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REVIEW ARTICLE

Processing Influences on Composition and Quality Attributes of Soymilk and its Powder

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Abstract Soymilk is the aqueous extract of whole soybeans, resembling dairy milk in physical appearance and composition. The basic steps of its preparation include selection of soybeans, soaking in water, wet grinding, separation of soymilk from fiber (okara), cooking to inactivate lipoxygenase and trypsin inhibitors, formulation and fortification, and packaging. The properly processed soymilk and its derivatives offer many neutraceutical and health benefits. The type of processing and ensuing processing conditions such as high or low temperatures, short or prolonged temperature and cooking time, ultra high temperature, spray-drying parameters, processing treatment combinations with alkali or other chemicals etc. affect the properties of soymilk. The present article focuses on various processing aspects like soaking, blanching, heat treatments, chemical and enzyme treatments, fermentation, homogenization, filtration, spray-drying etc., and their effects on the composition, anti-nutrients, physico-chemical properties, sensory attributes, microbial load, and shelf-life of liquid and powdered soymilk. The applications of some novel techniques such as high-pressure processing, pulsed electric field, ultra high-pressure homogenization, ultrasound, and membrane separations on soymilk processing are also discussed.

Keywords Soybean · Soymilk · Thermal processing · Isoflavones · Viscosity · Color

Introduction

Soybean has been known as the best source of plant protein containing about 40 % of protein (dry basis), the highest protein content among legumes and cereals, and is also rich in nutritive minerals and dietary fiber. Soy proteins are highly digestible after proper heat treatment, and their amino acid profile is well balanced to meet the requirements for human nutrition. Soybean foods have become increasingly popular since the Food and Drug Administration (FDA) approved the soy protein health claim in 1999 [20]. According to that claim, consumption of foods derived from soybean has been associated with many health benefits and 25 g of soy protein per day may reduce the risk of heart disease**.** Several studies have demonstrated the associated advantages of the use of soy products in preventing heart disease, obesity, blood cholesterol, cancer, diabetes, kidney disease, osteoporosis, and blood pressure regulation [21, 30, 53, 78]. Unlike other legumes, soybeans contain a variety of nutritional components that provide health-promoting benefits [5, 84].

Soybean composition includes varying amounts of protein content (38–40 %), carbohydrates (30 %) and fat (18 %). Glycinin and b-conglycinin are the most important soy proteins, as in combination they represent more than 70 % of the total soy proteins [53]. Most fatty acids in soybean and its derivatives are unsaturated and, therefore, susceptible to oxidation [69, 71]. Other components include varying concentration of isoflavone; high levels of minerals including iron, calcium, zinc; vitamins including α-tocopherol, niacin, pyridoxine, and folacin. Soybeans are also a source of anti-nutrient factors such as saponins, phospholipids, protease inhibitors, phytates, and trypsin inhibitors.

Soybeans are transformed into many different varieties of foods to create versatility and provide tasty and easily

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digestible products. Among these soy foods, soymilk has gained much popularity as a healthy food drink. Soymilk has been an important high-quality protein source in the diet of Eastern people for a long time. In Western countries, soymilk has been used mainly as an important replacer of milk for lactose-intolerant people, as well as a low-cost source of good-quality protein and energy, mainly in developing countries [39, 45, 53]. Soymilk contains high amounts of protein, iron, unsaturated fatty acids, and niacin, but low amounts of fat, carbohydrates, and calcium as compared with cow milk and human milk. Containing about 3 % proteins, 2 % fat and 2 % non-lactose carbohydrates (Table 1), soymilk is less likely to be a factor in development of diabetes in later life as per some studies. More importantly, the soymilk contains polyunsaturated and monounsaturated fats, which do not lead to deposition of fats in blood vessels including those in heart and are therefore do not lead to heart disease. Soymilk has high concentration of lecithin and vitamins. It also contains isoflavones, which are strong antioxidants. The properly processed soymilk and its derivatives offer many neutraceutical and health benefits.

Commercial soymilk is produced mainly by conventional technologies using high-temperature heating, homogenization etc. High temperatures, however, may cause undesirable chemical changes, which include destruction of amino acids and vitamins, browning reactions, and development of cooked flavors. A few emerging technologies such as ultra high-pressure homogenization (UHPH), high-pressure processing (HPP), pulsed electric field (PEF) treatment have also been attempted for obtaining soymilks with good nutritional quality, long shelf-life and high colloidal stability. Different processing methods and conditions, applied in preparation of soymilk, ultimately affect their composition, nutritional value, and other functional properties. This review aims at providing a brief overview about different processing technologies used in preparation of liquid and powdered soymilk and their effect on different parameters of soymilk.

Manufacturing Processes for Soymilk

Soymilk is the aqueous extract of whole soybeans, closely resembling dairy milk in physical appearance and composition [71]. Also, called vegetable milk or *Fu Chang* in Chinese, it was reportedly developed in China before the Christian era [73]. The traditional soymilk is a simple water extract of soybean made from soaking the beans in water overnight, wet grinding the beans, steaming the wet mash to improve flavor and nutritional value, and filtering [32] (Fig. 1). Soymilk can also be prepared by reconstituting full-fat soy flour in water or by combining edible-quality soy protein solids, soybean oil, and water. More recently, modified modern flavored versions have hit the mainstream market as meal replacement beverages and cow milk replacer. Apart from its beverage form, soymilk is used as a base in a wide variety of soy dairy analogues, including tofu, soy yogurt/curd, ice cream, and soy-based cheeses [23].

The basic steps of preparation of soymilk include selection of soybeans, adding water, wet grinding and separation of soymilk from fiber (okara), cooking to inactivate lipoxygenase and trypsin inhibitors, formulation and fortification, and packaging of the soymilk. Wilkens et al. [102] developed the "Cornell process," and later the "Illinois

Table 1 Nutritional values of soymilk (per 100 g)

Component	Amount	Component	Amount
Water	93.0 (g)	Sodium, Na	12.0 (mg)
Energy	138.0 (kJ)	Zinc, Zn	0.23 (mg)
Protein	2.8 (g)	Copper, Cu	0.12 (mg)
Fat (total lipid)	2.0 (g)	Manganese, Mn	0.17 (mg)
Fatty acids, saturated	0.214 (g)	Selenium, Se	1.3 (μg)
Fatty acids, mono-unsaturated	0.326 (g)	Vitamin C (ascorbic acid)	0.0 (mg)
Fatty acids, poly-unsaturated	0.833 (g)	Thiamin (vitamin B1)	0.161 (mg)
Carbohydrates	1.8 (g)	Riboflavin (vitamin B2)	0.070 (mg)
Fiber	1.3 (g)	Niacin (vitamin B3)	0.147 (mg)
Ash	0.27 (g)	Panthotenic acid (vitamin B5)	0.048 (mg)
Isoflavones	8.8 (mg)	Vitamin B6	0.041 (mg)
Calcium, Ca	4.0 (mg)	Folic acid	1.5 (μg)
Iron, Fe	0.58 (mg)	Vitamin B12	0.0 (μg)
Magnesium, Mg	19.0 (mg)	Vitamin A	3.0 (μg)
Phosphorus, Mg	49.0 (mg)	Vitamin E	0.010 (mg)
Potassium, K	141.0 (mg)		

Source Soya [91]

