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The effects of freezing on soybean microstructure and qualities of soymilk

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ABSTRACT

This study was to investigate the effects of freezing on soybean microstructure and the qualities of soymilk made from the soybean by direct grinding in water without soaking. Transmission electron microscopic (TEM), particle size distribution determination and SDS–PAGE were used for the examination. TEM images showed that freezing treatment could induce the coalescence of protein storage vacuoles (PSVs) and oil bodies. Particle size distribution showed a trend that prolonging of freezing could decrease the average particle size of soymilk and increase the extraction efficiency of soybean. SDS–PAGE showed that extractable proteins were mainly composed of glycinin and β-conglycinin even after freezing. After freezing, soybean could be easily stored and could be used to make soymilk without soaking. The soymilk had a higher solid content and better stability than the soymilk from soybean without treatment. Thus, this study provided a convenient and short-time way to process soymilk at home.

1. Introduction

Soybeans and soy foods are very popular in Asian countries, and gradually consumed by western countries. This was owing to its nutritional and functional properties (FAD, 1999). Recent researches had demonstrated that soy products were associated with prevention of heart disease, certain types of cancer, osteoporosis, obesity and controlling cholesterol-lowering (Anderson et al., 1999; Garcia et al., 1997; Gullett et al., 2010; Sarkar and Li, 2003; Messina and Messina, 1991; Zhang et al., 2008; Velasquez and Bhathena, 2007; Orgaard and Jensen, 2008).

Soymilk is a kind of traditional and popular beverage made from soybean and could be used to prepare Tofu and Yuba. The invention of the soymilk grinder provides people the convenient and inexpensive way to make soymilk at home. In recent years, the health benefits of soy products and the popularity of soymilk grinder have contributed to an increase in soymilk consumption in China. It is said that there are about 70 million sets of family soymilk grinder now and about 20 millions sets are sold each year in China because many Chinese used to drink soymilk in their breakfast (China Household Electrical Appliances Association, 2010).

In soymilk making, soaking is the most time–cost processing which usually needs 12–24 h for home making. Many works have been done to shorten the soaking time either by choosing soybean cultivars with high water-absorbability or increasing soaking temperature by heating. However, soaking is still a neck for homemade soymilk. Some survey showed that about 61% of home grinders were expected to shorten the time in processing of soymilk (China Household Electrical Appliances Association, 2010).

Generally, freezing may destruct food cell and decreased the food quality so should be avoided in food processing. But sometimes, freezing may be used to alter the mechanical properties of the cell material because tissue damage could modify the texture of food and improve processing properties. Freezing has previously been proved to show positive effects on soybean for cooking or processing. It gives soybean better taste and reduces half of the cooking time when soybean was cook with rice (Lee et al., 1992). It could significantly improve the water absorption rate in rehydration and decrease cooking hardness of black beans (Gao et al., 2011). Tofu, prepared from frozen soybeans, also exhibits a more orderly and denser network structure because soymilk made from frozen soybeans coagulates faster and forms uniform structured gels (Noh et al., 2005). Soybean was also frozen before fermentation so as to improve the efficiencies of yeast. But there is not any research on frozen soybean for soymilk making and the effects of freezing on structure of soybean also have not been reported.

Based on the principles that the growth of ice crystals during freezing could damage the internal structure of soybeans and some gaps or channels might be formed in the air-drying process due to the water evaporation, which could help water enter the cell issue while the protein and lipid release into soymilk. In this study, we tried to develop a new kind of instant soybean for homemade

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soymilk, which was frozen and 45 °C air-dried. The effects of freezing on soybean microstructure and the qualities of soymilk were investigated.

2. Materials and methods

2.1. Preparation of freezing and air-drying treated soybean (FADTS)

Soybean (Zhonghuang No.13, protein 39%, lipid 17%, moisture content 14%), harvested in October 2009, were obtained from Chinese Academy of Agricultural Sciences. They were stored at 4 °C until use. In order to find a suitable freezing course, the freezing characteristics curve of freezer was tested before preparation of freezing and air-drying treated soybean. The soybean sample (100 ± 0.01 g) was placed in a freezer (BCD-278AZ, Hefei MeiLing Co., Ltd., China). A temperature recorder (179-T2, Apresys, Lnc., USA) was placed inside the sample to monitor the temperature during freezing. The sample was frozen once placed in the freezer and the temperature continuously was recorded and freezing curve was prepared.

About 100 g soybeans were washed and soaked in the de-ionized (DI) water at 20 °C for 10 h. The soaked beans were placed in a plastic draining basket (diameter 200 mm) to remove excess water and then frozen at a −5 °C refrigerator for 1 day, 2 days and 4 days respectively. The soybeans were dried at 45 °C in an air-dryer (DGX-9073 B-1, Shanghai FuMa Text Equipment Co., Ltd., China) until weight became to 100 ± 0.01 g.

