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Physico-chemical and Antioxidative Properties of Whey Protein Beverages

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Abstract

Whey, the liquid left after milk casein precipitation, was utilized, and its physicochemical properties were assessed. The aim of the research was to analyze the availability of nutrients in whey by preparing a modified whey protein beverage. Natural and artificial whey protein beverages were prepared. We studied the shelf life of these beverages over a period of 0 to 11 days and compared them based on their acidity, viscosity, pH, and organoleptic properties. It was studied by using students to test that, that natural whey beverage was curdled on day 7, whereas artificial whey beverage remained unaltered till day 11 of the study. The observation revealed that the natural whey beverage initially tasted better but curdled by the 7th day. In contrast, the artificial whey beverage, though initially unpalatable, improved in taste over time during storage. Among whey components, lactose is likely the least valuable and the most challenging to utilize. Different fruit concentrates, like apples, peaches, cherries, and berries, that are well-known sources of iron, magnesium, and antioxidants, are useful for the production of whey beverages with improved nutritional importance. Whey powder, whey concentrate, reduced mineral whey, whey protein concentrate, and lactose are used in human food; soil mixtures are mainly used in animal feed. Furthermore, the biological properties of whey protein have shown promise in the treatment of chronic diseases such as HIV, cancer, and cardiovascular diseases, and the nutrient-rich whey protein can be used in infant formula, adult formula, and sports nutrition.

Keywords: Whey protein, lactose, casein, beverage, cardiovascular disease, functional food

INTRODUCTION

Whey is the liquid fraction that remains in the manufacture of cheese, chhana, paneer, and casein. Whey is estimated to produce about 165 million tons worldwide, of which the maximum production of cheese whey consists of about 95%. In India the maximum production of whey is from dairy products such as chhana and paneer [1]. As an inexpensive source of high protein, whey is known to play a major role in weight management through its effects on short-term and long-term control of food intake, but the specific function of whey consumption of certain proteins and peptides on food intake is poorly understood. Clay-derived peptides affect the renin-angiotensin system and have insulinotropic properties [2]. 45 to 90 tons of clay soil per acre can be used for tillage. Beyond this limit, however, foul odors emerged. Fortified soil proteins improve soil fertility by providing essential nutrients and filling open, porous soils that increase drainage [3]. Whey is a substantial pollutant, with a high biochemical oxygen demand (BOD) ranging from 30,000 to 50,000 mg/l and a chemical oxygen demand (COD) ranging from 60,000 to 80,000 mg/l. As whey protein concentrate has its potent polluting strength, discarding whey plays a significant role in the loss

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of potential nutrients and energy. In addition, the economic condition of the dairy industry is affected by several treatment costs due to improper disposal of whey. A significant amount of the global cheese-whey production is currently disposed of as effluent. Disposing of cheese as waste poses significant pollution challenges for the surrounding environment [4–7]. Soil is now seen not as a waste product but as a valuable source of nutrient-rich resources. The economic benefits of dairy nutrients have increased the importance of supplementing the human diet with soil products [8].

Additionally, whey proteins can be produced through methods such as ultrafiltration and lactose hydrolysis, and whole whey or whey permeate can be used as fermentation feedstock [4]. Numerous dairy researchers have conducted studies on the production, processing, and utilization of whey for food and animal feed, as well as investigating its nutritive, therapeutic, and functional properties.

Whey proteins consist of various proteins, namely, α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, and proteose-peptones [9]. Beta-lactoglobulin consists of about 50% of total whey protein. Whey proteins do not precipitate with acid or rennin; instead, they can coagulate when exposed to heat. Whey also contains minor quantities of lactoferrin and serum transferrin. Through a process involving ultrafiltration, whey protein concentrate is produced.

The aim of the research was to enhance the nutritional value of whey to make a whey protein beverage, evaluate the physico-chemical properties of whey water, and utilize the valuable nutrients and antioxidant properties present in whey water.

