1-1-2003

Process optimization for solid extraction, flavor improvement and fat removal in the production of soymilk from full fat soy flakes

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Process optimization for solid extraction, flavor improvement and fat removal in the production of soymilk from full fat soy flakes

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Lester A. Wilson, Major Professor
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Iowa State University

Ames, Iowa

2003

Graduate College
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This is to certify that the master's thesis of

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has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

I would like to dedicate this thesis to my parents, Brata and Ryan who has made it possible for me to be here and help me to get through rough times.

Stanley Praviradjaja

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ABSTRACT

Traditionally soymilk has been made with whole soybeans; however, there are other alternative raw ingredients for making soymilk, such as soy flour or full-fat soy flakes. U.S. markets prefer soymilk with little or no beany flavor. Modifying the process or using lipoxygenase-free soybeans can be used to achieve this. Unlike the dairy industry, fat reduction in soymilk has been done through formula modification instead of by conventional fat removal (skimming). This project reports the process optimization for solids and protein extraction, flavor improvement and fat removal in the production of 5, 8 and 12 °Brix soymilk from full fat soy flakes and whole soybeans using the Takai soymilk machine. Proximate analyses, and color measurement were conducted in 5, 8 and 12 °Brix soymilk. Descriptive analyses with trained panelists (n = 9) were conducted using 8 and 12 °Brix lipoxygenase-free and high protein blend soy flake soymilks.

Rehydration of soy flakes is necessary to prevent agglomeration during processing and increase extractability. As the rehydration temperature increases from 15 to 50 to 85 °C, the hexanal concentration was reduced. Enzyme inactivation in soy flakes milk production (measured by hexanal levels) is similar to previous reports with whole soybeans milk production; however, shorter rehydration times can be achieved with soy flakes (5 to 10 minutes) compared to whole beans (8 to 12 hours). Optimum rehydration conditions for a 5, 8 and 12 °Brix soymilk are 50 °C for 5 minutes, 85 °C for 5 minutes and 85 °C for 10 minutes, respectively.

In the flavor improvement study of soymilk, the hexanal data showed differences between undeodorized HPSF in contrast to triple null soymilk and no differences between deodorized HPSF in contrast to deodorized triple null. The panelists could not differentiate

between the beany, cereal, and painty flavors. However, the panelists responded that the overall aroma of deodorized 8 °Brix triple null and HPSF soymilk are lower than the undeodorized triple null and HPSF soymilk. The triple null soymilk was perceived to be more bitter than the HPSF soymilk by the sensory panel due to oxidation on the triple null soy flakes. This oxidation may produce other aroma that was not analyze using the GC but noticed by the panelists. The sensory evaluation results did show that the deodorizer was able to reduce the soymilk aroma in HPSF soymilk so it would be similar to triple null soymilk at 8 °Brix level.

Regardless of skimming method and solids levels, the fat from whole soybean milk was removed less efficiently than soy flake milk (7 to 30% fat extraction in contrast to 50 to 80% fat extraction respectively). In soy flake milk, less fat was removed as the % solid increases regardless of the processing method. In whole soybean milk, the fat was removed less efficiently at lower solids level milk using the commercial dairy skimmer and more efficient at lower solids level using the centrifuge-decant method. Based on the Hunter L, a, b measurement, the color of the reduced fat soy flake milk yielded a darker, greener and less yellow colored milk than whole soymilk (α <0.05), whereas no differences were noticed in reduced fat soybean milk (α <0.05). Color comparison of whole and skim cow's milk showed the same trend as in the soymilk.

INTRODUCTION

Soymilk is made by a water extraction of soybeans. Its visual appearance is similar to cow's milk, and nutritionally more superior to other legumes (Philip and Helen 1973; Steinkraus and others 1978). In China, soybeans are called the "greater beans" due to their many health benefits (Simoons 1991). Soybeans have been proposed to be useful as an anti-carcinogen, a cholesterol-lowering agent, prevent calcium lost, and a phytochemical source (Messina 1995). Soybeans are an inexpensive source of high quality protein. The high quality protein of soybeans is useful as a protein substitute or a supplement for people in developing countries (Messina 1995; Iwuoha and Umunnakwe 1996). In the U.S., the Food and Drug Administration (FDA) recently approved a health-labeling claim for products containing soy protein in October 1999. Daily consumptions of 25 g (6.25 g per serving) of soy protein may reduce the risk of heart diseases, due to the cholesterol lowering effects of soy protein (Henkel 2000).

Based on the market research conducted by SoyaTech in 1999, the sales of soy foods in the U.S. were projected to increase from $2.1 billion to $3.57 billion by 2002 (Soya Tech 1999). In 2002, Soya Tech released a new report showing that the soy food industry had already reached $3.2 billion in sales by 2001. Soymilk sales alone in 2001 has reached $550 million and were projected to reach $1 billion in the coming three to five years (Soyatech 2002).

The acceptance of soy foods in the western market is affected, in part by its off-flavor (Wilson 1985; Feng and others 2001). The off-flavor of soy foods is caused by the activity of lipoxygenase (Wilkens 1967). This enzyme oxidizes the lipids in soybeans and the products produced by lipoxygenase produces flavors that are describes as grassy, painty, and beany.

Many methods have been developed in order to eliminate this off-flavor, such as processing modifications or eliminating the lipoxygenase enzyme through genetic modifications (Wilson 1996; Kwok 1995). Processing modifications include rapid enzyme deactivation using heat, such as the Cornell method, Illinois method, Rapid Hydration Hydrothermal Cooking (RHHTC), cold grind under vacuum method (ProSoya™), deodorization, antioxidant addition and alkali treatment methods (Wilson 1985; Kwok 1995; Liu 1997).

Most soymilk processes use whole soybeans as the starting material. However, there are other raw forms of soybeans that can be used to make soymilk; such as soy flour or full fat soy flakes (Wilson 1985; Moizuddin et al, 1999). Most studies have looked at optimizing the production of soymilk using whole soybeans. The use of soy flakes for soymilk production has not been studied extensively. The purpose of the first study is to optimize the use of soy flakes for soymilk production based upon solids, protein and flavor properties.

The development of lipoxygenase-free soybeans opened up new opportunities in creating no "off"-flavored soy foods. Previous studies have shown that these lines of soybeans can improve the flavor of soy products (Kobayashi and others 1995; Wilson 1996; Torres 2001). In the dairy industry, it is a common practice to deodorize cow's milk to remove any undesirable off-flavors using a flash vacuum-steam deodorizer (Farrall 1980). The same practice could be utilized on soybean milk. Thus, the objective of the second study is to evaluate the differences in the flavor of soymilk made from lipoxygenase-free soy flakes and vacuum deodorized soymilk.

Soymilk has been consumed as a substitute for cow's milk for centuries. While cow's milk with various lipid contents (whole, 2%, 1%, and skim) has been available for sometime, soybean milk counterparts have not. The production of low fat or non-fat soymilk would

provide more variety for the consumer. Moizuddin and others (1999) has reported the production of a lower fat tofu made from soyflakes. However, little research has looked at fat removal in soymilk. The optimum conditions for fat removal in soymilk have not been determined. Thus, the objective of the third study is to evaluate the efficiency of fat removal from soymilk produced from whole soybeans and soyflake at three solid levels.

THESIS ORGANIZATION

The thesis consists of a general introduction, a literature review and three manuscripts. The first manuscript is entitled "The influence of temperature and rehydration time on the production of soymilk made from full fat soy flakes"; the second "Comparison of Lipoxygenase-free soymilk with deodorized soymilk"; and the third "Efficiency in lipid removal from soymilk made from full fat soy flakes or whole soybeans at three solid levels". These papers will be submitted to the Journal of Food Science.

LITERATURE REVIEW

HISTORY

In Asia, soybeans have been consumed for thousands of years. Chinese people have consumed them for two millennia, where as in Japan it has only been consumed about 1000 years (Shurtleff and Aoyagi 1983). In that part of the world, soybeans have been used as one of the main sources of protein.

In the U.S., soybeans were introduced in 1800 but its many uses were not utilized until the twentieth century. At the beginning, the use of soy was limited as animal feed. It was not until 1945 that soybeans were utilized to produce both feed and food oil. From then on, soybeans production has grown considerably (Snyder and Kwon 1987). Based on the market research conducted by SoyaTech in 1999, the sales of soy foods in the U.S. were projected to increase from $2.1 billion to $3.57 billion by 2002 (Soya Tech 1999). In 2002, Soya Tech released a new report showing that the soy food industry had already reached $3.2 billion in sales by 2001. Soymilk sales alone in 2001 has reached $550 million and were projected to reach $1 billion in the coming three to five years (Soyatech 2002).

Some examples of soy foods that are available are given in Table 1.

Table 1. Examples of soy products

Name	Origin	Description
Soymilk	Japan, China, Korea	Filtered water extract of soybean
Tofu	Japan, China, Korea, Indonesia, Malaysia	Soybean curd
Tempeh	Japan, Indonesia, Malaysia	Fermented whole soybean
Miso	Japan, China, Korea, Indonesia, Malaysia	Fermented soy paste
Yuba	Japan, China, Indonesia, Malaysia	Soymilk Film

SOY FOODS NUTRITIONAL QUALITY

The excellent quality of soybeans protein has been utilized as a source of protein in many Asian countries for a long time. Recently, there has been some evidence of soybeans health benefits. To name a few, soybeans are thought to relieve the effect of postmenopausal symptoms, osteoporosis and cancer prevention activities for women. Soybeans contain more protein and are superior in quality to other legumes. Soybeans contain about 20% oil and 43 % protein (Karmas and Harris 1988). Soybeans are rich in the essential amino acid lysine, but low in sulfur containing amino acids. This is why soybeans are sometimes used to complement other legumes or cereals in food for non-human species. The superior quality of soybeans has led the use of soybeans as a substitute for animal protein with the advantage of having less fat in soy foods than animal products. The major applications of soybeans, soy protein or combination of the two are for soymilk and tofu production, infant formula, medical nutrition products, animal product substitutes, and bakery products (Snyder and Kwon 1987; Slavin 1991, Liu 1997).

In the U.S., the Food and Drug Administration (FDA) approved a health-labeling claim for products containing soy protein in October 1999. Daily consumptions of 25 g (6.25 g per serving) of soy protein may reduce the risk of heart diseases, due to the cholesterol lowering effects of soy protein. A study conducted at Wake Forest University Baptist Medical Center reported that soy protein can reduce plasma concentration of total Low Density Lipoprotein (LDL) cholesterol, but does not decrease the levels of High Density Lipoprotein (HDL) or the good cholesterol. HDL level has been reported to decrease the chance of heat disease. (Sirtori and others 1995; Henkel 2000)

Soybeans contain phytochemical compounds called isoflavones. There are three major estrogenic compounds that are included in the isoflavone family; genistein, daidzein, and glycitein. In soybeans, isoflavones have 12 isomers; the aglycons: genistin, genistein, daidzin, daidzein, glycetin, glycetein; the acetylglucosides: 6"-O-acetyldaidzein, 6"-O-acetylgenistein, 6"-O-acetylglycetein; the malonylglucosides: 6"-O-malonyldaidzein, 6"-O-malonylgenistein, and 6"-O-malonylglycetein (Kudou and others 1991). The amount of isoflavones in soybeans varies considerably, from as little as 400 to 2,500-μg/g due to environmental factors. In soymilk, the isoflavones contents range from 10 to 200-μg/g (USDA-Iowa State University Database on the Isoflavone Content of Foods - 1999). The amount of isoflavones in various foods is also dependent upon the method of processing. Alcohol washed soy flakes, used to produce soy concentrates, usually has greatly reduced levels of isoflavones. Heat treatment changes the form of the malonyl isoflavones into the acetyl form. In soymilk processing, all isoflavone from the soybeans is extracted into the soymilk. However, per serving consumption of soymilk, as expected, would have a lower concentration of isoflavones due to dilution effect (Wang and Murphy 1994 and 1996).

There are several indications that isoflavones have health benefits, but their contributions are not yet fully known. Isoflavones have a weak estrogenic activity and in pre-menopausal women, isoflavones competes with the human estrogen. The ability of isoflavones to compete with human estrogen is thought to be one of the mechanisms of isoflavones to prevent cancer. Estrogen is a cell-promoting hormone, which stimulates the growth and replication of cells. When cells are replicating, there is a chance of making an error in genetic replication, which then causes cancer cells. By blocking the estrogen hormone activity, isoflavones can then help reduce the number of replications of the cells,

which may prevent the occurrence of cancer. Isoflavones have also been shown to prevent the occurrence of colon and prostate cancer (Messina and Messina 1991). However, other studies also had shown that isoflavones had no effect in preventing cancer or showing any esterogenic activity. This uncertainty on the role of isoflavones in human body caused FDA not to make any ruling on health claim related to isoflavones.

Trypsin inhibitor (TI) has been known to be the major anti-nutritional compound in soybeans. There are two kinds of trypsin inhibitors: Kunitz and Bowman-Birk TI. Kunitz TI is less heat stable than the Bowman-Birk chymotrypsin inhibitor and trypsin inhibitor, because it has fewer disulfide linkages in the structure. Based on animal studies Kunitz TI binds to the trypsin enzyme and inactivates it. The inactivation of trypsin causes increase production of more trypsin by the pancreas, which leads to the enlargement of the pancreas. The action of TI decreases the digestion of protein in animal studies, and reduces protein absorption. However, in 1994, Kennedy has shown that the Bowman-Birk TI was able to prevent the occurrence of cancer cells in hamsters. The results suggested that the Bowman-Birk TI might have an anticancer activity that contributed to the overall health benefits of soy.

Soybeans contain about 20 % oil with about 80 % of the oil being unsaturated fatty acids. Linoleic acid (18:2) is the most predominant unsaturated fatty acid in soybeans, followed by oleic acid (18:1) (Table 2).

Table 2. Soybean fatty acid composition*

Fatty Acid	Percentage (%)
Saturated (Total):	*14.4*
- Myristic (C 14)	0.1
- Palmitic (C 16)	10.3
- Stearic (C 18)	3.8
Unsaturated (Total):	*80.6*
- Oleic (C 18:1)	22.8
- Linoleic (C 18:2)	51.0
- Linolenic (C 18:3)	6.8
Other:	*5.0*

* USDA National Nutrient Database for Standard Reference. Search keyword: Oil, soybean, salad or cooking.

THE FLAVOR OF SOYBEAN AND THE LIPOXYGENASE ENZYME

Soybeans have a unique and distinct flavor. Berczeller in 1924, described the flavor of soybean as "evil" tasting. Soybeans contain flavor that is described as beany, grassy, green, painty, astringent, and bitter (Wolf 1975; King and others, 2001). In China and some Asian countries, these flavors are favorable in soy foods. However, in Japan and in most western countries, these flavors are considered as "off"-flavors and undesirable. The off-flavors are the main factors in inhibiting the utilization of soy as food, despite its known health benefits.

The "off"-flavors of soybeans are caused by an oxidase enzyme called lipoxygenase (Lox). This enzyme is widely distributed in the plant kingdom, and it has been demonstrated in animal tissue as well (Samuelsson 1972; Nugteren 1975). Soybeans are known to contain the highest concentration of this enzyme in the plant kingdom (Axelrod 1974). The presence of high amounts of unsaturated fatty acid in soybeans makes a perfect substrate for the lipoxygenase enzyme to react. Any time the soybean cells are ruptured lipoxygenase

enzymes work almost instantaneously. Once the fatty acids are oxidized, the unique flavors of soybeans are produced.

Hexanal is thought to be the major compound that contributes to the "beany" flavor of soymilk. Based on the AEDA (Aroma Extract Dilution Analyses), Kobayashi and others (1995) reported that hexanal has a low flavor threshold. The report supports previous study by Fujimaki in 1965 that hexanal concentration of 10 ppm. is enough to contribute to the green bean flavor of soybean.

Lipoxygenase is an iron-containing enzyme (Roza and Francke 1973). The native state of lipoxygenase contain ferrous ion (Fe^{2+}) at the active site. When the ion is activated by oxygen or hydroperoxide, the active site is oxidized to the ferric ion (Fe^{3+}). The enzyme can now bind to the fatty acid by abstracting its hydrogen. From here, the lipoxygenase can participate in an aerobic or anaerobic reaction. In the aerobic reaction, the enzyme produces fatty acid hydroperoxide. The hydroperoxide will then react with other fatty acids and start an autoxidation process, which lead to the production of off-flavors. In the anaerobic reaction of lipoxygenase, the hydroperoxide fatty acids terminate the propagation step by reacting with other free radicals. The major product from the anaerobic reaction yields different kinds of volatile compounds and thus a different flavor profile.

Lipoxygenase has three isozymes; they are denoted as lipoxygenase 1, 2, and 3 (Lox1, Lox2, and Lox3). Each lipoxygenase isozymes differ in substrate specificity, reaction products, optimum condition and mobility in SDS gels. Among the three lipoxygenase, L2 is thought to be the major enzyme to produce the beany flavor in soybean (Matoba and others 1985). Lipoxygenase 3 has been proposed to decrease the amount of hexanal produced by competing with L1 and L2 in the breakdown of fatty acid (Hildebrand and others 1990). The

by-product of lipid oxidation produces a complex array of volatile compounds that contributed to the off-flavor.

Listed in Table 3 are some examples of compounds that have been found in the headspace and other flavor found in soymilk.

Table 3. Aroma and flavors of soymilk

Aroma and Flavor Description	Identified Compounds	Reference
Grassy	2-pentyl pyridine	Boatright and Lei 1999
	2-pentyl furan	Boatright and Lei 1999
	1-hexen-1-ol	Torres and Reitmeier 2001
Beany (Raw), Green	Acetophenone	Boatright and Lei 1999
	Hexanal	Torres and Reitmeier 2001
	Ethyl vinyl ketone	Mattick and Hand 1969
Sulfurous, Green Onion	Dimethyl trisulfide	Boatright and Lei 1999
Painty	Higher alka-2,4-dienals	Mcleod and Ames 1988
Cereal/Pasta/Flour Flavor	Hexanal	Boatright and Lei 1999
Floral	2-heptanone	Boatright and Lei 1999
Mushroom	1-octen-3-one	Boatright and Lei 1999
	1-octen-3-ol	Badenhop and Wilkens 1969
Astringency	Phenolic acids	Arai, and others 1966
Bitterness	Phenolic acids	Arai, and others 1966

Other flavor problem with soy protein is its flavor binding properties. Soy protein was shown by Arai and others (1970) to have the ability to bind flavors. Since then many studies have been conducted to study the mechanisms of flavor binding in soy protein. The proposed mechanisms of flavor-protein binding are through van der Waals, hydrophobic and hydrogen bonding (Aspelund and Wilson 1983; O'Keefe and others 1991a,b). Aspelund and Wilson (1983) reported that in dry conditions, binding of flavor-protein occurs through specific and non-specific interactions; in addition, the ligand's functional group is important in binding. In aqueous system, flavor-protein interaction occurs spontaneously due to hydrophobic binding. The number of binding sites is greater in glycinin than β-conglycinin (Damodaran and Kinsella 1981, O'Keefe 1991a,b).

Based on the experiment conducted by O'Keefe et.al. (1991a), the binding curve never reached saturation up to 1000 ppm of ligands (ketones, hexanol and hexane) which indicates that soy protein has high binding capacity. In some studies, there are some indications that ligand binding also changes the conformational structure of the protein and increases the binding sites (Kinsella and Damodaran 1980; Damodaran and Kinsella 1981; Thissen 1982). Likewise heat may also changes the conformational structure of the soy protein and exposing the nonpolar regions and increasing the binding sites (Crowther and others 1980).

The autoxidation property of lipoxygenase enzyme has been reported to be beneficial in certain applications. In 1934, Haas and Bohn reported the addition of soy flour to wheat flour (0.75 – 2 % addition) is useful to bleach carotene pigment in wheat flour. The addition of enzyme active soy flour also has been reported to be beneficial in improving over mixing and increasing dough stability (Faubion and Hoseney 1981).

SOYMILK AND SOYMILK PROCESSING METHODS

Soymilk is a water extract of soybeans. It contains about 2.75 % protein, 1.91 % fat and 1.81 % carbohydrate (USDA National Nutrient Database 2002); the nutritional contents of soybeans depend on the % solid of soymilk being produced. In soy yogurt, tofu or yuba production, soymilk is the intermediate step for producing these products. The traditional Chinese method for soymilk production is soaking the soybeans for 8-12 hours in cold water, wash, grinding, filtering and then cooking. Heating, in soymilk is an important step because it helps in the flavor development, pasteurization, and improving the nutritional quality (Wilson, Murphy and Gallagher 1992).

As discussed previously, soymilk has a strong off-flavor that is unpleasant to most western consumers. The benefits of utilizing soy as food products prompted researchers to develop new ways of processing soy to improve its flavor. Several methods have been proposed to improve the flavor of soymilk, through processing modifications or modifying the raw ingredient of soymilk. Some examples of processing modifications are Cornell, Illinois, Rapid Hydration Hydrothermal Cooking (RHHTC), cold-grind under vacuum (ProSoya), deodorization, antioxidant addition and alkali treatment methods. The steps of some of the methods are described in figures 1 to 5.

