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Shaikh Ajaj
Department of Food Process Technology, MIP College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

GM Machewad
Department of Food Microbiology and Safety College of Food Technology Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

SK Sadawarte
Department of Food Process Technology, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

SS Shaikh
Department of Food Process Technology, MIP College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

AR Sawate
College of Food Technology Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

AK Singh
Department of Food Process Engineering, SHUATS, Allahabad, Uttar Pradesh, India

Corresponding Author:
GM Machewad
Department of Food Microbiology and Safety, College of Food Technology Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Studies on preparation of soya milk fortified with moringa leaves extract

Shaikh Ajaj, GM Machewad, SK Sadawarte, SS Shaikh, AR Sawate and AK Singh

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Abstract
Soya milk was prepared by blending soybeans and moringa leaf extract. Further, soya milk was packed in PET bottles. The physiochemical characteristics soya milk prepared by blending soy milk (100mL) and moringa leaf extract (2.5mL) were found to contain fat (6.15%), protein (9.13%), carbohydrate (12.48%), calcium (9.9mg/100g) and iron (4.72mg/100g). The shelf life of soya milk was more than 30 days. Soya milk fortified with moringa leaf extract will provide novel uses for underutilized plants. Further it will provide consumer with new alternatives for only milk as a healthy value added soya milk. Moreover the research will bring to light the potential to the underutilized plants for food product development.

Keywords: Soya milk, soybeans, moringa leaf extract, shelf life

Introduction
In India 233.3 million will under nourished comprising about 24% of the total population (FAO, 2002). In this context soybean (*Glycine max*) with 40% protein and 20% fat assumes the most predominant position in solving the nutritional imbalances prevailing. It contains 20% non-cholesterol oil and 45% protein compared to 20% and 13% protein content in meat and egg, respectively. Food products fortified with soya beans are considerably cheaper than those fortified using other sources of high-quality protein such as fish, meat, milk and other protein-rich legumes. The cost of protein from soya bean is only about 10-20% of the cost of protein from fish, meat, eggs or milk. Soybean is a very rich source of essential nutrients and possesses good quality protein which is comparable to other protein foods and is suitable for all ages, infants to the elderly. The soy protein is highly digestible (92-100%) and contains all the essential amino acids except methionine which is relatively low but good source of lysine. Soybean protein products also contain a high concentration of iso-flavones, up to 1 g/kg [20]. The health benefits of soya proteins have been documented, relate to the reduction of cholesterol levels and menopause symptoms and the reduction of the risk for several chronic diseases such as cancer, heart disease and osteoporosis. The addition of soya protein in diet or replacing animal protein in the diet with Soy lowers blood cholesterol [5]. Moreover, soy protein is acceptable in almost all diets due to no cholesterol and absence of lactose. Every 1% reduction in cholesterol values is associated with an approximate 2-3% reduction in the risk of coronary heart disease [3]. Daily intake of 20-50 grams of isolated soy protein could result in a 20-30% reduction in coronary disease risk [4]. An important link between soy consumption and a reduced risk of certain types of cancer [10]. World soybean production in the 2009/2010 harvest will roughly 260 million tons, and the major producers will the United States, Brazil and Argentina, producing 91.4, 69.0 and 57.0 million tons, respectively. Soybean milk has been prepared for hundreds of years in the orient by a standard method of soaking beans in water for several hours, followed in order by grinding with filtration and cooking for about 30min [21]. Although this conventional process method is relatively simple the techniques and conditions have not been thoroughly investigated for producing a soymilk with a blend or desirable aroma. The soymilk off flavor problem mainly associated with volatile compound. Preliminary experiment [15]. Utilizing gas chromatography instrumentation demonstrated that the volatiles responsible for off flavor, derived only from soymilk which is prepared from whole fat soybeans.

