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QUALITY IMPROVEMENT AND SENSORY EVALUATION OF SOYA MILK PREPARED BY GERMINATED SOYBEANS

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ABSTRACT

This study focused on improving and diversifying the food and nutrition status of soy milk prepared by germinating the soyabeans prior to soy milk preparation and evaluating its quality and potential for acceptance. The pH, moisture, fat, protein, ash and total solid content of raw material soyabean were 6.76, 12,17.67,39.6, 5.3, 88 %. Non-Germinated and Germinated Soy milk (from short- time Soyabeans (28 hours)) were produced . Soya Milk samples were analyzed for protein, moisture, ash, pH, total solids, carbohydrates, fat , total phenolic content and sensory attributes. The pH ,moisture, protein, fat, ash, carbohydrate and total solid contents of germinated soy milk were 6.85, 95, 3.11,1.28 ,0.56, 0.50 and 5 % respectively while those of non- germinated soymilk were 7.05, 93 ,2.64, 1.50, 0.65, 2.21 and 7 %, respectively. From the results, comparing the germinated and non-germinated soybean, the total phenolics content was increased significantly from to 10.67 ± 0.434 to 13.76 ± 0.294 after germination. The germinated soy milk was liked much by the people as per its taste, colour, texture ,flavor and mean score for all attributes as per overall acceptability was 7.46 out of 10. Soy milk made under 28 hours germination conditions imparts great nutritional value as germination increases protein digestibility and decreases fat, carbohydrate, ash , total solids, pH and Anti- nutrient like total phenolic content. Thus, Soy Milk developed by incorporating short time germination of soyabeans have enhanced functional attributes and improved the food quality.

Key words: Soybean, Germination, Phenolic compounds, Organo leptic.

INTRODUCTION

About 840 million people were undernourished and 799 million were from the developing countries (FAO, 2002). In India 233.3 million were 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 soyabeans 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 soyabean is only about 10-20% of the cost of protein from fish, meat, eggs or milk.

NUTRITIONAL ATTRIBUTES OF LEGUME-SOYBEAN

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 isoflavones, up to 1 g/kg (Setchell et al. 1987). The health benefits of soy proteins have been documented, relate to the reduction of cholesterol levels and menopause symptoms and the reduction of the risk for several chronic diseases, i.e., cancer, heart disease, and osteoporosis. The addition of soy protein in diet or replacing animal protein in the diet with Soy lowers blood cholesterol (Carroll, 1991). Moreover, soy protein is acceptable in almost all diets due to no cholesterol and absence of lactose. Anderson (1995) found that every 1% reduction in cholesterol values is associated with an approximate 2-3% reduction in the risk of coronary heart disease. Daily intake of 20-50 grams of isolated soy protein could result in a 20-30% reduction in coronary disease risk (Bakhit et al1994). Lee et al. (1991) reported an important link between soy consumption and a reduced risk of certain types of cancer. World soybean production in the 2009/2010 harvest was roughly 260 million tons, and the major producers were the United States, Brazil and

Argentina, producing 91.4, 69.0 and 57.0 million tons, respectively (USDA, 2011).

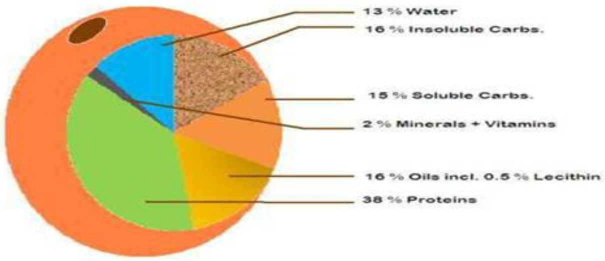

Fig 1. Soy composition by world soy foundation and national soy research lab

In India about 9.3 million tones of soybeans are produced annually and 80% is utilized for oil extraction. Only 10% is available for direct food uses. People are more health conscious now. Lactose intolerance is on the increase. Soymilk was first referred to in the United States by Trimble in 1896 in the American and the first commercial soymilk in the US was produced by J.A Chard soy products in New York (Gavin and Wettstein , 1990). Generally, soymilk has total solids of 8-10%, protein content of about 3.6%, fat 2.0%, carbohydrate 2.9% and ash 0.5% (Liu, 2005). 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. However, the presence of natural anti-nutrients, such as trypsin inhibitors (TI), lectins, phytic acids and indigestible oligosaccharides, has limited its consumption. 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, compounds, like phytic acid, which interferes with the availability of calcium, is not reduced effectively (Onuorah et al. 2007). 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.

