IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 3, Issue 5, May 2015, 1-8

EFFECT OF TECHNOLOGICAL PROCESSING AND FERMENTATION OF SOY MILK ON THE CONTENT OF ISOFLAVONES AND ANTIOXIDANT STATUS

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ABSTRACT

This study reports the effect of technological processing and fermentation of soy milk on the content of isoflavones and antioxidant status. HPLC determination method was used to monitor the concentration changes of two major dietary phytoestrogens present in soya such daidzein and genistein. Also, the changes in total phenolic content and antioxidant activity were determined. Results showed that the concentration of the two aglycones, daidzein and genistein was affected by heat treatment 780.3 µg/g and 31.86 µg/g respectively. While, the remain of daidzein was 239.3 µg/g in soymilk after fermentation. The processing steps also decreased in content of total phenolics and antioxidant activity which were 41.15% and 68.8% in soymilk from the quantity in whole seeds respectively. The percent of losses in fermented products were 16.6 % and 22.4 % from soybean seeds respectively.

KEYWORDS: Soybean Seed, Soy Milk, Processing, Fermentation, Isoflavones and Antioxidants Status

INTRODUCTION

Soybeans contains the highest quality protein of any plant sources (Smith and Circle, 1972) and is an inexpensive source of protein and calories for human consumption and is seen as a low cost substitute for dairy milk for poor in the developing countries. Being free of cholesterol, gluten and lactose, soymilk is also a suitable food for lactose intolerant consumers, vegetarians and milk allergy patients (Chou and Hou, 2000), and only a small quantity of saturated fatty acids (Scalabrini *et al.* 1998). The fermentation of soymilk with various organisms, especially lactic acid bacteria, has been attempted to overcome the problem of being flavor and flatulence to increase both acceptability and nutritional value (Mattick & Hand 1969; Thananunkul *et al.* 1976 and Salem *et al.* 1994).

Soybeans have high concentration of isoflavones in many studies, these soy isoflavones have been shown to have some healthy and enhancing properties, such as the prevention of certain cancer (Farina *et al.* 2006 and Miura *et al.* 2002). Lowering the risk of cardiovascular diseases (Goodman and Kritz-Silverstein 2001), an improvement of bone health (Weaver and Cheong 2005) and many other functional substances like phenolic acids (Zhang *et al.* 2003).Soybean contains three families of isoflavones as four distinct chemical structures, and most of the isoflavones in natural food materials exist in glycosylated form. However, the effective biological moieties of isoflavones are their aglycones, such as daidzein and genistein (Chien *et al.* 2006, Choi *et al.* 2002 and Izumi *et al.* 2000).

The objective of the present study was extended to our knowledge about the change of various Isoflavones compounds and Antioxidants status in soybean seed during processing of soymilk, and its fermentation according to the processing steps treatment.

MATERIALS AND METHODS

Materials

Dry, whole soybean seeds were obtained from Alexandria Company for extracted oil and their products. The bacterial cultures were: *Streptococcus thermophiles* CH10011105 Hensen (commercial) and *Lactobacillus delbrueckii* sabsp. *bulgaricus* 15 Hensen (commercial). Sodium carbonate, ethanol, methanol, Hydrochloric acid, acetonitrile, gallic acid, BHT, potassium dihydrogen phosphate and sodium carbonate were purchased from BDH Company. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and Folin-Ciocalteus phenol reagent was purchased from Sigma–Aldrich Inc. (St Louis, MO, USA).

METHODS

Preparation of Soybean Milk and its Fermentation

The soymilk (10 % T.S) was prepared according to Salem (1984) as follow: the whole soybeans were washed and blanched for 30 min in 0.25 % sodium bicarbonate solution (1:5) at 100 °C. The beans were washed and soaked in water (1:3) at room temperature (20±5 °C) over night, then de-hulled beans manually, washed and ground in warring blender for 5 min with previously boiled water. The ratio of beans to water was 1:3 (w/v) and the temperature of water during grinding between 50-60 °C. The resulted suspension was filtered by centrifugation at 3000 rpm for 3 min. The homogenized resultant soymilk at 4000 PSI (two stages) was dispersed in 150 ml screw cap bottles, autoclaved for 15 min at 121 °C and held at 5 °C until used.

Soy milk was fermented by starter of yoghurt *St. thermophiles* and *Lactobacillus delbrueckii* sabsp. *bulgaricus* which used according to Salem *et al.* (1994) after coagulation, it was filtrated with cheese cloths to obtained the fermented concentrated products.

