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# Evaluation of Antibacterial and Antioxidant Properties of Soy Whey based Beverages

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**ABSTRACT: The present study was aimed to utilization of soy whey obtained from tofu using *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactococcus lactis* cultures. As the soy is rich in isoflavones which are bioactive compounds, soy whey with fermentation would result in a functional drink. The soy whey beverages added with different levels of sugar at 4, 5, and 6%. At the 6% sugar level, the soy whey beverage prepared was found to be best with respect to sensory attributes. In case of antibacterial activity of soy whey and its beverages, among the tested organisms used soy whey beverage showed inhibitory zone 17 sq.mm, 18 sq.mm, and 4 sq.mm against *E. coli, Salmonella* sp. and *Staphylococcus aureus* respectively. Supernatant of soy whey beverage showed inhibitory zone18 sq.mm, 15 sq.mm and 4 sq.mm against *E. coli, Salmonella* sp. and *Staphylococcus aureus* respectively. CFE of soy whey beverage showed inhibitory zone 22 sq.mm, 19 sq.mm and 10sq.mm against *E. coli, Salmonella* sp. and *Staphylococcus aureus* respectively. The antioxidant activity (% DPPH) values for the soy whey, soy whey beverage and CFE of soy whey beverage were 63.87, 65.59, and 66.02% respectively. The optimized soy whey beverage was stored at refrigeration temperature (7 ± 1$^0$C). The control cow milk whey beverage remained in an acceptable condition with the pH 6 and acidity 1.2% of lactic acid while coliform and yeasts and molds were nil at the end of 9$^{th}$ day. In case of the soy whey beverage, it remained in acceptable condition with the pH 5.5 and acidity of 0.27% of lactic acid while Coliform and yeasts and molds were nil at the end of 6$^{th}$ day. The prepared soy whey beverage is a highly nutritious and functional drink for the consumers.**

**Keywords:** Soy whey, lactic cultures, soy whey beverages, antibacterial property, antioxidant property.

## INTRODUCTION

Soybean occupies a premier position among agricultural crops, being the most important source of good quality concentrated proteins as well as vegetable oil. Seeds of soybean have been used in Asia and other parts of the world for many centuries to prepare a variety of fresh, fermented and dried foods. Soy-based nutritious food products such as tofu, soy milk, soy sauce, miso, etc. have been developed for human consumption while oil extracted soy meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil finds multifarious uses in industries related to production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics. Recently, use of soy oil as biodiesel has opened up another possibility of renewable sources of energy for industrial uses. As a legume crop, soybean is capable of utilizing atmospheric nitrogen through biological nitrogen fixation and is therefore less dependent on synthetic nitrogen fertilizers (Aditya *et al*., 2012). World soybean production in 2019-20 is estimated as 333.67 million tonnes (MT) from a total area of 120.50 million hectares. India ranks fourth in area with 11.34 million hectares (28.02 million acres) accounting for 9.14% of the world area. Soybean development is highly sensitive to environmental fluctuations and water is the major factor having great impact on its productivity.

The fermented foods can benefit consumers compared to simple foods in terms of antioxidants, production of peptides, organoleptic and probiotic properties, and antimicrobial activity. It also helps in the levels of anti-nutrients and toxins level. The quality and quantity of microbial communities in fermented foods vary based on the manufacturing process and storage conditions/durability. Fermentation is the conversion of carbohydrate through homo or hetero fermentation to organic acids or alcohol and carbon dioxide, using lactic acid bacteria (Sharma *et al*., 2020).

Soymilk is a cheap, decent protein source that does not contain lactose. It replaces bovine milk as well. Though, soymilk protein is less digestible and more anti-nutrient than milk protein, it resolves the lactose intolerance problem and act as a vegan milk supply. In soymilk bioactive compounds like isoflavones, genistein, daidzein, and glycitein can also be minimized by the thermal processing.

The medicinal and nutritional properties of various fermented foods have been experienced by several generations. Recently, importance has been given to produce fermented milk products. A fermented milk product has been defined by the International Dairy

Federation as the milk product prepared from skimmed milk or not with specific cultures. Although soymilk has a composition similar to cow's milk, the oil-to-protein ratio is lower. For this reason, reconstituted soymilk is often supplemented with oil. In addition, soymilk may be supplemented with sugar to enhance its palatability. These soymilk beverages often contain fruit juice, cocoa, flavours (artificial or natural), stabilizers, and other ingredients to enhance customer acceptance by masking soy flavours (H E Snyder and L A Wilson 2013).

