Compositional analysis of developed whey based fructooligosaccharides supplemented low- calorie drink

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Revised: 4 July 2014 / Accepted: 25 August 2014 / Published online: 10 September 2014
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Abstract Array of evidences have indicated that the supplementation of diet with functional and nutritional components to provide numerous health benefits. In this context, fortification with novel constituents as prebiotics i.e. fructooligosaccharides (FOS) is an encouraging trend all over the world. In the current exploration, FOS was used as a prebiotic in whey based functional drinks. For drink formulation, four samples were prepared i.e. whey based drink (T_1) and FOS supplemented whey drinks @ 0.5, 1.0 and 1.5 % referred as T_2 , T_3 and T_4 , respectively. The formulated drinks were evaluated for compositional analysis with special reference to amino acids and mineral profiles. The functional drinks showed momentous impact on total solids (TS) whilst, pH, acidity, crude protein and fat were affected non-significantly. However, pH, TS, fat and protein contents of prepared drinks were decreased substantially ($p < 0.05$) during storage period. However, prepared drinks showed non-significant variations in essential and non-essential amino acids.

Keywords Whey · Fructooligosaccharides · Amino acid profile · Mineral profile · Low-calorie drink

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Introduction

Milk is assumed as nature's balanced food hence dairy beverages holds promising position in all segments of population worldwide. Likewise, whey based drinks have gained immense attention due to increased awareness regarding their health potential (Smithers 2008). Whey, a by-product of cheese manufacturing industry has enormous potential for the development of innovative products owing to its balanced amino acids profile and biologically valuable proteins (Yadav et al. 2010). However, it is still considered as industrial waste dumped oftenly despite of its high nutritional profile. It is estimated that in developed countries almost 95 % of whey is utilized in food products, whereas in developing economies like Pakistan major portion is going as waste. Dumping of whey (both paneer and cheese whey) causes unfriendly environment whilst its drying creates financial burden on small scale producers. In this context, development of whey based functional products is one of the prudent ways for its efficient utilization (Cruz et al. 2009).

Functional/nutraceutical foods contain specific dietary fiber, vitamins, minerals, fatty acids, bioactive substances such as phytochemicals or antioxidants, probiotics and prebiotics (Sloan 2000). Amongst, fiber is an emerging nutraceutical constituent that effectually clipped consumers' attention because of its high therapeutic potential. Numerous epidemiological studies have linked increased fiber consumption with reduced cardiac risks, diabetes and obesity (Kanakupt et al. 2011).

In current scenario, most of the individuals are consuming comparatively less dietary fiber as recommended by the American Dietetic Association 25–38 g/day for total and 5–10 g/day of soluble fiber. In order to meet the basic needs relatively higher amount of fiber should be consumed by the target population. Presently, the development of fiber fortified drinks especially containing soluble fiber is the prime

requirement. In this milieu, fructooligosaccharides and inulin have attained core attention as a source of soluble fiber. They can be utilized along with non-nutritive sweeteners in beverage formulations for specific health concerns (Babio et al. 2010; Alexiou and Franck 2008). Considering the facts, supplementation of liquid whey with fructooligosaccharides (FOS) as soluble fiber is one of the judicious approaches to enhance the functional/nutraceutical potential of developed drink in cost effective manner.

Fructooligosaccharides (FOS) are diverse group of carbohydrates comprising fructose residues as key monomers (Bonnema, et al. 2010). Fructooligosaccharides (FOS) synonymously called as oligofructose or oligofructan are oligosaccharide fructans. Short chain fructooligosaccharides (scFOS or FOS) are mixture of $1^F\text{-(1-}\beta\text{-fructofuranosyl)}_{n-1}$ sucrose oligomers, where n varied from 2 to 4 (Birkett and Francis 2009). The most commonly used source for fructooligosaccharides and inulin production is chicory (*Cichorium intybus*) belonging to family *Compositae*. It is native to Europe and consumed as vegetable since Roman and Greek antiquity (Wilson and Robert 2004).

The ultimate objective of the study was to develop a prebiotic supplemented whey based drink using fructooligosaccharides and characterization of formulated four drinks for nutritional quality.

