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Development and quality of apple -whey based herbal functional ready-to-serve beverage

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Abstract
Apple is one of the widely consumed fruits which constitute an important part of the human diet as a source of sugars and dietary fibres. But, it is poor in proteins and some minerals like calcium. On the other hand, whey- a by-product of dairy industry is an excellent source of proteins and calcium. Therefore, in the present study, apple-whey based functional ready-to-serve (RTS) beverage was developed by blending apple juice with whey and *jaljeera* extract and was evaluated for quality under ambient and refrigerated conditions during storage. The incorporation of whey had increased the calcium content from 3.96 to 15.64 mg/100 mL and total proteins from 0.07 to 0.29 % in the developed beverage. Further addition of *jaljeera* extract not only enhanced the sensory quality of the beverage, but also improved the ascorbic acid content (10.57 mg/100g) total phenols (37.86 mg/100g) and antioxidant activity (40.34 %). The developed product was stored safely for a period of sixty days under refrigerated storage conditions with minimum changes in chemical and sensory attributes.

Keywords: Apple, Calcium, Functional beverages, RTS, Whey

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INTRODUCTION

Fruits and vegetables have been reported to play an important role in human nutrition and health because of the abundance of bioactive and functional compounds like polyphenolics, vitamins-C, folic acid, dietary fibres and minerals present in them (Wargovich, 2000; Sharma *et al*., 2012). Regular consumption of fruits and vegetables both fresh and processed has been associated with the reduction of various degenerative diseases such as cardiovascular diseases, diabetes, obesity and certain cancers (Kaur and Kapoor, 2001). Now a days, there is a growing demand for functional fruit beverages as an alternative to traditional beverages and soft drinks which provide specific taste profile and disease preventing properties beyond general nutrition, popularly known as functional beverages (Kausar *et al*., 2012; Sharma and Tandon, 2015).

Among various fruits and vegetables, apple (*Malus ×domestica* Borkh.) is one of the most frequently consumed fruits in many regions across the world, constituting an important part of the human diet as a source of sugars, minerals and dietary fibres (Betoret *et al*., 2012). Despite presence of number of bioactive compounds mentioned above, apple contains insignificant amount of protein and is a poor source of minerals such as potassium and calcium (Lee, 2012). However, these lacking compounds can be incorporated in apple juice from other natural sources especially dairy. Whey, a by-product obtained during preparation of *channa*, *paneer*, cheese and casein have been reported to contain almost all water soluble nutrients present in milk, particularly lactose, protein, vitamins, and minerals (Goyal and Gandhi, 2009). It has anti-bacterial and anti-viral properties, improves immune system and potentially helps to reduce the risk of chronic diseases like

cancer, cardiovascular, arthritis etc. (Bhat and Singh, 2014). However, it has not been utilized beneficially and large quantity of whey is being drained out, which poses a serious damage to environment because of its high biological oxygen demand (Yadav *et al*., 2010). So, blending apple juice with whey seems to be an alternative tool not only to develop healthy nutritious beverage but also to provide an economical solution to utilize whey.

Furthermore, several researchers have suggested use of herbs/spices extract to enhance the palatability of fruit based beverages (Barwal and Sharma, 2001; Bhat *et al.,* 2013; Sharma and Tandon, 2015). These extracts add germicidal, medicinal and appetizing properties to the beverage and at the same time make the product more palatable and acceptable to larger group of consumers (Sirohi *et al.,* 2005). Keeping the above facts in view, the present study was conducted to develop apple-whey based herbal ready to serve (RTS) beverage and to evaluate its quality during storage.

MATERIALS AND METHODS

Extraction and preservation of apple juice: Apple fruits used in the study were sorted and washed thoroughly under running tap water to remove dirt and dust. Fruits were cut into pieces and the juice was extracted in the hydraulic press after grating the pieces. The extracted juice was filtered through the muslin cloth, heat preserved in glass bottles and stored at ambient temperature for further use.

