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Nutritional and physicochemical characterization of soymilk

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Abstract

Soybean (*Glycine max)* is a multifarious functional food due to the presence of various bioactive molecules *i.e.*isoflavones and phytosterols. The current rsearch work has been designed to explore nutritional significance of soy milk. Soy milk was prepared by soaking and grinding of soybeans and analyzed for pH, acidity specific gravity, total soluble solids, milk solid not fat, proximate composition and mineral contents. Results of soymilk physicochemical analysis such as pH (6.50), Acidity (0.67%), specific gravity (1.02), TSS (11.68%) and milk solid not fat (8.0%). Chemical analysis of soy milk showed that it contains moisture (88%), crude fat (3.68%), crude fiber (1.10%), ash (0.35%), NFE (2.35%) and crude protein (4.20%). Minerals contents such as Na (40.7mg), K (120.29mg), Zn (0.29mg), Mg (19.06mg) and Ca (23.57mg) were present in soy milk. Total phenolic and flavonoids contents were (400.27 mg) and (071.40mg).It contains high amount of protein and less amount of fat.So, it might be an alternate plant based protein as well as beneficial for CVD, diabetic and obese patients.

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Introduction

Soybean (*Glycine max*) belongs to a legume family Poacea is an important edible crop for many people all over the world. The word top producers of soy are United States, America, Brazil, India and China while japan is a major importer. Soybean cultivation started in East Asia but it is well adopted by tropical and subtropical areas of the world. In Pakistan, soybean cultivation started on small scales in northern areas and average production is low as compared to other countries. In last 5 years yield was only 351kg/hector is registered. Soybean is used as a major source of protein and oil so this is grown as a source of soy protein and soy oil for human and animal feed market. It has a concentration of protein 39% and oil is 21%. Pakistan is an agricultural country but still has low production of edible oil crop. Soybean is introduces as a new oil producing crop. Asian people consume more isoflavones then western countries and have low bone fractures ratios compare to other countries because of large amount of phytoestrogens present in Asian's diet.

Studies have shown that soybean phytosterols are beneficent to inhibit the absorption of cholesterol which is helpful for reduction of blood cholesterol level. It contains Soy isoflavones, soponins, protease inhibitors and some antioxidants which acted to eradicate mutagens and modulate initiation of tumors Soybean saponins work as soybean phytosterols which cause increase in its excretion and prevent its absorption results depletion of body cholesterol. Isoflavons present in soybean are naturally hetrocyclic phenols group. These isoflavons found in soybean approximately 0.1-5 mg/g. It is also present in the form of phytoestrogens mostly in plant foods. Soy and its products like supplements of isoflavone when consumed in higher quantities have resulted to eradicate cholesterol from blood serum.

It is also a source of many other nutrients such as fiber, vitamins oligosaccharides and protein. Presence of oligosaccharides which meet prebiotic standards and stimulate the growth of beneficial microorganism in large intestine (Jello *et al.*, 2011).

Soybean acts as a complete protein food for vegetarians as well as others. They provide not only some nutritional benefits but also has therapeutic effects especially for people who do not consume animal based protein. Soy protein is a good substitute of animal protein. Soybean contain high quality proteins (40-50%), carbohydrates (25-30%), fat (20-30%), dietary fiber (15%) and other foods (10%) as well as micronutrients like vitamins and minerals.It contains numerous phytochemicals (isoflavones, saponins, phytosterols, phytic acid and bioactive peptides) which reduce the cholesterol level by disrupting the absorption of cholesterol. An about 25g consumption of soy protein per day play important role to lower the risk of CVDs. It is important because they reduce menopausal symptoms as well as having potential roles in treatment, reduction and prevention of diseases like osteoporosis, cancer and kidney diseases (Mater *et al.,* 2010).

Material and methods

Procurement of raw materials

Soybeans *(Glycine max)* were procured from the local market of Faisalabad, Pakistan. All reagents were procured from Merck (Merck KGa A, Darmstadt, Germany) and Sigma-Aldrich (Sigma Aldrich, Tokyo, Japan).

Preparation of soy milk

Soybeans were cleaned manually by removing foreign objects. The cleaned beans were soaked in1L water for 14-16 h at 4 °C.The soaked beans were drained and blended with 1 L of water in a blender machine at low speed for 4 minutes. The resulted suspension was heated for 10 minutes at 80°C. The resulting material was filtered through a muslin cloth. Soy milk was cooled at room temperature (Pathomrungsiyounggul *et al.,* 2010).