It will postulated that soymilk could be produced with a minimum of flavor development by developing technique and conditions involving a high temperature – hydration grindings [17]. Indicated that the thermal in activations of lipoxygenase will pH. Off flavor development will suppressed in legumes at pH 3.85 and below [9].Extracted soymilk at pH 2 and found there will no lipoxygenase activity even when the product will neutralized to pH 6.4 [13]. A method of preparing soymilk resulted in a colloidal stable, bland product of very good acceptability [14]. With this method no filtration or dehulling is used, so protein recovery is essentially 100% and the beans are hydrated and blanched to eliminate the off flavor producing enzymes and growth inhibitors. However industrial equipment such as a homogenizer is required.

The leaves contain nutrients especially essential amino acids, vitamins, minerals and β-carotene [22]. For this reason, it is used as an alternative source for nutritional supplements and growth promoters in some countries [2]. Apart from nutritional benefits, *Moringa oleifera* is reported to be used for the treatment of rheumatism, ascites, infection, hiccough influenza and internal abscess [16]. Many recent reports on disease prevention by *Moringa oleifera* have been reported. Dietary fibre has shown beneficial effects in the prevention of several diseases, such as cardiovascular diseases, diverticulosis, constipation, irritable colon, colon cancer, and diabetes [18]. The fruit fibre has a better quality than other fibre sources due to its high total and soluble fibre content, water and oil holding capacities, and colonic ferment ability, as well as a lower phytic acid and caloric value content [7]. A high dietary fibre content of (about 50g/100 g) is indicative of a good source of dietary fibre. The maturation of banana fruits has shown to impact the dietary fibre compositions of Cellulose, lignin, and hemicelluloses contents of, the components of the insoluble dietary fibre fractions, varied from 7-12g/100 g, 6.4-9.6 g/100g and 6.48.4g/100g, respectively, where as pectin contents, a component of the soluble dietary fibre ranged from 13-21.7g/100g [6]. The concentrations of hydrogen cyanide, an extremely poisonous substance and oxalate contents in swill found to be 1.33 mg/g 0.51mg/g respectively, falling within the safety limits [1]. These results indicated that swill safe and valuable functional ingredients for human consumption. Soy milk, the water extract of soybeans, is typically produced by grinding the soaked soybeans with water. As an inexpensive and convenient source of high quality proteins, soy milk is one of the most important traditional beverages that are consumed widely in Asian countries, including China, Japan, Korea, Singapore and Thailand. In recent decades, extensive evidence has indicated the strong relationships between soy food consumption and health promoting effects. Soy milk possesses a balanced nutrient combination, which is similar to cow's milk, but free of cholesterol, gluten and lactose, plus favorable phyto-chemical compounds linked to health. Consequently, it has drastically spread its popularity to Western consumers in European countries, Australia and the United States, especially among vegetarians, milk allergy patients or people of lactose-intolerance, who use soy milk as a dairy alternative. In response to a gradual increase in sales and consumption, various new products have been introduced into the soy milk market. Some basic changes are made to the flavor or the soybean source. However, the most recent innovations are focusing on producing "functional soy milk". Functional soy milk can be considered as soy milk that contains extra bioactive components and may help to enhance health or lower risk of diseases. Soybean is a good source of phenolic compounds with antioxidant properties and has an extraordinarily high amount of isoflavones, a group of phyto-estrogens that have been reported to possibly lower the risk of hormonal and age-related diseases. However, the presence of natural anti-nutrients, such as trypsin inhibitors, lectins, phytic acids and indigestible oligosaccharides, has limited its consumption. Thus, modifying the processing methods could be an effective way to improve the health-promoting bioactive components and/or reduce the undesired compounds originally present in soybeans, to support functional soy milk product development. The heating process during conventional soy milk making considerably destroys most of the anti-nutritional factors in soy milk and improves the digestibility of soy protein, as well. However, compound like phytic acid, which interferes with the availability of calcium, is not reduced effectively. At the same time, over-heating to eliminate trypsin inhibitor activity to a great extent can cause damage to amino acids, as well as loss in the overall nutritional value of soy milk. Lately, incorporating the fermentation process into soy milk production has become a popular method to improve the acceptability and health properties of soy milk. Research has shown that soy milk fermented with properly selected microbial species had major advantages in decreasing indigestible oligosaccharides, like raffinose and stachyose, and beany flavor (undesirable for most Western consumers). However, with respect to other anti-nutritional factors, such as trypsin inhibitors, no improvements were observed. In this study, a change was made to the conventional soy milk making method by starting the process with short-time germinated soybeans in an attempt to overcome some of these limitations with soy milk Soy beans have been an integral part of the diet of people in the Far East for more than 5,000 years. However the history of soy protein products is relatively short. In early years soy protein products were mainly used to meet nutritional needs, but more recently they have been used primarily for the irunique functionality with steadily improving production. Increases of vitamin contents (e.g., vitamin C and riboflavin) and mineral availability (e.g., calcium) [8] improvement of protein digestibility [11] hydrolysis of flatulent-causing oligosaccharides reducing levels of trypsin inhibitors, lectin, phytic acid and lipoxygenase activity, which lead to undesirable beany flavor [23] enrichment of phenolics, iso-flavone aglycones and sponging glycosides [11]. The research will bring to light the potential to the underutilized plants for food product development. The research will broaden understanding of sensory characteristics and preferences of soya milk fortified with moringa leaf extract in general. It will further advance in healthy soya milk development.

