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FORMULATION AND STATISTICAL EVALUATION OF A READY-TO-DRINK WHEY BASED ORANGE BEVERAGE AND ITS STORAGE STABILITY

FORMULACIÓN Y ESTADÍSTICA DE UNA BEBIDA DE NARANJA PREPARADA A BASE DE SUERO Y SU ESTABILIDAD DE ALMACENAMIENTO

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Abstract

A value-added functional beverage is formulated utilizing unprocessed liquid whey. Whey has excellent nutritional qualities and bland flavors; it is easy to digest and has a unique functionality in a beverage system. The ready-to-drink beverage is formulated with concentrated whey, orange juice along with an adequate amount of sugar, stabilizer, citric acid and flavor. Orange juice is used since the acidic flavor of whey is compatible with citrus flavors and particularly orange. The health and nutritional benefits of orange further imparts the value to the formulated beverage. Nine blend formulations are prepared by varying the dry matter of whey, fruit juice and sugar content. Based on a statistical analysis of the sensory evaluation of the drinks, the optimal formulation is found to have a ratio 3:2 for concentrated liquid whey and orange juice followed by an addition of 8% sugar (w/v) and 0.1% stabilizer (w/v). The shelf-life of the final product is carried out both at room temperature (30±2ºC) and refrigeration temperature (7±1ºC) with and without addition of preservatives. The product remains in good condition up to eleven days at room temperature and up to three months under refrigeration condition with addition of 150 ppm of sodium benzoate.
Keywords: whey protein, functional beverage, formulation, sensory evaluation, storage stability.

Resumen

Se realizó una bebida funcional de valor agregado, utilizando suero líquido sin procesar. El suero tiene excelentes cualidades nutricionales y sabores insulsos; es fácil de digerir y posee una funcionalidad única en un sistema de bebidas. La bebida preparada se formuló con suero de leche concentrado, zumo de naranja, una cantidad adecuada de azúcar, estabilizador, ácido cítrico y saborizante. Los beneficios nutricionales y saludables de la naranja le imparten aún más el valor a la bebida formulada. Nueve formulaciones de mezcla se prepararon mediante la variación de la materia seca de suero de leche, jugo de fruta y el contenido de azúcar. Basado en un análisis estadístico de la evaluación sensorial de las bebidas, se encontró que la formulación óptima tiene una relación de 3:2 para el suero líquido concentrado y jugo de naranja, seguido por la adición del 8% (w/v) de azúcar y 0.1% de estabilizador (w/v). El tiempo de almacenamiento del producto final se llevó a cabo tanto a temperatura ambiente (30±2°C) y temperatura de refrigeración (7±1°C) con y sin la adición de conservadores. El producto se mantiene en buenas condiciones hasta once días a temperatura ambiente y hasta tres meses bajo condiciones de refrigeración con la adición de 150 ppm de benzoato de sodio.
Palabras clave: proteína de suero, bebida funcional, formulación, estabilidad de almacenamiento, análisis estadístico.

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1 Introduction

Whey or milk plasma is a greenish yellow, semi translucent liquid that separates from the curd during the cheese making process. It is one of the major problematic disposals for dairy industry because of high Biological Oxygen Demand (BOD) value ranging from 39,000 to 48,000 ppm (Divya and Kumari, 2009) and its stringent environmental regulatory acts. It consists of 45 to 50% of total milk solids, 70% of lactose, 20% of milk proteins, 70 to 90% of milk minerals and almost all the water-soluble vitamins originally present in milk (Kinsellan, 1984; Horton, 1995). Whey protein comprises of four major protein fractions and six minor protein fractions. Major protein fractions include beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), bovine serum albumin (~8%) and immunoglobulins (~2%) (Flores-Andrade *et al*., 2013). Minor fractions include lactoferrin, lysozyme, lactoperoxidase and glyco macro peptides (Walzem *et al*., 2002; Marshall, 2004). Whey protein contains essential AAs and branched-chain AAs such as isoleucine, leucine and valine which are important in tissue growth and repair, regulation of muscle protein synthesis and glucose homeostasis (Irena *et al*., 2008). Whey proteins are one of the best quality food proteins having a high protein efficiency ratio, stable below pH 4 and a less chalky mouth feel compared to other protein sources, making it an ideal protein source in developing value-added functional beverages (Beristain *et al*., 2006). Lactose, one of the essential constituents of whey dry matter, also possesses beneficial effects such as stimulation of peristaltic activities in the digestive tract, alleviation of calcium and phosphorus absorption and establishment of lightly acidic environment in the gut which inhibits the growth and expansion of pathogens (Irena *et al*., 2008). Water-soluble vitamins like riboflavin, folic acid and cobalamine are also found in whey in significant amount (Irena *et al*., 2008; Naik *et al*., 2009; Sakhale *et al*., 2012).