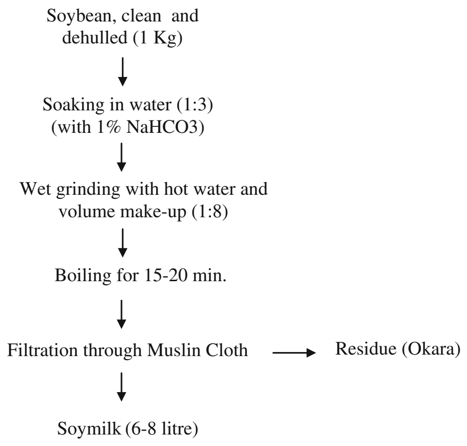


Fig. 1 Traditional soymilk manufacturing process

process" was developed by Nelson et al. [66] for the manufacture of soymilk. Though these conventional soymilk manufacturing processes basically involve the soaking, grinding, filtering, and cooking steps, many process modifications have taken place over the years with the advancement of new processing technologies and considering consumer requirements. In large-scale production, continuous high-temperature short-time processes often substitute normal low-temperature, long-time thermal processes. The advent of ultra high-temperature (UHT) heating and aseptic packaging has further contributed to the production of long-life soymilk packaged aseptically in paper-plastic cartons. They are more convenient for transportation, distribution, and storage [24].

Soy food processing has been reviewed in detail [18, 53]. Basically, there are four basic methods for making soymilk viz. the traditional cold-grind method, the hot-grind method, the hot-blanch method, and the Canadian method. Main steps for making soymilk with these processes are given in Table 2, along with a comparison of product quality and basic plant cost [27].

1. *Cold-grind method*: The age-old Chinese and Japanese method (traditional method) uses cold grinding of well-soaked and rinsed soybean in a stone mill with water. The resulting puree is mixed with additional water and cooked in an open kettle or in a pressure cooker for an adequate time to make it digestible. The cooked slurry is filtered through a filter bag to extract soymilk. The okara (residue in the bag) is pressed to squeeze out remaining soymilk. The squeezed okara is mixed with water and filtered and pressed again to obtain thin soymilk. This thin soymilk is either mixed with the soymilk already extracted or used in place of water in the stone mill when grinding soybean in order to improve soymilk yield. This method gives soymilk with excellent mouthfeel and good yield. However, the soymilk has very high level of the rancid-oil-like smell, which is rather repulsive, especially when soymilk is consumed as a cold beverage rather than as a hot drink.
2. *Hot-grind method*: It generally involves grinding soybeans (with or without hulls, soaked or dry) with hot, almost boiling water, sometimes accompanied with steam injection, to make ground soybean slurry. Most hot-grind methods use sodium bicarbonate or caustic soda to increase the pH of the water to make it significantly alkaline. High temperature and pH substantially inactivate the lipoxygenase enzyme and reduce the rancid-oil-like taste in soymilk [16]. Once the soymilk is extracted, its alkalinity is neutralized by adding hydrochloric or some other acid. Soymilk extracted with this method has significantly less rancid-oil-like off-flavor but suffers from a chalky

Table 2 A comparison of basic soymilk manufacturing processes

Comparison of constituents	Traditional	Hot grind	Hot blanch	Canadian+
Beans	Whole, any type	Whole or dehulled, high quality	Whole or dehulled, good quality	Whole or dehulled, any type
Processing chemicals	None	$NaHCO_3$/HCl, etc.	$NaHCO_3$/HCl, etc.	None
Soaking	Yes	Optional	Hot Blanch	Preferred
Grinding	Cold grind/ filter/cook	Hot grind/filter	Hot grind and high-pressure homogenization	Airless/cold grind/ filter or filter/cook
Soymilk	Dissolved solids	Mostly dissolved solids	Mostly suspended solids	Dissolved solids
Odor	Rancid	Less rancid	Roasted nut	Cereal
Mouthfeel	Smooth	Chalky	Very chalky	Smooth
Protein yield	70–90 %	60–80 %	98 %	70–90 %
Basic plant cost	Low	Medium to very high	High	Low to medium

mouthfeel resulting from the adverse affect of heat on the protein solubility.

3. *Hot-Blanch method*: This is a further improvement over hot-grind method, where the beans are thoroughly blanched in boiling water or alkaline solution for a duration that is long enough to completely inactivate the lipoxygenase enzyme. This hot-blanch method eliminates the rancid-oil-like flavor completely at the cost of adding roasted nut flavor to the product and making the protein almost completely insoluble in water. The blanched soybeans are ground very fine in water, usually in a colloid mill, and the resulting fine slurry is passed through a high-pressure homogenizer. The soymilk thus obtained is a suspension of fine soybean particles in water and in spite of its pleasant taste has very chalky mouthfeel. When alkaline solution is used, the soymilk is neutralized with an acid to achieve a pH in the range of 6.7–7.2.
4. *The Canadian airless cold-grind method:* This method has been developed to keep the virtue of the traditional cold-grind method (the smooth mouthfeel) while eliminating its rancid-oil-like off-flavor in the making of soymilk. In this method, the enzyme lipoxygenase is unable to catalyze the reaction among the reactants (oxygen, water, and oil) to produce off-flavor volatiles like aldehyde, ketones, furans, etc., for one of the reactant (oxygen) is kept out of the reaction volume. No heat treatment is required to partially or fully inactivate the enzyme for controlling or eliminating the off-flavor. Any heat treatment to improve the protein efficiency ratio (PER) and to inactivate the trypsin inhibitor enzyme is differed until soy protein and other solubles are already in the water. Such heat treatment in the later stage of processing is desirable to make the product digestible and to denature any anti-nutritional factors. The chalky taste is thus eliminated without compromising the mouthfeel. No chemicals are at all are required in this method. For good mouthfeel of soymilk, the rule is to avoid heat-treating soybeans above 50 °C prior to or during the grinding process.

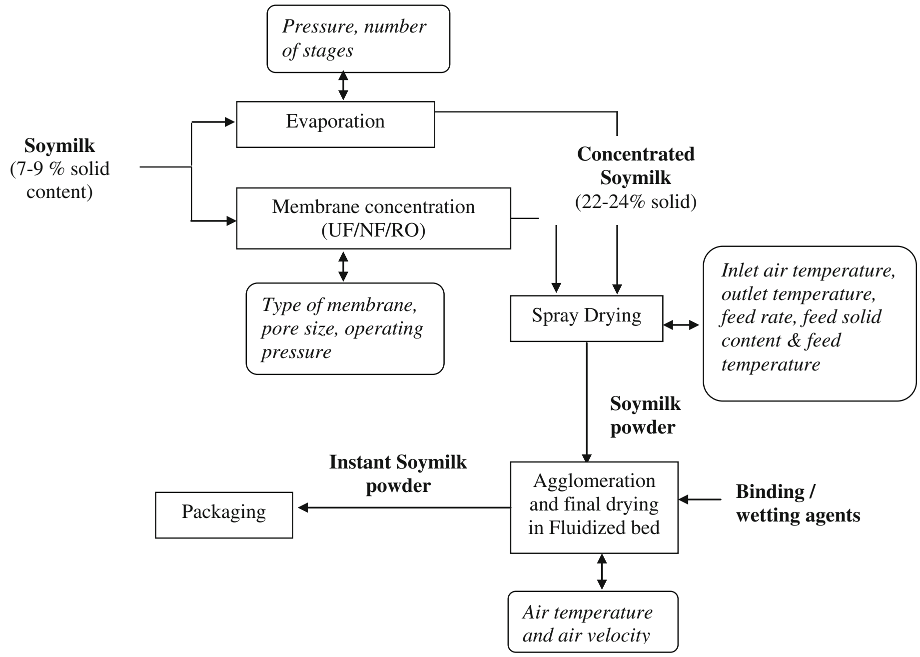
Manufacturing Processes for Soymilk Powder

Soymilk powder is the product obtained by removal of water from liquid soymilk, or by the blending of edible-quality soy protein and soybean oil powders. Soymilk powder shall contain not less than 38.0 % soy protein, not less than 13.0 % soy fat and not less than 90 % total solids [7].