MATERIALS AND METHODS

pH Determination in whey Sample

According to the AOAC method, the pH of the sample was determined by using the Deluxe pH meter-101; serial number-1603159 [10].

Determination of specific gravity by using specific gravity bottle

According to the AOAC method, the specific gravity of the said sample was detected by using a Riviera specific gravity bottle [10]. The specific gravity of the whey protein was calculated as follows:

Specific gravity of whey = $(W_2 – W_1) \setminus (W_3 – W_1)$

Where W_1 = weight in g of the empty specific gravity bottle.
W_2 = weight in g of the specific gravity bottle along with whey at 20^oC.
W_3 = weight in g of the specific gravity bottle with distilled water, 20^oC.

Determination of viscosity by Ostwald viscometer

According to the AOAC method, the viscosity of the whey beverage was determined at 20^oC, and the calculation is as follows:

$n_1 = n_2 \times (d_1t_1 / d_2t_2)$

where, n_1 = viscosity of whey, n_2 = viscosity of water, t_1 = average time for the flow of whey sample, t_2 = average time for the flow of water, d_1 = density of whey sample, d_2 = density of water [10].

Determination of fat content by Gerber method

By using a Gerber centrifuge (LMMCO, manufactured by AMARDEEP INDUSTRIES, Delhi-41), the fat content of the whey protein was measured.

Using this method, the butrometer was filled with 10 ml of Gerber sulfuric acid. Then 10.75 ml of milk was carefully transferred to a butyrometer without reacting with Gerber's acid. One milliliter of isoamyl alcohol was then added.

The butyrometer was inverted for 2, 3 times and the contents were properly mixed. Then the butyrometer was paced in the Gerber centrifuge, and the machine was balanced properly. Centrifugation was carried out at 1200 rpm for five minutes. The stopper was adjusted to read the test sample until the lower end of the grease column coincided with the zero point. The percent read was then recorded at the top of the section [11].

Estimation of protein content by Kjeldahl's method

1gram of whey was transferred into a Kjeldahl digester flask. Added 2.5 ml of concentrated sulfuric acid (H_2SO_4) and 1 g of copper sulfate ($CuSO_4$) and potassium sulfate (K_2SO_4) in a ratio of 1:20 were mixed in the Kjeldahl flask. The flask was placed on a heater in a fume chamber and heated gently until frothing ceased. Heating was continued until boiling until the liquid was clear and free from a yellowish color. Heating was continued further for 2 hours and then the mixture was allowed to cool and diluted with distilled water.

The mixture was then transferred into the micro-Kjeldahl distillation assembly. A conical flask containing 10 ml of boric acid was placed at the same time under the condenser outlet. Then 15 to 20 ml of 50% sodium hydroxide (NaOH) (w/v) was carefully added down the neck of the distillation tube. The mixture was allowed to absorb the ammonia in the condenser outlet (all the ammonia has been absorbed by boric acid, as indicated by the collection of about 50 ml of distillation). Distillate was then titrated with 0.2N hydrochloric acid (HCl) till the green color changed to grey (in boric acid, the color is blue, which changes to green on absorption of ammonia). Blank was determined under identical conditions by using 1 g of pure sucrose in place of whey.

CALCULATION

1ml of 0.02 N HCL = 0 .00028 gm of N_2,
Nitrogen present by weight = (A - B) × 0.O28 \ W,
Protein present by weight = (A – B) .028 × 6.38 \ W.

Where, A = volume in ml of 0.02N HCl used for sample in the titration, B = volume in ml of 0.02N HCl used for blank in the titration and W = weight in gram of whey sample [7].

Determination of Titratable Acidity

10 ml of the whey sample was pipetted into two 100 ml conical flasks. A blank sample was prepared by adding 1 ml of resaniline acetate solution to 10 ml of whey in one of the conical flasks. 1 ml of phenolphthalein indicator solution was added to the 10 ml of whey sample. 0.1 N NaOH was added slowly from a burette; the sample was stirred and continued to be added drop by drop until, by comparison, the colors matched the relatively paramagnet pink tint of the blank. The experiment was repeated to get concordant values.

