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Studies on quality evaluation of soya milk from *Moringa oleifera* fortified with moringa extract

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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), iron (4.72mg/100g) and total dissolved solids 778S/cm). 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
Many of these are closely related to soy milk quality and can be key attributes of soy milk. Thus, germination can be a very potent and efficient step for producing functional soy milk, as it covers the major benefits produced from other modifications, like fermentation, but possesses even more advantageous characteristics. Water extractable soybean proteins can be divided in whey proteins (10%) and globulins (90%). Soybean globulins consist of four fractions, 2S (15%), 7S (34%), 11S (41.9%) and 15S (9.1%) [6]. Several trypsin inhibitors and cytochrome c exist in the 2S fraction; other minor proteins may also be present [18]. Soybean [*Glycine max* (L.) Merrill] is one of the nature's wonderful gifts. Soybean represents an excellent source of high quality protein; it has a low content in saturated fat, it contains a great amount of dietary fiber and its isoflavone content makes it singular among other legumes. [1]On an average, dry soybean contains 40% protein, 20% oil, 35% Soluble and insoluble (dietary fiber) carbohydrate and 5% ash [10]. Soybean oil is low in saturated fat, rich the essential fatty acids and is an excellent source of vitamin E. The ratio of polyunsaturated fatty acid to saturated fatty acid is 82:18, which is highly conducive to lower the blood cholesterol [26]. Soybean and soy foods have been used as beneficial dietary and nutritive materials in several. Asian countries due to their high quality proteins and many other functional substances like omega-3, omega-6, phenolic acids and isoflavones [29]. Whey is a valuable dairy by-product obtained during cheese/paneer and casein manufacture. It is presently being drained off as such due to the absence of economically viable methods for its utilization, which not only causes loss of precious nutrients but also creates environmental problems due to its high Biological Oxygen Demand (38,000 to 46,000 ppm). These amounts are 100 times higher than the effluent load from an average household. Further, the treatment of whey before disposal is a very costly affair [25]. The use of soy milk was first reported about 2000 years ago in China. Soy milk was the first plant-based milk which serves the purpose of providing nutrients to the population where the milk supply was inadequate [21]. It was also popular amongst the populations who are allergic to milk proteins and are lactose intolerant. Soy milk is a good source of essential monounsaturated and polyunsaturated fatty acids which are considered good for cardiovascular health. It serves as an inexpensive, refreshing and nutritional beverage to the consumers. Isoflavones appeared to be the functionally active component responsible for the beneficial effects of soybean. Isoflavones are well known for their protective effect against cancer, cardiovascular disease, and osteoporosis [15]. Genistein is the most abundant isoflavone in soybean and is proposed to be the most biologically active [5]. Apart from isoflavones, soy proteins are also known to provide protective and therapeutic benefits against several diseases. Soy foods are also found to be rich in phyto-chemicals such as phytosterols, which are recognized for their cholesterol lowering properties [8]. On average, dry soybean contains roughly 40% protein, 20% oil, 35% carbohydrate, and 5% ash [10].

Traditional process of soy milk preparation leaves a product with limited shelf life and a characteristic beany flavor. Modern soy milk production utilizes advanced technologies and equipments to maximize nutritional value, shelf-life and convenience with reduced beany flavor. In view of its popularity, a wide array of variants is available in the market. Based on solids concentration, available variants are light, dairy like and rich soy milk, with respect to formulation, sweetened, original and flavored soy milk, with respect to fortification, regular, enriched, and blended soy milk [10]. Several methods were tested for their efficacy in removal of off flavor or beany flavor. Some of the widely accepted methods in order to remove or deodorize the beany flavor of soy milk are: vacuum treatment at high temperature which results in stripping off of most volatile compounds (short chain fatty acids, sterols, sulphur compounds etc.), leading to a better acceptable product to the consumers, Cornell hot grinding method where soaked soybeans were ground with boiling water or steam to obtain a slurry at a temperature of 80 ºC, the slurry was then kept at this temperature for 10 min in order to inactivate lipoxygenase, pre-blanching method where soaked soybeans were blanched in boiling water to inactivate lipoxygenase. Apart from these methods, alkaline soaking, use of defatted flour, soy protein isolates and concentrates have reduced beany flavor to some extent. Nowadays, foods are intended not only to satisfy hunger and provide necessary nutrients for humans, but to prevent nutrition-related diseases and improve consumers' physical and mental well-being [21]. Moreover, there is plenty of scientific literature that demonstrates the close connection between diet and health, particularly related to chronic diseases, which have encouraged the development of growing spectrum products such as nutraceuticals, medifoods and vita foods.

