Discover Food

Discover

Research

Impact of different processing techniques (chemical, heating and sonication) on physicochemical and microbial characterization of kinnow-whey based beverage

Farzana Siddique[1] · Ashiq Hussain[1,2] · Saima Noreen[1] · Muhammad Rehan Arif[3] · Shazia Yaqub[1] · Syeda Ayesha Batool[2] · Aqsa Iqbal[1] · Haya Fatima[3] · Faiza Iftikhar Gorsi[2] · Ammad Iqbal[1] · Sameh A. Korma[4] · Amer Ali Mahdi[5]

Received: 20 July 2023 / Accepted: 18 October 2023
Published online: 23 October 2023

Abstract

Whey beverages that have been added with fruits pulp have always been remained the priority demand of food consumers, but loss of nutritional contents and microbial safety of such beverages is always matter of concern of food producers. To overcome these issues food experts are always in try to find out the processing and preservation techniques for safety of these beverages. In present study kinnow-whey beverage was prepared by addition of kinnow concentrate and acidic whey, at optimum concentrations. Effect of different processing techniques on the physicochemical properties, microbial inactivation, and sensory characteristics of beverage were investigated during 90 days of storage. The outcomes of pasteurization were compared with non-thermal techniques, such as use of preservatives and sonication (20 kHz frequency, 70% amplitude and 5, 10 and 15 min). pH values of all the treatments were reduced, whereas titratable acidity was increased significantly, during storage. There was a significant increase in the brix, viscosity and total solids, with the increase in the storage period. Highest values obtained for brix, viscosity and total solids were T_3 (14.32°), T_3 (15.13 cP), and T_3 (15.13 mg/L) after 90 days storage, respectively. Total plate count (TPC) and yeast and mold count were increased during prolong storage in all treatments. Results showed that the maximum TPC was noted in T_0 (2.9×10^4 cfu/ml) at 90 days, while minimum TPC was observed in T_5 (0.8×10^4 cfu/ml) at start of study. Similarly, maximum yeast and mold count were noted in T_1 (3.2×10^4 cfu/ml) at 90 days whereas, minimum yeast and mold count was observed in T_5 (0.1×10^4 cfu/ml) at start of study. Overall acceptability of beverage was highest in 15 min sonicated treatments. It was concluded that the beverages processed with sonication (15 min) demonstrated better stability and sensory attributes compared to other techniques.

Highlights

- Kinnow-whey beverage processed with different techniques
- Sonication, an effective technology for beverages processing

✉ Ashiq Hussain, ashiqft@gmail.com; ✉ Amer Ali Mahdi, amer.alimahdi@yahoo.com; Farzana Siddique, farzana.siddique@uos.edu.pk; Saima Noreen, binteislam31@gmail.com; Muhammad Rehan Arif, rehanarif5272@gmail.com; Shazia Yaqub, shaziaft743@gmail.com; Syeda Ayesha Batool, ayeshasyed39@gmail.com; Aqsa Iqbal, aqsa0057@gmail.com; Haya Fatima, hfkhan512@gmail.com; Faiza Iftikhar Gorsi, faiza.gorsi@yahoo.com; Ammad Iqbal, ammadiqbal@gmail.com; Sameh A. Korma, sameh.hosny@zu.edu.eg | [1]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan. [2]Punjab Food Authority, Lahore, Punjab, Pakistan. [3]PMAS Arid Agriculture University, Rawalpindi, Punjab, Pakistan. [4]Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt. [5]Department of Food Science and Nutrition, Faculty of Agriculture, Food and Environment, Sana'a University, Sana'a, Yemen.

- Application of sonication for 15 min at 20 kHz, provided promising result
- During 90 days of storage, sonicated beverage exhibited lowest microbial growth
- Higher acceptability of sonicated beverage, compared to other processing techniques.

Keywords Whey beverage · Kinnow · Pasteurization · Sonication · Preservatives: Microbial

1 Introduction

Phytochemistry of fruits and vegetables provide us the base of blending their parts and derived value-added products, loaded with antioxidants, responsible to counter the attack of pathogens [1]. Fruits pulp, concentrate and juices, are the base for development of food formulations, which boost the nutritional value of developed products [2].

The net global whey production of cheese whey (liquid) in 2008 was reported to be 187 million metric tons. Around 3.2 million metric tons, which was about 1.7% of total production was used by different food processing industries for manufacturing of enriched products, such as whey powder (85 million tons), whey protein concentrates, and whey isolates etc. There was an increase of 6.3% in production of whey protein in 2009. The sale of energy drinks ($1.03 million) is rising day by day. Roughly 4100 food and beverage products enriched with whey protein were launched in U.S. market in last 6 years [3].

Kinnow is among the most popular fruits for production of beverages among consumers. The kinnow is extensively cultivated in Punjab Province of India and Pakistan. It is mandarin hybrid famous for its high yielding properties. It is hybrid of two cultivars of citrus termed as 'King' (*Citrus nobilis*) & 'Willow Leaf' (*Citrus deliciosa*). Kinnow was for the first time developed by Howard B. Frost in the University of California Citrus Experiment Station. The kinnow was assessed and released as emerging variety of citrus hybrid for cultivation at commercial level in 1935. Pakistan is producing about 2.5 million tons of Kinnow each year and exporting it to round about 50 countries which include Russia, Indonesia, Ukraine, Sri Lanka, Gulf States, Philippines, United Arab Emirates, Saudi Arabia, Iran, Tajikistan and Uzbekistan [4]. Whether fresh or processed, orange constitutes a considerable source of antioxidants in the diet, vitamin C and polyphenolic compounds are considered to be among the prominent [5]. Ascorbic acid, folate, and other vitamins, as well as secondary metabolites, including limonoids, flavones, phenolics, flavonoids and carotenoids are all abundant in kinnow juice. The way that kinnow juice is processed and stored affects how many phytochemicals are present in it [6].

In contrast to traditional heat treatment, non-thermal food processing technology known as sonication has attracted a lot of interest for its ability to preserve food's nutritional value and original freshness while using less energy and having a higher level of sensory appeal [7]. One of the non-thermal food processing techniques that can be employed in place of thermal food processing is ultrasound. The energy generated by sound waves of different frequencies that is too high for the human ear to detect, or above 16 Kz, is referred to as ultrasound. It is a cutting-edge technology that shortens processing times and lowers costs while increasing and guaranteeing product quality. It has many benefits when paired with microwave technology. Food quality has been effectively impacted by factors like texture, colour, antioxidants, and polyphenolic characteristics [8]. Sonication treatment appeared to be effective for increasing many quality characteristics as well as microbial decontamination [9, 10]. As a green processing technology, ultrasound has great potential in food applications [11].

The taste of the whey is pretty much unappealing, highly acidic and lactose to glucose ratio is fairly high especially in acid whey therefore number of procedures and techniques have been developed to improve its characteristics to enhance its acceptability for utilization in human nutrition [12]. Orange and citrus flavored drinks have been the most frequently proposed products in the market for whey blended products [13]. However, a variety of other fruits were also used in the process, such as tropical fruits such as mango, grapefruit, banana, and papaya [14]. Other fruits such as apple, cherry, pear, & melon are also used for the manufacturing of useful products from whey [15].

To successfully manufacture whey-fruit based beverages there have been numerous attempts. Dhamsaniya and Varshney [16] used over ripened bananas to produced whey-based banana beverages. Ahmed et al. [17] and Sakhale et al. [18] used different ratios of whey and mango pulp and blended them to formulate whey-based mango beverages. Baljeet et al. [19] and Shukla et al. [20] prepared whey-based pineapple beverages. Naik et al. [21] manufactured whey-based watermelon beverage, whereas Singh et al. [22] and Singh et al. [23] developed utilized paneer (cottage cheese) with whey and extracts of guava to produce soft beverage, authors also analyzed the shelf life and quality of the finished product. Abu-Ghoush et al. [24] created a beverage recipe using orange juice, carboxymethyl cellulose, and whey

protein isolate. However, these studies were inadequate to present the chemical and microbial safety patterns of these formulations, which identified the gaps to be covered by this study in a comprehensive approach. Further, in current study, different preliminary trials were also performed to find out the best formulation of kinnow concentrate and whey.