Lactic acid bacteria (LAB) play a major role in determining the positive health effects of fermented milk. LAB are the Gram positive, facultative anaerobic, non-spore former, catalase negative, litmus milk reducers. They utilize lactose as sole source of carbon and in turn release the lactic acid as the principle acid. The health benefits of fermented milks include prevention of gastrointestinal infections, reduction of serum cholesterol levels in turn reducing atherosclerosis and possess antimutagenic activity which may be due to their antibacterial property. Soy milk supports healthy muscles and organs which is rich in omega-3-fatty acids, they are the healthy fats which body cannot form on its own. Omega-3-fatty acids reduce risks of dementia and Alzheimer's disease. The fermented milks are recommended for consumption by lactose intolerant individuals as most of lactose will be metabolized by LAB.

Whey is a by-product obtained during the manufacture of products like tofu, etc. which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste thus building the BOD content of the dairy sewage. Production of whey- based beverages started in 1970's and until today a wide range of different whey beverages has been developed. Whey is a genuine thirst quencher, unlike most soft drinks. In order to reduce the BOD level of the whey and to acquire therapeutic nutrient-rich whey can be utilized for the production whey beverage. Whey beverages are suitable for wide range of consumers from children to the oldest ones (Basant *et al*., 2017). As soy whey contains a certain amount of nutrients and has high water content, it requires further treatment before it can be disposed of into the sewage and this wastewater treatment is an expensive process that contributes significantly to the tofu manufacturing cost. The nutrient content in soy whey, which consists of carbohydrates, nitrogen compounds and minerals, supports microbial growth (Belen *et al*., 2013). This study was conducted to develop a new soy-based food product utilizing whey and maximize the health benefits of whey for the consumer as a functional drink.

## MATERIAL AND METHODS

The experiment was conducted at Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bangalore – 560024. The samples and cultures were collected and maintained in The Department of Dairy Microbiology.

**Preparation of Soy Milk.** The clean, dried and off-white coloured soybean of 100 gm quantity were soaked in water for 10-12 hours or overnight. Soaked soybean's husk were removed by means of pressure of two hands and cleaned with continuous flow of fresh water. Then clean dehulled soybeans were ground with water by blending machine for 10-15 min at low speed. The homogenized mass was strained through a sterile muslin cloth to separate milk from residue. The separated milk was diluted with distilled water in 1:4, 1:5 and 1:6 ratios. Soymilk was then pasteurized at 63°C for 30 minutes in hot water bath (Wasima *et al*., 2016).

**Preparation of Soy Whey.** Soy whey was obtained as per the procedure of (Jian *et al*., 2019) with slight modification. Soy whey can be generated via tofu production. Tofu was produced firstly grinding the soaked soybeans with water to obtain soy milk. The soy milk was then heated up followed by inoculating the soy milk with 10% of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactococcus lactis* cultures which were maintained in a reconstitute sterile skim milk and was incubated at 37° till the milk was set. The coagulated milk was transferred to the previously sterile muslin cloth and tied with the thread and hanged at room temperature 30° for 10-12 hr. Then the whey obtained from the tofu was collected in a sterile conical flask and used for further analysis or stored at 4° until further analysis.

**Preparation of Soy Whey Beverage.** Soy whey beverage was prepared by adding sugar (granular sugar) to the whey sample at 4, 5 and 6 per cent levels, then the well mixed whey samples were transferred to the previously sterile bottles and subjected for heating in a water bath maintained at 85° for 5 minutes and cooled to room temperature and stored at refrigeration temperature (7°).

