Original Article

Change in oligosaccharides during processing of soybean sheet

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Oligosaccharides have been credited with many health-promoting functions, which had been identified in many clinical studies, such as promoting the growth of *Bifidobacterium* in human intestine and balance of intestinal bacteria, modulating the immune response, inhibition of cancer and tumor, stimulation of mineral absorption. In this study the effect of processing unit operations on the levels of soybean oligosaccharides during production of soybean sheet were investigated. The concentrations of oligosaccharide in initial raw soybean were: sucrose 43.05 g/kg, raffinose 7.52 g/kg and stachyose 41.32 g/kg (in dry matter). Oligosaccharide losses in the soaking water, in the first filtrating stage, in the second filtrating stage and finally in the sheet formation stage were 0.68, 10.3, 8.15 and 47.22 g/kg (initial dry soybean) respectively, representing 0.74, 11.21, 8.87 and 51.39% of the total oligosaccharides present in the initial soybeans. The recovery of oligosaccharides in the final soybean sheet from the initial soybean was 27.92%. The loss of soybean oligosaccharides in different processing stages, especially in the by-product, the sweet slurry, was considerable. The loss of oligosaccharides was mainly associated with water/matter removal in production process. The analysis of loss profile implied possible ways to improve the technology for production of oligosaccharides-enriched soy-sheets.

Key Words: soybean sheet, loss of oligosaccharides, raffinose, stachyose, sweet slurry

Introduction

Soybean oligosaccharides are a group of soluble low molecular weight oligosaccharides in soybean seeds, which include sucrose, stachyose and raffinose. Soybean oligosaccharides are defined as non-digestible oligosaccharides (NDOS) or non-digestible sugars (NDS) except sucrose,[1,2] since human gastrointestinal tract does not possess α-galactosidase enzyme essential for hydrolysis of the α-1, 6 galactosyl linkages.[3] Therefore oligosaccharides are supposedly involved in flatulence.[4] The presence of these oligosaccharides impedes the full utilization of the soybean products. Many researches have been carried out to reduce the oligosaccharides content in legume seeds or in soybean products by processing techniques such as soaking, cooking, irradiation, germination, fermentation and enzyme treatment.[5-7]

However, many clinical researches have suggested that oligosaccharides, with approximately 30 to 50% caloric value of sucrose, may contribute to the growth of beneficial bacteria in the intestines,[8] prevention of cancer,[9] lowering the levels of blood cholesterol and reducing the risk of coronary heart disease,[2] modulating the immune response,[10] and stimulation of minerals absorption.[11]

With the progress of oligosaccharides researches, it was found that soybean oligosaccharides are not the direct causes of flatulence. Modern safety tests have proven that oligosaccharides are safe for human consumption.[12]

Soybean consumption in Asian countries has a history of several thousand years and many forms of soybean products were evolved. Some of the examples are non-fermented soybean foods such as tofu, soybean sprouts, dried tofu, soybean sheet (in Chinese) or Yuba (in American), and fermented soybean foods, such as soy sauce, sufu, tempeh, natto and miso. Soybean sheet formed on the surface of heated soymilk with the evaporation of surface vapor is a traditional dried soybean food in the far East.[13] It is an edible coating or film of soybean proteins. As a vegetarian food ingredient, it has considerable potential in western markets.

During the preparation of soybean food, processing methods have great impact on the nutritional compositions of the final products. In case of production of tofu, large quantity of water-soluble protein, saccharides and isoflavones were lost in whey waste water.[14] In some practical production steps the oligosaccharide contents usually decreased to various extent with different processing methods.[15-17]

Processing unit operations, such as soaking, dehulling, grinding, washing and heat treatment alter the distribution and content of oligosaccharides, which caused much of

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raffinose and stachyose loss in final soybean products.[7] Fermentation with R. *oligosporus* for production of tempeh resulted in reduction of more than 80% stachyose and 50% raffinose respectively.[7] Decrease of stachyose and raffinose by hydrolysis with α-galactosidase enzyme, germination and irradiation is also well-documented.[18] These researches emphasized the effect and importance of processing techniques on the oligosaccharides changes. Many similar researches have been done in tempeh fermentation.[7,17] However no report is available about oligosaccharides changes in soybean sheet production. The aim of this study is to investigate the changes of oligosaccharides during the preparation of soybean sheet and provide a basic loss profile for further study.