Material and Methods

Procurement of raw materials

Fresh leaves of were collected from healthy and uninfected tree in Mahewa east area. Soybeans, sugar, essence were purchased from local market of Allahabad.

Preparation of the moringa leaf extract

The Fresh raw moringa leaves were selected by visual appearance of fresh and dark coloured, fully matured without any physical damage, on the surface. Then the collected moringa leaves were washed by pure water for removing of the dust. After then the fresh leaves grinded to obtain moringa leaves extract.

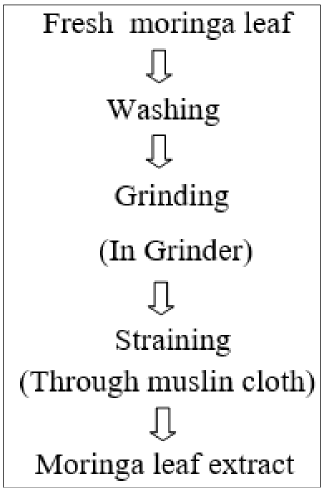

Fig 1: Flow sheet for moringa leaf extract

Preparation of soybean milk

Soya milk can be made from whole soybeans or full-fat soy flour. The dry beans are soaked in water overnight or for a minimum of 10h or more depending on the temperature of the water. The rehydrated beans then undergo wet grinding with enough added water to give the desired solids content to the final product. The ratio of water to beans on a weight basis should be about 3:1. The resulting slurry or purée is brought to a boil in order to improve its nutritional value by heat inactivating soybean trypsin inhibitor, improve its flavor and to sterilize the product. Heating at or near the boiling point is continued for a period of time, 15-20 min for 80-95 ^{0}C, followed by the removal of an insoluble residue (soy pulp fiber or *okara*) by filtration.

Hot extraction

The method as development by INTSOY (International soybean program) and modified by. The soybeans were cleaned in order to remove dirt's and also some impurities like other seed stalks etc. The beans were blanched in hot water for 30 min for the following reason; to soften the seeds and aid in see coat removal to removal, to reduce the beany flavor and eliminates the anti-nutritional factors. The beans were the dehulled and milled with water using the Kenwood blender, about 3 parts of water was added to the slurry and filtered using a Muslim cloth, the filtrate was allowed to simmer on fire about 10 minutes it was then bottled and allowed to cool.