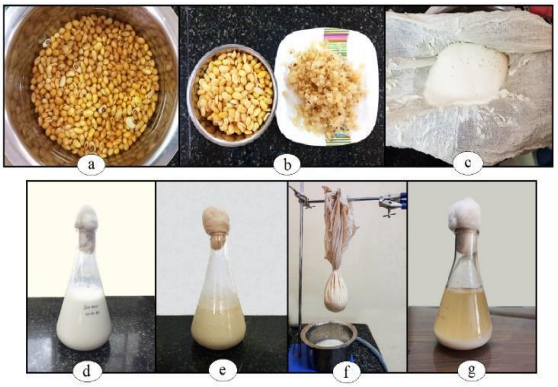
**Chemical Analysis of Soy Milk, Soy Whey and Whey Beverage**

**1. Determination of Titratable Acidity in Soy Milk (ISI:SP18 PART XI, 1981)**

Samples of soy milk and whey beverage each of 10ml was transferred to clean dry beakers, added 2-3 drops of phenolphthalein indicator. Mixed well and titrated against 0.1N NaOH till pale pink colour persisted for 30 sec. The titratable acidity of the test sample was calculated by,

Percent lactic acid (Percent LA) =9 x 0.1 x V/W

V = Titre value; W = Weight of the sample




(a) Soaking of Soybeans; (b) De-husking of Soybeans (c) Extraction of Soymilk; (d) Soymilk; (e) Fermented Soy Curd; (f) Draining of Soy Whey; (g) Soy Whey

**Fig. 1.** Preparation of Soy Whey.

**2. Determination of Protein Content in Soy Milk by Pyne's Method (ISI:SP18 PART XI, 1981)**

Sample of 10 ml was transferred to clean dry conical flask; 1ml of phenolphthalein indicator and 0.4 ml saturated potassium oxalate solution was added. The mixture was left for 2 min at room temperature and titrated against 0.1 N NaOH. Neutral formaldehyde solution of 2ml was transferred and further titrated against 0.1 N NaOH. Protein content in the sample was calculated and expressed as per cent.

Per cent protein = V x 1.7 (V=Titre value)

**3. Fat Estimation of Soy Milk by Gerber's Method (ISI: SP18 PART XI, 1981)**

Gerber Sulphuric acid (90 per cent) 10ml was taken into clean and dry butyrometer, pipetted 10.75 ml of samples separately into it and finally 1ml of amyl alcohol was added. Stopper was tightened and contents were mixed by shaking at 45° angle, placed the butyrometer in the Gerber centrifuge and balance it, centrifuged for 5 mins. Fat column was adjusted within the scale of butyrometer and reading was noted. Fat was expressed as per cent.

**4. Determination of Total Solids (FSSAI, 2016)**

About 5 ml of the sample was transferred into a beaker, warmed slowly to 35-40°C on a water bath with careful mixing to incorporate any cream adhering to the sample. The sample was cooled quickly to room temperature. A dish was heated with its lid alongside in the drying oven at least 1hour. The lid was placed on the dish and immediately transferred to a desiccator and allowed cooling to the room temperature (at least for 30 mins) and weighing to the nearest 0.1mg. 5ml of prepared sample was added, to the lid on the vigorously boiling water bath in such a way that the bottom of the dish is directly heated by the steam. Heating was continued till most of the water was removed. The dish was removed from the water bath, the underside was wiped and placed in the oven alongside the lid and dry in the oven for about 2 hours. The lid was placed and transferred to the dessicator. The dish was allowed to cool and was weighed to the nearest 0.1mg. Again, the dish was heated with its lid alongside in the oven for 1 hour. The lid was placed on the dish and immediately transferred to the dessicator followed by cooling and weighing again. The operation was repeated again until the difference in the two consecutive weighing does not exceed 1mg. The lowest mass was recorded.

**Calculation**

Total Solid Content= $M_2$-$M_0$/ $M_1$-$M_0$ x 100

Where

$M_0$= mass in g of dish + lid

$M_1$=mass in g of dish + lid and test portion

$M_2$=mass in g of dish + lid and dried test portion

**Microbiological Analysis of Whey Samples (Harrigan,1998)**

**1. Serial Dilution.** Sample of 11 g was weighed aseptically and transferred to the sterile 99 ml of phosphate buffer diluent to make 1st dilution. Further required ($2^{nd}$ & $3^{rd}$) dilutions were prepared serially using $1^{st}$ dilution.

**2. Pouring of the Media.** Violet Red Bile Agar (VRBA), Malt Extract Agar (MEA) media were used for Coliforms and Yeasts and molds, then the media were allowed to solidify.