2.2. Transmission electron microscope (TEM) detection

Soybean samples were dissected into small pieces and immediately fixed in 2.5% glutaraldehyde buffered at pH 7.2 with 50 mM sodium phosphate. Primary fixation was performed at room temperature (about 25 °C) for 4 h. After several washes with water, some tissues were post-fixed with 1% aqueous osmium tetroxide for 1 h. Excess osmium tetroxide was removed by washing the samples in water three times. The samples were dehydrated in either a graded ethanol or acetone series. Then the tissues were incubated in propylene oxide and infiltrated gradually in Spurr's resin. Thin sections of all embedded tissues were sectioned with a diamond knife and collected on 200 mesh copper grids. Thin sections were stained individually with Sato's triple lead citrate solution and examined in a JEOL 1200 EX (Tokyo, Japan) transmission electron microscope at 80 kV.

2.3. Soymilk preparation

Soybean (100 g) or FADTS (100 g) were added into a soymilk grinder (Model FSM-100, Shenyang Machinery No. 3 Factory, China), and DI water were added to make the total weight of 1000 g. This was ground for 5 min without soaking. The slurry was filtered through a 100-mesh sieve and the filtrate was deemed as raw soymilk. Raw soymilk was heated at approximately 95 °C for 5 min by an Ohmic heating equipment (Frontier Engineering Co., Japan).

2.4. Internal space of soybean and FADTS determination

One thousand kernels were selected at random for soybean and FADTS (1 day, 2 days, 4 days) respectively. The sample mass was measured with an electronic balance (YP 10002, Shanghai YuePing Equipment Co., Ltd., China) of 0.01 g sensitivity. Sample volume was measured by a hexane-displacement method. Hexane 500 ml was putted into a 1000 ml cylinder. Sample was lightly poured into the cylinder and all the beans were fell on cylinder bottom after about 20 s. The final volume was then measured by the measuring cylinder. Each of the experiments had 3 replicates. The ratio of volume to weight of sample was calculated using the following formula:

$$\text{Ratio of volume to weight(ml/g)} = (\text{final volume(ml)} - 500(\text{ml}))/\text{Sample mass(g)} \quad (1)$$

2.5. Protein, lipid and solid determination

Protein was determined by the Kjeldahl method (AACC, 2000a), employing a nitrogen-to-protein conversion factor of 6.25.

Lipid was determined with AACC method 30-25 (AACC, 2000b). The freeze-dried soymilk was extracted for 6 h with petroleum ether with an Automated Soxhlet Extractor (SZF-06C, Zhejiang TuoPu Equipment Co., Ltd., China).

Solid content was determined by the method of Khursheed and Suthar (Khursheed and Suthar, 2010).

2.6. Analysis of soymilk particle size

The particle size of soymilk was measured using a laser light scattering instrument (LS 320, Beckman Coulter, Lnc., USA) with a Small Volume Module sample platform. The refractive index was 1.333. DI water was poured into the instrument sample chamber and was circulated at 50 l/min. Each sample was measured in triplicate and expressed as a percentage of volumetric particle size distributions using Beckman Coulter LS version 3.29 analytical software.

2.7. SDS–PAGE

Sucrose was put into soymilk to the concentrations of 20%. They were stirred for 20 min and centrifuged (50,000g, 30 min). Soymilk was separated into three fraction, floating fraction (oil bodies), supernatant fraction (protein fraction), and precipitate fraction (a little). The protein fraction was used for SDS–PAGE.

SDS–PAGE was conducted with the method of Laemmli (1970) with the concentrations of the stacking and running gels being 5% and 12.5%, respectively. The buffer in the reservoir contained 0.025 M Tris, 0.192 M glycine and 0.1% SDS, while the buffers in the stacking and running gels were 0.125 M Tris–HCl (pH 6.8) and 0.38 M Tris–HCl (pH 8.8), respectively. Samples 0.01% contained 0.25 M Tris–HCl (pH 6.8), 1% SDS, 2% 2-mercaptoethanol,

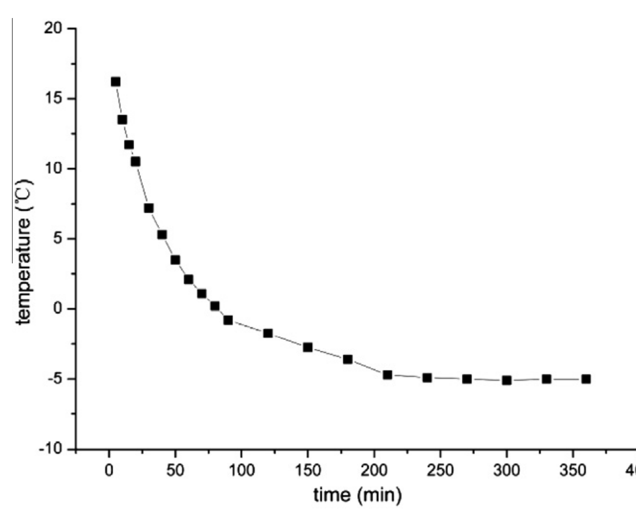

Fig. 1. The effect of freezing time on the soybean temperature.