Soymilk powder has a white to light brown color and mixes readily with warm or cold water. It can be made of plain soymilk or can contain additional ingredients, such as sugar, flavors, and calcium. While it is not as common as cow milk powder, soy milk powder can be found manufactured in number of countries including China, Japan, Malaysia, USA, Vietnam, Germany, Brazil etc.

The basic steps in manufacturing of soymilk powder along with process control parameters have been shown in Fig. 2. Spray-drying is the most widely used commercial method for drying milks because the very short time of heat contact and the high rate of evaporation give a high-quality

Fig. 2 Process flow chart for manufacturing of soymilk powder



product with relatively low cost [53]. Spray-drying has been introduced for production of dehydrated soymilk powders by Wijeratne [101]. Evaporation/concentration of the soymilk prior to spray-drying must be less than the usual cow milk since soymilk viscosity would otherwise be too much for the process. Although the main part of the spray-drying equipment for traditional milk powder is used, some equipment modification is necessary to be able to spray-dry the soymilk. Concentration of the soymilk by ultrafiltration (UF) membrane has also been attempted [6, 35]. Suherman [93] developed a new technology for producing soymilk powder materials from suspension, namely fluidized bed drying of inert particle. The drying of pastes and suspensions in fluidized bed of inert particles is a lower cost alternative to the spray-drying, with the same level of product quality.

Agglomeration or instantization is a further process, which allows the finished powdered milk to be quickly reconstituted and dissolved in water. Agglomeration of the spray-dried powders with maltodextrin as an aqueous binder solution using a fluidized bed agglomerator improved the handling and reconstitution properties of the powders. The optimum binder concentration was found to be 10 % w/v maltodextrin, which resulted in the largest particle size of the agglomerated powder (260 μm) having a good flow ability and low cohesiveness [35].

Effect of Processing on Different Parameters of Liquid and Powdered Soymilk

Some of the parameters/quality measures that are important for developing soymilk specifications and for statistical process control are composition/nutritional profile, color, viscosity, particle size and stability, microstructure, volatiles or aroma, microbiological safety, shelf-life and sensory attributes. The quality of soymilk can be measured using both sensory and instrumental techniques [31, 64]. Sensory measures are useful for identifying the product attributes that consumers like, while instrumental measures provide more objective measures. HPLC and other spectrophotometers have been used for determination of components like isoflavones, lipoxygenase, and trypsin inhibitors [40, 51, 104], while instrumentations like Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) are used to examine the microstructure of soymilk [17]. Volatile chemicals in soymilk affect taste and are identified and measured using gas chromatography (GC). The chemicals found in soymilk having the strongest odors are hexanal, acetaldehyde, methanethiol, dimethyl trisulphide (DMTS), and 2-pentyl furan [13]. These chemicals cause off-flavors (beany and sulphurous) in soymilk.

The type of processing and processing conditions such as high or low temperatures, short or prolonged temperature and cooking time, ultra high temperature (UHT), processing treatment combinations with alkali or other chemicals etc. all affect the nutritional, physico-chemical, and sensory properties of soymilk and its powder. The effect of processing on different constituents and quality parameters of liquid and powdered soymilk is presented in Table 3 and discussed in the following sections.

Processing Effects on Proximate Composition and Changes in Nutrient Content

Processing methods and conditions result in varying composition and nutrient levels of soymilk. Khaleque et al. [41] investigated the effect of presoaking soybeans in chemical solutions on the chemical composition and reduction in beany flavor in soymilk. Soymilk prepared from beans presoaked in carbonate contained more protein and had a higher viscosity than milks prepared from beans presoaked in water or sodium hydroxide. Beans presoaked in carbonate were easier to process than the other two. However, there was no significant difference in the amino acid pattern of the proteins in the soymilks prepared by the three methods. Of the chemicals used, sodium carbonate and sodium hydroxide had a significant effect on the reduction in beany flavor in soymilk. Sodium carbonate soaking at 0.4 M concentration for 18–24 h was significantly better than any other presoak treatment.

The effects of heat treatment on nutrient content do not seem to be significant, except when prolonged cooking at extreme temperature is applied. However, excessive heat can cause protein denaturation with concomitant loss of its functionality and generates additional volatile organic compounds responsible for cooked or toasted off-flavors [28]. It is also known that the denaturation of protein by heating increases hydrophobicity [90]. The interaction between protein and lipids are important in achieving acceptable soy product characteristics. Soy lipids play an important role in physical characteristics such as texture and sensory quality of products made from soymilk [15, 105]. A lot of the soy neutral lipids are in particulate phase of unheated soybean milk. With heating, half of the phospholipids are retained in the particles and half move to the soluble phase. When heated at 75 °C, lipids in the soluble and particulate fractions are liberated and shift to the floating fractions. Almost all lipid shifts to the floating fraction at 90 °C then proteins in the fractions are rearranged by heating [68, 85].

Iwuoha and Umunnakwe [34] demonstrated that processing method, storage temperature, and storage duration have significant combined effects on the proximate chemical composition, physicochemical, and sensory attributes of soymilk. Processing variables included in the study included

Table 3 Effect of chemicals/processing methods on quality parameters of soymilk

Agent	Type/conditions	Used in	Effect on product	References
Chemicals	Sodium carbonate (0.4 M)	Soaking of soybeans	More protein content and higher viscosity of soymilk; easier to process; reduction in beany flavor; accelerate the rate of inactivation of trypsia inhibitor during steaming/boiling	[41]
Enzymes	Celluclast 1.5L, 1.2 % (v/v) CGTase,	As a treatment to soymilk	Reduction in the size of particles present in the whole soymilk; lower level of chalkiness and higher physical stability compared to the single or double-homogenized products; increase in viscosity of soymilk	[81]
			Increases isoflavone components and the formation of isoflavone aglycones, higher sensory scores for taste preference than control soymilk	
Heat	80–150 °C for various durations	Cooking	Denaturation of soy protein; cooked or toasted off-flavors; decline in lysine content (when heated for prolonged period); movement of soy lipids to soluble phase; decrease in the particle size distribution and improved its stability; increase in viscosity; inactivate the enzyme lipoxigenase (LO) and trypsin inhibitors	[8, 15, 36, 48, 49, 55, 67, 68, 85, 95, 105]
High hydrostatic pressure	300–700 MPa	Pre-treatment of the hydrated beans	Protein denaturation and increase in insolubility; increase in viscosity of soymilk; increased shelf-life of soymilk up to two weeks in refrigerated storage	[38, 50, 89, 109]
Pulsed electric field	18–22 kV cm^{-1} and number of pulses from 0 to 100	As a treatment to soymilk	Increase in soy protein dispersion and viscosity; inactivation of soybean lipoxygenase	[51, 103]
Ultra high-pressure homogenization	300 MPa	As a treatment to soymilk	Less particle settling; protein denaturation and increased protein stability; considerably reduced initial counts, spores and enterobacteria counts	[17, 74]
Ultrafiltration	20,000 molecular weight cutoff	Filtration	Removal of low-molecular-weight anti-nutritional factors, in particular the oligosaccharides, raffinose and stachyose, and phytic acid	[6]

