doi.org/10.36721/PJPS.2020.33.1.REG.191-197.1

Development of nutritious probiotic sportsman drink

Sheraz Ahmed*[1], Mian Kamran Sharif[2], Masood Sadiq Butt[2], Zeeshan Akram[2], Shumaila Yousaf[3], Muhammad Imran[4], Muhammad Nadeem[5], Muhammad Rizwan Yousuf[6]

[1]Department of Food Sciences, Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur
[2]Faculty of Food, Nutrition and Home Sciences, University of Agriculture Faisalabad, Pakistan
[3]Nuclear Institute for Agriculture & Biology, Faisalabad, Pakistan
[4]University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Pakistan
[5]Department of Environmental Sciences, COMSATS Institute of Information Technology, Vehari, Pakistan
[6]Department of Theriogenology, University of Veterinary & Animal Sciences, Lahore, Pakistan

Abstract: Whey plays an important role in the sports nutrition because of high quality proteins and essential amino acid profile. Nine formulations of sportsman drinks were made using Cheddar, Mozzarella and Paneer whey with normal as well as additional fermentation. The developed sportsman drinks were evaluated for physico-chemical analyses, amino acid profile, viscosity and total plate count along with sensory response during two month storage. Drink having Cheddar whey (T_4) with additional fermentation was better in terms of quality and nutrition. Furthermore, amino acid profile considered it a complete and balanced source of essential and non-essential amino acids. Amongst essential amino acids, highest values was recorded for branched chain amino acids like leucine (73.16±3.09) followed by lysine (61.56±0.61) and valine (44.13±1.86)mg/g protein. The dietary significance of sportsman drink can be enhanced through additional fermentation using *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophillus*.

Keywords: Nutrition, whey, probiotic, sportsman.

INTRODUCTION

Whey is the greenish translucent liquid residue of milk obtained when it is converted into the curd by using rennet or edible acidic substance (Zimecki and Kruzel, 2007).

It was considered as waste product of cheese industry but the discovery of its nutritional status raised its importance from by-product to co-product of the manufacturing process (Gill and Rutherford, 2000).

Depending upon the raw material (enzyme, rennet or acid) used in the coagulation of milk; it is broadly classified into sweet whey/rennet whey and acid whey (Stanciuc *et al*., 2010).

Liquid whey contains 50% of total solids of the whole milk. It has lactose 5%, soluble proteins 0.6 to 0.8%, lipids 0.5% and minerals 8 to 10%. Whey salts contain more than 50% of potassium chloride and sodium chloride and salts of calcium (phosphates) (Marwaha and Kennedy, 1988). Whey proteins are approximately 20% of the milk proteins, 50% of which are β-lactoglobulin, 20% are α-lactalbumin, 10% are bovine serum albumin (BSA) and 10% other minor proteins, such as lactoferrin, peptone, immunoglobins, prolactin, proteose, folate, calmodulin and binding proteins. Whey salts contains more than 50% of potassium chloride and sodium chloride and salts of calcium (phosphates) etc. It also contains significant quantities of lactic acid (0.05% w/v), citric acid, NPN compounds and vitamin B complex (Juan *et al*., 2009).

Whey proteins have found a number of applications in the industry because of certain functional and nutritional properties. Functional properties like solubility, whip ability, emulsification, viscosity, gelation, foam formation, firmness, creaminess to end product and heat stability has made it useful component in beverage industry, confectionary, desserts manufacturing and dairy and bakery products (Otte *et al*., 2007).

Whey is a famous nutritional supplement that helps to regulate immune system, increase muscle strength and prevent a number of diseases like heart problem and weakened bones. In addition, whey has the ability to inhibit oxidation, hypertension and tumor formation and also act as hypolipidemic and chelating agent (Park *et al*., 2007).

Whey protein considered a useful ingredient in infant formula because of its low allergen causing ability, weight reduction, weight gain and diets, protein fortified fruit juices and other healthy foods and drinks (O.mole *et al*., 2012). It also contains vitamins and minerals comprising high bioavailability along with branched-chain amino acids such as valine, isoleucine and leucine (Ha and Zemel, 2003).