Physicochemical analysis of soy milk

pH

pH of milk sample was directly measured from digital pH meter electrical digital type pH meter (WTW series pH-720) was used to determine pH of sample. pH meter was first calibrated using buffer solution of

pH 4 and 7. Milk sample was taken into beaker and adjusted to room temperature. Electrode of pH meter was immersed in milk sample and reading was taken after stabilization of pH meter.

Acidity

Acidity of samples was determined by titration method given in AOAC. Acidity is determined by taking 10g of milk sample in 100 mL Erlenmeyer flask and after adding 2-3 drops of phenolphthalein, it was titrated against 0.1N NaOH until endpoint of pink color was developed. The acidity was calculated by following formula:

$$\text{Acidity (\%)} = \frac{0.009 \times \text{volume used of M / 10 NaOH (mL)}}{\text{Weight of sample (g)}} \times 100$$

Specific gravity

Specific gravity of soy milk was calculated by using Lactometer. The soy milk sample was filled in a 100ml cylinder. Lactometer was introduced into the cylinder Readings were taken at which the soy milk touches the stem of the lactometer. Specific gravity was calculated using the formula as:

$$\text{Specific Gravity} = \frac{\text{CLR}}{1000} + 1$$

Where, CLR is correct Lactometer reading.

Total soluble solids

The total solids in each sample were determined according to standard procedure given in AOAC (2011). About 3g milk sample was taken in china dish and placed in water bath for 15 min at 65 ^{o}C. It was then placed in hot air oven for 3.5hours at 100 ^{o}C. Sample was placed in desiccator for half hour to cool and was weighed on digital weighing balance. Percent residue were calculated as

$$\text{Total soluble solids (\%)} = \frac{\text{Residue after drying}}{\text{Weight of sample (mL)}} \times 100$$

Milk solid not fat

The milk solid not fat content was determined by Kirk and Sawyer (1991). A 10g sample of soy milk was taken in a porcelain dish and 1mL of phenolphthalein indicator was added. The sample was then titrated with 0.1M NaOH solution. Then 3mL of formalin was added to the neutralized mixture and titrated to neutrality against 0.1M NaOH solution (titration= XmL) 3 mL of formalin solution was titrated separately (titration= Y mL)

SNF% = 5.67(X-Y)

Proximate analysis

Moisture content

Moisture content in samples was determined by using the hot air oven (Memmert 200, Germany) according to method No. 44-15A [5]. Sample (2g) was taken in pre-weighed china dish and placed in oven at 100±5^{o}C for 24 hours. After attaining the constant weight the sample was removed from the oven, cooled in desiccator and weighed.

$$\text{Moisture (\%)} = \frac{\text{Wt. of original sample (g)} - \text{wt. of dried sample}}{\text{Wt. of original sample (g)}} \times 100$$

Crude fat

Crude 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^{o}C for 1 hour and weighed.

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

Crude fiber

Crude fiber in the samples was estimated by using Labcono Fibertech (Labcono Corporation, Kanas, USA.) and the standard procedure as given in method No. 32-10 (AACC, 2000). The fat free sample (2g) was subjected to crude fiber determination by digesting the sample in glass beaker attached to the Fiber tech for 30 min in 200 mL boiling H_2SO_4 (1.25%). The acid was drained out and digested sample was filtered and washed thrice with distilled water to make it acid free.

Then it was again digested for 30 min in 200mL of boiling NaOH (1.25%). The alkali was drained off and digested sample was filtered and washed thrice with distilled water to make it alkali free. The residue obtained was dried in hot air oven at100±5°C for 24 hours to get oven dried weight. Dried residue was further ignited in Muffle Furnace at 600°C to get ash content.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of oven dried residue (g)} - \text{Weight after ashing (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude 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 ($CuSO_4$, $FeSO_4$, K_2SO_4 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.

$$\text{Nitrogen (\%)} = \frac{\text{Volume of 0.1N } H_2SO_4 \text{ used (mL)} \times \text{Volume of dilution (mL)} \times 0.0014}{\text{Weight of sample (g)} \times \text{Volume of aliquot sample (mL)}} \times 100$$

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.