Fig. 1 - Traditional soymilk production

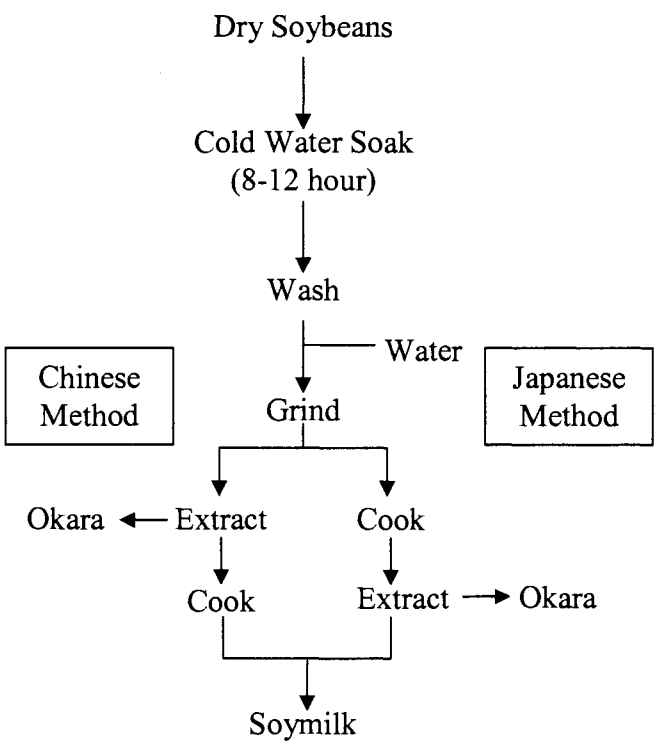


Fig. 2 - Cornell method

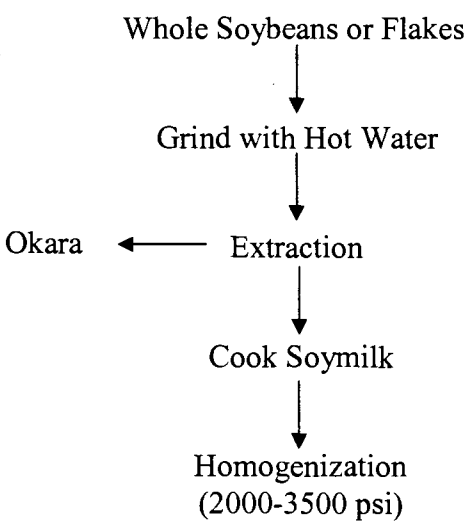


Fig. 3 - Illinois method

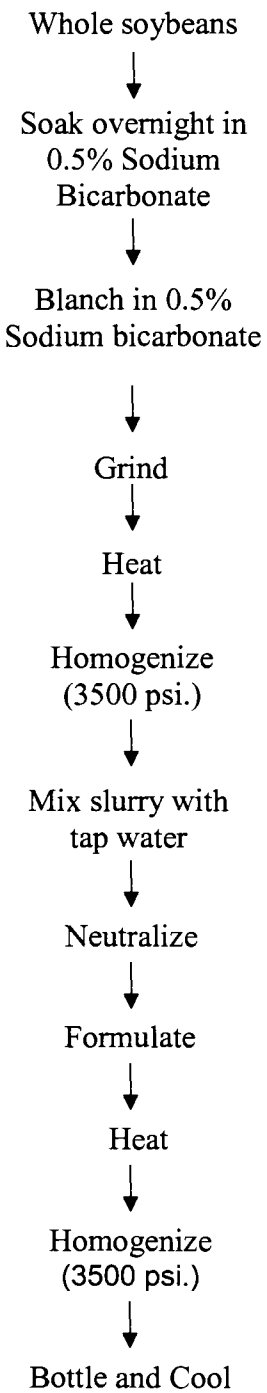


Fig. 4 - RHHTC method

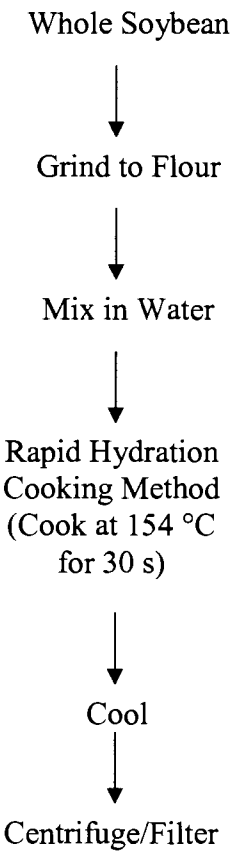
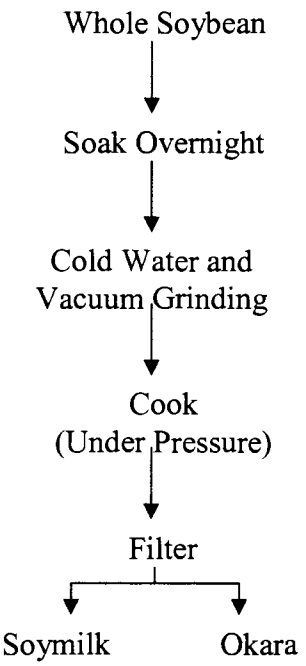


Fig. 5 - ProSoya method



The Illinois and the RHHTC method incorporate all of the soybean parts into the resulting soymilk due to high shear of the process. However, the RHHTC method uses a higher temperature (154 °C for 3 s) than the Illinois method (82 °C). The ProSoya and the Cornell method is a modification of the traditional methods. In the ProSoya method, the soybeans are ground with cold water under vacuum to prevent the incorporation of oxygen into the fatty acid by lipoxygenase. In the Cornell method, enzyme inactivation is accelerated by inactivation of the enzyme using wet heat (80 °C and above) during grinding. (Kwok and Niranjan 1995)

Even though it was reported that the processing modifications reduced off-flavor development, each method has its own advantages and disadvantages in terms of protein and solids recovery. The traditional and Cornell method yield the lowest % solid extraction (55-65% wt/wt), where the Illinois and the RHHTC method yield the highest % solid extraction (86-89% wt/wt). For protein extraction the traditional, Cornell and the ProSoya method extracted about 70-80 % (wt/wt) of protein and the Illinois and RHHTC method extracted about 90-93 % (wt/wt) protein. In the Illinois and the RHHTC method, all of the soybeans including the okara are included in to the soymilk and therefore increases its % solid and % protein extraction (Golbitz 1995; Kwok and Niranjan 1995). In the Illinois method, where soybeans are blanched for 30 minutes, Johnson and Snyder (1978) showed that the initial blanching step would heat fixed the protein bodies in the soybeans and make it insoluble even after the grinding step. If the soybeans are ground with hot water, such as in the Cornell method, the protein fixation does not occur and better extraction can be obtain. Another disadvantage of the Illinois and RHHTC method is that the suspended insoluble solids may settle out.

Some research suggest that modifying the processing methods by the addition of extra ingredients such as antioxidants, sodium bicarbonate, oxidase enzymes or masking agents can improve the flavor of soymilk. It was assumed that the additions of antioxidants capture the free radicals that are form by the lipoxygenase enzyme, and hence reduces the lipid oxidation and the lipid oxidations byproducts. In 1991, Vijayvaragiya and Pai evaluated the use of several antioxidants in preparing soymilk through lipoxygenase enzyme assay. Among all of the antioxidants that were evaluated, it was found that propyl gallate in combination with citric acid and ascorbic acid showed the most inhibition of lipoxygenase I isozymes.

Another processing modification employs the use of sodium alkali (sodium hydroxide, sodium carbonate, and sodium bicarbonate) in treating the soybeans either before or after soymilk making. An example where soybeans were treated before the process is in the Illinois method where soybeans were soaked and ground using sodium bicarbonate. The other application of sodium alkali is by adding it into the soymilk itself (Bourne, and others, 1976). In this case, Bourne reported that the pH change in soymilk was not the one responsible for the improvement of flavor but instead it was the concentration of the sodium ions. The addition of an oxidase enzyme was suggested for flavor removal through oxidation. The addition of the enzyme would oxidize the already present off-flavor to reduce the amount of off-flavor (aldehydes to carboxylic acids). A study conducted by Maheshwari and others (1997) uses porcine liver aldehyde oxidase (PAO) enzyme to reduce the amount of 'off' flavor in an aqueous defatted soy flour system. The result from the study showed that the addition of the enzyme would decrease the amount of 'off' flavors. Last, the additions of sugars and flavorings could also be used to mask the presence of beany flavor (Torres-Penaranda and others 2001).

Soy flavor improvement can also be done through breeding techniques. Soybeans plants can be crossbred to produce soybeans that lack the lipoxygenase (Lox1, Lox2 and Lox3) enzymes. Soybeans plants that lack the Lox1, Lox2, Lox3, Lox12, Lox23, Lox13 or Lox123 can now be found. Flavor improvement of soymilk has been reported with these varieties (Kobayashi and others 1995; Wilson 1996; Torres 2001).

As the famous phrase says, "there is more than one way to skin a cat", there is more than one-way to make soymilk. Ingredients such as soy flakes, soy flour (full fat or defatted), soy powder, or soy protein isolate can be used to make soymilk (Johnson and others 1981;Yazici and others 1997; Moizuddin and others 1999). Some advantages of using these alternative ingredients are by saving time and cost. These ingredients have a larger surface area and therefore it would need shorter rehydration time to process, thus saving energy (no grinding step), materials (soak water), sanitation time and cost in comparison to using whole soybeans (Moizuddin 1999). As with any powdered products, processing difficulties would be preventing agglomeration during dispersion in liquid.

In the study conducted by Moizuddin and others (1999), they evaluated the use of whole soybean and soy flakes for tofu production using direct and indirect heat treatment. They reported that tofu made with soy flakes has lower fat content (26% db) and the okara has higher fat content than the tofu (40% db) and okara made with whole soybean in both processing methods. They proposed that the hulls from whole soybeans might play a role as a filtering aid during pressing by providing channels for the fat to escape, where in soy flakes, the absence of the hull cause caking of the insoluble matter and preventing the fat to escape (Moizuddin and others 1999).

The 'off'-flavors of soymilk could be removed from the soymilk through a deodorization process. The deodorization process is a common procedure in processing dairy milk. In dairy milk, off-flavors could be caused from a carry over from the cows' diet. Dairy milk is commonly deodorized with a flash steam-vacuum deodorizer. The process is done after the pasteurization step. The deodorizer works by creating a large thin layer of milk along the inside wall of the deodorizer and the vacuum would volatilize and remove the off-flavors. Inside the deodorizer, steam is usually added into the system to compensate for the loss of moisture during deodorization and to optimize flavor extractions (Farrall 1980). Shurtleff (1979) describe the use of a vacuum pan (with 40 cm Hg or 7.7 psi. vacuum pressure) to remove the off-flavor of soymilk which was prepared using the pre-blanch method. The deodorization step can be applied several times before the formulation process. In practice, to achieve an acceptable flavored soymilk several combinations of processing method can be used to prevent the formation of the off-flavors.

To improve the nutritional quality and to provide varieties of soymilk to the consumer, reduced fat soymilk can be produced. Little research has looked at the production of reduced fat soymilk. Conceivably, the fat of soymilk can be removed using the same method as cow's milk. In cow's milk, the cream is separated from the milk using centrifugal force. During centrifugation, the lower density fat will move inwards whereas the higher density skim milk and other particles will move outwards of the axis of rotation. A commonly used cream separator is a disc-bowl centrifuge (Fig. 6).

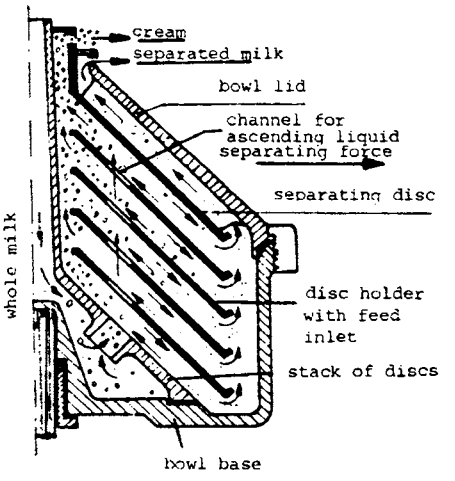


Figure 6 - Disc Bowl Centrifuge (source: Kessler, 1981)

The disc-bowl centrifuge consists of a bowl base, disc holder, discs stack, separating disc, bowl lid, feed inlet and outlet. The purpose of the conical discs is to allow higher throughput of the milk by increasing the clarifying area and allowing the particles to move in the centrifugal field. By having the distance between the discs as small as possible, the milk-particles would be able to flow more efficiently in the centrifugal fields ensuring the particles are not affected by the fluid turbulence to achieve better separations. A larger distance between the discs is needed if there is a possibility of clogging. The cow's milk is separated at 45-55 °C; higher temperature would cause the milk protein to precipitate. The % milk can be adjusted by carefully mixing a certain amount of cream back into the separated milk. Using a similar principle, fat can be removed using a centrifuge. This method managed to remove most of the fat by using a very high centrifugal force (156000 g) (Shibasaki and others 1972; Ono and others 1996).

The fat of soymilk, similar to the dairy milk counterpart, has unstable oil in water emulsion. The emulsion stability of dairy milk can be disrupted by agitation or by lowering its temperature. Both agitation and temperature reduction deformed the fat globules of milk

and causing flocculation of fat globules and causes separation of fat. A destabilization of an emulsion is caused by a collision of fat droplets in a continuous phase. The collisions would cause a separation, flocculation or coalescence of the fat droplets. If after the collision separation occurs, it means a stable emulsion system has been achieved. If the collisions produce flocculation or coalescence of fat droplets, the fat droplets form a larger structure, which cause separation of the fat or an unstable emulsion system. The larger the radius of the fat droplets the faster is the rate of sedimentation or separation. Homogenization reduces the size of fat globules and increases its emulsion stability. During homogenization process of dairy milk, the fat globules incorporate the whey protein and casein into the structure yielding a stable emulsion of milk. (Buchheim and Dejmek 1997).

The collisions of the fat droplets can be prevented by reducing their kinetic energy or by having an energy barrier between them. Reducing the kinetic energy of the fat droplets can be achieved by increasing the viscosity of the continuous phase, for example by adding gum into the system. An energy barrier between the fat droplets can be achieved by protecting the fat droplets with an outer layer and hence prevent flocculation and coalescence; a good example is by adding emulsifiers into the system. (Friberg 1997). Flocculation may also occur by protein bridging, which happens when the tail of the protein binds to another exposed surface of fat globules and forming a fat droplets cluster.

Protein load is defined as the amount of protein that is absorbed in mg/m^2. The protein load diminishes with the fat surfaces, which means that the smaller the fat droplet size the higher the probability of the proteins to unfold on the surface of the fat droplets and forming a thin layer. The protein load may or may not be affected with the method of

emulsion preparation; for example, an emulsion prepared using a blender vs. a valve homogenizer (Tornberg and others 1997).

Soy protein is known to have a good emulsifying capability. It has been added into various food systems to provide emulsion stability, such as in comminuted meats, coffee whiteners, mayonnaise, etc. The emulsion properties of the β-conglycinin and glycinin soy proteins have been studied by several researchers (Kanamoto and others 1977; Aoki and others 1980). Kanamoto et al (1977) reported that phosphatidylcholine can form complexes to the soy protein through sonication and bound nonspecifically to either the β-conglycinin or the glycinin globulin. Aoki and others reported that β-conglycinin globulin has higher emulsion stability than glycinin globulin. They also reported that soy protein showed the lowest emulsion stability and capacity when the soy protein is at the isoelectric region (pH 4-4.5).

In a study conducted by Guo and others (1997), the movement of lipid during soymilk heating was observed. In this study, heated soymilk at different temperatures was separated into particulate, soluble and floating fractions through centrifugation. They concluded that fat migration occurs in two stages. In the first stage, fat is released into the soluble fraction at 65-75 °C and then the fat migrated from the soluble to the floating fraction at temperature above 75 °C in the second stage. In this study, they also reported that the release of fat from the particulate to the soluble fraction is due to the denaturation of the glycinin protein. These findings correspond to the results reported by Aoki and others (1980), where they reported that emulsifying capacity and stability decreases with heat with the lowest was observed at 85 °C.

COLOR, GAS CHROMATOGRAPHY AND SENSORY EVALUATION

Hearing, taste, touch, smell and vision are all the senses that we use to evaluate food and all of these senses play a roll in the acceptance of food. Appearance is one of the first sensory inputs when we examine foods. This sensory input gives us clues on what to expect from the food. For example, a brown banana indicates over ripeness of the food or clumps in the milk indicate spoiled milk. The appearance judgment is something that we have been trained since the day human were able to see and therefore it varies from one person to another or from one culture to another. Appearances are classified into two areas; color (blue, red, yellow, etc.) and geometric attributes or appearance (size, shape, glossy or surface texture). (Hunter and Harold 1987).

The organ that makes it possible for human to see is the eyes (Fig. 7). The eyes act as a receptor of light and allow us to see. The light that enters the eyes goes through a flexible lens that allowed the light to be focused onto the fovea part of the retina. The retina is the light detecting membrane and fovea is the region at the center of the retina. The amount of light that goes through the eyes are controlled by the iris, which acts like a diaphragm in a camera; the less intense is the light the more the iris is opened and the opposite for more intense light. (MacDougall 2002).

The light that is focused into the fovea is then transmitted to the optic nerves and carried to the brain, which then translates and interprets as a visual image. There are two light-detecting cells within the retina, the rod and cone cells. The rod cells are more sensitive than the cone cells, and that is why it is used for in response to darkness or lightness condition; however it does not have the ability to detect color. The cone cells are responsible

for color perception and the rod cells are responsible for low light visual perception. Human color visualization is a trichromatic (three colors) detection system because three types of cone cells have been identified in the human eyes. The three cone cells are differentiated

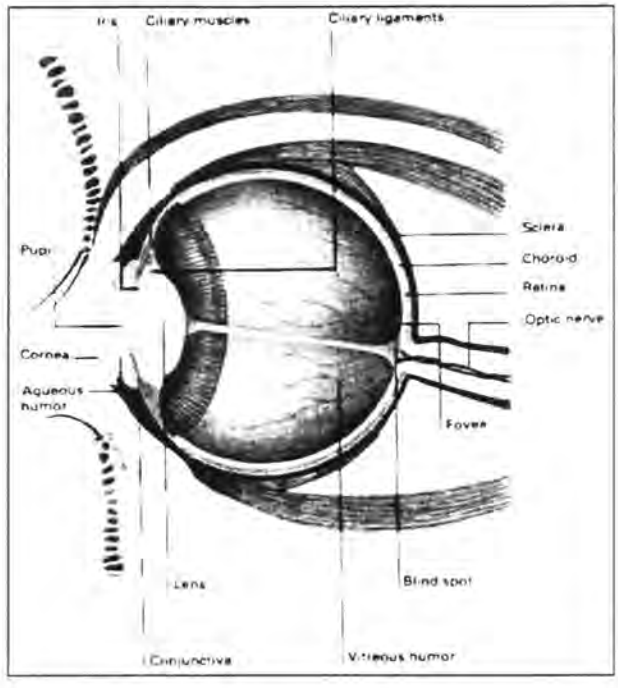


Figure 7 - Eye Anatomy
(Picture was taken from: www.aao.org/news/eyenet)

based on its maximum sensitivity to a certain light wavelength. Based on this classification the cone cells are differentiated to blue (B), green (G), and red (R) cone pigments. The cone cells are located at the fovea and occupies <2 ° of the visual field. Located at 10 ° from the fovea is the region where the cone cells and the rod cells are mixed that is useful for eyes accommodation from dark to lightness and vice versa. (MacDougall 2002)

Based on the understanding of the human eye and how it is influenced by the environment, several color systems have been developed. Some commonly used color systems are the Munsell, Ostwald and Opponent-colors/Hunter Lab/ CIE Lab color system.

The color systems are based on 3 dimensional color scale. The vertical scale is a measure of value or brightness or lightness-darkness (terms are based on the individual systems); and the horizontal scale is a measure of color (in this case the term used could be hue and chroma, hue and depth or yellowness-blueness and redness-greenness) (Hunter and Harold 1987). The location of the rod and cone cells within the retina is also used as the basis of the CIE standard colorimetric observer. Currently there are two accepted standard observer: 2° (proposed in 1931) and 10° (proposed in 1964) standard observer (MacDougall 2002). Since the type of light being used to illuminate the samples can affect the perceived color appearance, several standard illuminations have been proposed; such as Illuminant C and D65. The illumination standard is based on the temperature of the light source. The illuminant C has a light source temperature of 6,770 K and the D65 has a light source temperature of 6,500K. The commonly use illumination and standard observer is D65 and 10° standard observer. (MacDougall 2002)

Instrumental measurements to measure the appearance of various food products employ the use of uses direct color measurement or photoelectric measurement. Direct color measurement employs the use of color atlases and compared directly to the samples being measured. Therefore, in this method the color is measured both objectively and subjectively. The commonly used color atlases are the Munsell and Swedish NCS atlases. A Lovibond Tintometer is an instrument that is created to match the color of the sample using colored filters. The other type of color measurement instruments are the photoelectric instruments. There are two photoelectric instruments that have been developed for color measurement: the trichromatic colorimeters and the spectrophotometer system. An example of trichromatic colorimeters is an instrument developed by Hunter in 1940s. The instruments consist of a

light source, three wideband red, green, and blue filters to match 2 ° standard observer and CIE standard illuminant C. Currently more advanced version of Hunter are available as well as the handheld versions. In a spectrophotometer, instead of using a filter as in the colorimeters, it uses an integrating spheres and a diffraction grating to measures all spectrum of visible light (380-700 nm). The result is expressed as the ratio between the reflected light from the sample and a reference light standard and usually expressed as a percentage. In the modern spectrophotometer, an extra reference beam is used to minimized error and ensuring stability. With a spectrophotometer, the surface of the sample plays an important role in the results; i.e. a glossy surface vs. a rough surface. Extra care need to be done when using the spectrophotometer because the sphere is prone to be contaminated by the sample. (MacDougall 2002)

Instrumental measurement of aroma is available through the invention of gas chromatography. Chromatography is derived from the word "chroma" which means color and "graphein" which means to draw. The concept is derived when a black ink is blotted on a piece of water absorbing paper, and the paper separates the colors that are present in the black ink into its individual colors. This basic principle is the common principle that drives the development of various chromatography methods, such as thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC). Chromatography methods have the same basic components, which consists of mobile and stationary phase. The development of various chromatography methods has created a powerful tool of analysis. Chromatography can be use for the detection of vitamins, pesticides, sugars, fatty acids, amino acids, food additives, flavor and odor compounds. For flavor and odor analyses, gas chromatography is a commonly chosen method of analyses.

Capillary GC columns consist of fused silica tubing and the stationary phase. The tubing provides structural support and protection, whereas the stationary phase is responsible for sample separations. Fused silica tubing is made using high purity synthetic quartz (SiO_2) and covered with polyimide for protective coating; and to minimize sample interaction with the tubing, the inner surface is chemically treated. The polyamide coating has an upper temperature limit of 360 °C or short term at 380 °C (McNair 1998, Agilent 2002).

The stationary phase can be made using numerous materials; the most common ones are polysiloxanes and polyethylene glycol. The polysiloxanes has a siloxane backbone with each silicon atom having two functional groups. The functional groups attached to the backbone differentiate the uses of the column and the column properties. The most common functional groups are methyl, cyanopropyl, trifluoropropyl and phenyl. Some examples of this type of columns are the DB-1 and the DB-5 columns. The DB-1 column has all of the polyxsiloxanes backbone substituted with methyl groups. The DB-5 column contains (5%-Phenyl)-methylpolysiloxanes, which means that phenyl, substituted 5% of the backbone and the other 95% is substituted with methyl functional groups. The properties of DB-1 and DB-5 column are (1) non-polar, (2) excellent general-purpose column, (3) low bleed, (4) wide range of applications, (5) high temperature limits, (6) bonded and cross-linked, and (7) solvent rinsable. The DB-1 and DB 5 column can be used for semivolatiles, alkaloids, drugs, FAMEs, halogenated compounds, pesticides and herbicides. The different between these two columns are the degree of polarity of the stationary phase. (McNair 1998, Agilent 2002).

The polyethylene glycol stationary phase is usually non-substituted. These stationary phases are less stable, less robust and have lower temperature limits than the polysyloxanes

stationary phase, however, it has unique separation properties. An example of this type of column is the DB-Wax column. (McNair 1998, Agilent 2002).

Once the sample is ready to be injected, the sample is desorbed from the syringe by heating the injection port. During the injection of the sample, three methods of injection can be applied: split, splitless and in column injection mode. A split mode is used when only high concentration compounds are of interest and usually this method is used when doing compound quantification. A splitless mode is used when the compound of interest is available in trace amounts or the presence of excess amount of solvent. Using the splitless method, a large volume of samples is needed. With the split method, recommended samples should have narrow boiling point ranges, not thermally labile and are non-absorptive on high surface area supports. Whereas in the splitless method, recommended samples should not contain low boiling point compounds, thermally labile and samples which tend to adsorb on glass surfaces. (Varian manual 1989, McNair 1998).