The current world production of whey is about 125 million tons (MMT), 64% of which is produced in

*Corresponding author: e-mail: sheraz1080@yahoo.com

European countries and 24% in North America. The Channa and Paneer whey is the major contributor (80%) to total whey production in the world. Almost 55% of total whey is treated and converted into various valuable food products (Naik *et al*., 2009). Pakistan is the fifth largest milk producing country, but contributes only 0.4% of the total world whey production per year.

This indicates the potential of whey processing in Pakistan (PDDC, 2006).

Drinks are essential component of daily diet which not only quench the thirst but also are valuable source of water and electrolytes. Sports drinks are principally recommended for people undertaking strength exercises. These are the outcome of wide range of scientific and nutritional investigations to back up their efficiency and safety (Burke, 2001).

Probiotic are beneficial to increase nutritional profile of drinks for sportsman. The promising health benefits associated with probiotic are improvement in the gastrointestinal micro flora, stimulation of immune system and reduced risks of pathogens. The additional of whey based functional drinks with strains of mixed cultures comprising *Lactobacillus delbrueckii subsp. lactis* and *Streptococcus thermophilus* can impart aroma as well as enhance the probiotic in the gut. Moreover, drinks developed by consuming diverse cultures of *Lb. delbrueckii subsp. lactis* and *Streptococcus thermophilus* have shown greater storage strength (Bulatovic *et al*., 2014).

The present project was designed to develop a whey based sportsman drink through fermentation to enhance nutritional profile. The probiotic enriched drinks of Cheddar, Mozzarella and Paneer whey were fermented by *Lactobacillus delbrueckii subsp*. *bulgaricus* and *Streptococcus thermophillus.* Moreover, the study determined the consumer acceptability of these fermented drinks. The results obtained from this study will help development of new whey based fermented products, increase economic benefit to dairy processors, as well as help decreasing environmental pollution caused by dairy wastes.

MATERIALS AND METHODS

Procurement of Raw Materials

Cheddar and Mozzarella cheese whey were procured from Noon Dairies Bhalwal, Sargodha (Pakistan) while Paneer whey was procured from Gourmet Foods, Lahore (Pakistan).

The refrigeration conditions were maintained during transportation and storage to ensure whey quality. Other raw materials required for the preparation of sportsman drinks were obtained from the local market. Starter cultures (*Lactobacillus delbrueckii subsp. bulgaricus* and S*treptococcus thermophillus*) were obtained from Chr. Hansen's Laboratory (Ireland) Ltd, Cork, Ireland. All reagents (analytical and HPLC grade) & standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Characterization of whey

Cheddar, Mozzarella and Paneer whey samples were analyzed for various physicochemical parameters including pH, acidity, total solids, fat, crude protein, ash, lactose, mineral analysis and amino acid profile. Whey samples were also evaluated for microbial assay *i.e* .total plate count (TPC).

pH

Electronic digital pH Meter (EUTECH pH 510, Cyberscan, Singapore) was used.

Acidity

Whey samples were analyzed for acidity through the titrimetric method following the procedure described in AOAC (Association of official analytical chemists) Method No. 947.05(AOAC, 2006).

Fat

Fat content was calculated by Gerber method following the protocol (Djuric *et al*., 2004).

Crude protein

Crude protein was determined by Kjeldahl method using Kjeltech apparatus (TechnikGmbh, Behr Labor, Germany) following the procedure elaborated in AOAC Method No. 991.20(AOAC, 2006).

Ash content

Ash in whey was estimated by incinerating samples in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) following the protocol given in AOAC Method No. 945.46(AOAC, 2006).

Lactose

The lactose was determined through enzymatic method as explained (AOAC, 2006).

Total solids

Total solids assessed according to AOAC Method No. 925.23(AOAC, 2006).

Mineral profile

Whey was analyzed for mineral assay (calcium, magnesium, sodium and potassium) following the procedure described (AOAC, 2006).

Amino acid profile

Amino acid profile of whey samples was determined following the procedure (Walsh and Brown, 2000).

Total plate count
Total plate count was determined by the procedure (Yousaf and Calstrom, 2003).