Calculation

1 ml of 0.1 N NaOH = 0.009 g of lactic acid. Thus, titrable acidity (in terms of percentage lactic acid) = 9AN / W. Where A is the volume in ml of 0.1N NaOH required for the sample, W is the weight in g of the whey sample, and N is the normality of NaOH [7].

Determination of Ash Content

10 g of the whey sample was poured into a previously ignited, cooled, and weighed silica crucible. The sample was evaporated until dryness by placing the crucible in the hot water bath. The crucible

was then transferred into a muffle furnace at about 550^{o} C and left until a white and light gray ash resulted. The crucible was taken out of the muffle furnace, allowed to cool in a desiccator, and then weighed. This process was repeated until the difference between two consecutive weighings was less than 1 mg.

Calculation

Ash, percent by weight = $(W_2 – W) \times 100 / (W_1 - W)$

Where W = weight in g of empty crucible, W_1 = weight in g of whey sample with crucible, and W_2 = weight in g of whey sample with crucible after ashing [8].

Determination of Total Solid

10 ml of whey sample was taken in a previously weighed dry aluminum dish. The dish was kept in an air oven maintained at 101 ±1^{o} C for 2 hours to get the content dried, cooled in a desiccator, and weighed. The process was repeated until the difference between two consecutive weights taken was less than 1 mg [8].

Calculation

If W = weight of residue after drying (g), W_1 = weight of sample taken for testing (g), total solids percentage = $100W / W_1$.

Analysis of Moisture Content in whey Beverage

Instruments used: hot air oven; SL. NO. 1501004.

Samples weighing 2 grams each were placed in a pre-weighed flat-bottomed dish and placed in a hot air oven at 101±1°C for 3 hours. After 1 hour, the dish was weighed and returned to the oven for an additional 30 minutes of drying [7]. The moisture content was then calculated as follows:

Moisture content = $(W_2 – W_3 / W_2 – W_1) \times 100$

Where W1 = weight of empty dish, W2 = weight of dish + sample, and W3 = weight of dish + dried samples.

Preparation of Natural Whey Beverage

For this study, we used “Red Cow Creamy Delight” milk (100 ml), i.e, homogenized and pasteurized. Three different milk samples were prepared as follows:

- *Sample 1:* In 100 ml of whey, 10 ml of mango extract and 5 g of sugar were added.
- *Sample 2:* In 100 ml of whey, 15 ml of mango extract and 5 g of sugar were added.
- *Sample 3:* In 100 ml of whey, 20 ml of mango extract and 5 g of sugar were added.

Among these three, Sample 2 was accepted after sensory analysis, and further study was done on it. sample 1 and sample 3 were discarded due to bad sensory results. All the samples were stored at 5-7℃.

Preparation of Artificial whey Beverage

Three different samples were prepared, which are:

- *Sample 1:* In 100 ml of whey sample, 5 g of sugar, 2 ml of artificial flavor, and 3 drops of artificial color were added
- *Sample 2:* In 100 ml of the sample, 5 g of sugar, 3 ml of artificial flavor, and 3 drops of artificial color were added.
- *Sample 3:* In 100 ml of sample, 5 g of sugar, 5 ml of artificial flavor, and 3 drops of artificial color were added.

Among these three samples, sample 1 was accepted after sensory analysis, and further study was done on it. Sample 2 and sample 3 were discarded after sensory estimation. All the samples were stored at 5-7°C.

Sensory Evaluation

Sensory analysis was done on both natural and artificial whey beverages, according to Srilakshmi.

