Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 99 (2006) 231–237
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture

Molamma P. Prabhakaran, Conrad O. Perera *

Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 4, Singapore 117543, Singapore

Received 4 January 2005; received in revised form 28 June 2005; accepted 28 June 2005

Abstract

The objective of this study was to compare the effect of hot and cold grinding as well as the effect of direct and indirect ultra high temperature (UHT) treatment conditions on the level of isoflavones during the manufacture of soymilk. Soymilks were manufactured from dehulled soybeans by hot grinding or cold grinding processes. After inactivation of lipoxygenase at 85 °C, the resulting slurries were decanted and supernatants were held at 120 °C for 80 s to inactivate the trypsin inhibitor. The decanted soya bases were cooled and subjected to different temperature/time regimes by direct and indirect UHT treatments. Samples were drawn at different points in the processing operation and a reversed phase high performance liquid chromatography method was used to determine the concentration of isoflavones. Results showed that hot grinding caused a higher extraction of isoflavones into the soymilk than the cold-grinding process. However, direct or indirect heating in the UHT process did not significantly influence the concentration of isoflavones.

Keywords: Soymilk; Isoflavone; UHT; Cold grinding; Hot grinding

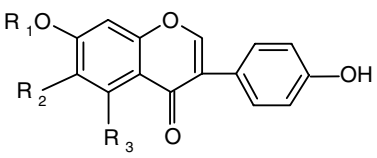
1. Introduction

Isoflavones are a group of naturally occurring heterocyclic phenols found in soybean and foods derived from soy. The major isoflavones (Fig. 1) in soybeans are genistin and daidzin and their corresponding aglucones, genistein and daidzein. The third kind of isoflavone includes the glycitein and its 7-*O*-β-glucoside, glycitin (Wang & Murphy, 1994). Acetylated (6″-*O*-acetylgenistin, 6″-*O*-acetyldaidzin and 6″-*O*-acetylglycitin) and malonylated (6″-*O*-malonylgenistin, 6″-*O*-malonyldaidzin and 6″-*O*-malonylglycitin) isoflavones were also found in soybeans (Kudou et al., 1991). These compounds are particularly important due to their diverse pharmacological and antioxidant properties. It has been hypothesized that isoflavones reduce the risk of several types of cancers including the breast, prostate and colon (Messina & Barnes, 1991; Wu et al., 1998), heart disease (Ho et al., 2000; Wong et al., 1998), menopausal symptoms (Chiechi, 1999) and bone health (Anderson & Garner, 1997). Moreover, in 1999, the US Food and Drug Administration (US-FDA) approved a health claim for the cholesterol-lowering effects of soy protein, which are associated with isoflavones.

Soybeans are transformed into many different varieties of foods to create versatility and provide tasty and easily digestible products. Among these soy foods, soymilk has gained much popularity as a healthy food drink. Wilkens, Mattick and Hand developed the "Cornell process" in Wilkens, Mattick, and Hand (1967) and later Nelson, Steinberg and Wei developed the "Illinois process" in Nelson, Steinberg, and Wei (1976) for the manufacture of soymilk. Though these conventional soymilk manufacturing processes basically involve the

* Corresponding author. Tel.: +65 6874 2842; fax: +65 6775 7895.
E-mail address: chmpco@nus.edu.sg (C.O. Perera).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.06.055



R_1	R_2	R_3	Compounds
H	H	OH	Genistein
Glucose	H	OH	Genistin
H	H	H	Daidzein
Glucose	H	H	Daidzin
H	OCH_3	H	Glycite in
Glucose	OCH_3	H	Glycitin

Fig. 1. Chemical structures of three parent isoflavones and their respective glucosides in soy samples.

soaking, grinding, filtering and cooking steps, many process modifications have taken place over the years with the advancement of new processing technologies and considering consumer requirements. In large-scale production, continuous high-temperature, short time processes often substitute normal low-temperature, long-time thermal processes. The advent of UHT heating and aseptic packaging has further contributed to the production of long-life soymilk packaged aseptically in paper-plastic cartons. They are more convenient for transportation, distribution and storage (Gosta, 1995). However, the fate of isoflavones on extraction and UHT treatment conditions during such type of soymilk manufacturing is almost unknown. An understanding on the concentration of these beneficial phytochemicals in the soymilk prepared by different processing methods was therefore considered essential. Though there is an extensive literature available on the analysis of isoflavones in soy foods (Coward, Barnes, Setchell, & Barnes, 1993; Jackson et al., 2002), little information is available on the effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. Here, we have considered two different grinding processes for the preparation of soymilks.