Cold Extraction

The soybeans were cleaned to remove dirt's and impurities. Water was added to the beans in the ratio of 1.3 (beans to water) and the beans were allowed to soak for 10 h under refrigeration temperature. This was done in order to prevent fermentation of the beans and growth of microbes. The soybeans were then dehulled and the chaff removed by adding water and decanting. Milling was done using a Kenwood blender. About 3 parts of water was added to the slurry and the mixture was allowed to simmer on fire for 10 min. It was then bottled while still hot and allowed to cools.

Soaking before hot extraction

Soybeans were cleaned to remove stories iron and other impurities or unwanted particles. The beans were put into a pot of water at a ratio of 1:3 (beans to water) and allowed to soak for 18h in a refrigerator. This was to soften the seeds, to present fermentation of the beans and reduce the anti-nutritional compounds. The beans were drained and blanched for 20 min without decorating. The seed coats were then removal (de-hulling) and milling was done by using a Kenwood blender. The slurry was filtered using a musclin cloth. The filtrate (soymilk) was allowed to simmer on fire for commutes. It is then bottled while hot and cooled.

Soya milk

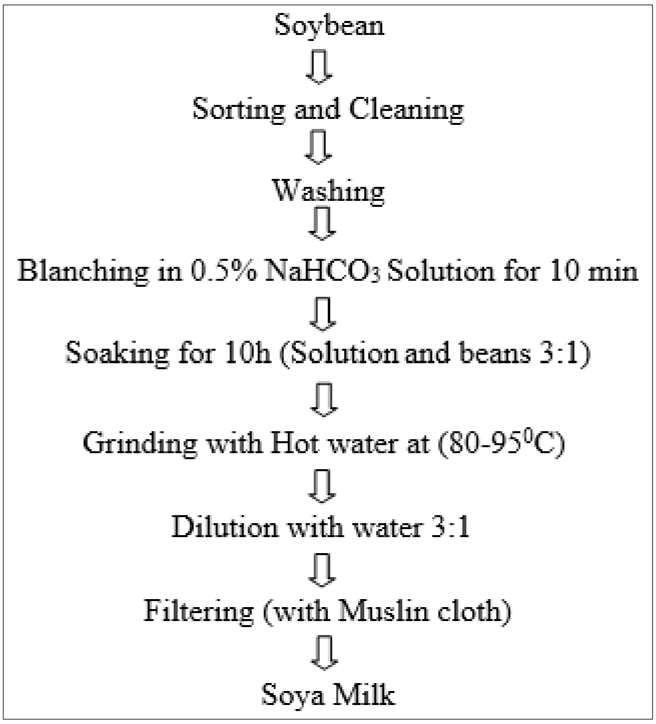

Fig 2: Flow sheet for preparation of soya milk

Formulation of soya milk

The standardized recipe for the development of soya milk fortified with moringa based leaves extract in order to increase acceptability (Table 1).

Table 1: Ingredient formulation of soya milk

Ingredient	T_0	T_1	T_2	T_3
Soya milk	100	100	100	100
Moringa extract	-	2.5	3	4
Sugar	20g	20g	20g	20g
Vanilla essence	3drops	3drops	3drops	3drops

Chemical characteristics soya milk

Fat

Fat was determined by solvent extraction method No. 30-25 using Soxhlet apparatus (HTZ 1O45 Extraction Unit, Hoganas, Sweden) as mentioned in (AACC, 2000). Moisture free sample (2 g) was taken in a thimble followed by addition of n-hexane (50 mL) into the flask which was attached to the Soxhlet apparatus. The sample was subjected to extraction of fat for 2-3 hours by adjusting the rate of 3-4 drops of hexane per second. After 6-7 siphons thimble was removed, placed in an oven at 100±5 ºC for 1h and weighed.