RESULTS AND DISCUSSION

The physico-chemical properties of whey protein were analyzed, and the results are shown in Table-1. Two different types of whey beverages, i.e., an artificial whey beverage (using artificial color and artificial flavor and whey) and a natural whey beverage (using natural mango extract and whey itself), were prepared. Their shelf lives were studied on the basis of their acidity, viscosity, and pH (results are given in Table 2), and on the basis of organoleptic properties, the sensory evaluation was prepared on the basis of a "9-point hedonic scale." The shelf life was studied for 5 days (0, 3rd, 5th, 7th, and 11th days) (Table 3).

In the case of natural and artificial whey beverages, it was revealed from Table 2 that the acidity and viscosity of the beverage were gradually increasing, and the pH of the beverage was gradually decreasing as the H^+ concentration was gradually increasing. Table 3 shows that the appearance, color, and flavor of the artificial whey protein beverage stored at 2^{o}C were consistent throughout the 11 days.

Table 3 shows that the appearance, color, and flavors of the natural beverage gradually decreased. The shelf life of this beverage was studied until 7th day; after that, the beverage got cuddled, and further study was not able to be performed.

Table 1. Physico-chemical parameters of whey protein in cow milk samples.

Parameters	**Sample 1**	**Sample 2**	**Sample 3**	**Average**
Moisture content	92%	91.6%	93.0%	92.2%
Total solids	8%	8.4%	7 %	7.8%
Specific gravity	1.01	1.01	1.01	1.01
Viscosity	1.14	1.11	1.11	1.12
Acidity	0.09	0.09	0.09	0.09
Fat content	0.1 %	0.1%	0.1%	0.1%
Protein	0.80	0.80	0.79	0.796
pH	5.60	5.75	5.58	5.64
Ash content	0.14%	0.9%	0.13%	0.39%

The experiment was carried out in triplicate.

Table 2. Physico-chemical properties of artificial and natural whey protein beverages.

Whey protein beverages	**Parameters**	**Day 0**	**Day 3**	**Day 5**	**Day 7**	**Day 11**
Artificial	Acidity	0.09	0.108	0.126	0.135	0.18
	Viscosity	1.378	1.384	1.581	1.652	1.962
	pH	5.60	5.50	5.25	5.10	4.99
Natural	Acidity	0.09	0.11	0.17	0.25	-
	viscosity	1.55	1.71	2.38	-	-
	pH	5.75	5.47	5.22	5.13	-

The experiment was carried out in triplicate.

Table 3. Sensory evaluation of natural and artificial whey protein beverages.

Sensory attributes	**Natural whey beverages**					**Artificial whey beverages**				
	Day 0	*Day 3*	*Day 5*	*Day 7*	*Day 11*	*Day 0*	*Day 3*	*Day 5*	*Day 7*	*Day 11*

Appearance	9	7	7	5	-	9	8	8	8	8
Color	9	8	7	5	-	8	8	8	8	8
Flavor	9	8	6	4	-	5	5	7.5	7	7
Overall acceptability	9	8	7	4.5	-	7	7	8	8	8

CONCLUSION

In the present study, preliminary investigations were carried out to ascertain the difference between the two types of beverages, i.e., artificial whey beverage and natural whey beverage. The comparison was done on the basis of their organoleptic, physico-chemical and sensory analyses. On the basis of the above results, it was concluded that initially, natural whey beverages tasted much better than those of artificial whey beverages, but as the days passed, natural whey beverages deteriorated more quickly than artificial whey beverages because of no preservative were added to them, so the acidity of the beverages gradually increased. As the days passed, the taste of artificial whey beverages somewhat improved and became more acceptable. The natural whey beverages was cuddled after the 5th day due to a rapid increase in acidity and was discarded. Artificial whey beverage was studied till the 11th day and that was more or less in good condition.

Future Aspect of the Study

Comparisons on the basis of more parameters could be done in the future and more physico-chemical properties (freezing point, curd tension, thermal conductance, heat capacity, heat stability, cholesterol, phospholipid, free fatty acid content, etc.) could be detected and vitamin and mineral content could be measured.

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