Preparation of whey from cow milk: Whey was prepared as per the method given by Sakhale *et al.* (2012). Fresh cow milk was heated in stainless steel vessel at 82^{o}C for 10 minutes, cooled to 70^{o}C and acidified by adding 2 per cent citric acid solution with continuous stirring resulting in complete coagulation of milk protein (casein). It was then, filtered through two-fold muslin cloth. The resultant liquid whey was collected and centrifuged at 5000 rpm for 10 minutes to separate the fat before its use in product development.

Apple-whey RTS beverage: The apple juice and whey with varying levels (0 to 50 %) of fruit part (blend) and total soluble solids (TSS) were tried for optimization of a suitable combination for the preparation of palatable apple-whey blended functional beverage. The acidity (as % citric acid) was kept constant (i.e. 0.30 %) in all the treatments. The treatment T_1 was kept as control which was having 100 per cent of apple juice (13 ^{o}B TSS) whereas the treatment T_{20} was 100 per cent whey (13 ^{o}B TSS). The rest of the treatments were prepared by blending the both constituents in a ratio (apple: whey) with TSS level of 10, 13 and 15 ^{o}B in each consecutive proportion blends viz. T_2-T_4 (90:10), T_5-T_7 (80:20), T_8-T_{10} (75:25), T_{11}-T_{13} (70:30), T_{14}-T_{16} (60:40) and T_{17}-T_{19} (50:50).

Herbal apple-whey beverage: *Jaljeera* extract was prepared by mixing of *jaljeera* powder (MDH®) in boiling water in the ratio of 1:1 followed by straining through muslin cloth and used for the product development. The jaljeera extract was used as 0, 1.0, 1.5, 2.0, 2.5 and 3.0 per cent for the preparation of herbal apple whey RTS beverage. According to manufacturer, 100g *jaljeera* packet (MDH) consists of iodized salt, black salt, unripe dry mango, cumin, seedless tamarind, citric acid, mint leaves, kachri, dried ginger, yellow chillies, black pepper, turmeric, cloves and asafetida in descending order by weight.

Packaging and storage: 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^{o}C) as well as low temperature (4-7^{o}C) for a period of 2 months. The beverages were analyzed for various physico-chemical and sensory characteristics at different intervals of 0, 15, 30, 45 and 60 days during storage both at ambient and low temperature conditions.

Physico-chemical and sensory evaluation: Various physico-chemical parameters viz. TSS, sugars, titratable acidity, ascorbic acid, protein and lactose content of beverage were determined according to the standard procedures as described by Ranganna (2009). The pH of fruit, juice, milk, whey and prepared products was determined by using a digital pH meter (CRISON Instrument, Ltd. Spain). Total solids were estimated by lactometer by using Richmond formula (Thompkinson and Sabikhi, 2006). The total calcium content in the samples was analyzed by digestion method (Jackson, 1973). The amount of total phenolics compounds in apple and developed products were determined with Folin- Ciocalteu reagent according to the method of Bray and Thorpe (1954). Antioxidant activity (Free radical scavenging activity) of juice and beverage was measured as per the method of Brand-Williams *et al.* (1995) by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a source of free radical. Whereas, the antimicrobial activity of the developed beverages against *E. coli* was measured by well diffusion method (Aneja, 2003). Fat content of milk and whey was determined by Gerber method (BIS, 1977). The 9-point Hedonic rating method as given by Pimentel *et al*. (2016) 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.

Statistical analysis: All the analytical parameters were recorded in replicates and the mean values of each parameter has been described. The data

of quantitative estimation of physico-chemical characteristics from different experiments 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 Mahony (1985).The significance ($p< 0.05$) or otherwise of data obtained from various experiments was judged with the help of F-Table using OPSTAT software.

RESULTS AND DISCUSSION

Physico-chemical characteristics of fresh apple juice and whey: The analysis of apple juice indicated that it contained 12.51°B total soluble

Table 1. Physico-chemical characteristics of fresh apple juice and fresh whey.