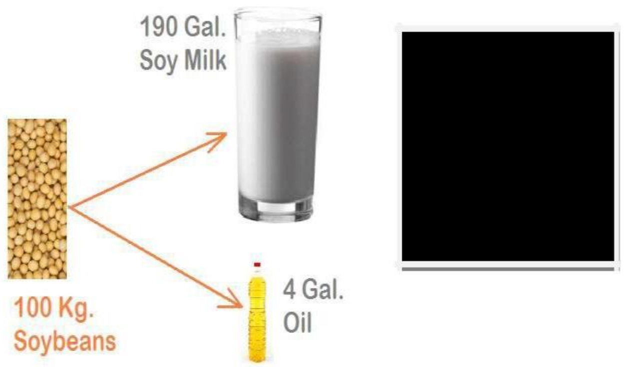

Fig 2. Dimensional Comparative Analysis by World Initiative for Soy in Human Health

The fact that germination confers reveal beneficial properties to soyabeans can be utilized in preparation of nutritionally more potent soy milk. Thus, this project aimed to prepare soy milk from soybeans germinated at optimized conditions and to evaluate its functional properties. Keeping this in mind, following objectives were designed:

OBJECTIVES

The objectives of the study is to Germinate the Soyabeans, to prepare Soy Milk from Germinated and Non-Germinated Soyabeans, to investigate physicochemical properties of Germinated and Non-Germinated Soy Milk and Antinutritional properties of soyabean and Germinated Soyabeans.

MATERIALS AND METHODS

SOYBEAN MATERIALS

Yellow soybeans (*Glycine max*) (harvested in 2013) were purchased from a local grocery in Chandigarh, India. A few seeds with defects were removed from samples.

GERMINATION OF SOYBEAN

Soybeans were cleaned and rinsed three times with tap water before being soaked overnight (~12 h) at room temperature with a water to dry bean ratio of 10:1, v/w. The soaked beans were drained, rinsed and placed in moist cloth and water was applied everyday to provide moisture to the seeds during sprouting process. The germination process was carried out at room temperature (~25 °C) for 28h, 50h and 72h, respectively. The Germinated Soyabeans are used for soy milk preparation while Non-Germinated Soy Milk was prepared after soaking process only.

PREPARATION OF SOY MILK

For each batch of soy milk, 200 g of non-germinated or germinated soybeans underwent the washing and soaking procedures (dry weight basis), were drained, rinsed and mixed with tap water 1800 ml (water/dry bean = 9:1, v/w). The mixture was subjected for blanching for 20 minutes and then drained and dehulled. Grinding was done in household blender and blended for 5 min at low speed. The cooked soy slurry was filtered through a mesh screen to obtain soy milk. Then , obtained slurry was boiled for 10 min. with addition of sugar. The cooked soy milk was immediately placed in an ice bath to cool down rapidly followed by addition of Butter Scotch flavor. For quality attribute analysis, liquid soy milk was sampled; the rest of the soy milk was used for Sensory Analysis. The lower value is most probably comes from the different water-to-bean ratio selected in soy milk production (8:1 in Harjai and Singh's study), but may also rise from the cultivar difference of the soybeans used.

DETERMINATION OF QUALITY ATTRIBUTES

pH VALUE

The pH of soy milk was measured with a calibrated digital pH meter .

TOTAL SOLIDS AND MOISTURE CONTENT

Total solids content was determined according to procedures described by Liu and Chang (2012). For raw material Soyabean, Moisture content was determined by Infrared Moisture Analyser and the Moisture content was determined as moisture %.