Materials and methods

Procurement of raw material

Sweet liquid whey was procured from Noon Dairies, Bhalwal and brought to the *Functional and Nutraceutical Food Research Section*, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad. The refrigeration conditions (4 °C) were maintained during storage to ensure whey quality. Fructooligosaccharides (90 % pure with active ingredients: oligofructose or sucrose trisaccharide) were obtained from Organic Herbs, China.

Development of functional drinks

During product development module, four treatments were prepared given. The first drink (T₁) was comprised of whey based drink whilst, T₂, T₃ & T₄ additionally contained 0.5, 1.0 & 1.5 % fructooligosaccharides (FOS). To formulate whey based drink, initially Cheddar cheese whey was clarified through cheese cloth to eliminate casein particles. Further, ingredients including citric acid, carboxymethyl cellulose (CMC) and aspartame (sweetener) were added. Afterwards, the heated whey drink was cooled and homogenized @ 7000 U/min with lab scale homogenizer, considered

as T₁. For the preparation of functional drinks T₂, T₃ and T₄, resultant mixture was pasteurized @ 65 °C for 30 min in a water bath. Next, preservative (sodium benzoate), food grade color (orange red) and orange flavor were added to enhance the hedonic response of the prebiotic FOS was added @ 0.5, 1.0 & 1.5 %, respectively to the formulated whey based drink. The formulated drinks were filled in pre-sterilized transparent 500 mL plastic labeled bottles and stored at 5 °C in the refrigerator.

Functional drink analysis

Compositional assay

The developed functional drinks were analyzed for various physicochemical parameters including pH, acidity, total solids, fat, crude protein, mineral profile, amino acid profile and by their respective protocols mentioned herein on fortnightly basis during 2 months storage.

pH

For the pH estimation, electronic digital pH Meter (Inolab pH 720, WTW 82362) was used. The drink sample was taken in a 50 mL flask to measure this trait. After calibration, pH was directly recorded from digital display.

Acidity

The prepared drinks were analyzed for acidity through the titrimetric method. Acidity was determined by titrating raw liquid whey (25 mL) against N/10 NaOH until pH 8.3 remain stable for 10 s using phenolphthalein as indicator following the guidelines of AOAC (2006), Method Number 947.05.

$$\text{Acidity(\%)} = \left[\frac{0.009 \times \text{volume of NaOH used} / \text{weight of the sample (g)}}{\times 100} \right]$$

Total solids

Total solids of drinks were assessed according to the AOAC (2006) Method No. 925.23. The sample was heated for 15 min in water bath followed by drying in hot air oven at 100±5 °C till constant weight. The total soluble solids were calculated by the following expression.

$$\text{Total solids(\%)} = \frac{\text{residue after drying (g)}}{\times 100 / \text{weight of the sample (g)}}$$

Fat

Fat content was calculated by Gerber method following the protocol of Djuric et al. (2004). For the purpose, 10 mL of sulfuric acid (H_2SO_4) was poured into the butyrometer. The sample (10.94 mL) and isoamyle alcohol (1–2 mL) was added. Afterwards, butyrometer was closed with a rubber stopper. The contents of butyrometer were mixed carefully by rotating the tube at 45° and placed in a water bath at 65°C for 5 min. Butyrometer was then immediately centrifuged @1,100 rpm for 5 min. The resultant fat percentage was read out from the butyrometer graduated column.

Crude protein

Crude protein was determined by Kjeldahl method using Kjeltex Apparatus (Model: D-40599, Behr Labor Technik, GmbH-Germany) following the procedure elaborated in AOAC (2006) Method No. 991.20. Accordingly, 1 mL drink sample was digested in a digestion tube using 25 mL sulfuric acid and 2–3 g of digestion mixture (CuSO_4 : KSO_4 : FeSO_4 : = 5:94:1). Digested sample was distilled with 40 % NaOH followed by titration against 0.1 N HCl. The protein content in milk was estimated by multiplying with factor 6.38.

Mineral profile

The drink samples were analyzed for mineral assay following the guidelines of AOAC (2006). Purposely, Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) was used to determine sodium and potassium whilst calcium and magnesium through Atomic Absorption Spectrophotometer (Varian AA240, Australia).