The objective of this study was to determine if there was any loss or improvement in the extraction of isoflavones into the soymilk by hot grinding of soybeans compared to cold grinding during the soymilk preparation. Studies were further carried out to evaluate the fate of isoflavones during the UHT treatment process. Two different UHT conditions were applied for this study. Analyses were performed to understand if a direct or indirect UHT treatment to soya base could cause any loss of isoflavones.

2. Materials and methods

2.1. Chemicals

Isoflavone standards such as daidzin, glycitin, genistin, daidzein, glycitein and genistein were purchased from LC Laboratories (Woburn, MA, USA). All solvents used were of HPLC grade and were purchased from Sigma Chemical Co. (Jefferson City, MO, USA).

2.2. Pilot plant scale preparation of soymilk

Identity preserved soybean seeds (Harovinton) were obtained from a local seed supplier who provided the same cultivar for all the trials. Dehulled soybeans were either hot ground or cold ground, using a disc mill with water at 95 or 45 °C, respectively. Soybeans were added at the rate of 70 kg/h and water was added at the rate of 300 l/h. The resulting slurry was passed through a colloid mill and then through a holding tube at 85 °C to inactivate the lipoxygenase (EC 1.13.11.12), an iron containing dioxygenase which otherwise can cause the oxidation of fatty acids resulting in off-flavour development. Further it was passed through a decanting centrifuge for separation of the insoluble residue (okara). The soymilk thus obtained was held at 120 °C for 80 s to inactivate approximately 80% of the Kunitz trypsin-inhibitor and thereby improving its soy protein digestibility (Kwok, Qin, & Tsang, 1993), flash-cooled to 75 °C and further cooled to 25 °C. The cooled soymilk base was subjected to two different UHT treatment conditions: one was a direct UHT process and the other was an indirect UHT process. This was mainly to understand the influence of the type of UHT process on the level of isoflavones in the end product. For the direct UHT system, steam was injected into the product and soya base came in direct contact with the heating medium. The direct UHT process was carried out at a temperature of 143 °C for 10 s. The indirect system was based on tubular heat exchangers whereby heat was transferred from the heating medium to the product through a heat exchanger. The indirect UHT process was carried out at a temperature of 140 °C for 4 s. These conditions were chosen from the normal industry practices used for UHT treatment of soymilk in the region.

The flow diagram (Fig. 2) shows the processing of soybeans to soymilk. The sampling points at different stages of the manufacturing process are also indicated. Soymilk preparations were carried out with the replicate trials being further carried out to confirm the reproducibility of the results obtained. Every trial involved either a direct UHT process (injection of steam into the product at 143 °C for 10 s) or an indirect UHT process (using a heat exchanger at 140 °C for

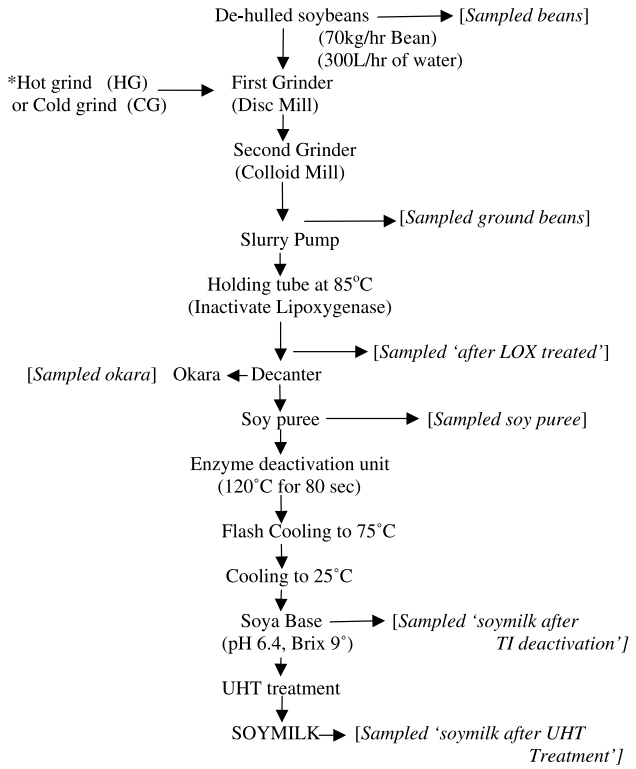


Fig. 2. Flow diagram for processing of soybeans to soymilk.