Total ash

Ash was determined by incineration of sample as inorganic matter by following the procedure as mentioned in method No. 08-01 (AACC, 2000). The sample (1g) was taken in a pre-weighed crucible and charred on burner before incinerating in the Muffle Furnace (MF-1/02, PCSIR, Pakistan) where a temperature of 600°C was maintained till the sample was converted to grayish white ash. The crucible was weighed after cooling in desiccator.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Nitrogen free extract (NFE)

The NFE was calculated by using the following formula:

$$\text{NFE (\%)} = 100 - (\%\text{Moisture} + \%\text{Crude protein} + \%\text{Crude fat} + \%\text{Crude fiber} + \%\text{Ash})$$

Mineral analysis

Soy milk was analyzed for minerals by using wet digestion method. Sample (0.5) was first digested at low temperature (60-70°C) with 10mL of HNO_3 for 20 min in 100mL conical flask on hot plate, then it was digested at high temperature (190°C) with 5mL 60% $HClO_4$ till the contents of flask became clear. The digested samples were transferred to 100mL volumetric flask and volume was made with double distilled and de-ionized water and then filtered. The filtered sample solution was run through atomic absorption spectrophotometer (AA240 Varian K, Australia). Samples of known strength were first run for each mineral to obtain standard curve. The mineral contents of the samples were determined by using the respective standard curve prepared for each element (AACC, 2000). All samples were analyzed for sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu) and iron (Fe) contents using flame photometer (Sherwood Flame Photometer 410, Cambridge, UK) according to method given in AOAC (2011).

Total phenolic and flavonoids

Total phenolic and flavonoids of soy milk were estimated by following the modified protocol as stated by Rani and Pradeep (2015).

Extraction of is flavones

The freeze dried samples 10 g were extracted with 20 ml of 80% ethanol in a flask and were stirred for 1 h at 60°C. The extracted solutions were centrifuged at

5000× g for 10 min, and the supernatants were dried by evaporation. Following the evaporation of aqueous ethanol, the insoluble residue was dissolved in 10 ml of 80% ethanol. The extract obtained was used for further antioxidant activity.

Total phenol content

Total phenol contents (TPC), of the extracts were determined by method as developed by Benherlal and Arumughan (2007). Gallic acid is used as a standard; stock solution of gallic acid was prepared by dissolving 0.5 g gallic acid in 10 ml of ethanol in a 100 ml volumetric flask and diluting to volume with double distilled water. To prepare a calibration curve 0, 1, 2, 3, 5 and 10 ml of gallic acid stock solution were added into 100 ml volumetric flask separately and then diluted to volume with double distilled water. The resultant solutions contained concentrations of 0, 50, 100, 150, 250 and 500 mg/l gallic acid.

The sample 40µl was pipetted into separate cuvette and 3.16 ml of double distilled water was added. FolinCiocalteu's reagent 200µ was added and mixed well. After 8 min, 600µ of 20% sodium carbonate solution was mixed thoroughly. The solution was allowed to stand at 40 ^{o}C for 30 min and absorbance of each solution was noted at 765 nm against the blank (without phenolic solution).

Results and discussion

The results pertaining to various aspects of the study are discussed below:

Physicochemical analysis of soy milk

Soy milk was analyzed for pH, acidity, specific gravity, total soluble solids and milk solid not fat by using their respective procedures. Mean values of all parameters are given in Table 1.

pH

Mean values of pH of soy milk was 6.5-7.5±1.50 (Table1). It has direct influence on the flavor perception in the dairy products. Product pH is influenced by biochemical changes and its composition.

Acidity

Means for acidity of soy milk was 0.67±0.002. The result pertaining to mean values given in Table1.

Table 1. Mean values of physicochemical analysis of soy milk.

Compositional Parameter	Mean values±SD
pH	6.50±1.50
Acidity	0.67±0.12
Specific gravity	1.02±0.05
Total soluble solids	11.68±1.50 %
Milk solid non fat	8.00±2.00 %

Specific gravity

Specific gravity of soy milk was 1.02±0.05 (Table 1). A comparative study of soy paneer prepared from soy milk, blends of soy milk and skimmed milk was designed to develop processed paneer by partial addition of soy milk and skim milk. Sample A was prepared with 100% soy milk using citric acid at a concentration of 1.5% as a coagulant whereas sample B and C prepared with skimmed milk and soy milk at a concentration of 1.5% citric acid as a coagulant.

The samples were analysed for proximate analysis and specific gravity. Specific gravity was ranged from 0.98 to 2.35according to study (Krishna *et al.*, 2003; Park *et al.*, 2005).

Total soluble solids

The mean value of total soluble solids of soy milk was 11.68±1.50% given in Table1. Total solids in frozen product play a major role in their quality.