TS= 100 - Moisture content

ASH CONTENT

Ash content was determined after burning in a muffle furnace at 550°C for 12 hours (AOAC Method,900.02 A or B,920.117,923.03)

$$\%\text{ Ash (dry basis)} = \frac{\text{weight after ashing- weight of empty crucible}}{\text{Original sample weight – dry matter coefficient}}$$

where dry matter coefficient= %solid/100

FAT CONTENT ESTIMATION

Fat content were determined using Mojonnier method (AOAC Method, 989.05) .

$$\%\text{Fat} = \frac{100 *[\text{(wt of dish+ fat)-wt of dish}}{\text{wt of sample}}$$

In raw soyabean sample ,fat content was determined using Soxhlet Method (FAO, Food and Nutrition papers,1986).

$$\%\text{ Fat} = \frac{\text{(weight of beaker+ fat-weight of beaker)} \times 100}{\text{weight of sample in grams}}$$

TOTAL SOLUBLE SOLID ESTIMATION

Abbe Refractometer was used to calculate TSS. A drop of distilled water was placed on prism to check accuracy . The brix of the sample was taken using same procedure and TSS is expressed as ◦Brix.

PROTEIN CONTENT

Crude protein in food sample digested wth sulphuric acid in presence of catalyst at 380◦C (Willis et al. 1996). The nitrogen released from proteins and non- proteins constituents of the sample is converted to ammonium sulphate. The ammonia nitrogen reacts with salicylate-nitroprussde reagent in presence of soduim hypochloirte to form a green coloured complex whose absorbance is measured at 685nm. The crude protein concentration was calculated by multiplying the concentration of nitrogen obtained with a factor of 6.25. 100 mg of defatted food sample was taken in a Kjeldahl digestion flask. Added 2gm of digestion mixture along with 2ml of conc. Sulphurc acid and it was properly mixed. The contents were digested at 380◦ C for one hour. After digestion , contents were cooled and transferred in 100 ml volumetric flask and made up to volume with distilled water. Suitable aliquots were taken i.e 0.25,0.5,0.75,1 ml to determine nitrogen content in sample.

A set of standards were run in range of 4-20 µg N. The caliberation curve was constructed by plotting concentration of nitrogen on x-axis and absorbance at 685 nm on y- axis. The concentration of nitrogen was computed in the sample from standard curve. Crude protein in the sample was calculated by following equation:

Crude protein(%)= N×6.25* (for cereals)

CARBOHYDRATE CONTENT

Carbohydrate content was calculated by subtracting the moisture, protein, fat and ash content from the total mass. (Liu and Chang, 2012)

Carbohydrate content = (100- [moisture + crude protein + fat + ash]).

SENSORY EVALUATION

Sensory evaluation is the method used to identify the market susceptibility in the beverage based products. Without appropriate sensory analysis there is high risk of market failure. Sensory evaluation may be seen as the scientific discipline which looks at how measurements evoked and interpret the characteristics of food and materials as permitted by the senses. Human judges are used to measure the flavor or sensory characteristics of food. Soy milk from short time germination of soyabeans flavoured with Butter scotch with increased nutritive value was prepared as described in procedure and served for organoleptic quality testing. A panel of trained judges as well as 15 member sensory panel rated for different quality attributes on Hedonic Rating Test Scale as shown below. Sensory evaluation of the soy products was done by a 15 member sensory panel. The panel members were requested to evaluate the milk samples in terms of taste, colour, texture, flavour and overall acceptability on a 9 point hedonic scale ranging from 1= dislike very much to 9= Like extremely .

Table 1. Hedonic rating test scale

Degree of preference	Score
Like extremely	9
Like very much	8
Like much	7
Like moderately	6
Neither like nor dislike	5
Slightly dislike	4

Dislike moderately	3
Dislike much	2
Dislike very much	1

ASSAY FOR TOTAL PHENOLIC CONTENT

SAMPLE

Soyabean samples were washed and cleaned with tap water before soaked for 6 hoursat room temperature (28°C). After 6 hours, samples were put under wet muslin cloth and left germinated for 28 hours at room temperature (28°C) without direct contact with sun light (Yasmin et al. 2008).While soyabean sample without germination was also taken for analysis.

Extraction was done following the method of Xu and Chang (2008) with a slight modification. About 0.5 g ground germinated and non germnated samples were accurately weighed in centrifuge tubes. Five mililitres of acetone/ water (50:50 v/v) extraction solvent were added to the samples. The tubes were capped and vortexed (Boeco, Germany) for 15 min before centrifuged at 3000 rpm for 10 min (Hettich, Germany).Total phenol content was determined using the method of Makkar et al. (1993). Samples (50 µL) were put in test tubes and the volume was made up to 500 µL using distilled water. Then, 250 µL of Folin-Ciocalteu reagent was added into the test tube followed by 1.25 mL of sodium carbonate solution. The tubes were vortexed before incubated in the dark for 40 min. Total phenolic were expressed as gallic acid equivalents through the calibration curve of gallic acid (Sigma, USA) with the concentration range of 0-100 mg/ml. Absorbance was read at 725 nm using spectrophotometer (Shimadzu, Australia).