Preparation of sportsman drinks
The whey drink (100-mL) was formulated by whey (72-mL), banana pulp (15-mL), sugar (12-g), sodium benzoate as preservative (0.005-g), stabilizer (0.05-g), citric acid (0.05-g), color and flavor. The whey was pasteurized at 70°C for 15 s and homogenized. Cooling was done at 4°C. All the ingredients were mixed to prepare sportsman drinks and filled in PET bottles. Nine formulations of sportsman drinks were made using Cheddar, Mozzarella and Paneer whey with normal as well as additional fermentation. The drinks were stored at refrigeration temperatures for 60 days and analyzed for pH, acidity, total solids, fat, crude protein, ash, lactose, mineral analysis and amino acid profile.

Fermentation of sportsman drinks
Starter cultures (0.02%) of *Lactobacillus delbrueckii subsp. bulgaricus* and S*treptococcus thermophillus* were added in formulations for additional fermentation at 37°C for 24 h according to the method explained by Pescuma *et al*., (2010).

Viscosity
Rheological measurements of sportsman drinks were carried out through Viscometer (LVDVE 230) as explained by Gassem and Frank, (1991).

Sensory evaluation
The prepared sportsman drinks were assessed for sensory profiling using 9-Point Hedonic score system following the procedure of Meilgaard *et al*., (2007).

STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis to check the level of significance as described by Steel *et al*., (1997) by using software Minitab version 15.1.

RESULTS

In the instant project liquid Cheddar, Mozzarella and Paneer whey procured from local cheese processing industry were exploited for various quality attributes. The results along with comprehensive debate are given below:

The effect of different whey formulations and storage intervals on physico-chemical characteristics of sportsman drinks revealed significant variations among the treatments with respect to pH, acidity, fat, lactose and total solids except crude protein and ash content. Similarly only crude protein showed non-significant effect of storage whereas interaction between drink formulations and storage was non-significant for all parameters.

Overall, the range of pH, acidity, fat, protein, lactose and total solids range in all formulations having normal as well as additional fermentation as depicted in table 1. After 60 days storage, pH, fat, crude protein, lactose and total solids were decreased, while acidity was increased as shown in table 1. The means for viscosity at initiation and termination of the study were (10.38±0.29) and (4.28 ± 0.21), respectively. In the developed drinks, the total plate count was increased as a function of storage from 1.42 ± 0.07- 6.45±0.34 x l04 cfu/mL. This significant increase in total plate count of sportsman drink was due to additional fermentation. The mineral content of sportsman drinks indicated significant effect of treatments but non-significant effect on storage and their interaction on Ca, Mg, Na and K concentrations.