Materials and methods

Materials

Soybean was bought at a local supermarket, Hangzhou, Zhejiang, China. HPLC grade acetonitrile was purchased from Tianjin (China), double distilled deionized water was used for all HPLC analyses. Glucose, fructose, sucrose, raffinose and stachyose standards were from Sigma Chemical Co, St. Louis, MO, USA. Other reagents were of analytical grade.

Production of soybean sheet

Soybean sheet was prepared following the general processing technology as described by Li.[13] Three batches of soybeans (20 g each) were sorted, rinsed, soaked in 46 mL deionized water at 20℃ for 12 to 14 hours, and then washed. 200 mL deionized water was added to the soaked beans, and the mixture was blended completely with a commercial blender. The soymilk slurry was then filtrated with sterile gauze for the first time. The raw soymilk was heated up to 100℃ (with stirring) and held at the same temperature for 3 to 5 min. Then the cooked soymilk was filtrated again to eliminate large particles. The heated soymilk was then poured into a flat-bottom pan and kept at 80 to 90℃ in water bath. The surface of the soymilk was under cold air circulation with a speed of 3 m/s. Soybean sheet was harvested from the surface of the soymilk in about every 10 min until no further film formation occurred. Then the soybean sheets were dried at 35 to 45℃ for 8 to 10 hours. At each stage, the volumes of water used and wastes drained were recorded and samples retained for analysis. The production flow of soybean sheet was shown in Figure 1.

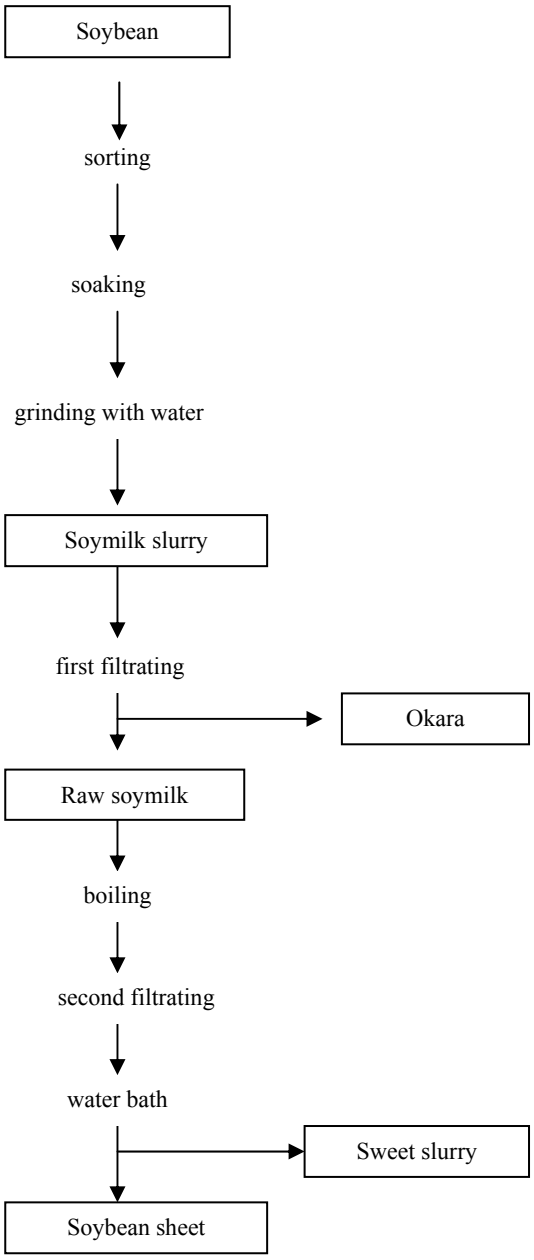

Figure 1. General flow of soybean sheet production.