The addition of sugar and other ingredients like rice flour cause overall increase the total solids. A study was conducted to improve and diversify the food and nutrition status of low income families in developing world by initiating the production of soy corn milk followed by evaluating its quality and sensory attributes. Soy corn milk was prepared from soybeans and maize with 75% and 25%, respectively. Milk sample was analyzed for different physicochemical analysis and total solid contents were 12.2% (Kolapo*et al.*, 2006).

Milk solid non fat

Mean value of milk solid nonfat contents of soy milk were 8.00±2.00% given in Table 1. In a study sesame ice cream was prepared by using soy milk and analyzed for different parameters. Milk solid nonfat contents were higher in ice cream prepared with skim milk (8.9%) as compared to ice cream prepared with soy milk (7.5%). According to a research, effect of skim milk and soy milk blends on the quality of ice cream was examined. Four types of soy milk blends were prepared from soy flour or soy powder for the formation of new variety of ice cream. The physicochemical sensory as well as micro element detection of ice cream was performed. According to results milk solid non-fat contents were ranged from 9.25-14% (Abdullah *et al.,* 2010).

Table 2. Mean values of proximate analysis of soy milk.

Parameters	Mean values±SD
Moisture	88.25±2.00
Crude fat	3.68±1.00
Crude protein	4.20±1.00
Crude fiber	1.10±0.50
Ash	0.35±0.02
NFE	2.35±1.15

Proximate analysis of soy milk

Moisture

Means values for moisture of soy milk in Table 2 According to others, moisture contents were found 88.25±2.00 in soy milk. A research was performed for the comparison of physicochemical and functional properties of soy milk with the addition of onion. For this purpose, whole onion with peel added into soy milk for the production of soy milk with whole onion and peeled onion for the production of whole peel onion. Control soy milk was revealed 90.54% moisture and soy milk with onion peel 90.21% (Jhang *et al.*, 2009).

Crude fat

Means values for crude fat of soy milk was 3.68±1.00 as in Table 2. It has been reported that low fat prevent the blockage of arteries and affects food perception including mouth feel, appearance and texture. Low fat under such formulations is preferably being offered to diet conscious persons. Physicochemical and sensory characteristics of chocolate prepared from soy milk was determined.

In this research, beany flavor of soy milk was reduced by washing and autoclaving. Various combinations of ingredients were added for the preparation of chocolate. The soy milk was analyzed for carbohydrates moisture, fat and total ash. Fat content in soy milk was found 2.6% (Raja *et al.*, 2014).

Crude protein

The result pertaining for means values of crude protein of soy milk was 8.00±1.00 given in Table 2. The presence of proteins in a product represents its quality index. Protein is consist of amino acids. By measuring the amount of nitrogen in a sample the quantity of crude protein is determined. Total nitrogen contributes to the flavor, body and texture to the finish product.

It is also essential for the formation of small stable air cells. Total nitrogen is also a source of calories. Total nitrogen is responsible for variation in solubility index as well as process variation. A study was done to determine the physical, chemical and sensory properties of soy milk, tofu and doughnuts made from specialty full fat soy flour. Several specialty soy flours were used to prepare soy milk and analysed for proximate analysis. The protein contents of soy milk was ranges from 6-9%(Raja *et al.*, 2014).

Crude fiber

The result pertaining to mean values of crude fiber of soy milk was shown in Table 2. The mean value of Crude fiber was 1.10±0.50. A study done for the comparison between the effect of soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia.

In this research soy milk and non-fat cow milk were analyzed for compositional analysis and crude fiber contents were observed 0.25 (Mcdowell *et al.*, 2002).

Ash

The result pertaining to means values of ash contents of soy milk was 0.35±0.02 are shown in table 2. The ash content is an inorganic residue remaining after the removal of water and organic matter by heating in the presence of oxidizing agents. This gives a measure of the total amount of minerals in a food. Ash in dairy product is an important source of many minerals and vitamins and in low calorie density. Basic purpose of this study was to prepare soy milk with improved food quality and to enhance the functional quality attributes by incorporating short term germination into the processing. In the subsequent evaluation on the quality attributes under the optimum germination condition, soy milk made from 28 h-germinated soybeans presented enhanced nutritional value and comparable physicochemical properties to conventional soy milk. The ash content was ranged from 0.33±0.1 to 0.35±0.01. The results of the current research project were in agreement with the findings of a study which has been conducted previouslyMartinez *et al.* (2011).