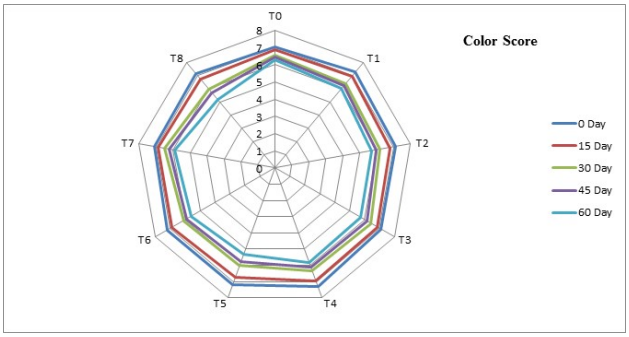

Fig. 1: Color score of sensory evaluation

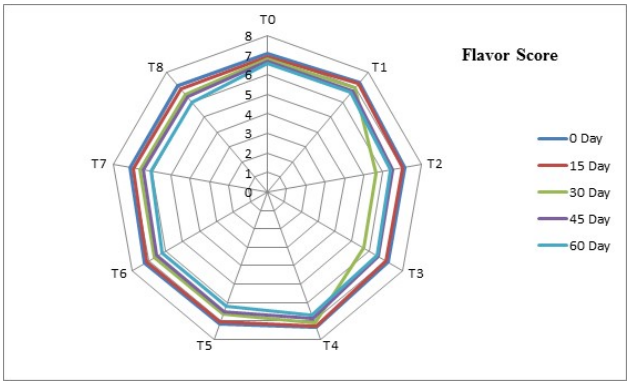

Fig. 2: Flavor score for sensory evaluation

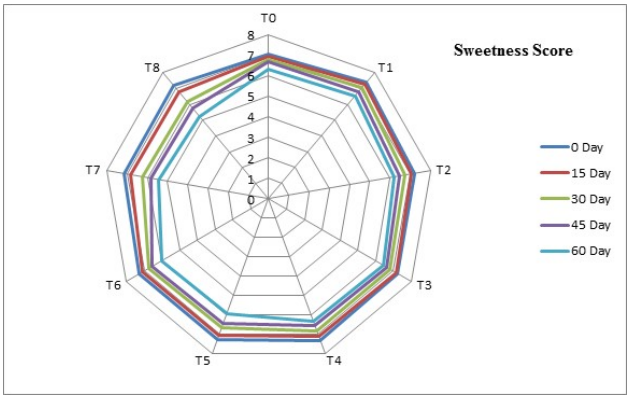

Fig. 3: Sweetness score for sensory evaluation

Amino acid profile of developed drinks depicted significant variations among essential and non-essential amino acids due to different types of whey. In developed fermented sportsman drink, among essential amino acids *i.e.* leucine (73.16±3.09), lysine (61.56±0.61), threonine (38.02±1.48), isoleucine (41.52±2.04), valine (44.13± 1.86), methionine (18.89±0.85), phenylalanine (18.07± 0.67), tryptophan (13.61±0.43) and histidine (15.19± 0.54) mg/g protein (table 2) whereas among non-essential amino acids alanine (38.04±1.16), arginine (16.15±0.71), cysteine (11.45±0.52), aspartic acid (77.15±1.15), glutamic acid (116.71±3.56), glycine (11.17±0.28), proline (46.92±3.08), serine (42.21±2.51) and tyrosine (22.13±1.54) mg/g protein (table 3) were also present in substantial quantities. As a result of additional fermentation there was increase in amino acid contents.

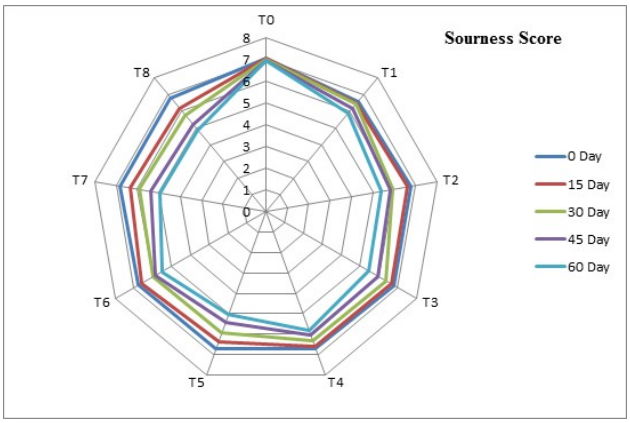

Fig. 4: Sourness score for sensory evaluation

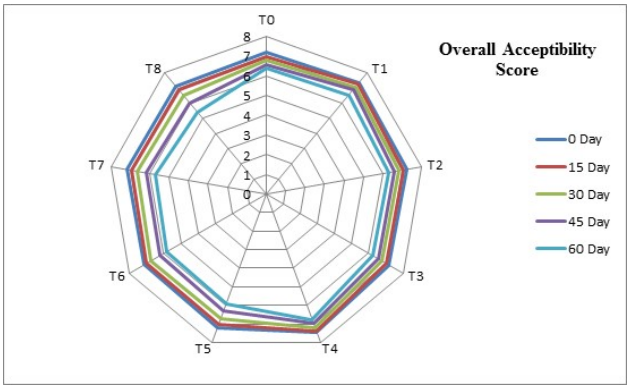

Fig. 5: Overall acceptability score for sensory evaluation

The developed sportsman drinks were evaluated for consumer acceptability for various sensory attributes like color, flavor, sourness, sweetness and overall acceptability using 9-point hedonic scale system. Mean squares revealed significant differences among the sportsman drink due to different types of whey and storage intervals. The score obtained by the drink for color, flavor, sweetness, sourness and overall acceptability are depicted in fig. 1,2,3,4 and 5, respectively.