Parameters	(Mean*± SE)
Fresh apple juice	
TSS (°Brix)	12.51 ± 0.05
Titratable acidity (%)	0.32 ± 0.06
pH	4.32 ± 0.01
Reducing sugars (%)	6.78 ± 0.01
Total sugars (%)	10.92 ± 0.01
Ascorbic acid (mg/100g)	7.69± 0.02
Calcium (mg/100mL)	3.98±0.04
Protein (%)	0.08±0.03
Total phenols (mg/100g)	35.58 ±0.06
Antioxidant potential (% free radical scavenging activity)	28.37 ± 0.01
Fresh whey	
Total solids (%)	6.12±0.02
Titratable acidity (%)	0.22± 0.01
pH	4.78± 0.02
Fat (%)	0.20 ±0.03
Protein (%)	0.79±0.04
Lactose (%)	4.90 ±0.02
Calcium (mg/100mL)	58.70 ± 0.08

*Each value is average of three determinations (n=3); SE = Standard error

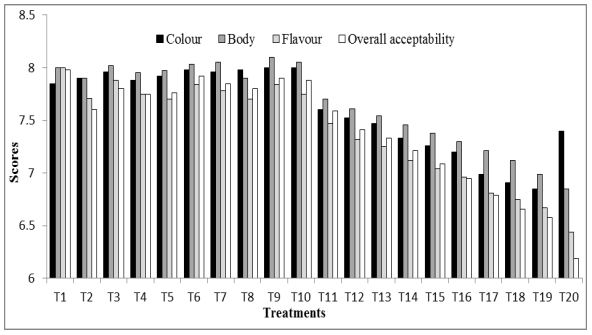

Fig. 1. *Sensory evaluation of different apple -whey blended beverages.*

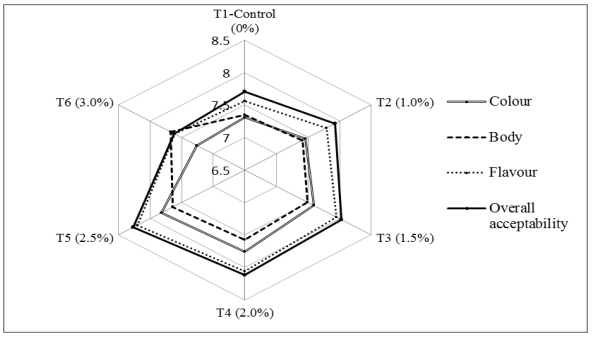

Fig. 2. *Effect of different concentrations of jaljeera extract on sensory attributes of herbal apple-whey beverages.*

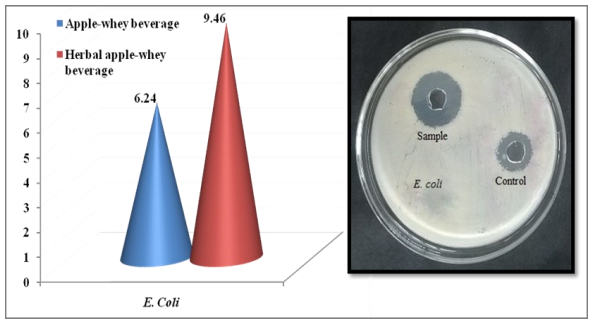

Fig. 3. *Antimicrobial activity of apple-whey beverage and herbal apple-whey beverage against E. coli.*

Table 2. Physico-chemical characteristics of apple, apple-whey and herbal apple-whey beverage.

Parameters	Mean* ± SE		
	Apple beverage (100 % apple juice)	Apple-whey beverage (A_{75}:W_{25})	Herbal apple-whey beverage (*jaljeera* @ 2.50 %)
TSS (°Brix)	13.00±0.01	13.00 ± 0.01	13.10 ±0.02
Titratable acidity (%)	0.30± 0.02	0.30± 0.02	0.30 ±0.03
pH	4.29±0.01	4.30 ± 0.03	4.32 ±0.03
Reducing sugars (%)	6.21±0.03	7.46 ± 0.03	7.48 ±0.01
Total sugars (%)	10.94±0.01	11.21 ± 0.02	11.23±0.08
Ascorbic acid (mg/100g)	6.81±0.04	6.64±0.03	10.57±0.06
Total phenols (mg/100g)	26.83± 0.04	25.64 ± 0.02	37.86±0.05
Antioxidant potential (% free radical scavenging activity)	24.24±0.01	23.12 ± 0.01	40.34±0.02
Lactose (%)	ND	1.21 ± 0.01	1.21±0.02
Calcium (mg/100mL)	3.96±0.09	15.64±0.05	15.68±0.05
Total proteins (%)	0.07±0.01	0.29 ± 0.01	0.29 ±0.01

