

# Optimization of Fructooligosaccharide Fortified Low Calorie Apple-Whey Based RTS Beverage and Its Quality Evaluation during Storage

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## Authors' contribution

This work was carried out in collaboration among all authors. Author RS designed the study and the research work was carried out by author RC under the guidance of author RS. All authors has helped in preparation of manuscript, analysis of data and approved the final manuscript.

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## ABSTRACT

Apple based beverages are rich source of sugars and dietary fibres but deficit in proteins and some minerals like calcium. Whey- a major environmental pollutant from dairy industry is an excellent source of proteins and calcium. Non-nutritive sweeteners can be used for the development of low calorie hypoglycaemic beverages. Therefore, the present work was planned to optimize and evaluate the effect of fructooligosaccharide (FOS) incorporation on physico-chemical, nutritional and sensory characteristics of apple-whey blended ready-to-serve beverage. Herbal apple-whey blended beverage was prepared by using 75% apple juice+25% whey with 2.5% *jaljeera* extract and 13°B TSS. Results revealed that the beverage with 75% sweetos (mixture of fructooligosaccharide and sucralose) was found most acceptable with overall acceptability score of 8.59±0.26. Selected beverage had 12.20±0.01°Brix TSS, 0.30±0.01% acidity, 6.28±0.03% reducing sugar, 9.43±0.06% total sugars, 10.57±0.05 mg/100 g ascorbic acid, 37.84±0.03 mg/100 g total

phenols,  $15.64 \pm 0.02$  mg/100 mL calcium,  $0.28 \pm 0.03\%$  protein and  $1.59 \pm 0.03\%$  FOS thus depicting enhanced nutritional value. Beverages were stored successfully for a period of 60 days under ambient and refrigerated conditions. However, various quality parameters of RTS beverage were retained higher under refrigerated storage conditions. Conclusively, this creates a scope for better health beverage as well as efficient utilization of whey.

**Keywords:** Apple; whey; fructooligosaccharide; low calorie beverages; RTS; storage.

## 1. INTRODUCTION

Bioactive compounds derived from natural foods like fruits and vegetables can exert functional and health-promoting effects through bioactivity beyond the basic nutrient composition [1]. Further, there has been a growing demand for beverages enriched with bioactive compounds having health-promoting characteristics besides quenching one's thirst and providing nutrition [2, 3]. The fruit juice-based beverage is a fast growing sector within the beverage market and ready- to- serve beverages are more popular among these beverages [4]. Among various fruits, apple is one of the most frequently consumed fruit in the world constituting an important part of the human diet as a source of sugars, minerals and dietary fibres. Despite presence of number of bioactive compounds, apple contains insignificant amount of protein and is a poor source of minerals such as potassium and calcium [5]. Whey is a by-product obtained during preparation of cheese which have been reported to contain nutritional components like lactose, protein, vitamins, and minerals and mostly drained out unutilized [6]. The dairy beverages formulated with cheese whey have gained prominence in the global dairy market and are widely accepted by the consumers of different age groups because of their enhanced nutritional value [7]. Various researchers have also suggested use of whey in the development of healthy nutritious fruit based beverages [8-10]. Moreover, whey based fruits beverages have been considered more suitable for health as compared to other drinks [11].

On the other hand, the present day consumers wish to enjoy the pleasure of sweetness without extra calories, hence, non-nutritive sweeteners like sucralose, fructooligosaccharides (FOS) could be suitable alternative for the development of such beverages [12]. Among various sweeteners, *sweetos* is a low calorie sweetener that contains fructo-oligosaccharides and sucralose. It imparts sweetness equivalent to sugar and contributes less than one calorie/gram energy. The FOSs have attracted special

attention because of their prebiotic properties and also their sweet taste being very similar of sucrose [13]. These are short chains of fructose molecules which are excellent source of soluble fibres and do not add calories because they are not digestible by stomach enzyme [14,15]. Keeping the above facts in view, the present study was conducted to optimize fructooligosaccharide fortified apple-whey based herbal RTS beverage and to evaluate its quality during storage.

## 2. MATERIALS AND METHODS

### 2.1 Procurement of Raw Material and Preparation

Apple fruits (*Malus x domestica* Borkh.) and toned milk were procured fresh from local market. Apple juice was extracted in the hydraulic press after grating the pieces. The extracted juice was heat preserved in glass bottles and stored at  $5 \pm 1^\circ\text{C}$  until use. Whereas, whey was prepared by simple acid coagulation method [9].