Storage contributed momentous decline in color scores from 7.04±0.31- 5.18±0.20 at initiation till termination of the study. Judges liked the flavor of sportsman drink having cheddar whey with additional fermentation (7.36± 0.16) followed by (7.32±0.31) sportsman drink having Cheddar whey with normal fermentation and (7.23±0.32) sportsman drink having Cheddar and Mozzarella whey (50:50) combination with additional fermentation whereas drink having Paneer whey with additional fermentation showed minimum acceptance as indicated by (6.39± 0.26) lowest score followed by (6.53±0.24) drink having Paneer whey with normal fermentation.

DISCUSSION

Whey proteins are responsible for nutritious quality of sports drinks due to availability of high quality essential amino acids. The present study investigated the nutritional quality of probiotic enriched sports drinks using Cheddar, Mozzarella and Paneer whey for athletes. There is inverse relation between elevation in acidity and decline in pH of whey based drinks. Whey based guava drink was prepared using 67.5% of whey and 20% guava pulp. The pH of all formulations varied from 3.39-4.15.While during 90 days storage pH was slightly decreased (Singh *et al*., 2014). Likewise, an inverse link between increase in acidity and decrease in pH of whey based fermented pineapple drink (Shukla *et al*., 2013), whey based mixed herbal beverage (Baljeet *et al*., 2013).

Storage has profound effect on fat, protein and total solid contents of whey based drink (Singh *et al*., 2014). The decrease in fat and protein contents of beverage during storage is might be due to the production of fatty acids and proteolysis that ultimately reduces the total solid contents (Yadav *et al*., 2010). The fermentation of whey drink caused by the addition of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophillus at 37^{o}C for 24h and subsequently stored for 60 days resulted in decreased pH. All the proteins have specific isoelectric point which is strongly influenced with changes in pH. The drop in pH resulted because of fermentation caused proteolysis in the whey drink, which decreases the quantity of crude proteins of drink, thus resulted in release of amino acids from the polypeptide chains. Storage decrease protein content of whey based orange nectar drink, whey based pear nectar drink, whey based peach nectar drink and whey based apple juice drink (Djuric *et al*., 2004). There was non-significant effect of ash content of whey based orange, pear, peach and apple drinks (Djuric *et al*., 2004).

Storage has significant effect on lactose content of whey based fruit drinks (Saravana and Manimegalai, 2005). There was decrease in apparent viscosity of fermented lactic beverage during entire storage study (De-Oliveira *et al*., 2001). There was decrease in apparent viscosity of

Table 1: Changes in Physico-chemical characteristics of sportsman drinks

Physico-Chemical Characteristics	0 Day	60 day
pH	5.14 ± 0.21 - 4.28±0.26	5.20 ± 0.29 - 4.02 ± 0.18
Acidity	0.41±0.02- 0.53 ± 0.01%	0.26 ± 0.01 - 0.57 ± 0.04%.
Fat	0.26 ± 0.02 - 0.19 ± 0.02%	0.25 ± 0.03 - 0.15 ± 0.01%
Protein	0.90 ± 0.02 - 0.75 ± 0.03%	0.87 ± 0.01 - 0.62 ± 0.01%
Lactose	4.58 ± 0.23 – 4.32 ± 0.18 %	4.37 ± 0.24 - 3.30 ± 0.25 %
Total solids	16.34 ± 0.99 - 15.75 ± 0.99 %	16.02 ± 0.88 - 14.78 ± 0.94 %