4 s). Two pilot runs were carried out on each of the trials, one involving hot grinding (grinding of dehulled soybeans with hot water at 95 °C) and the other involving cold grinding (grinding of dehulled soybeans with water at 45 °C).

2.3. Extraction and analysis of isoflavones

The extraction procedure followed the method of Klump, Allred, MacDonald, and Ballam (2001), which used 80% methanol as the extraction solvent (AOAC Method No. 2001.10). A mild saponification step was further carried out to convert the isoflavone glucoside ester forms to their corresponding glucoside forms, leaving the three aglucones intact. This permits the analysis of both the β-glucosides and the aglucones in a single chromatographic run. Hence, a total of six isoflavones were quantified from the extracted test samples. Using the Klump's method for extraction allowed a direct comparison of stable and readily available isoflavone glucoside and aglucone reference standards. All extraction experiments and analysis were performed in triplicate and each isoflavone value is reported as the mean value as obtained. UHT process may cause changes in the acetyl or malonyl isoflavone conjugates, however, it is the total amount of isoflavones that were evaluated in this study to understand the effects of processing.

2.4. HPLC instrumentation and chromatographic conditions

The HPLC apparatus was a Waters 2695 separations module with inline degasser, a Waters 2996 photodiode array detector capable of monitoring UV absorbance from 200 to 350 nm and the Waters "Empower Software System" (Waters Corp., Milford, MA, USA). Reversed phase HPLC analyses of isoflavones were carried out on a YMC-pack ODS-AM 303 (5 μm, 25 cm × 4.6 mm i.d.) column (Waters Corp., Milford, MA, USA).

The mobile phase consisted of (A) 0.1% glacial acetic acid in filtered MilliQ water and (B) 0.1% glacial acetic acid in acetonitrile. The injection volume was 20 μl, and the components were eluted using the following solvent gradient: from 0 to 5 min 10% B; from 5 to 50 min 10–35% B; then held at 35% B for another 10 min and re-equilibrated back to 10% B. The flow rate was 0.8 ml/min and UV detector wavelength was set at 260 nm. The total run time was 80 min including the equilibration of column.

Stock solutions were prepared for the six isoflavone standards and calibration standards were further prepared by diluting the stock solutions. Linear responses were obtained for the six standards after injection on HPLC and calibration curves were plotted by using peak area vs. corresponding isoflavones concentration. The identity and purity of isoflavones in the samples were confirmed by matching the retention times and mass spectrum analysis of the standards. This was performed on a Finnigan Quadrupole Mass Spectrometer (San Jose, CA, USA). Isoflavones were separated by reversed-phase HPLC on YMC pack ODS AM-303 column using a gradient run. No significant difference in the chromatographic resolution was observed between HPLC-UV detection and HPLC-MS. Selected peaks were then extracted using positive and negative acquisition mode over an m/z range of 160–800. Results of analysis showed that an HPLC-MS run using ESI was sufficient to obtain the most sensitive and structurally useful information about the isoflavone conjugates.

2.5. Moisture determination

The moisture contents of soybean seed powders were measured in triplicate as the loss in weight of a 2 g ground sample after 24 h at 105 °C in an air oven. Moisture contents of all other samples were determined using vacuum-oven method (AOAC, 2002, Method No. 925.09).

2.6. Statistical analysis

Isoflavone values reported are the means of three determinations with their standard deviations (±SD). Test for analysis of variance were carried out using SPSS

version 12.0 (SPSS, Inc., Chicago, USA) to determine the significant differences between the isoflavone values in soymilk obtained by hot and cold grinding methods as well as between the two UHT treatments. p Value of 0.01 or less was considered significant. On comparison of isoflavone levels in hot-grind vs. cold-grind soymilks, a highly significant relationship ($p < 0.01$) was observed.

3. Results and discussion

In this study, the concentrations of isoflavones in soy samples were expressed as micromoles per gram of dry matter (μmol/g DM). Seeds used for the trials had nearly the same concentration of isoflavones. Soybean seeds used for the direct and indirect UHT trials had isoflavone concentrations of 4.50 μmol of glucosides and 0.25 μmol of aglucones (Table 1).