Amino acid profile

Amino acid profile of whey drinks was determined following the protocol of Walsh and Brown (2000). In this context, sample (5 mL) was centrifuged at 5,000 g for 15 min at 4°C to separate the fat. Hydrochloric acid (6 M) was added to the

sample vial for a final concentration of 5 mg of protein/mL of HCl. Hydrolysis vial was placed in an ultrasonic cleaner and flushed with nitrogen gas before sealing under vacuum. Sample was placed in a heating block for 4 h at 145°C . Afterwards, samples was removed from the heating block and allowed to cool before filtration through 0.2 μm filter. Sample was dried with N_2 and dissolved in a 1 M Beckman sample dilution buffer. The prepared whey sample was analyzed for amino acid profile by running through Automated Amino Acid Analyzer (Model: L-8500 A, Hitachi, Japan). Areas of amino acid standards were used to quantify each amino acid in representative sample.

Statistical analysis

The resulting data were subjected to statistical analysis using completely randomized design (CRD) by statistical software Cohort version 6.1. Moreover, analysis of variance (ANOVA) technique was applied to determine the level of significance (Steel et al. 1997).

Results & discussion

The effect of FOS on pH, acidity, fat and crude protein contents of functional drinks showed non-significant, except for total solids. However storage significant affected all these traits.

pH and acidity

The pH values of formulated functional drinks i.e. T_1 , T_2 , T_3 and T_4 were recorded as 4.96 ± 0.29 , 4.92 ± 0.24 , 4.90 ± 0.21 and 4.89 ± 0.25 , respectively (Tables 1 and 2). However, storage exhibited significant decline in pH from 5.45 ± 0.31 to 4.25 ± 0.22 at 0 to 60th day, respectively. Similarly, acidity in functional drinks T_1 , T_2 , T_3 and T_4 was noticed as 0.43 ± 0.02 , 0.45 ± 0.01 , 0.46 ± 0.03 and 0.47 ± 0.02 , respectively. During

Table 1 Effect of fructooligosaccharides supplementation and storage on pH of whey based low-calorie drink. Values are mean $\pm$ SD ($n=3$)

Means carrying different letter varied significantly ($p<0.05$); (T_1 =whey drink; T_2 =whey drink+0.5 % FOS; T_3 =whey drink+1.0 % FOS; T_4 =whey drink+1.5 % FOS)

Storage (Days)	Treatments				Means
	T_1	T_2	T_3	T_4	
0	5.49 ± 0.23	5.47 ± 0.14	5.45 ± 0.21	5.44 ± 0.25	5.45 ± 0.31^a
15	5.33 ± 0.19	5.30 ± 0.23	5.30 ± 0.26	5.31 ± 0.29	5.31 ± 0.26^b
30	5.05 ± 0.20	5.05 ± 0.29	5.02 ± 0.12	5.01 ± 0.18	5.03 ± 0.23^c
45	4.59 ± 0.22	4.58 ± 0.26	4.54 ± 0.20	4.53 ± 0.19	4.56 ± 0.20^d
60	4.36 ± 0.16	4.25 ± 0.14	4.22 ± 0.17	4.20 ± 0.21	4.25 ± 0.22^e
Means	4.96 ± 0.29	4.92 ± 0.24	4.90 ± 0.21	4.89 ± 0.25	

Table 2 Effect of fructooligosaccharides supplementation and storage on acidity (%) of whey based low- calorie drink. Values are mean±SD (*n*=3)

Means carrying different letter varied significantly (*p*<0.05); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS)

Storage (Days)	Treatments				Means
	T ₁	T ₂	T ₃	T ₄	
0	0.30±0.02	0.31±0.03	0.32±0.01	0.33±0.01	0.33±0.01 ^e
15	0.39±0.01	0.42±0.02	0.43±0.02	0.44±0.02	0.41±0.02 ^d
30	0.46±0.02	0.45±0.01	0.48±0.03	0.49±0.03	0.47±0.03 ^c
45	0.49±0.03	0.50±0.03	0.52±0.04	0.54±0.02	0.51±0.02 ^b
60	0.53±0.01	0.55±0.03	0.56±0.02	0.57±0.01	0.55±0.04 ^a
Means	0.43±0.02	0.45±0.01	0.46±0.03	0.47±0.02	

60 days storage, acidity of the functional drinks increased momentarily from 0.33±0.01 to 0.55±0.04 % (Table 4). The decline in pH with enhanced acidity might be due to conversion of lactose to lactic acid present in drinks. Additionally, the acidic nature of artificial sweetener may also contribute towards increased acidity during storage.