Table 2: Essential amino acids (mg/g protein) in sportsman drinks

Amino acids	Sportsman Drinks									F value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Isoleucine	0.006 ±0	30.98 ±1.76	29.55 ±0.83	28.82 ±1.45	41.52 ±2.04	37.08 ±0.85	39.09 ±1.15	30.03 ±0.99	35.41 ±1.23	19.26 **
Leucine	0.007 ±0	64.60 ±2.46	63.52 ±1.81	62.70 ±2.26	73.16 ±3.09	71.24 ±3.46	72.34 ±2.67	61.56 ±1.99	68.23 ±3.55	41.03 **
Threonine	0.002 ±0	37.59 ±0.98	36.48 ±1.75	36.71 ±1.34	38.02 ±1.48	37.12 ±2.19	37.83 ±0.72	35.23 ±1.56	36.15 ±1..54	29.6 *
Valine	0.005 ±0	30.29 ±0.55	29.17 ±0.86	29.52 ±1.22	44.13 ±1.86	39.47 ±1.83	41.54 ±1.77	28.32 ±0.92	35.05 ±1.01	24.65 **
Lysine	0.002 ±0	60.30 ±0.95	59.71 ±1.15	58.89 ±1.17	61.56 ±0.61	60.23 ±1.51	60.58 ±0.85	57.15 ±0.96	57.02 ±0.59	36.8 *
Methionine	0.002 ±0	17.34 ±0.64	17.25 ±0.89	17.01 ±0.55	18.89 ±0.85	17.96 ±0.76	17.91 ±0.49	16.62 ±0.48	17.09 ±0.72	15.24 *
Phenylalanine	0.009 ±0	18.42 ±0.65	18.36 ±0.33	18.30 ±0.81	18.07 ±0.67	17.73 ±0.93	17.82 ±0.78	16.08 ±0.63	16.91 ±0.51	19.2 *
Tryptophan	0.002 ±0	13.87 ±0.23	13.03 ±0.32	13.56 ±0.29	13.61 ±0.43	12.98 ±0.52	13.08 ±0.71	12.93 ±0.56	13.01 ±0.63	20.7 *
Histidine	0.008 ±0	13.20 ±0.41	13.09 ±0.37	12.78 ±0.29	15.19 ±0.54	13.76 ±0.71	14.34 ±0.80	12.06 ±0.69	13.17 ±0.22	18.59 *

T_0 : Drink without whey as control
T_1 : Sportsman drink having Cheddar whey with normal fermentation
T_2 : Sportsman drink having Mozzarella whey with normal fermentation
T_3 : Sportsman drink having Cheddar and Mozzarella cheese whey 50:50 normal fermentation
T_4 : Sportsman drink having Cheddar whey drink with additional fermentation
T_5 : Sportsman drink having Mozzarella whey drink with additional fermentation
T_6 : Sportsman drink having Cheddar and Mozzarella cheese whey 50:50 additional fermentation
T_7 : Sportsman drink having Paneer whey with normal fermentation
T_8 : Sportsman drink having Paneer whey with additional fermentation

whey based mango drink. The decrease in viscosity was might be due to decrease in total solids of the drink. Whey proteins such as β-lactoglobulin, α-lactalbumin and bovine serum albumin have electrophilic properties which keep the lipids and water portions of the drink intact. With storage the inherent proteins keeps on degrading which results in reduced electrophilic properties of these proteins resulting in release of lipids and water. This results in reduction in viscosity.

In an another study, there was momentous increase from 1.93 to 2.73 x 104 cfu/mL in total plate count of whey based mango drink during one month storage study (Sakhale *et al*., 2012).

Amino acid profile of sportsman drink: Whey based fermented beverage was prepared using probiotic (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) to obtain a fermented product with high amino acids concentration. During fermentation, Lactobacillus delbrueckii subsp. bulgaricus was the most proteolytic strain released 14 amino acids and displayed highest amino acids concentration after 12 h incubation. Among the essential amino acids like leucine, lysine and valine were present in highest concentration whilst among non-essential amino acids glutamic acid, serine, histidine and glycine were present in highest concentration (Pescuma *et al*., 2010). Amino acids oxidation is increased by