Table 3. Means values of mineral contents of soy milk (mg/100g).

Mineral contents	Mean values/100g±SD
Na	40.37±5.00
Ca	23.57±3.02
K	120.29±2.00
Mg	19.06±2.30
Zn	0.29±1.50

NFE

The means values of carbohydrates of soy milk were 2.35 accessible in Table 2. In a study, comparison of nutritional and chemical parameters of soy milk and cow milk was done and showed that soy milk contains 4.67g fat, 0.52g of fatty acids, 6.73 of protein, 4.43 of carbohydrate and 0.00 of lactose. Present results were strongly agreed with this study as the mean value of nitrogen free extract was 4.25 (Rehamn *et al.,* 2007).

Mineral contents

Sodium is an important mineral present in body and controls the blood pressure. It regulated the function of nerves and muscles that's why sodium concentration carefully controlled by the body.

It also works in concert with potassium to maintain normal body fluids. Means values of sodium contents of soy milk was 40.37±5.00 mg shown in Table 3.Potassium is an important mineral that conduct electricity in the body along with sodium chloride, calcium and magnesium. It is crucial to heart functions and plays a key role in skeletal and smooth muscle concentration. Mean values of potassium of soy milk was 120.29±2.00 mg shown in Table 3. Making it important for normal digestive and muscular function (Park *et al.*, 2005).

The means values of Zn of soy milk were 0.29±1.50 mg given in Table 3. Zn is necessary for body's immune system to work properly and a good wound healer. It play important role in cell division, cell growth and breakdown of carbohydrates. It is also needed for smell and taste. Means values of Magnesium was 19.06±2.30 mg (Table 3).

It works as an enzyme cofactor. It helps in the formation of DNA and RNA. It regulates the cholesterol production in the body.The mean value of calcium of soy milk was 23.57±3.02 mg (Table 3) body use 99 percent of its calcium to keep bones and teeth strong and healthy. It supports skeletal structure and function.

The rest of the calcium in body plays a key role in cell signaling, blood clotting, muscle contraction and nerve functions. A study was conducted on improving the mineral availability in soy milk. A double blind randomized study was conducted on 60 patients and soy milk was given to these patients to determine the comparison of effect of soy milk and non- fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia. For this reason, soy milk was analyzed for proximate analysis and mineral analysis. Result range for sodium from 37±4.2mg for potassium 139±1.5, for Zn 0.22±0.1, for Mg 20±1.1 and for calcium 7±1.5(Liliana *et al.*, 2004).

Table 4. Means values of total phenolic contents and flavonoids in soy milk.

	Mean±SD
Total phenolic contents	400.27 ± 0.52 mg GAE/100 g
Total flavonoids contents	71.40±3.42mg GAE/100 g

Total phenolic and flavonoids

Means values of total phenolic contents and flavonoids of soy milk were 400.27±0.52 and 71.40±3.42 respectively shown in Table 4. A study was conducted to determine antioxidant capacity, phenolic and flavonoids in soybean by products. High protein and carbohydrate contents were found in grade A soy milk powder as compared to grade B soy milk powder and husk powder. Ash and total fiber were found highest in soy husk. Antioxidant capacity was assessed by using beta carotene bleaching assay was in order of soy husk > GBSP > GASP (Prior *et al.,* 2005).

Flavonoids ware abundantly present in fruits, vegetables and grains. As the secondary metabolites in plants, flavonoids are beno- γ-pyrone derivatives composed of polyphenol and pyrane rings has health promoting effects. Use of foods contain active ingredients in considerable quantity minimize the strength of metabolic ailments. The risk of diseases like stroke, GIT dysfunctions, diabetes mellitus, CVDs, obesity and hypertension are controlled by dietary interventions. However dietary fiber intake help in reduction of glucose level, serum lipid concentrations, blood pressure and improving body's defense system. Conclusively, many life threating disorders are treated sustainably by diet based therapies and knowledge about active ingredients is required for successful strategy and target population (Steyn *et al.*, 2008).

Conclusion

Soybean represents an excellent source of high quality protein with a low amount of saturated and Trans fatty acids along with the raised quantity of dietary fibre. The research has shown the possible reduction in the incidence of cardiovascular diseases and it also improves the glycemic load. The isoflavones of the soybean is also associated with prevention and treatment of various types of cancers and other multiple disorders. Thus, it would be beneficial to consider the replacement of animal based food items for the soybean foods in order to achieve the health and nutritional benefits.

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