Once the samples are separated in the column into each individual compounds, the samples are then detected by the detector. In G.C., the available detectors are Thermal Conductivity Detector (TCD), Flame Ionization Detectors (FID), Electron Capture Detector (ECD), Flame Photometric Detector (FPD) and Nitrogen-Phosphorous Detector (NPD). TCD has poor sensitivity and is used less in food applications. In food applications, FID is the most widely used detectors; this detector has good sensitivity for organic compounds. ECD is widely used in pesticides residue determination and has the ability to detect halogen, nitrogen, phosphorous, sulfur, metals or conjugated double bonds compounds. FPD and NPD are selective detectors for sulfur/phosphorous and phosphorous/nitrogen compounds detection (Reineccus 1994, McNair 1998). The responses that are created by the detector is

then converted into electrical response and with the help of an interface, the time needed for the compound concentration to reach the detector can be recorded as retention time (RT) and the intensity of the compound as peak height or area. The samples can be identified by comparing the RT of flavor standards or with the help of a mass spectrophotometer.

Gas chromatography in conjunction with sensory analyses of foods can provide important information about the flavor properties of a food product. Equilibrium headspace is defined by Wyllie, and others (1978) as "... the gaseous mixture surrounding a sample within a closed system at equilibrium". Headspace analysis is the analyses of the equilibrated headspace above the sample for its constituents. In studying the aroma of foods, headspace analysis is the method of choice because it measures compounds that are responsible for the aroma of foods. Another advantage is in the measurement of low boiling point flavor compounds; in most solvent extraction methods, these low boiling point flavor compounds would be lost during solvent removal. In addition, the presence of the high solvent peak may hide the some of the low boiling point volatile compounds. Equilibrium headspace sampling is a rapid and efficient method and the compounds of interests are less likely to be modified during sample collection (Maarse, 1991). Dynamic headspace analyses method resembles the flavor release during the ingestion of foods. During the ingestion of foods, the foods are macerated and aromas are released at a certain rate (samples). These aromas are then carried to the nose cavities and capture by the various nose receptors (detectors) and the signals are transmitted to the brain for interpretation.

The disadvantages of using headspace sampling are that volatiles are usually present in small samples concentrations and water vapor disrupts measurement. Unlike our nose receptors, which are able to detect very small quantities of volatile compounds, instrumental

analysis has not been able to match the sensitivity of the nose. Therefore, in headspace analyses, a pre-concentration method is needed in order for the G.C. to detect the compounds. An example of a pre-concentration method is through cryogenic focusing or using absorbent materials (ex. Charcoal, Tenax, or SPME). In cryogenic focusing, as the sample is injected, the sample is concentrated/freeze/liquidized into a thin layer within the column with the help of cryogenic coolant (ex. Liquid nitrogen, dry ice and acetone).

The most sensitive sensory identification instruments do not tell the kind of sensory stimulus that is perceived by humans. Therefore, instrumental measurements are more useful when combined with sensory evaluation methods for flavor determination. Sensory evaluation is defined by Lawless and Heymann (1998) as "a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing". A sensory food scientist must be able to master all the attributes stated by the definitions, which is to "evoke, measure, analyze and interpret", in order to gain useful sensory information from a product.

Sensory analysis testing methods can be classified into discrimination, descriptive attribute, difference and affective tests. A discrimination test is a test to test for differences between products; some examples are triangle test, duo-trio test, and sequential test. A descriptive test is a more detailed sensory test, where the product is analyze and measured for its specific sensory properties (eg. flavor, aroma, texture, and color). Some examples of descriptive test are flavor profile®, quantitative descriptive analyses (QDA®), texture profile (TPA), time intensity descriptive analyses, and sensory Spectrum®. Attribute difference tests are similar to discrimination test, except it is differentiating specific sensory attributes between products; some examples are paired comparison, simple ranking, and pair wise

ranking test. Affective test are tests to measure the degree of liking or disliking of a product, there are two types of this test acceptance and preference test. (Lawless and Heymann 1998, Meilgaard, Civille, Carr 1991)

Choosing the type of sensory test to be used depends on the type of information wanted. Some sensory tests cannot be combined with other tests. For example, preference test cannot be combined with descriptive test; the trained panelists in the descriptive test are too inform with the product and may bias their preference results on the product. A Descriptive analyses test is used when more specific sensory information are needed from a product. This test involves familiarizing a group of tester/panelists with the product and having them describe and measure the flavor intensity of the product. The group of panelists is lead by a panel leader, which will lead the panelists to be train with the sensory attributes of the product. The panelists can be screened based on their enthusiasm about the project, their commitment and their taste sensitivity. (Lawless and Heymann 1998, Meilgaard, Civille, Carr 1991)

The methods by which the panelists are trained are varied. The panelists can describe their own set of descriptive terms or they could be provided with a list of descriptive terms from which they could choose. Once the set of terms are chosen, the panel leader will then need to calibrate the panelists with the perceived sensory attributes. One way to achieve this is by providing the panelists with a set of standards, which the panelists need to familiarize and reach a consensus among the trained panelists on the intensity of the standards on a scale. In developing the descriptive terms, it is necessary to avoid redundant terms or terms that describes similar flavor. (Lawless and Heymann 1998, Meilgaard, Civille, Carr 1991)

Training is the most important part of descriptive analysis, because it is the step where the human panelists are being calibrated as an instrument for measurement. Depending on the method, the panel leader can be an active role during the training step, such as in the flavor profile method, or a passive role, such as in the QDA method. Over a long period of testing period, the panelists need to be recalibrated to maintain consistency. (Lawless and Heymann 1998, Meilgaard, Civille, Carr 1991)

The trained panelists then record their responses in a scale that could be analyzed statistically. An example of a commonly use scales is the line scale. The line scale is a 15 cm horizontal line anchored with the descriptive words generated by the panelists, located at both sides of the line placed 1 cm from the ends. The purpose of the anchors is to reduce the tendency of the panelists to use the center part of the line. Once the data are collected, they can be analyzed statistically using analysis of variance or multivariate statistical techniques. (Lawless and Heymann 1998, Meilgaard, Civille, Carr 1991)

CONCLUSION

Based on the previous studies discussed in this literature review, three research objectives are proposed for improvement in soymilk processing and new product development. The purpose of the first study is to optimize the use of soy flakes for soymilk production based upon solids, protein and flavor properties. The objective of the second study is to evaluate the differences in the flavor of soymilk made from lipoxygenase-free soy flakes and deodorized soymilk. The third study is to evaluate the efficiency of lipid removal from soymilk produced from whole soybeans and full fat soy flakes at three solid levels.

THE INFLUENCE OF TEMPERATURE AND REHYDRATION TIME ON THE PRODUCTION OF SOYMILK MADE FROM FULL FAT FLAKES

A paper to be submitted to the Journal of Food Science

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ABSTRACT

Traditionally, soymilk has been made with whole soybeans. However, there are other alternative raw ingredients for making soymilk, such as soy flour or full-fat soy flakes. The preferable soymilk flavor for U.S. markets is soymilk with little or no beany flavor. Modifying the process or using lipoxygenase-free soybeans can be used to achieve this desired trait. Most studies have looked at processes using whole soybeans. This project reports the optimized production of soymilk using full-fat soy flakes by modifying the water temperature and rehydration time soy flakes were used to make soymilk using a commercial Takai Soymilk machine. Three different percent solid soymilks (5, 8, and 12 °Brix) were prepared on separate days. On each processing day, soymilk was prepared by rehydrating flakes at 0, 5, and 10 minutes with 15, 50 and 85 °C water. Proximate and trypsin inhibitor analyses were done on the soymilk, okara, and soy flakes. Headspace analyses using gas chromatography was used to measure hexanal levels. Prior rehydration of soy flakes is necessary to prevent agglomeration during processing and to increase extractability. As the rehydration temperature increases from 15 to 50 to 85 °C, the hexanal concentration was reduced. The effect of heat on enzyme inactivation (measured by hexanal levels) is similar to

previous reports of the influence of heat during soymilk processing with whole beans. However, the rehydration times are shorter for the flakes (5 to 10 minutes) compared to whole beans (8 to 12 hours). The optimum rehydration conditions for a 5, 8 and 12 °Brix soymilk are 50 °C for 5 minutes, 85 °C for 5 minutes, and 85 °C for 10 minutes respectively.

Key words: soymilk, soy flakes, rehydration time, optimization, hexanal, soy protein

INTRODUCTION

Soymilk is made by a water extraction of soybeans. Its appearance is similar to cow's milk, and nutritionally superior to other legumes (Philip and Helen 1973; Steinkraus, and others 1978). In China, soybeans are called the "greater beans" due to their many health benefits (Simoons 1991). Soybean consumption has been proposed to be useful as an anti-carcinogenic activity, a cholesterol-lowering agent, to prevent calcium lost in bones, and as a phyto-estrogen source (Messina 1995). Soybeans are an inexpensive source of high quality protein. The high quality protein of soybeans is useful as a protein substitute or a supplement for people in developing countries (Messina 1995, Iwuoha and Umunnakwe 1996). In the U. S., the Food and Drug Administration (FDA) recently approved a health-labeling claim for products containing soy protein in October 1999. Daily consumption of 25 g (or 6.25 g per serving) of soy protein may reduce the risk of heart disease, due to the cholesterol lowering effects associated with soy protein (Henkel 2000).

Based on the market research conducted by SoyaTech in 1999, the sales of soy foods in the U.S. were projected to increase from $2.1 billion to $3.57 billion by 2002 (Soya Tech 1999). In 2002, Soya Tech released a new report showing that the soy food industry had

already reached $3.2 billion in sales by 2001. Soymilk sales alone in 2001 has reached $550 million and were projected to reach $1 billion in the coming three to five years (Soyatech 2002).

The acceptance of soy foods in the western market is affected in part due to its off-flavor (Wilson 1985; Feng and others 2001). The off-flavor of soy foods is caused by the activity of lipoxygenase enzyme (Wilkens 1967). Many methods have been developed in order to eliminate this off-flavor such as processing modifications or eliminating the lipoxygenase enzyme through genetic modifications (Wilson 1996, Kwok 1995). Processing modifications involve in rapid enzyme deactivation using heat, such as the Illinois method, hot grind method, rapid hydrothermal cooking (RHC) and pH adjustment methods (Kwok 1995; Liu 1997).

Most soymilk processes use whole soybeans as the starting material, however there are other raw forms of soybeans that can be used to make soymilk, such as soy flour or full fat soy flakes (Moizuddin and others 1999). Most studies have looked at optimizing the production of soymilk using whole soybeans. The use of soy flakes for soymilk production has not been studied extensively. The purpose of this study is to optimize the use of full fat soy flakes for soymilk production based on solids, protein and flavor properties.

MATERIALS AND METHODS

Materials

XLRB soy flakes were provided by MicroSoy Corporation (Jefferson, Ia, U.S.A.). XLRB is a blend of three IA high-protein identity preserve cultivars of soybeans blended for

soy flakes production. All chemicals used for analyses were reagent grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.).

Soymilk production

Soymilk was prepared at the Center for Crops Utilization Research (CCUR) pilot plant at Iowa State University (Ames, Ia, U.S.A.). The soymilk was produced using the Takai Automated Soymilk and Tofu System (Takai Tofu and Soymilk Equipment Inc. Japan), using the method of Moizuddin and others. (1999). The ratio of flakes to water used depends on the percent solids that were planned on each day of processing. On each processing day, only one level of solids was made. Soy flakes were rehydrated with a rotating paddle mixer using 25, 50 or 85 °C water. Timing was started at the first contact of flakes with water. At 0 minute rehydration, the flakes were placed directly into the cooking tank. Randomization was applied in the order of rehydration time and temperature to rehydrate the soy flakes.

After rehydration at each temperature was completed, the soymilk slurry was heated to 95 °C, the soymilk was then held at this temperature 7 minutes for 5° and 8 °brix soymilk and 10 minutes for the 12 °brix soymilk to allow pasteurization and reduction of trypsin inhibitor levels. The hot slurry was then pumped into a 120 mesh, horizontal rotating cylindrical screen to separate the insoluble solids. The remaining insoluble solids were roller-pressed over a 100-mesh screen drum. The soymilk was then homogenized at 7000 psi and collected in capped 2L plastic bottles. All soymilk samples were immediately sealed and refrigerated until analyses the following day.

The percent soymilk and okara yield of the process is calculated using the following equation:

$$\% \text{ Soymilk / Okara Yield} = \frac{Okara / Soymilk\ Wt.}{Water\ Wt. + Soy\ Flakes\ Wt.} \times 100\%$$

Gas chromatography

Headspace analysis (Wilson and others., 1992) was conducted for all soymilk samples using a Varian 3740 Gas Chromatography (GC) equipped with dual flame ionization detector (FID). The temperature of the injector and detector was held constant at 150 °C and 230 °C respectively. The initial column temperature is 50 °C. A DB5 fused glass silica column (J&W Scientific, Palo Alto, CA) was then programmed to heat at a rate of 10 °C/min until the column temperature reaches 230 °C and held at this maximum temperature for 3 minutes. Hydrogen and nitrogen gas flow rate was set at 30 ml/min and oxygen flow rate was set at 300 ml/min. The output from the gas chromatograph was recorded using a Hewlett Packard integrator model 3390A (Fisher Scientific, Fair Lawn, NJ). Hexanal peak was identified using a hexanal standard (Sigma Aldrich, St. Louis, MO)

Headspace analyses method

Samples for headspace analyses were prepared by placing twenty five grams of soymilk into a clear glass bottle and sealed with a Teflon coated septa and standard aluminum seal (Supelco, Inc., St. Louis, MO). Samples were incubated with a water bath at 37 °C with continuous stirring for at least 30 minutes. Liquid nitrogen was used to cryo-focus the headspace sample in the column. Two ml of headspace was sampled using a 5 ml Hamilton gas-tight syringe and injected at a rate of 1 ml/min. Duplicates of headspace analysis were done on each samples.

Color measurement

The color of soymilk was determined using a 5100 LabScan (Hunter Color Lab, Fairfax, VA). Soymilk samples were placed into 60 X 15 mm diameter plastic petri dishes (Fisher Scientific, Fair Lawn, NJ) and measurements were taken on the soymilk surface using a 0.25-inch sampling port under D65 illumination and 10 ° standard observer. Three measurements of each sample were done at three different sites on the surface of the soymilk.

Proximate analyses

Moisture was analyzed using AOAC method 925.19. (AOAC 2000). Crude protein was determined using the micro Kjeldahl AOAC methods 955.04(c) and 954.01(AOAC 2000), with Kjeltab TCT was used as the catalyst instead of HgO_2. Percent fat content of the samples was determined by Woodson-Tenent Laboratories Inc. (Des Moines, IA) using acid hydrolyses AOAC method 989.05 (AOAC 2000).

Statistical analyses

Data were analyzed using one-way analyses of variance (ANOVA) and differences among treatment means were analyzed by least significant difference (LSD). The optimum point of rehydration and hexanal peak were determined using response surface regression analysis. SAS System 8.02 (SAS Institute Inc., Cary, NC) statistical program was used for the statistical calculation.

RESULTS AND DISCUSSION

Processing data

The three different solid levels of soymilk were chosen because they represent the commonly used solid level in the production of various soy products. Five °Brix soymilk is commonly produced for the production of firm style tofu; 8 °Brix soymilk is the solid level commonly produced for commercial soymilk; and 12 °Brix soymilk is the solid level commonly produced for the production of base milk for transport efficiency. Traditional soybean processing method uses cold water for grinding and soaking the soybeans, whereas in the modified version of soymilk processing uses hot water grinding. Therefore, 15 °C rehydration was chosen to represent the traditional method; 85 °C rehydration was chosen to represent the hot water grinding, and 50 °C rehydration was chosen as the middle temperature point.

Table 1 shows the yield data collected during the processing stage. It is observed that the efficiency of the liquid extraction process decreased at 12 °Brix soymilk and at 85 °C rehydration. The decrease in the extraction efficiency at the higher solids level may be caused by the inability of the pump to transport the soymilk into the separator and therefore cause a decrease in soymilk yield.

The okara produced in 8 °Brix soymilk at 85 °C rehydration is higher than at 15 and 50 °C rehydration. These results suggested that the okara retains more water or some protein bodies was left in the soy flakes due to the higher rehydration temperature and therefore reduces the soymilk yield. During the 0 minute rehydration test, soy flake agglomerates were found and some remained inside the cooking tank with the most severe agglomeration found for 12 °Brix soymilk which would also reduce production efficiency.

Rehydration optimization of soymilk at the 5 °Brix Level

At 5 °Brix level, the % moisture, % protein and % fat of soymilk shown in Figures 1a, 1b and 1c show that there were no differences in the moisture and protein (Table 2) content between rehydration times at 15 and 50 °C (α<0.05). The % moisture of soymilk were found to be lower at the higher rehydration temperature (50 °C). There was no statistical significant difference in the % protein of soymilk at all rehydration times and temperatures.

However, there is a statistical difference (α<0.05) between the % moisture data of the okara (Table 2). The highest amount of moisture (or lowest amount of solids) in the okara was found at rehydration at 15 °C for 5 minutes and rehydration at 50 °C for 10 minutes. Lower % solids in the okara are an indication that the flakes had a better extraction at that time and temperature. However, the additional solids extracted were diluted in the larger volume of soymilk so that even though there was a numerically higher extraction at both temperatures it did not produce a statistical difference in the soymilk. There was no statistical difference (α<0.05) found in the okara % protein and % fat data (Table 2).

The results show that at 5 °Brix, the flakes to water ratio is low enough to allow proper rehydration of the flakes. The steam injection along with the agitator inside the cooking tank is sufficient to rehydrate the flakes without any additional rehydration time. However, the production of lower solids okara is desirable from the processing standpoint, because it reduces the amount of waste being generated.

Rehydration optimization of soymilk at the 8 °Brix Level

The results show that at 15 °C rehydration, there is no statistical difference (α<0.05) of soymilk solids, protein and fat content between rehydration times (Table 3). At 50 °C

rehydration, the highest % moisture (or lowest % solids) and protein content of soymilk was found at 0 minute rehydration (α<0.05). At this rehydration temperature, the analyses of the soymilk fat content did not show any statistical differences. At 85 °C rehydration, the lowest amount of % moisture (or highest amount of % solids) and % protein was found at 5 minutes rehydration (α<0.05), but there was no statistical difference (α<0.05) between 0 and 5 minutes rehydration. The fat content of soymilk showed no statistical differences (α<0.05) at all temperature and rehydration condition.

The % moisture, % protein and % fat of the okara (Table 3) showed that there is no statistical difference (α<0.05) between 15 °C and 50 °C rehydration across all rehydration times. Lower amount of moisture (or higher amount of solids), protein and fat content of the okara (α<0.05) were found at 85 °C rehydration. There was no difference in the moisture content of okara between rehydration times at 85 °C. However, the lowest amount of protein was found at 0 minute rehydration time at this temperature.

When the amount of each nutrient content (water, protein, fat, carbohydrate and ash) of okara are calculated, the okara from 85 °C rehydration retains more water, protein, and fat (Fig. 1a, 1b, and 1c). In theory, the more solids, protein and fat components left in okara, the fewer the amounts of solids, protein and fat that go into the soymilk. However, within the same rehydration time, the % protein, % solid and % fat of soymilk does not show a significant difference between them. Since, the okara retains more moisture at 85 °C rehydration, the amount of water that went into the soymilk is reduced as well, and therefore concentrating the soymilk showing higher % composition. These results confirm previous findings by Johnson and Snyder (1978) that blanching of soybeans with temperature greater than 85 °C would cause poorer protein extraction of soybeans. The higher rehydration

temperature heat fixed the protein within the cell; in addition, along with the protein trapping, water and fat globules are trapped as well.

In order to find the optimum rehydration point statistically, the data were analyzed with a response surface regression analysis. The analysis indicates that optimum solid and protein extraction can be achieved at 30 °C rehydration for 6 minutes and optimum fat extraction can be achieved at 40 °C rehydration for 6 minutes (Fig. 2a, 2b, and 2c).

Rehydration optimization of soymilk at the 12 °Brix Level

There were no statistical differences ($\alpha < 0.05$) in % moisture, % protein and % fat of soymilk between rehydration times at 15 °C (Table 4). At 50 °C rehydration, there is a statistical difference ($\alpha < 0.05$) in % moisture, % protein and % fat of soymilk between 0 minute rehydration and the 5 to 15 minutes rehydration; with the 0 minute having the highest amount of moisture (or the lowest amount of solids) and the lowest amount protein. At 85 °C rehydration, statistical differences ($\alpha < 0.05$) of % moisture of soymilk were found between rehydration times. The % moisture of soymilk decreases (or % solid of soymilk increases) as the rehydration time increases (from 5 – 15 minutes). There were no statistical differences ($\alpha < 0.05$) in % protein of soymilk at this rehydration temperature.

At 15 °C rehydration, there is no statistical difference ($\alpha < 0.05$) in the % moisture, % protein and %fat content of okara (Table 4). At 50 °C rehydration, 0 and 5 minutes rehydration has lower moisture (or higher solids) and lower fat content than 10 and 15 minutes rehydration ($\alpha < 0.05$), with no statistical difference ($\alpha < 0.05$) in the % protein. At 85 °C rehydration, there is no difference in the % moisture content of okara. However, the % protein of okara decreases over time at this rehydration temperature. The % protein of okara

at 85 °C rehydration was higher ($\alpha < 0.05$) than rehydration at 15 and 50 °C, which supports the previous data of protein fixation in the soy flakes cell.

At 0-minute rehydration the flakes were noted to agglomerate severely during processing, which would reduces the extraction efficiency. The decrease in extraction efficiency seen in 8 °Brix level was not found at this solid level. These results suggest that at 12 ° Brix, the flakes to water ratio is high enough to protect the flakes from severe protein denaturation and additional times allow better solids and protein extraction. The response surface regression analysis indicates that optimum solid and protein extraction can be achieved at 55 °C rehydration for 10 minutes and optimum fat extraction can be achieved at 40 °C rehydration for 10 minutes (Fig. 3a, 3b, and 3c).

GC and color analyses results

Figure 4a, 4b and 4c show hexanal concentration (shown as peak area) for 5, 8, and 12 °Brix soymilk. At 5 °Brix (Fig. 4a), there is no statistical difference ($\alpha < 0.05$) between rehydration temperatures at the same rehydration time. However, 5 minutes of rehydration produced a significantly higher hexanal level at 15 °C.

At 8 °Brix (Fig. 4b), the hexanal content at 15 °C increases over time ($\alpha < 0.05$), whereas at 50 °C the hexanal peak increases and then stays the same after 5 minutes of rehydration ($\alpha < 0.05$). The combination of enzyme activity and enzyme inactivation at 50 °C overall produces a lower level than 15 °C. At 85 °C the hexanal peak remains constant across rehydration times, however the values are lower than at 15 or 50 °C ($\alpha < 0.05$). The results support previous studies by Wilkens and others. (1967), where the development of off-

flavors was prevented by the blanching step. At 15 °C rehydration, the enzyme was not inactivated and therefore continues to develop more hexanal over time.