Table 3: Non-essential amino acids (mg/g protein) in sportsman drinks

Amino acids	Sportsman Drinks									F value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Alanine	0.004 ±0	37.67 ±0.35	37.58 ±0.82	37.41 ±0.41	38.04 ±1.16	37.62 ±0.97	38.01 ±1.39	36.98 ±0.83	37.07 ±1.65	59.1*
Arginine	0.003 ±0	16.10 ±0.19	15.85 ±0.39	15.93 ±0.65	16.15 ±0.71	15.45 ±0.38	15.72 ±0.70	13.37 ±0.54	15.05 ±0.63	22.13*
Cysteine	0.002 ±0	11.28 ±0.52	11.03 ±0.61	11.14 ±0.38	11.45 ±0.52	10.34 ±0.44	10.68 ±0.35	10.40 ±0.27	11.01 ±0.46	19.27*
Aspartic acid	0.006 ±0	75.97 ±2.45	74.50 ±1.76	74.63 ±0.96	77.15 ±1.15	76.24 ±2.45	76.4± 1.57	72.70 ±1.63	73.10 ±1.91	62.48*
Glutamic acid	0.007 ±0	108.38 ±3.44	106.17 ±3.27	107.78 ±2.85	116.71 ±3.56	111.35 ±2.81	113.06 ±1.82	102.25 ±2.89	104.23 ±3.74	80.07**
Glycine	0.005 ±0	10.06 ±0.36	8.49 ±0.71	8.91 ±0.62	11.17 ±0.28	10.07 ±0.31	11.35 ±0.98	7.65 ±0.84	9.23 ±0.36	23.6*
Proline	0.004 ±0	46.56 ±2.11	45.83 ±2.28	45.91 ±2.15	46.92 ±3.08	45.07 ±1.07	46.11 ±1.40	44.25 ±0.99	46.19 ±1.02	31.05*
Serine	0.003 ±0	33.12 ±1.80	33.34 ±2.04	32.09 ±1.15	42.21 ±2.51	37.54 ±1.77	39.78 ±2.26	31.92 ±1.81	35.29 ±.05	27.34**
Tyrosine	0.001 ±0	22.55 ±0.92	21.84 ±0.81	21.05 ±2.11	22.13 ±1.54	21.82 ±0.98	21.97 ±0.92	21.06 ±1.19	21.19 ±2.16	18.55*

numerous dynamics like exercise strength and period and synthesis of muscle glycogen. The incorporation of dietary amino acids increases synthesis of muscle protein during retrieval from strength exercise. Fermentation increased branched chain amino acids required by sportsman for muscles strength (Burd *et al*., 2011).

The results of hedonic response of sportsman drinks are supported by the earlier findings of various researchers. Panelists liked flavor, sourness and overall acceptability of freshly prepared drinks whereas momentous decline in score was obtained during 60 days storage. This was due to proteolytic and lipolytic changes among the formulation ingredients. The addition of fermentation improved the hedonic response of sportsman drinks. There was momentous decline in the sensory attributes of whey based fermented pineapple drink (Shukla *et al*., 2013), mango drink (Sakhale *et al*., 2012), banana herbal drink (Yadav *et al*., 2010) and guava drink (Divya and Kumari, 2009) during different storage duration.

CONCLUSIONS

Whey should be promoted as a valuable and cheap source of protein in daily diet. Novel processing technologies are adopted to introduce health supportive edibles from dairy industrial byproducts. Further studies should be carried out to manufactures value added products from whey through novel processing technologies for diverse application.

REFERENCES

AOAC (2006). Official Methods of Analysis. The Association of Official Analytical Chemists.Inc.18th Ed. Arlington, USA.

Baljeet SY, Ritika BY and Sarita R (2013). Studies on development and storage of whey-based pineapple (Ananas comosus) and bottle gourd (*Lagenariasiceraria*) mixed herbal beverage. *Int. Food Res. J.,* **20**(2): 607-612.

Bulatovic MLJ, Marica BR, Ljiljana VM, Svetlana BN, Maja SVS and Aleksandra PDV (2014). Improvement of production performance of functional fermented whey based beverage. *Chem. Ind. Chem. Eng. Q.,* **20**(1): 1-8.

Burd NA, West DW, Moore DR, Atherton PJ, Staples AW and Prior T (2011). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J. Nutr.,* **141**(4): 568-573.

Burke LM (2001). Energy needs of athletes. *Can. J. Appl. Physiol.,* **26**(1): 202-219.

De-Oliveira MN, Sodini I and Corrieu G (2001). Manufacture of fermented lactic beverages containing probiotic cultures. Department of Pharmaceutical and Biochemical Technology, Sao Paulo University, 580. Sao Paulo, Brazil.

Divya and Kumari A (2009). Effect of different temperatures, timings and storage periods on the physic-chemical and nutritional characteristics of whey guava beverage. *World J. Dairy Food Sci.,* **4**(2): 118-122.

Djuric M, Caric M, Milanovic S, Tekic M and Panic M (2004). Development of whey based beverages. *Eur. Food Res. Technol.,* **219**(4): 321-328.

Gassem MA and JF Frank (1991). Physical properties of yoghurt made from milk treated gesellschaft. USDA. United States Department of Agriculture. 2003.