Investigations on different whey-based beverages, which have been reported previously were limited to common processing and preservation techniques, which were the possible drawbacks for microbial food safety of stored beverages. Further, physicochemical and sensory aspects of the beverages were not discussed in detail. Keeping in view this research gaps, the present study was designed to implement and compare advanced green processing technology, sonication at different time periods keeping frequency same, to assess the changes in quality, nutrition and microbial safety of whey-kinnow beverage during 90 days of storage. While a comparison of sonication with chemical-based preservatives added and pasteurized beverages was also made to find out the most effective technique for processing and preservation.

2 Materials and methods

2.1 Procurement of raw material

Whey was procured in liquid form from a local dairy industry located in Sargodha, Pakistan. Kinnow was collected in the form of kinnow concentrate from Fauji Foods Limited, Bhalwal. All other materials were purchased from the local market of Sargodha.

2.2 Preparation of raw materials

Freshly drained whey, which from the cheese manufacturing process of industry was collected, was further pasteurized to remove remaining casein. After the process the whey was stored in refrigerated temperature. Kinnow concentrate, which was acquired for beverage formulation, was already processed.

2.3 Preparation of formulated beverage

Blended beverages were prepared using pasteurized liquid whey (having pH 6.36), kinnow concentrate (having pH 3.65 and Brix 65), and sugar in different combinations, to attain 16 Brix in the final product. Guar gum was added in all formulations as stabilizer (0.15 g/100 ml of beverage), as it forms thick structure when placed in water for few minutes. Guar gum helps to increase the stability of kinnow whey beverage during thermal treatment. Artificial food grade orange flavor was added at the ratio of 1 ml/litter beverage, followed by addition of artificial orange color. For this purpose, 1 g of color was added in 10 ml of solvent, to prepare color and then 1 ml color solution was added in each 200 ml sample. The artificial flavor and color greatly enhance the overall acceptability of product [24]. All the ingredients are mixed using blender/juicer, then poured into bottles and finally corked and stored. All the bottles were thoroughly sanitized before filling. Five different compositions of kinnow whey beverage were prepared (95:5, 96:4, 97:3, 98:2 and 99:1) as given in Table 1. Sensory analysis was performed for all the compositions to select best composition for further physicochemical and microbial testing.

Table 1 Formulation for preliminary trial of Kinnow whey beverage

Trial samples	Whey (ml)	Kinnow conc. (ml)	Sugar (g)	Guargum (g/100 ml)	Brix
1	99	1	10	0.15	16
2	98	2	10	0.15	16
3	97	3	10	0.15	16
4	96	4	10	0.15	16
5	95	5	10	0.15	16

2.4 Treatment plan for study

After conducting the preliminary trials and selecting the best treatment for further study, six treatments of kinnow whey beverage were prepared in triplicates, each having 95% whey and 5% kinnow concentrate. Different processing techniques were applied on each treatment as shown in Table 2. Chemical preservation was applied on sample T_0, having a combination of preservatives KMS 75% and sodium benzoate 25% using electronic weighing balance (ELECTRONIC SCALE JJ224BC), whereas, sample T_1 was treated with thermal processing technique, as sample was pasteurized at 85 °C for 15 min for preservation (using water bath for heating and laboratory thermometer was used to monitor the temperature), and sample T_2 was treated with both processes, preservatives and pasteurization (the sample was first pasteurized and then preservatives were added in similar ratio as in sample T_0). Non-thermal sonication technique was applied using sonicator (UP400S by Hielscher ultrasound technology, Germany) on remaining three samples at ½ cycle and amplitude 70% for different time periods with 20 kHz frequency. The probe size of the sonicator used was 25 mm. Sample T_3 was given sonication at given amplitude and cycle for 5 min, which caused its temperature to increase by 1 °C (initial temp 18 °C and final temp 19 °C). T_4 was sonicated at given amplitude and cycle for 10 min, which caused its temperature to increase by 2 °C (initial temp 17.5 °C and final temp 19.5 °C). Similarly, T_5 was processed at given amplitude and cycle for 15 min, which caused its temperature to increase by 2.5 °C (initial temp 21 °C and final temp 23.5 °C).

2.5 Physicochemical analysis

2.5.1 pH analyses of formulated beverage

The pH value was determined by using digital pH meter (Model PH-209B by Lutron) as described by AOAC method no. 981.12 [25]. The electrode of pH meter was directly dipped into the sample to be analyzed and the value on the meter, which illustrates the pH of the sample, was noted after performing analysis three times.

2.5.2 Titratable acidity of formulated beverage

Titratable acidity of beverages was determined using the titration method as illustrated in the AOAC [25] and was expressed as g of citric acid/100 ml of the sample. Briefely explaining, to prepare 0.1N NaOH solution, 2 g of NaOH was dissolved in 500 ml of distilled water. Similarly, phenolphthalein indicator was prepared by dissolving 1 g of phenolphthalein in 100 ml ethanol. Then 10 ml of kinnow-whey beverage was taken as sample from each treatment and 2–3 drops of indicator was added into it. Sample was titrated against 0.1N NaOH solution until pink color was obtained. Acidity factor used for acidity determination was 0.064, and below given formula was used to determine titratable acidity.

$$\text{Titratable acidity} = \text{amount of NaOH used} \times 0.064.$$

2.5.3 Brix of formulated beverage

Total soluble solids were measured using a hand refractometer (RHB-32ATC) of 0–32°B. Brix was obtained by adding few drops of sample solution on the prism of refractometer and closing the lid, under method 932.12 of AOAC [25]. To see

Table 2 Treatment plan for research

Treatments	Whey: Kinnow conc. (ml)	Sugar (g)	Guargum (g/100 ml)	Variables
T_0	95:5	10	0.15	Preservatives
T_1	95:5	10	0.15	Pasteurization
T_2	95:5	10	0.15	Preservatives and Pasteurization
T_3	95:5	10	0.15	Sonication (5 min)
T_4	95:5	10	0.15	Sonication (10 min)
T_5	95:5	10	0.15	Sonication (15 min)

the internal scale, refractometer was held perpendicularly to a source of light. The Brix reading were clearly seen, where the light area meets the dark areas on the scale.

2.5.4 Viscosity of formulated beverage

Prior to the measurement of viscosity, the kinnow-whey beverage samples were conditioned at 4 °C. A rotator type viscometer (LAMY RHEOLOGY instruments) was used to determine the viscosity of beverage samples as earlier reported by Aggarwal et al. [26], with some modifications. Viscosity was determined in triplicate to find out the mean value, by using spindle number 5 and the speed was set at 50 rpm. The results of the viscosity were expressed in centipoises (cP).

2.5.5 Total solids of formulated beverage

Total solids of kinnow-whey beverage were determined according to the method reported in AOAC [25], using conventional oven method, as was earlier determined by Tanwar et al. [27], with required modifications. Briefly describing, 10 g sample of beverage was placed in an air oven at 105 °C in sample dish. The weight of the samples was monitored every 4 h until the consecutive weight difference was less than 1 mg/g solids. Total solids were determined in triplicate, and were calculated using the following formula:

$$\text{Total solids} = \text{W2/W1} \times 100$$

where: W_1 = initial weight of the sample; W_2 = final weight of the sample.

2.6 Microbial analysis of formulated beverage

Microbial quality of kinnow-whey beverage was monitored periodically after every month, during the whole storage study. For this purpose, 10 ml sample was taken from each treatment, which was aseptically mixed with 90 ml of distilled water and shaken properly to homogenize the sample. Subsequent dilutions were prepared for each treatment and samples were incubated for 24–48 h at 37 °C. Total plate count in the samples was determined according to the guidelines of American Public Health Association [28], using nutrient agar, while yeast and mold count was calculated using potato dextrose agar according to APHA [28]. TPC results were calculated from the petri dish as colonies cfu/g. All the trials were performed in triplicate to find out the mean values.

2.7 Sensory evaluation of formulated beverage

Sensory parameters of kinnow-whey beverage, such as flavor, color, aroma and overall acceptability were evaluated using ordered nine-point hedonic scale. The 9-point hedonic scale indicates a scale of grading the sample on the basis of its sensory characteristics from liked extremely (9), liked very much (8), liked moderately (7), liked slightly (6), neither liked nor disliked (5), disliked slightly (4), disliked moderately (3), disliked very much (2), to disliked extremely (1). Sensory assessment was planned for 80 participants according to the guidelines given by Lawless and Heymann [29]. From both genders 80 people participated in the process of evaluation. Complete process was performed in separate booths; participants were presented with 150 ml of chilled beverage in transparent cups and distilled water for rinsing purpose.