**3. Incubation.** Coliforms plates were incubated at 37°/ 18-24 hours and yeast and molds plates were incubated at 30°C for 3-5 days.

**4. Colony Counting.** After the completion of the incubation period, the typical colonies were counted in the countable plates ranging between 30-300 using colony counter and average count was expressed as cfu/ml of the product.

**Sensory Analysis.** Sensory attributes of soy whey-based beverage were carried out by the expert panel in this area by using a nine-point hedonic scale to judge the quality of the product with respect to body and texture, colour and flavour and overall acceptability.

**Determination of Antibacterial Activity of Soy Whey Beverage**

**1. Preparation of Soy Whey Fractions for the Antibacterial Activity**

The whey fractions were prepared as described by Aneja *et al*. (2002) and used immediately after the preparation. Whey beverage was prepared essentially as described by Babar *et al*. (2008) from fermented soy milk. The suspension was centrifuged (10,000 rpm for 20 min at 5 °C) and the supernatant was filtered using a 0.45µm filter (Uugantsetseg *et al*., 2014). Then the whey, supernatant and CFE fractions were checked for antibacterial activity against the test pathogens *E. coli*, *Salmonella* sp. and *Staphylococcus aureus*.

**2. Antibacterial Activity of Whey Fractions**

The seeded agar plates were prepared by pouring 20ml of Molten Muller Hinton agar and added 0.2ml of pathogen culture which was previously adjusted to get 10° cells/ml, then the plates were incubated at refrigeration temperature for 30 mins. Wells of 7mm were made using the sterile seven mm diameter borer, 50µl of soy whey, whey beverage, whey supernatant and CFE were transferred to the respective wells and incubated at 37°C without inverting the plates for 24 hours. The inhibitory zone area was measured around the well (Prabha *et al*., 2008). The zone formation around the wells were measured as indicative of antibacterial activity. Sample producing inhibitory zone formation around the well was considered as having antibacterial activity against the particular pathogens.

**Determination of Antioxidant Property of Soy Whey Beverage**

**1. Preparation of Whey Beverage for Antioxidant Activity Test**

The whey fractions were prepares as described by Virtanen *et al*. (2012) and used immediately after the preparation. Whey (15 ml) was collected from the fermented okra and the pH was adjusted to 4.6 with 1M HCl. The suspension was centrifuged (10,000 rpm for 20 minutes at 5°C and the supernatant was filtered on a 0.45µm filter.

**2. DPPH Radical Scavenging Activity of Whey Fraction** (Uugantsetseg *et al*., 2014)

DPPH (2,2-diphenyl-1-piccrylhydrazyl) was added as a stable radical. A volume of 2ml of DPPH radical solution (0.004 percent, W/V) in 95 percent ethanol was added to 2ml of the whey samples, mixed vigorously and allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured at 517nm using the calibrated spectrometer. Ethanol was used as blank,

while DPPH solution in ethanol served as the control. The radical scavenging activity of the samples was expressed as, percent inhibition of DPPH absorbance:

Inhibition = [(A control-B test)/A control] x 100

Where:

A - Control is the absorbance of the control sample (DPPH solution without whey fraction)

B - Absorbance of test sample (DPPH solution plus whey fraction)

**Storage Study of Soy Whey Beverage**

Optimized soy whey beverage obtained from soy milk using lactic cultures along with the control samples were stored in the sterile glass bottles at refrigeration temperature (7 ± 1°) till visual as well as flavour and microbial defects were noticed in the tested samples. The samples were drawn from the refrigeration temperature stored samples once in 3 days for sensory, chemical and microbiological analysis. Cow milk whey was carried for comparison.

## RESULTS AND DISCUSSION

Soybean is a base material for soy whey functional drink preparation, which is famous in Madhya Pradesh. It is a fermented product prepared by setting the milk by using lactic cultures. During the preparation, the whey obtained is normally discarded through having plenty of nutrients. Some of the nutrients express therapeutic benefits like antibacterial and antioxidant property. In this study an attempt is made to develop soy whey-based beverage to analyze the sensory, chemical, microbial, antibacterial and antioxidant properties of soy whey beverages, with shelf life study of optimized whey beverage. The results obtained were statistically analyzed wherever required and presented in the form of table, graph and photographs.