At 12 °Brix (Fig. 4c), temperature and time effects were noted for the hexanal content of soymilk. At 15 °C, the highest hexanal content occurs at 5 minutes rehydration, whereas at 50 °C the highest hexanal content occurs at 15 minutes rehydration. Across temperature within the same rehydration time, statistical differences were found at 5 and 15 minutes of rehydration. The trend that was found at these rehydration times was that lower hexanal peaks were found at higher rehydration temperature ($\alpha<0.05$). The lower hexanal content at higher temperature (85°C) was similar to the 8 ° Brix results. The response surface regression analysis does not indicate which time and temperature combination would produce the least amount of hexanal being generated at 8 and 12 °brix soymilk. The analyses indicates that the least amount of hexanal would be produced at 0 minute and at 0 °C temperature, which would be reasonable theoretically because the enzyme reaction is prevented by increasing its energy of activation and reducing the reaction time (Fig. 5a and 5b). However, it is not possible to produce soymilk under that condition.

The Hunter color measurement of the soymilk does not show any significant differences between the treatments of the soymilk (data not shown).

CONCLUSION

Similar to many powdered raw material alternatives to whole soybeans, immediate introduction to the water would cause the powder to agglomerate. Soy flakes are no difference than its powder counterparts. Initial rehydration step is needed in order to process soy flakes, unless the equipment is able to break the agglomeration of the product.

High temperature rehydration, similar to previous study with whole soybeans, would causes protein denaturation, which would heat fixed the protein within the soy flakes. Protein fixation in soy flakes is followed by fat and water fixation as well. Based on the GC data, flavor improvement can be achieved through this high temperature rehydration (pre blanching step) with no significant color changes to the soymilk. Based on this study, optimum condition for flavor improvement and extraction of soy flakes processing for 5, 8 and 12 °Brix soymilk production can be achieved by rehydration 5 minutes at 50 °C, 5 minute at 85 °C and 10 minute at 85 °C respectively.

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TABLES AND GRAPHS

Table 1 - Soymilk processing data summary

Processing Type	Rehydration Time (Min)	Water Temp (°C)	Flake:Water Ratio	°Brix	Soymilk Yield (%)	Okara Yield (%)
5 °Brix	0	15	1:13	5.33 ± 0.23 ^{a}	88.75 ± 3.13 ^{a}	4.54 ± 0.43 ^{a}
	5			5.10 ± 0.10 ^{a}	90.45 ± 1.35 ^{a}	4.46 ± 0.40 ^{a}
	10			5.27 ± 0.42 ^{a}	89.37 ± 5.95 ^{a}	4.56 ± 0.38 ^{a}
5 °Brix	0	50	1:13	5.03 ± 0.06 ^{a}	87.10 ± 3.93 ^{a}	4.80 ± 0.64 ^{a}
	5			5.33 ± 0.58 ^{a}	87.10 ± 1.55 ^{a}	4.44 ± 0.30 ^{a}
	10			5.30 ± 0.10 ^{a}	87.67 ± 2.04 ^{a}	4.43 ± 0.56 ^{a}
8 °Brix	0	15	1:8	8.03 ± 0.49 ^{abc}	87.49 ± 3.68 ^{a}	8.19 ± 0.61 ^{c}
	5			8.10 ± 0.66 ^{ab}	87.04 ± 2.01 ^{ab}	8.02 ± 0.84 ^{c}
	10			8.15 ± 0.35 ^{ab}	86.94 *	9.16 ± 2.97 ^{bc}
8 °Brix	0	50	1:8	7.27 ± 0.92 ^{abc}	80.32 ± 2.75 ^{ab}	7.60 ± 2.39 ^{bc}
	5			8.47 ± 0.40 ^{a}	81.16 ± 1.21 ^{b}	8.13 ± 0.79 ^{c}
	10			8.33 ± 0.85 ^{ab}	82.79 ± 1.43 ^{ab}	8.11 ± 0.58 ^{c}
8 °Brix	0	85	1:8	6.30 ± 0.14 ^{d}	65.36 ± 5.98 ^{c}	12.34 ± 1.50 ^{a}
	5			7.00 ± 0.28 ^{dc}	71.22 ± 2.52 ^{c}	10.68 ± 0.79 ^{ab}
	10			7.30 ± 0.42 ^{bcd}	70.18 ± 2.31 ^{c}	10.08 ± 1.19 ^{abc}
12 °Brix	0	15	1:5	10.93 ± 0.12 ^{abcd}	76.25 ± 1.47 ^{a}	12.35 ± 2.02 ^{a}
	5			11.83 ± 0.80 ^{abc}	74.23 ± 1.28 ^{a}	12.45 ± 0.98 ^{a}
	10			11.87 ± 0.42 ^{abc}	76.20 ± 0.96 ^{a}	12.44 ± 1.18 ^{a}
12 °Brix	0	50	1:5	10.17 ± 1.67 ^{d}	71.79 ± 1.23 ^{ab}	12.01 ± 0.75 ^{a}
	5			11.83 ± 0.38 ^{abc}	73.10 ± 1.21 ^{ab}	13.06 ± 0.66 ^{a}
	10			12.13 ± 0.42 ^{ab}	73.67 ± 2.26 ^{a}	12.86 ± 0.49 ^{a}
	15			10.70 ± 0.71 ^{cd}	68.34 ± 15.63 ^{abc}	14.03 ± 0.46 ^{a}
12 °Brix	5	85	1:5	10.80 ± 0.28 ^{bcd}	59.40 *	7.88 ± 11.15 ^{a}
	10			12.00 ± 0.00 ^{abc}	57.15 *	7.39 ± 10.45 ^{a}
	15			12.3 ± 0.14 ^{a}	62.57 ± 8.46 ^{bcd}	13.80 ± 0.20 ^{a}

* No standard deviation can be calculated.

Results are expressed as means ± SD (n = 3).

$^{a-d}$ Means with the same code within the same column and solid level are not significantly different ($\alpha<0.05$).

Table 2 - % Moisture, % protein, % fat, % carbohydrate and ash of soymilk and okara at 5 °Brix soymilk level

Soymilk

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	94.79 ± 0.18^{bc}	2.52 ± 0.07^{a}	1.33 ± 0.05^{a}	1.22 ± 0.15^{a}
5		94.77 ± 0.10^{bc}	2.54 ± 0.12^{a}	1.39 ± 0.10^{a}	1.15 ± 0.06^{a}
10		94.88 ± 0.02^{c}	2.46 ± 0.15^{a}	1.34 ± 0.15^{a}	1.25 ± 0.07^{a}
0	50	95.03 ± 0.36^{ab}	2.44 ± 0.32^{a}	1.39 ± 0.07^{a}	1.15 ± 0.07^{a}
5		94.63 ± 0.13^{a}	2.59 ± 0.19^{a}	1.45 ± 0.05^{a}	1.33 ± 0.07^{a}
10		94.92 ± 0.37^{a}	2.38 ± 0.26^{a}	1.48 ± 0.09^{a}	1.23 ± 0.23^{a}

Okara

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	77.34 ± 0.90^{ab}	5.06 ± 0.80^{a}	3.14 ±0.35^{a}	14.42 ± 1.33^{a}
5		78.49 ± 1.59^{c}	5.13 ± 0.54^{a}	2.95 ± 0.42^{a}	14.53 ± 3.81^{a}
10		77.28 ± 0.89^{ab}	5.60 ± 1.35^{a}	3.40 ± 0.16^{a}	13.88 ± 1.66^{a}
0	50	76.28 ± 1.05^{a}	6.00 ± 0.64^{a}	3.49 ± 0.31^{a}	14.23 ± 1.35^{a}
5		76.59 ± 1.42^{a}	5.48 ± 1.09^{a}	3.29 ± 0.53^{a}	14.63 ± 2.10^{a}
10		77.88 ± 0.58^{bc}	5.05 ± 0.70^{a}	3.25 ± 0.41^{a}	13.82 ± 1.02^{a}

Results are expressed as means ± SD (n = 3).

$^{a-c}$ Means with the same letter code within the same column in the soymilk or okara table are not significantly different (α<0.05).

Table 3 - % Moisture, % protein, % fat, % carbohydrate and ash of soymilk and okara at 8 °Brix soymilk level

Soymilk

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	91.96 ± 0.15^{ab}	3.81 ± 0.15^{ab}	2.15 ± 0.03^{a}	2.06 ± 0.07^{a}
5		91.97 ± 0.22^{ab}	3.81 ± 0.28^{a}	2.19 ± 0.06^{a}	2.04 ± 0.11^{a}
10		91.92 ± 0.14^{ab}	3.75 ± 0.15^{ab}	2.13 ± 0.06^{a}	2.12 ± 0.01^{a}
0	50	92.67 ± 1.40^{c}	3.49 ± 0.58^{c}	1.99 ± 0.43^{a}	1.72 ± 0.45^{a}
5		91.57 ± 0.18^{a}	3.99 ± 0.29^{a}	2.30 ± 0.02^{a}	2.14 ± 0.14^{a}
10		91.71 ± 0.13^{a}	3.96 ± 0.27^{ab}	2.27 ± 0.04^{a}	2.06 ± 0.15^{a}
0	85	92.38 ± 0.12^{bc}	3.41 ± 0.16^{bc}	2.18 ± 0.02^{a}	2.03 ± 0.06^{a}
5		91.84 ± 0.52^{ab}	3.80 ± 0.29^{ab}	2.23 ± 0.26^{a}	2.13 ± 0.03^{a}
10		93.22 ± 1.37^{d}	3.11 ± 0.55^{c}	1.94 ± 0.25^{a}	1.61 ± 0.38^{a}

Okara

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	75.91 ± 1.09^{c}	5.41 ± 0.47^{c}	3.45 ± 0.22^{de}	16.26 ± 0.83^{a}
5		75.70 ± 1.20^{c}	5.53 ± 0.52^{c}	3.28 ± 0.21^{de}	16.35 ± 0.65^{a}
10		75.92 ± 0.62^{c}	5.64 ± 0.46^{c}	3.20 ± 0.00^{e}	16.11 ± 0.84^{a}
0	50	75.19 ± 0.89^{bc}	6.42 ± 0.22^{c}	3.93 ± 0.13^{dc}	17.63 ± 4.64^{a}
5		75.57 ± 1.14^{c}	5.62 ± 0.07^{c}	3.44 ± 0.21^{de}	18.13 ± 3.70^{a}
10		75.63 ± 0.61^{c}	5.49 ± 0.14^{c}	3.55 ± 0.20^{de}	16.26 ± 1.58^{a}
0	85	74.69 ± 0.47^{ab}	7.52 ± 3.28^{b}	4.58 ± 0.38^{bc}	13.21 ± 2.43^{a}
5		73.88 ± 0.41^{a}	9.06 ± 1.04^{a}	4.86 ± 0.76^{ab}	12.20 ± 2.20^{a}
10		73.88 ± 0.29^{a}	8.32 ± 0.31^{ab}	5.46 ± 0.10^{a}	12.25 ± 0.08^{a}

Results are expressed as means ± SD (n = 3).

[a-e] Means with the same letter code within the same column in the soymilk or okara table are not significantly different ($\alpha<0.05$).

Table 4 - % Moisture, % protein, % fat, % carbohydrate and ash of soymilk and okara at 12 °Brix soymilk level

Soymilk

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	88.58 ± 0.15^{bc}	4.98 ± 0.12^{e}	3.14 ± 0.18^{ab}	3.05 ± 0.28^{a}
5		88.40 ± 0.06^{abc}	5.26 ± 0.25^{cde}	3.28 ± 0.10^{a}	3.08 ± 0.20^{a}
10		88.43 ± 0.11^{abc}	5.24 ± 0.15^{bcde}	3.23 ± 0.08^{a}	3.04 ± 0.05^{a}
0	50	89.28 ± 0.78^{d}	4.94 ± 0.43^{de}	3.00 ± 0.23^{bc}	2.78 ± 0.25^{a}
5		88.40 ± 0.48^{abc}	5.44 ± 0.22^{ab}	3.29 ± 0.15^{a}	3.31 ± 0.42^{a}
10		87.99 ± 0.27^{ab}	5.61 ± 0.09^{a}	3.29 ± 0.16^{ab}	3.09 ± 0.12^{a}
15		88.34 ± 0.01^{ab}	5.35 ± 0.07^{abcd}	3.12 ± 0.01^{a}	3.25 ± 0.02^{a}
5	85	89.25 ± 1.31^{d}	5.09 ± 0.50^{cde}	3.29 ± 0.01^{a}	3.09 ± 0.20^{a}
10		88.69 ± 0.48^{c}	5.30 ± 0.07^{abcde}	2.84 ± 0.01^{c}	3.18 ± 0.56^{a}
15		88.12 ± 0.40^{a}	5.46 ± 0.27^{abcd}	3.32 ± 0.05^{a}	3.16 ± 0.10^{a}

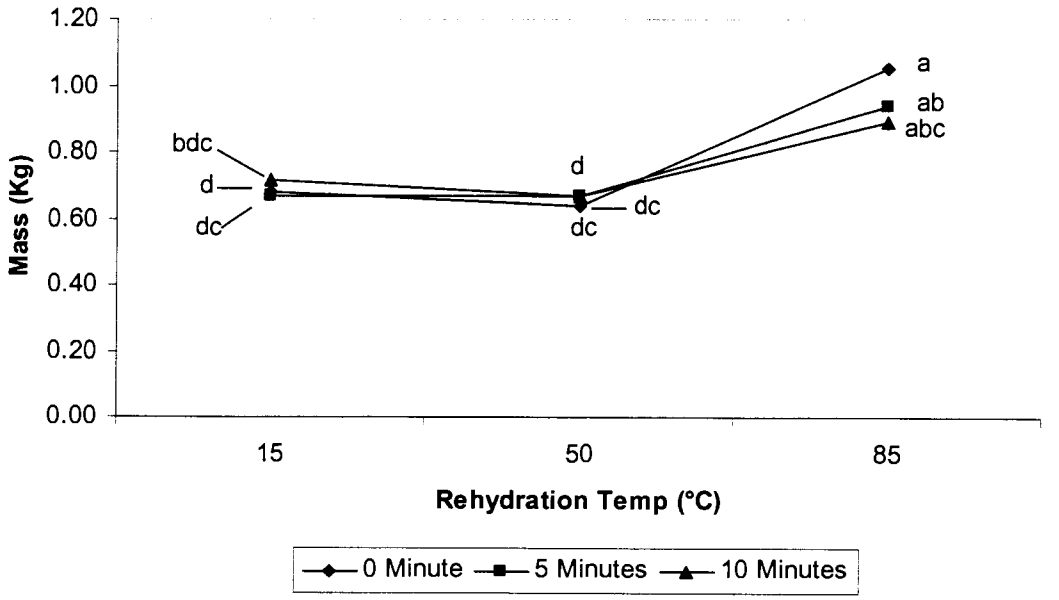
Okara

Rehydration Time (Min)	Water Temp (°C)	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate and Ash (%)
0	15	74.56 ± 1.27^{a}	7.08 ± 0.20^{cd}	3.94 ± 0.41^{cd}	13.68 ± 1.44^{a}
5		75.87 ± 0.86^{abc}	6.27 ± 0.57^{d}	3.40 ± 0.21^{d}	13.93 ± 1.04^{a}
10		75.95 ± 0.89^{abc}	6.31 ± 0.19^{d}	3.53 ± 0.18^{d}	14.21 ± 1.12^{a}
0	50	74.67 ± 0.67^{ab}	7.16 ± 0.89^{cd}	4.13 ± 0.15^{bc}	14.04 ± 0.66^{a}
5		76.78 ± 2.44^{abc}	6.67 ± 1.07^{cd}	3.73 ± 0.25^{cd}	17.25 ± 4.67^{a}
10		76.15 ± 0.92^{d}	5.95 ± 0.19^{d}	3.52 ± 0.38^{d}	14.38 ± 1.25^{a}
15		77.08 ± 0.54^{dc}	6.81 ± 1.26^{bcd}	3.72 ± 0.21^{d}	12.04 ± 0.01^{a}
5	85	83.09*	8.03 ± 2.93^{a}	3.73*	12.83*
10		79.89*	8.65 ± 1.53^{ab}	4.76 ± 0.21^{a}	10.49 ± 0.83^{a}
15		80.39 ± 0.84^{abc}	7.91 ± 0.45^{bc}	4.54 ± 0.13^{ab}	11.32 ± 0.18a

* No standard deviation can be calculated. Results are expressed as means ± SD (n = 3).

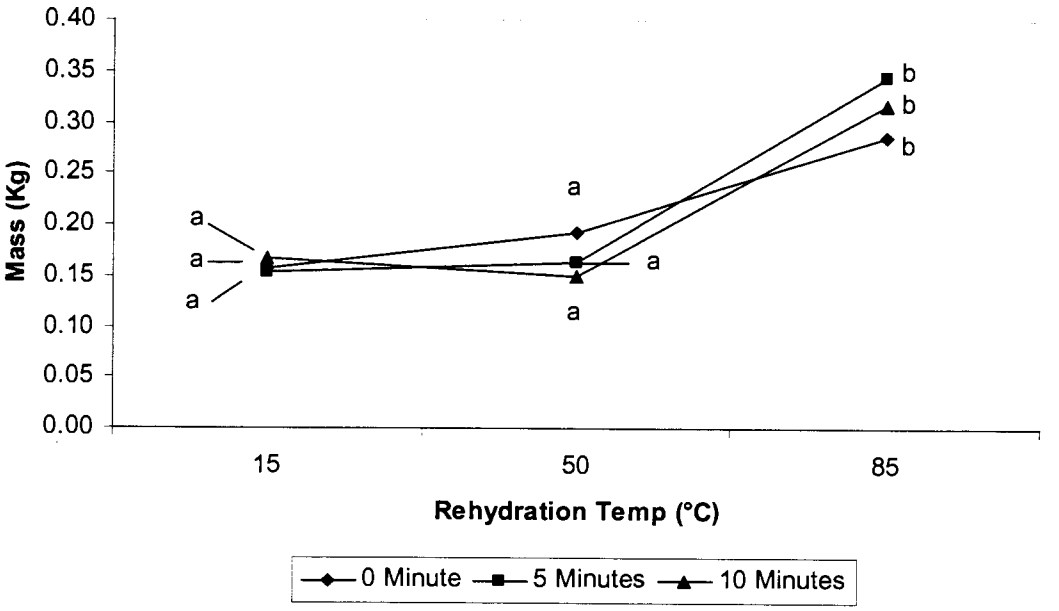
[a-e] Means with the same letter code within the same column in the soymilk or okara table are not significantly different (α<0.05).

Figure 1a - Solid content of Okara at 8 °Brix



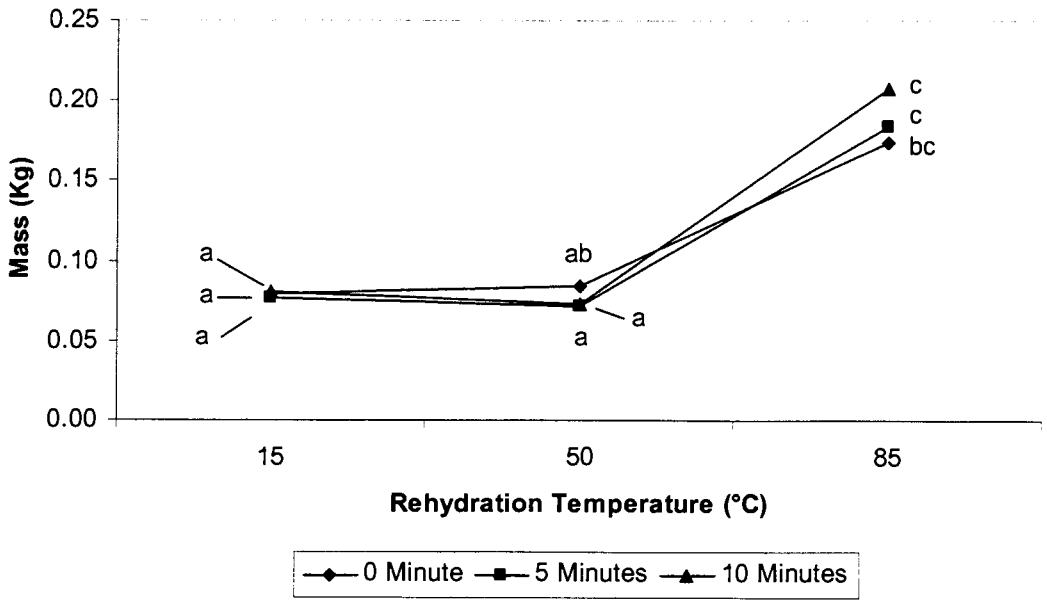
[a-d] Means with the same letter code are not significantly different ($\alpha < 0.05$).

Figure 1b - Protein content of Okara at 8 °Brix



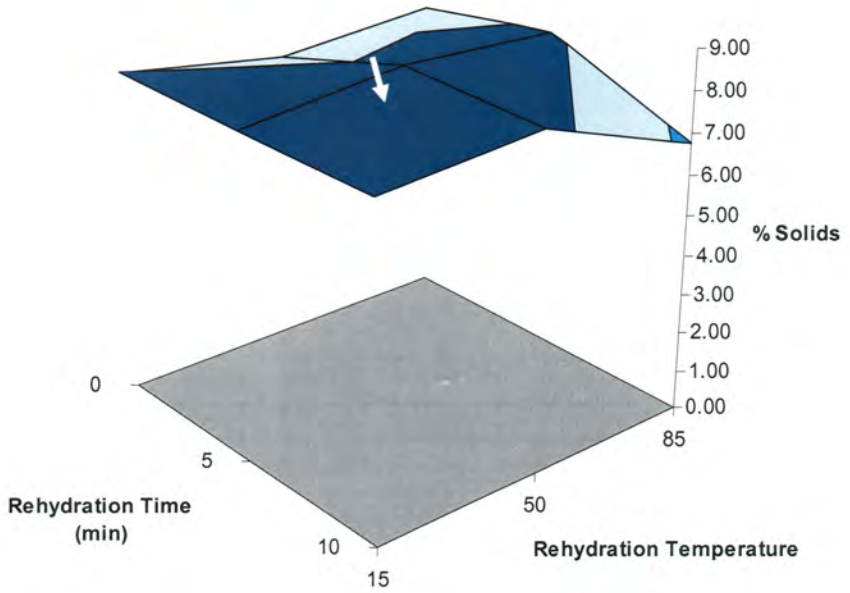
[a-b] Means with the same letter code are not significantly different ($\alpha<0.05$).

