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Research Article

Ultrasound-Assisted Optimal Development and Characterization of Stevia-Sweetened Functional Beverage

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Nowadays, blend of different fruit juices as functional beverages is highly recommended to retain maximum concentration of nutrients due to their integrated effect against processing, to utilize underconsumed fruits and/or vegetables, and to provide healthy product to the consumer. The novelty in this particular work is optimization of blend using stevia-based low caloric extract as sweetener to replace high caloric sugar contents. For this purpose, juices of apple and carrot along with the extract of stevia leaves underwent the process optimization using a statistical approach of D-optimal mixture design. The best beverage formulation with high sensory scores was composed of apple juice 70%, carrot juice 27%, and stevia extract (5 g/100 ml) 3%. The optimized beverage was then processed with conventional pasteurization at 95°C for 1 min and compared with ultrasound processing for 15 min at four different amplitudes (20%, 40%, 60%, and 80%). According to the observation, the treatment US_{60} (60% amplitude for 15 min) showed better retention for total phenolic content (78.65 ± 1.37 mg GAE/100 mL), total flavonoid content (23.53 ± 0.15 mg CE/100 mL), DPPH (66.43 ± 1.45 %), and FRAP (65.17 ± 1.10 mmol Trolox/100 mL) as compared to pasteurized and control samples while keeping other physicochemical properties in range. The research therefore indicates that ultrasound is good alternative to pasteurization and stevia to sugar in terms of functional beverage development.

1. Introduction

Functional foods possess such food components that provide health benefits beyond fulfillment of basic nutrients. Fruit beverages are considered as the most active functional food group being rich source of vitamins, bioactive components, fiber, and/or minerals [1]. Beverages are also in high demand due to their thirst quenching, nutritious, easily digestible, appetizing, and highly refreshing properties for human intake [2]. Among them, fruit juices are important for good health and are equally essential for the people of all age groups as they are an essential constituent of a wholesome diet. They are far better than the synthetic or artificial beverages that are at hand in the market. Different methods are used to improve the nutritional quality of juices. Blending of different fruit juices is one of the best methods as by changing the kind and quality of fruits, nutrients (e.g., vitamin and mineral) of the juices can be enhanced [3]. Pure or concentrated juices are less popular than blended or mixed fruit juices as mixed fruit juices have high value of natural aroma and health benefits [4]. Mixed fruit beverage is in great demand in society for daily use as all types of fruits even those having low total soluble solids in it yet have high potential as they are more acidic, highly bitter, and having strong flavor which can be masked in beverage by blending with other juicy fruits. The functionality of these fruit blends

can further be increased by introducing different plant extracts with the objective to target any health aspect, for instance, development of apple, blueberry, and cranberry juice together with water extracts of ginger [5], formulation of jamun, mango, pineapple, pomegranate, and purple grapes drinks by adding stevia aqueous extract as natural sweetener [6], and preparation of ginger-lime ready-to-serve (RTS) functional beverage sweetened by Palmyra sugar candy [7].

Improvements in the food processing techniques have become the need of hour because of the increasing demand of food either in terms of quality and quantity over the past few years [8]. Product safety, quality, and its shelf life depend largely on the processing methods that have been used in their manufacturing. Thermal processing techniques cause losses of vital nutrients along with the assurance of product safety and extended shelf life. Nevertheless, effects of heat are not specific and can cause depletion of sensory and nutritive qualities of food products and damage their functional properties [9]. To avoid these changes, alternative methods have been developed including osmotic dehydration, ultrasound, membrane filtration, high pressure treatment, ozone treatment, pulse electric field, irradiation, and active packaging [10]. Presently, ultrasound is among the emerging novel processing technologies that are being applied on foods to process them at low temperatures and/or for short time, thus minimizing the undesirable modifications induced by heat [11]. Different studies from the past few years have confirmed that this novel method is moderate food preservation technique competent of inactivating deteriorative microbes and enzymes and providing safe and fresh-like products with least modifications to their physicochemical, nutritional, and sensorial properties [12–17]. Moreover, they appear to be more environment friendly than the traditional ones because of their hygienic processing of food products.

Seeing the significance of the previous literature, the current study was conducted to explore the effect of ultrasound processing and conventional heating on physicochemical attributes, functional characteristics, radical scavenging activity, and organic acids profile of mixed fruit juice developed from apple and carrot juices using stevia as natural sweetener.

2. Materials and Methods

2.1. Reagents and Raw Materials. Sigma-Aldrich (Sigma-Aldrich, Tokyo, Japan), Merck (Merck KGaA, Darmstadt, Germany), and Uni-Chem (China) were the sources of all chemicals and reagents. Apple (*Pyrus malus* Linn) and carrot (*Daucus carota*) were purchased from local growers of Faisalabad and stevia (*Stevia rebaudiana*) leaves were procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan, and stored at freezing temperature until further processing.