The processing system used for this study had a particular advantage of grinding the soybeans directly with cold or hot water rather than a 'soak and grind process' used conventionally. The traditional soymilk making process, termed as the 'soak and grind process' involves the soaking of soybeans in water for a prescribed period of time (4–12 h) followed by grinding them in fresh water. Earlier Wang and Murphy (1996) did a mass balance study during the preparation of soymilk, where the raw soybeans and soaked beans were analyzed for their isoflavone concentrations. Soaked beans contained a lesser concentration of isoflavones than the raw soybean and the differences in their content of isoflavones was explained as due to the leaching of isoflavones into the soaking water. Such losses of isoflavones in soaking water were avoided by using the direct grinding process utilized in this study. However, this was not a mass balance study of isoflavone fractionation during UHT processing, but a study on the comparison of isoflavone concentrations between hot grinding and cold grinding of soybeans, as well as between direct and indirect UHT treatments. Moreover, there is a difficulty in comparing the isoflavone levels in the soybeans with those found in the final soymilk on a dry weight basis, because the composition of dry matter in the whole beans and soymilk differ as a result of removal of okara in the soymilk manufacturing process. Hence, a comparison of isoflavone levels between two different grinding procedures (hot vs. cold) and between the different UHT treatment methods (direct vs. indirect) were carried out to understand the effects of processing on the isoflavone levels in soymilks obtained.

3.1. Isoflavone levels in soymilk prepared by hot-grind vs. cold-grind method

The type of grinding process most probably caused a difference in the activity of glucan *endo*-1,6-β-glucosidase, popularly known as the β-glucosidase. Grinding of soybeans at a lower temperature resulted in an increase in the action of β-glucosidase, as shown by the increase in the concentration of aglucones. HPLC data showed that the samples of 'ground soybeans' along with the 'LOX treated beans' and the 'okara' obtained from the cold-grind process had a higher concentration of total aglucones (Table 2). The respective samples from a hot-grind procedure had very minimum amount of total aglucones in them. The same trend was observed in every trial carried out. The cold grinding of soybeans at a temperature of 45 °C favors an enhanced activity of the β-glucosidase, whereby the unconjugated isoflavone aglucones,

Table 1
Isoflavone concentrations reported as μmol/g of the sample (on dry matter basis) during the hot-grind process trial

Sample names (hot-grind process trial)	M (%)	Glucosides			Aglucones			Total [a] (μmol/gDM)	
		Din	Gln	Gin	Dein	Glen	Gein	Glucosides	Aglucones
Soybeans for indirect process	10.0	1.94	0.41	2.20	0.08	0.18	ND	4.55 ± 0.07^{a}	0.26 ± 0.004^{a}
Ground beans	86.4	2.46	0.50	2.33	ND	ND	ND	5.29 ± 0.08^{b}	ND
LOX treated	85.8	2.44	0.47	2.30	0.03	ND	0.04	5.21 ± 0.01^{b}	0.07 ± 0.010^{b}
Okara	73.0	1.50	0.32	1.48	0.08	ND	0.06	3.30 ± 0.01^{c}	0.13 ± 0.002^{bc}
Soy puree	90.0	3.36	0.61	2.98	0.08	ND	0.05	6.95 ± 0.02^{de}	0.13 ± 0.004^{bc}
Soymilk after TI deactivation	90.8	3.30	0.64	3.13	0.02	ND	0.04	7.08 ± 0.02^{e}	0.05 ± 0.001^{b}
Soymilk after indirect UHT	90.4	3.05	0.62	3.06	0.03	ND	0.04	6.74 ± 0.02^{d}	0.06 ± 0.004^{b}
Soybeans for direct process	10.0	1.92	0.42	2.19	0.09	0.19	ND	4.53 ± 0.07^{a}	0.27 ± 0.007^{a}
Ground beans	84.5	2.63	0.44	2.40	0.04	ND	0.03	5.47 ± 0.12^{b}	0.07 ± 0.007^{b}
LOX treated	84.6	2.68	0.44	2.45	0.04	ND	0.03	5.57 ± 0.03^{b}	0.07 ± 0.001^{b}
Okara	73.8	1.79	0.01	1.76	0.05	0.02	0.04	3.57 ± 0.02^{c}	0.10 ± 0.014^{bc}
Soy puree	90.0	3.58	0.61	3.46	0.05	ND	0.03	7.65 ± 0.09^{e}	0.08 ± 0.003^{b}
Soymilk after TI deactivation	91.0	3.91	0.62	3.43	0.05	ND	0.04	7.96 ± 0.02^{e}	0.09 ± 0.001^{b}
Soymilk after direct UHT	90.6	3.16	0.56	3.06	0.04	ND	0.03	6.78 ± 0.02^{d}	0.08 ± 0.001^{b}

M (%), moisture%; Din, daidzin; Gln, glycitin, Gin, genistin; Dein, daidzein; Glen, glycitein; Gein, genistein.
Means with different letters in the same column are significantly different at $p < 0.05$. ND, not detected.
[a] Mean of three determinations with standard deviation.