### 2.2 Optimization of Fructooligosaccharide Fortified Low Calorie Apple-Whey RTS Beverage

For optimization of fructooligosaccharide fortified low calorie apple-whey RTS beverage, the formulation optimized by Sharma et al. [16] consisting of apple (75%) + whey (25%) with 2.5% jaljeera extract and 13°B TSS was used to prepare apple-whey blended RTS beverage. *Jaljeera* extract was prepared by mixing of *jajjeera* powder (MDH) in boiling water in the ratio of 1:1 followed by straining through muslin cloth and used for the product development. Whereas, different proportions of non-nutritive sweetener '*sweetos*' (mixture of fructooligosaccharide and sucralose) viz. 0, 25, 50, 75 and 100 per cent were tried by replacing sugar sweetness for optimization of a suitable combination. The acidity (as % citric acid) was kept constant (i.e. 0.30%) in all the treatments. The prepared beverages were filled hot into pre-

sterilized glass bottles (200 mL capacity) after adding suitable and recommended stabilizer (i.e. carboxy methyl cellulose @ 0.1%) and stored at ambient temperature (12-25°C) as well as low temperature (4-7°C) for a period of 60 days and analyzed for various physico-chemical and sensory characteristics at different intervals of 0, 15, 30, 45 and 60 days during storage.

### **2.3 Physico-chemical and Sensory Evaluation**

The physico-chemical parameters of RTS beverage viz. TSS, sugars, titratable acidity, ascorbic acid, protein and lactose content were determined according to the standard procedures as described by Ranganna [17]. The pH of the beverage was determined by using a digital pH meter (CRISON Instrument, Ltd. Spain). The total calcium content in the samples was analyzed by digestion method [18]. Whereas, total phenolics content was determined with Folin-Ciocalteu reagent according to the method of Bray and Thorpe [19]. Antioxidant activity (Free radical scavenging activity) was measured as per the method of Brand-Williams et al. [20] by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a source of free radical. Fructooligosaccharide (FOS) content in the beverages was determined by HPLC method described by Sangeetha et al. [21]. Energy value of the RTS beverage was measured in a bomb calorimeter (Model Toshival DT-100). Whereas, total plate count of microorganisms was done by aseptically inoculating 0.1 g of serially diluted sample in standard plate count agar medium [17]. The 9-point Hedonic rating method as given by Pimentel et al. [22] was followed for conducting the sensory evaluation of the developed beverages. A panel of ten judges comprising of faculty members and post graduate students of the Department of Food Science and Technology were selected to evaluate the products. Each sample was evaluated for various sensory attributes viz. colour, body, flavour and overall acceptability on 9-point Hedonic scale.

### **2.4 Statistical Analysis**

All the analytical parameters were recorded in replicates (n=5) and expressed as means± SE. The data of quantitative estimation of physico-chemical characteristics was assessed as per the analysis of variance (ANOVA) for Completely Randomized Design (CRD) whereas; the data pertaining to sensory evaluation were analyzed by Randomized Block Design (RBD) as given by

Cochran and Cox [23]. The significance ( $p < 0.05$ ) or otherwise of data obtained from various experiments was judged with the help of F-Table using OPSTAT software.

## **3. RESULTS AND DISCUSSION**

### **3.1 Optimization of Fructooligosaccharide Fortified Low Calorie Apple-Whey RTS Beverage**

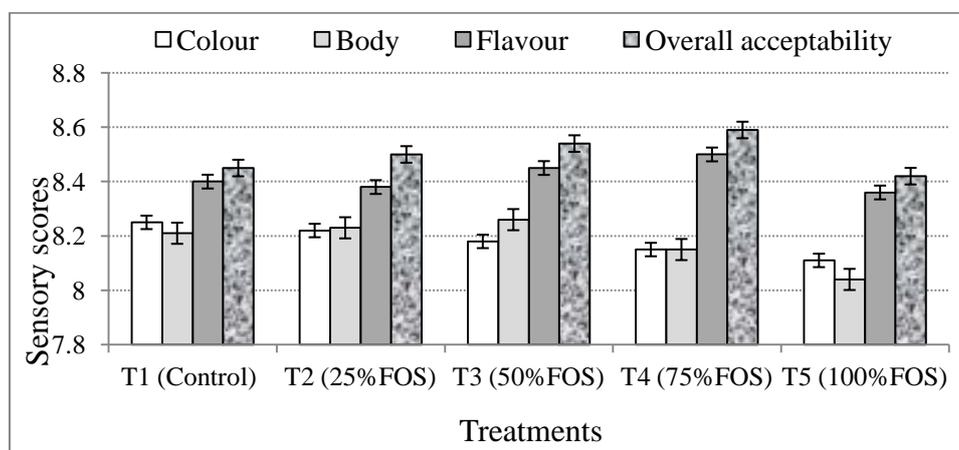
The suitability of supplementing apple-whey based beverage with FOS was evaluated by replacing sucrose with equivalent sweetness level of sweetos and its effect on various sensory attributes is presented in Fig. 1. The colour score of fructooligosaccharide fortified apple-whey RTS beverage decreased non-significantly ( $p < 0.05$ ) with the increased proportions of sweetos. It was noticed that with the increase in proportion of sweetos up to a level of 75 per cent, the body score increased, beyond which it decreased due to the fact that fructooligosaccharides act as a bulking agents and might have added turbidity to the prepared beverages at higher concentration [24,25]. The flavour score of apple-whey based low calorie beverages were non-significantly affected by substitution of sucrose with different proportion of sweetos. Whereas, the overall acceptability score of prepared beverages exhibited a gradual increase with substitution of sucrose with sweetos up to 75 per cent. Among various treatments, beverage containing 75 per cent sweetos (T<sub>4</sub>) obtained highest rating score (8.59) for overall acceptability on 9-point Hedonic scale (Fig. 1) and hence was optimized.