2.8 Storage study of formulated beverage

Kinnow-whey beverage was stored at refrigerated temperature and subjected to above mentioned quality tests (physicochemical, sensory and microbial) during the storage period of 90 days.

2.9 Statistical analysis of the data obtained after experiments

Data for all measurement are presented as means of triplicate ± standard deviation. Significance of treatments were determined using ANOVA and means were ranked using the HSD Tukey test ($p \leq 0.05$). Statistical analysis was performed on Statistix 8.1 (Analytical Software for Windows Tallahassee, USA) by following the procedures described by Steel and Torrie [30].

3 Results and discussion

3.1 pH

Mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum pH was noted in T_0 (6.31) at 0 day, while the minimum pH was observed in T_2 (3.01) at 60th day of study, as presented in Table 3. From the results presented, it was evident that sonication for longer time periods caused less decrease in pH during storage, which was speedier in other blends during 90 days. This less decrease in pH as a result of sonication was attributed to less disturbance in organic acids and other molecules of beverage, which was high in pasteurized and chemically treated samples. One significant factor that describes the stability of bioactive chemicals in fruit juice is pH [31]. The pH values of all the treatments were reduced significantly during storage. This may be due to the fact of increasing titratable acidity, which increases due to production of secondary acidic metabolites such as organic acids during different reactions. The acidity and pH are inversely related. Majumdar et al. [32] showed similar outcomes for a juice combination of bottle guard and basil leaves, and Jan and Masih [33] reported similar outcomes for a pineapple juice blend with carrot and orange. Acidification reduces the activity of undesirable microorganisms and their capacity for proliferation, and this causes reduction of pH which benefits beverage preservation [34]. Similar findings were reported by Herrera-Ponce et al. [35], in which ultrasound treatment significantly reduced pH during storage of whey-oat beverage. Supportive results were also found in the experiments of Purewal et al. [36], when pH during 90 days of storage was decreased in kinnow based beverage.

Ultrasounds (15 min, 40 kHz and 240 W) have been proved useful in inactivation of various kind of microbes without any significant changes in the juice quality, by increasing the pH of sugarcane juice [37]. Adulvitayakorn et al. [38] compared thermal processing with microwave and thermosonication for sugarcane juice processing and reported slight decrease in pH of sugarcane juice processed at 700W through microwave technology and thermosonicated at 80 °C. Further they linked this variation in pH with variation in temperature of the juice samples under treatment. Previous studies by Khandpur and Gogate [39] unequivocally demonstrated that pH is crucial to the quality and safety of juices. Low pH or acidic conditions increase the anti-microbial effectiveness of sonication, which may be related to increased hydroxyl radical generation. Juices with lower pH values are therefore more sensitive to ultrasonic therapy.

3.2 Titratable acidity

Results presenting interaction between five different treatments and storage days revealed that sonication significantly lowered down the titratable acidity of beverages, when compared with preservatives and pasteurized, but change in sonication time had not affected acidity significantly. The maximum titratable acidity was noted in

Table 3 Effect of various treatments and storage period on pH of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	4.77 ± 0.01^{i}	4.02 ± 0.01^{m}	3.53 ± 0.008^{s}	3.13 ± 0.008^{u}	3.86 ± 0.64^{E}
T_1	4.82 ± 0.008^{h}	4.17 ± 0.008^{l}	3.76 ± 0.008^{p}	3.22 ± 0.008^{t}	3.99 ± 0.61^{C}
T_2	4.92 ± 0.008^{g}	3.92 ± 0.004^{o}	3.71 ± 0.004^{q}	3.07 ± 0.008^{v}	3.90 ± 0.69^{D}
T_3	5.91 ± 0.004^{c}	5.31 ± 0.004^{f}	4.22 ± 0.008^{k}	3.62 ± 0.008^{r}	4.84 ± 1.05^{B}
T_4	5.96 ± 0.004^{b}	5.62 ± 0.008^{e}	4.82 ± 0.008^{h}	3.92 ± 0.008^{o}	5.16 ± 0.92^{A}
T_5	5.98 ± 0.004^{a}	5.76 ± 0.004^{d}	4.62 ± 0.008^{j}	3.97 ± 0.004^{n}	5.17 ± 0.96^{A}
Mean ± SD	5.55 ± 0.74^{A}	4.80 ± 0.80^{B}	4.11 ± 0.49^{C}	3.48 ± 0.37^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

T_0 (0.98) at 90th day, while the minimum acidity was observed in T_5 (0.12) at 0 day of study, as can be seen from the Table 4. Beverage made with whey was shown to be more acidic the longer it was stored. This may be explained by the presence of acidic polyphenol such mangiferin, gallic acid, benzoic acid, and other phenols and flavonoids in fruit. This could be the result of bacteria from lactose fermenting organisms spoiling the food [27]. Santhirasegaram et al. [40] discovered comparable results, when applying thermal treatments of 30 and 60 s at 90 ℃ and 80 ℃ and ultrasound treatments of 15, 30, and 60 min at a frequency of 40 kHz to mango juice.

Adulvitayakorn et al. [38] compared conventionally heat-treated sugarcane juice with microwave and thermosonicated juice samples and calculated their titratable acidity. Thermosonication resulted non-significant effect of sugarcane juice samples while microwave heating exhibited significant increase in titratable acidity. The effects of the treatments on the juice's look and quality might be better understood if you are aware of how the pH and titratable acidity alter. The acidic hydrolysis of polysaccharides utilizes the acid for the conversion of non-reducing sugars into reducing sugars, due to which there is a significant decrease in titratable acidity [31]. Orange juice that had been pasteurized and kept for up to 14 weeks by Kaanane et al. [41], in which ascorbic acid breakdown appeared to be the primary cause of non-enzymatic browning. Furfural formation during storage was much elevated at higher temperature and long-time storage.

3.3 Brix

Total soluble solids (Brix) of pasteurized and chemically preserved beverages were significantly increased during storage, whereas as of those sonicated, it was significantly decreased during storage period. The mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum brix was noted in T_3 (14.32) at 90th day while the minimum brix was observed in T_5 (10.02) at 90th day of study, as presented in Table 5. Total soluble solids were expressed as brix. The total soluble solids of all the treatments were increased with storage time except the ultrasound treated beverage in which the brix was decreased during storage. The increase in the brix was due to the breakdown of monosaccharide and other sugars during prolonged storage of the beverage. The results were in agreement with the findings of Barwal et al. [42] and Yadav et al. [43], as was observed increase in brix of fruits drink during storage.

Similar findings were also present in the experiments of Purewal et al. [36], as during 90 days of storage a significant rise in TSS of kinnow-amla beverage was observed. Brix can affect the viscosity characteristics, consistency and integrity of the beverage. Mukhtar et al. [37] used ultrasonic waves at 15 min, 40 kHz and 240 W for sugarcane juice processing and observed slight increase in TSS, total sugars and reducing sugars. Further they explained this increase linked with greater extraction ability of ultrasounds due to which conversion of disaccharides to monosaccharides may have taken place.