STATISTICAL ANALYSIS

Total phenolics content results were expressed as mean ± standard deviation (SD). Comparison of the difference in total phenolics between germinated and non-germinated soy bean was made.

RESULTS AND DISCUSSION

SELECTION OF OPTIMUM GERMINATION CONDITION

Germination effect of soybeans depends on several factors, including soaking time, humidity, germination time and temperature , and even under the same germination condition. There was an abrupt increase in radicle growth after the initial 28 h. Generally, it takes four to six days for soybean sprouts to mature, but the germination time considered in this study is only confined to the germination (1.6 cm sprout) at room temperature (~25 °C) for about 28 h was selected as the optimum germination condition for germinated soybean milk production, taking into account all aspects. The weight of different criteria for selecting the optimum condition can change with the purpose of product development. first three days, since a shorter time will theoretically favor better retention of the original soy milk perception and minimize the production cost and labor caused by the additional germination step in soy milk manufacturing. The sensory quality of soy beverages produced from germinated soybeans declined with increasing germination time, and two days was the limit without any significant changes in the sensory attributes. Germination (1.6cm sprout) at room temperature (~25 °C) for about 28h was selected as the optimum germination condition for germinated soybean milk production, taking into account all aspects. Of course, the weight of different criteria for selecting the optimum condition can change with the purpose of product development.

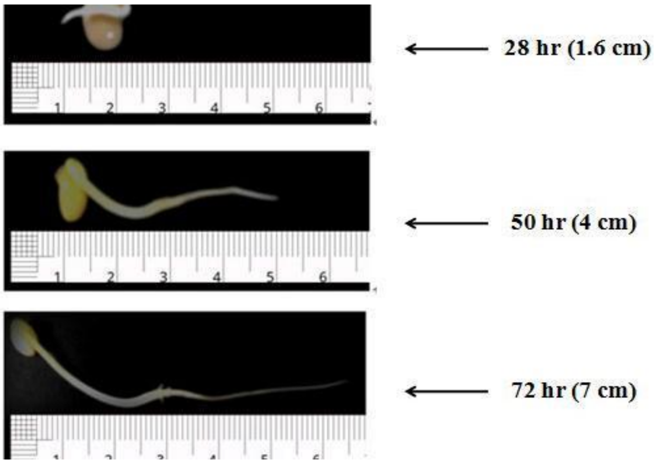

Fig 3. Radical development of soyabeans germinated for different time

PROFILE OF SOY MILK MADE FROM GERMINATED SOYBEAN

pH AND TOTAL SOLUBLE SOLIDS

The nutritional profile and some physicochemical properties of 28 h-germinated soybean milk are exhibited in relation to the non-germinated group. The pH of non-germinated soy milk is within the normal range (~ 7) i.e 7.05 , whereas it decreased a little in germinated soybean milk i.e 6.85.The small change is not supposed to greatly affect soy milk flavor, however, the pH change may have some effect on the success of the process using soy milk produced to make other soy products (e.g., tofu). The pH of raw soyabean was found to be 6.76 .Total Soluble Solids in case of non- germinated soy milk is 58% while in case of germinated soy milk TSS% is 57%. The pH of controlled soy milk is within the normal range (~ 6.7) determined by Liu and Chang (Liu and Chang. 2004) , whereas it decreased a little in germinated soybean milk, possibly affected by the changed ash content.

TOTAL SOLIDS, MOISTURE AND ASH CONTENT

After germination, Total solid content dropped significantly from 7% in case of non-germinated Soy Milk to 5% in germinated soy milk while the Moisture content increases from 93 to 95% from Non-germinated soy milk to germinated soy milk. Also in case of raw material used i.e soyabean the TS% is found to be 88% with moisture content equal to 12%. After germination, total solid content dropped significantly ($p < 0.05$) in the Germinated soy milk, as a combined effect of the soybeans absorbing water and utilizing the constituents for germination and seedling growth (Zeilenski et al. 2003). Liu and Chang. 2012) found that total solids in soy milk might positively relate to carbohydrate content. Ash content also significantly reduced from 0.65% in non-germinated soy milk to 0.56% in germinated soy milk , which could result from leaching into the water. In case of raw material soyabean Ash content is 5.3%.