Rather being considered as a waste product, nowadays whey is formulated to prepare various valued products that are rich in nutritional and functional properties. This includes infant formulas (Irena *et al*., 2008), food supplements for exercise performance and enhancement (Marshall, 2004; Morris *et al*., 2008), soups and beverages (Marshall, 2004) designed to meet a variety of health goals for people with a wide age distribution. From a functional perspective, whey proteins are appropriate for beverage formulation as it has a fresh, neutral taste and good solubility (Flores-Andrade *et al*., 2013). Any flavor that is imparted from the whey protein lends itself well to citrus and fruit-flavored drinks. Nutritive benefits of whey can be utilized with fruit juice, pulp or concentrate in the development of a value added beverage. This would be the most logical and economical way of utilization of whey nutrients in human food chain (Goyal and Gandhi, 2009). Whey drinks are light, refreshing and less acidic than fruit juices as well as nutritious. Beverages based on fruits and milk products are currently receiving considerable attention due to their growing market potential (Beristain *et al*., 2006). Several attempts have been made to use whey for successful production of fruit-whey based beverages. Shekilango *et al*. (1997) and Dhamsaniya and Varshney (2013) produced whey-banana beverages from overripe bananas. Singh *et al*. (1994), Sikder *et al*. (2001), Ahmed *et al*. (2011) and Sakhale *et al*. (2012) formulated whey based mango beverages by blending different ratios of whey and mango pulp. Whey based pineapple beverages were prepared by Baljeet *et al*. (2013) and Shukla *et al*. (2013). Naik *et al*. (2009) developed whey based watermelon beverage, while Singh *et al*. (1999) and Singh *et al*. (2014) formulated a soft beverage utilizing paneer (cottage cheese) whey and guava extracts and analyzed the quality and shelf life. Abu-Ghoush *et al*. (2009) developed a beverage formula utilizing whey protein isolate, carboxymethyl cellulose and orange juice. They also proposed a model for the beverage viscosity using neuro-fuzzy inference approach. Oranges constitute a significant source of antioxidants (mainly vitamin C), polyphenol compounds (hydroxyl cinnamic acid and flavanones), phyto-chemicals (hesperidins and narigenin) and various vitamins and minerals. These components exhibit therapeutic properties such as anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities (Klimczak *et al*., 2007). In most of these works, unprocessed liquid whey or whey protein isolate is used for formulation of the beverage. However, in the present study, concentrated whey is used which helps in removal of typical off-flavour of whey thus yielding improved sensorial appreciation with nutritional enrichment. To the best of our knowledge, optimization of concentrated whey with orange juice has not been formulated till date. Thus orange-whey beverage is an interesting and innovative product in the developing market as functional foods which can be used as a tool by the food processors to produce a high quality beverage product.

2 Materials and methods

Double toned milk for the preparation of whey, oranges of Nagpur (India) variety, sugar and flavor are obtained from the local market. All the chemicals are purchased from HIMEDIA® and are of analytical grade.

For preparing the whey, milk is heated until the boiling point and then cooled to75°C, coagulated using 2% calcium lactate solution followed by continuous stirring which results in the complete coagulation of the milk protein (casein). After separation, the whey is filtered using muslin cloth. The prepared whey is concentrated using rotary vacuum pan evaporator (Eyela, Model No. 1000S-N) at a temperature of 50°C and at a pressure of 760 mm mercury up to about 50% of original volume before blending with the fruit juice. For preparing the orange juice, the oranges are peeled off and the seeds are removed. The juice is extracted in a juice extractor and filtered through the muslin cloth.