Table 4 Effect of various treatments and storage period on titratable acidity of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	0.41 ± 0.004^{j}	0.72 ± 0.01^{f}	0.92 ± 0.01^{b}	0.98 ± 0.004^{a}	0.76 ± 0.23^{E}
T_1	0.43 ± 0.004^{j}	0.62 ± 0.01^{h}	0.87 ± 0.008^{c}	0.96 ± 0.02^{a}	0.72 ± 0.21^{C}
T_2	0.32 ± 0.01^{k}	0.62 ± 0.008^{h}	0.83 ± 0.01^{d}	0.91 ± 0.004^{b}	0.67 ± 0.23^{D}
T_3	0.12 ± 0.008^{n}	0.32 ± 0.008^{k}	0.62 ± 0.01^{h}	0.78 ± 0.009^{e}	0.46 ± 0.26^{B}
T_4	0.13 ± 0.02^{n}	0.28 ± 0.004^{l}	0.52 ± 0.008^{i}	0.73 ± 0.01^{f}	0.41 ± 0.23^{A}
T_5	0.12 ± 0.008^{n}	0.22 ± 0.008^{m}	0.42 ± 0.008^{j}	0.68 ± 0.004^{g}	0.36 ± 0.22^{A}
Mean ± SD	0.25 ± 0.14^{D}	0.46 ± 0.20^{C}	0.69 ± 0.19^{B}	0.84 ± 0.12^{A}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

Table 5 Effect of various treatments and storage period on Brix of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	12.82 ± 0.008^{k}	13.32 ± 0.008^{g}	13.92 ± 0.008^{e}	14.22 ± 0.008^{b}	13.57 ± 0.56^{A}
T_1	12.32 ± 0.008^{m}	12.98 ± 0.008i	13.72 ± 0.008^{f}	14.02 ± 0.008^{c}	13.26 ± 0.69^{C}
T_2	12.42 ± 0.008^{l}	13.22 ± 0.01^{h}	13.98 ± 0.004^{d}	14.32 ± 0.008^{a}	13.48 ± 0.76^{B}
T_3	12.82 ± 0.008^{k}	12.41 ± 0.004^{l}	11.34 ± 0.02o	10.22 ± 0.008^{t}	11.69 ± 1.05^{D}
T_4	12.92 ± 0.008^{j}	11.42 ± 0.008^{n}	11.02 ± 0.008^{q}	10.32 ± 0.008^{s}	11.42 ± 0.99^{E}
T_5	12.97 ± 0.004^{i}	11.22 ± 0.008^{p}	10.86 ± 0.01^{r}	10.02 ± 0.008^{u}	11.26 ± 1.12^{F}
Mean ± SD	12.71 ± 0.25^{A}	12.47 ± 0.86^{C}	12.42 ± 1.45^{B}	12.18 ± 2.06^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

3.4 Viscosity

Data presenting interaction among five treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and 90 days storage (0, 30, 60, and 90) expressed that use of chemical preservatives and pasteurization resulted in increase in viscosity of beverage, whereas sonication caused significant decrease in it. The maximum viscosity was noted in T_3 (15.13) at 90th day while the minimum viscosity was observed in T_5 (8.96) at 90th day of study, as results have been presented in Table 6. The viscosity of food systems is typically increased by adding thickening agents such as dietary fibre that generate viscous solutions. Dietary fibres that are water soluble, such as -glucans, pectin, and plant gums, create colloidal solutions that have a higher viscosity. The results of the treatment without using ultrasound are in line with findings of Saxena et al. [44]. Change in viscosity of jujube extract incorporated beverages as a results of thickening agents and sonication, in the study of Farahani et al. [45], were closely related to the current findings. The existence of more concentration of hydrocolloids in the product may lead to enhance the viscosity parameter because the hydrocolloids can form bonds with water molecules in the specimen. Silva et al. [46] studied the effects of ultrasound technique on the physical

Table 6 Effect of various treatments and storage period on viscosity of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60.com	90	
T_0	12.33 ± 0.008^{l}	13.22 ± 0.008^{i}	13.98 ± 0.004^{f}	14.62 ± 0.008^{c}	13.53 ± 0.89^{C}
T_1	12.43 ± 0.01^{k}	13.82 ± 0.008^{g}	14.22 ± 0.008^{e}	14.96 ± 0.02^{b}	13.85 ± 0.95^{B}
T_2	12.92 ± 0.008^{j}	13.77 ± 0.01^{h}	14.42 ± 0.009^{d}	15.13 ± 0.008^{a}	14.06 ± 0.85^{A}
T_3	11.94 ± 0.01^{m}	10.88 ± 0.008^{o}	10.02 ± 0.008^{q}	9.72 ± 0.008^{s}	10.64 ± 0.90^{D}
T_4	10.98 ± 0.008^{n}	10.04 ± 0.01^{q}	9.53 ± 0.02^{t}	9.13 ± 0.008^{u}	9.92 ± 0.72^{E}
T_5	10.62 ± 0.008^{p}	9.82 ± 0.008^{r}	9.13 ± 0.008^{u}	8.96 ± 0.02^{v}	9.63 ± 1.12^{F}
Mean ± SD	11.87 ± 0.83^{D}	11.92 ± 1.77^{B}	11.88 ± 2.41^{C}	12.08 ± 2.91^{A}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

properties and functional attributes of reconstituted whey protein powders. They reported that ultrasonic treatment of whey protein gels' microstructure produced a compact network of tightly packed whey protein aggregates.

3.5 Total solids

Total solids (soluble and insoluble) sowed similar trend as Brix and were higher in chemically preserved and pasteurized samples as compared to sonicated samples. Sonication longer time posed more decrement in total solids than lesser time, as the values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum total solid contents were noted in T3 (15.13) at 90th day while the minimum total solid contents were observed in T_5 (10.03) at 90th day of study, as shown in Table 7. In the T_0, T_1, and T_3 treatments, the total solid values rise in comparison to one another over the course of the storage trial. The greater total solids were much more apparent in the interaction between storage time and treatment. The results are according to the research of Tanwar et al. [27], in which total solids of mango whey beverage was increased during storage. Whey contains almost half of the total solids present in the original whole milk. These solids include whey proteins, lactose, vitamins, minerals and some fat. Total solids of ultrasound treated beverages were decreased during storage in current study. The results are according to findings of Sattar et al. [47]. Pareek et al. [48] conducted a study on the carbonation of manually prepared whey-based fruit juice. The manufactured orange juice carbonated whey drink at a 70:30 ratio was the best in terms of total and soluble solids.

Ismail et al. [49] performed experiments on the storage study of mango whey beverage. Results clearly indicated that there was a change in the physicochemical parameters. According to Jeličić and Božanić [50], whey in its liquid form contains of 93% water, and roughly 50% of total solids are present.

3.6 Sensory evaluation

3.6.1 Flavor

Sonication for 5 min provided best flavor scores, which were closely related with samples on which synergism of pasteurization and chemical preservation was applied, and as the sonication time was increased flavor scores were decreased. The mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum score was noted in T_3 (7.98) at 0 day while the minimum score was observed in T_5 (4.93) at 90th day of study as presented in Table 8. The flavor of all the treatments was observed in acceptable limit. While the ultrasound treated sample showed minimum score when exposed to prolong storage. The current findings are correlated according to the results obtained by Zinoviadou et al. [51]. Fagnani et al. [52] studied on enhancing the sustainability of industry by converting whey into highly value-added products. Although without other ingredients, whey (which is naturally sour) has tasteless flavor. By combining whey with orange juice's citrus taste, the sensory difficulty was reduced.

Table 7 Effect of various treatments and storage period on total solids of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	13.22 ± 0.008^{k}	13.98 ± 0.004^{g}	14.32 ± 0.008^{d}	14.92 ± 0.008^{b}	14.11 ± 0.64^{B}
T_1	13.28 ± 0.004^{j}	13.93 ± 0.02^{h}	14.13 ± 0.008^{f}	14.84 ± 0.01^{c}	14.04 ± 0.57^{C}
T_2	13.62 ± 0.01^{i}	14.25 ± 0.05^{e}	14.93 ± 0.008^{b}	15.13 ± 0.004^{a}	14.48 ± 0.62^{A}
T_3	13.22 ± 0.008^{k}	12.74 ± 0.01^{n}	11.92 ± 0.008^{p}	11.22 ± 0.01^{s}	12.27 ± 0.79^{D}
T_4	13.02 ± 0.01^{l}	12.32 ± 0.008^{o}	11.52 ± 0.008^{q}	10.82 ± 0.008^{u}	11.92 ± 0.86^{E}
T_5	12.94 ± 0.01^{m}	11.32 ± 0.008^{r}	10.98 ± 0.008^{t}	10.03 ± 0.01^{v}	11.31 ± 1.09^{F}
Mean ± SD	13.21 ± 0.22^{A}	13.09 ± 1.08^{B}	12.96 ± 1.58^{C}	12.83 ± 2.22^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