Extraction of oligosaccharides

The samples to be analyzed include the soybean, the soaking water, the raw soymilk, the soybean sheet, and the sweet slurry. 2 g of finely weighed raw soybeans were ground into powder with a muller, sieved through sieve (100 mesh), defatted with hexane, and then homogenized in aqueous ethanol (70%, 20 mL) at 70℃ for one hour. The mixture was centrifuged at for 10 min, and the supernatant was condensed to 20 mL using a vacuum rotary evaporator with a water bath.[19] The soaking water (10 mL) was treated with saturated acetic plumbum solution (about 30%) to precipitate large molecules, and the mixture was centrifuged, then added with oxalic acid solution to eliminate the surplus of plumbum ion, centrifuged again for 10 min, and the pH of the supernatant liquid was adjusted to 7 with NaOH and HCl solutions.[20] The raw soymilk was treated with the method of Chaturvedi *et al.*[21] 5 mL of the raw soymilk were first centrifuged for 45 mins at 4℃. Add 20 mL of ethanol (66.7%) to the aqueous layer, and left overnight at 4℃. And then centrifuged for 15 min at 4℃. The soybean sheet was treated like the initial soybean powder. A solid to solvent ration of 1:10 was used. The sweet slurry was first adjusted pH to 9 with NaOH or HCl solutions and then stirred at 90℃ for 30 min. After centrifuging, the supernatant liquid was adjusted pH 4.5 with HCl solutions and centrifuged again.[22] The supernatant was ready for analysis. All the samples preparation and analysis were repeated three times.

Analyses of oligosaccharides

High Performance Liquid Chromatograph method (HPLC) was used to analyze the oligosaccharides. The method

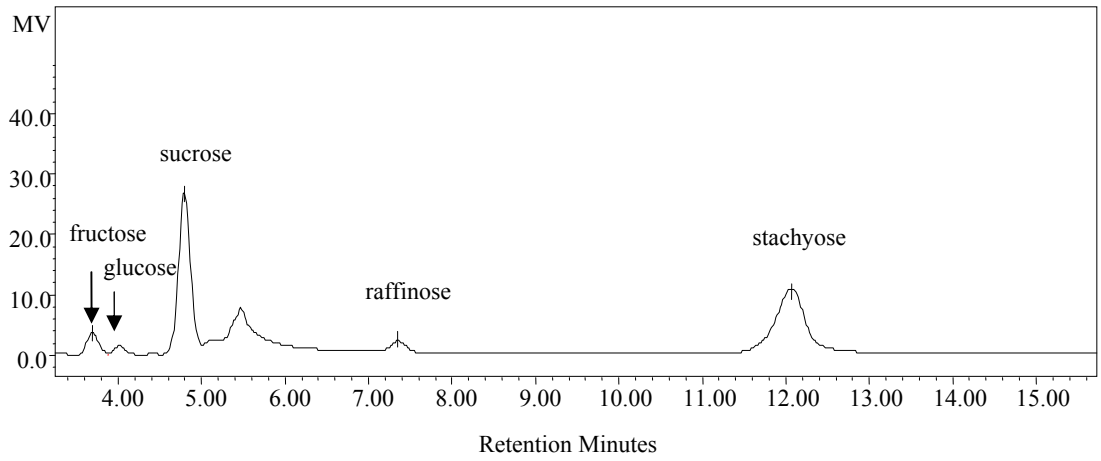

Figure 2. Chromatograms profiles of monosaccharides and oligosaccharides detected using HPLC method from soybean powder.