2.1 Preparation of formulated beverage

The blended beverages are prepared using concentrated whey, extracted juice and ground sugar at different ratios. In all the formulations, a stabilizer (0.1% w/v sodium alginate) is added at 60°C followed by an artificial orange flavor at the rate of about 1ml per liter beverage. Sodium alginate forms a gel network in acidic environment and has the capacity to withstand thermal stress during boiling or cooking of the product (Draget, 2000; Yavorska, 2012). Therefore the addition of this hydrocolloid in the formula helped to increase the stability of whey proteins against precipitation during heat treatment. The addition of food grade artificial orange flavor also increased the overall acceptability of the product (Abu-Ghoush *et al*., 2009). All ingredients are mixed with a shaker, filtered, bottled and finally corked. Before storing the beverages, bottles are pasteurized at a temperature of 65-70°C for 15 minutes and then cooled to room temperature. The storage stability of the optimized beverage sample is determined at room temperature (30±1ºC) and refrigeration temperature (7±1ºC) with and without addition of sodium benzoate (150 ppm) as preservative.

2.2 Analytical tests

The determination of pH is carried out using a digital pH meter (Model 5633, ECIL, India). Titratable acidity is determined according to the AOAC (2000). Total soluble solids are measured using a hand refractometer of 0-32°B (Model LE-354, ERMA, Japan) and the density is examined using a specific gravity bottle. Lactose, total sugars, vitamin C and β-carotene contents are determined by the method described by Ranganna (2004). Ash content, total fat and moisture are determined according to the AOAC (2000) methods. Protein estimation is done by the Kjeldahl method (BIS, 1961).

2.3 Microbial tests

Microbiological quality of the beverage samples are periodically analyzed during storage taking 10 ml representative samples which are aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions are prepared with the same diluents and in all cases duplicate-counting plates are prepared of appropriate dilutions. The total viable counts and yeast and mold counts in the samples are determined according to American Public Health Association (APHA) using nutrient agar and potato dextrose agar respectively.

2.4 Sensory evaluation

Formulated beverages of various combinations are chilled before evaluation. A trained panel evaluated the sensory characteristics such as color, appearance, taste, flavor, body, consistency and overall acceptability as described in detail by Poste *et al.* (1991).

2.5 Statistical analysis

The beverage is formulated with different levels of concentrated whey, orange juice and sugar. As seen in Table 1, in formulas F1, F2 and F3 the ratio of concentrated whey and orange juice are 3:2, 1:1 and 2:3 respectively along with 7% sugar, while in formulas F4, F5 and F6 the same ratios are considered along with 8% sugar, while for formulas F7, F8 and F9 9% sugar is considered. Segment diagrams and boxplots are used to visualize the sensory evaluation of the formulated beverages and a Principal Component Analysis (PCA) is conducted to understand the relationship between the different sensory characteristics of the beverages. The effect of the different beverage formulas is assessed through a mixed model where the panelists are considered as random and the formulas as fixed. Normal QQ-plots and residual plots are constructed to assess the model assumptions together with the Breusch-Pagan and the Shapiro-Wilk test (see e.g. Kutner *et al*., 2005; De Neve *et al*., 2014). Pairwise comparisons between formulas are conducted with the paired t-test (paired according to panelist) and the Holm method is considered to adjust for multiplicity. All tests are performed at 5% level of significance. Scatterplots are used to visualize the changes in physicochemical properties over time during storage. All analyses are conducted by using the R statistical software package (R, 2014).

3 Results and discussion

3.1 Formulation of beverage

The different formulations as indicated in Table 1 are analyzed for sensory properties by a panel comprising of 7 judges drawn from faculty members as well as industry professionals. The panelists are asked to record their observations on the sensory sheet based on a 9 point Hedonic scale (ranging from 1: dislike extremely to 9: like extremely). All formulas are prepared in triplicate and these triplicate scores are averaged for each panelist. It is worthwhile to note that the order of tasting these formulae is randomized to avoid any bias. The segment diagrams (Figure 1) indicate that the panelists, on average, prefer the formulas with 8% sugar (F4, F5 and F6). From these, F4 is preferred on average for flavor, taste, consistency and overall acceptability. The panelists also appreciate F5 and F6 for the sweetness and the color, but less for the consistency. The boxplots (Figure 2) confirm this and indicate a small variability among the scores for each formula. Figure 3 shows the biplot of a principal component analysis (PCA). The first two principle components explain 90% of the total variability. The biplot illustrates that the first principle component (PC 1) corresponds to an average of the 6 sensory characteristics where more weight is given to the sweetness, consistency and overall acceptability. Larger negative values indicate a more preferred formula suggesting that F4 is preferred over the others. The second principle component (PC 2) is mainly a contrast between color, sweetness and overall acceptability relative to consistency, taste and flavor. For F4, consistency, taste and flavor were more appreciated by the panelists, while for F5 and F6 the opposite holds. The mixed effects model indicated a significant difference between formulas in terms of the average overall acceptability ($p<0.001$). Formula F4 had a significant higher overall acceptability than formulas with 7% and 9% sugar ($p<0.001$) and F5 ($p=0.0088$), while there was no significant difference compared to F6 ($p = 0.11$). To summarize, it can be concluded that F4 - made with 3 parts of concentrated whey, 2 parts of orange juice, sugar comprising of 8% of the total volume of the formula followed by addition of 0.1% sodium alginate and 1 ml/L artificial orange flavor - is the most appreciated. Hence this formula is used to evaluate its nutritional profile and storage stability of the beverage with or without addition of preservative during storage at room and refrigeration temperature.