Table 8 Effect of various treatments and storage period on flavor of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	7.91 ± 0.004^{c}	7.01 ± 0.004^{g}	6.51 ± 0.004^{k}	5.83 ± 0.008^{p}	6.81 ± 0.79^{B}
T_1	7.94 ± 0.01^{b}	6.83 ± 0.008^{i}	5.92 ± 0.008^{o}	5.02 ± 0.008^{r}	6.42 ± 1.13^{E}
T_2	7.98 ± 0.004^{a}	6.96 ± 0.01^{h}	14.93 ± 0.01^{n}	5.84 ± 0.02^{p}	6.70 ± 0.88^{C}
T_3	7.98 ± 0.004^{a}	7.22 ± 0.008^{f}	6.04 ± 0.008^{j}	6.22 ± 0.008^{m}	7.05 ± 0.67^{A}
T_4	7.88 ± 0.004^{d}	6.96 ± 0.01^{h}	6.03 ± 0.008^{n}	5.82 ± 0.008^{p}	6.67 ± 0.85^{D}
T_5	7.82 ± 0.008^{e}	6.32 ± 0.008^{l}	5.62 ± 0.008^{q}	4.93 ± 0.008^{s}	6.17 ± 1.11^{F}
Mean ± SD	7.92 ± 0.06^{A}	6.88 ± 0.28^{B}	6.15 ± 0.39^{C}	5.61 ± 0.48^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

Balaji and Prasad [53] combined the nutrients of the two fruits, kinnow and aonla, into a blended beverage. The findings demonstrated that combining kinnow concentrate produced better outcomes for flavour of formulation. In recent past years, a large number of patents having recipes for manufacturing of beverages based on whey by adding fruit concentrates or fruit juices have been registered. Thus, beverages with citrus flavors and those with the scents of other tropical fruits, such as mango, banana, or papaya, have often been recommended since they have been successful in masking the offensive smell of cooked food and the salty-sour fresh whey flavor [54].

3.6.2 Color

The mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum score was noted in T_1 and T_2 (7.98) at 0 day while the minimum score was observed in T_5 (4.73) at 90th day of study, as results have been shown in Table 9. The color scores of all the treatments were reduced during storage. The minimum score was observed at 90th day of storage. The high intensity and prolong treatment of ultrasound may affect the color of the beverage. The change in color is due to the Maillard's reaction. The results are in line with

Table 9 Effect of various treatments and storage period on color of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	7.85 ± 0.01^{c}	6.34 ± 0.02^{h}	5.44 ± 0.02^{m}	4.96 ± 0.02^{o}	6.15 ± 1.15^{F}
T_1	7.98 ± 0.004^{a}	6.82 ± 0.008^{f}	5.95 ± 0.02^{j}	5.02 ± 0.008^{n}	6.44 ± 1.14^{C}
T_2	7.98 ± 0.004^{a}	6.95 ± 0.02^{e}	6.02 ± 0.008^{i}	5.42 ± 0.008^{m}	6.59 ± 1.01^{B}
T_3	7.92 ± 0.008^{b}	6.83 ± 0.008^{f}	6.02 ± 0.008^{i}	5.96 ± 0.03^{j}	6.68 ± 0.82^{A}
T_4	7.83 ± 0.008^{c}	6.94 ± 0.008^{e}	5.83 ± 0.008^{k}	4.92 ± 0.008^{p}	6.38 ± 1.15^{D}
T_5	7.73 ± 0.008^{d}	6.77 ± 0.01^{g}	5.63 ± 0.008^{l}	4.73 ± 0.008^{q}	6.21 ± 1.18^{E}
Mean ± SD	7.88 ± 0.09^{A}	6.77 ± 0.21^{B}	5.81 ± 0.22^{C}	5.16 ± 0.42^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

the findings of Tanwar et al. [27]. Aggarwal et al. [26]. investigated the effect of different hydrocolloids on stability of beverages and kinnow juice which results showed CMC cause more stability and suspending than other hydrocolloids.

Liu et al. [55] identified total 34 volatile components in ultrasonic treated melon juice and the composition of these volatile components were consistent with those present in control juice samples, which explored that sonication did not have any negative effect on juices quality. Similar experiments by Farahani et al. [45], provided variation in color of jujube extract incorporated beverages treated with sonication and other chemicals.

3.6.3 Aroma

The mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum score was noted in T_3 (7.99) at 0 day, while the minimum score was observed in T_5 (4.83) at 90th day of study as shown in Table 10. The highest score was observed in sample treated with 3 min sonication. While all the scores were significantly reduced during the prolong storage of beverage. Milk that has been sonicated appears to have an increasingly odd flavor and odor with increasing sonication time. The results are in line with the findings of Carrillo-Lopez et al. [56].

Similar findings were also present in the experiments of Purewal et al. [36], as sensory evaluation of kinnow-amla beverage got good scores even after 60 days of storage, and it might be consumed as an antioxidant-rich beverage with health benefits. Given the ideal processing conditions (concentration temperature of 65 °C) for potential commercial use, an experimental study demonstrated that it was possible to mix whey and concentrated watermelon juice for the production of bioactive-rich popsicles, the developed formulations were of good quality in terms of flavor and aroma [57].

3.6.4 Overall acceptability

Overall acceptability of sonicated samples was not much different from others, except those sonicated for 5 min, which provided best scores even after 90 days of storage. Mean values of interaction between treatments and storage days represented that the maximum score was noted in T_3 (7.98) at 0 day while the minimum score was observed in T_5 (4.93) at 90th day of study, as depicted in Table 11. All the treatments showed score within an acceptable level. The sample with 5 min sonication treatment provides highest score. When creating milk beverages, Komes et al. [58] heated goat's milk with HIU (100 W for 5 and 10 min) and added plant extracts. The product had much better sensory qualities than plain goat's milk, with samples that had been enhanced with mint and rosemary scoring highest overall acceptance.

Bohora [59] studied the utilization of pineapple for the development of whey-based beverage. Eight various formulations of beverage were produced from whey and pineapple in the range of 50–100% and 0–50%, respectively, and results were supportive to current ones. In another study, excellent nutritional properties of whey were used along with orange juice to formulate 9 different blends of beverages at different ratios, by Chatterjee et al. [60]. Use of preservatives

Table 10 Effect of various treatments and storage period on aroma of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	7.97 ± 0.009^{a}	6.83 ± 0.008^{h}	5.98 ± 0.004^{l}	4.98 ± 0.004^{q}	6.44 ± 1.14^{D}
T_1	7.93 ± 0.008^{b}	6.92 ± 0.01^{g}	5.93 ± 0.02^{m}	4.77 ± 0.01^{s}	6.39 ± 1.22^{E}
T_2	7.98 ± 0.008^{a}	6.95 ± 0.01^{f}	5.95 ± 0.008^{m}	5.03 ± 0.004^{p}	6.47 ± 1.15^{C}
T_3	7.99 ± 0.008^{a}	7.47 ± 0.008^{e}	6.78 ± 0.008^{i}	6.32 ± 0.004^{j}	7.13 ± 0.66^{A}
T_4	7.88 ± 0.004^{c}	6.96 ± 0.01^{f}	6.03 ± 0.008^{k}	5.73 ± 0.008^{n}	6.65 ± 0.88^{B}
T_5	7.82 ± 0.008^{d}	6.32 ± 0.008^{j}	5.62 ± 0.008^{o}	4.83 ± 0.008^{r}	6.14 ± 1.14^{F}
Mean ± SD	7.92 ± 0.06^{A}	6.90 ± 0.34^{B}	6.05 ± 0.36^{C}	5.27 ± 0.57^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

Table 11 Effect of various treatments and storage period on overall acceptability of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	7.92 ± 0.008^{a}	6.67 ± 0.004^{i}	5.83 ± 0.008^{o}	4.77 ± 0.008^{t}	6.31 ± 1.22^{D}
T_1	7.94 ± 0.03^{b}	6.72 ± 0.008^{h}	5.46 ± 0.01^{q}	4.92 ± 0.008^{s}	6.26 ± 1.22^{E}
T_2	7.96 ± 0.02^{ab}	6.82 ± 0.01^{g}	5.94 ± 0.008^{m}	5.01 ± 0.004^{r}	6.43 ± 1.13^{C}
T_3	7.98 ± 0.008^{c}	7.22 ± 0.008^{f}	6.72 ± 0.008^{h}	6.22 ± 0.008^{k}	7.02 ± 0.65^{A}
T_4	7.88 ± 0.004^{d}	6.83 ± 0.004^{g}	6.03 ± 0.008^{l}	5.88 ± 0.004^{n}	6.65 ± 0.82^{B}
T_5	7.82 ± 0.008^{e}	6.32 ± 0.008^{j}	5.68 ± 0.008^{p}	4.93 ± 0.008^{s}	6.18 ± 1.11^{F}
Mean ± SD	7.91 ± 0.05^{A}	6.76 ± 0.27^{B}	5.94 ± 0.40^{B}	5.29 ± 0.56^{D}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

as compared to untreated juices was proved effective in this study, but current study demonstrated that use of ultrasonic was much effective than other techniques for preservation of juices.