CRUDE PROTEIN , FAT AND CARBOHYDRATE

Raw material Soyabean was found to contain crude protein and fat as 39.6 %and 17.07. Fat content had reduced a little i.e 1.50% in case of non-germinated soy milk and 1.28% in case of germinated soy milk, but the change is not significant. On the other hand, protein content increased significantly i.e 2.64% in case of non-germnated soy milk while 3.17% was obtained in germinated soy milk made from 28 h-germinated soybeans, which is reasonable, since germination of soybeans tends to increase their protein content as a result of a net synthesis of enzyme proteins, especially during the early stage of germination, when hydrolysis of storage protein in cotyledons is minimum.

Short-time germination is also shown to improve the protein quality in soybeans, as it promoted *in vitro* protein digestibility and generated a higher protein efficiency ratio (Martinez,2011). As protein is the most critical nutrient component in soy milk and daily consumption of 25 g of soy protein is recommended to reduce the risk of coronary heart disease. However, it is not clear in the present research whether the improved protein value is an effect of increased non-protein nitrogen, which was also observed to increase during soybean germination (Mostafa et al.1987). Total carbohydrate content in case of non-germinated soy milk is 2.21% but in case of germinated soy milk it remains 0.50%. Hence the loss during germination is primarily the result of a decrease in simple sugars, low molecular weight oligosaccharides and starch, which are quickly consumed in the metabolic processes during germination (Mostafa et al. 1987).This may improve the nutritional value of soy milk produced in terms of carbohydrate combination and eliminate flatulent problems related to soy milk consumption caused by raffinose and stachyose However, generally, changes with 28 h-germinated soybean milk composition provide comparable and even superior nutritional quality. The proximate composition of controlled soy milk is comparable with Harjai and Singh's investigations (Harjai et al. 2007) in which soy milk contained about 6% total solids, 3% protein and 1.75% fat. Fat content had reduced a little, but the change is not significant.

Table 2. Physicochemical properties of soy milk made from non-germinated and germinated (28 h) soybeans

Physicochemical properties	Non-germinated soybean milk	Germinated Soy Milk
pH	7.05	6.85
Total solids (g/100 g liquid soy milk)	7.0	5
Total soluble solids	58%	57
Moisture (g/100 g liquid soy milk)	93	95
Ash (g/100 g liquid soy milk)	0.65	0.56
Protein (g/100 g liquid soy milk)	2.64	3.11
Fat (g/100 g liquid soy milk)	1.50	1.28
Carbohydrate (g/100 g liquid soy	2.21	0.50

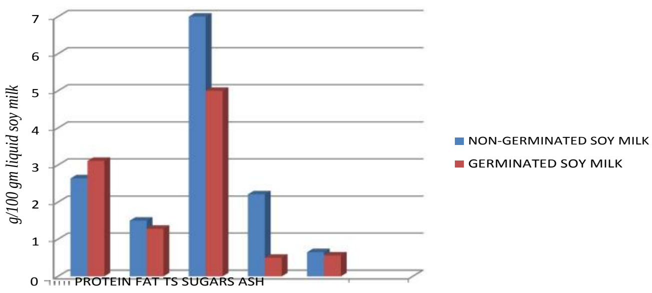

Fig4 :Representation of proximate compositions of soyabean obtained during analysis

TOTAL PHENOLIC CONTENT

The total phenolics in germinated and non-germinated soybean was determined. Among the two samples, non-germinated soy bean contained total phenolics (10.67 ± 0.43) and germinated soy bean contained (13.76 ± 0.294) total phenolics content . Comparing the germinated and non-germinated soy bean, the total phenolics content was increased significantly from 10.67 ± 0.434 to 13.76± 0.294 . During the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena etal. 2003; Dharmalingam and Nazni, 2013; Khandelwal et al. 2010).