Table 1. Compositions of orange juice, concentrated whey and sugar for the different beverage formulations used in this study

Formulations	Concentrated whey (ml)	Orange Juice (ml)	Sugar (% w/v)	Stabilizer (% w/v)	Flavour (ml/L)
F_1	60	40	7	0.1	1
F_2	50	50	7	0.1	1
F_3	40	60	7	0.1	1
F_4	60	40	8	0.1	1
F_5	50	50	8	0.1	1
F_6	40	60	8	0.1	1
F_7	60	40	9	0.1	1
F_8	50	50	9	0.1	1
F_9	40	60	9	0.1	1

Table 2. Physicochemical analysis (mean ± standard deviation) of whey (plain and concentrated), orange juice and formulation F4.

Characteristics	Plain whey	Concentrated whey	Orange juice	Formulation F4
Moisture (%)	93.75 ± 0.47	56.17 ± 0.33	85.31 ± 0.36	85.5 ± 0.79
Fat (%)	0.19 ± 0.03	0.75 ± 0.03	-	0.73 ± 0.4
Protein (%)	0.46 ± 0.05	1.1 ± 0.02	-	1.05 ± 0.22
Lactose (%)	4.57 ± 0.35	5.63 ± 0.04	-	1.17 ± 0.08
Total Sugar (%)	-	-	6.46 ± 0.07	4.52 ± 0.35
Ash (%)	0.53 ± 0.025	0.64 ± 0.015	0.38 ± 0.035	0.67 ± 0.06
Titratable Acidity (%)	0.193 ± 0.041 (in terms of lactic acid)	0.34 ± 0.017 (in terms of lactic acid)	0.69 ± 0.023 (in terms of citric acid)	0.52 ± 0.02
pH (-)	6.27 ± 0.12	6.47 ± 0.04	3.92 ± 0.04	4.78 ±0.17
Total soluble solids (%)	-	-	9.13 ± 0.21	14.43 ± 0.25
Specific gravity	-	-	1.28 ± 0.06	1.022 ± 0.009
β-Carotene (mg/100ml)	-	-	1.017 ± 0.035	0.517± 0.01
Vitamin C (mg/100ml)	-	-	38.37 ± 1.32	20.19 ± 2.16

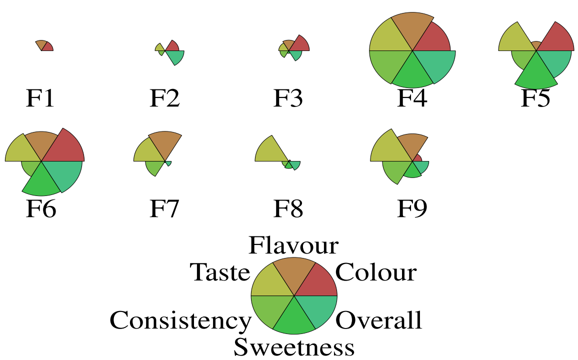

Fig. 1. Segment diagrams of the six sensory characteristics for the nine beverage formulations (labeled as F1 to F9) as mentioned in Table 1.