Table 2
Isoflavone concentrations reported as μmol/g of the sample (on dry matter basis) during the cold-grind process trial

Sample names (cold-grind process trial)	M (%)	Glucosides			Aglucones			Total [a] (μmol/gDM)	
		Din	Gln	Gin	Dein	Glen	Gein	Glucosides	Aglucones
Soybeans for indirect process	10.0	1.94	0.41	2.20	0.08	0.18	ND	4.55 ± 0.07^{a}	0.26 ± 0.004^{a}
Ground beans	86.2	0.84	0.23	0.98	1.54	0.25	0.10	2.05 ± 0.02^{b}	2.79 ± 0.022^{b}
LOX treated	85.0	1.01	0.21	1.07	1.06	0.20	0.75	2.30 ± 0.04^{b}	2.01 ± 0.046^{b}
Okara	74.0	0.87	0.21	0.97	0.68	0.10	0.39	2.05 ± 0.01^{b}	1.17 ± 0.003^{c}
Soy puree	89.4	2.79	0.49	2.70	0.29	0.03	0.19	5.97 ± 0.06^{c}	0.50 ± 0.007^{d}
Soymilk after TI deactivation	90.4	2.79	0.49	2.66	ND	0.02	0.12	5.95 ± 0.02^{c}	0.14 ± 0.003^{a}
Soymilk after indirect UHT	90.8	2.93	0.52	2.76	0.29	0.03	0.20	6.21 ± 0.01^{d}	0.51 ± 0.008^{d}
Soybeans for direct process	10.0	1.92	0.42	2.19	0.09	0.19	ND	4.53 ± 0.06^{a}	0.27 ± 0.007^{a}
Ground beans	84.2	1.39	0.23	1.36	1.13	0.19	0.78	2.98 ± 0.02^{b}	2.06 ± 0.011^{b}
LOX treated	85.2	1.30	0.21	1.27	1.13	0.19	0.78	2.77 ± 0.03^{b}	2.10 ± 0.022^{b}
Okara	74.6	0.76	0.16	0.81	0.88	0.18	0.60	1.73 ± 0.01^{e}	1.67 ± 0.029^{c}
Soy puree	90.2	3.50	0.57	3.21	0.27	0.03	0.17	7.29 ± 0.03^{f}	0.47 ± 0.050^{d}
Soymilk after TI deactivation	91.2	3.30	0.55	3.09	0.29	0.03	0.18	6.93 ± 0.03^{df}	0.49 ± 0.001^{d}
Soymilk after direct UHT	91.0	2.88	0.47	2.80	0.27	0.03	0.17	6.16 ± 0.03^{d}	0.47 ± 0.001^{d}

M (%), moisture%; Din, daidzin; Gln, glycitin, Gin, genistin; Dein, daidzein; Glen, glycitein; Gein, genistein.
Means with different letters in the same column are significantly different at $p < 0.05$. ND, not detected.
[a] Mean of three determinations with standard deviation.

daidzein, genistein and glycitein are formed. This resulted in higher aglucone concentrations in samples of the cold-grind process. Studies by Matsuura, Obata, and Fukushima (1989) observed an enhanced β-glucosidase action under special conditions of processing, such as soaking. The optimum condition for β-glucosidase activity was also observed at a temperature of 45 °C by these researchers (Matsuura, Sasaki, & Murao, 1995). Our studies shows that a soaking process was not necessarily required, but the grinding of soybeans with water at 45 °C was reasonably enough for an enhanced action of β-glucosidase. The length of time for which the soybeans resided at 45 °C during the grinding of bean was lesser than that would have happened during a soaking procedure. However, this short period of time was found enough for β-glucosidase to cause its action. Our studies shows that the temperature of contact of soybeans (45 °C) have had a major influence towards the enhanced action of the enzyme resulting in the formation of aglucones.

