lead to increase in syneresis. The statistical analysis showed a significant difference (\( p < 0.05 \)) between the samples.

Color

Color is an important quality attribute of a product like yogurt. The results of color measurement in the soybean yogurt samples are expressed according to the CIELAB system, and the values L*, a*, b* and ∆E* were determined.

| Sample  | pH     | WHC (%) | Syneresis (%) |
|---------|--------|---------|---------------|
| US-280  | 3.50 ± 0.03ab | 49.42 ± 0.48c | 69.04 ± 0.78bc |
| SM-20   | 3.38 ± 0.01ab  | 49.02 ± 0.19bc | 65.11 ± 0.61bc |
| HPH-100 | 3.32 ± 0.02ab  | 48.42 ± 0.40bc | 60.18 ± 0.74bc |
| C       | 3.34 ± 0.03ab  | 48.13 ± 0.28bc | 62.96 ± 0.70bc |

Data show in mean ± standard deviation. Different letters in the same column indicate significant differences (\( p < 0.05 \)). Los datos muestran promedio ± desviación estándar. Las distintas letras en la misma columna indican diferencias significativas (\( p < 0.05 \)).
Table 2. L*, a*, b* color coordinates and color difference (ΔE) of soybean yogurt samples treated by ultrasonication, shear mixing and high pressure homogenization.

| Sample    | L*      | a*      | b*      | ΔE     |
|-----------|---------|---------|---------|--------|
| US-280    | 87.92 ± 0.03<sup>b</sup> | -2.60 ± 0.08<sup>c</sup> | 11.49 ± 0.03<sup>a</sup> |        |
| SM-20     | 88.48 ± 0.08<sup>c</sup> | -2.39 ± 0.09<sup>bc</sup> | 12.45 ± 0.04<sup>a</sup> | 13.29 ± 0.07<sup>bc</sup> |
| HPH-100   | 88.71 ± 0.10<sup>d</sup> | -2.70 ± 0.09<sup>bc</sup> | 12.60 ± 0.05<sup>ab</sup> | 13.38 ± 0.06<sup>ab</sup> |
| C         | 87.12 ± 0.06<sup>a</sup> | -3.05 ± 0.04<sup>c</sup> | 11.09 ± 0.03<sup>b</sup> | 12.74 ± 0.03<sup>b</sup> |

Data show in mean ± standard deviation. Different letters in the same column indicate significant differences (p < 0.05).

Rheological properties of soybean yogurt

Some studies related to the yogurt have been focused on rheological parameters and sensory properties can devote the additional quality characteristics of the yogurt (Cier, Gündüz, Yılmaz, & Memeli, 2015). In the present study, the effect of homogenization treatment to soybean milk on rheological behavior of the yogurt was investigated. The empirical data obtained from the Brookfield viscometer were converted into viscosity functions (apparent viscosity and shear rate). The apparent viscosity of the soybean yogurt samples decreased as the rate of shear increased (Figure 1). The SM-20 sample had the highest apparent viscosity value and the US-280 had the lowest value. Rheological constants were predicted to describe this behavior by means of fitting the rheological model of non-Newton to experimental data (Table 3). Non-Newton model estimation was in the range of 0.15–0.23 for flow behavior index (n), and in the range of 7.59–11.32 for consistency coefficient (k) of soybean yogurt with different homogenization treatment conditions. Since n values were all lower than 1, the soybean yogurt samples were found as a pseudoplastic fluid nature. Similarly, the rheological studies on fermented soy milk beverages with the addition of apple juice revealed that it behaved as a thixotropic fluid and flow textural properties.

Figure 1. Changes of apparent viscosity of yellow soybean yogurt treated by ultrasonication (US-280), shear mixing (SM-20) and high pressure homogenization (HPH-100).