3.2 *Physicochemical properties of whey and orange juice*

The physicochemical characteristics of whey and orange juice used in the manufacture of the beverage are represented in Table 2 where all results are obtained in triplicate. From the table it is clear that almost all the lactose present in milk is recovered in whey. The orange juice as expected is rich in the vitamin C content. The results were found to be approximately similar to the experiments of Sikder *et al*. (2001), Del Caro *et al*. (2004), Kumar and Manimegalai (2005) and Klimczak *et al*. (2007). The data on chemical composition of the final beverage are given in the last column of Table 2. The average titratable acidity was 0.52% in terms of citric acid, which is almost similar to the results obtained for probiotic whey-pineapple beverage by Shukla *et al*. (2013). The pH of orange whey beverage ranges from 3.95-4.28, which is slightly higher than the value obtained for whey enriched guava beverage by Divya and Kumari (2009) and for whey and bael fruit beverage by Singh and Nath (2004). The lower pH value of 4.78 of the final beverage certainly increases the storage stability of the final product by impeding undesirable microorganism growth including coliform bacteria (Abu-Ghoush *et al*., 2009). The total soluble solids and specific gravity of the formulated beverage are in the range 14.2-14.8% and 1.016-1.032 respectively. The beverage has lower carotene content than the juice, which is 0.5167 μg/100 ml. The average vitamin C content is 20.19mg/100 ml.

3.3 *Storage stability of formulated beverage*

Beverage F4 is stored both at room temperature (30±2°C) and refrigeration temperature (7±1°C) to determine its storage stability with and without addition of preservative. When the beverage is stored at room temperature, it is acceptable up to 5 days without addition of preservative and up to 11 days after addition of preservatives. When stored at refrigeration temperature it is acceptable up to 49 and 91 days without and with the addition of preservative, respectively.