blanching, moist- and dry-heating, wet- and dry-dehulling, wet- and dry-milling. Higher protein value was found in the dry-dehulled and dry-milled samples compared to the blanched and wet-milled samples. It was attributed to the fact that dry heat has a less damaging effect on protein than moist heat. Soymilk samples stored at ambient temperature (29 ± 1 °C) showed a reduction in protein and fat, and an increase in moisture content and carbohydrate. Results of the quality parameters studied showed that longevity, stability of physical and chemical characteristics, and organoleptic acceptance of the soymilk were better under refrigeration (10 ± 2 °C) and best under frozen (−3 ± 1 °C) storage. According to them, soymilk produced from flour produces better nutritional profile and more desirable physicochemical properties than milk produced from wet blanched beans. The most affected parameters of soymilk during processing include protein, fat, fiber, viscosity, and flavor, while the least affected are moisture, carbohydrates, specific gravity, and mouthfeel.

With respect to essential amino acid content, even at higher temperatures, no significant changes were observed in available lysine during a 3-h heating period at 95 °C [49]. In fact at elevated temperatures of 120 and 140 °C optimum heat processed soymilk had higher levels of available lysine than did soymilk processed at 95 °C. But, prolonged heating at these temperatures (120 and 140 °C) caused a decline in available lysine content.

High-pressure processing (HPP), a novel food processing and preservation technology with many advantages [12], has also been tried as an alternative to thermal processing for soymilk production, and effects of high-pressure processing have been investigated on changes in soy protein [38, 50, 109]. High pressure could denature soy protein completely and exposed hydrophobic regions. Denaturation occurred at 300 and 400 MPa for 7S and 11S globulins in soymilk, respectively. These indicated that soy proteins were dissociated by high pressure into subunits, some of which aggregated and became insoluble [109]. The content of protein decreased and fat increased in soymilks prepared from pressurized soybeans with increasing pressure level.

Low-speed centrifugal fractionation can be applied to separate the large oil-protein particles (2–60 µm) from raw soymilk and can be used as an innovative method for preparing low-fat soymilk and 11S protein-enriched ingredients [56]. The large particles were separated by centrifuging raw soymilk for 5–30 min at a low gravitational force ranging from 96 to 2,410 g. Chemical analysis showed that 80–90 % of the total lipids and 30–40 % of the total proteins were located in the precipitated fraction. The supernatant fraction had a dramatically higher protein-to-lipid ratio than the whole soymilk.

In case of powdered soymilk, the effect of spray-drying on the protein profile, amino acids composition, carbohydrate level, and nitrogen solubility index of the powder has been studied [3]. The protein dispersibility index (PDI) of the spray-dried powder was altered by changing the processing conditions used during manufacture or spray-drying. The PDI of the spray-dried soymilk base was higher when ammonium bicarbonate rather than sodium bicarbonate was used for blanching the cotyledons. The PDI of the spray-dried powder increased when the soybean slurry was homogenized at high pressures and when sodium bisulfite was added to the soymilk base before drying. Sodium bisulfite was the most effective single treatment for increasing PDI. Increasing the pH of the soymilk base to 9 before spray-drying increased the PDI of the soy powder.

Spray-drying studies were also carried out on UF soymilk concentrates [6]. The nitrogen solubility index (NSI) of the spray-dried powder improved with percent water removal during UF and also by the addition of sucrose to the concentrate prior to spray-drying. There is also very little, if any, detectable difference in the taste and flavor. Ultrafiltration increased total solids, protein, and fat contents, but decreased carbohydrate and ash contents of soymilk, leading to an increase in particle size, wettability, and dispersibility of the resultant spray-dried powders [35].

Processing Effects on Isoflavone Composition

Isoflavones are a group of naturally occurring heterocyclic phenols found in soybean and foods derived from soy [94]. The major isoflavones in soybeans are genistein and daidzein and their corresponding aglucones, genistein, and daidzein. The third kind of isoflavone includes the glycitein and its 7-O-β-glucoside, glycitein [97]. Acetylated (600-O-acetylgenistein, 600-O-acetyldaidzin and 600-O-acetylglycitein) and malonylated (600-O-malonylgenistein, 600-O-malonyldaidzin and 600-O-malonylglycitein) isoflavones were also found in soybeans [43]. These compounds are particularly important due to their diverse pharmacological and antioxidant properties.

The processing operations and conditions applied for production of soy-based products and ingredients determine the final content and profile of isoflavones [54, 65, 97]. Extraction in water is the first important processing step in the recovery of isoflavones from soy matrices to produce soymilk, tofu, and soy protein isolate (SPI). During soymilk and SPI production, isoflavones can be partially retained in the fiber fractions. These losses in the fiber fractions can be minimized by adjusting temperature and pH [75, 79, 80, 92]. The adjustments should be done carefully as an increase in extracted isoflavone can negatively affect the extractability of protein [10]. The conversions of isoflavones during processing are dictated by both their chemical structure, and other parameters such as pH, temperature, moisture, and activity of endogenous b-glucosidases [33]. Reduced levels of isoflavone in products like protein isolate (600–1,000 µg) in comparison with soybeans and flour is a result of aqueous processing, mainly aqueous alcohol.

Soymilk contains approximately 4–7 mg total isoflavones/100 g with considerable variations both in composition and content [42, 65]. Interestingly, the isoflavone content of soymilk made from soy protein isolate (SPI) is much lower than that made from whole soybeans because the mild alkali extraction used in the production of SPI causes isoflavone losses of approximately 53 % [98]. According to Murphy et al. [65], aseptically processed (i.e. ultra high temperature) soymilk contains predominantly concentrations of β-glucoside isomers whereas pasteurized soymilk contains mainly isoflavone concentrations as malonylglucosides. A detailed kinetic study of the rearrangement of the different forms of isoflavone during heat processing of soymilk indicates that in raw soymilk, the predominant forms are the malonylglucosides [63]. If the soymilk is not heat-processed, less than 1 % isoflavone glucosides are hydrolyzed by native soybean glucosidases to the aglycones. Immediate heat processing of soymilk (after extraction from soybeans) to model aseptic heat processing results in conversion of malonylglucosides to glucosides. Few acetylglucosides are produced until soymilk is exposed for longer than 60 min at 80 °C. Yen and Kao [107] investigated the effects of processing conditions on the changes in isoflavone content of black soymilk and soymilk film (*yuba*). When black soybeans were soaked in water at 30 and 50 °C for various periods of time, the contents of daidzein and genistein increased with increased soaking time while daidzin and genistein decreased. No significant differences ($P > 0.05$) were observed in the contents of isoflavones in black soybean under soaking conditions at 30 °C for 12 h and at 50 °C for 6 h. But, the amounts of isoflavones in black soybean changed markedly under the soaking conditions at 20–60 °C for 8 h. The change in the isoflavone contents in black soybean during soaking was attributed to its beta-glucosidases activity. The effect of soaking temperature on beta-glycosidase activity of black soybean was in the order of 50 > 40 > 60 > 30 > 20 °C. The contents of daidzein and genistein in *yuba* prepared by soaking soybeans at 50 °C for 6 h were about 2.5 times of that prepared by soaking soybeans at 30 °C for 12 h, which suggested that change.