### **3.2 Physico-Chemical and Nutritional Quality of Apple-Whey Based Functional Beverages**

The physico-chemical and nutritional characteristics of best rated FOS fortified low calorie apple-whey beverage prepared by using sweetos were compared with that prepared without FOS (Table 1). The analysis of data revealed that higher total soluble solids (TSS) contents 13.10°B were recorded in apple-whey beverage containing 100 per cent sucrose while lower value of 12.20°B was observed in apple-whey beverage containing 75% FOS. The variation in TSS is in correspondence to the decreased sugar concentration and subsequently increased concentration of sweetos. It might be due the fact that non-nutritive sweeteners including sweetos do not add to the TSS of the finished product [26,27]. The titratable acidity and

pH were same in both the products as same amount of fruit part and citric acid was added to maintain the acidity around 0.3 per cent. Data given in Table 1 showed significant variations in reducing and total sugars content among both the beverages. Higher reducing sugars (7.48%) were recorded in 100 per cent sucrose sweetened beverage whereas, lower contents (6.28%) were recorded in FOS fortified low calorie apple-whey beverage. Similar trend was also observed for total sugars. The results depicting decrease of sugars (reducing and total) with the increase in per cent share of non-nutritive sweetener (sweetos) is in corroboration with the earlier findings [12,15,28]. Whereas, non-significant variations in ascorbic acid, phenols, anti-oxidant potential, lactose, calcium and protein content might be due to the fact that

equal proportion of fruit part (juice+whey) was used for the preparation of apple-whey based beverages. Calculated energy value of 40.54 Kcal/100 g was recorded in FOS sweetened low calorie RTS beverage compared to 50.15 Kcal/100 g noted for 100 per cent sucrose sweetened RTS beverage. The reduced energy value in fructooligosaccharide sweetened beverage might be due to the fact that non-nutritive sweeteners are non-calorific [27]. Our results are in conformity with earlier findings [28,29]. Estimation of FOS content in apple-whey beverages by HPLC method revealed that apple-whey beverage with 75 per cent FOS contained 1.59 per cent FOS content as compared to negligible amount of 0.04 per cent recorded in control sample (without FOS) (Fig. 2).

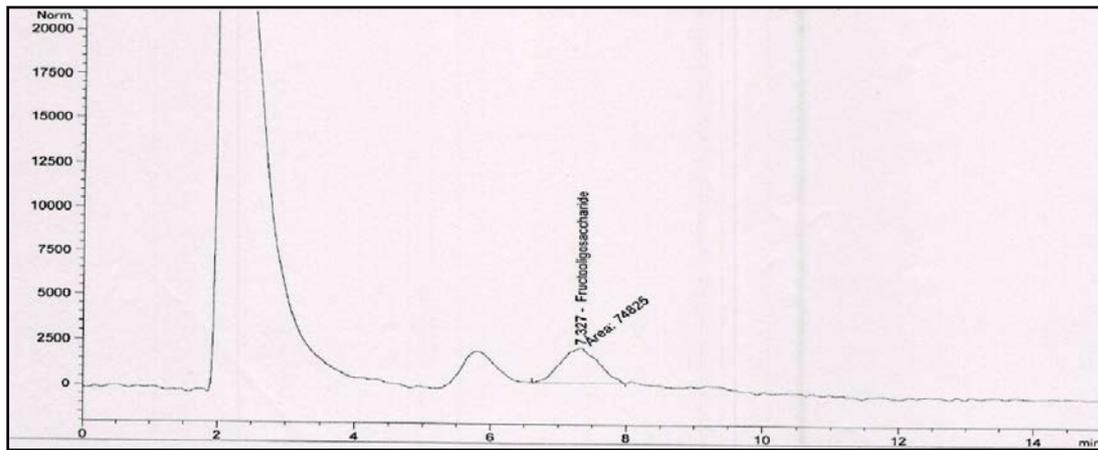


**Fig. 1. Effect of FOS fortification on sensory quality of apple-whey blended beverages (error bars indicates standard error of the average)**