In México [12], data from three national surveys conducted in 1988, 1999 and 2006, using the International Obesity Task Force classification system, described the upward trends on overweight and obesity in school-age children and teenagers at a national level [3]. Besides, increased world population, and the continuous rise of morbid obesity and other nutritional diseases have led to the employment of protein from vegetal sources, along with the preference of low-fat dairy products in consumers from our country. Therefore, people are constantly pursuing better life quality by eating low-fat dairy products which may reduce the risk of stroke or coronary heart disease [15]. Also, the remarkable sales in soy-based products can be attributed to the beneficial health properties of soy-derived foods. Diabetes mellitus is a largely occurring endocrine disorder in many countries [7]. In diabetes, due to defects in the production of insulin or its action, blood glucose levels become elevated. Also impaired is the functioning of the macronutrient metabolism, leading to long-term health complications [13]. In addition, free radicals generated during long-term hyperglycemia impair the body's antioxidant defense system [23]. Diabetes treatment is based on pharmacological hypo glycaemic agents and insulin; however, the efficacy of these therapies is limited due to their many side effects. Therefore, finding natural compounds is essential for overcoming these problems [11]. Moringa is an easily propagated plant which thrives well in harsh environmental conditions. It is increasingly gaining global attention due to an excellent profile of nutrients and antioxidants. Moringa leaf is rich in minerals, amino acids, vitamins and carotene. It also contains a rare combination of health-promoting antioxidants: zeatin, quercetin, sitosterol, caffeoylquinic acid and kaempferol.

Were evaluated after mixing of Moringa extract. The storage study was carried out in the intervals of 10, 20 and 30 days. In which all the effect were check during storage condition like carbohydrate, fat, protein, pH, Acidity, Total Dissolve Solids (TDS). The Microbial analyses were carried out to observe the growth of microbes.

The following results well observed from the study.

- In developed soya milk the nutritional composition of calcium and iron was found high.
- The protein, fat, carbohydrates, and iron content of developed Moringa based soya milk was found.
- The shelf life of Moringa based soya milk was more than 30 days.

From the above results it can be conclude that

1. Developed Moringa based soya milk having treatment T1 was found best on the basis of sensory evaluation.
2. The combination of different herbs gave a good result for sensory parameters.

The chemical parameters determine the nutritional importance of consuming the moringa based soya milk.

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 optain moringa leaves extract.

Fresh Moringa leaf
⇩
Washing
⇩
Grinding
(In Grinder)
⇩
Straining
(Through muslin cloth)
⇩
Moringa leaf Extract

Fig 1: Flow sheet for Moring 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 10 hours 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 minutes for 80-95 ºC, 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 30minutes 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 10hrs 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

Soybean 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 18hrs 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 minutes without decorating. The seed coats were then removal (dehaling) and milling was done by using a Kenwood blender. The slurry was filtered using a Muslim cloth. The filtrate (soymilk) was allowed to simmer on fire for commutes. It is then bottled while hot and cooled.

Soya milk

Soya bean
⇩
Sorting Cleaning
⇩
Washing
⇩
Blanching in 0.5% $NaHCo_3$ Solution for 10 min
⇩
Soaking for 10h (Sol^n and beans 3:1)
⇩
Grinding with Hot water at (80-95 °C)
⇩
Dilution with water 3:1
⇩
Filtering (with Muslin cloth)
⇩
Soya Milk

Fig 2: Flow sheet for preparation of soya milk

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	3drop	3drop	3drop	3drop

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 (2g) was taken in a thimble followed by addition of n-hexane (50mL) 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 °C for 1 hour 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. H2SO4 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

The carbohydrate content of soy milk was determined by the formula,

Carbohydrate % = 100-(protein % + fat % + moisture content % + ash %)

Total Dissolved Solids

A determination of a solution's conductivity provides a first indication of the concentration of electrolytes dissolved in the sample solution. The term total dissolved solids (TDS) are used in water analysis.