The effect of hot and cold grinding as well as the effect of direct and indirect ultra high-temperature (UHT) treatment conditions on the level of isoflavones during the manufacture of soymilk was studied [75]. Soymilks were manufactured from dehulled soybeans by hot grinding or cold-grinding processes. After inactivation of lipoxygenase at 85 °C, the resulting slurries were decanted and supernatants were held at 120 °C for 80 s to inactivate the trypsin inhibitor. The decanted soya bases were cooled and subjected to different temperature/time regimes by direct and indirect UHT treatments. Results showed that hot grinding caused an improvement in the extraction of isoflavones into the soymilk compared to cold grinding and there was no apparent difference in the loss of isoflavones due to direct UHT heating compared to indirect UHT heating.

Kao et al. [40] studied the stability of isoflavone glucosides during processing of soymilk and tofu. The recoveries of all the isoflavones in soybean, soaked bean, soymilk, and tofu ranged from 65 to 91 % based on the extraction and HPLC method used in the study. Results showed that the concentrations of the three aglycones, daidzein, glycitein, and genistein increased with increasing soaking temperature from 25 to 45 °C and soaking time from 0 to 12 h, while a reversed trend was found for the other nine isoflavones, nine glucosides malonylglucosides malonyldaidzin, malonylglycitein, and malonylgenistein. After soaking at 45 °C for 12 h, the concentrations of daidzein, glycitein, and genistein increased by 403.1, 190.9, and 779.9 μg/g, respectively, from their initial values probably because of conversion from daidzin, glycitein, and genistein. However, the concentrations of total isoflavones followed a decreased order for the increase in soaking temperature and time. In addition, soaking temperature had a greater impact on the yield of total isoflavones than soaking time.

The effects of high-pressure processing have been investigated on isoflavone content [37], in which high hydrostatic pressure was applied to hydrated soybeans (100–700 MPa, 25 °C) and soymilk (400–750 MPa; 25 and 75 °C). Neither pressure level nor initial treatment temperature affected soymilk isoflavone content. However, combined pressure and mild thermal treatment modified the isoflavone distribution. At 75 °C, the isoflavone profile shifted from malonylglucosides toward β-glucosides, which was correlated with the effect of adiabatic heating. When pressure was applied to the hydrated soybeans, the soymilk isoflavone concentration varied between 4.32 and 6.06 μmol/g. The results showed that high-pressure processing applied as a pre-treatment of the hydrated beans in soymilk production does not increase the water-extractability of isoflavone during soymilk production and that overall only small changes in isoflavone profile and content occur in these conditions.

Fermentation of soymilk with probiotic lactic acid bacteria and yeasts have been found to improve antioxidant activity, which are closely related to the conversion of isoflavones in the soybeans from glycosides to aglycones [52, 77, 99]. The β-glucosidase enzyme that hydrolyzes glucosides to aglycones is very sensitive to molecular structure and thus the profile of the isoflavones can affect their rate of hydrolysis. In fermented soymilk both the inhibition of ascorbate autoxidation, and the reducing activity and scavenging effect of superoxide anion radicals varied with the starters used, but nevertheless are significantly higher than those found in unfermented soymilk. Antioxidative activity in soymilk fermented with lactic acid bacteria and bifidobacteria simultaneously was significantly higher than that fermented with either individually [99]. The activity increased as the fermentation period was extended. All the lactic acid bacteria produced β-glucosidase enzyme, which hydrolyzed isoflavone glucosides to aglycones at a significant level in the fermented soymilk [77].

To compare the antioxidative activity of soymilk powder, the fermented and unfermented soymilk was subjected to both spray-drying and freeze-drying [99]. Spray-drying was found to cause a significantly ($P < 0.05$) higher reduction rate of antioxidative activities than freeze-drying. The destruction of superoxide dismutases (SOD), glutathione, isoflavones, and other antioxidative-related enzyme and compounds might have occurred during the process of dehydration of soymilk and resulted into the reduced activities. While these effects were more pronounced with spray-drying than freeze-drying and thus led to an increased reduction in antioxidative activities observed with the spray-dried fermented soymilk.

Processing Effects on Anti-nutrients in Soymilk

Lipoxygenase Enzyme

This enzyme is responsible for causing the development of characteristic “beany” off-flavor and bitterness in soymilk. Lipoxygenases (LOX) are dioxygenases that catalyze the addition of molecular oxygen to polyunsaturated fatty acids containing the group *cis, cis* -1,4-pentadiene. The bad taste is imparted by the catalytic action of lipoxygenase enzyme in synthesizing off-flavor, causing volatiles from lipids in the bean in the presence of water and oxygen [11, 19]. Lipoxygenase, distributed throughout the soybean cotyledons, becomes active as soon as their cell structure is broken. Therefore, the control of off-flavors has traditionally been done by inactivating the enzyme, such as by heating and/or altering pH of the aqueous medium in which the seeds are disintegrated. The problem with these treatments has been that they tend to insolubilize the soybean protein and thereby reduce soymilk yield and make it chalky in mouthfeel [2, 14, 51]. The degree of enzyme inactivation required to reduce the off-flavors to acceptable level leads to an unacceptably low protein solubility. An approach of tackling the problem is to only partially inactivate the enzyme, remove most remaining off-flavor by deodorization, and mask any residual off-flavor by flavoring. In another approach, the enzyme is totally inactivated prior to disintegrating the beans, and the resulting insoluble soybean protein is dispersed in water by fine grinding and high-pressure homogenizing.

Trypsin Inhibitor

Protease inhibitors (PIs) are generally considered the main antinutritional factors in soybeans. Soybean PIs belong to a broad class of proteins that inhibit proteolytic enzymes, such as trypsin and chymotrypsin. Both compounds are important animal digestive enzymes for splitting proteins to render dipeptides and tripeptides [83]. However, the specificity of these inhibitors is not necessarily restricted to trypsin and chymotrypsin but also to elastase and serine proteases for which serine constitutes the active site. Unless thoroughly inactivated, this enzyme could limit the growth of growing humans and animals as inferred from studies on laboratory animals. Trypsin inhibitor (TI) can be inactivated by heat and chemical treatment.

The many different methods by which soybeans are processed usually reduce TIs content by up to 90 % [29, 95]. Inactivation of TIs in whole soybeans can be achieved by atmospheric steaming (15 min) if the initial moisture content is about 20 %. If beans are soaked in water overnight to reach 60 % of moisture, 5 min in boiling water is sufficient to inactivate trypsin inhibitors [82]. The rate of inactivation of TI in soy milks prepared from carbonate presoaked beans was faster than that of the water presoaked preparation when processed at 98 °C, and this effect was primarily associated with the change that occurred in the pH of the former system [96]. The effect of alkaline pH's at 98 °C on the inactivation of trypsin inhibitor was examined, and it was found that the rate of inactivation was changed from zero order at pH 6.8 to first-order kinetics at pH 9.9.