Figure 1c - Fat content of Okara at 8 °Brix



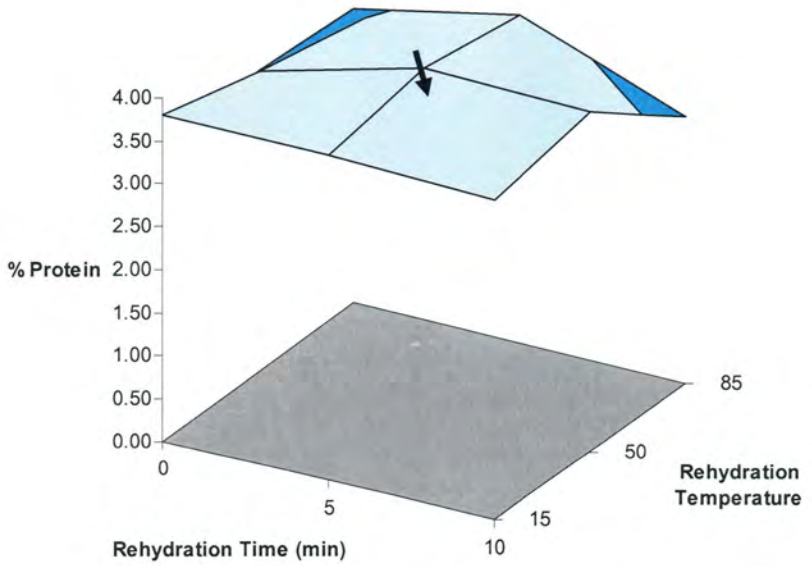
[a-c] Means with the same letter code are not significantly different (α<0.05).

Figure 2a - The response surface regression plot of % solid content of soymilk at 8 °Brix



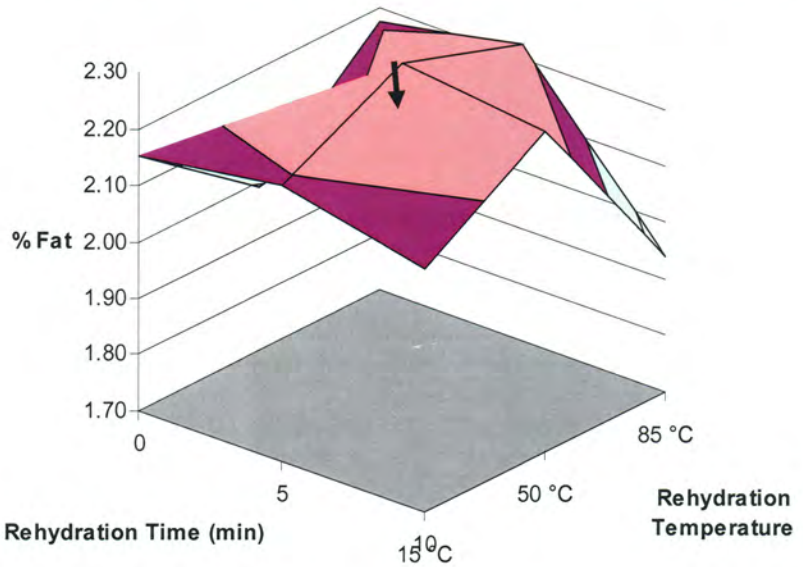
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 2b - The response surface regression plot of % protein content of soymilk at 8 °Brix



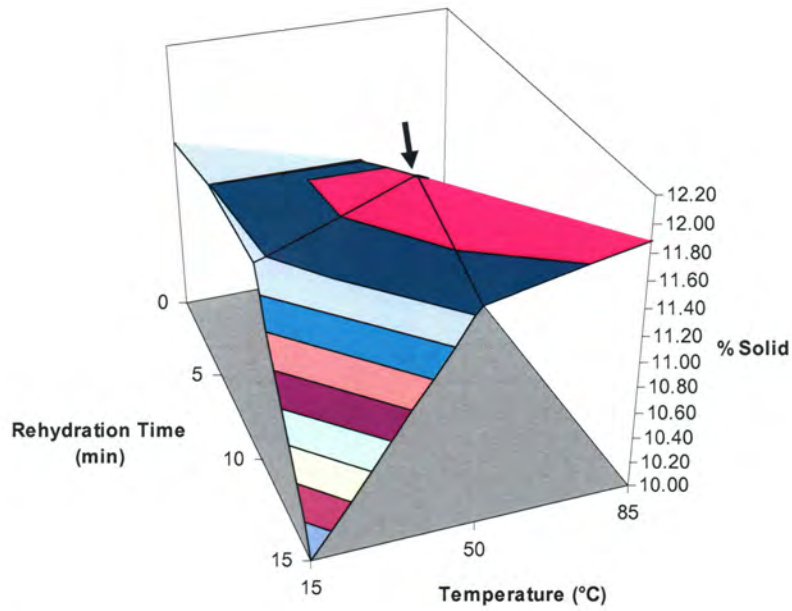
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 2c - The response surface regression plot of % fat of soymilk at 8 °Brix



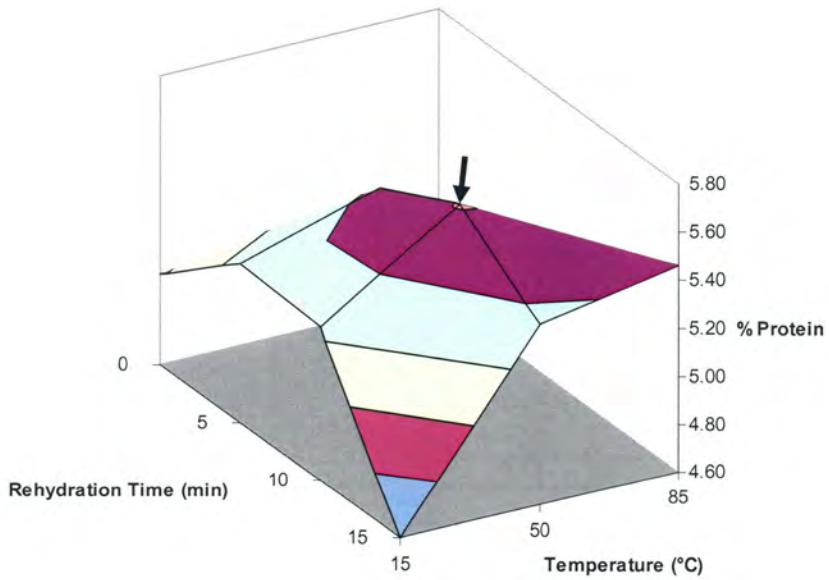
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 3a - The response surface regression plot of % solid content of soymilk at 12 °Brix



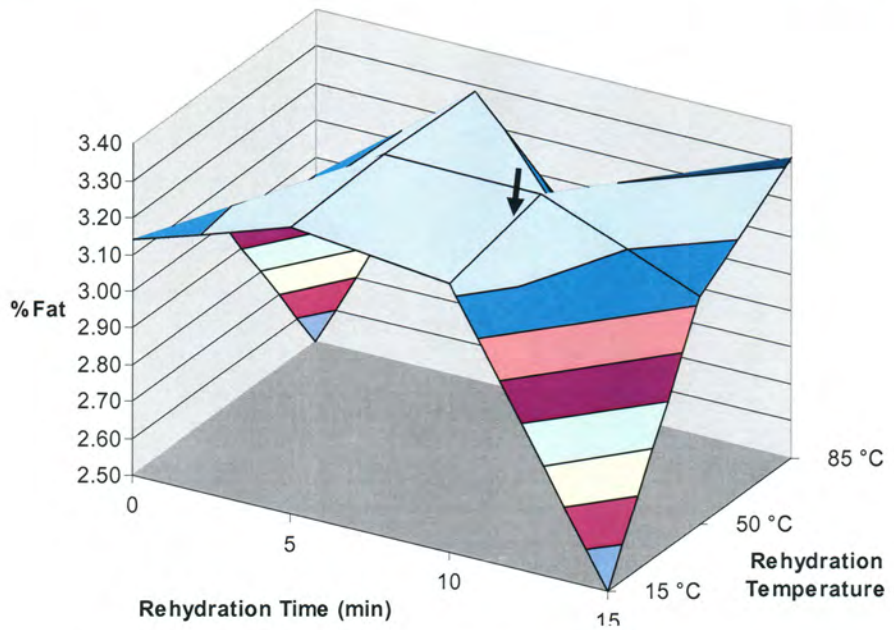
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 3b - The response surface regression plot of % protein content of soymilk at 12 °Brix



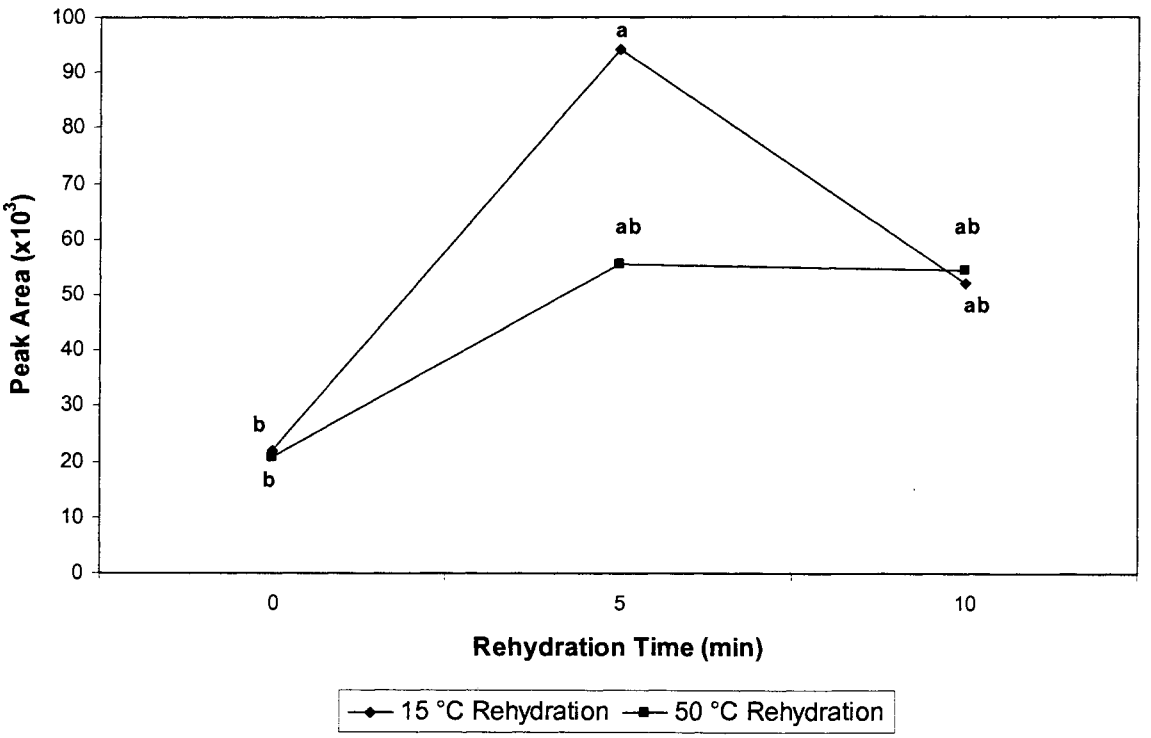
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 3c - The response surface regression plot of % fat of soymilk at 12 °Brix



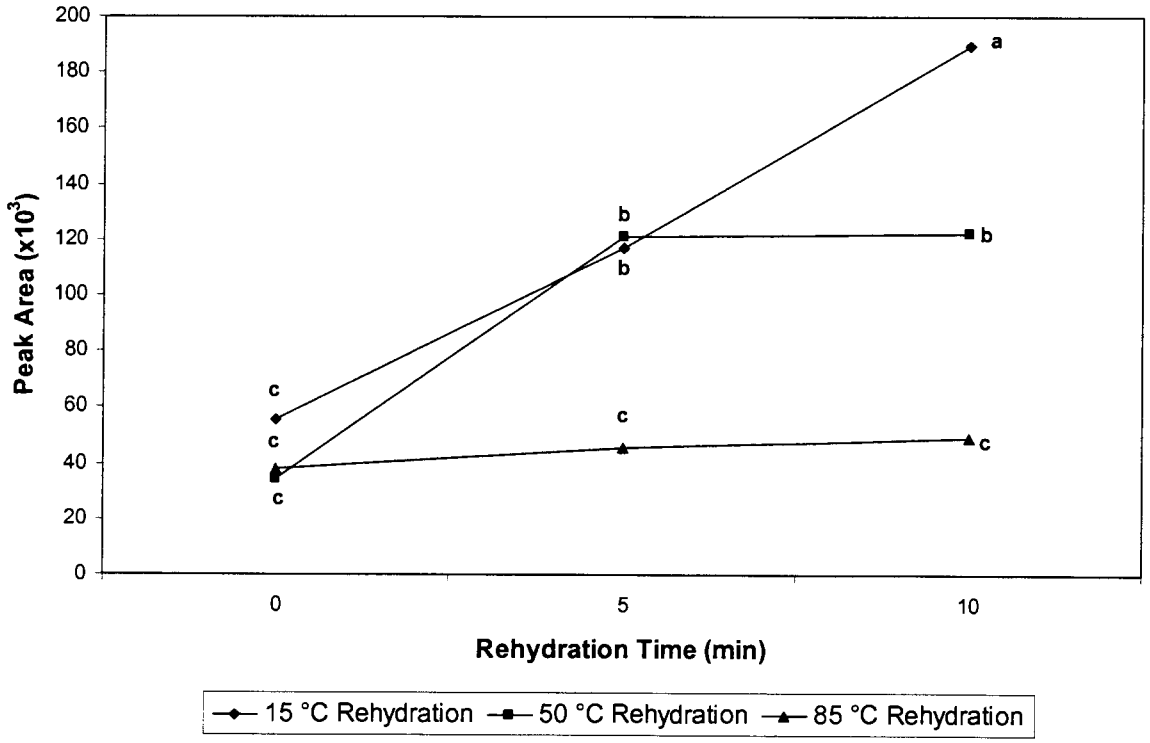
Arrows indicates the point of optimum extraction based on the response surface regression analyses.

Figure 4a - Hexanal concentration at 5 °Brix soymilk level



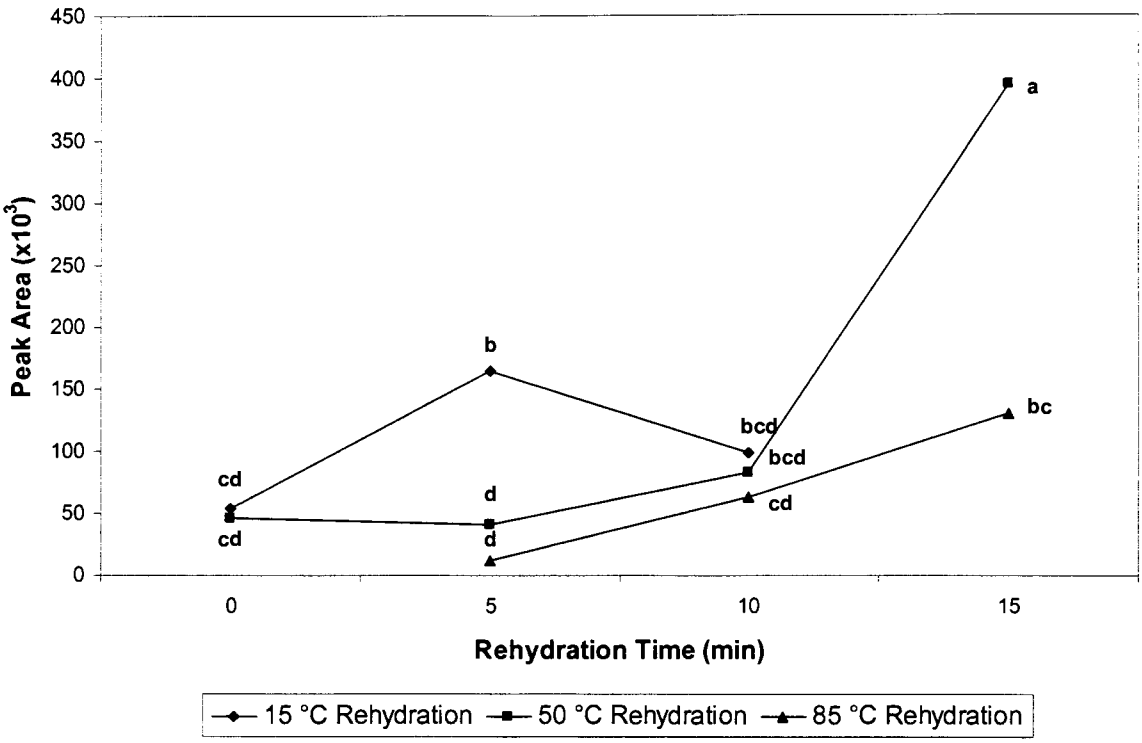
[a-b] Means with the same letter code are not significantly different (α<0.05).

Figure 4b - Hexanal concentration at 8 °Brix soymilk level



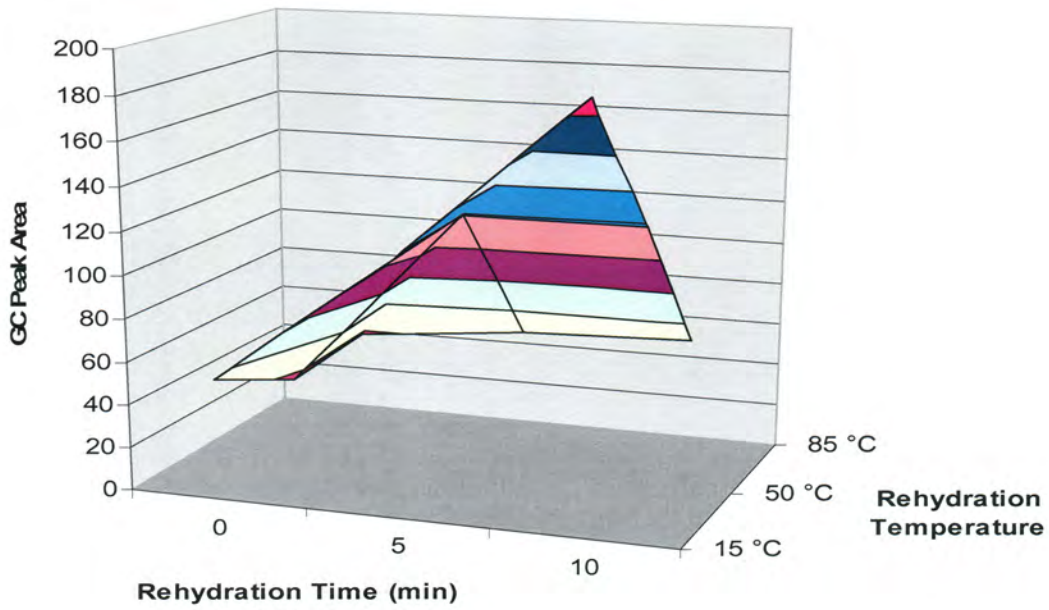
[a-c] Means with the same letter code are not significantly different (α<0.05).

Figure 4c - Hexanal Concentration at 12 °Brix Soymilk Level



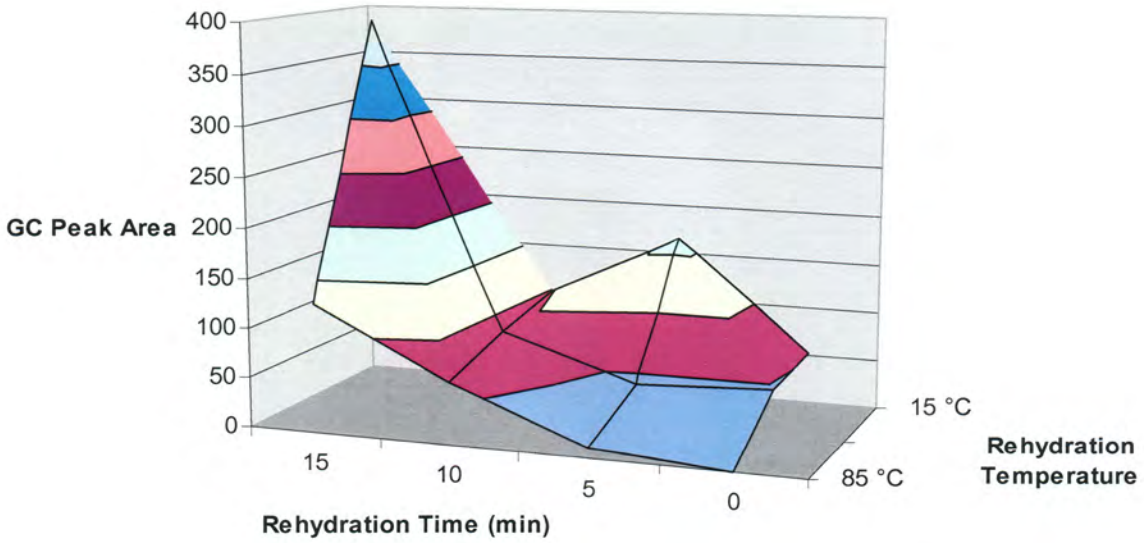
[a-d] Means with the same letter code are not significantly different (α<0.05).

Figure 5a - The response surface regression plot of hexanal peak area of soymilk at 8 °Brix



Arrows indicates the point of lowest level of hexanal content based on the response surface regression analyses.

Figure 5b - The response surface regression plot of hexanal peak area of soymilk at 12 °Brix



Arrows indicates the point of lowest level of hexanal content based on the response surface regression analyses.

COMPARISON OF LIPOXYGENASE FREE SOYMILK WITH DEODORIZED SOYMILK

A paper to be submitted to the Journal of Food Science

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ABSTRACT

The differences in the flavor of soymilk made from lipoxygenase-free (triple null) soy flakes and deodorized high protein soy flake (HPSF) soymilk were evaluated. Soymilk at 8 and 12 °Brix were produced and deodorized. The sensory characteristics of the soymilk were analyzed using instrumental methods (GC and Hunter color measurement) and trained sensory panelists. Hexanal data showed differences between undeodorized HPSF in contrast to triple null soymilk and no differences between deodorized HPSF in contrast to deodorized triple null. The panelists could not differentiate between the beany, cereal, and painty flavors. However, the panelists responded that the overall aroma of deodorized 8 °Brix triple null and HPSF soymilk are lower than the undeodorized triple null and HPSF soymilk. The triple null soymilk was perceived to be more bitter than the HPSF soymilk by the sensory panel due to oxidation on the triple null soy flakes. This oxidation may produce other aroma that was not analyze using the GC but noticed by the panelists. The sensory evaluation results did show that the deodorizer was able to reduce the aroma in HPSF soymilk so it would be similar to triple null soymilk at 8 °Brix level.

Key words: soymilk, deodorizer, lipoxygenase-free, triple null.

INTRODUCTION

The problem of 'off' flavors that is found in soybeans has been known to the western world for a while. Berczeller, in 1924, described the flavor of soybean as "evil" tasting. Soybeans contain flavor that is described as beany, grassy, green, painty, astringent, and bitter (Wolf 1975 and King and others 2001). In China and some eastern Asian countries, these flavors are favorable in soy foods. The "off"-flavors of soybeans remain the main factors in limiting the utilization of soy for food, despite its known health benefits. The "off"-flavors of soybeans are caused by breakdown of the products of an oxidase enzyme called lipoxygenase. Soybeans are known to contain the highest concentration of this enzyme in the plant kingdom (Axelrod 1974). The presence of high amounts of unsaturated fatty acids in soybeans makes them perfect substrate for the lipoxygenase enzyme to react. Any time the soybean cells are ruptured, lipoxygenase work almost instantaneously. Once the fatty acids are oxidized, the unique flavors of soybeans are produced.