Total bacterial count and yeast and mould count

To obtain direct counts, 15-20mL sterile, molten (45-50 °C) 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.

Results and discussion

	0 days	10 days	20 days	30 days
T0	8.76	8.64	8.53	8.44
T1	9.13	9.02	8.95	8.86
T2	9.48	9.72	9.58	9.47
T3	10.47	10.29	10.17	9.98
T4	10.83	10.75	10.58	10.42
F- test	S	S	S	N/S
SED±				0.017
C.D. (5%)				0.143

Effect of storage on carbohydrate of Soya milk

The protein content of Soya milk samples for sample of T_0 was 8.76 on 0 days, 8.64 on 10 days, 8.53 on 20 days, 8.44 on 30 days. T_1 was 9.13 on 0 days, 9.02 on 10 days, 8.95 on 20 days, 8.86 on 30 days. T_2 was 9.48 on 0 days, 9.72 on 10 days, 9.58 on 20 days, 9.47 on 30 days. T_3 was 10.47 on 0 days, 10.29 on 10 days, 10.17 on 20 days, 9.98 on 30 days, and T_4 was 10.83 on 0 days, 10.75 on 10 days, 10.58 on 20 days, 10.42 on 30 days. Similarly on 10 days, 20 days and 30 days shows increase in the protein content in the sample. (Table 4.2; Fig. 4.1).

Table 2: Effect of storage on carbohydrate content of Soya milk

Treatment	0 days	10 days	20 days	30 days
T0	12.87	12.62	12.43	12.27
T1	13.42	13.27	13.13	12.97
T2	12.15	12.02	11.92	11.88
T3	12.10	12.02	11.97	11.91
T4	13.00	12.92	12.87	12.78
F-test	S	S	S	N/S
SED±				0.0025
C.D. (5%)				0.110

The Carbohydrate content of Soya milk samples for sample of T_0 was 12.87 on 0 days, 12.62 on 10 days, 12.43 on 20 days, 12.27 on 30 days. T_1 was 13.42 on 0 days, 13.27 on 10 days, 13.13 on 20 days, 12.97 on 30 days. T_2 was 12.15 on 0 days, 12.02 on 10 days, 11.92 on 20 days, 11.88 on 30 days. T_3 was 12.10 on 0 days, 12.02 on 10 days 11.97 on 20 days, 11.91 on 30 days, and T_4 was 13.00 on 0 days, 12.92 on 10 days, 12.87 on 20 days, 12.78 on 30 days. Similarly on 10 days, 20 days and 30 days shows increase in the moisture content in the sample. Decrease in the Carbohydrate content in the sample. (Table 4.3; Fig. 4.2).

Effect of storage on calcium content of Soya milk during storage

Table 4: Effect of storage on calcium content of Soya milk during storage

Treatment	0 days	10 days	20 days	30 days
T0	9.77	9.61	9.51	9.44
T1	9.90	9.81	9.73	9.69
T2	9.99	9.87	9.77	9.65
T3	10.12	9.99	9.73	9.66
T4	10.10	10.02	9.98	9.87
F- test	S	S	S	N/S
SED±				0.0002
C.D.(5%)				0.080

The Ca (mg/100g) content of Soya milk samples for sample of T_0 was 9.77 on 0 days, 9.61 on 10 9.69 days, 9.51 on 20 days, 9.44 on 30 days. T_1 was 9.90 on 0 days, 9.81 on 10 days, 9.72 on 20 days, on 30 days. T_2 was 9.99 on 0 days, 9.87 on 10 days, 9.77 on 20 days, 9.65 on 30 days. T_3 was 10.12 on 0 days, 10.02 on 10 days, 9.89 on 20 days, 9.66 on 30 days, and T_4 was 10.10 on 0 days, 10.02 on 10 days, 9.98 on 20 days, 9.87 on 30 days. Similarly on 10 days, 20 days and 30 days shows decrease in the calcium content in the sample. (Table 4.4; Fig. 4.3)

Effect on fat content of soya milk during storage

Table 6: Effect on fat content of soya milk during storage

Treatment	0 days	10 days	20 days	30 days
T0	6.02	5.97	5.87	5.81
T1	6.15	6.09	6.00	5.92
T2	6.12	6.03	5.93	5.75
T3	6.20	6.12	6.09	6.01
T4	7.00	6.91	6.87	6.81
F- test	S	S	S	N/S
SED±				0.021
C.D.(5%)				0.058