**1. Principle chemical components of raw soy milk used for whey preparation**

The raw soy milk was prepared by grinding the clean dehulled soybeans with water. The homogenized mass was strained through a sterile muslin cloth to separate milk from residue and the milk was collected in a sterile container, pasteurized and stored at refrigeration temperature (7°) till analysed. The raw soy milk used in this study was analysed for the principal chemical components (Table 1). The milk had 0.11, 2.80, 2.4, and 7.7 per cent acidity (lactic acid), protein, fat, and total solids respectively. Odour of raw milk was found to be satisfactory.

**Table 1: Principle chemical components of raw soy milk used for whey preparation.**

| Components | Percent |
|---|---|
| Titrable acidity (Lactic acid) | 0.11% |
| Protein | 2.80% |
| Fat | 2.4% |
| Total solids | 7.7% |

**1. Chemical quality of freshly prepared soy whey**

The freshly prepared soy whey was analysed for the chemical components**.** The whey was obtained from tofu using 10% of lactic cultures consisted of (Table 2) 0.64, 2.80, 2.5 per cent of acidity (lactic acid), protein, and total solids respectively and was fat-free. The pH of the soy whey was observed as 5.5.

**Table 2: Chemical quality of freshly prepared soy whey.**

| Components | Percent |
|---|---|
| Titrable acidity (Lactic acid) | 0.64% |
| Protein | 2.80% |
| Fat | 0% |
| Total solids | 2.5% |
| pH | 5.5 |

**3.Chemical quality of freshly prepared soy whey beverage**

Soy whey beverage was prepared by adding sugar (granular sugar) to the whey sample at 4, 5 and 6 per cent levels, then the well mixed whey samples were transferred to the previously sterile bottles and subjected for heating in a water bath maintained at 85℃ for 5 minutes and cooled to room temperature and stored at refrigeration temperature (7°). The freshly prepared soy whey beverage was analysed for the chemical components (Table 3). The soy whey beverage had 0.24, 6.8, and 4.24 per cent acidity (lactic acid), protein, and total solids respectively and was fat-free. The pH was observed to be 5.5. Odour of soy whey beverage was found to be satisfactory.

**Table 3: Chemical quality of freshly prepared soy whey beverage.**

| Components | Percent |
|---|---|
| Titrable acidity (Lactic acid) | 0.24% |
| Protein | 6.8% |
| Fat | 0% |
| Total solids | 4.24% |
| pH | 5.5 |

**4. Effect of different levels of sugar addition on the sensory characteristics of soy whey beverage obtained from tofu prepared using lactic cultures**

In order to prepare the soy whey beverage sugar was addedto improve the palatability. In present study sugar at 4, 5 and 6 per cent levels were tried to optimize the sensory quality of whey beverage (Table 4 and Fig. 2).

The average sensory scores given by panel of judges for control (cow milk whey beverage) sample for color and appearance, taste, aroma, consistency and overall acceptability were 7.7, 8.0, 8.9, 9.0, 8.5 of the 9 point hedonic scale respectively. Among the sugar added soy whey beverage 4 per cent sugar added whey beverage sample for color and appearance, taste, aroma, consistencyand overall acceptability were 7.2, 5.0, 4.5, 7.0, 4.2 of the 9 point hedonic scale respectively. 5 per cent sugar added whey beverage sample for color and appearance, taste, aroma, consistency and overall acceptability were 7.2, 6.0,3.0, 7.6, 5.0 of the 9 point hedonic scale respectively. 6 percent sugar added whey beverage sample for color and appearance, taste, aroma, consistency and overall acceptability were 8.0, 9.0, 6.5, 7.0, 7.5 of the 9 point hedonic scale respectively. Based on the sensory score for color and appearance, taste,

aroma, consistency and overall acceptability, the sugar level was optimized to be 6 per cent.

**5. Antibacterial activity of soy whey beverages and its fractions**

To determine the antibacterial activity of the soy whey beverage and their whey fractions, the whey samples were tested for their antibacterial activity against the different test indicator organisms.

In this study the soy whey, soy whey beverage, supernatant of soy whey beverage and Cell Free Extract (CFE) of soy whey beverages prepared from soy whey from tofu using *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactococcus lactis* cultures were tested for antibacterial activity against the test pathogens *E. coli*, *Salmonella* sp. and *Staphylococcus aureus* by agar well diffusion method (Fig. 3).