Processing temperature and cooking time have been demonstrated to inactivate the enzyme lipoxigenase (LOX) and other antinutrient factors such as trypsin inhibitors [48]. However, thermal destruction of these compounds depends on temperature, heating time, particle size, and physical condition of the product. The inactivation can be accelerated or delayed by the addition of basic or acid compounds [8, 76]. Lipoxigenase was mostly inactivated by heating with residual activity of 14 % when 0.01 M sodium carbonate was used compared to water (residual activity 46 %). Lipoxigenase can be destructed completed by a short heating time from 70 °C to boiling temperature of 96 °C. But, TI is not fully inactivated at 95 °C for many minutes [22]. Trypsin inhibitor is only inactivated to acceptable levels in soymilk in the final UHT step at 135 °C for 2 min. Several studies have reported to have successfully destroyed TI within temperature ranges of 100 °C [59] to 121–154 °C and at time intervals of 10–90 s [46]. TI in soymilk was satisfactorily destroyed to 10 % of original concentration at 143 and 154 °C with 62 and 29 s heating time, respectively. Though not quite significant, but conventional heating other than the microwave heating also decreases trypsin inhibitor activity in soymilk.

Lipoxygenases activities and hence beany flavors in soymilk could be reduced with hot water blanching and grinding at temperature above 80 °C. However, hot water blanching also affected the non-beany aromas of soymilk. A suitable blanching and grinding time is thus necessary to achieve a balance of soymilk flavors [58]. When the soaked soybeans were blanched and ground with hot water for 2–6 min, the LOX activity was between 38 and 57 % of the initial value. For these processing times, the non-beany compounds could be largely maintained.

Other established technologies, such as the ultra high-temperature (UHT), pulsed electric field (PEF), and high-pressure processing (HPP) process, have also been reported to inactivate lipoxygenase and TIs. In UHT process, the holding times required to inactivate 90 % of trypsin inhibitors in soymilk (pH 6.5) were 60 min, 56 s, and 23 s at 93, 143, and 154 °C, respectively [48]. It is also recommended by the authors that heat-inactivation of trypsin inhibitors in soymilk at temperatures below 100 °C prior to the UHT process because prolonged heating at higher temperatures may destroy lysine, sulfur amino acids, and vitamins.

Pulsed electric field treatment is a good alternative to heat pasteurization and enzyme inactivation for liquid food preservation. PEF allows the inactivation of deleterious enzymes at different levels, depending on the enzyme itself and the medium in which it is suspended [61]. Li et al. [51] studied the effects of pulsed electric field (PEF) parameters on soybean LOX by exposing soymilk to PEF strengths from 20 to 42 kV/cm, up to 1,036 µs treatment time, with 400 Hz of pulse frequency and 2 µs of pulse width in square wave pulse of bipolar mode. LOX activity was measured using a continuous spectrophotometric rate method based on the enzymatic oxidation of linoleic acid to the hydroperoxide of linoleic acid. One unit of LOX activity was defined as a change of 0.001 units of absorbance per minute per milliliter of enzyme extract. The percentage of residual activity $RA(\%)$ of soybean LOX was defined as

$$RA(\%) = 100 \times (A/A_0) \tag{1}$$

where A is the soybean LOX activity of PEF-treated and A_0 is the initial soybean LOX activity of un-treated soymilk.

Residual activity of soybean lipoxygenase decreased with the increase in PEF treatment time and strength. The maximum inactivation of soybean LOX achieved was 88 % at 42 kV/cm, 1,036 µs, 400 Hz of pulse frequency and 2 µs of pulse width at 25 °C. Moreover, pulse frequency (100–600 Hz) and pulse width (1–5 µs) were also tested at constant pulse strength of 30 kV/cm and treatment time of 345 µs. In comparison with different kinetic inactivation models, Weibull distribution function (Eq. 2) was most suitable model describing the inactivation of soybean LOX as a function of PEF process parameters [51]. The results indicated pulse frequency and pulse width significantly influenced inactivation of soybean LOX ($P < 0.05$).

$$RA = RA_0 \times e^{-(p/\alpha)\gamma} \tag{2}$$

where RA is the residual activity (%) of soybean LOX PEF-treated, RA_0 the initial enzyme activity of un-treated soymilk (100 %), p the PEF parameters (time, strength, pulse frequency and width), α is the scale factor, and γ is the shape parameter.

High hydrostatic pressure (HHP) processing in combination with thermal treatment (250/50, 550/19, 550/65, and 550/80 MPa/ °C) was applied to soymilk made from previously soaked soybeans (in distilled water or 0.5 % sodium bicarbonate solution) [25]. Treatment combination at higher pressures and temperatures for selected holding times resulted in an increased inhibition rate of trypsin inhibitors in soymilk. Residual trypsin, at 550 MPa and 80 °C, was high at higher HHP holding times. The highest percentage of residual trypsin (76 %) was estimated after a 15-min holding time. The use of sodium bicarbonate for soaking of soybeans synergistically decreased the trypsin inhibitor activity in soymilk in comparison with residual trypsin using distilled water alone.

Ultrafiltration (UF) technology has been used for the removal of low-molecular-weight anti-nutritional factors, in particular the oligosaccharides, raffinose and stachyose, and phytic acid [6]. Using a membrane having a 20,000-molecular weight cutoff, the rejection rates of the oligosaccharides and phytic acid increased during ultrafiltration. At 60 % water removal, over 80 % of each of the oligosaccharides was removed. In the case of phytic acid, however, only 50 % could be removed. This could be because phytic acid exists as phytates or associated with native protein by salt linkages, and thus complete, or near complete, removal of this would be difficult to achieve even if multiple-stage ultrafiltration is employed.

Processing Effects on Particle Size and Protein Stability

Soymilk contains colloidal fat and protein and in some cases cellular particles. The size and distribution of these particles, which affects mouthfeel, can be measured with a particle size analyzer. Creaming and sedimentation of soymilk are undesirable, and potential particle instability in soymilk can be identified using turbidity measurements, based on changes over time of the back-scattering of light. The chalkiness attribute is related to the sensation of chalky powder in the mouth after the ingestion of the product, which has been attributed to the particles present in soymilk that are retained in the pores and in the mucous membranes of the mouth during ingestion. Many researchers have tried to solve this problem by introducing a filtration stage through a decanter or continuous filtration in the process of soymilk manufacture, in order to remove the particles suspended in the product [100].

The effect of heat treatment at 95–100 °C for 5 min and homogenization on the physicochemical properties of soymilk was studied [67], determining the particle size distribution and the amount and type of protein present after step-wise centrifugation. Soymilk was prepared according to the procedure of Mullin et al. [62] with slight modifications. A portion of soymilk so prepared was heated at 95–100 °C for 7 min and then cooled to room temperature in an ice-water bath (heated soymilk). A portion of the heated soymilk was passed through a valve homogenizer at 69 MPa at room temperature for four passes (heated-homogenized soymilk). A step-wise centrifugation procedure was applied to soymilk from each of these treatments to obtain four different supernatant phases by centrifuging the soymilk at 8,000, 15,000, 40,000, and 122,000g at 20 °C for 30 min. Differential scanning calorimetry of soy protein showed three thermal transitions for unheated soymilk at 54, 70, and 94 °C, which were attributed to 2S, 7S and 11S, respectively. These thermal transitions were absent from heated (and homogenized) soymilk. Unheated soymilk showed a large average particle size, a broad size distribution, and significant protein precipitation with centrifugation. Heating of soymilk decreased the particle size distribution and improved its stability. Homogenization also resulted in a decrease in particle size, with a narrower size distribution compared to heated soymilk. During step-wise centrifugation, changes in the ratios of 11S (glycinin) and 7S (b-conglycinin) in the supernatants were noted, and they depended on the treatments applied to soymilk [67]. Particle size showed significant differences between soymilks homogenized by one pass and two pass [87].