Isoflavone levels in the final soymilks obtained by hot-grind and cold-grind processes are shown in Tables 1 and 2, respectively. It was found that soymilk prepared by hot-grind process contained significantly higher ($p < 0.01$) isoflavone concentration than that prepared by a cold-grind process. For example, the total amount of isoflavone glucosides in soymilk prepared by a hot-grind process was 6.74 μmol/g DM and that from a cold-grind process was 6.21 μmol/g DM. Such a trend was observed in the main trials as well as in the replicate trials (data not shown) independent of whether it was direct UHT treated or indirect UHT treated. ANOVA results showed high MSE values (0.661) proving significant differences in the level of isoflavones in soymilk of a hot-grind vs. cold-grind process. Hot grinding caused an increase in the extraction of isoflavones into the soymilk compared to cold grinding. This improvement in the extraction of isoflavones into the soymilk could be due to the high solubility of isoflavones in hot water than in cold water extract (Gugger & Grabiel, 2000). This highlights the need for soymilk manufacturers to give a greater preference for hot grinding over cold grinding from the point of view of an improvement in the extraction of isoflavones into the soymilk.

3.2. Isoflavone levels in soymilk subjected to direct vs. indirect UHT processes

Soymilk prepared by hot-grind or cold-grind processes were subjected to direct/indirect UHT treatments and were analyzed for their isoflavone concentrations. The total isoflavone glucoside amounts in soymilk obtained by the hot extraction process followed by an indirect UHT treatment was 6.74 μmol/g DM, while the same by corresponding direct UHT treatment was 6.78 μmol/g DM (Table 1). In a similar way the total isoflavone glucoside amounts in soymilk prepared by a cold extraction process followed by an indirect UHT treatment was 6.21 μmol/g DM, while the corresponding soymilk subjected to a direct UHT treatment was 6.16 μmol/g DM (Table 2). Statistical analysis showed no significant differences ($MSE = 5 \times 10^{-5}$) in isoflavone levels in the soymilk subjected to indirect vs. direct UHT treatment ($p < 0.01$). Hence preference to any one UHT method is therefore not necessary, where either a direct or an indirect UHT treatment is good enough for soymilk processing.

3.3. Okara and isoflavone losses

A large proportion of isoflavones were found to be lost in the soy residue (okara) after a hot-grind or

cold-grind process followed by decanting. The loss of isoflavones into the okara during the traditional soymilk making process was also determined in our studies. Traditionally, soymilk is made by soaking soybeans in excess water followed by grinding, filtering, and cooking (Liu, 1997). The soy pulp residue or okara is removed during the filtration process. The 'soak and grind process' of soymilk making was carried out in a local soymilk manufacturing factory under industrial conditions. Soybeans were soaked in water at ambient temperature for a period of 5 h, followed by grinding with water (bean:water ratio of 1:4), extraction and cooking to obtain soymilk. Results showed that the amount of isoflavones fractionated into the okara during the traditional soymilk manufacturing process was lower than the amount of isoflavones lost in the okara during a 'no soak procedure' followed in our pilot plant study. The comparison of isoflavone levels in soybean seeds and okara for the pilot plant processes and the traditional process, after calculating them on the same basis of moisture content is shown in Fig. 3. Wang and Murphy (1996) reported as recovering high levels of isoflavones in the soymilk by pressing the okara, similar to what we found in the traditional industrial process that we employed for comparison. This highlights the need to evaluate automated and advanced processing systems used for soymilk preparations. It is therefore imperative to conduct further studies on the ways to minimize the loss of isoflavones into the okara and maximize the extraction of isoflavones into the soymilk. Okara is not utilized fully as food or feed sources, until recently, okara powder has been used to partially replace wheat flour for bread making.