*Each value is average of three determinations (n=3); SE = Standard error; ND = Not detected; A= apple; W= whey

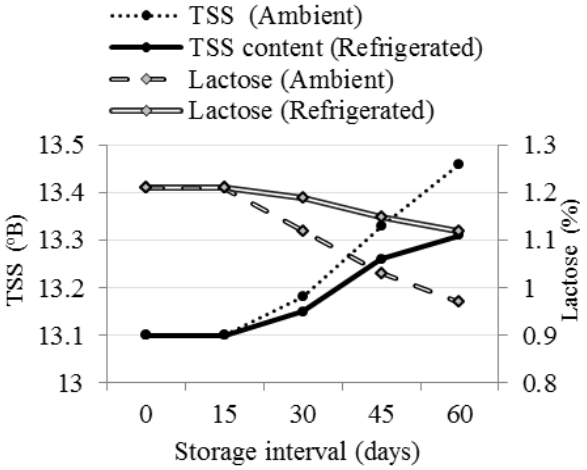

Fig. 4. *Effect of storage on TSS and lactose content of herbal apple-whey RTS beverage.*

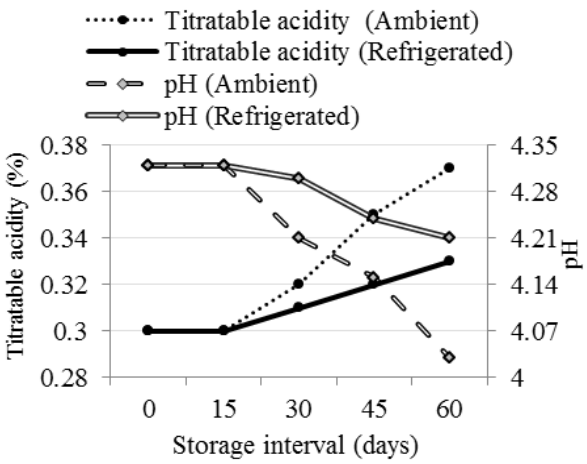

Fig.6. *Effect of storage on titratable acidity and pH content of herbal apple-whey RTS beverage.*

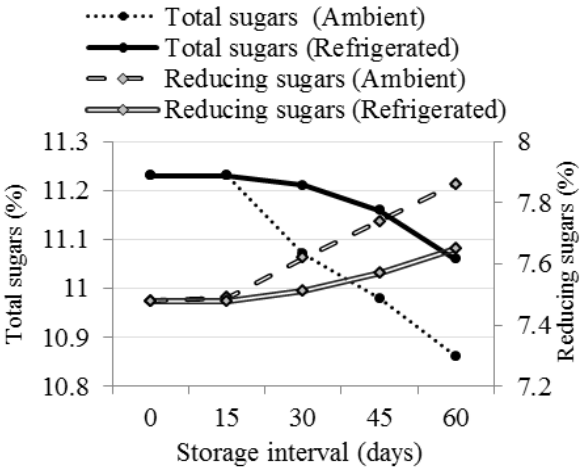

Fig. 5. *Effect of storage on total sugars and reducing sugars content of herbal apple-whey RTS beverage.*