Ultra high-pressure homogenization (UHPH) produced a highly physically stable product although with color changes in comparison with control base product (BP) and Ultra high-temperature (UHT) soymilk [17, 74]. Physical stability of soymilk was measured on days 1, 30, and 60 after processing, and the stability index was expressed as the % (w/w) of particles sedimented after centrifugation. The higher the value of this parameter, the lower the stability of soymilk, as a consequence of particle sedimentation. This index could also be related to the graininess perceived in the mouth [17]. UHPH-processed samples showed less particle settling than BP and UHT soymilks, and these differences were also observed at days 30 and 60 of storage at 4 °C (Fig. 3). Differential scanning calorimetry analysis indicated that soymilk proteins were partially denatured by 200 MPa pressure, whereas UHPH treatment at 300 MPa showed the same extent of denaturation as UHT soymilk.

In another study, the effects of high pressure (400, 500, and 600 MPa), dwell time (1 and 5 min), and temperature (25 and 75 °C) on protein stability of soymilk were evaluated [89]. After processing and during storage, there were significant differences in protein stability between untreated (control) and pressurized samples. In addition, the stability of pressurized soymilk can be maintained for 28 days at 4 °C.

The use of enzymes has also been tried during soymilk production in order to reduce the particle size and chalkiness sensation, as well as to improve the physical stability of the milk [81]. Enzymatic treatment (Celluclast 1.5L and Pectinex ultra sp from Novozymes Biotech, Inc., and several Rohalases from Rohm Enzyme GmbH) was carried out in the homogenized sample (single run), using enzyme concentration of 1.2 % (v/v) and 3 h of treatment. Celluclast 1.5L treatment and filtration in a 20-µm pore size tissue filter resulted in an effective reduction in the size of particles present in the whole soymilk. Such reductions originated products with lower level of chalkiness and higher physical stability compared to the single- or double-homogenized products. Fermentation of soybean with *Monascus* yeasts prior to soymilk manufacturing has been found to increase the solubility of soy proteins within the acidic region [52].

The particle size soymilk powder mainly depended on inlet heated-air temperature of the spray dryer, and a decrease in particle size was observed with increasing inlet temperature [72].

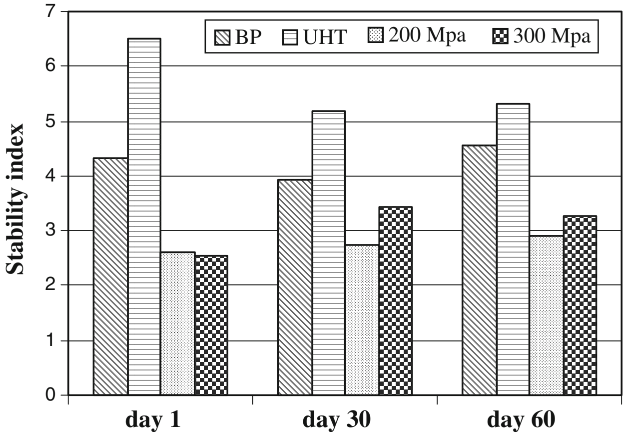


Fig. 3 Stability index of BP, UHT, and UHPH-treated soymilks during storage (adapted from Cruz et al. [17])

Processing Effects on Color

Non-enzymatic browning, mainly due to the Maillard reaction, occurs in soymilk during heat treatment. The measurement of browning is useful in evaluating the quality of soymilk due to the fact that proteins participate in the reaction. The degree of browning is used as a quality index to estimate protein damage in soymilk. The Hunter L-values of soymilk processed at 93 and 121 °C for various times and also spray-dried at different drying temperatures were measured and found that the L-values decreased with heating time indicating that browning of the soymilk was induced by heat [95]. Johnson et al. [36] also evaluated the effect of direct steam infusion cooking on chemical browning of soymilk at various temperatures (99–154 °C) at pH 6.7 and 9.5. In this processing method, the color change in the soymilk was influenced by the heat effect as well as by the yield of solids in the soymilk fraction. Studies on the browning of ultra high-temperature (UHT) processed soy beverage stored at different temperatures (5–45 °C) revealed that the change in the reflectance of UHT soy beverage at 450 nm followed first-order kinetics with an activation energy equal to 41.2 kJ/mol [4].

The effect of temperature and time of heating on color changes in soymilk has been studied using both instrumental analysis and sensory evaluation methods [47]. The heating temperatures and times used covered a range from 80 to 140 °C and 0.5–180 min, respectively. The color L*-value decreased with the increase in heating temperature and time, following first-order kinetics. Unlike cow milk, soymilk did not exhibit an initial whitening phenomenon upon heating. The a*- and b*-values increased with heating time and temperature following zero order kinetics. The increase in b*-value indicates an increase in the yellowness of the color. Both a*- and b*-values can serve as indicators of the browning reactions. Compared with previously reported results for cow milk, the changes in the CIELAB values in soymilk due to heat appeared to follow similar patterns, but the reactions were slower and less temperature sensitive. As compared to cow milk, soymilk is less sensitive to chemical browning. The slower browning rate in soymilk could be explained by its very low reducing sugar content (glucose) compared to the relatively high lactose content in cow milk, which favors sugar–amine reaction. Another factor that could possibly affect the kinetics of Maillard browning is the structure and conformation of the protein molecules, which participate in the reaction. The whey proteins in cow milk are heat sensitive and readily denatured (unfolded) by heat to enable more amino side groups to become available for the browning reaction. On the other hand, there are many evidences that soy proteins are rather heat stable, which permits the use of a more intense heat treatment in soymilk processing.

Processing Effects on Viscosity

Viscosity of soymilk has been found to be affected by processing methods and conditions. Soymilk prepared from beans presoaked in carbonate contained more protein and had a higher viscosity than milks prepared from beans presoaked in water or sodium hydroxide [41]. Fortification of soymilk with calcium gluconate and calcium lactate significantly increased its viscosity [70]. The effects of selective thermal denaturation (STD) on soymilk viscosity and physical properties of tofu were investigated [55] with three soybean samples and varied soymilk solid contents (10–12 %). Compared to one-step heating at 95 °C for 5 min, two-step heating (75 °C, 5 min and then 95 °C, 5 min) increased the soymilk viscosity by up to 6 times. The maximum viscosity was reached when the first-step heating temperature was 70 °C. DSC results showed that separate denaturation of *β*-conglycinin and glycinin during two-step heating was related to the increase in soymilk viscosity. The differences in viscosity between two-step and one-step heating decreased with decreasing soymilk protein content.

Rheological characterization of both soymilk treated with Celluclast 1.5L and filtered in the 20-μm pore size tissue is given [81]. The increase in the soymilk viscosity with the enzymatic treatment can justify the highest physical stability degree and the sensorial body attribute. As expected, the filtrated soymilk presented a smaller value of viscosity than the control due to the removal of insoluble solids during the process. However, the lower level of insoluble solids in the filtered soymilk, which also confers a higher physical stability of the product, led to a smaller value of body attribute in the sensory analysis.

The rheological properties of soymilk were affected by PEF treatment [103]. The apparent viscosity of soymilk increased from 6.62 to 7.46 (mPa.s) by increasing the intensity of electric field from 18 to 22 kV cm^{-1} and number of pulses from 0 to 100. The viscosity of soymilk was also found to increase with increasing pressure treatments from 100 to 500 Mpa [109] (Fig. 4). The same phenomenon happened with heated samples. Soy protein dispersion increases the viscosity after heating and undergoes an irreversible change to the progel state [106]. The changes in viscosity and phase of soymilk after high-pressure treatments indicated that the soy proteins in soymilk had been modified to form colloidal phase.