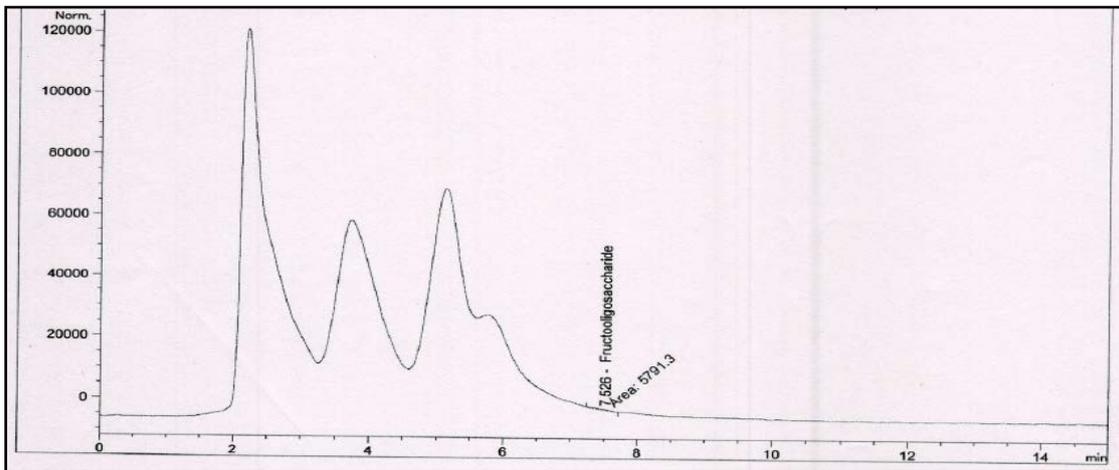
**Table 1. Physico-chemical characteristics of sucrose sweetened and FOS sweetened apple-whey functional beverages**

Parameters	Mean* ± SE	
	Apple-whey beverage (100 % sucrose)	Apple-whey beverage (75% FOS)
TSS (°Brix)	13.10±0.02	12.20±0.01
Titrateable acidity (%)	0.3±0.03	0.30±0.01
pH	4.32±0.01	4.32±0.03
Reducing sugars (%)	7.48±0.04	6.28±0.03
Total sugars (%)	11.23±0.08	9.43±0.06
Ascorbic acid (mg/100 g)	10.57±0.06	10.57±0.05
Total phenols (mg/100 g)	37.86±0.05	37.84±0.03
Antioxidant potential (% free radical scavenging activity)	40.34±0.04	40.32±0.07
Lactose (%)	1.21±0.02	1.19 ± 0.06
Calcium (mg/100 mL)	15.68±0.05	15.64 ± 0.02
Protein (%)	0.29 ±0.01	0.28±0.03
Fructo-oligosaccharides (%)	0.04±0.02	1.59±0.03
Energy value (Kcal/100 g)	50.15±0.01	40.54±0.03

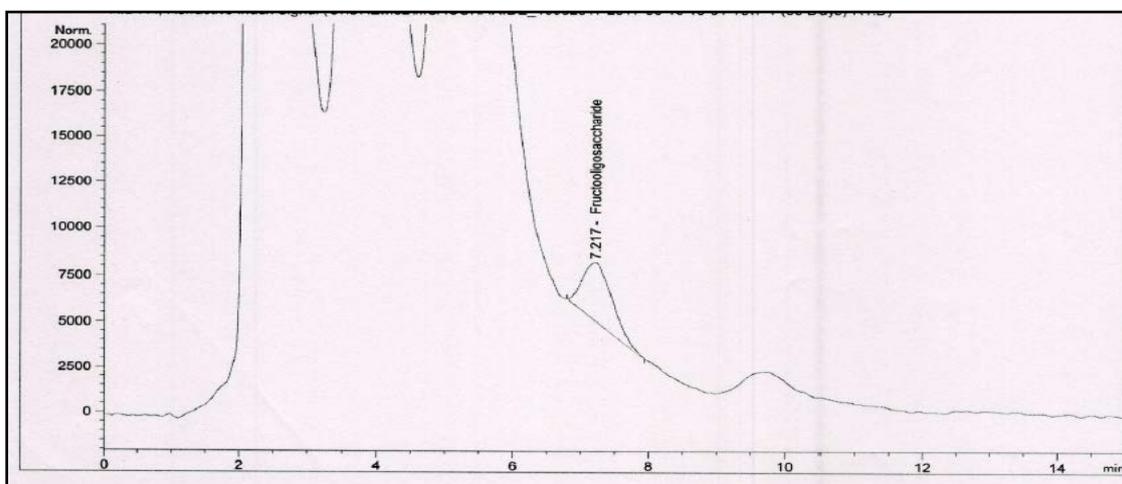
\* The values are mean of three replicates (n=5); SE= Standard error