3.7 Microbial analysis

3.7.1 Total plate count

Sonication had significant effect on TPC of beverages and increase in sonication time provided best results for microbial safety of beverage, even during 90 days of storage. On the other hand, chemical preserved beverages exhibited increase in TPC during storage. The mean values regarding interaction between treatments (T_0, T_1, T_2, T_3, T_4, and T_5) and days (0, 30, 60, and 90) depicted that the maximum total plate count were noted in T_0 (2.9×10^4) at 90th day while the minimum total plate count was observed in T_5 (0.8×10^4) at 0 day of study. The total plate count of all treatments showed lower results and the count was increased during prolong storage as can be seen from Table 12. Fruits and their parts have loads of bioactives, responsible for antimicrobial activities, and when food formulations are developed by using pulp

Table 12 Effect of various treatments and storage period on total plate count (cfu/g) of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	1.82 ± 0.008^{i}	2.13 ± 0.01^{e}	2.82 ± 0.08^{b}	2.96 ± 0.03^{a}	2.43 ± 0.49^{A}
T_1	1.73 ± 0.01^{j}	1.98 ± 0.004^{g}	2.33 ± 0.01^{d}	2.95 ± 0.02^{a}	2.25 ± 0.48^{B}
T_2	1.68 ± 0.008^{k}	1.92 ± 0.008^{h}	2.02 ± 0.008^{f}	2.57 ± 0.01^{c}	2.04 ± 0.34^{C}
T_3	1.22 ± 0.008^{o}	1.43 ± 0.01^{n}	1.74 ± 0.008^{j}	1.82 ± 0.004^{i}	1.55 ± 0.25^{D}
T_4	1.12 ± 0.008^{p}	1.42 ± 0.008^{n}	1.63 ± 0.008^{l}	1.74 ± 0.02^{j}	1.47 ± 0.24^{E}
T_5	0.83 ± 0.008^{q}	1.22 ± 0.008^{o}	1.56 ± 0.008^{m}	1.72 ± 0.004^{j}	1.331 ± 0.35^{F}
Mean ± SD	1.40 ± 0.37^{A}	1.68 ± 0.35^{C}	2.01 ± 0.45^{B}	2.29 ± 0.56^{A}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

and concentrates from these fruits, the active components play their role in encountering the growth of microbes thus extending the shelf life of food products if properly stored [1].

The current findings were similar to the results obtained by Tanwar et al. [27]. The ultrasound treated samples were showed the lowest microbial count. The physical phenomena of cavitations that occur when applying ultrasound treatments could be used to explain the ultrasound-treated samples' considerable decreases in the rate of microbial growth [61]. Cavitation can break molecules or particles through a variety of mechanisms, which can happen separately or in combination. These mechanisms include the production of free radicals, micro streaming and implosion shock waves produced by mechanical stresses; thermal effects caused by bubble implosion. The recent findings are according to the result obtained by Tomadoni et al. [62]. Microbial growth was accelerated over the storage time in kinnow-banana RTS under normal storage condition, without any advanced treatment, was reported by Kumar and Singh [63], highlighting the drawback of conventional processing and storage, as compared to green processing technology, which was applied in current study.

3.7.2 Yeast and mold count

Mean values regarding interaction between five different treatments and 90 days of storage revealed significantly different results for all treatments. It was clear that the maximum yeast and mold count were noted in T_1 (3.2×10^4) at 90th day, whereas, the minimum yeast and mold count was observed in T_5 (0.1×10^4) at 0 day of study. The yeast and mold count were significantly lower in all the samples except untreated, but in ultrasound treated samples these were very much under control, due to effectiveness of sonication, as evident from the Table 13. An earlier study discovered that using ultrasound in *Aloe Vera* juice could replace the heat treatment method because sonication may be able to render the microorganisms inactive [64]. The recent findings were also related to the results obtained by de Albuquerque et al. [65].

Leon-Lopez et al. [66] studied the manufacturing of a whey-based beverage by adding hydrolyzed collagen in different concentrations. The findings demonstrated that hydrolyzed collagen served as an antimicrobial agent, as a result there were no microorganism activity present in the treated drinks. In a much similar study Fagnani et al. [67] evaluated orange juice and whey beverage that had not undergone any enzymatic processing. Four whey and orange juice formulations were stored at 5 °C for a period of 28 days, during which the beverage, maintained stability and had high microbiological quality. In a similar fashion study, Bohora [59] pasteurized whey beverage enriched with pineapple concentrate at 82.5 °C for 20 min, and storage was done at normal temperature (25 ± 5 °C) in 250 ml plastic bottles and refrigerated temperature (7 ± 1 °C) for time period of 20 days. The effects of time of storage and temperature on microbial parameters, specifically TPC, yeast and mold count were noted and on the basis of results of all the analysis, they observed that beverage could be kept perfect for up to 20 days without using preservatives.

Table 13 Effect of various treatments and storage period on yeast and mold count (cfu/g) of Kinnow whey beverage

Treatments	Storage period (days)				Mean ± SD
	0	30	60	90	
T_0	1.73 ± 0.008^{g}	1.98 ± 0.008^{f}	2.32 ± 0.008^{e}	2.66 ± 0.01^{d}	2.17 ± 0.36^{C}
T_1	1.52 ± 0.008^{a}	1.96 ± 0.03^{e}	2.64 ± 0.02^{d}	3.22 ± 0.008^{a}	2.33 ± 0.67^{A}
T_2	1.22 ± 0.01^{i}	1.98 ± 0.008^{f}	12.72 ± 0.008^{c}	3.04 ± 0.01^{b}	2.24 ± 0.73^{B}
T_3	0.32 ± 0.008^{q}	0.53 ± 0.008^{o}	0.83 ± 0.01^{l}	0.98 ± 0.008^{j}	0.66 ± 0.26^{D}
T_4	0.27 ± 0.008^{r}	0.43 ± 0.008^{p}	0.63 ± 0.01^{n}	0.92 ± 0.01^{k}	0.56 ± 0.25^{E}
T_5	0.12 ± 0.008^{s}	0.33 ± 0.01^{q}	0.73 ± 0.01^{m}	0.82 ± 0.008^{l}	0.50 ± 0.30^{F}
Mean ± SD	0.86 ± 0.66^{D}	1.20 ± 0.79^{C}	1.64 ± 0.94^{B}	1.94 ± 1.07^{A}	

Means carrying different letters are statistically significant

T_0 = Kinnow whey beverage with preservatives

T_1 = Kinnow whey beverage with pasteurization

T_2 = Kinnow whey beverage with preservatives and pasteurization

T_3 = Kinnow whey beverage with 5 min sonication

T_4 = Kinnow whey beverage with 10 min sonication

T_5 = Kinnow whey beverage with 15 min sonication

4 Conclusion

Development of innovative and nutritional beverages based upon dairy by products are always area of research for food processors. In this research work, whey was effectively used to produce a kinnow-whey beverage having acceptable sensory and nutritional properties as well as good shelf life. Physicochemical and sensory parameters of whey beverage were observed at refrigerated storage temperature (4 °C) for 90 days. During storage, decline in total solids, viscosity and titratable acidity, were observed, while pH, total plate count, and yeast and mold count were increased. Among different processing and preservation techniques tested, sonication was proved useful for retaining optimum quality of the beverage, with minimum physical and chemical deterioration of the beverage. Whereas, untreated beverage samples lost their quality and safety quickly, during the storage. Overall acceptability of beverage was highest for the beverage that was sonicated for 15 min. It was discovered that among different treatments, beverage that was sonicated for 15 min can be stored up to 3 months at refrigerated temperature without addition of any preservatives.

5 Recommendations

Functional beverages are expanding in the world and since fruits are valuable food sources and are known as sources of antioxidant and phenolic compounds, their extracts and juices in these beverages can be used in different forms. Kinnow whey beverage is a fascinating and innovative food product in the growing market which can be used by food processing industries as a productive and high-quality functional food. Awareness among consumers is growing their attention towards nutritional value and potential health benefits of food. Further phytochemical profiling and antioxidant potential of this food formulation can be carried out before commercialization of this innovative functional beverage in the market.