The results regarding pH and acidity of functional drinks in current exploration are in agreement with the findings of Yadav et al. (2010), they used acid whey produced by acid coagulation of milk and observed an inverse association between pH and acidity of whey based banana herbal beverage during study. They explicated that pH changed significantly from 5.47±0.02 to 5.01±0.01 after 20 days storage at refrigeration temperature. Similarly, acidity varied from 0.38 to 0.49 during the entire period. Recently, Sakhale et al. (2012) also recorded an increase in acidity of acid whey (2 % citric acid) based ready to serve (RTS) mango beverage during 30 days of storage. The observed values for acidity of fructooligosaccharides (FOS) supplemented functional drinks are also in accordance with the outcomes of Divya (2009) who assessed an increase in total acidity of whey guava beverage with the passage of time. Likewise, Saravana and Manimegalai (2005) also noticed decline in pH and elevation in acidity of whey based papaya beverage stored at refrigeration temperature for 3 months. The decrease in pH is due to the production of amino & organic acids from proteins and lactose during storage.

Table 3 Effect of fructooligosaccharides supplementation and storage on total solids (%) of whey based low- calorie drink. Values are mean±SD (*n*=3)

Means carrying different letter varied significantly (*p*<0.05); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS)

Storage (Days)	Treatments				Means
	T ₁	T ₂	T ₃	T ₄	
0	6.69±0.31	7.12±0.28	7.85±0.35	8.07±0.43	7.43±0.39 ^a
15	6.64±0.29	7.05±0.41	7.75±0.49	8.03±0.46	7.36±0.40 ^b
30	6.51±0.35	6.99±0.32	7.55±0.37	7.90±0.44	7.23±0.31 ^c
45	6.46±0.32	6.85±0.37	7.45±0.39	7.57±0.40	7.08±0.35 ^d
60	6.27±0.36	6.79±0.26	6.92±0.41	7.01±0.46	6.74±0.27 ^e
Means	6.51±0.29 ^d	6.96±0.34 ^c	7.50±0.45 ^b	7.71±0.42 ^a	

Total solids, fat and crude protein

Means for total solids contents in different treatments varied from 6.51±0.29 to 7.71±0.42 % (Table 3) as a function of FOS addition in the drinks. In contrary, with the passage of storage interval, total solids decreased noticeably from 7.43±0.39 at initiation to 6.74±0.27 % at the termination of study.

Similarly, functional drinks T₁, T₂, T₃ and T₄ had fat contents 0.21±0.02, 0.20±0.01, 0.19±0.02 and 0.18±0.01 %, respectively. A substantial decrease from 0.23±0.03 to 0.15±0.01 % in fat contents for all treatments was noticed throughout the storage span (Table 4). Likewise, means for protein content of functional drinks T₁, T₂, T₃ and T₄ were 0.74±0.04, 0.73±0.03, 0.71±0.03 and 0.69±0.02 %, respectively (Table 5). Two months storage resulted in a significant decline in protein content from 0.80±0.02 to 0.66±0.02 %.

Earlier studies of Yadav et al. (2010) strengthened the current results, reported significant effect of storage on protein, fat and total solids contents of whey based beverages during storage. They further elucidated that the decrease in fat and protein content of beverages during storage is might be due to the production of fatty acids and proteolysis that ultimately reduce the total solid contents. The similar findings were also documented by Dave et al. (2003) that the protein and fat content of dairy products decreased significantly throughout the study. In another study, Kindstedt

Table 4 Effect of fructooligosaccharides supplementation and storage on fat (%) of whey based low- calorie drink. Values are mean±SD (*n*=3)

Means carrying different letter varied significantly (*p*<0.05); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS)

Storage (Days)	Treatments				Means
	T ₁	T ₂	T ₃	T ₄	
0	0.25±0.01	0.24±0.02	0.23±0.04	0.22±0.02	0.23±0.03 ^a
15	0.23±0.01	0.23±0.01	0.22±0.01	0.21±0.01	0.22±0.02 ^{ab}
30	0.22±0.02	0.22±0.01	0.20±0.02	0.19±0.02	0.20±0.01 ^b
45	0.20±0.01	0.19±0.03	0.17±0.04	0.16±0.02	0.18±0.01 ^c
60	0.18±0.02	0.16±0.01	0.15±0.01	0.14±0.01	0.15±0.01 ^d
Means	0.21±0.02	0.20±0.01	0.19±0.02	0.18±0.01	

et al. (1995) elucidated that the fat and protein contents of mozzarella cheese decreased momentarily during 2 months storage under refrigeration conditions. The biochemical reactions and exposure to light & oxygen during storage of dairy products may cause oxidative damage to the lipids and proteins consequently decreasing their concentration in the final products (Lucey et al. 2003). The present results are also in accordance with the outcomes of Djuric et al. (2004). They prepared whey based fruit beverage and observed the values for fat and protein as 0.45 & 0.71 % respectively.