Based on the market research conducted by SoyaTech in 1999, the sales of soy foods in the U.S. were projected to increase from $2.1 billion to $3.57 billion by 2002 (Soya Tech 1999). In 2002, Soya Tech released a new report showing that the soy food industry had already reached $3.2 billion in sales by 2001. Soymilk sales alone in 2001 have reached $550 million and were projected to reach $1 billion in the coming three to five years (Soyatech 2002). This growth in sales is partly contributed due to the advancement in soy foods flavor improvement. Flavor improvement can be done through process modifications, flavoring additives and breeding techniques. Process modifications, such as the Cornell and rapid hydration hydrothermal cooking (RHHTC), have been developed to achieve immediate inactivation of the lipoxygenase enzyme (Kwok 1995; Wilson 1996).

In dairy milk processing, a method of "off"-flavor removal has been used for many years, using the method called deodorization. In dairy milk, off-flavors could be caused by the cows' diet. Dairy milk is commonly deodorized with a flash steam-vacuum deodorizer. The process is done continuously immediately after the pasteurization step. The deodorizer works by creating a large thin layer of milk along the inside wall of the deodorizer and the vacuum would volatilize and remove the off-flavors. Inside the deodorizer, steam is usually added into the system to compensate for the loss of moisture during deodorization and to optimize flavor extractions (Farrall 1980). Shurtleff (1979) describe the use of a vacuum pan (with 40 cm Hg or 7.7 psi. vacuum pressure) to remove the off flavor of soymilk which was prepared using the pre-blanch method. The deodorization step can be applied several times before the formulation process. In a study conducted by Hashim and Chaveron (1995), several commercial, undeodorized and deodorized soymilk was evaluated for its off flavor component, they reported that in the deodorized samples, about 70% of the hexanal was removed because of the process. However, this study did not specify the deodorization method that was used. They also reported that the panelists preferred the deodorized soymilk in comparison with other soymilk. In practice, to achieve an acceptable flavored soymilk several combinations of processing method can be used to prevent the formation of the off-flavors.

Inactivation of soy lipoxygenase can also be done through breeding techniques. Soybeans plants can be crossbred to produce soybeans that lack the lipoxygenase enzyme. Soybeans plants that lack the lipoxygenase isozymes can now be found. Flavor improvement of soymilk has been reported with these varieties (Kobayashi and others 1995; Wilson 1996; Torres 2001).

The effectiveness of the two methods has been individually studied. However, the effectiveness of the two methods has not been compared. Thus, the purpose of this study is to evaluate the differences in the flavor of soymilk made from lipoxygenase free soy flakes and deodorized soy flakes milk.

MATERIALS & METHODS

Materials

High protein blend soy flakes (HPSF) and lipoxygenase-free (triple null) soy flakes were provided by the MicroSoy Corporation (Jefferson, Ia., U.S.A.). High protein blend soy flake is a blend of three IA high-protein identity preserve cultivars of soybeans blended for soy flakes production. The lipoxygenase-free cultivar that is used for flaking is IA 2032 cultivar. All chemicals used for analyses were reagent grade (Fisher Scientific, Fair Lawn, N.J., U.S.A.).

Soymilk production

Soymilk was prepared at the Center for Crops Utilization Research (CCUR) pilot plant at Iowa State University (Ames, Ia, U.S.A.). The soymilk was processed using the Takai Automated Soymilk and Tofu System (Takai Tofu and Soymilk Equipment Inc. Japan), using the method of Moizuddin and others (1999). Two levels of solid level are produced for this study (8 and 12 °Brix). For 8 °Brix soymilk, 3.7 kg of flakes were used with 30 L of water; and for 12 °Brix soymilk, 5.52 kg of flakes were used with 30 L of water. These levels of soymilk are produced and deodorized in a day. The soy flakes were

rehydrated with a rotating paddle mixer using 85 °C water for 5 minutes. Timing was initiated at the first contact of flakes to water.

After the soymilk has reached 95 °C, the soymilk was then held for 7 minutes at this temperature when producing 8 °brix soymilk and 10 minutes for the 12 °brix soymilk to allow pasteurization and reduction of trypsin inhibitor levels. The hot slurry was then pumped into a 120 mesh, horizontal rotating cylindrical screen to separate the insoluble solids. The remaining insoluble solids were roller-pressed over a 100-mesh screen drum. The soymilk was then homogenized at 7000 psi and collected in 2 L plastic bottles (undeodorized soymilk). The remaining soymilk was then deodorized. All soymilk samples were immediately refrigerated for analyses the following day.

Soymilk deodorization

Soymilk was deodorized using a pilot plant scale ProSoya VS40 deodorizer (ProSoya Inc., Ottawa, Canada). To reproduce the treatment used in a typical soymilk plant, soymilk was deodorized twice. Soymilk was reheated by steam injection until it reached 80 °C in the ProSoya unit. Once the desired temperature has been reached, vacuum is pulled in the deodorizer tank at the same time steam is introduced inside the deodorization tank. Vacuum is maintained inside the tank at 15-psi vacuum pressure. Soymilk is introduced inside the tank as slow as possible to maintain optimum volatilization of aromas. The process is repeated again for the second deodorization step. The soymilk was then homogenized at 7000 psi and collected in 2 L plastic bottles. All soymilk samples were immediately refrigerated until analyses the following day.

Gas chromatography (GC)

Headspace analysis (Wilson and others 1992) was conducted for all soymilk samples using a Varian 3740 Gas Chromatography (GC) equipped with dual flame ionization detector (FID). The temperature of the injector and detector was held constant at 150 °C. The initial column temperature is 50 °C. The fused glass silica column was then heated at a rate of 10 °C/min until the column temperature reaches 230 °C and held at this maximum temperature for 3 minutes. Hydrogen and nitrogen gas flow rate was set at 30 ml/min and oxygen flow rate was set at 300 ml/min. The output from the gas chromatograph was recorded using a Hewlett Packard integrator model 3390A (Fisher Scientific, Fair Lawn, N.J., U.S.A.).

Headspace analyses method

Sample for headspace analyses were prepared by placing twenty five grams of soymilk into a clear glass bottle and sealed with a Teflon coated septa and standard aluminum seal (Supelco, Inc.). Samples were incubated with a water bath at 37 °C with continuous stirring for at least 30 minutes. Liquid nitrogen was used to cryo-focus the headspace sample in the column. Two ml of headspace was sampled using a 5 ml Hamilton gas-tight syringe and injected to the GC at a rate of 1 ml/min. Duplicates of headspace analysis were done on each samples. Hexanal peak was identified by comparing the retention time of a hexanal standard (Sigma Aldrich, St. Louis, Mo., U.S.A.).

The % reduction or % difference of hexanal peak area is calculated using the following equation:

$$\% \text{ Reduction} = \frac{(\textit{Undeodorized peak area} - \textit{deodorized peak area})}{\textit{Undeodorized peak area}} \times 100\%$$

$$\% \text{ Difference} = \frac{\textit{Differences between the compared peak area}}{\textit{Peak area being compared}} \times 100\%$$

Sensory evaluation

Sensory evaluation for panelists training was conducted based on the method described by Lawless and Heymann (1998). Human subject approval for conducting the panel was obtained through the Human Subject Research office at Iowa State University (Ames, Ia, U.S.A.). The panelists were consisted of 9 graduate students (3 Asian Americans, 1 Latin Americans, and 5 Caucassian Americans) from the Food Science and Human Nutrition Department at Iowa State University. The panelists were exposed to several variety of soymilk that is produced at the Center for Crops Utilization Research (CCUR) pilot plant as well as a commercial sample. The panelists were asked to develop the sensory terms based on some provided terms as well as the panelists' terms.

Once the panelists have familiarized themselves with the flavor standards, the panelists were screened based on a triangle test in differentiating the standards and in identifying the intensity of the standards. Those who were successful in identifying the flavor standards were accepted to participate in this study. The panelists that passed the initial screening were then further train in order to reach a consensus between the panelists on the intensity of each flavor standards.

The panel is conducted in partitioned booth under white light, and the samples are served at refrigeration temperature. Each samples of soymilk are presented in a white plastic cup labeled with three digit random numbers. Thirty ml of soymilk were presented per samples. Within the same solid level, the °Brix of the soymilk samples were adjusted to the

same °Brix by diluting them with distilled water prior to the panel. On each panelist day, the panelists were asked to evaluate four samples of soymilk. The panelists recorded their responses in a 15 cm line scale, which are anchored with the intensity description located at 1-cm of the beginning and the end of the line. The soymilk samples are evaluated for their appearance (whiteness to yellowness), aroma and flavor (weak to strong overall flavor), cereal, beany, painty flavor, astringency, bitterness and sweetness. The panelists' responses were measured with a ruler and reported in mm. All data were collected and analyzed statistically.

Color measurement

The color of soymilk was determined using a 5100 LabScan (Hunter Color Lab, Fairfax, VA, U.S.A.). Soymilk samples were placed into 60 X 15 mm diameter plastic petri dishes (Fisher Scientific) and measurements were taken on the soymilk surface using a 0.25-inch sampling port under D65 illumination and 10° standard observer. Three measurements of each sample were performed at three different sites on the surface of the soymilk.

Proximate analyses

Moisture was analyzed using method 925.19 (AOAC 2000). Crude protein analysis was determined using the micro Kjeldahl AOAC methods 955.04(c) and 954.01 (AOAC 2000), with Kjeltab TCT was used as the catalyst instead of HgO_2. Percent fat content of the samples were determined by Woodson-Tenent Laboratories Inc. (Des Moines, IA, U.S.A.) using acid hydrolysis AOAC method 989.05 (AOAC 2000).

Statistical analyses

Sensory analyses were analyzed using general linear model procedure. GC and color data were analyzed using one-way analyses of variance (ANOVA). SAS System 8.02 (SAS Institute Inc., Cary, NC) statistical program was used for the statistical calculation.

RESULTS & DISCUSSION

Soymilk composition

There are no statistical differences ($\alpha < 0.05$) found in the % moisture, % solid, % protein and % fat between the deodorized and undeodorized soymilk (Table 1) within the same solid level. The results showed that during the deodorization process, the steam that was incorporated or condensed in the deodorizer did not dilute the soymilk, which might affect the sensory evaluation of soymilk.

Gas chromatography and sensory evaluation of deodorized and undeodorized soymilk

The results from hexanal analyses of soymilk with gas chromatography showed that undeodorized triple null soymilk has a lower hexanal content than undeodorized HPSF soymilk ($\alpha < 0.05$) at 8 and 12 °Brix. However, there is no statistical difference ($\alpha < 0.05$) between the deodorized HPSF soymilk and the undeodorized triple null soymilk at 8 and 12 °Brix (Table 2). The deodorized triple null soymilk showed no statistical difference with the undeodorized triple null and deodorized HPSF soymilk. Based on these results, the deodorizer was able to reduce the amount of hexanal in HPSF soymilk to the level similar to the hexanal in triple null soymilk.

Although statistically there is no difference between the deodorized and undeodorized soymilk, the % reduction or % difference data shown in Table 2 shows the best estimate of reduction from the deodorization process or differences between samples. Higher % reduction of hexanal was found from the 12 °Brix HPSF soymilk compared to the 8 °Brix HPSF soymilk. With the triple null soymilk, 39% of hexanal reduction was found at the 8 °Brix level and 25% reduction was found at the 12 °Brix level. At 8 °Brix soymilk, there is a difference of 44% between undeodorized HPSF and undeodorized triple null soymilk and at 12 °Brix the difference is 79%.

The amount of hexanal at the end of the process were similar between the 8 and 12 °Brix deodorized soymilk, this result may indicate that this is the maximum odor removal that can be achieved by the deodorizer. Other possibilities would be that the soy protein binds to the flavor and the amount detected from the deodorized samples were the hexanal that is still bound by the soy protein.

The color measurements of soymilk were reported in Table 3. There is no statistical significant difference between the color of undeodorized and deodorized soymilk within the same soymilk type ($\alpha < 0.05$). However, the triple null soymilk has a significantly higher 'b' value (more yellow) than the HPSF soymilk. Moizuddin and others (1999) evaluated the color of tofu made from soy flakes and whole soybeans processed under direct and indirect heating processes. They reported that ΔL of 4, Δa of 0.5 and Δb of 1 were enough for the trained panelists to observe a significant difference in the color of tofu. Assuming that the color difference of tofu and soymilk perceived similarly by the trained panelists, the 'b' value difference between the HPSF and triple null soymilk should be enough for the panelists to

see a difference. As will be discussed in the sensory data, the panelists were able to tell a difference between the soymilk samples.

Based on the sensory data, the panelists were unable to differentiate the sweetness, cereal, beany and painty aroma between all samples at both solid levels (Table 4). However, the panelists were able to differentiate the soymilk aroma of soymilk at 8 °Brix level but not at the 12 °Brix level. The trained panelists were also able to differentiate the bitterness and the appearance of the soymilk. The panelists indicated that at 8 °Brix, soymilk aroma of undeodorized HPSF ($\alpha < 0.05$) is stronger than the undeodorized triple null. In addition, the panelists reported that the deodorized HPSF soymilk and triple null soymilk are not significantly different between each other, but both are less strong in soymilk aroma than the undeodorized HPSF and triple null. For bitterness and appearance of the soymilk, the panelists reported that the triple null soymilk was more bitter and yellow than the HPSF soymilk (Table 4).

The panelists' responses correspond to the GC data at 8 °Brix soymilk. Based on the GC data, the undeodorized HPSF soymilk has significantly higher amount of hexanal compared to the deodorized HPSF soymilk, undeodorized and deodorized triple null soymilk. However, the hexanal data at 12 °Brix do not correspond to the sensory data. Twelve-°Brix soymilk is not the concentration that is normally consumed by the consumer (commercially is at 8 °Brix concentration) and it maybe that the flavor is too strong to be differentiated by the panelist. It is also noted by the panelists that the triple null soymilk perceived to be more bitter and yellow compared to the HPSF soymilk. Torres-Penaranda and Reitmeier (2001) reported that in the evaluation of soymilk made from triple null soybeans stored for 15 months, there is a significant increase in the bitterness in the soymilk. This bitterness may be

developed through the oxidation of the soybeans or in this case soy flakes. The intense bitterness in the triple null soymilk may distract the panelist in differentiating the beany, cereal and painty flavor of soymilk. The oxidation may also oxidize the lipid in the soybeans and produces other flavor compounds other than hexanal.

CONCLUSION

Based on the GC data, the deodorizer was effective in reducing the original amount of hexanal in HPSF soymilk to the level found in undeodorized triple null soymilk. The sensory evaluation results did show that the deodorizer was able to reduce the soymilk aroma in HPSF soymilk so it would be similar to triple null soymilk at 8 °Brix level.

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TABLES

Table 1 - Proximate analyses results of soymilk samples

	8 °Brix HPSF		8 °Brix Triple Null	
	Undeodorized	Deodorized	Undeodorized	Deodorized
% Moisture	91.86 a	91.48 a	91.87 a	92.87 a
%Solids	8.14 a	8.52 a	8.13 a	7.13 a
%Protein	3.54 a	3.76 a	3.90 a	3.41 a
%Fat	2.34 a	1.87 a	2.14 a	1.80 a

	12 °Brix HPSF		12 °Brix Triple Null	
	Undeodorized	Deodorized	Undeodorized	Deodorized
% Moisture	88.15 a	89.06 a	90.00 a	91.29 a
%Solids	11.85 a	10.94 a	10.00 a	8.71 a
%Protein	5.49 a	5.23 a	4.76 a	4.18 a
%Fat	3.37 a	3.12 a	2.54a	1.78 a

Means were calculated based on three replications.

a Means with the same letter code showed no significant differences (α <0.05).

Table 2 - Hexanal peak area of undeodorized and deodorized soymilk at 8 and 12 °Brix level

Sample	Fresh (Peak Area)	Deodorized (Peak Area)	%Reduction
8 °Brix HPSF	31,714 a	20,037 ab	37%
8 °Brix Triple null	17,635 b	10,833 b	39%
%Difference	44%	46%	
12 °Brix HPSF	49,227 a	16,984 b	65%
12 °Brix Triple null	10,193 b	7,644 b	25%
% Difference	79%	55%	

Means were calculated based on three replications.

a,b Means with the same letter code showed no significant differences (α < 0.05) within the same solid level.

Table 3 - L, a, b values of undeodorized and deodorized soymilk at 8 and 12 °Brix level

	Undeodorized			Deodorized		
Sample	**L**	**a**	**b**	**L**	**a**	**b**
8 °Brix HPSF	79.80 a	-1.33 a	12.40 a	78.79 a	-1.76 a	12.21 a
8 °Brix Triple null	78.89 a	-2.16 a	14.46 b	78.92 a	-2.15 a	14.11 b
12 °Brix HPSF	83.53 a	-0.66 a	13.96 a	79.81 a	-0.69 a	13.83 a
12 °Brix Triple null	80.61 a	-0.62 a	16.83 b	80.34 a	-0.46 a	15.99 b

* L = 100 light & L = 0 dark; a = + red & a = - green; b = + yellow & b = - blue

a, b Means with the same letter code showed no significant differences (α<0.05) within L, a, or b value and soymilk solid level.

Table 4 - Sensory results of deodorized and undeodorized HPSF and lipoxygenase-free (triple null) soymilk

Sensory Attributes*	8 °Brix HPSF		8 °Brix Triple null	
	Undeodorized	Deodorized	Undeodorized	Deodorized
Appearance	69 a	63 a	80 b	75 b
Soymilk aroma	81 c	49 b	78 a	55 b
Cereal flavor	61 a	79 a	66 a	63 a
Beany flavor	59 a	65 a	73 a	57 a
Painty	41 a	36 a	36 a	35 a
Astringent	50 c	47 ac	54 c	33 b
Bitter	49 c	38 a	73 d	68 bcd
Sweetness	30 a	24 a	24 a	19 a

Sensory Attributes*	12 °Brix HPSF		12 °Brix Triple null	
	Undeodorized	Deodorized	Undeodorized	Deodorized
Appearance	87 b	69 a	91 b	86 b
Soymilk aroma	88 a	75 a	66 a	72 a
Cereal flavor	85 a	81 a	75 a	76 a
Beany flavor	69 a	66 a	77 a	74 a
Painty	34 a	34 a	38 a	33 a
Astringent	50 ab	38 a	60 b	43 a
Bitter	40 a	34 a	78 b	84 b
Sweetness	35 a	35 a	20 b	25 ab

* Responses means is measured in mm based on 150 mm line scale. For all attributes 0 mm is no sensory attributes and 150 mm is strong sensory attributes, except in the appearance attribute 0 mm is whiteness and 150 mm is yellowness.

a-d Means with the same letter code showed no significant differences ($\alpha < 0.05$) within the same sensory attributes.

EFFICIENCY IN LIPID REMOVAL FROM SOYMILK MADE FROM FULL FAT SOY FLAKE OR WHOLE SOYBEANS AT THREE SOLID LEVELS

A paper to be submitted to the Journal of Food Science

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ABSTRACT

Soymilk has been consumed as a substitute for cow's milk for centuries and is the fastest growing soy food in the U.S. Unlike the dairy industry, fat reduction in soymilk has been done through formula modification instead of by conventional fat removal techniques (skimming). The objective of this study is to evaluate the efficiency of fat removal from soymilk produced from whole soybeans and full-fat soy flakes at three solid levels; 5, 8, and 12 °Brix. Whole soybeans and soy flakes were used to make soymilk using a commercial Takai Soymilk machine. Soymilk fat was removed using either a commercial dairy skimmer or a centrifuge-decant method. Proximate analyses were determined on all fractions of the skimming process (whole and skimmed soymilk, centrifuge precipitate, and cream). The color of soymilk was measured using a Hunter color LabScan 5100. Regardless of skimming method and solids levels, the fat from whole soybean milk was removed less efficiently than from soy flake milk (7 to 30% fat extraction in contrast to 50 to 80% fat extraction respectively). In soy flake milk, similar amounts of fat could be removed from 5 and 8% solids milk (75% fat extraction) but only 60% fat extraction from 12% solids milk using the commercial dairy skimmer. In whole soybean milk, the fat was removed less efficiently at

lower solids level milk using the commercial dairy skimmer and more efficient at lower solids level using the centrifuge-decant method. The L, a, b value of the reduced fat soymilk showed that soymilk made from soy flakes yielded a darker, greener and less yellow color milk than whole soymilk ($\alpha < 0.05$). Less observable differences were noticed in reduced fat whole soybean milk ($\alpha < 0.05$). Color comparison of whole and skim cow's milk showed the same trend as in the soymilk.

Key words: soymilk, reduced fat and full fat soymilk

INTRODUCTION

In eastern Asia, soymilk has been consumed as cow's milk substitute for centuries. In the US, only until recently has there been an increase in the use of soy as a protein source. Based on the market research conducted by SoyaTech in 1999, the sales of soy foods in the U.S. were projected to increase from $2.1 billion to $3.57 billion by 2002 (Soya Tech 1999). In 2002, Soya Tech released a new report showing that the soy food industry had already reached $3.2 billion in sales by 2001. Soymilk sales alone in 2001 has reached $550 million and were projected to reach $1 billion in the coming three to five years (Soyatech 2002).

Cow's milk with various fat contents (whole, 2%, 1%, and skim) has been available for sometime. Currently not many reduced fat soymilks are available in the grocery store. The reduced fat soymilk can be made through formula modification. Soymilk can be produced from soybeans and then additional protein (solids) materials can be added into the soymilk to increase its solids content followed by dilution of the product. In the end, the soymilk would have compositionally reduced fat but with the same amount of protein

compared to regular soymilk. Conceivably, the fat of soymilk can be removed using the same method as cow's milk. In cow's milk, the cream is separated from the milk using centrifugal force. In which, during centrifugation, the lower density fat will move inwards whereas the higher density skim milk and other particles will move outwards of the axis of rotation. A commonly used fat separator in dairy industry is a disc-bowl centrifuge.

In the study conducted by Moizuddin and others (1999), they evaluated the use of whole soybean and full fat soy flakes for tofu production in using both direct and indirect heat treatments. They reported that tofu made with soy flakes had lower fat content and the okara has higher fat content than the tofu and okara made with whole soybeans in both processing methods. The hulls from whole soybeans may play a role as a filtering aid during pressing by providing channels for the fat to escape, where in soy flakes, the absence of the hull caused caking of the insoluble matter and prevented the fat from escaping (Moizuddin and others 1999).

Fat removal in soymilk has also been studied using a centrifuge method, in which it was found that most of the fat could be separated as a floating layer which contains a few proteins (Shibasaki and others 1972; Ono and others 1996). In a study conducted by Guo and others (1997), the movement of lipid during soymilk heating was observed. In this study, heated soymilk at different temperatures was separated into particulate, soluble and floating fractions through centrifugation. They concluded that fat migration occurs in two stages. In the first stage, fat is released into the soluble fraction at 65-75 °and then the fat migrated from the soluble to the floating fraction at temperatures above 75 °C in the second stage. In this study, they also reported that the release of fat from the particulate to the soluble fraction is due to the denaturation of the glycinin protein. These findings correspond to the results

reported by Aoki and others (1980), where they reported that emulsifying capacity and stability decreases as heat increased with the lowest emulsifying capacity was observed at 85 °C.