Acknowledgements This manuscript has been derived from the diisseration of Mr. Ammad Iqbal, entitled "Impact of different processing techniques on physicochemical characterization of kinnow whey beverage", which was submitted to Higher Education Commission (HEC) of Pakistan for fulfilment of degree requirements.

Author contributions AH, Conceptualization; FS and SAK, Data curation; AI and HF, Formal analysis, Funding acquisition and Investigation; SAB, Methodology and, Project administration; SN, Resources; FIG, Software; AI, Supervision; FS, Validation; HF, Visualization; AH and AAM, Roles/Writing—original draft; AH and SAK, Writing—review and editing.

Funding No funds in any form were availed for this research work.

Data availability Data relevant to this study can be provided upon request.

Declarations

Competing interests The authors have declared no conflicts of interest for this article.

References

1. Hussain A, Kausar T, Sehar S, Sarwar A, Quddoos MY, Aslam J, Liaqat A, Siddique T, An QU, Kauser S, Rehman A, Nisar R. A review on biochemical constituents of pumpkin and their role as pharma foods; a key strategy to improve health in post COVID 19 period. Food Prod Process Nutr. 2023;5(1):1–14.
2. Hussain A, Kausar T, Sehar S, Sarwar A, Ashraf AH, Jamil MA, Noreen S, Rafique A, Iftikhar K, Aslam J, Quddoos MY, Majeed MA, Zerlasht M. Utilization of pumpkin, pumpkin powders, extracts, isolates, purified bioactives and pumpkin based functional food products; a key strategy to improve health in current post COVID 19 period; an updated review. Appl Food Res. 2022;2: 100241.
3. Chavan RS, Shraddha RC, Kumar A, Nalawade T. Whey based beverage: its functionality, formulations, health benefits and applications. J Food Process Technol. 2015;6(10):1–20.

4. Waqar M, Gu R, Nie F. Mapping ICT use along the citrus (Kinnow) value chain in Sargodha District. Pak Sustain. 2018;10(12):4743.
5. Klimczak I, Małecka M, Szlachta M, Gliszczyńska-Świgło A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J Food Compos Anal. 2007;20(3–4):313–22.
6. Matsumoto H, Ikoma Y. Effect of different postharvest temperatures on the accumulation of sugars, organic acids, and amino acids in the juice sacs of Satsuma mandarin (*Citrus unshiu* Marc.) fruit. J Agric Food Chem. 2012;60(39):9900–9.
7. Salehi F. Physico-chemical properties of fruit and vegetable juices as affected by ultrasound: a review. Int J Food Prop. 2020;23(1):1748–65.
8. Nehra M, Duhan J, Khan M, Sandhu K, Ansariee MJ. Synergistic effects of sonication and microwave on safety and quality of foods. In Ultrasound and Microwave for Food Processing. Academic Press; 2023, 363–378.
9. Rawson A, Tiwari BK, Patras A, Brunton N, Brennan C, Cullen PJ, O'donnell C. Effect of thermosonication on bioactive compounds in watermelon juice. Food Res Int. 2011;44(5):1168–73.
10. Bhat R, Kamaruddin NSBC, Min-Tze L, Karim AA. Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. Ultrason Sonochem. 2011;18(6):1295–300.
11. Taha A, Mehany T, Pandiselvam R, Siddiqui SA, Mir NA, Malik MA, Sujayasree OJ, Alamuru KC, Khanashyam AC, Casanova F, Xu X, Pan S, Hu H. Sonoprocessing: mechanisms and recent applications of power ultrasound in food. Crit Rev Food Sci Nutr. 2022. https://doi.org/10.1080/10408398.2022.2161464.
12. Husain Q. β Galactosidases and their potential applications: a review. Crit Rev Biotechnol. 2010;30(1):41–62.
13. Varzakas T, Özer B. Application of sweeteners in food and drinks (bakery, confectionery, dairy products, puddings, fruit products, vegetables, beverages, sports drinks, hard candies, loukoumia, marmalades, jams, jellies, baked goods, sorbet). Sweeteners: Nutr Aspects Appl Prod Technol. 2012; 209.
14. Joshi VK, Kumar A, Kumar V. Antimicrobial, antioxidant and phyto-chemicals from fruit and vegetable wastes: a review. Int J Food Ferment Technol. 2012;2(2):123.
15. Mirabella N, Castellani V, Sala S. Current options for the valorization of food manufacturing waste: a review. J Clean Prod. 2014;65:28–41.
16. Dhamsaniya NK, Varshney AK. Development and evaluation of whey based RTS beverage from ripe banana juice. J Food Process Technol. 2013;4(2):2157–7110.
17. Ahmed T, Sabuz AA, Mohaldar A, Sazzad Fardows HM, Inbaraj BS, Sharma M, Rana MR, Sridhar K. Development of novel whey-mango based mixed beverage: effect of storage on physicochemical, microbiological, and sensory analysis. Foods. 2023;12(2):237.
18. Sakhale BK, Pawar VN, Ranveer RC. Studies on development of soymil- based mango RTS beverage. Elec J Env Agricult Food Chem Title. 2012;11(5):523–8.
19. Baljeet SY, Ritika BY, Sarita R. Studies on development and storage of whey-based pineapple (*Ananas comosus*) and bottle gourd (*Lagenaria siceraria*) mixed herbal beverage. Int Food Res J. 2013;20(2).
20. Shukla M, Jha YK, Admassu S. Development of probiotic beverage from whey and pineapple juice. J Food Process Technol. 2013;4(206):1–4.
21. Naik YK, Khare A, Choudhary PL, Goel BK, Shrivastava A. Studies on physico-chemical and sensory characteristics of whey-based watermelon beverage. Asian J Res Chem. 2009;2(1):57–9.
22. Singh D, Singh R, Bhatt F. Development, quality evaluation and shelf life studies of whey guava beverage. Int J Curr Eng Technol. 2014;4(3):2171–5.
23. Singh P, Sahota PP, Bhadra F, Singh RK. Optimization, production and scale up of debittered kinnow beverage by α-L-rhamnosidase producing yeast. Emirates J Food Agric. 2015;27:548–55.
24. Abu-Ghoush M, Al-Mahasneh MA, Samhouri M, Al-Holy M, Herald T. Formulation and fuzzy modeling of viscosity of orange beverages fortified with carboxymethylcellulose-whey protein isolate emulsions. Jordan J Biol Sci. 2009;2(3):109–18.
25. AOAC (Association of Official Agricultural Chemists) (2000). In W. Horwitz, & G. Latimer (Eds.). The official methods of analysis of AOAC International (20th ed.). Gaithersburg: AOAC International.
26. Aggarwal P, Kumar V, Yaqoob M, Kaur S, Babbar N. Effect of different levels of hydrocolloids on viscosity and cloud stability of kinnow juice and beverages. J Food Process Preserv. 2020;44(10): e14802.
27. Tanwar TK, Wagh RV, Mehta N, Malav OP, Kour S, Kumar P. Preparation of functional beverage from whey-based mango juice. Pharma Innov J. 2022;11(7):4710–6.
28. APHA. Frances, PD and Keith, I. (Eds.), Compendium of Methods for the Microbiological Examination of Foods. Washington, DC; 2001.
29. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices, vol. 2. New York: Springer; 2010.
30. Steel RGD, Torrie JH. Principles and procedures of statistics, a biometrical approach (No. Ed. 2). McGraw-Hill Kogakusha, Ltd.; 1980.
31. Bhardwaj RL, Pandey S. Juice blends—a way of utilization of under-utilized fruits, vegetables, and spices: a review. Crit Rev Food Sci Nutr. 2011;51(6):563–70.
32. Majumdar TK, Wadikar DD, Vasudish CR, Premavalli KS, Bawa AS. Effect of storage on physico-chemical, microbiological and sensory quality of bottlegourd-basil leaves juice. Am J Food Technol. 2011;6(3):226–34.
33. Jan A, Masih ED. Development and quality evaluation of pineapple juice blend with carrot and orange juice. Int J Sci Res Publ. 2012;2(8):1–8.
34. Thakkar P, Vaghela B, Patel A, Modi HA, Prajapati JB. Formulation and shelf-life study of a whey-based functional beverage containing orange juice and probiotic organisms. Int Food Res J. 2018;25(4).
35. Herrera-Ponce AL, Salmeron-Ochoa I, Rodriguez-Figueroa JC, Santellano-Estrada E, Garcia-Galicia IA, Vargas-Bello-Pérez E, Alarcon-Rojo AD. Functional properties and consumer acceptance of whey-oat beverages under different ultrasonication times and inulin concentration. J Food Process Preserv. 2022;46: e16907.
36. Purewal SS, Kamboj R, Sandhu KS, Kaur P, Sharma K, Kaur M, Salar RK, Punia S, Siroha AK. Unraveling the effect of storage duration on antioxidant properties, physicochemical and sensorial parameters of ready to serve Kinnow-Amla beverages. Appl Food Res. 2022;2(1): 100057.
37. Mukhtar K, Nabi BG, Arshad RN, Roobab U, Yaseen B, Ranjha MMAN, Muhammad Aadil R, Ibrahim SA. Potential impact of ultrasound, pulsed electric field, high-pressure processing, microfludization against thermal treatments preservation regarding sugarcane juice (*Saccharum officinarum*). Ultrasonics Sonochem. 2022;90: 106194.
38. Adulvitayakorn S, Azhari SH, Hasan H. The effects of conventional thermal, microwave heating, and thermosonication treatments on the quality of sugarcane juice. J Food Process Preserv. 2020;44(2): e14322.