Table 3. Total Phenolic content of Non- Germinated and Germinated Soyabeans(as expressed as mg gallic acid equivalent/mg dry weight)

Sample	Total Phenolic Content
Non-germinated soy bean	Germinated soy bean
10.67±0.434*	13.76±0.294*

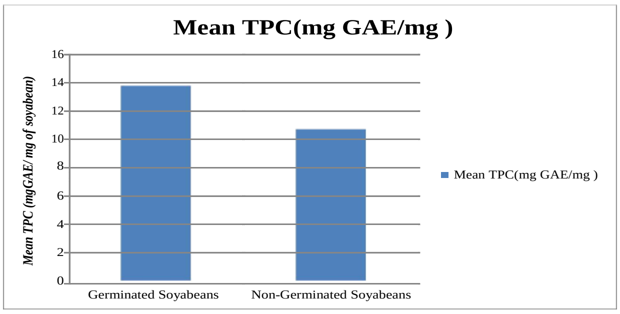

Fig5. Total Phenolic Content in extracts of Germinated and Non- Germinated Soyabeans

Fig5. Total Phenolic Content in extracts of Germinated and Non- Germinated Soyabeans

SENSORY EVALUATION

Result of sensory attributes' evaluation (figure.) showed that the addition of germination step for the production of soy milk significantly improved the taste ,texture, flavour as well as acceptability but the colour was not affected and the result are taken as mean values for each attribute. From overall acceptability, it is found that Soy milk made with short time Germination has been liked much by sensory panel as its score is equivalent to 8. Likewise, for taste, colour, texture, flavor the scores obtained from mean values are 8.45, 8.15, 8.45,8.75 out of 10.

Table 4. Sensory Evaluation Scores for Germinated Soy Milk

Taste	Colour	Texture	Flavour	Overall Acceptability
8.5	8.15	8.45	8.75	7.46

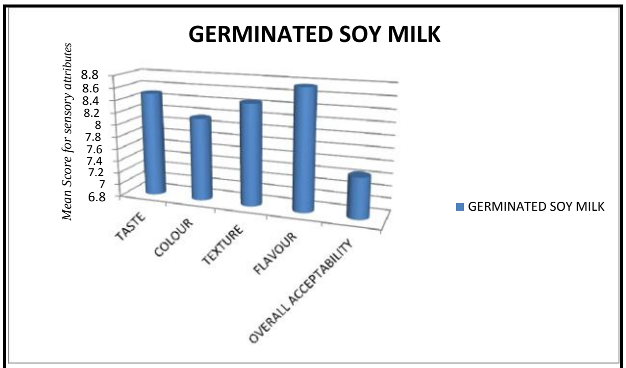

Fig 6. Result of sensory evaluation of Germinated Soy Milk (Mean score of Sensory attributes)

Fig 6. Result of sensory evaluation of Germinated Soy Milk (Mean score of Sensory attributes)

Although the sensory attributes of the value-added soy milk produced in this project are not supposed to drastically deviate from traditional soy milk, it is necessary to set up a sensory panel to evaluate these attributes and to ensure acceptability by consumers; the stability of formulation is another factor to be considered. As mentioned earlier, germination, a single process, serves as a pool of possibilities for raw material improvement in soy milk production, since many of the improved attributes during soybean germination are valuable for soy milk.

CONCLUSION

Extensive biochemical reactions take place during the process of soybean germination. Many of them lead to changes that are closely related to and can be key attributes in determining soy milk quality. In this research, improvements in bioactive components like total phenolics were investigated from short-time germinated soybeans. Radical development of soyabean germinated at different time was also seen. Optimum germination time was selected, and soy milk made from 28 h-germinated soybeans under optimum germination conditions showed significantly higher protein content and moisture content and a lower amount of fat, carbohydrate, ash, TS and carbohydrate and a generally comparable physicochemical profile to Non- Germinated soy milk. While the pH of The current approach seems to be a feasible and convenient way for the industry to develop functional soy milk products and provides practical data for industrial settings. Meanwhile, it offers a safer and more nutritious alternative for household soy milk consumers. Germination process was shown to increase the Total phenolic contents .The reduction of antinutrients may improve the nutritional quality of legumes .Besides, the findings in this study also imply that short-time germinated soy milk was liked much by the people as per its taste, colour, texture,flavor. Hence, Germination could be a potential pre-treatment method to promote the functional properties in various soybean-derived food products.

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