The test organisms ($10^5$ cell/ml) were seeded with Muller Hinton agar. Wells of 7 mm were made by a sterileborer, 50µl each of soy whey, soy whey beverage, supernatant of soy whey beverage and Cell Free Extract (CFE) of soy whey beverage were transferred to the respective wells and incubated overnight at 37° without inverting the plates. The clear zone around the wells indicated inhibitory activity against the particular test organism (Table 5). Among the tested organisms used soy whey beverage showed inhibitory zone 17 sq.mm, 18 sq.mm, and 4 sq.mm against *E. coli*, *Salmonella* sp. and *Staphylococcus aureus* respectively. Supernatant of soy whey beverage showed inhibitory zone 18 sq.mm, 15 sq.mm and 4 sq.mm against *E. coli*, *Salmonella* sp. and *Staphylococcus aureus* respectively. Cell Free Extract (CFE) of soy whey beverage showed inhibitory zone 22 sq.mm, 19 sq.mm and 10 sq.mm against *E. coli*, *Salmonella* sp. and *Staphylococcus aureus* respectively. But soy whey did not show any inhibitory zone against the test organisms.

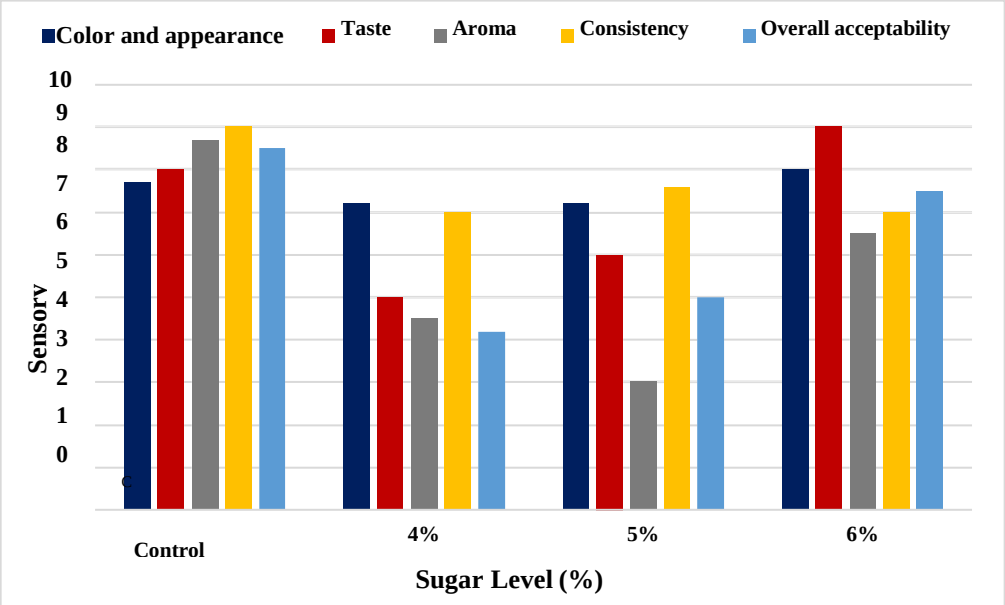


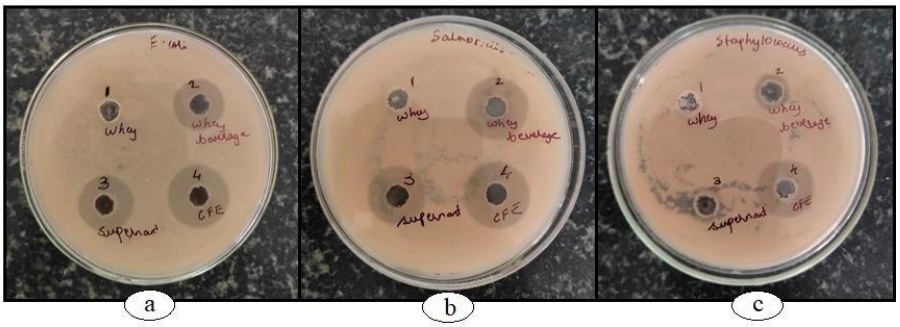

**Fig. 2.** Effect of different levels of sugar addition on the sensory characteristics of soy whey beverage obtained from tofu prepared using lactic cultures.