Our study reveals the fate of isoflavones during the extraction and UHT processing of soymilk. Such data are very useful for process optimization during a soymilk preparation. Nowadays, in large-scale production of soymilks, automated extraction and UHT systems are being used. Though such systems offer time and energy savings, the question still remains whether there is a conflict between an ideal system and optimal retention of important functional and nutritional components in the manufacture of soymilk. Much of the research to date has been done on the understanding of the availability of lysine, thiamine and riboflavin of soymilk during heat treatment (Kwok & Niranjan, 1995; Kwok, Shiu, Yeung, & Niranjan, 1998). But, to our knowledge, this is the first study on a continuous pilot plant unit using different extraction methods and UHT treatments to process soymilk. Moreover, the results on the fate of

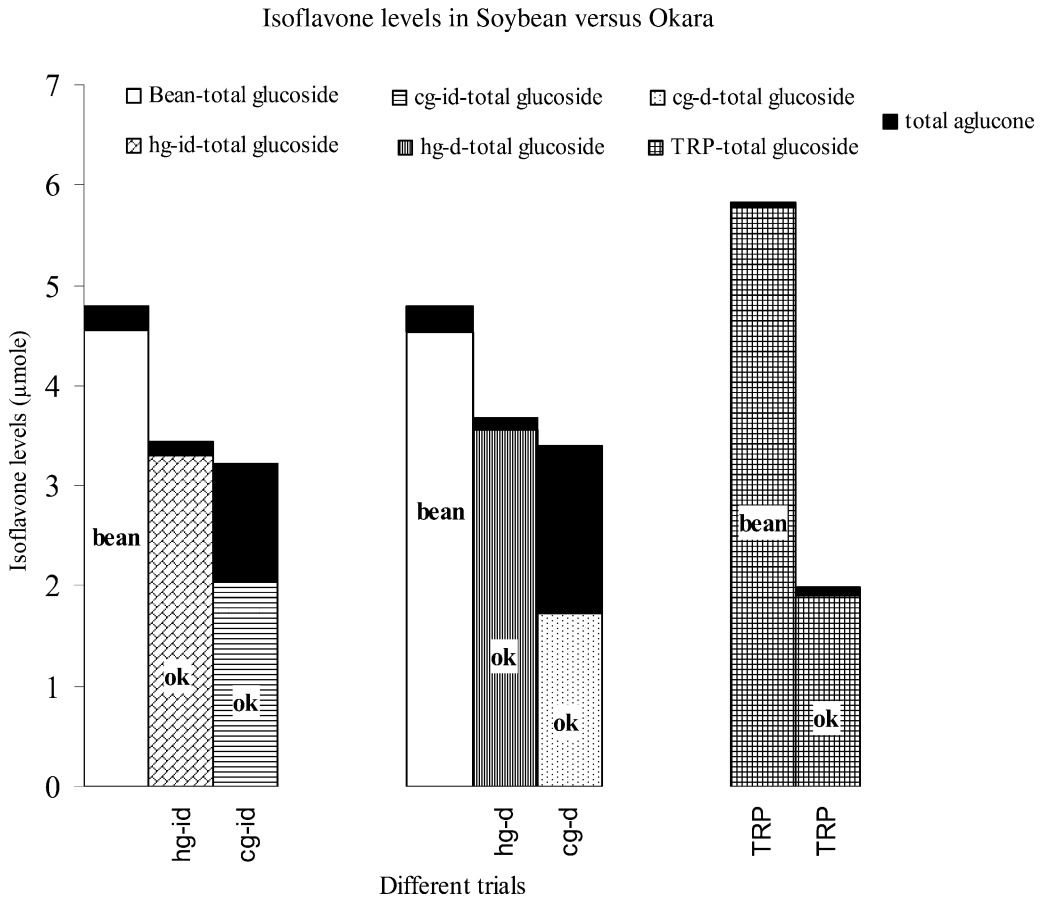


Fig. 3. Comparison of isoflavone levels in soybean seed and okara during the hot and cold grinding trials and from a traditional soymilk process.

isoflavones are also useful while defining mathematical and kinetic models to understand the maximum retention of nutrients in the soymilk prepared by UHT process.

4. Conclusion

In conclusion, we found that hot grinding caused an improvement in the extraction of isoflavones into the soymilk compared to cold grinding and there was no apparent difference in the loss of isoflavones due to direct UHT heating compared to indirect UHT heating. However, the isoflavone analysis method utilized in this study was insensitive to soy isoflavone conjugates other than the ones mentioned. The distribution of isoflavones in the bean and okara highlight the need to consider optimization of the retention of these functionally important components in the soymilk. This study further highlights the need to utilize okara and converting it to palatable food products suitable for human consumption.

Acknowledgement

This research was supported by Grant # R-143-000-179-112 from the National University of Singapore.

References

Anderson, J. J. B., & Garner, S. C. (1997). The effects of phytoestrogens on bone. *Nutrition Research, 17*(10), 1617–1632.