Table 3. Statistical evaluation of the compatibility of the non-Newton model (viscosity) with experimental stress-shear rate data and texture profile analysis of soybean yogurt samples treated by ultrasonication, shear mixing and high pressure homogenization.

| Sample    | k      | n      | R<sup>2</sup> | Hardness | Adhesiveness | Cohesiveness |
|-----------|--------|--------|--------------|----------|--------------|--------------|
| US-280    | 7.59 ± 0.03<sup>a</sup> | 0.23 ± 0.04<sup>ab</sup> | 0.999 ± 0.0001<sup>bc</sup> | 0.28 ± 0.07<sup>bc</sup> | -1.11 ± 0.11<sup>bc</sup> | 0.46 ± 0.06<sup>a</sup> |
| SM-20     | 11.32 ± 0.07<sup>c</sup> | 0.15 ± 0.01<sup>b</sup> | 0.9995 ± 0.0002<sup>bc</sup> | 0.28 ± 0.03<sup>bc</sup> | -1.22 ± 0.06<sup>bc</sup> | 0.46 ± 0.06<sup>a</sup> |
| HPH-100   | 8.79 ± 0.10<sup>d</sup> | 0.19 ± 0.01<sup>b</sup> | 0.9991 ± 0.0003<sup>ab</sup> | 0.20 ± 0.03<sup>c</sup> | -0.53 ± 0.07<sup>bc</sup> | 0.51 ± 0.04<sup>a</sup> |
| C         | 8.30 ± 0.04<sup>b</sup> | 0.21 ± 0.01<sup>c</sup> | 0.9994 ± 0.0001<sup>c</sup> | 0.27 ± 0.03<sup>b</sup> | -1.05 ± 0.13<sup>bc</sup> | 0.50 ± 0.05<sup>a</sup> |

Data show in mean ± standard deviation. Different letters in the same column indicate significant differences (p < 0.05). Los datos muestran promedio ± desviación estándar. Las distintas letras en la misma columna indican diferencias significativas (p < 0.05).
curves showed that it has shear-thinning properties (Lcjer et al., 2015).

**Texture profile analysis**

Texture means physical properties of the yogurt that come from its structural elements, which can be perceptible by human senses. The main texture parameters of yogurt are: hardness, adhesiveness and cohesiveness (Domagala, Sady, Grega, & Bonczar, 2006). Texture profile data of all the soybean yogurt samples are presented in Table 3. No differences (\(p > 0.05\)) in hardness were observed between the control and treated samples. The HPH treatment caused a general decrease of hardness and adhesiveness in comparison to other samples. Even though without statistical significance (\(p > 0.05\)), there was a tendency for an increased hardness and decrease cohesiveness in US-280 and SM-20. Serra et al. reported that HPH of milk at 100–130 MPa decreased significantly the firmness of such yogurt and lower than that of yogurt made from conventionally treated milk fortified with skim milk powder (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007). That is in agreement with the current research. However, the findings were contradictory to what was expected for high pressure effects on texture and WHC of soybean yogurt. Serra et al. also observed that improvement in firmness and WHC only when the pressure applied were higher than the pressure used (i.e. ≥200 MPa).

Higher pressure for soybean milk treatment can increase the number of particles and amount of denatured proteins that could interact with each other, leading to the production of firmer and more stable yogurt (Huang & Kuo, 2015).

**Sensory evaluation**

The sensory evaluation scores are reported in Table 4. On the whole, overall acceptability scores of all the samples were above 6 on a scale of 1–10 indicating acceptability yogurts with the homogenization treatments of the soybean yogurts. The HPH-100 showed better sensory quality than others. The lowest scores were given to the C sample signifying that the homogenization treatments had positive effects on soybean yogurt production. The soybean yogurt had a lower score in flavor (below 5 on a scale of 1–10), which might be partly due to the objectionable beany flavor still present in yogurt samples. The soybean milk was heated before use to remove or reduce the raw soybean odor and deactivate physiological harmful substances, such as trypsin inhibitor, and hemagglutinin (Nishinari, Fang, Guo, & Phillips, 2014). However, the treatment does not meet the panelists’ demands. The highest scores for texture were given to the HPH-100 sample. There was no difference in texture between US-280 and SM-20 yogurt samples. In addition to flavor and texture, the difference in acidity may also affect the overall acceptability. The acidity in US-280 yogurt was higher than other samples and got the lowest score. In general, acidity more than 1.8% led to unpleasant acid taste and that titratable acidity about 1.15% was considered as an optimum range (Park et al., 2005).

**Conclusions**

In the present study, different treatments-US, high shear mixing and HPH were performed on soybean milk to produce set-type soybean yogurt. The US- and HPH-treated samples had improved mechanical characteristics and water holding capacity when compared with yogurts made from soy milk using conventional heat treatment. Besides, they received good results in the overall acceptability of sensory evaluation. Our results suggest that US and HPH have the potential to be used to improve the texture and stability of soybean yogurt.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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