The instability of soy flake milk fat emulsions has been observed for three years in the previous storage study of soy flakes milk conducted by Iowa State University's (ISU) food processing class. Where in this class, various levels of homogenization pressure on soy flakes milk (0 – 2000 psi.) was compared to whole soybean milk with the same level of homogenization (0 – 2000 psi.). The class reported that no floating material was found on the unhomogenized whole soybean milk until the 7^{th} day, while floating material (a bright yellow layer containing fat and protein) was found on the unhomogenized soy flake milk the next day. These results suggest that a traditional skimming method must be successful to remove this fat

These mentioned studies have all been under lab scale processing conditions and the optimum condition for fat removal in soymilk has not been determined. Hence, the objective of this study is to evaluate the efficiency of fat removal from soymilk produced from whole soybeans and soy flake at three different solid levels under pilot plant conditions.

MATERIALS & METHODS

Materials

High protein blend soy flakes (HPSF) soy flakes were provided by MicroSoy Corporation (Jefferson, IA, U.S.A.). Vinton 81 soybeans grown in 2001 were provided by Pattison Bros (Fayette, IA, U.S.A.). All chemicals used for analyses were reagent grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.).

Soymilk production

Soymilk was prepared at the Center for Crops Utilization Research (CCUR) pilot plant at Iowa State University (Ames, IA, U.S.A.). The soymilk was produced using the Takai Automated Soymilk and Tofu System (Takai Tofu and Soymilk Equipment Inc. Japan). The ratio of soy flakes or soybeans to water used depends on the percent solids (Table 1).

Table 1 - Amount of soy flakes, whole soybeans and water used for soymilk production

Soymilk Type	Soybeans Soaking Time (hour)	Soy flakes Rehydration Time (min)	Whole Soybean or soy flake Wt. (Kg)	Total Amount of Water (L)
5 °Brix	12	2.5	2.3	30
8 °Brix	12	5	3.7	30
12 °Brix	12	5	5.5	30

A slurry of soy flake was made by rehydrating soy flakes with 85 °C water in the Takai mixing tank, for the times in Table 1. The Takai mixing tank was equipped with a rotating paddle mixer. The whole soybean slurry was prepared by grinding the soaked whole soybeans (Table 1) twice (0.2 and 0.05 mm grinder head) with a Stephan grinder MC 15 (Stephan Machinery Corp., Columbus, OH, U.S.A.). For each grinding step, the soybeans were ground with 10 L of 85 °C water.

The slurry was then pumped using a steam injector to push the slurry, into the cooking tank. The slurry tank was rinsed with 1.2 L of water and added to the cooking tank. The temperature of the slurry was monitored using a Fisherbrand® Traceable® Total-Range Digital Thermometer (Fisher Scientific, Fair Lawn, NJ, U.S.A.). In the cooking tank, the soy slurry was cooked using direct steam injection until it reached 95 °C. After the soymilk has

reached 95 °C, the soymilk was then held at this temperature for 7 minutes (Moizuddin and others 1999). The hot slurry was then pumped into a 120 mesh, horizontal rotating cylindrical screen to separate the insoluble solids from the soymilk. The remaining insoluble solids were roller-pressed over a 100-mesh stainless steel screen drum. The finished soymilk was then collected into a 35-L stainless steel milk can. The finished soymilk produced from the Takai soymilk machine will be designated as "whole soymilk" throughout this paper.

Fat removal of soymilk was done using two methods, dairy creamer and centrifuge-decanter method. The first method uses a Westfalia Separator AG type LWA205, a pilot plant scale dairy creamer (Oelde, Germany). Four liters of 5 °Brix soymilk and 2 L of 8 and 12 °Brix soymilk were put into the holding bowl and the temperature of soymilk was adjusted to 65 -70 °C. A lower amount of soymilk was used for the 8 and 12 °Brix soymilk to compensate for the larger amount of precipitate that may hinder the skimming process. The skimmer is set to its maximum speed and the soymilk then entered the skimmer with a flow rate of 880 ml/min. The cream and reduced fat milk was collected in stainless steel buckets and the weight was measured. The weight of the centrifuge bowl of the dairy creamer was measured before and after the skimming process. The material inside the centrifuge bowl was collected in a plastic container. Additional hot water was used to collect all the materials from the centrifuge bowl. The leftover materials found in the centrifuge were designated as "centrifuge matter" which consists of precipitate, soymilk, and cream. The soymilk was then homogenized at 5000 psi (Moizuddin and Wilson 2003a) and collected in a capped 2 L plastic bottle. All soymilk samples were immediately refrigerated until analyses the following day.

The second technique used was the centrifuge method described by Moizuddin and others (2003b). The soymilk that was produced from the Takai was collected into 1 L Nalgene centrifuge bottles and kept refrigerated overnight. The bottle was then centrifuge for 1 hour at 3500-rpm with the temperature held constant at 4 °C using a Sorvall RC 3b plus centrifuge (Kendro Lab., Newtown, CT, U.S.A.). After 1 hour centrifugation a fat layer, soymilk and precipitate layer can be seen in the soymilk. The soymilk was separated from the lipid by decanting it through four layers of cheesecloth. The resulting lipid layer was solid enough to be retained by the cheesecloth and it was not washed away by the soymilk. All soymilk samples were immediately refrigerated for analyses.

The percent recovery and percent reduction of the process was calculated using the following equation:

$$\% \text{ Recovery} = \frac{Output\ Wt.}{Input\ Wt.} \times 100\%$$

$$\% \text{ Reduction} = \frac{Mass\ of\ lost\ components}{Mass\ of\ Initial\ components} \times 100\%$$

Moisture, protein and fat measurements

Moisture was analyzed using AOAC method 925.19 (AOAC 2000). Crude protein was determined using the micro Kjeldahl AOAC methods 955.04(c) and 954.01 (AOAC 2000), with Kjeltab TCT was used as the catalyst instead of HgO_2. Percent fat content of the samples was determined using Babcock acid hydrolysis for whole and skim milk (Marshall RT 1993), modified by adding n-butanol into the soymilk to increase fat collection similar to

the method described in Laboratory manual; methods of analysis of milk and its products(1959).

Color measurement

The color of soymilk was determined instrumentally using a 5100 LabScan (Hunter Color Lab, Fairfax, VA, U.S.A.). Soymilk samples were placed into 60 X 15 mm diameter plastic petri dishes (Fisher Scientific) and measurements were taken on the soymilk surface using a 0.25-inch sampling port under D65 and 10 ° standard observer illumination. Three measurements of each sample were done at three different sites on the surface of the soymilk.

Statistical analyses

Proximate analyses data were analyzed using split plot design, and differences among treatment means were analyzed using proc mixed based on the split plot design. Comparison between two samples is calculated using paired t-test method. SAS System 8.02 (SAS Institute Inc., Cary, NC, U.S.A.) statistical program was used for the statistical calculation.

RESULTS & DISCUSSION

Processing of soybean and soy flake milk

The three different solid level of soymilk were chosen because they represent the commonly used solid level in the production of various soy products. Five °Brix soymilk is commonly produced for the production of firm style tofu; 8 °Brix soymilk is the solid level commonly produced for commercial soymilk; and 12 °Brix soymilk is the solid level commonly produced for the production of base milk for transport efficiency. The soymilk at

the same solid levels that was produced using soybeans and soy flakes showed no statistical significant difference in composition (Table 2).

Inside the centrifuge bowl of the dairy creamer, a precipitate layer can be found on the inside cover of the centrifuge. The amount of centrifuge matter was consistently found to be the same in soybean and soy flake milk at all solid levels. From this process, the % recovery of soymilk was about 80% at the 8 and 12 °Brix level and 90% at the 5 °Brix level for both soymilk sources (Table 2).

The higher °Brix level should have a greater amount of fat than the lower °Brix level and therefore more cream output should be observed. However, cream was only produced from soy flake milk at 5 and 8 °Brix level, no cream was produced from whole soybean milk at all solid levels. The different amount of sample that is used for 8 and 12 °Brix whole soybean milk and soy flake milk at 12 °Brix can be one of the reasons why the soymilk has no cream output. However, if this were the case, soy flakes milk at 8 °Brix should not produce a cream output. Further analyses of the soymilk, which will be discussed in the next section, would explain this observation.

The soymilk produced was saved for centrifugation the next day. Upon refrigeration for a day, two separate layers, which consist of a yellow floating layer and soymilk, were observed on the soy flake milk; no separation is observed on the soybean milk. This observation corresponds to the soymilk storage study conducted by the ISU food processing class. The amount of the precipitate after the centrifuge process was found to be higher in the soy flake milk. In both processes, the resulting cream from soy flakes has a bright yellow color, whereas the cream from the whole soybean has a white color.

Fat removal using the commercial dairy skimmer and the centrifuge method

Inside the centrifuge, centrifuge matter were collected and analyzed for its solid, protein and fat content. The centrifuge matter from soy flake milk processing showed a higher % solid, % protein and % fat than from soybean milk at the 5 and 8 °Brix solid level. At the 12 °Brix solid, the centrifuge matter of soy flake milk only has higher % solid and % fat compared to soybean milk centrifuge matter (data not shown). These results support the observation noted earlier in the centrifuge-decant method, which showed larger amount of precipitate layer found from the centrifuged soy flake milk (Table 2).

Using the dairy skimmer, more fat can be removed from soy flake milk than from whole soybean milk (Table 3). The amount of fat removed from soy flake milk is about 2-5 fold more than what is removed from whole soybean milk. Across the different °Brix levels of soy flake milk, there was no statistical difference ($\alpha < 0.05$) found between the % fat extracted from 5 and 8 °Brix solids level, but the % fat extracted was found significantly lower for 12 °Brix solids soymilk. In whole soybean milk, 5 °brix soymilk had significantly lower fat extracted than either 8 or 12 °Brix soymilk with no statistical significant difference found between them ($\alpha < 0.05$). The same trend is found in % solid extracted as in the % fat extracted (Table 3). Within the same solid level, there is no statistically significant different between soy flake and whole soybean milk ($\alpha < 0.05$) in the % protein extracted (Table 3). Across all solid levels, there was no significant difference in the % protein extracted in soy flakes ($\alpha < 0.05$).

The results from the centrifuge-decant method showed similar results to the dairy skimmer method. The amount of fat extracted is greater in soy flake milk than in soybean milk (Table 3). In soy flake milk, the highest % fat extracted is obtained at the 8 °Brix level,

and the lowest at the 12 °Brix level. The opposite result is found in whole soybean milk; the lowest level of % fat extracted is found at the 8 and 12 °Brix level and the highest is at 5 °Brix level. Compare to the result of soybean milk using the dairy skimmer, the % fat extracted in this procedure does not follow the same trend (Fig. 3). In the dairy skimmer, the % fat extracted increases as the solids level of soybean milk (8 and 12 °Brix) increases, where in the centrifuge-decant method the % fat extracted decreases as the solids level increases. Within the same soymilk type (soy flakes milk or whole soybean milk), there is no statistical significant difference ($\alpha < 0.05$) across all °Brix level for protein extracted using the centrifuge-decant method. However, more protein is extracted from soy flake milk than whole soybean milk ($\alpha < 0.05$) (Table 3).

Emulsion stability can be improved by increasing the viscosity or by adding an emulsifier (Friberg 1997). Based on this theory, as the amount of solids in soymilk increase, the viscosity of the milk would also increase and hence would increases the emulsion stability of milk; and therefore, the fat would be harder to be removed at higher % solid. Soy protein is known to be a good emulsifier. An increase of soy protein in the milk should increase the emulsion stability as well. Since there is no difference in the amount of protein and fat between soy flake and whole soybean milk, they both have the same protein to fat ratio. The same protein to fat ratio between the two types of milk suggests the amount of protein interacting with the fat globules should be similar. Therefore, the fat removal of soymilk should be depended on other factors such as the solids level or processing. The result of soy flake milk seems to support this hypothesis, where there is a decrease in % fat extraction as the % solid increases (Fig. 3). Whole soybean milk results showed two different

and opposite trends in the % fat extraction between the two methods (Fig. 3). Therefore, the same hypothesis cannot be applied in whole soybean milk.

The lower emulsion stability of soy flake milk helps in the removal of fat from soymilk (shown by separation upon storage). This lower emulsion stability of soy flakes may be explained based on the analyses of the centrifuge matter. Protein extraction by the dairy skimmer was greater in soy flake milk than soybean milk. The larger amount of protein indicates that soy flakes protein in soymilk is more prone to denaturation than whole soybean milk protein and this denaturation of protein releases the fat more readily. As indicated by Guo and others (1997), the denaturation of protein, which is found in the particulate matter of soymilk, was followed by the increase of fat into the floating layer as the soymilk is being heated. The denaturation of soy flake milk protein perhaps occurs during the hot rehydration step of making the soymilk.

In addition, there is a possibility that the different method of making soymilk in soybean and soy flakes (grinding in soybean against no grind in soy flakes) played a significant role in the emulsion stability of the soymilk. Since the soybean milk was prepared through grinding the whole soybeans twice, the small opening at the grinder head (0.05 mm) may partly homogenized the soybean milk and therefore increase the emulsion stability of the soymilk. An incorporation of more protein into the fat globules during grinding might increases the soymilk emulsion stability property. The influences of processing method on emulsion stability have also been reported by Tornberg and others (1977).

In soy flake milk, within the same °Brix level, there was no statistical difference found between the two methods (Fig. 3); except at the 5 °Brix, where a higher % fat extraction was found using the dairy skimmer. In both methods, the least amount of fat

extraction was found at the 12 °Brix solid level. In soybean milk, the trend using both methods seem to be opposite of each other (Fig. 3). Using the dairy skimmer, more fat was extracted with increasing °Brix level where with the centrifuge method less fat was extracted with increasing °Brix level.

Hunter color Lab measurement of reduced fat milk

According to the Hunter Lab color measurements, the color of the soy flake milk is similar to the color of whole soybean milk within the same solid level. The color of reduced fat soy flake milk is found to be darker, less yellow and greener than the whole milk (Table 4). This result shows that in soy flake milk, fat influence the color of the soymilk. However, in whole soybean milk, there is no statistical significant difference between the color of whole and reduced fat soymilk, because less fat was removed. The measurements showed a slight decrease in the L, a, b values; however, it is not enough to show significant differences.

To explore the color changes in reduced fat milk, dairy milk at different % fat level was purchased and the color was determined using the same method. In dairy milk, similar results were found, the "L" and "b" value decreases and the "a" value increases when whole milk and skim milk was compared. Less apparent differences were found between the whole and 2% milk. These two comparisons suggest that there is a correlation between the amounts of fat remove on the color of milk. The bright yellow color of soy flakes cream suggested that in soy flake milk, removal of fat could cause a detrimental change in color of the milk.

Moizuddin and others (1999) evaluated the color of tofu made from soy flakes and whole soybeans processed under direct and indirect heating processes. They reported that ΔL of 4, Δa of 0.5 and Δb of 1 were enough for the trained panelists to observe a significant

difference in the color of tofu. Assuming that the color difference of tofu and soymilk perceived similarly by trained panelists, then the color of whole soymilk and reduced fat milk should be noted to be different if it would undergo a trained panelist sensory evaluation.

CONCLUSION

In both processes, soy flake milk yield a better extraction of fat than soybean milk, these findings may be attributed to the processing effect of the soy flakes. There is an indication of a decrease in fat removal with increase of solid levels of soymilk made from soy flakes. However, the result was not found to be true in soybean milk. The removal of fat from soymilk is followed by a change in the color of soymilk, and the changes are more pronounced as more fat is removed.

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TABLES AND FIGURES

Table 2 - Proximate analyses data of soymilk made from soy flakes and whole soybeans

		Soymilk			
Sample	°Brix	%Moisture	%Protein	%Fat	%Carbohydrate and %Ash
Soy Flakes	5	94.99 ± 0.06	2.14 ± 0.05	1.21 ± 0.05	2.78 ± 0.06
	8	92.05 ± 0.14	3.65 ± 0.03	1.98 ± 0.04	4.20 ± 0.15
	12	88.14 ± 0.11	5.46 ± 0.43	3.03 ± 0.04	6.21 ± 0.20
Whole Soybean	5	94.78 ± 0.02	2.63 ± 0.07	1.16 ± 0.01	2.50 ± 0.35
	8	92.00 ± 0.16	3.82 ± 0.26	1.81 ± 0.02	3.94 ± 0.46
	12	88.85 ± 0.32	5.71 ± 0.13	2.52 ± 0.25	5.27 ± 0.62

Results are expressed as means ± S.D (n = 2).

Table 3 - Yield data of from the dairy skimmer and centrifuge-decant method using soy flakes and whole soybeans

		Dairy skimmer			Centrifuge-decant	
Sample	°Brix	Soymilk recovery (%)	Centrifuge matter (%)	Cream (%)	Soymilk recovery (%)	Precipitate & cream (%)
Soy Flakes	5	89.44 ± 1.95	8.45 ± 0.71	3.73 ± 2.81	96.06 ± 0.30	3.95 ± 0.30
	8	79.90 ± 3.10	15.04 ± 1.35	1.35	92.75 ± 0.52	7.27 ± 0.52
	12	79.14 ± 2.15	16.22 ± 1.98	-	90.64 ± 0.65	9.35 ± 0.65
Whole Soybean	5	89.09 ± 0.06	8.16 ± 0.32	0.22	98.25 ± 0.49	1.75 ± 0.49
	8	80.01 ± 0.35	15.86 ± 0.59	-	97.60 ± 0.00	2.40 ± 0.00
	12	79.05 ± 1.15	16.19 ± 0.95	-	95.79 ± 0.29	4.20 ± 0.29

Results are expressed as means ± S.D (n = 2).

Table 4 - % Solid, % protein, and % fat extracted from soy flake milk and whole soybean milk process using dairy skimmer and centrifuge-decant method

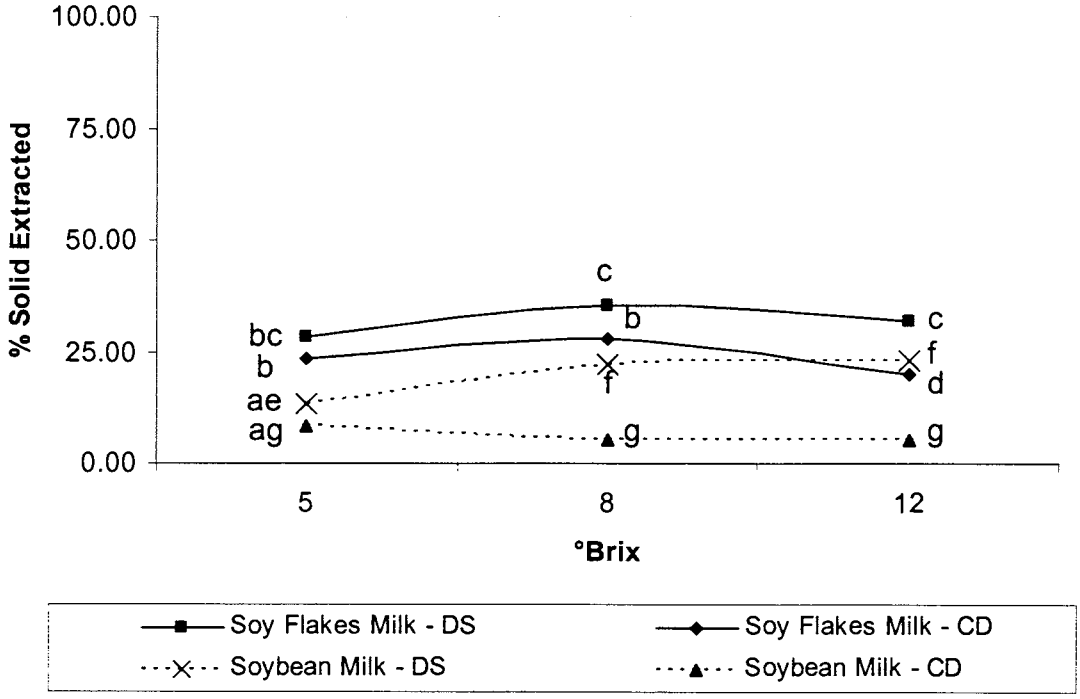
Sample	° Brix	%Solid Extracted		%Protein Extracted		% Fat Extracted	
		DS*	CD*	DS	CD	DS	CD
Soy flake milk	5	28.35 ± 3.61 [bc]	23.35 ± 3.27 [b]	14.60 ± 8.88 [a]	17.77 ± 3.65 [a]	75.12 ± 4.46 [b]	66.03 ± 5.29 [a]
	8	35.39 ± 2.48 [c]	27.91 ± 0.71 [b]	26.97 ± 5.54 [a]	19.10 ± 0.57 [a]	75.17 ± 2.60 [b]	76.50 ± 2.30 [b]
	12	32.05 ± 1.69 c	19.79 ± 8.16 [d]	24.93 ± 3.27 [b]	15.80 ± 1.67 [a]	58.82 ± 2.13 [ac]	52.29 ± 0.54 [c]
Whole Soybean	5	13.39 ± 0.78 [ae]	8.21 ± 1.99 [ag]	10.72 ± 0.85 [c]	5.10 ± 3.06 [b]	15.15 ± 5.92 [f]	24.20 ± 3.76 [d]
Milk	8	22.26 ± 0.61 [f]	5.25 ± 1.13 [g]	18.79 ± 2.46 [b]	0 [b]	23.39 ± 1.15 [g]	7.82 ± 3.83 [e]
	12	22.80 ± 0.26 [f]	5.43 ± 3.64 [g]	22.62 ± 1.66 [b]	4.51 ± 8.09 [b]	26.27 ± 5.92 [g]	9.01 ± 6.01 [e]

Results are expressed as means ± S.D (n = 2)

* DS = Dairy Skimmer; CD = Centrifuge-Decant

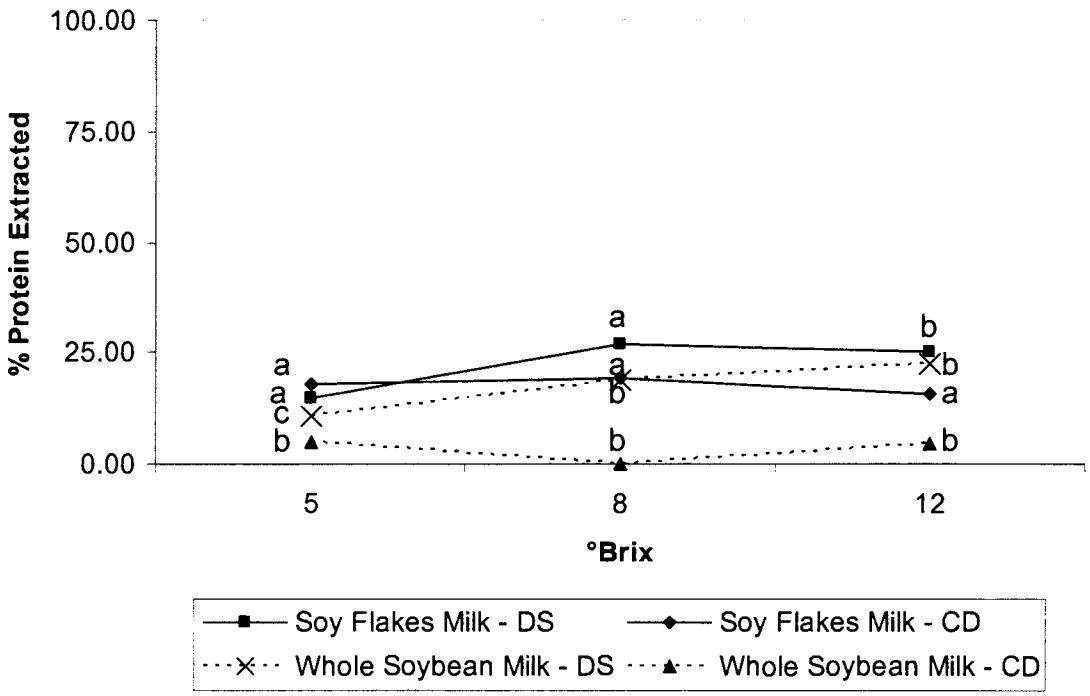
[a-g] Means with the same letter code is not significant different ($\alpha < 0.05$) within the same extracted component.