2.2. Juice Formulations. The fruits were washed and peeled and the juice of apple and carrot was extracted using household juicer (OT-MJ176 A, Guangdong, China). Stevia extract was prepared by stirring 5 g of dried stevia leaves' powder in 100 ml distilled water on a hot plate (Corning 6798–420D) for 2 hours at 60°C and 1000 rpm and filtered for further use in juice formulation. The ratio (5 g/100 ml) of stevia extract was finalized after various preliminary trials. Different formulations of juices and stevia extract were developed and optimized using D-optimal mixture design (Table 1). The application of a mixture design in product development is an efficient method to find out the optimum blend of various mixture components. Best formulation based on sensory attributes was further processed for physicochemical and functional studies.

2.3. Sensory Analysis. The sensory evaluation was carried out for juices on the basis of aroma, taste, mouthfeel, texture, color, aftertaste, and overall acceptability by a panel of 20 semitrained members on a 9-point structured hedonic scale (where 9 = excellent, 8 = very good, 7 = good, 6 = below good above fair, 5 = fair, 4 = below fair above poor, 3 = poor, 2 = very poor, and 1 = extremely poor) [18].

2.4. Processing of Optimized Juice Formulations

2.4.1. Thermal Processing. A 100 mL of optimized juice blend was heated at 95°C for 1 min on a flame and then cooled down to room temperature by placing it in water bath [19] and stored at 0°C until analysis.

2.4.2. Ultrasound Processing. The optimized juice blend (100 mL) was processed using sonication apparatus VCX750 (Sonics & Materials, Inc. Newtown, CT, USA) at 750 W and constant frequency of 20 kHz. The ultrasound probe was inserted up to a depth of 25 mm in the juice sample and sonicated at four different amplitude levels: 20% (US_{20}), 40% (US_{40}), 60% (US_{60}), and 80% (US_{80}) for time duration of 15 min [16]. The processed juice samples were stored at 0°C until analysis.

2.5. Determination of pH, Total Soluble Solids (TSS), and Titratable Acidity (TA). The pH of treated and untreated samples was determined through benchtop pH meter (WTW InoLab, Germany). Total soluble solids (TSS) were estimated as Brix with a handheld refractometer (WYA-2 W, Shanghai Precision & Scientific Instrument Co. Ltd., China) at room temperature (25 ± 1°C). To determine the titratable acidity (TA), 20 mL juice sample was diluted with 80 mL distilled water in a beaker. This solution was then titrated with standard solution of 0.1 N NaOH by using phenolphthalein as indicator (pH 8.2 ± 0.1). The volume of NaOH was expressed as % malic acid [20].

2.6. Functional Profile

2.6.1. Total Phenolic Contents (TPC). Folin–Ciocalteu reagent was used to determine TPC in juice sample [21]. For this purpose, 0.5 mL of juice sample was incorporated in 1 mL of Folin–Ciocalteu reagent (1 N) and left for 6 min, and

Table 1: Mean hedonic response of the sensory evaluation of different beverage formulations ($n = 20$ participants).

Formulation	Composition			Sensory attributes						
	Apple juice (% v/v)	Carrot juice (% v/v)	Stevia sol. (% v/v)	Aroma	Taste	Mouthfeel	Texture	Color	Aftertaste	Overall acceptability
F_1	80	18	2	6.60 ± 0.84	6.42 ± 0.84	5.73 ± 0.75	6.32 ± 0.92	6.47 ± 0.85	5.64 ± 0.99	6.20 ± 0.41
F_2	60	37	3	5.41 ± 0.74	6.10 ± 0.80	5.74 ± 0.78	5.34 ± 0.72	5.24 ± 0.72	6.30 ± 0.85	5.69 ± 0.43
F_3	80	17	3	6.63 ± 0.87	6.43 ± 0.84	6.34 ± 0.85	6.36 ± 0.81	6.15 ± 0.81	6.84 ± 0.92	6.46 ± 0.24
F_4	70	27	3	7.42 ± 1.05	7.01 ± 1.02	6.40 ± 0.87	6.44 ± 0.84	7.36 ± 1.06	7.22 ± 1.04	6.98 ± 0.45
F_5	80	19	1	6.54 ± 0.92	5.82 ± 0.76	5.37 ± 0.73	6.52 ± 0.85	6.44 ± 0.82	5.82 ± 0.73	6.09 ± 0.48
F_6	70	28	2	7.12 ± 1.02	6.72 ± 0.89	6.60 ± 0.98	6.45 ± 0.84	7.25 ± 1.06	6.74 ± 0.86	6.81 ± 0.31
F_7	75	23.5	1.5	6.66 ± 0.85	6.80 ± 0.99	6.44 ± 0.92	6.23 ± 0.81	6.64 ± 0.85	5.77 ± 0.79	6.42 ± 0.38
F_8	60	38	2	5.34 ± 0.72	5.43 ± 0.78	5.57 ± 0.75	6.20 ± 0.81	5.58 ± 0.73	5.17 ± 0.72	5.55 ± 0.35
F_9	80	17	3	6.51 ± 0.84	6.42 ± 0.83	6.21 ± 0.82	6.47 ± 0.84	6.56 ± 0.83	6.64 ± 0.85	6.47 ± 0.15
F_{10}	80	19	1	6.72 ± 0.97	5.71 ± 0.77	5.54 ± 0.74	6.72 ± 0.86	6.64 ± 0.85	6.10 ± 0.80	6.24 ± 0.53
F_{11}	65	33.5	1.5	6.14 ± 0.81	6.20 ± 0.82	6.25 ± 0.83	5.94 ± 0.79	5.83 ± 0.78	5.64 ± 0.77	6.00 ± 0.24
F_{12}	70	29	1	7.39 ± 1.05	6.93 ± 0.86	6.33 ± 0.81	6.73 ± 0.88	7.10 ± 1.02	6.67 ± 0.85	6.86 ± 0.37
F_{13}	60	39	1	5.42 ± 0.82	5.15 ± 0.75	5.74 ± 0.78	5.35 ± 0.73	5.84 ± 0.79	5.24 ± 0.71	5.46 ± 0.28
F_{14}	60	37	3	5.58 ± 0.85	6.13 ± 0.81	5.91 ± 0.78	5.38 ± 0.74	6.21 ± 0.82	5.75 ± 0.75	5.83 ± 0.32