**Table 4: Effect of different levels of sugar addition on the sensory characteristics of soy whey beverage obtained from tofu prepared using lactic cultures.**

| Levels of sugar addition to soy whey | Color and appearance | Taste | Aroma | Consistency | Overall acceptability |
|---|---|---|---|---|---|
| | Score (9 point hedonic scale) | | | | |
| **Control** | 7.7 | 8.0 | 8.7 | 9.0 | 8.5 |
| **4%** | 7.2 | 5.0 | 4.5 | 7.0 | 4.2 |
| **5%** | 7.2 | 6.0 | 3.0 | 7.6 | 5.0 |
| **6%** | 8.0 | 9.0 | 6.5 | 7.0 | 7.5 |

**Table 5: Antibacterial activity of soy whey beverages and its fractions.**

| Type of whey beverage fraction | Test Organisms | | |
|---|---|---|---|
| | *Escherichia coli* | *Salmonella* sp. | *Staphylococcus aureus* |
| | Area of inhibitory zone (sq.mm) | | |
| Soy whey | NIL | NIL | NIL |
| Soy whey beverage | 17 | 18 | 4 |
| Supernatant of soy whey beverage | 18 | 15 | 4 |
| Cell free extract of soy whey beverage | 22 | 19 | 10 |




(a) Antibacterial Activity against *E. coli;* (b) Antibacterial Activity against *Salmonella;* (c) Antibacterial Activity against *Staphylococcus aureus*

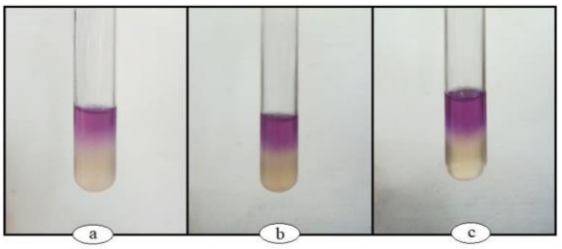
**Fig. 3.** Antibacterial Activity of soy whey fractions against test cultures.

**6. Antioxidant activity of soy whey beverages and itsfractions**

The soy whey, soy whey beverage and CFE of soy whey beverage were evaluated for the antioxidant properties withhelp of the most commonly used method DPPH activity test. The antioxidant activity (% DPPH) values for the soywhey, soy whey beverage and CFE of soy whey beverage were 63.87, 65.59, and 66.02 per cent respectively. The antioxidant activity of cow milk whey sample was 71.16 per cent.

**Table 6: Antioxidant activity of soy whey beverages and its fractions.**

| Type of whey beverage fraction | Absorbance | TransmissionPercentage | Antioxidant Activity(%) |
|---|---|---|---|
| Soy Whey | 0.168 | 32.3 | 63.87 |
| Soy whey beverage | 0.160 | 31.0 | 65.59 |
| Cell free extract of soy whey beverage | 0.158 | 32.5 | 66.02 |




(a) Antioxidant Activity of Soy Whey*;* (b) Antioxidant activity of soy whey beverage: (c) Antioxidant Activity of CFE of Soy Whey Beverage

**Fig. 4.** Antioxidant Activity of soy whey fractions.

The coliform and yeast and molds were nil counts on 0, 3, and 6$^{th}$ day and on 9$^{th}$ day the coliform and yeast and molds count were 1 × $10^2$ cfu/ml and 15×$10^2$ cfu/ml respectively. Therefore, the present study reveals that the soy-based food product utilizing could be considered whey for the consumer as a functionaldrink.

**7. Storage study of soy whey beverage**

Optimized soy whey beverage obtained from soy milk using lactic cultures along with the control samples were stored in the sterile glass bottles at refrigeration temperature (7 ± 1°C) (Fig. 9) till visual as well as flavourand microbial defects were noticed in the tested samples. The samples were drawn from the refrigeration temperature stored samples once in 3 days for sensory, chemical and microbiological analysis. Cow milk whey was carried for comparison.