Sampling points at different stages of the manufacturing process is shown in **Figure (1)** as follow:

Sample 1: Dry, whole soybean seeds

Sample 2: The seeds after blanched in sodium bicarbonate

Sample 3: The cooking solution

Sample 4: Raw Soymilk (Extracted solution)

Sample 5: The residual after centrifugation (Okara)

Sample 6: The Final product (soymilk after homogenization and sterilization)

Sample 7: Fermented product (soymilk after fermentation and filtration)

Determination of Isoflavones Content during Processing Steps

Isoflavones such daidzein and genistein in soybean seeds during soymilk processing and after fermentation were

hydrolyzed and determined according to Ewald *et al.* (1999) as follow: 0.5 gm of soybean seeds powder and soy based products was hydrolyzed in 40 ml of 62.5 % aqueous methanol with 2 mg/ml of BHT and 10 ml of 6 M HCL at 90 °C with reflux for 2 h. Methanol was added to all samples to 100 ml after hydrolysis, and the samples were finally sonicated for 5 min to remove oxygen before subjected to analysis using HPLC. The samples were separated using HPLC with reversed phase column manufactured by Shimadzu, Japan. The mobile phase consisted of 30 % of acetonitrile in 0.025 M potassium dihydrogen phosphate (pH 2.4) with flow rate 1.3 ml/min. The compounds were detected by a UV detector set at 330 nm.

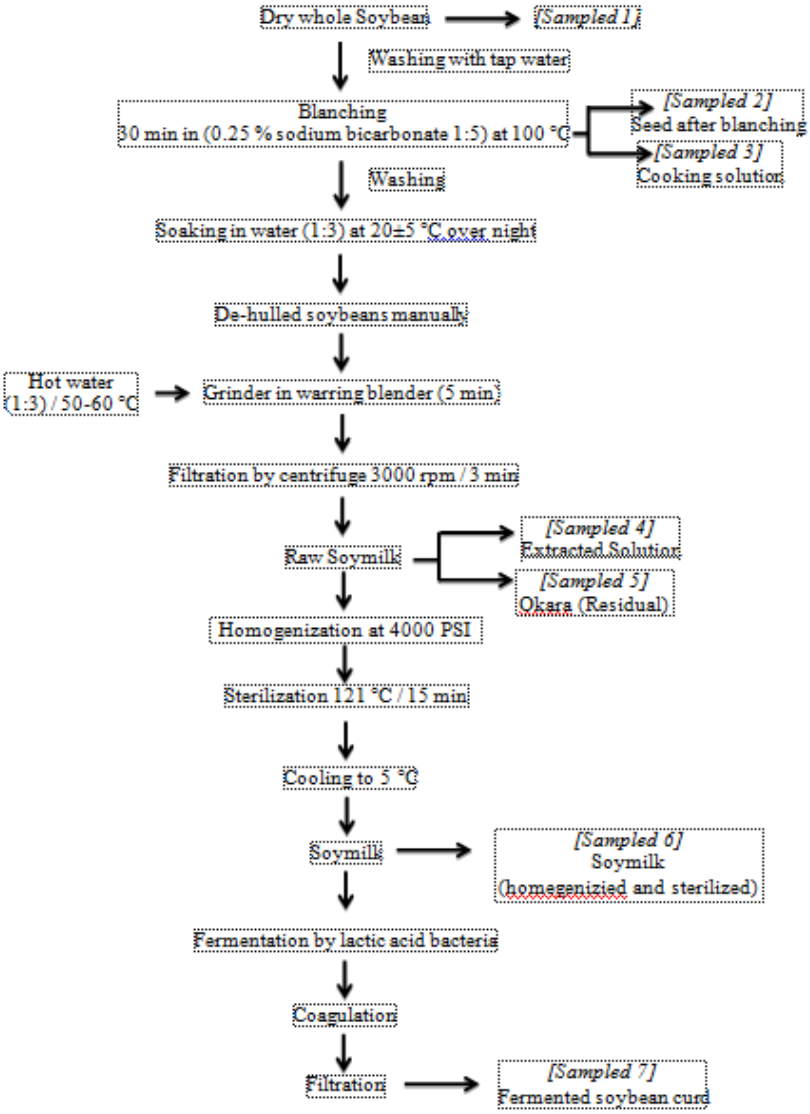

Figure 1: Flow Diagram for Processing of Soybeans to Soymilk and its Fermentation

Determination of Total Phenolic Content

The total phenolic content of ethanolic extract of 0.5 g dried powder from soybean seeds and soy based products was determined colorimetrically, using the Folin-Ciocalteu method, as described by Makkar *et al* (1997). Aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. The mixture

was stirred and allowed to stand in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank using a model UV/VIS 1201 spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as gallic acid equivalents from a calibration curve.

Assay of Antioxidant Activity

The free radical scavenging activity using the 1.1-diphenyl-2-picryl- hydrazil (DPPH) reagent was determined according to Brand-Williams *et al.* (1995). The powdered seeds and soy based products (1 g) was extracted with 50% methanol: water, then 1.5 ml of freshly prepared methanolic DPPH solution (20 µg ml^{-1}) was added and stirred to 0.75 ml of the extract sample. The decolourizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control. The standard curve was linear between 25 and 800 µM Trolox.

Statistical Analysis

Data expressed as mean of triplicate samples and were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $P \leq 0.05$ to determine the significant differences in results using JMP 4.0 statistical software package (SAS, 2000).