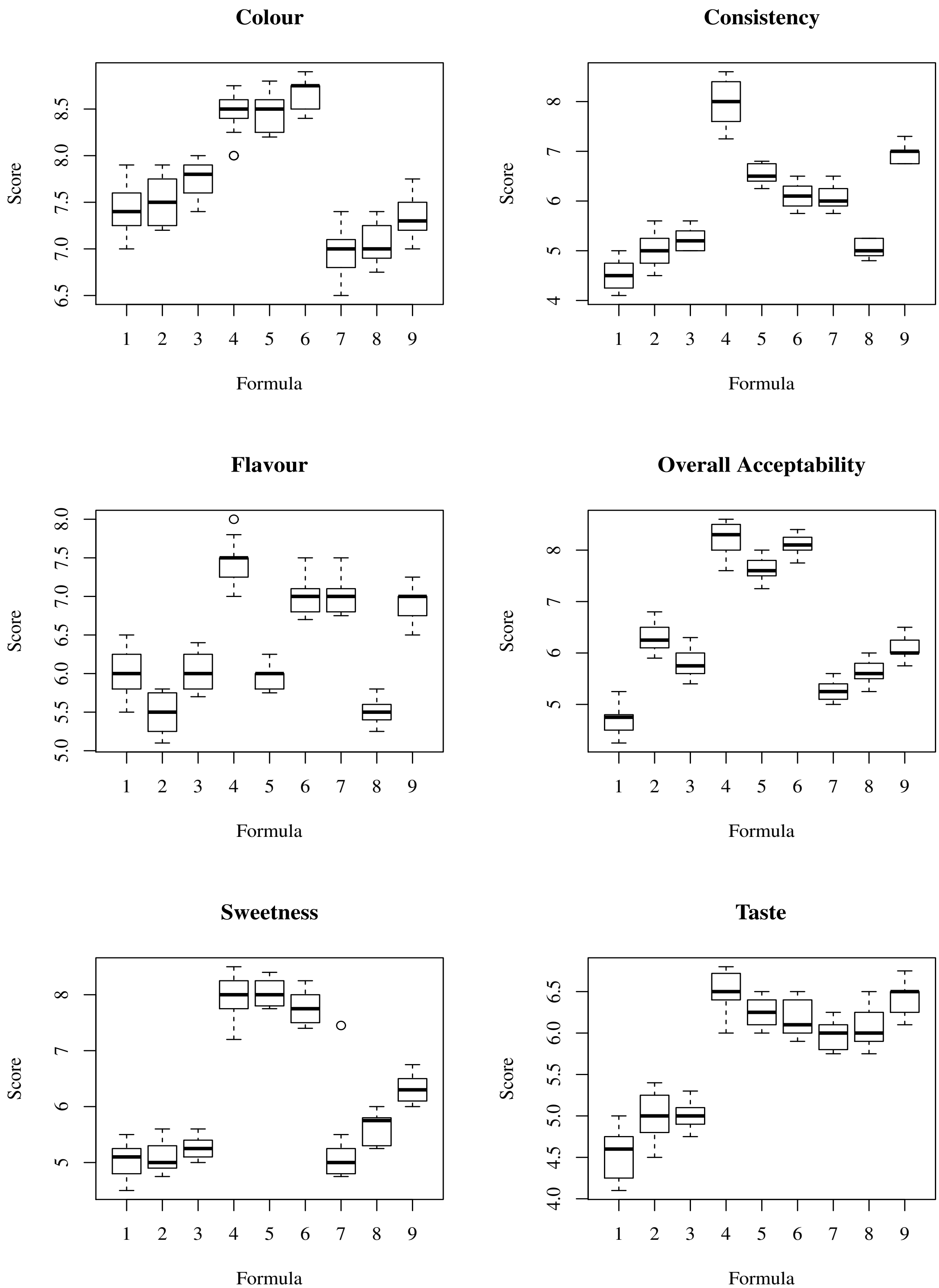

Fig. 2. Boxplots of the six sensory characteristics for the nine beverage formulations (numbered as 1 to 9) as mentioned in Table 1.

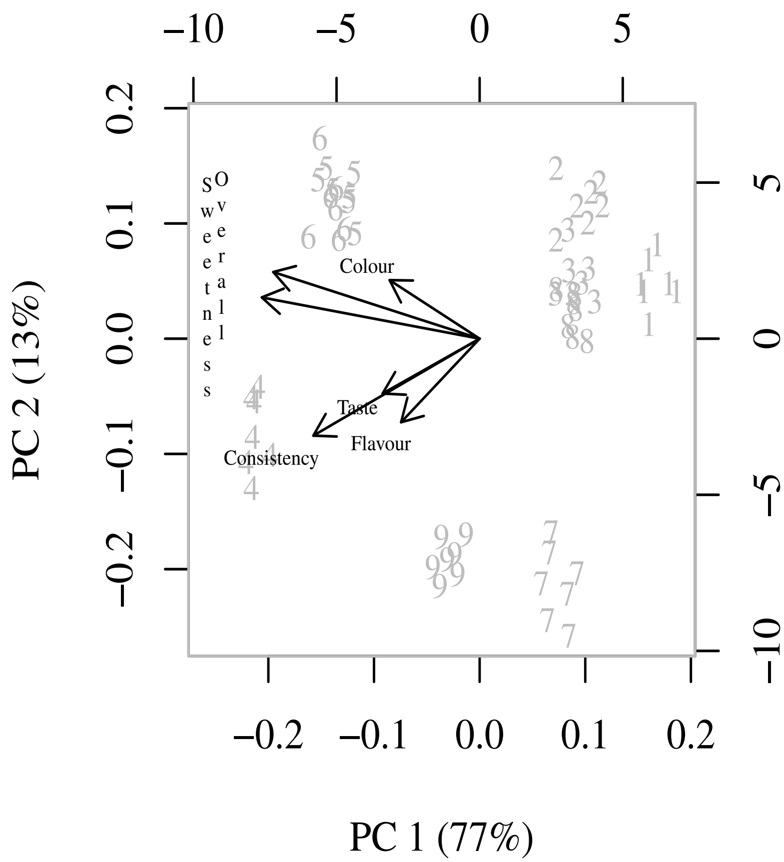

Fig. 3. Biplot of a principle component analysis of the six sensory characteristics for the nine beverage formulations (numbered as 1 to 9) as mentioned in Table 1.

During storage the changes in pH, titratable acidity and total soluble solids (TSS) as well as the total bacterial count together with the yeast and mold count are measured and analyzed.

3.3.1 Changes during storage at room temperature

Figure 4a shows that the pH of the whey based beverage stored without preservative declines from an initial value of 4.76 to 4.23 after 5 days of storage, whereas the beverage stored with preservative is acceptable up to 11 days with the pH declining from 4.8 to 4.16 on the final day. The titratable acidity of the beverage under non refrigerated storage condition increases from an initial value of 0.107% to 0.128% in terms of citric acid after 5 days when stored without preservative; while similar results are observed for the beverage having preservative in which the acidity increased from 0.112% to 0.152% in terms of citric acid after 11 days of storage (Figure 4b). An increase in acidity is rapid in the samples stored without preservative. The changes in the total soluble solids (%TSS) of the beverages kept at room temperature are shown in Figure 4c. The change in TSS is not so prominent during the initial days of storage. However as the storage days increase, TSS increases progressively. The total bacterial count is 1.73×10^4 and $1.92\ \times10^4$cfu/ml for the beverage sample with and without preservative respectively as seen in Table 3. With storage time, the microbial count increased gradually until it reached to 3.88 $\times10^4$ cfu/ml in samples without preservative after 5 days of storage at room temperature. In the samples containing preservative, the mold count after the end of the storage period is 4.18×10^4 cfu/ml. At the first day of storage, the initial yeast and mold count are14 and 12 respectively in the beverage sample without and with preservative. At the final day of storage, this increased to 86 and 107 respectively.