Table 1. Sugar concentration in soybean products and by-products (g/kg dry weight)[a]

Products	Fru + Glu[b]	Sucrose	Raffinose	Stachyose
Raw soybean	1.40 ± 0.03	43.1 ± 2.52	7.52 ± 0.91	41.3 ± 3.61
Soaked water	0.39 ± 0.07	0.16 ± 0.11	0.25 ± 0.15	0.27 ± 0.10
Soaked soybean	NC[c]			
Raw soymilk	0.85 ± 0.25	36.1 ± 3.45	6.87 ± 2.78	37.9 ± 3.64
Boiled soymilk	NC[c]			
Soybean sheet	2.38 ± 1.05	11.3 ± 4.51	4.05 ±1.20	10.2 ± 3.23
Sweet slurry	2.79 ± 0.56	20.0 ± 4.85	4.57 ± 0.57	22.6 ± 5.26

[a] From means ± standard deviation of three duplicates for each sample. [b] Fructose + glucose. [c] Not collected.

used in this research was described by Martínez-Villaluenga *et al.*[23] with slight modification. HPLC system (Waters 2695, Marlborough, MA) consists of multi solvent delivery system, 717 plus auto sampler, 2414 refractive index detector. An amino-bonded column (Hypersil, 250 mm × 4.6 mm i.d.) connected with a guard column (Hypersil, 4 mm×50 mm) packed with C_{18} Porasil was used to analyze the oligosaccharides. The samples were filtrated through millipore membrane (0.45 μm) prior to HPLC analysis. The mobile phase acetonitrile-water (75:25 v/v, HPLC grade) was ultrasonicated for 30 min before chromatographic analyses. Chromatogram conditions were 40℃ column temperature, 10 μL injection volume and 1.5 mL/min flow rate.

Calibration curves and recovery

Standard sugars were dried at 70℃ in vacuum oven and stored in a desiccator at room temperature prior to testing. Sugars were first dissolved in purified water and then diluted with 75% acetonitrile to get a similar condition with the mobile phase. The concentration of the standards solutions was 10 mg/mL. The standard curves were generated from eight different concentrations of sugars. The regression coefficients of the curves for sucrose, raffinose and stachyose as well as fructose and glucose were greater than 0.990. Quantification of individual sugars was achieved by comparison of the peak areas with standards. The HPLC graph and retention time of sucrose, raffinose and stachyose as well as fructose and glucose is illustrated in Figure 2.

Statistical analyses

All experimental treatments were repeated 3 times. Values shown in this paper were the average of three replicates ± standard deviation (SD) in all the results tables. Statistical significance was analyzed using the Statistical Package for Social Science,Version 11.5 (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

Results

Oligosaccharide levels in raw soybeans

The oligosaccharides and monosaccharides contained in the raw soybean were identified mainly as sucrose, raffinose and stachyose as well as fructose and glucose (Figure 2). Sucrose (4.34%), raffinose (0.75%) and stachyose (4.13%) are the predominant sugars in soybean (Table 1).

Effect of soaking on content of oligosaccharides

The concentration of oligosaccharides left in the soaked soybeans was calculated by the initial oligosaccharides amount in the raw soybeans minus those lost in the soaking water. Soaking the soybean for 12 to 14 hours at 20℃ did not reduce the sugar content too much. The total loss of sucrose, raffinose and stachyose in the soaking water (g/kg dry matter) represented 0.74% of the total oligosaccharides in starting material (Table 1). Accordingly, the content of sucrose, raffinose and stachyose in the soaked beans were 42.89, 7.27 and 41.05 (g/kg dry matter) respectively, representing 99.26% of the total oligosaccharides content in the raw soybean. The loss amount of fructose and glucose was similar to the result