Figure 1 - % Solid extracted from soy flakes and whole soybean milk using the dairy skimmer and centrifuge-decant method at 5, 8 and 12 °Brix



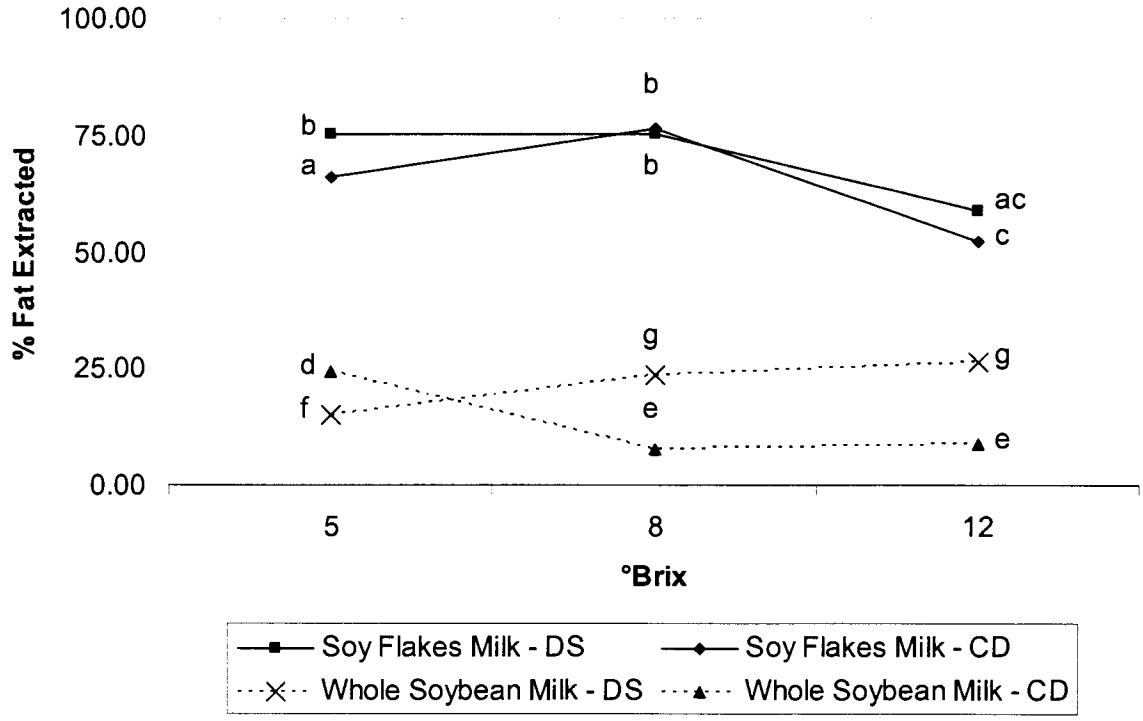
[a-g] Means with the same code is not significantly different ($\alpha < 0.05$).

Figure 2 - % Protein extracted from soy flakes and whole soybean milk using the dairy skimmer and centrifuge-decant method at 5, 8 and 12 °Brix



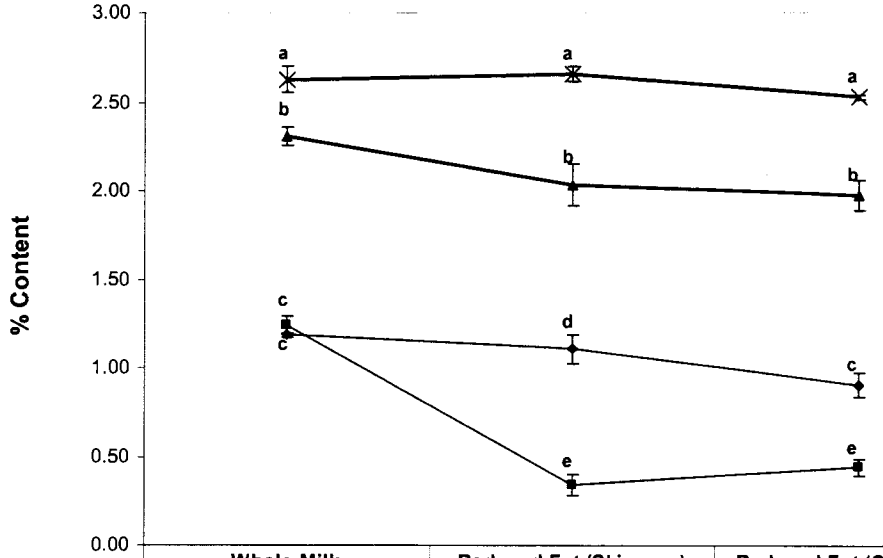
[a-c] Means with the same code is not significantly different ($\alpha < 0.05$).

Figure 3 - % Fat extracted from soy flakes and whole soybean milk using the dairy skimmer and centrifuge-decant method at 5, 8 and 12 °Brix



[a-g] Means with the same code is not significantly different ($\alpha < 0.05$).

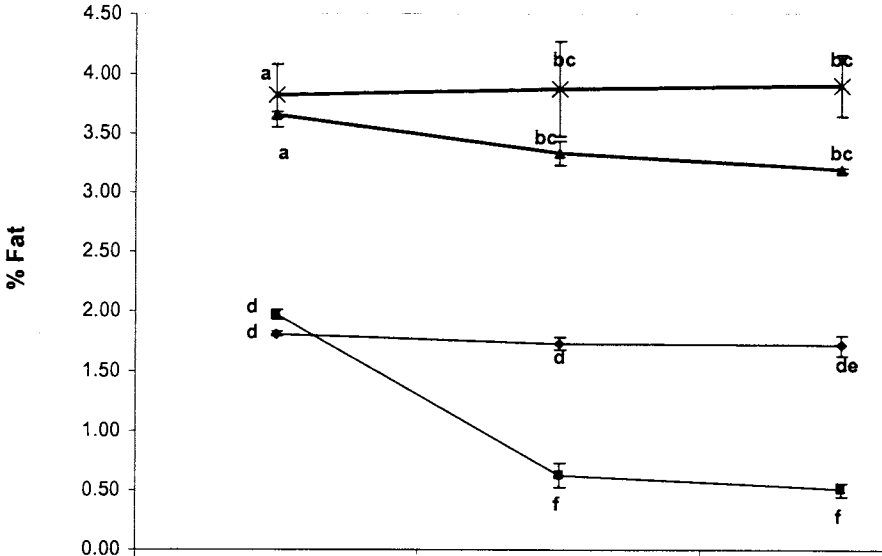
Figure 4 - % Fat and % Protein of whole milk, and reduced fat milk from dairy skimmer and centrifuge-decant method from soy flake and whole soybean milk at 5 °Brix level.



	Whole Milk	Reduced Fat (Skimmer)	Reduced Fat (Centrifuge)
% Fat - Soy Flakes	1.24	0.35	0.44
% Fat - Soybeans	1.19	1.11	0.91
% Protein - Soy Flakes	2.31	2.04	1.97
% Protein - Soybeans	2.63	2.66	2.53

[a-e] Means with the same code is not significantly different ($\alpha < 0.05$).

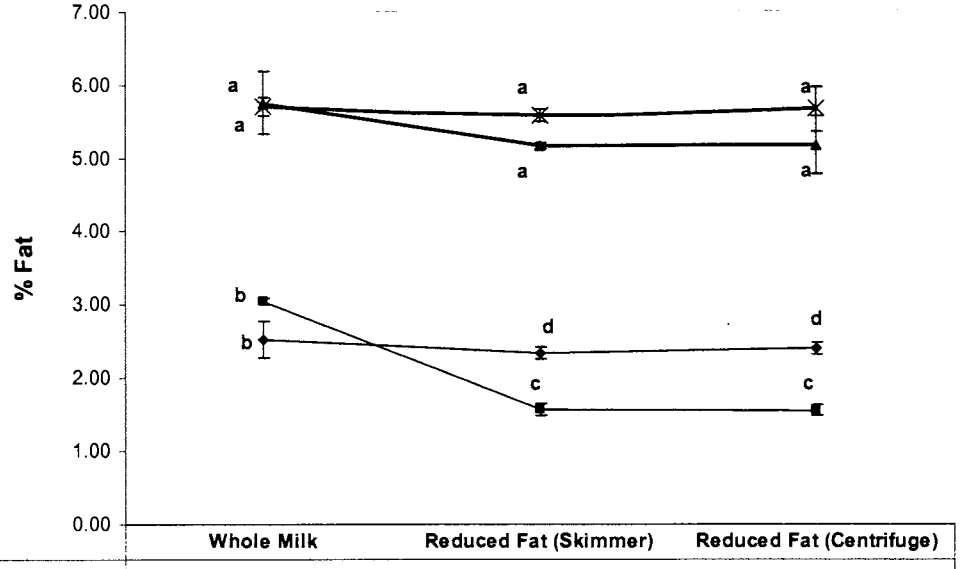
Figure 5 - % Fat and % Protein of whole milk, and reduced fat milk from dairy skimmer and centrifuge-decant method from soy flake and whole soybean milk at 8 °Brix level.



	Whole Milk	Reduced Fat (Skimmer)	Reduced Fat (Centrifuge)
% Fat - Soy Flakes	1.98	0.63	0.51
% Fat - Soybeans	1.81	1.74	1.71
%Protein - Soy Flakes	3.65	3.34	3.19
% Protein - Soybeans	3.82	3.88	3.90

[a-f] Means with the same code is not significantly different ($\alpha < 0.05$).

Figure 6 - % Fat and % Protein of whole milk, and reduced fat milk from dairy skimmer and centrifuge-decant method from soy flake and whole soybean milk at 12 °Brix level.



	Whole Milk	Reduced Fat (Skimmer)	Reduced Fat (Centrifuge)
% Fat - Soy Flakes	3.05	1.56	1.55
% Fat - Soybeans	2.52	2.34	2.39
% Protein - Soy Flakes	5.76	5.17	5.17
% Protein - Soybeans	5.71	5.59	5.66

[a-d] Means with the same code is not significantly different ($\alpha < 0.05$).

Table 5 - Color measurement results of whole and reduced fat soymilk

			Whole soymilk			Reduced fat soymilk		
Method	**Sample**	**°Brix**	**L***	**a***	**b***	**L***	**a***	**b***
Dairy Skimmer	Soy Flakes	5	75.97a	-2.86b	10.19a	60.65a	-4.34b	2.88a
		8	79.57a	-1.67b	13.55a	72.05b	-3.97b	9.33a
		12	80.75a	-1.17a	15.01a	68.20a	-1.83a	12.66a
	Whole Soybean	5	78.32a	-3.42a	8.68a	64.64a	-2.46a	9.40a
		8	81.39a	-2.31a	13.36a	80.05a	-2.14a	12.61a
		12	80.53a	-1.11a	15.16a	78.19a	-0.70a	15.43a
Centrifuge	Soy Flakes	5	74.75a	-2.69a	11.51a	55.68b	-4.29b	1.17b
Method		8	79.72a	-1.53a	13.74a	67.18a	-4.50b	7.15b
		12	81.82a	-1.67a	15.07a	72.44b	-2.10a	16.19a
	Whole Soybean	5	81.63a	-2.71a	10.65a	79.43a	-2.83a	11.97a
		8	81.39a	-2.31a	13.36a	78.34a	-1.14a	15.14a
		12	80.53a	-1.11a	15.16a	77.89a	-1.33a	14.28a

* L = 100 light & L = 0 dark; a = + red & a = - green; b = + yellow & b = - blue.

a,b Means with the same letter code showed no significant differences (α <0.05) within L, a, or b value, soymilk solid level and process.

CONCLUSIONS

From the first study, it was concluded that as with other powdered raw material alternatives to whole soybeans, immediate introduction to the water would cause agglomeration to the powder. Soy flakes is no difference than its powder counterparts. Initial rehydration step is needed in order to process soy flakes, unless the equipment is able to break the agglomeration of the product without homogenizing the milk.

High temperature rehydration, similar to previous study with whole soybeans, would causes protein denaturation, which would heat fixed the protein within the soy flakes. Protein fixation in soy flakes is followed by fat and water fixation as well. Based on the GC data, flavor improvement can be achieved through this high temperature rehydration (pre blanching step) with no significant color changes to the soymilk. The optimum condition for flavor and extraction of soy flakes processing for 5 °Brix soymilk can be achieved at 50 °C for 5 minutes. As for optimum processing condition of 8 and 12 °Brix soymilk, it can be achieved by rehydration at 5 and 10 minute at 85 °C respectively.

In the flavor improvement of soymilk study, the GC data showed that the deodorizer was effective in reducing the original amount of hexanal in HPSF soymilk to the level found in undeodorized triple null soymilk. The sensory evaluation results did show that the deodorizer was able to reduce the soymilk aroma in HPSF soymilk so it would be similar to triple null soymilk at 8 °Brix level.

The study on the fat reduction of soymilk leads to the conclusion that in both processes, soy flake milk yields a better extraction of fat than soybean milk. These findings may be attributed to the processing effect on the soy flakes. There is an indication of a

decrease in fat removal with increase of solid levels of soymilk made from soy flakes. However, the result does not find to be true in soybean milk. The removal of fat from soymilk is followed by a change in the color of soymilk, and the changes are more pronounced as more fat is removed.

Based on these completed studies several future studies are recommended:

1. The effect of high temperature rehydration of soy flakes on yield and quality of tofu.
2. The quality of soy flakes protein (NSI, structure and so forth) compared to whole soybean protein.
3. Evaluation of how fat removal changes the sensory properties of reduced fat soymilk.
4. Color changes during the production of soy flakes should be investigated.
5. The quality of tofu made from the reduced fat soymilk.
6. Sensory evaluation of undeodorized and deodorized soymilk should be re-done with a fresh lipoxygenase-free (triple null) soy flakes or whole soybeans.

APPENDIX I - CONSENT FORM FOR SOYMILK SENSORY EVALUATION

Iowa State University
Soymilk Sensory Evaluation
Consent Form

You are being asked to evaluate the flavors of soymilk. You will be asked to place the soymilk sample into your mouth and taste the flavors and complete a short evaluation form. The time involved in completing each panel will be about 15 minutes. The frequency of the panel is once a week. This study would last until Summer 2002. Your participation is strictly voluntary, and will provide research data for graduate studies thesis.

These tests may pose risks or discomforts to some people; a list of all possible ingredients is included below in the event you know of a substance (soy) to which you have allergic reactions or intolerance. Other discomfort feeling that you might have is from the unpleasant flavor of soybeans, therefore a waste cup is provided, so you would not have to ingest it. You may voluntarily withdraw from this sensory evaluation test at any time throughout testing with out penalty.

No reference will be made to individual judges in any presentation of discussion of data, and no record will remain once data are analyzed.

The evaluation will be done in the CCUR test kitchen at the Food Science Building. Please direct any questions or concern you may have about the project or the tests to Stanley Prawiradjaja at (515) 294 –1873 or you can email me at sprawira@iastate.edu

Your willingness to participate in this panel is greatly appreciated.

List of ingredients included in this test:
Soybean (soy proteins) and water.

Signature_____ **Date**_____

APPENDIX II - SENSORY EVALUATION INSTRUCTION

Evaluation Instruction!

- Please be seated and turn on the light switch in front of you to let us know that you are here.
- Please put your panel number and the date on the forms in front of you.
- Please cleanse your mouth by drinking a glass of water provided before you taste the samples.
- Some flavor standards have been provided as intensity references. Rinse your mouth thoroughly with the water provided in between standards and sample.
- Evaluate the samples from left to right.
- Evaluate the appearance first, and then smell the aroma of the soymilk for 'soymilk aroma' attributes.
- Drink the soymilk and evaluate its aroma, mouth-feel and taste.
- Cleanse your mouth with water or crackers in between samples to remove the flavors from the previous samples.
- When you are done, turn off the light switch and leave the forms in the booth.
- **Don't forget to take the treats and thank you for your participation!**

APPENDIX III - SOYMILK SENSORY EVALUATION FORM

Sensory Evaluation of Soymilk

Sample Number: _____ Panelist Number: _____

Date: _____

Appearance

Yellowness

White — Yellow

Aroma & Flavor

Soymilk Aroma

No soymilk Aroma — Strong soymilk Aroma

Flour/Cereal/ Pasta Flavor

No Cereal Flavor — Strong Cereal Flavor

Beany Flavor (beany and grassy)

No Beany Flavor — Strong Beany Flavor

Painty

No Painty Flavor — Strong Painty Flavor

Mouthfeel

Astringency

Not Astringent — Very Astringent

Taste

Bitterness

Not Bitter — Very bitter

Sweetness

Not Sweet — Very Sweet

Please put any comments below:

APPENDIX IV - SAMPLE SAS PROGRAMS

A. Least Significant Difference (LSD)

```
options formdlim ='-';

Data okara;
Input sample $ gc;
Cards;
;
proc sort;
By sample;
run;

Proc glm alpha=.05;
class sample;
model gc = sample;
means sample/LSD lines;
run;
```

B. Proc glm for sensory

```
options formdlim ='-';

Data soymilk;
input batch temp $ rehydrate panel $ appear aroma pasta beany painty astrin bitter
sweet;
cards;

;

proc sort;
 by temp rehydrate;

Proc glm;
class panel rehydrate temp;
model appear aroma pasta beany painty astrin bitter sweet = rehydrate;

by panel;
output out= outmean2 p = appear aroma pasta beany painty astrin bitter sweet;
run;

proc print data = outmean2;
run;

data mean2;
set outmean2;
keep batch temp $ rehydrate panel $ appear aroma pasta beany painty astrin bitter
sweet;
```

```
if rep=2;

proc print data = mean2;
run;

proc plot;
plot appear*batch = panel;
plot aroma*batch = panel;
plot pasta*batch = panel;
plot beany*batch = panel;
plot painty*batch = panel;
plot astrin*batch = panel;
plot bitter*batch = panel;
plot sweet*batch = panel;
run;

proc glm;
class panel temp rehydrate;
model appear aroma pasta beany painty astrin bitter sweet= panel temp rehydrate
temp*rehydrate;
lsmeans temp rehydrate temp*rehydrate/stderr;

lsmeans temp*rehydrate/pdiff adjust=t;
run;
```

C. 1 way ANOVA

```
options formdlim = '-';

data twelvebrix;
  input rehydration $ solid protein;
cards;

;
/* calculate ANOVA table and printout means and s.e. for each group */
proc glm;
  class rehydration;
  model protein = rehydration;
  lsmeans rehydration /stderr;

/* slightly more complicated - output residuals and plot diagnostics */
proc glm;
  class rehydration;
  model protein = rehydration;
  output out=resids p = yhat r = resid;

proc plot;
  plot resid*yhat;
```

```
  title 'Predicted vs residual plot';

proc univariate plot;
  var resid;

proc print;  /* N.B. not needed if all you want is the residual plot */

/* now add estimates and multiple comparisons procedures to proc glm */
proc glm;
  class rehydration;
  model protein = rehydration;
  /*estimate 'spock - rest ' code 6 -1 -1 -1 -1 -1 -1 /divisor = 6;
  estimate 'BAD: spock - rest' code 6 -1 -1 -1 -1 -1 -1;*/

  /*contrast 'spock - rest' code 5 -1 -1 -1 -1 -1;*/
  contrast 'between treatments' rehydration 1 -1 0 0 0 0,
                                rehydration 0 1 -1 0 0 0,
                                rehydration 0 0 1 -1 0 0,
                                rehydration 0 0 0 1 -1 0,
                                rehydration 0 0 0 0 1 -1;
run;
```

D. Proc mixed

```
options formdlim = '-';

data soymilk;
input source $ brix day sr fat prot;

cards;
;
proc mixed method = type3;
 class source brix day;
 model sr = source brix source*brix/ddfm = satterth;
 random day(source);

lsmeans source*brix/pdiff adjust=tukey;

proc mixed method = type3;
 class source brix day;
 model fat = source brix source*brix/ddfm = satterth;
 random day(source);

lsmeans source*brix/pdiff adjust=tukey;


proc mixed method = type3;
 class source brix day;
```

```
 model prot = source brix source*brix/ddfm = satterth;
 random day(source);

lsmeans source*brix/pdiff adjust=tukey;
```

E. Response surface regression

```
options formdlim = '-';

data twelvebrix;
  input temp rehydration $ milksolid milkprotein;
cards;

;
proc sort;
by brix;

proc means noprint;
 by brix source day;
 var sr fatext;\output out=means mean = sr fatext;

proc rsreg;
by brix;
model milksolid milkprotein = temp rehydrate;

/* To plot surface */
proc rsreg out=preds noprint;
by brix;
model milksolid milkprotein = temp rehydrate;

proc plot;
plot temp*rehydrate = milksolid/contour = 6;
plot temp*rehydrate = milkprotein/contour = 6;
```

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ACKNOWLEDGEMENTS

MAJOR PROFESSOR:
I would like to thank Dr. Lester Wilson
for being my mentor and major professor.
For all the trust, guidance and supports
that you have given.

P.O.S. COMMITTEE:
Dr. Philip Dixon in your patience and
assistance in statistical analyses.

Dr. Patricia A. Murphy for being my
POS committee member and helping
me with my thesis.

SPONSORS:
MicroSoy Inc. Jefferson, Ia
Center for Crops Utilization and Research, Jefferson, Ia

CO-WORKERS, COLLEAGUES
AND PANELISTS:
Thank you for all the patience, energy
and help from all of my undergraduate student
helpers and panelists in your assistance in
helping me completing this study.
Also, Fransiska Yulianti and Mak Ping Shing
for their advices.

ADMINISTRATIVE SUPPORTS:
Thank you for all the help in sending, purchasing,
and administrative support from
the secretaries from 2312 Food Science Building.
(Mildred M. Peterson, Marylou Weigel,
Brenda Emery, Nancy Holcomb, Lani McKinney and Jim Henderson)

FRIENDS:
My special thanks go to Fong Ching-Ching
for helping me to get through the thesis writing process.