(a) Fructo-oligosaccharide standard peak



(b) Apple-whey beverage (100% sucrose)



(c) Apple-whey beverage (75% fructooligosaccharide)

Fig. 2. HPLC chromatogram for FOS estimation; (a) standard peak, (b) apple-whey beverage (100% sucrose) and (c) apple-whey beverage (75% FOS)

### 3.3 Changes in Quality Characteristics of FOS Fortified Low Calorie Apple-Whey Beverages during Storage

#### 3.3.1 Physico-chemical characteristics

##### 3.3.1.1 Total soluble solids

On storage, TSS of beverages increased during entire storage period with slightly higher increase in beverages stored under ambient conditions (Fig. 3a). The increase in total soluble solids might be due to hydrolysis of polysaccharides into soluble disaccharides and monosaccharides in the presence of acid during storage [30]. Increase in TSS had also been reported in whey based banana and pineapple herbal beverage [10,31].

##### 3.3.1.2 Titratable acidity

Statistically ( $p < 0.05$ ) significant increasing trend in titratable acidity of beverages during entire storage was observed (Fig. 3b). The increase in acidity might be due to the conversion of lactose to lactic acid and formation of organic acid by ascorbic acid present in beverage. Our results were in accordance with the findings of Panghal et al. [11] in whey-papaya RTS beverage.

##### 3.3.1.3 Ascorbic acid

Data presented in Fig.3c revealed that the ascorbic acid content of apple-whey based functional beverages decreased significantly ( $p < 0.05$ ) during storage period which was more prominent in ambient storage conditions compared to refrigerated storage conditions. During 60 days of storage period, the initial ascorbic acid content of 10.57 mg/100 g in treatment  $T_1$  and  $T_2$  decreased to 8.51 and 8.57 mg/100 g during ambient storage conditions and 9.41 and 9.44 mg/100 g during refrigerated storage conditions, respectively. Analogue results have also been reported in whey-guava beverage, bitter gourd aonla blended squash and whey based pineapple beverage [10,32,33].

##### 3.3.1.4 Reducing sugars

The reducing sugars content of beverages increased with increase in storage intervals from 15 to 60 days (Fig. 3d). On the preparation day, reducing sugar content as 7.48 per cent was observed in treatment  $T_1$  (100% sucrose) and 6.28 percent in treatment  $T_2$  (75% fructooligosaccharide) which increased to 7.86 and 6.65 per cent during ambient storage conditions and 7.65 and 6.47 per cent during refrigerated storage conditions, respectively after

60 days of storage. The increase in reducing sugars was attributed due to the inversion of non-reducing sugars to reducing sugars under acidic conditions. Similar findings were also reported by Sharma et al. [16] and Hamid et al. [34] in herbal apple-whey RTS beverage and mulberry RTS, respectively during storage.

##### 3.3.1.5 Total phenols

Effect of storage on total phenolic contents of FOS fortified apple-whey based functional beverage revealed that the total phenolic content decreased with increase in storage intervals from 15 to 60 days (Fig. 3e). The decrease in the total phenolic content of beverages during storage might be due to their involvement in the formation of polymeric compounds by complexation with protein and their subsequent precipitations. These results are in well coherence with Sharma et al. [16] in herbal apple-whey RTS beverage, Sharma and Thakur [33] in bitter gourd-aonla blended squash and Thakur et al. [35] in wild aonla RTS beverage.

##### 3.3.1.6 Total proteins

Statistically significant decrease in protein content of beverages was recorded during entire storage period (Fig. 3f) which is attributed to proteolysis that ultimately reduces the total solid contents. On the preparation day, total protein content as 0.29 per cent was observed in treatment  $T_1$  and 0.27 per cent in  $T_2$  which decreased to 0.15 per cent (in both treatments) during ambient storage conditions and 0.22 and 0.21 per cent during refrigerated storage conditions, respectively after 60 days of storage. Our results are in conformity with the findings of Yasmin et al. [15] in whey based low calorie drink during storage.