The Fat content of Soya milk samples for sample of T_0 was 6.02 on 0 days, 5.97 on 10 days, 5.87 on 20 days, 5.81 on 30 days. T_1 was 6.15 on 0 days 6.09 on 10 days, 6.00 on 20 days, 5.92 on 30 days. T_2 was 6.12 on 0 days, 6.00 on 10 days, 5.93 on 20 days, 5.75 on 30 days. T_3 was 6.20 on 0 days, 6.12 on 10 days, 6.09 on 20 days, 6.01 on 30 days, and T_4 was 7.00 on 0 days, 6.91 on 10 days, 6.87 on 20 days, 6.81 on 30 days. Similarly on 10 days, 20 days and 30 days shows decrease in the fat content in the sample.

Effect on Iron of soya milk during storage

Table 7: Effect on Iron of soya milk during storage

Treatment	0 days	10 days	20 days	30 days
T0	4.92	4.81	4.73	4.69
T1	4.72	4.65	4.61	4.54
T2	4.57	4.52	4.49	4.33
T3	4.99	4.87	4.81	4.69
T4	4.82	4.81	4.19	4.12
F- test	S	S	S	N/S
SED±				0.050

The Iron Fe (mg/100g) content of Soya milk samples for sample of T_0 was 4.92 on 0 days, 4.81 on 10 days, 4.73 on 20 days, 4.69 on 30 days. T_1 was 4.72 on 0 days, 4.65 on 10 days, 4.61 on 20 days, 4.54 on 30 days. T_2 was 4.57 on 0 days, 4.52 on 10 days, 4.49 on 20 days, 4.33 on 30 days. T_3 was 4.99 on 0 days, 4.87 on 10 days, 4.81on 20 days, 4.69 on 30 days, and T_4 was 4.82 on 0 days, 4.81 on 10 days, 4.19 on 20 days, 4.12 on 30 days. Similarly on 10 days, 20 days and 30 days shows increase in the moisture content in the sample. (Table 4.5; Fig. 4.4).

Sensory Evaluation

The judgment was made by rating product on a 9-point hedonic scale with corresponding descriptive term ranging from 9 “like extremely” to “dislike extremely” to determine the pleasurable and un pleasurable feel of Soya milk. 10 untrained panelists aged between 18-35 years participated in the consumer test conducted at the department of Food Process Engineering, Sam Higginbottom University of Agriculture, Sciences and Technology, Allahabad.

Result of Organoleptic Evaluation of Soya milk

Organoleptic quality parameters of a product assume pivotal role in anticipating the consumer response to the product. On the basis of organoleptic evaluation of soya milk samples were selected as best sample. The percent of Moringa was used separately in different proportions (2.50ml, 3m, 4ml) in soya milk with different proportion the formulated Moringa based soya milk was further organoleptically analyzed for quality attributes like colour, flavour, taste and overall acceptability. The data pertaining to organoleptic evaluation of Moringa based soymilk are presented in Table-4.3

Note: T_1 = 2.5% Moringa extract; T_2 = 3% Moringa extract; T_3 = 4% Moringa extract; T_4 = 5% Moringa extract.

The data pertaining to the sensory scores of appearance, colour, flavor, taste, texture and overall acceptability of soya milk prepared of with different proportions is given in the Table-9. A significant difference was observed for the scores obtained for *soya milk* for all the sensory parameters. Mean color scores ranged from 6.5 to 9 with a mean of 7.55. The mean flavour scores ranged from 6.1 to 9, with mean of 7.15. The mean taste score ranged from 6.3 to 9, with a mean of 7.2. The mean overall acceptability scores ranged from 6.4 to 9, with mean of 7.36.

Table 8: Organoleptic characteristics

SS Sample	Colour	Taste	Flavour	Appearance	Over All
T0	9.000	9.000	9.000	9.000	9.000
T1	7.600	7.389	7.200	7.100	7.489
T2	7.211	6.944	6.778	6.689	6.989
T3	7.000	7.000	6.767	6.800	7.189
T4	7.156	7.000	6.822	6.900	7.389
F-test	S	S	S	S	N/S
SED±					0.055
C.D. (5%)					0.154

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