Mineral assay

The mineral content of functional drinks indicated non-substantial effect of treatments, storage and their interaction on Na, K, Ca and Mg. The mean values (Table 6) recorded for sodium were 34.48±0.75, 33.39±0.96, 33.07±0.85 and 31.96±0.95 mg/100 g for therapeutic drinks i.e. T₁, T₂, T₃ and T₄, correspondingly. Likewise, the observed values for potassium were 100.34±2.37 and 97.39±2.36 mg/100 g for T₁ and T₄. Similar non-significant declining tendency was observed for calcium content of functional drinks and the values noted for T₁, T₂, T₃ and T₄ were 24.89±0.54, 24.54±0.62, 23.08±0.55 and 22.38±0.49 mg/100 g, respectively. The means for magnesium ranged from 4.99±0.10 (T₁) to 4.54±0.19 (T₄) mg/100 g.

Whey contains numerous valuable micronutrients like, sodium, potassium, chloride, magnesium, citrate and phosphate that help to enhance the functionality of whey proteins (Zemel 2004). Whey is also an excellent source of bioavailable calcium that improves bone health. The composition of whey salts expounded low sodium to potassium ratio that is essential for maintenance of blood pressure. Moreover, calcium from the whey is readily absorbed in the intestine, facilitated by the presence of lactose (Smithers 2008). The instant results for minerals contents in whey based functional drink are in conformity with the previous findings of Goyal and Gandhi (2009). They recorded the values for sodium, potassium, calcium and magnesium for cheese whey as 35, 130, 48, 5.9 mg/100 g. Previously, Djuric et al. (2004) noticed similar non-momentous decrease for mineral content of whey based fruit beverages during storage.

Amino acid profile

The F values for amino acid profile of formulated drinks (Tables 7 & 8) depicted that the addition of fructooligosaccharides to the drinks did not impart any significant variation in both essential and non-essential amino acids. The data for essential amino acids indicated that leucine was present in the highest amount (96.09±5.65 mg/g protein) in T₁ followed by lysine (83.88±1.44 mg/g protein) and threonine (48.76±

Table 5 Effect of fructooligosaccharides supplementation and storage on crude protein (%) of whey based low- calorie drink. Values are mean±SD (*n*=3)

Means carrying different letter varied significantly (*p*<0.05); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS)

Storage (Days)	Treatments				Means
	T ₁	T ₂	T ₃	T ₄	
0	0.81±0.03	0.80±0.01	0.81±0.05	0.79±0.04	0.80±0.02 ^a
15	0.78±0.02	0.76±0.03	0.73±0.02	0.72±0.06	0.74±0.05 ^b
30	0.74±0.04	0.73±0.02	0.70±0.04	0.69±0.01	0.71±0.04 ^c
45	0.72±0.02	0.70±0.03	0.68±0.01	0.66±0.03	0.69±0.03 ^d
60	0.69±0.01	0.68±0.04	0.66±0.05	0.63±0.01	0.66±0.02 ^e
Means	0.74±0.04	0.73±0.03	0.71±0.03	0.69±0.02	

Table 6 Effect of fructooligosaccharides supplementation and storage on mineral profile of whey based low-calorie drink. Values are mean $\pm$ SD ($n=3$)