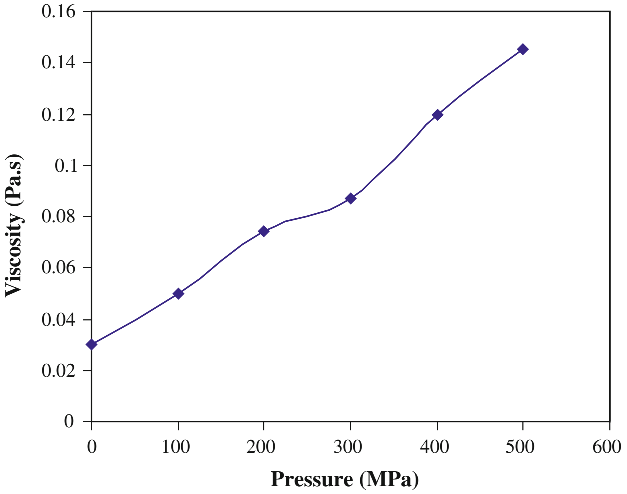


Fig. 4 Effect of high pressure on viscosity of soy milk (adapted from Zhang et al. [109])

Processing Effects on Sensory Attributes

The sensory attributes of perceived color and flavor are the most important characteristics in soymilk because they are readily assessed by consumers. The off-flavors of soymilk is generally described as: “beany,” “green,” “bitter,” “grassy,” “painty,” and “astringent.” The panel may describe poor texture as: “gritty,” “floury,” “chalky.” Soymilk when subjected to severe heating acquires a brown color and cooked flavor [44].

Application of different enzymes (CGTase, pullulanase, α-glucosidase, α-glucosidase, pectinase, naringinase, hesperidinase, and betaglucuronidase) in soymilk production not only increases isoflavone components and the formation of isoflavone aglycones, but the CGTase-treated soymilk, for instance, showed a higher taste preference than control soymilk. Several hydrolytic enzymes-Celluclast 1.5L, Pectinex ultra sp, Rohalases (SEP, F, 7069 e 7118) and filtration with different pore size tissues (20; 30; 85; 100 mum) independently studied, regarding their effect on the sensory quality of whole soymilk, indicated further that enzymatically treated soymilk resulted in a higher overall impression of flavor than the filtered product and products submitted to one and two homogenizations [81].

Processing Effects on Microbial Safety and Shelf-Life of Soymilk

Soymilk contains most of the soybean components including protein, lipid, and saccharides [26]. Consequently, fresh soymilk has a very short shelf-life. Thermal processing is the most common practice used to improve the microbial safety and extend the shelf-life of soymilk because it inactivates vegetative pathogens and many spoilage bacteria [45]. The traditional processing involving temperature of 90–100 °C, applied up to 30 min [108]. In some conditions, thermal processing detrimentally affects nutritional and quality attributes of soymilk and produces strong off-flavors [57]. It limits the development of soy foods that are appealing to consumers and negatively impacts the use of heat-treated soymilk as an ingredient [1, 44]. Because of these detrimental effects of thermal treatment on soymilk properties, other processing methods such as high-pressure homogenization, high-pressure throttling, and pulsed electric field have been applied to soymilk in order to improve its shelf-life [51, 86, 88].

Ultra high-pressure homogenization (UHPH) at 200 and 300 MPa considerably reduced initial counts, spores, and enterobacteria counts, thereby indicating the potential of this technology to process soymilk for its preservation [17]. Poliseli-Scopel et al. [74] found that UHPH treatments at 200 and 300 MPa were more effective than pasteurization against almost all the microorganisms. However, significant differences were detected between UHPH samples at 200 MPa (55 and 65 °C inlet temperature) and pressurized samples at 300 MPa. It was concluded that UHPH at 300 MPa and 75 °C inlet temperature was able to produce commercially sterile soymilks.

High-pressure processing (HPP) treatment was found capable of improving shelf-life without negatively impacting the stability of the soymilk [89] and thus can be an alternative commercial process to traditional thermal treatments for extending the shelf-life of refrigerated soymilk. Pressure at 400 MPa (5 min), 500, and 600 MPa (1 and 5 min) produced 100 % sub-lethal injury in surviving bacterial populations irrespective of temperature. After 28 days of refrigerated storage, both aerobic and anaerobic pressurized samples had better or similar stability as the control on day one of storage. Soymilk control samples were spoiled after 7 days whereas pressurization increased soymilk shelf-life by at least 2 weeks.

Morales-de la Pena et al. [60] compared the effects of high-intensity pulsed electric fields (HIPEF) treatment (35 kV/cm, 4 μs bipolar pulses at 200 Hz for 800 or 1,400 μs) and conventional thermal pasteurization (90 °C, 60 s) on the microbial stability of a soymilk–fruit juice beverage. A maximal microbial reduction of 5.44 and 5.09 log units was achieved after applying a HIPEF treatment for 800 or 1,400 μs to soymilk beverage inoculated with *Lb. brevis* or *L. innocua*, respectively. HIPEF processing for 800 μs ensured the microbial stability of the beverage during 31 days; however, longer microbial shelf-life (56 days) was achieved by increasing the treatment time to 1,400 μs or by applying a thermal treatment.

The efficacy of ultraviolet (UV) light on inactivation of *E. coli* W1485 and *B. cereus* spores in raw soymilk has been studied by Bandla et al. [9]. Coiled-tube UV reactors were used to investigate the influence of tube diameter and Reynolds number (Re) to inactivate *E. coli* W1485 and *B. cereus* spores in raw soymilk. Inactivation efficiency of both microorganisms increased with Re. Maximum reductions of 5.6 log10 CFU/ml of *E. coli* and 3.29 log10 CFU/ml of *B. cereus* spores were achieved in the 1.6 mm ID UV reactor. Inactivation efficiency was higher in the 1.6 mm ID UV reactor than the 3.2 mm ID UV reactor for both the organisms.

Conclusions

There are many variations on the basic soymilk processing steps, and the general aim of these processes are to produce a soymilk that has good sensory attributes, and that is convenient and safe to consume, produces little waste (okara) and is profitable to manufacture. Until recently, the only successful way of combating the off-flavor in soymilk was to kill the lipoxygenase enzyme, such as by thermal and/or chemical treatment. However, the processes also adversely affect a lot of good protein cells, making the extraction of soluble protein in water very difficult. Liquid soymilk produced from the traditional wet methods is most stable in sensory attributes when stored at very low temperatures. In general, the most affected parameters of soymilk include protein, fat, fiber, viscosity, and flavor, while the least affected are moisture, carbohydrates, specific gravity, and mouthfeel. In addition, enzymatically treated soymilks with hydrolytic enzyme have been shown to have enhanced soymilk flavor. In some instances, the development of novel fermented soymilk with probiotic bacteria does alter functional, antioxidant, and sensory attributes of soymilk. The antinutrient factors, trypsin inhibitor, lipoxigenase, saponins, and phytic acids are destroyed or inactivated at an elevated temperature and/or by processes like high-pressure, pulsed electric filed treatment, and membrane separation. These novel processing techniques have great potential for future applications in soymilk industries in order to produce safe soymilk with maximum retention of nutrients.

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