#### 3.3.2 Sensory characteristics

The data given in Fig. 4a shows there was a general decreasing trend of colour score during storage and the colour score were retained higher under refrigerated storage conditions. The decrease in colour score during storage might be due to chemical reactions which have led to the formation of brown pigments hence made the appearance of the product less acceptable by the panellists [36]. The body score also decreased with progression of storage time (Fig. 4b). Decrease in body score during storage might be due to co-polymerization, interaction between phenolics, degradation of colloidal particles and protein as well as the formation of complexes with pectin and phenolics during storage [12].

Flavour score of the beverages declined significantly ( $p < 0.05$ ) during entire storage period of 60 days. However, the scores were retained better under refrigerated storage conditions. Decrease in flavour scores (Fig. 4c) might be attributed to the possible loss of volatile aromatic substances during storage at ambient conditions [29,34]. Similarly, overall acceptability score declined for all the beverages, however quality deterioration was higher in case of ambient

storage conditions (Fig. 4d). The loss of colour due to browning, increase in acidity and change in taste and flavour of the beverages during storage might have contributed to decline in overall acceptability scores. Similar observations have also been reported in fructooligosaccharide fortified fruit juice beverages, *Aloe vera*-aonla low calorie beverage, whey based pine apple beverage and wild jamun beverage [10,12, 37,38].