Table 3. Changes in microbiological quality during storage at room temperature (30±2ºC)

Days of storage	Total Bacterial Count (cfu/ml)		Yeast and Mold count (cfu/ml)	
	Without preservative	With preservative	Without preservative	With preservative
0	1.92×10^4	1.73×10^4	14	12
1	2.08×10^4	1.90×10^4	38	26
3	3.16×10^4	2.42×10^4	64	41
5	3.88×10^4	2.81×10^4	86	64
7	-	3.4×10^4	-	76
9	-	3.98×10^4	-	85
11	-	4.18×10^4	-	107

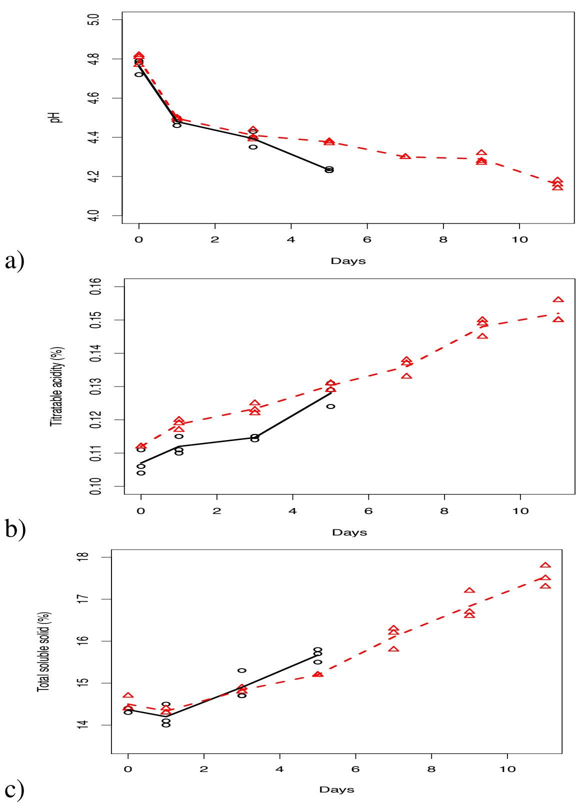

Fig. 4. Changes over time in pH (upper panel), titratable acidity (% in terms of citric acid, middle panel) and total soluble solid content (%, lower panel) of the beverage stored at room temperature (30±2°C) without preservatives (solid black line and bullets) and with preservatives (dashed red line and triangles).

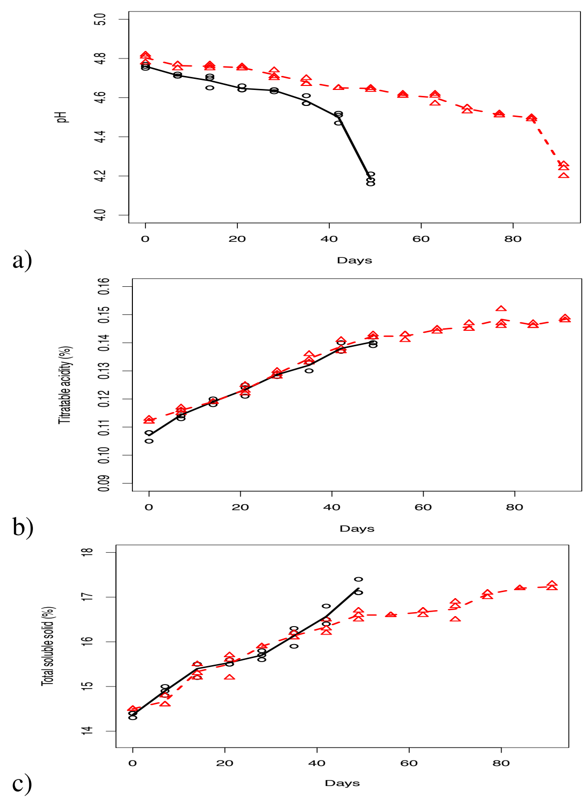

Fig. 5. Changes over time in pH (upper panel), titratable acidity (% in terms of citric acid, middle panel) and total soluble solid content (%, lower panel) of the beverage stored at refrigeration temperature (7±1°C) without preservatives (solid black line and bullets) and with preservatives (dashed red line and triangles).