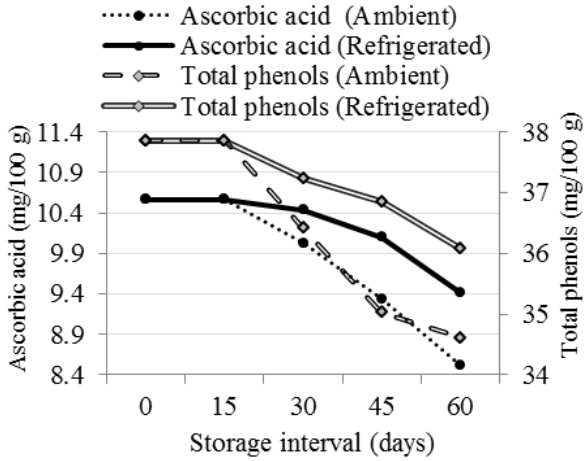

Fig. 7. *Effect of storage on ascorbic acid and total phenols content of herbal apple-whey RTS beverage.*

solids (TSS), 0.32 per cent titratable acidity (as % malic acid), 7.69 mg/100g ascorbic acid, 6.78 per cent reducing sugars, 10.92 per cent total sugars and 4.32 pH (Table 1). The calcium, total phenolic contents and antioxidant activity in apple juice were observed as 3.98 mg/100g, 35.58 mg/100g and 28.37 per cent, respectively. Nearly similar findings for these parameters in apple juice were also reported in the literature (Hyson, 2011; Leahu *et al.,* 2013).

Whereas, the analysis of freshly extracted whey indicated that, it contained 6.12 per cent total solids, 0.22 percent of titratable acidity and 4.78 pH. The fat, protein, calcium and lactose content of whey was recorded as 0.20 per cent, 0.79 per cent, 58.70 mg/100mL and 4.90 per cent, respectively (Table 1). The total solids, acidity, pH and lactose content of 7.37 per cent, 0.32 per cent, 5.51 and 4.05 per cent in whey had also been reported by Sakhale *et al*. (2012) and Yadav *et al.* (2010).

Development of apple-whey RTS beverage: The data pertaining to the effect of different proportions of apple juice and whey with varying levels total soluble solids (TSS) on the sensory quality attributes of apple-whey blended functional beverages is presented in Fig. 1.

The colour scores of the apple-whey blended beverage varied from 6.85 to 8.00 which remained statistically significant among various treatments. It was also observed that with the increase in proportion of whey up to 25 per cent, the colour score of prepared beverages increased significantly ($p<0.05$), however, it decreased significantly beyond 25 per cent. Similarly, the body score for different treatments increased with increase in the proportion of whey up to 25 per cent, beyond which it decreased consistently.

In the present study, efforts were made to incorporate whey in apple juice to the maximum acceptable level. Though, the flavour score decreased with increase in proportion of whey, but addition of whey up to a level of 25 per cent was adjudged acceptable by the panellists. The highest score was obtained by the pure apple juice (T_1) which remained statistically at par with treatment T_3, T_6

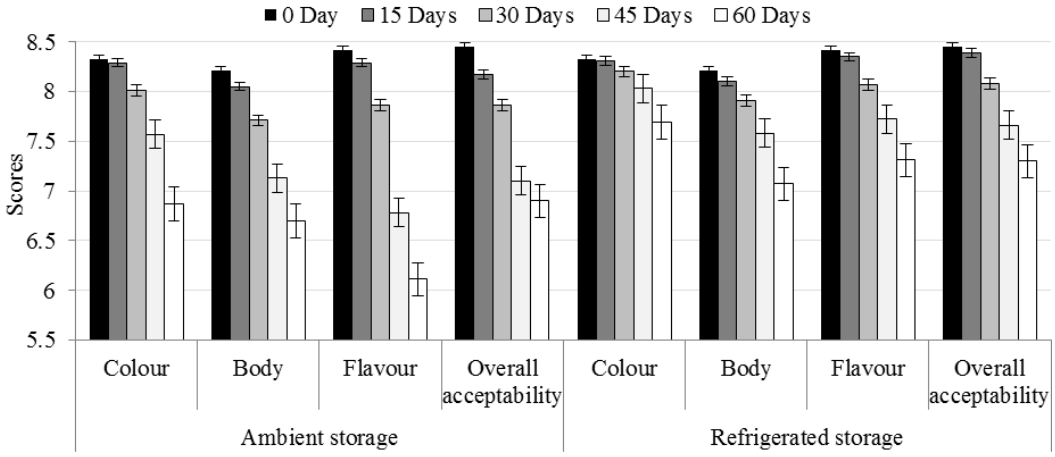

Fig. 8. *Effect of storage on sensory quality of herbal apple-whey RTS beverage.*