Gill HS, Rutherford KJ and Cross ML (2000). Bovine milk. A unique source of immunomodulatory ingredients for functional foods. *In*: Buttriss J. Saltmarsh M, editors. Functional Foods II-Claims and Evidence. Cambridge, Royal Society of Chemistry Press, England, pp.82-90.

Ha E and Zemel MB (2003). Functional properties of whey, whey components and essential amino acids: Mechanisms underlying health benefits for active people (Review). *J. Nutr. Biochem.,* **14**(5): 251 -258.

Juan W, Qing-He Z, Zhi-Hua W and Hong-Mei L (2009). Determination of Major Bovine Milk Proteins by Reversed Phase High Performance Liquid Chromatography. *Chinese J. Anal. Chem.,* **37**(11): 1667-1670.

Marwaha SS and Kennedy JF (1988). Review: whey pollution problem and potential utilization. *Int. J. Food Sci. Technol.,* **23**(4): 323-336.

Meilgaard MC, Civille GV and Carr BT (2007). Sensory Evaluation Techniques, 4th Ed. C.R.C. Press, New York.

Naik YK, Khare A, Choudhary PL, Goel BK and Shrivastava A (2009). Studies on physico-chemical and sensory characteristics of whey based watermelon beverage. *Asian J. Res. Chem.,* **2**(1): 57-59.

Omole, Olusola J, Ighodaro, Macdosnald O and Mathew MO (2012). Proximate composition of whey from South West Nigeria. *Adv. Biores.,* **3**(1): 14-16.

Otte J, Samah MAS, Zakoraand Nielsen De MS (2007). Fractionation and identification of ACE-inhibitory peptides from α-lactalbumin and b-casein produced by thermolysin-catalysed hydrolysis. *Int. Dairy J.,* **17**(12): 1460-1472.

Park, YW, Juarez M, M. Ramos and Haenlein GFW (2007). Physico-chemical characteristics of goat and sheep milk. *Small Rumin. Res.,* **68**(68): 88-113.

PDDC (2006). Pakistan Dairy Development Company White Paper on Pakistan's Dairy Sector. Dairy Pakistan Township, Kot Lakhpat, Lahore, Pakistan.

Pescuma M, Herbert EM, Mozzi F and De-Valdez GF (2010). Functional fermented whey based beverage using lactic acid bacteria. *Int. J. Food Microbiol.,* **141**(1): 73-81.

Sakhale BK, Pawar VN and Ranveer RC (2012). Studies on the development and storage of whey based RTS beverage from mango cvkesar. *J. Food Process Technol.,* **3**(3): 41-44

Saravana KR and Manimegalai G (2005). Studies on storage stability of whey based papaya juice blended RTS beverage. *J. Food Sci. Technol.,* **42**(2): 185-188.

Shukla M, JY Kumar and Shemelis A (2013). Development of probiotic beverage from whey and pineapple juice. *J. Food Process Technol.,* **4**(2): 91-95

Singh D, Singh Rand Bhatt F (2014). Development, quality evaluation and shelf life studies of whey guava beverage. *Int. J. Curr. Eng. and Technol.,* **4**(3): 2171-2175.

Stanciuc N, PlanckenI Van Der, Rotaru G and Hendrickx M (2010). Denaturation impact in susceptibility of b-lactoglobulin to enzymatic hydrolysis: A kinetic study. *Revue Roumaine De Chimie.,* **53**(10): 45-51.

Steel RG, Torrie J Hand Dickey DA (1997). Principles and Procedures of Statistics-A Biometrical Approach. 3rd Ed. McGraw Hill Book Co. Inc. New York.

Walsh MK and Brown RJ (2000). Use of amino acid analysis for estimating the individual concentrations of proteins in mixtures. *J. Chromatogr. A.,* **891**(2): 355-360.

Yadav RB, Yadav B Sand Kalia N (2010). Development and storage studies on whey-based banana herbal (*Menthaarvensis*) beverage. *Am. J. Food Technol.,* **5**(2): 121-129.

Yousaf AE and Calstrom (2003). Food microbiology a laboratory manual. Wiley-Interscience. A John Wiley and Sons Inc., New Jersey, USA.

Zimecki M and Kruzel ML (2007). Milk-derived proteins and peptides of potential therapeutic and nutritive value. *J. Exp. Therap. Oncol.,* **6**(2): 89-106.