**Table 7: Effect of refrigeration (7±1°C) storage on the quality of soy whey beverage obtained fromtofu prepared using lactic cultures.**

| Control | | | | |
|---|---|---|---|---|
| Days of Storage | Parameters Analyzed | | | |
| | Titrable Acidity(% Lactic Acid) | pH | Coliform (cfu/ml) | Yeast & Molds(cfu/ml) |
| 0 | 0.81% | 5.5 | NIL | NIL |
| 3 | 0.81% | 5.5 | NIL | NIL |
| 6 | 0.85% | 6 | NIL | NIL |
| 9 | 1.2% | 6 | NIL | NIL |
| **Soy Whey Beverage** | | | | |
| Days of Storage | Parameters Analyzed | | | |
| | Titrable Acidity(% Lactic Acid) | pH | Coliform (cfu/ml) | Yeast & Molds (cfu/ml) |
| 0 | 0.26% | 5.5 | NIL | NIL |
| 3 | 0.26% | 5.5 | NIL | NIL |
| 6 | 0.27% | 5.5 | NIL | NIL |
| 9 | 0.26% | 5.5 | 1 x $10^2$ | 15 x $10^2$ |

**8. Effect of refrigeration (7±1°C) storage on the quality of soy whey beverage obtained from tofu preparedusing lactic cultures**

The control whey beverage sample, the initial pH was 5.5 and acidity 0.81 per cent remains unchanged up to 3$^{rd}$ day but further storage increased the pH to 6 and same for the rest of the storage days. However, acidity got increased to 0.85 per cent LA on 6$^{th}$ day, 1.2 per cent LA on 9$^{th}$ day. Thecoliforms and yeast and molds were nil counts on 0, 3, 6, and 9$^{th}$ day.

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The control whey beverage sample, the initial pH was 5.5 and acidity 0.81 per cent remains unchanged up to 3rd day but further storage increased the pH to 6 and same for the rest of the storage days. However, acidity got increased to 0.85 per cent LA on 6th day, 1.2 per cent LA on 9th day. Thecoliforms and yeast and molds were nil counts on 0, 3, 6, and 9th day.

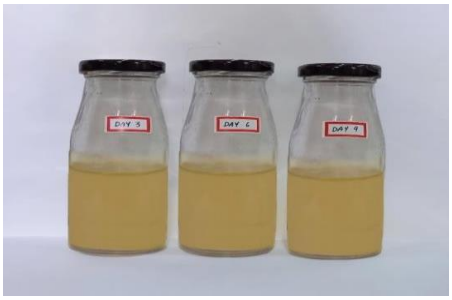

**Fig. 5.** Soy Whey Beverage Samples Stored in Glass Bottles.

In case of soy whey beverage sample, the initial pH was 5.5 and acidity 0.26 per cent remains unchanged up to 3rd day but further storage increased the pH remained same forthe rest of the storage days. However, acidity got increased to 0.27 per cent LA on 6th day, again got decreased to 0.26per cent LA on 9th day. The coliform and yeast and molds were nil counts on 0, 3, and 6th day and on 9th day the coliform and yeast and molds count were $1 \times 10^2$ cfu/ml and $15 \times 10^2$ cfu/ml respectively. Therefore, the present study reveals that the soy-based food product utilizing could be considered whey for the consumer as a functionaldrink.

## CONCLUSIONS

Soy Whey is a by-product obtained during the manufactureof tofu which for years was thought to be insignificant andwas either used as an animal feed or it was disposed of as waste thus building the BOD content of the dairy sewage. The soy whey obtained has nutritional, therapeutic and antioxidant properties. Soy Whey is a genuine thirst quencher, unlike most soft drinks. Utilization of soy wheyin the form of beverage has been attempted in the present study. The study was carried out to analyze sensory, chemical, microbial, antimicrobial and antioxidant activity of soy whey beverage in order to evaluate the keeping quality and storage study of optimized beverage. The soy whey samples produced inhibitory zone formation around the well and was considered as having antibacterial activity against certain pathogens. The antioxidant activity of the soy whey fractions was found to be more than 60 per cent. The present study reveals that the soy-based food product utilizing whey could be considered for the consumer as a functional drink.

## FUTURE SCOPE

The global soy protein ingredients market has witnessed significant growth in recent years and is expected to continue expanding at a steady pace. A market analysis of the global soy protein ingredients market reveals several key factors driving its growth and shaping its future prospects.

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**Conflict of interest.** None.

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