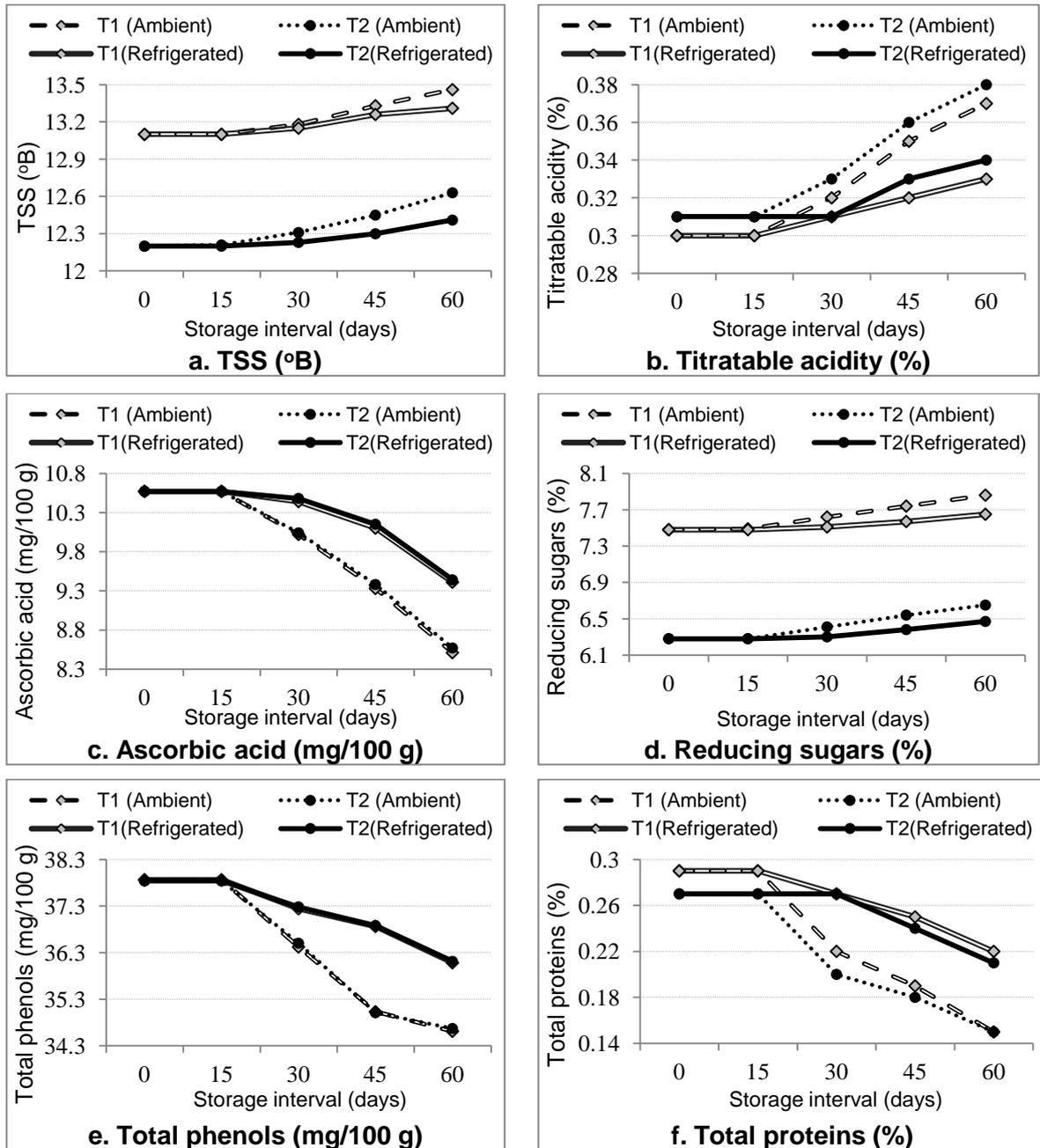
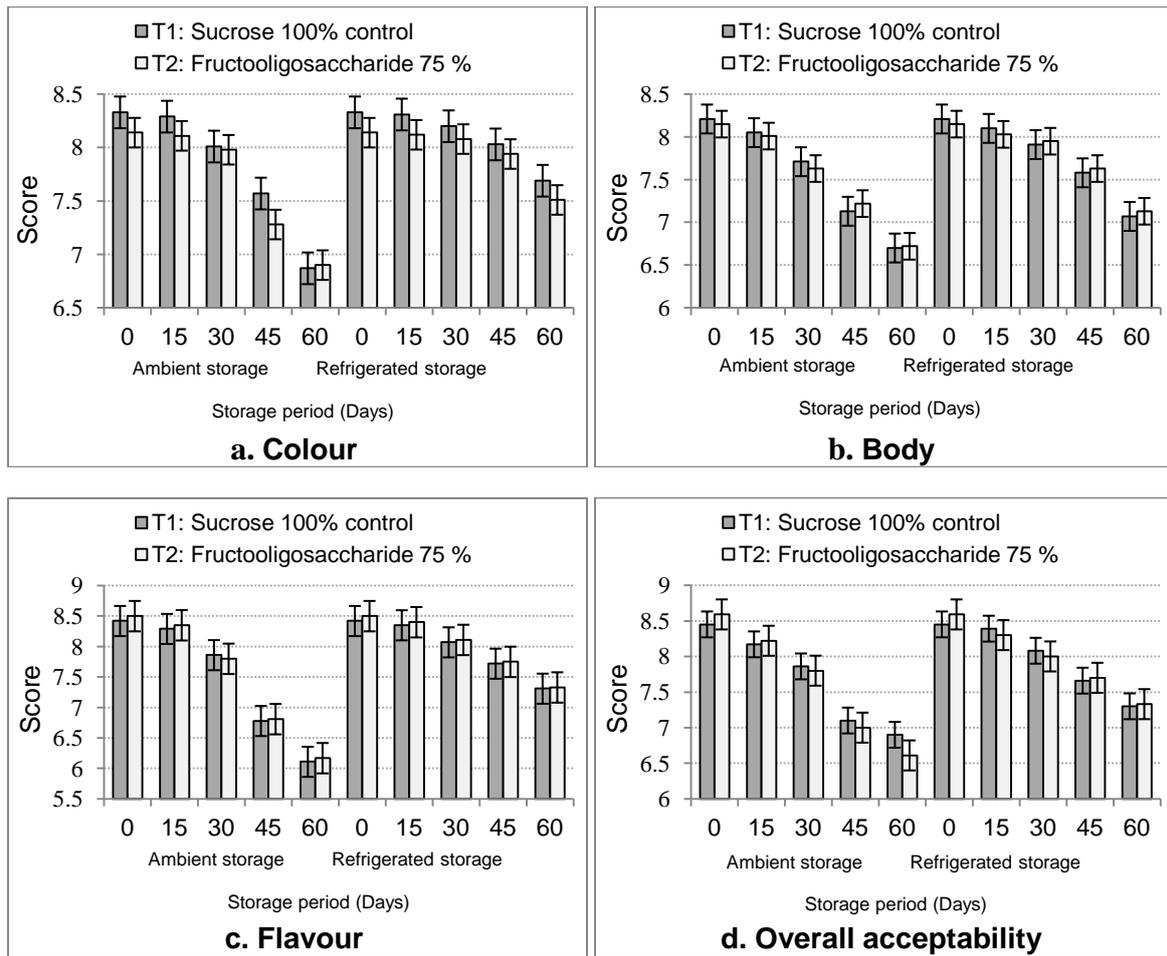
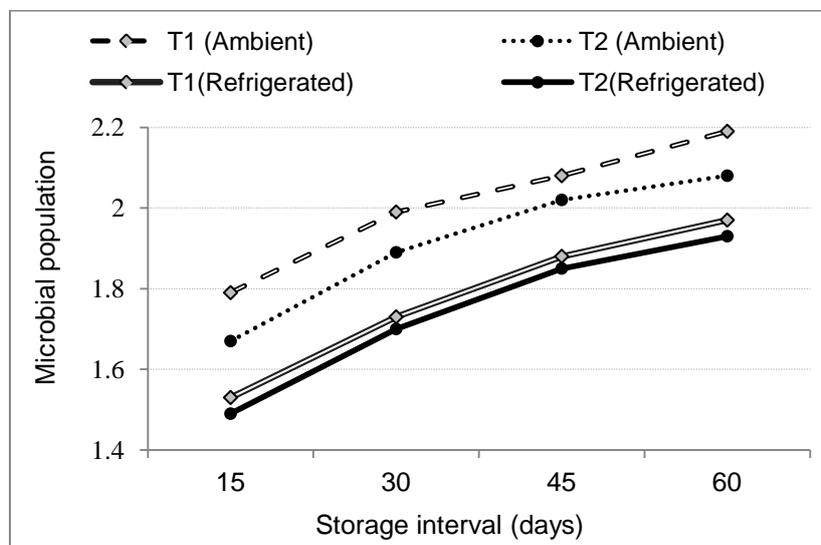