and T_9. It was also observed that the products having TSS (13°B) obtained better score for taste/ aroma compared to those having 10 °B and 15 °B TSS. Similarly, the higher overall acceptability score (7.98) was obtained by the pure apple juice (T_1) which was found to be statistically at par with treatment T_3, T_6, T_7, T_8T_9 and T_{10}. Whereas, the lowest overall acceptability score (6.19) was obtained by treatment T_{20} (100% whey). It was observed that, the addition of whey at higher level (>25 %) resulted in decrease in overall acceptability score of the products. Thus, incorporation of whey up to 25 per cent level was found acceptable. Hence, the treatment T_9 (75 % apple juice + 25 % whey with 13 °B TSS was selected for further studies.

Development of herbal apple-whey RTS beverage: The data pertaining to effect of addition of *jaljeera* extract at different concentrations on sensory characteristics of apple-whey beverages is presented in Fig. 2. It was observed that with the increase in the level of *jaljeera* extract the colour scores increased but, beyond 2.50 per cent it decreased. The highest colour score (7.82) was obtained by treatment T_5, which remained statistically at par with treatments T_4(7.76). The body score of *jaljeera* fortified apple-whey beverages varied from 7.35 to 7.67 with highest recorded for beverage having 3 per cent *jaljeera* extract which remained statistically at par with treatment T_5 (7.64). Whereas, the highest flavour score (8.20) was recorded for treatment T_5 having 2.50 per cent *jaljeera* extract. The flavour score showed a decreasing trend above a level of 2.50 per cent *jaljeera* extract due more spicy and salty taste of the beverage as reported by the respondents.

The mean score of overall acceptability for various treatments varied from 7.62 to 8.26. It was observed that with the addition of *jaljeera* extract upto a level of 2.50 per cent, the score for taste/ aroma increased which resulted in higher overall acceptability score of the products. While, the addition of *jaljeera* extract at higher levels (>2.50 %) resulted in decline of overall acceptability score of the products. In all, the treatment T_5 was observed the most acceptable by the panellists, which might be due to improved colour and body score with enhanced taste/aroma of the developed beverage. Increase in different sensory attributes by addition of different herbal extracts has also been reported earlier in whey based banana beverage, spiced pineapple beverage and milk based drink (Yadav *et al.,* 2010; Amaravathi *et al.,* 2014; Seth and Hirdyani, 2016).

Physico-chemical characteristics of apple-whey based beverage: The optimized/selected apple-whey blended beverages were analysed for various physico-chemical characteristics and compared with apple beverage (100% apple juice). A comparison of data presented in Table 2 revealed that the addition of whey had improved the nutritional quality of beverage as evident from its higher calcium (15.64 mg/100mL) and total proteins (0.29 %) content compared to the standard apple beverage (3.96 mg/100mL and 0.07 %), respectively. Further, the addition of 2.5 per cent *jaljeera* extract had improved the nutritional quality of the beverage as evident from its higher ascorbic acid (10.57 mg/100g), total phenolic contents (37.86 mg/100g) as well as antioxidant potential (40.34 % free radical scavenging activity) compared to apple-whey blended beverage (Table 2). The developed apple-whey herbal RTS beverage was also found to have higher (9.46 mm) antimicrobial activity against human pathogen *E. coli.* (Fig. 3), indicating its nutritional, antioxidant and antimicrobial properties. Similar results have also been reported by Kapoor and Ranote (2015) and Sharma *et al.* (2015) in pear-jamun blended juice and *Aloe vera* beverages.