2 mL of Na_2CO_3 (20%) was incorporated in the developed mixture. Mixture was further retained for 60 min at ambient temperature. At the end absorbance measured at 765 nm through spectrophotometer (Specord 200/plus, Germany). TPC were showed as mg gallic acid equivalents per 100 ml of juice sample (mg GAE/100 mL).

2.6.2. Total Flavonoid Contents (TFC). For the determination of TFC, Kim et al. [22] explained a method in which 1.25 mL of deionized water, 75 μL $NaNO_2$ solution (5%), and 0.25 mL of the juice sample were mixed together. The freshly developed solution was kept for 15 min, and then, 150 μL $AlCl_3$ solution (10%) was added; newly developed mixture was incubated for 5 min, and after that, addition of 0.5 ml of standardized NaOH (1 M) was made. At the end, distil water was added up to 2.5 mL and mixed well. Sample was kept in spectrophotometer (Specord 200/plus, Germany) for its TFC content determination at absorbance of 415 nm. Outcomes of TFC were showed as mg catechin equivalents per 100 ml of juice sample (mg CE/100 mL).

2.7. Radical Scavenging Activity

2.7.1. DPPH Assay. Khan [23] described a method to determine the DPPH assay of juice sample. In this method, 1 mL of DPPH (20 ppm) was mixed into 25 μL of filtered juice sample and kept it in dusky place for 30 min. After that, absorbance was taken at 517 nm. Formula used to calculate the DPPH % is as follows:

$$\text{DPPH\%} = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100. \quad (1)$$

where A_c represents the absorbance of the control sample and A_s represents the absorbance of the test sample.

2.7.2. FRAP Assay. Value of FRAP assay was determined by using the previously described method [24]. For this purpose, 300 mM acetate buffer (pH 3.6), 1 mM TPTZ solution, and 20 mM ferric chloride were mixed in the ratio of 10 : 1 : 1 and heated up to 37°C. Mixed fruit juice sample (6 μL) was added in the mixture and kept for 6 min. Afterwards, the absorbance was measured at 593 nm. Value of FRAP assay was expressed in mmol Trolox/100 mL of juice.

2.8. Statistical Analysis. The statistical software package Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used to optimize the experimental design as well as to analyze the data of sensory parameters for formulated fruit juices. The data obtained from treatments were further subjected to statistical analysis using SPSS 21 Statistical Software and expressed as means ± standard deviations. Results were analyzed for variance at a significance level of 5% and evaluated by LSD post hoc test.