3.3.2 Changes during storage at refrigeration temperature

Whey based orange beverage has a shelf-life of about 49 days when storing without preservative, while the beverage containing preservative remains fit for consumption up to 3 months. The initial pH of 4.8 decreases to 4.23 after 91 days of storage when stored with addition of preservatives, while the pH decreases from 4.76 to 4.18 without addition of preservative.

Table 4. Changes in microbiological quality during storage at refrigeration temperature (7±1ºC)

Days of storage	Total Bacterial Count (cfu/ml)		Yeast and Mold Count (cfu/ml)	
	without preservative ($\times 10^4$)	with preservative ($\times 10^4$)	without preservative	with preservative
0	1.92	1.73	14	12
7	1.96	1.78	16	12
14	2.04	1.85	23	18
21	2.4	1.96	41	32
28	2.67	2.04	66	43
35	3.08	2.3	73	56
42	3.52	2.56	95	70
49	4.1	2.91	112	86
56	-	3.16	-	98
63	-	3.45	-	106
70	-	3.72	-	118
77	-	3.9	-	128
84	-	4.13	-	141
91	-	4.46	-	147

The changes in pH of the beverage stored under refrigeration condition are shown in Figure 5a. A decline in pH during storage is observed which may be due to the action of citric and ascorbic acid on the sugar and protein component of the product. Production of organic acids and amino acids lead to an increase in acidity thereby a decrease in pH, as also reported for mango based beverages (Kalra *et al*., 1991; Sikder *et al*., 2001). The changes in titratable acidity are shown in Figure 5b. The whey based orange beverage has an initial titratable acidity of 0.112% in terms of citric acid and increases to 0.148% citric acid after 3 months of storage when stored after addition of preservative. As seen in Figure 5c, the changes in %TSS are almost similar in both the beverage samples. The gradual increase in TSS with storage might be due to the conversion of insoluble polysaccharides into reducing sugars. Increase in reducing sugars can be attributed to hydrolysis of sugars by acid, which might have resulted in degradation of disaccharides to monosaccharides. Another possibility for the increment in TSS content could be due to hydrolysis of sucrose to invert sugars, as previously reported for increased TSS value with storage time in the case of bitter gourd RTS (Barwal *et al*., 2005). The increase in TSS during storage is also reported in ready to serve beverage developed from guava nectar by Murari and Verma (1989). The value increases from 14.4% to 17.2% after 49 days of storage without addition of any preservative. While adding preservatives, TSS content increases from the initial 14.5% to 17.2% on day 91 of storage. The changes in microbiological quality of the beverage during storage at refrigeration temperature are indicated in Table 4. A rapid increase in total bacterial count as well as yeast and mold count is observed in samples without preservative. Note that the beverage sample got spoiled after 49 days, so no data are taken then after. The microbiological quality of the developed whey based orange beverage is similar with the results reported by Ahmed *et al*. (2011) and Sakhale *et al*. (2012) using whey based mango beverages. From the results obtained, it can be summarized that an acceptable ready to drink whey beverage could be prepared by optimizing proportion of ingredients. The product can be best stored for about three months after addition of permitted preservative under refrigerated condition.

Conclusions

Nowadays dairy industries are looking for new product ideas and technologies to meet the ever-increasing consumer requirement for healthy foods and to increase the profitability. Especially for poorly developed and developing countries, product development using whey as a water replacement with added nutrition can be used as an excellent source of good quality protein. In this study, whey is successfully utilized to develop an orange-based fruit beverage with optimum sensory and nutritional properties as well as good storage stability. The beverage possesses high color, flavor and stability properties. A nutritious beverage with better storage life is developed with the addition of whey, orange juice and sugar in appropriate proportion. When stored in the fridge and after adding prescribed limit of preservatives, the beverage can be stored for about three months. In view of the functional properties arising from bioactive constituents present in fruit and whey, it is proposed that orange based whey beverages with excellent nutritional, sensory and storage properties could be an interesting product in the constantly growing market for functional foods. This could potentially increase the commercial and economical value of whey as a functional food which is still considered as a by-product.

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