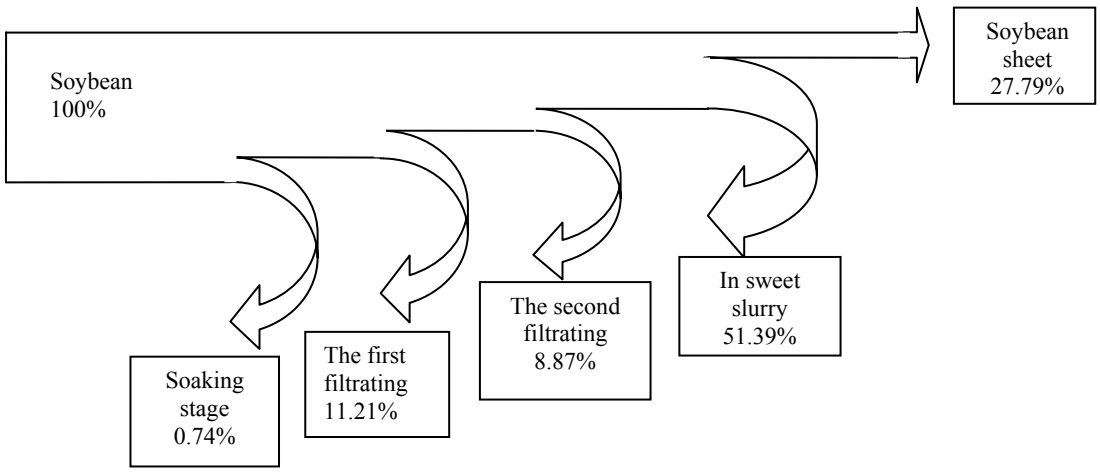

Figure 3. Oligosaccharides balance during soybean sheet processing.

Table 2. Oligosaccharides concentration in different soybean sheets (g/kg dry matter)[a]

Sugar	1	2	3	4
Fru + Glu[b]	2.05 ± 0.41	1.99 ± 0.25	1.88 ±0.64	2.07 ± 0.56
Sucrose	15.3 ± 2.12	19.3 ± 5.14	31.8 ± 6.43	43.1 ± 8.62
Raffinose	5.21 ± 1.89	5.53 ± .99	5.92 ± 1.31	7.55 ± 2.63
Stachyose	17.7 ± 1.76	22.9 ± 3.24	32.6 ± 5.26	43.6 ± 3.50
Total	40.2 ± 1.22*	49.7 ± .95*	72.1 ± 3.55*	96.3 ± 4.13 *

[a] From means ± SD of three duplicates for each sample. [b] Fru + Glu, fructose + glucose. 1, 2, 3, 4 represented the first, second, third and the fourth piece of soybean sheet respectively. * $p< 0.05$ level.

of Ruiz-Terán *et al.*[17]and Sánchez-Mata *et al.*[24]

Effects of washing, grinding and filtration on content of oligosaccharides

The oligosaccharides lost in this stage were estimated by the amount in the soaked soybeans minus those in the raw soymilk. Washing the soaked soybeans in tap water, grinding them in purified water, as well as filtrating the soybean slurry to obtain soymilk removed 11.21% of the total oligosaccharides.

Effects of cooking and the second filtrating on content of oligosaccharides

Cooking the raw soymilk and filtrating reduced oligosaccharides by 8.87% (relative to the total oligosaccharides in the initial soybean) (Fig 3). We observed that the content of fructose + glucose in soybean sheet increased a little compared with those in boiled soymilk (Table 1). However in general, the content of soybean oligosaccharides decreased gradually in each step (Table 1). This might be attributed to processing factors such as the hydrolysis of oligosaccharides by elevated temperatures[1].

Effect of sheet formation on content of oligosaccharides

A considerable loss of oligosaccharides occurred at sheet formation stage, accounting for 27.79% (relative to the total oligosaccharides content in starting material). The amount of oligosaccharides in single soybean sheet harvested at different time was different (Table 2). The concentration of fructose + glucose and the raffinose did not change a lot in different soybean sheets. However the sucrose and stachyose content increased gradually in different soybean sheets and attained the highest level in the last piece of soybean sheet. The total content of sugar detected in each soybean sheet was significantly different ($p<0.05$).