Sodium (mg/100 g)			Potassium (mg/100 g)					Means					
Days	Treatments		T ₁	T ₂	T ₃	T ₄	Days	Treatments	T ₁	T ₂	T ₃	T ₄	Means
0	35.08±0.98		34.97±0.87	34.97±0.87	33.98±0.93	32.08±0.96	0	101.15±3.23	101.15±3.23	100.62±2.36	99.73±2.36	99.18±2.69	100.17±3.97
15	35.07±0.97		33.45±0.96	33.45±0.96	33.65±0.93	32.77±0.96	15	100.78±2.97	100.78±2.97	100.03±2.36	99.35±2.43	98.75±2.23	99.73±2.65
30	34.99±0.98		33.19±0.96	33.19±0.96	32.93±0.93	32.01±0.95	30	100.54±2.21	100.54±2.21	99.74±2.36	99.10±2.29	97.35±2.68	99.18±2.35
45	33.98±0.97		33.24±0.95	33.24±0.95	32.96±0.93	32.02±0.95	45	99.97±2.15	99.97±2.15	99.25±2.35	98.57±2.35	96.22±2.45	98.50±2.56
60	33.25±0.97		32.12±0.95	32.12±0.95	31.83±0.93	30.90±0.95	60	99.25±2.53	99.25±2.53	98.17±2.35	98.03±2.47	95.49±2.12	97.73±2.11
Means	34.48±0.75		33.39±0.96	33.39±0.96	33.07±0.85	31.96±0.95	Means	100.34±2.37	100.34±2.37	99.56±2.60	98.96±2.58	97.39±2.36	
Calcium (mg/100 g)			Magnesium (mg/100 g)					Means					
Days	Treatments		T ₁	T ₂	T ₃	T ₄	Days	Treatments	T ₁	T ₂	T ₃	T ₄	Means
0	25.27±0.63		25.10±0.69	25.10±0.69	23.38±0.72	22.76±0.93	0	5.05±0.13	5.05±0.13	4.95±0.17	4.86±0.16	4.72±0.09	4.89±0.18
15	25.12±0.53		24.78±0.45	24.78±0.45	23.29±0.61	22.41±0.55	15	5.04±0.11	5.04±0.11	4.84±0.15	4.75±0.17	4.69±0.14	4.83±0.11
30	24.96±0.61		24.52±0.51	24.52±0.51	23.02±0.81	22.35±0.82	30	5.01±0.16	5.01±0.16	4.74±0.14	4.69±0.15	4.48±0.12	4.73±0.09
45	24.67±0.70		24.26±0.44	24.26±0.44	22.91±0.66	22.22±0.54	45	4.97±0.12	4.97±0.12	4.68±0.11	4.56±0.13	4.40±0.08	4.65±0.16
60	24.43±0.42		24.04±0.39	24.04±0.39	22.79±0.56	22.18±0.67	60	4.90±0.13	4.90±0.13	4.61±0.12	4.50±0.11	4.41±0.15	4.60±0.13
Means	24.89±0.54		24.54±0.62	24.54±0.62	23.08±0.55	22.38±0.49	Means	4.99±0.10	4.99±0.10	4.76±0.16	4.67±0.18	4.54±0.19	

Means carrying different letter varied significantly: T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS

Table 7 Essential amino acids (mg/g protein) of whey based fructooligosaccharides supplementation low- calorie drink. Values are mean±SD ($n=3$)

Treatments	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
T ₁	17.46±0.29	45.32±1.79	96.09±5.65	83.88±1.44	25.74±0.42	25.23±0.41	48.76±1.82	20.36±0.33	45.53±0.77
T ₂	17.36±0.40	44.87±0.77	96.47±3.66	83.80±1.45	25.11±0.93	24.44±0.67	48.11±1.93	20.17±0.94	45.23±0.93
T ₃	16.94±0.69	44.46±2.17	96.05±2.16	83.38±1.44	24.69±1.23	24.02±0.86	47.69±0.96	19.78±1.08	44.79±0.56
T ₄	16.56±0.94	44.07±1.96	95.66±6.63	83.08±1.43	24.31±0.99	23.63±0.98	47.31±2.32	19.21±0.82	44.08±1.76
F value	3.35 ^{NS}	2.09 ^{NS}	0.45 ^{NS}	0.72 ^{NS}	2.87 ^{NS}	3.23 ^{NS}	1.81 ^{NS}	0.98 ^{NS}	2.53 ^{NS}

Means carrying different letter varied significantly ($p<0.05$); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS); NS=Non-significant