39. Khandpur P, Gogate PR. Effect of novel ultrasound-based processing on the nutrition quality of different fruit and vegetable juices. Ultrason Sonochem. 2015;27:125–36.
40. Santhirasegaram V, Razali Z, Somasundram C. Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. Ultrason Sonochem. 2013;20(5):1276–82.
41. Kaanane A, Kane D, Labuza TP. Time and temperature effect on stability of Moroccan processed orange juice during storage. J Food Sci. 1988;53(5):1470–3.
42. Barwal VS, Sharma R, Singh TK. Development and evaluation of dietetic bitter gourd ready-to-serve drink. J Food Sci Technol. 2005;42(2):202.
43. Yadav RB, Yadav BS, Kalia N. Development and storage studies on whey-based banana herbal (*Mentha arvensis*) beverage. Am J Food Technol. 2010;5(2):121–9.
44. Saxena D, Chakraborty SK, Sabikhi L, Singh D. Process optimization for a nutritious low-calorie high-fiber whey-based ready-to-serve watermelon beverage. J Food Sci Technol. 2015;52(2):960–7.
45. Farahani ZK, Mousavi M, Ardebili MS, Bakhoda H. Stability, physicochemical, rheological and sensory properties of beverage containing encapsulated jujube extract with sodium alginate stabilized by sodium alginate and Gellan gum. Food Chem Adv. 2023;2: 100195.
46. Silva M, Bui THA, Dharmadana D, Zisu B, Chandrapala J. Ultrasound-assisted formation of double emulsions stabilized by casein-whey protein mixtures. Food Hydrocolloids. 2020;109: 106143.
47. Sattar S, Imran M, Mushtaq Z, Ahmad MH, Arshad MS, Holmes M, Maycock J, Nisar MF, Khan MK. Retention and stability of bioactive compounds in functional peach beverage using pasteurization, microwave and ultrasound technologies. Food Sci Biotechnol. 2020;29(10):1381–8.
48. Pareek N, Gupta A, Sengar R. Preparation of healthy fruit based carbonated whey beverages using whey and orange juice. Asian J Dairying Foods Res. 2014;33(1):5–8.
49. Ismail AE, Abdelgader MO, Ali AA. Microbial and chemical evaluation of whey-based mango beverage. Adv J Food Sci Technol. 2011;3(4):250–3.
50. Jeličić I, Božanić R, Tratnik L. Whey-based beverages-a new generation of dairy products. Mljekarstvo. 2008;58(3):257–74.
51. Zinoviadou KG, Galanakis CM, Brnčić M, Grimi N, Boussetta N, Mota MJ, Saraiva JA, Patras A, Tiwari B, Barba FJ. Fruit juice sonication: implications on food safety and physicochemical and nutritional properties. Food Res Int. 2015;77:743–52.
52. Fagnani R, Puppio AAN, Zanon EO. Sustainable alternative for the food industry: converting whey and orange juice into a micro-filtered beverage. Scientia Agricola. 2018;75:136–43.
53. Balaji V, Prasad VM. Studies on value added kinnow-aonla blended ready to serve beverage. J Food Process Technol. 2014;5(1):1–4.
54. Harmayani E, Anal AK, Wichienchot S, Bhat R, Gardjito M, Santoso U, Siripongvutikorn S, Puripaatanavong J, Payyappallimana U. Healthy food traditions of Asia: exploratory case studies from Indonesia, Thailand, Malaysia, and Nepal. J Ethnic Foods. 2019;6:1–18.
55. Liu X, Zhang C, Wang H, Wang Y, Zhu D, Liu H. Ultrasonic treatment maintains the flavor of the melon juice. Ultrason Sonochem. 2023;92: 106284.
56. Carrillo-Lopez LM, Garcia-Galicia IA, Tirado-Gallegos JM, Sanchez-Vega R, Huerta-Jimenez M, Ashokkumar M, Alarcon-Rojo AD. Recent advances in the application of ultrasound in dairy products: effect on functional, physical, chemical, microbiological and sensory properties. Ultrason Sonochem. 2021;73: 105467.
57. Martins CP, Ferreira MVS, Esmerino EA, Moraes J, Pimentel TC, Rocha RS, Freitas MQ, Santos JS, Ranadheera CS, Rosa LS, Teodoro AJ, Mathias SP, Silva MC, Raices RSL, Couto SRM, Granato D, Cruz AG. Chemical, sensory, and functional properties of whey-based popsicles manufactured with watermelon juice concentrated at different temperatures. Food Chem. 2018;255:58–66.
58. Komes D, Bušić A, Belščak-Cvitanović A, Brnčić M, Bosiljkov T, Vojvodić A, Dujmić F. Novel approach to the development of functional goat's milk-based beverages using medicinal plant extracts in combination with high intensity ultrasound treatment. Food Technol Biotechnol. 2017;55(4):484.
59. Bohora G. Preparation of whey based pineapple beverage and its storage quality evaluation (Doctoral dissertation); 2018.
60. Chatterjee G, De Neve J, Dutta A, Das S. Formulation and statistical evaluation of a ready-to-drink whey-based orange beverage and its storage stability. Revista mexicana de ingeniería química. 2015;14(2):253–64.
61. Vercet A, Sánchez C, Burgos J, Montañés L, Buesa PL. The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. J Food Eng. 2002;53(3):273–8.
62. Tomadoni B, Cassani L, Viacava G, Moreira MDR, Ponce A. Effect of ultrasound and storage time on quality attributes of strawberry juice. J Food Process Eng. 2017;40(5): e12533.
63. Kumar V, Singh SK. Quality and evaluation of banana and kinnow fruit based RTS beverage as influenced by blending ratio and their storage study at different conditions. Int J Agric Eng. 2013;6(2):409–14.
64. Chakraborty S, Uppaluri R, Das C. Optimization of ultrasound-assisted extraction (UAE) process for the recovery of bioactive compounds from bitter gourd using response surface methodology (RSM). Food Bioprod Process. 2020;120:114–22.
65. Albuquerque JG, Escalona-Buendía HB, de Magalhães Cordeiro AMT, dos Santos Lima M, de Souza Aquino J, da Silva Vasconcelos MA. Ultrasound treatment for improving the bioactive compounds and quality properties of a Brazilian nopal (*Opuntia ficus-indica*) beverage during shelf-life. LWT. 2021;149: 111814.
66. León-López A, Pérez-Marroquín XA, Campos-Lozada G, Campos-Montiel RG, Aguirre-Álvarez G. Characterization of whey-based fermented beverages supplemented with hydrolyzed collagen: antioxidant activity and bioavailability. Foods. 2020;9(8):1106.
67. Fagnani R, Battaglini APP, Beloti V, Urbano A, de Camargo Bronzol J. Alcohol stability of milk from the perspective of X-ray diffractometry. Food Biophys. 2016;11:198–205.