RESULTS AND DISCUSSIONS

Determination of Isoflavones during Processed Soybean Milk and Soy Fermented

Most studies of the biological properties of isoflavones have focused on genistein and daidzein and their glucosides. Therefore data in table (1) shows the effect of manufacturing processes steps of soybeans to soymilk and its fermentation on the content (µg/g) of two major dietary phytoestrogens present in soya such daidzein and genistein. Prior to blanching, whole soybean seeds were found to contain the following concentrations of isoflavones: daidzein 1071.7 µg/g) and genistein (39.7 µg/g).In general, the content of isoflavones can be varied depending on variet, location and season of harvest.Soybeans produced in USA has been reported to range from 1400 to 4200 µg/g for 12 forms of isoflavones (wang and Murphy, 1994). However, some soybeans were found to contain less than 1000 µg/g isoflavones (Simonne *et al.*, 2000). The recovery of two isoflavones after blanching ranged from 45.4% for daidzein and 52.8% for genistein, which were less than those (60–90%) reported in the literature (Coward, *et al.* 1993 and Murphy, 1981), probably because of the recovery can be affected by variety of foods matrix, method of extraction and separation (Kao and Chen, 2002). The concentrations of the two aglycones, daidzein and genistein were losing in the cooking solution by 31.78% may be due to high cooking temperature (100 °C) may inhibit the activity of β-glucosidase and the formation of aglycones was not observed (pandjaitan *et al*. 2000). After soaking at 20±5 °C over night and filtration, the raw soymilk contain higher concentrations of daidzein than genistein being 608.3 and 20.98 µg/g respectively. Apparently daidzein is the most susceptible to formation during soaking, followed by genistein. The differences in their content of isoflavones were explained as due to the leaching of isoflavones into the soaking water. While in the sample of the residual after centrifugation, the concentration of daidzein were 232.2 µg/g.In contrast, the isoflavones genistein showed not detected trend.The percentage of loses in our investigation was 21.6% which Less than reported by (Prabhakaran, 2005) which demonstrated that large proportion of isoflavones were lost in soy residue (okrara) during traditional soymilk making process by 33.9%. It is imperative to conduct further studies on the ways to minimize the loss of isoflavones into the okara and to maximize the isoflavones extraction into soymilk. Finally, the remain of isoflavon daidzein was 239.3 µg/g in

soymilk after fermentation due to the fermentation processes promoted a significant transformation of isoflavones glycoside into aglycones making it possible to use fermented soy as an ingredient in the formulation of foods with functional properties and have health benefits as reported by (Leomar *et al.*, 2011).

Table 1: Effect of Processing Steps of Soymilk and Soy Fermented on the Isoflavones Content

Samples	Daidzein (µg/G)	Genistein (µg/G)
1	1071.7[a]	39.7[a]
2	780.3[b]	31.86[b]
3	340.6[e]	18.37[d]
4	608.3[c]	20.98[c]
5	232.2[g]	ND
6	487.3[d]	ND
7	239.3[f]	ND

Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).
ND: not detected

1: Dry, whole soybean seeds	2: The seeds after blanched in sodium bicarbonate
3: The cooking solution	4: Raw Soymilk (Extracted solution)
5: The residual after centrifugation (Okara)	6: Soymilk after homogenization and sterilization
7: Soymilk after fermentation and filtration	

The Antioxidant Status during Processed Soybean Milk and Soy Fermented

Content and profile of phenolic compounds in soybean seed or soy food was affected by genetic factors, environmental factors or soy food processes (wang & Murphy, 1996). The data presented in table (2) showed the effect of processing steps of soymilk and the fermented soy on the antioxidant status which revealed that the dry, whole soybean seeds was characterized by high content of total phenols and had a great free radical scavenging activity. On the other hand by processing steps we found decreased in content of total phenolics and antioxidant activity which depending on the processing, thermal degradation, fermentation. About 35% of phenolic compounds were eliminated during cooking process of soybean. While, the percentages of antioxidant activity and total phenolics in soymilk was about 68.8 and 41.15% from the seeds respectively.After fermentation of soymilk by lactic acid bacteria the antioxidant activity and total phenolic compounds were decreased by 16.6 and 22.4 % respectively. These losses may be caused by enzymatic hydrolysis and these results are in agreement with those obtained by (Chung *et al.*, 2011).

Table 2: Effect of Processing Steps of Soymilk and Soy Fermented on the Antioxidant Status

Samples	Total Phenolics (Mg GAE /100g)	Antioxidant Activity (µm Trolox/100g)
1	673[a]	3321[a]
2	259[d]	2016[d]
3	239[e]	1818[e]
4	373[b]	2304[b]
5	159[g]	1512[g]
6	277[c]	2286[c]
7	231[f]	1773[f]

Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).

1: Dry, whole soybean seeds	2: The seeds after blanched in sodium bicarbonate
3: The cooking solution	4: Raw Soymilk (Extracted solution)
5: The residual after centrifugation (Okara)	6: Soymilk after homogenization and sterilization
7: Soymilk after fermentation and filtration	

CONCLUSIONS

It could be concluded that, the processing steps included blanching, soaking and fermentation affects the concentration of the two aglycones, daidzein and genistein and decreased the content of total phenolics and antioxidant activity. Also, it could be noticed that, the soymilk is a good source of total phenolics content and a great free radical scavenging activity.

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