1.82 mg/g protein) that decreased non-substantially to 95.66±6.63, 83.08±1.43 and 47.31±2.32, respectively in T₄. Similar non-momentous diminishing order was noticed for valine, isoleucine, histidine, methionine, phenylalanine and tryptophan that varied from 45.53±0.77 to 44.08±1.76, 45.32±1.79 to 44.07±1.96, 17.46±0.29 to 16.56±0.94, 25.74±0.42 to 24.31±0.99, 25.23±0.41 to 23.63±0.98 & 20.36±0.33 to 19.21±0.82 mg/g protein in T₁ to T₄, respectively. Moreover, the data pertaining to non-essential amino acids of functional drinks presented the maximum value for glutamic acid in T₁ as 151.21±6.60 mg/g protein that gradually declined to 151.00±4.87, 150.10±3.59 and 149.71±8.32 mg/g in T₂, T₃ and T₄, correspondingly. Likewise decreasing trend was noticed for alanine, arginine, aspartic acid, cystein, glycine, serine & tyrosine and their concentrations ranged from 47.37±0.81 to 46.40±2.30, 22.12±0.36 to 20.74±0.76, 104.38±3.78 to 103.01±3.45, 14.92±0.23 to 14.21±0.30, 16.44±0.78 to 15.58±0.36, 50.35±3.14 to 49.24±0.84 & 29.75±0.99 to 28.49±0.39 mg/g protein in functional drinks i.e. T₁ and T₄, respectively.

Whey proteins are easily digestible and promote the postprandial concentrations of amino acids in the plasma. Most of the whey amino acids act as direct insulin secretagogues, resultantly improve insulin response. The promising amino acids in this context are isoleucine, leucine, valine, lysine and threonine (Calbet and MacLean 2002; Krebs et al. 2003).

In a study, Nilsson et al. (2007) assessed the amino acid composition of whey proteins and reported the values for essential amino acids 79.8, 57.3, 59.3, 61.1 mg/g for leucine, isoleucine, valine and threonine, respectively. Amongst non-essential amino acids, the maximum concentration was recorded for glutamic acid (141.4 mg/g) whilst the minimum (13.8 mg/g) was noted for glycine. Later, it was reported that heating whey protein concentrates (WPC) significantly affects the availability of lysine alongside impairs enzymatic release of other amino acids depending on the intensity of heat treatment. Amino acid profile of whey protein showed that heating of whey at 121 °C for 80 min destroys significant proportion of cysteine, lysine and arginine. Alongside, availability of other amino acids i.e. aspartic acid, glutamic acid, proline, alanine, glycine, threonine and serine is particularly affected (Onwulata et al. 2008). In case of current findings, the time and temperature used for pasteurization did not impart any deleterious effect on the amino acid composition of whey and resultant drinks.

Conclusion

Considering the facts, it is rational to supplement the liquid whey with soluble fibers like fructooligosaccharides in order to enhance the functional characteristics of whey and to exploit this most neglected dairy by-product in a cost effective manner.

Table 8 Non-essential amino acids (mg/g protein) of whey based fructooligosaccharides supplementation low- calorie drink. Values are mean±SD ($n=3$)

Treatments	Alanine	Arginine	Aspartic acid	Cystein	Glutamic acid	Glycine	Proline	Serine	Tyrosine
T ₁	47.37±0.81	22.12±0.36	104.38±3.78	14.92±0.23	151.21±6.60	16.44±0.78	67.53±3.14	50.35±2.84	29.75±0.99
T ₂	47.28±1.51	22.03±0.28	104.29±4.90	14.89±0.66	151.00±4.87	16.34±0.49	67.43±3.65	50.08±1.76	29.30±1.21
T ₃	46.93±1.87	21.13±0.33	103.39±6.58	14.57±0.34	150.10±3.59	16.12±0.47	67.19±2.95	49.45±1.15	28.88±0.50
T ₄	46.40±2.30	20.74±0.76	103.01±3.45	14.21±0.30	149.71±8.32	15.58±0.36	66.85±1.14	49.24±0.84	28.49±0.39
F value	2.06 ^{NS}	2.98 ^{NS}	0.39 ^{NS}	3.46 ^{NS}	0.18 ^{NS}	3.99 ^{NS}	0.94 ^{NS}	2.03 ^{NS}	2.78 ^{NS}

Means carrying different letter varied significantly ($p<0.05$); (T₁=whey drink; T₂=whey drink+0.5 % FOS; T₃=whey drink+1.0 % FOS; T₄=whey drink+1.5 % FOS); NS=Non-significant

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