Changes in quality during storage

Physico-chemical characteristics: The TSS content of herbal apple-whey based RTS beverage increased slightly during storage (Fig. 4) and this increase during storage might be due to solubilisation of insoluble carbohydrates of the product due to presence of acid (ascorbic acid) during storage (Sirohi *et al*., 2005). Whereas, the lactose content in apple-whey based functional beverages experienced a significant decrease during storage (Fig. 4) and this decrease was more at ambient storage conditions as compared to refrigerated storage conditions. Our results are in conformity with the findings of Yadav *et al.* (2010) for whey based banana herbal beverage, Mohammed *et al.* (2014) for whey papaya beverage and Thakur *et al.* (2017) for box myrtle RTS beverage.

The total sugars of beverage showed a significant decrease during storage which was comparatively less in refrigerated storage conditions than in ambient conditions (Fig. 5). The Millard reaction and other chemical reactions of sugars with acids during storage also led to decrease in total sugar content (Yadav *et al*., 2010). An increase in reducing sugars content during storage from 15 to 60 days was observed which might be due to the inversion of non-reducing sugars to reducing sugars under acidic conditions (Aruna *et al*., 1997; Sharma *et al*., 2018). Similar findings were also reported by Kumar and Manimegalai (2005) in whey based papaya RTS and Hamid *et al.* (2017) in mulberry RTS beverage.

The beverage exhibited a slight increase in titratable acidity during storage and consequently the pH of the same decreased with storage period (Fig. 6). 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 whereas, with increase in acidity, pH correspondingly decreased. Similar, results have also been reported by Yadav *et al.* (2010) and Ismail *et al.* (2011) in whey-based banana and mango beverage, respectively.

Ascorbic acid content of developed RTS beverage decreased significantly during storage however, the decrease was lesser in refrigerated storage conditions than that of ambient (Fig. 7). The stability of ascorbic acid content is dependent on processing and storage conditions and lost during storage. Significant decrease in ascorbic acid during storage may be attributed to its degradation into dehydro-ascorbic acid, furfural and hydroxy furfural at ambient conditions (Aruna *et al*., 1997; Sharma and Thakur, 2017; Thakur *et al.,* 2018). Similarly, the total phenolic contents decreased with increase in storage intervals from 15 to 60 days (Fig. 7). Further, while comparing the overall effect of storage conditions, it was found that higher amount of total phenols retained under refrigerated storage conditions compared to ambient storage conditions. The decrease in the total phenolic content of beverages during storage might be due to their involvement in the formation of polymeric compounds by complexion with protein and their subsequent precipitations (Kumar and Manimegalai, 2005; Sharma and Thakur, 2017).

Sensory characteristics: The colour, body, flavour and overall acceptability scores of herbal apple-whey based RTS beverage decreased significantly during storage (Fig. 8) and this decrease was more pronounced under ambient storage conditions than refrigerated storage conditions. Retention of higher sensory scores in refrigerated conditions might be due to the slower rate of chemical reactions during storage. The colour score of the beverage decreased with the increase in storage intervals from 15 to 60 days which 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.

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 (Wilson and Burns, 1983). The possible reason for decrease in flavour scores might be attributed to the possible loss of volatile aromatic substances during storage. It was observed that overall sensory quality of the beverages stored under ambient temperature decreased drastically after 30 days of storage period, whereas, it remained well within the acceptable range (≥7.0) under refrigerated conditions. The loss of colour, body and flavour score of beverages during storage might have contributed to decline in overall acceptability scores. The results were in conformity with the finding of Yadav *et al.* (2010) and Sakhale *et al.* (2012) in whey based banana and mango RTS beverage, respectively.

Conclusion

Conclusively, it emerged from the present study that blending of apple juice with whey up to a level of 25% did not affect the sensory quality of apple juice. The addition of whey and *jaljeera* extract not only enhanced the sensory quality of the developed beverage but also improved the nutritional quality of blended beverage in terms of calcium content (15.64 mg/100mL), total proteins (0.29%), ascorbic acid (10.57 mg/100mL) and total phenols (37.86 mg/100mL). The beverages were safely stored for a period of 45 days under both storage conditions without many appreciable changes in quality characteristics. However, the changes were slower and lesser in refrigerated storage conditions as compared to that under ambient conditions.

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