AOAC, (2002). *Official methods of analysis of AOAC international* (17th ed., Vol. 2). Association of Official Analytical Chemists: Gaithersburg, MD, USA.

Chiechi, L. M. D. (1999). Dietary phytoestrogens in prevention of long term postmenopausal diseases. *International Journal of Gynecology and Obstetrics, 67*, 39–40.

Coward, L., Barnes, N. C., Setchell, K. D. R., & Barnes, S. (1993). Genistein, daidzein, and their beta glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *Journal of Agricultural and Food Chemistry, 41*, 1961–1967.

FDA (1999). Food labeling health claims, soy protein and coronary heart disease. Food and Drug Administration, 21 Code of Federal Regulations, Part 101, October 26.

Gosta, B. (1995). *Longlife milk in dairy processing handbook* p. 224. Lund, Sweden: Tetra Pak Processing Systems AB.

Gugger, E. & Grabiel, R. D. 2000. inventors; Archer Daniels Midland Company, assignee. (2000 March 7). *Process for production of isoflavone fractions from soy*. US Patent 6,033,714.

Ho, S. C., Woo, J. L., Leung, S. S. F., Sham, A. L. K., Lam, T. H., & Janus, E. D. (2000). Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. *Journal of Nutrition, 130*, 2590–2593.

Jackson, C. J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., et al. (2002). Effects of processing on the content and composition of isoflavones during the manufacturing of soy beverage and tofu. *Process Biochemistry, 37*, 1117–1123.

Klump, S. P., Allred, M. C., MacDonald, J. L., & Ballam, J. M. (2001). Determination of isoflavones in soy and selected foods containing soy by extraction, saponification and liquid chromatography: collaborative study. *Journal of AOAC International, 84*(6), 1865–1883.

Kudou, S., Fluery, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K., et al. (1991). Malonyl isoflavone glycosides in soybean seeds (*Glycine max Merrill*). *Agricultural and Biological Chemistry, 55*(9), 2227–2233.

Kwok, K. C., & Niranjan, K. (1995). Review: effect of thermal processing on soymilk. *International Journal of Food Science and Technology, 30*, 263–295.

Kwok, K. C., Qin, W. H., & Tsang, J. C. (1993). Heat inactivation of trypsin inhibitors in soymilk at ultra-high temperatures. *Journal of Food Science, 58*(4), 859–862.

Kwok, K. C., Shiu, Y. W., Yeung, C. H., & Niranjan, K. (1998). Effect of thermal processing on available lysine, thiamine and riboflavin content in soymilk. *Journal of Science and Food Agriculture, 77*, 473–478.

Liu, K. (1997). *Soybeans chemistry, technology and utilization* p. 137. New York: Chapman & Hall.

Matsuura, M., Obata, A., & Fukushima, D. (1989). Objectionable flavor of soymilk developed during soaking of soybeans and its control. *Journal of Food Science, 54*(3), 602–605.

Matsuura, M., Sasaki, J., & Murao, S. (1995). Studies on β-glucosidases from soybeans that hydrolyze daidzin and genistin: isolation and characterization of an isozyme. *Bioscience Biotechnology and Biochemistry, 59*(9), 1623–1627.

Messina, M., & Barnes, S. (1991). The role of soybean products in reducing the cancer risks. *Journal of National Cancer Institute, 83*, 541–546.

Nelson, A. I., Steinberg, M. P., & Wei, L. S. (1976). Illinois process for preparation of soymilk. *Journal of Food Science, 41*, 57–61.

Wang, H. J., & Murphy, P. A. (1994). Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year and location. *Journal of Agricultural and Food Chemistry, 42*, 1674–1677.

Wang, H. J., & Murphy, P. A. (1996). Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry, 44*, 2377–2383.

Wilkens, W. F., Mattick, L. R., & Hand, D. B. (1967). Effects of processing method on oxidative off-flavors of soybean milk. *Food Technology, 21*, 1630–1633.

Wong, W. W., Smith, E. O., Stuff, J. E., Hachey, D. L., Heird, W. C., & Pownell, H. J. (1998). Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *American Journal of Clinical Nutrition, 68*, 1385S–1389S.

Wu, A. H., Ziegler, R. G., Nomura, A. M. Y., West, D. W., Kolonel, L. N., Ross, P. L. H., et al. (1998). Soy intake and risk of breast cancer in Asians and Asian Americans. *American Journal of Clinical Nutrition, 68*, 1437S–1443S.