Most oligosaccharides were left in soymilk residue (the sweet slurry). This part accounted for 51.39% of the total oligosaccharides content in the initial soybean, and representing a single largest loss of oligosaccharides in the process flow of soybean sheet production. The total recovery of oligosaccharides in soybean sheet was as low as 27.79% when compared with the oligosaccharides content in the initial soybean (dry weight basis), and more than 70% of the oligosaccharides were lost in the process flow.

In summary, during the soaking, grinding and the first filtrating, the boiling and the second filtrating, and finally in the sheet formation, the losses of the oligosaccharides were 0.74%, 11.21%, 8.87% and 51.39% of the total oligosaccharides in the initial soybean respectively (Fig 3).

Discussion

Differences in oligosaccharides content of soybean are most likely due to differences in variety. Egounlety *et al.*[7] detected different contents of oligosaccharides in soybean variety TGX 536-02D. Trugo *et al.*[1] reported the total galactosides content in the Brazilian soya beans cultivars

ranged from 3.9 to 5.3 % and stachyose was the predominant component in all samples when the soya beans were extracted with methanol and water followed by actived charcoal clearing and acetonitrile dilution. They indicated that different extracting methods affected detected sugar contents.

However we found that both stachyose and sucrose were the dominant sugars in this soybean sample, whose concentrations was similar.

Egounlety *et al*.[7] observed soaking the soybean for 12 to 14 hours reduced about 25% raffinose and 20% stachyose, the loss of sucrose in soaking liquids was even more than that of raffinose when during the prepartion of tempeh. Prinyawiwatkul *et al*.[25] reported when soaking cowpea in 1: 6 water (cowpea: water, w/w) at 25℃ for 24 hours the sucrose and stachyose decreased by 39% and 18.4% respectively. Ruiz-Terán *et al*.[17] found that when the soybeans were hydrated in water at 100℃ for 30 min the oligosaccharides losses in the hydration water was 45 g/kg initial dry soybean.

Different results in oligosaccharides content detected in soaking water might be attributed to different varieties,[23,26] volume of soaked water, soaking temperature and soaking time.[7,25]

Soaking is an important operation during the preparation of soybean sheet. Soaking can make the dry beans absorb a certain volume of water, which facilitated the following grinding process. Li[13] believed soaking made the seed soft, the seed structure loose, the protein easily extracted, the seed components diffuse into water. This contributes to the detectable sugar in the soaked water.

Washing the soaked soybeans in water could flow away the foam and ash in the soaked soybeans, which is an usual practice in soybean sheet processing. Ruiz-Terán *et al*.[17] reported in their study that washing the soaked soybeans at 60℃ reduced the oligosaccharides content during preparation of tempeh. Egounlety *et al*.[7] reported the combined effect of soaking, dehulling, washing and cooking reduced more than half of oligosaccharides in preparation of bean tempeh.

Grinding method might influence the oligosaccharides concentration in soymilk. Lasekan *et al*.[27] reported that by a fine milling the sugar content in two sorghum cultivars would be increased.

Filtrating separated non-soluble soybean residue from soymilk which guaranteed the good quality of soybean sheet. A certain amount of oligosaccharides was left in the soy residue. Inevitably this lead to certain loss of oligosaccharides.

In the sheet formation process, as water evaporates with time dry matter in the soymilk increased and the film thickness as well as the weight of the soybean sheet increased too. This was the major reason accounting for the increasing tendency in oligosaccharides content in final soybean sheets recovered later in the process.

The results from the present study indicate that soybean oligosaccharides are easily subjected to loss with various treatments, including soaking, grinding, filtrating, boiling while the last step of sheet formation accounted for the highest loss.

The by-product in soybean sheet production, the sweet slurry which contains large amount of oligosaccharides, is not fully utilized. The analysis of loss profile implies possible ways to improve technology for production of oligosaccharides enriched soy-sheets, and whilst arouses attention to make full use of the by-products in the processing of the soybean sheet.

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