Fig. 3 (a-f). Effect of storage on physico-chemical characteristics of FOS sweetened apple-whey based functional beverages (T<sub>1</sub>: Sucrose 100%, T<sub>2</sub>: Fructooligosaccharide 75 %)



**Fig.4 (a-d): Effect of storage on sensory characteristics of fructooligosaccharide sweetened apple-whey based functional beverages (T<sub>1</sub>: Sucrose 100%, T<sub>2</sub>: Fructooligosaccharide 75 %)**



**Fig. 5. Microbial population ( $\times 10^4$  log cfu/mL) of FOS fortified apple-whey functional beverages during storage (T<sub>1</sub>: Sucrose 100%, T<sub>2</sub>: Fructooligosaccharide 75%)**

**Table 2. ANOVA for sensory attributes of apple-whey based low calorie beverage prepared by sweetos**

Source of Variation	Degree of Freedom	Mean Sum of Square (MSS)			
		Color	Body	Flavour	Overall acceptability
Treatment	4	0.010	0.023	0.010	0.014
Error	10	0.010	0.002	0.007	0.002

### 3.3.3 Microbial quality

The microbial load of RTS was analyzed periodically during storage (Fig. 5) and data revealed that, as the storage period proceeds the total plate count was also increased under both storage conditions up to 60 days storage. The total plate count of beverages reached  $2.19 \times 10^4$  (cfu/mL) for T<sub>1</sub> (100% sucrose),  $2.08 \times 10^4$  (cfu/mL) for T<sub>2</sub> (75% sweetos), respectively under ambient storage while,  $1.97 \times 10^4$  (cfu/mL) for T<sub>1</sub> (100% sucrose),  $1.93 \times 10^4$  (cfu/mL) for T<sub>2</sub> (75% sweetos), respectively under refrigerated storage temperature conditions. However, these microbial counts were in well within the acceptable range suggested by FSSAI [39] for micro flora in any milk based beverage (maximum  $50 \times 10^3$  cfu/mL). Most of the microbes were destroyed by heat processing and the product was further packed in pre-sterilised glass bottles to avoid contamination but milk based beverages contained their own micro-flora which increase during storage periods. Further, whey contains lactose sugar and proteins which favour growth of microorganism during storage. Similar results have also been reported by in whey based mango and whey-papaya RTS beverage [9,11].

## 4. CONCLUSION

In conclusion, the results suggest that fructooligosaccharide may be used as functional ingredient in formulating apple-whey blended beverage with improved nutritional and sensory features. The developed apple-whey beverage (75% sweetos) contained 1.59 per cent FOS content and 40.54 Kcal/100 g energy value, besides other nutritional compounds depicting its functionality as low calorie prebiotic beverage. The present study also suggests an important alternative for reusing whey generated during cheese production and provide an effective way of delivering health benefits of both apple juice and whey to the consumers in the form of a palatable and nutritious beverage with reduced energy value.

## CONSENT

Informed consent was obtained from all individual participants involved in the study for sensory analysis of the products.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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