$$\text{Fat (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

Protein

The protein percentage in the samples was estimated using the Kjeldahl's (Technick GmbH D-40599, Behr Labor, Germany)

method No. 46-10 (AACC, 2000). The sample was digested in the digestion tube for three to four hours with the aid of 30mL conc. H_2SO_4 in the presence of 5g of digestion mixture (CuSO4, FeSO4, K2SO4 in the ratio of 9:1:90) till the material attained transparent or light green color. The digested material was then transferred to 250mL volumetric flask and volume was made up to the mark with distilled water. 10mL of diluted sample was distilled with 10mL of 40% NaOH solution with the help of distillation apparatus. The ammonia released was collected in 4% boric acid having methyl red indicator. The solution was then titrated against 0.1N H_2SO_4. A blank (without sample) was also run in the same manner.
Protein % = Nitrogen (%) × Respective factor according to the type of the flour. The protein percentage was calculated by multiplying nitrogen contents of each sample with respective conversion factors i.e. 6.25 for meat, 5.70 for wheat flour bread, and 5.30 for seed and for gel.

Carbohydrate
Carbohydrate content of soya milk was calculated by subtracting the moisture, protein, fat and ash content from the total mass.

Calcium
Calcium was analyzed by atomic absorption spectrophotometry (model AA100; Perkin-Elmer, Norwalk, CT). The soya milk was first ashed at 600 °C and the ash was taken up in hydrochloric acid before analysis.

Total bacterial count and yeast and mould count
To obtain direct counts, 15-20mL sterile, molten (45 - 50 ℃) sterile media was poured into sterile Petri dishes containing 1mL volumes of diluted test sample. Inoculum was distribute throughout medium by rotating the plate in one direction, then in the reverse direction. The plate was allowed to cool and the lid was closed. This was carried out in a sterile condition in the laminar air flow.

Storage study
The samples were stored at refrigeration temperature (4–7 °C) and were drawn at 10 days interval upto 30 days. The samples were analyzed for their total bacterial count and Yeast and mould count during storage.

Result and Discussion
Chemical characteristics of Soya milk
The chemical characteristics of soya milk were found to contain fat content 6.02, 6.15, 6.01, 6.20 and 7% (Table 2). The protein content of soya milk was found to contain 8.76, 9.13, 9.84, 10.47 and 10.83%. The carbohydrate content was found to be 9.63, 12.48, 10.91, 12.74, and 12.87 per cent. The calcium content of soya milk was found to contain 9.77, 9.90, 9.99, 10.15 and 10.54mg/100g. The iron content of soya milk was found to contain 4.94, 4.72, 4.57, 4.99, and 4.28 mg/100g.

Table 2: Chemical characteristics of soya milk

Parameters	Values				
	T_0	T_1	T_2	T_3	T_4
Fat (%)	6.02	6.15	6.01	6.20	7.00
Protein (%)	8.76	9.13	9.84	10.47	10.83
Carbohydrate (%)	9.63	12.48	10.91	12.74	12.87
Calcium (mg/100g)	9.77	9.90	9.99	10.15	10.54
Iron (mg/100g)	4.92	4.72	4.57	4.99	4.28

Changes in microbial load in soya milk during storage
Microbial analysis was done to study the microbial quality of Soya milk by using pour plate technique. The analysis was done at the interval of one month after the preparation of Soya milk with concentration of was kept constant. Samples of Soya milk with 60 days storage period tend to have higher mean bacterial counts 3.8 x 10^5 CFU/g than those with 40 days 1.93 x 10^5 CFU/g and 20 days 1.48 x 10^3 CFU/g (Table 3).

Table 3: Microbial quality of Soya milk

Storage period (days)	Total bacterial count (CFU/g)	Yeast and mould count (CFU/g)
10	1.72 x 10^4	1.2 x 10^3
20	2.22 x 10^4	1.9 x 10^3
30	2.40 x 10^4	2.45 x 10^3

Conclusion
It is concluded that the moringa based soya milk was found high in calcium and iron content. Soya milk fortified with moringa leaf extract will provide novel uses for underutilized plants. Further, it will provide consumer with new alternatives for only milk as a healthy value added soya milk. Moreover, the research will bring to light the potential to the underutilized plants for food product development.

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