3. Results and Discussion

3.1. Sensory Analysis. According to the sensory evaluation (Table 1), all of 14 formulations of mixed apple-based beverage were acceptable to the panelists. However, only 3 formulated combinations showed higher overall sensory scores (>6.50) among the 14 combinations. It emphasizes that the incorporation of different juices had a significant effect on the sensory attributes of beverage. In the current study, the highest sensory scores for aroma, taste, color, aftertaste, and overall acceptability were obtained for formulation F_4 composed of 70% apple juice, 27% carrot juice, and 3% stevia extract (5 g/100 ml) (Table 1), while for mouthfeel and texture, highest values were obtained for formulations F_6 and F_{12}, respectively. Figure 1 shows the response surface plots of all sensory attributes affected by composition of mixed fruit beverage. The direction of curvature in each plot tells the response of sensory attribute against linear beverage commodity. Addition of stevia solution influenced the sensory parameters of mixed fruit juices and was found to be a good replacer for high caloric

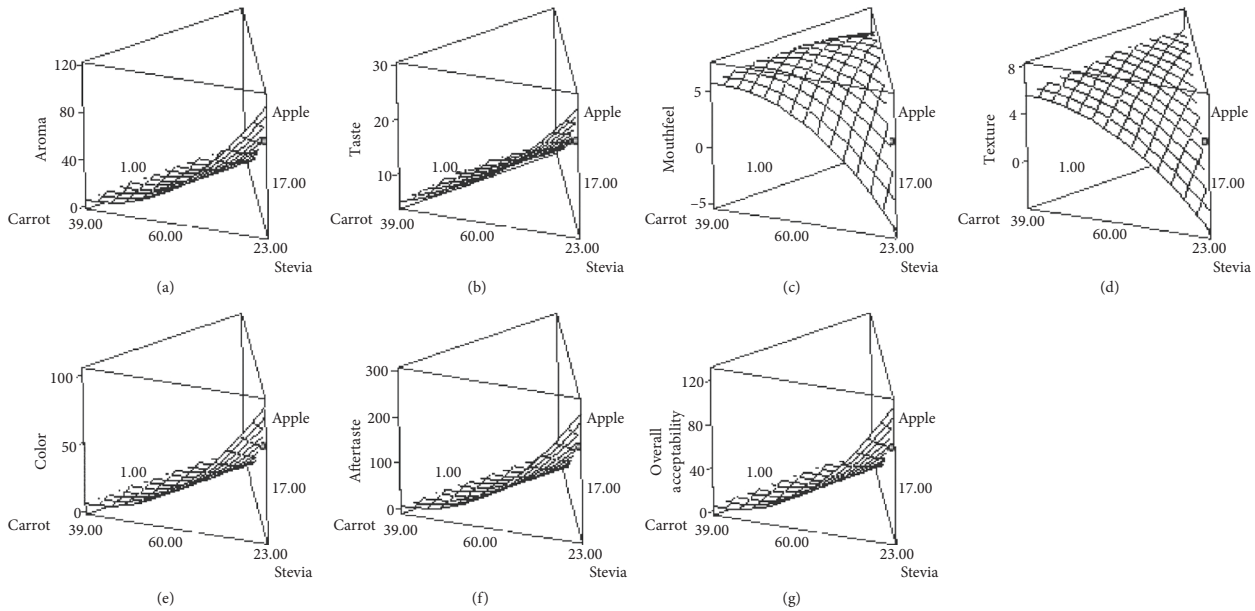

Figure 1: Mixed surface plot representation of sensory attributes.

Table 2: Physicochemical parameters, phenolic profile, and antioxidant activity for processed mixed fruit beverages

Treatments	Physicochemical parameters			Phenolic profile		Antioxidant activity	
	pH	TSS (°Brix)	Titratable acidity (% MA)	TPC (mg GAE/ 100 mL)	TFC (mg CE/ 100 mL)	DPPH (%)	FRAP (mmol Trolox/100 mL)
C	$3.89 \pm 0.03^{n.s.}$	$10.03 \pm 0.06^{n.s.}$	$0.34 \pm 0.04^{n.s.}$	54.02 ± 1.53^{abcd}	12.09 ± 0.13^{abc}	54.15 ± 1.61^{abcd}	53.64 ± 1.12^{abcd}
P	$3.82 \pm 0.02^{n.s.}$	$10.23 \pm 0.10^{n.s.}$	$0.41 \pm 0.05^{n.s.}$	30.90 ± 2.01^{abcde}	7.30 ± 0.11^{abcde}	36.89 ± 1.70^{abcde}	47.55 ± 1.03^{abcd}
US_{20}	$3.88 \pm 0.03^{n.s.}$	$10.07 \pm 0.09^{n.s.}$	$0.33 \pm 0.05^{n.s.}$	61.11 ± 1.58^{abcde}	14.36 ± 0.15^{abc}	49.43 ± 1.91^{abcde}	59.70 ± 0.94^{abcd}
US_{40}	$3.86 \pm 0.01^{n.s.}$	$10.20 \pm 0.05^{n.s.}$	$0.29 \pm 0.03^{n.s.}$	69.60 ± 0.89^{abcde}	16.91 ± 0.14^{abcd}	57.96 ± 1.69^{abcd}	62.28 ± 1.41^{abc}
US_{60}	$3.87 \pm 0.01^{n.s.}$	$10.13 \pm 0.05^{n.s.}$	$0.35 \pm 0.06^{n.s.}$	78.65 ± 1.37^{abcde}	23.53 ± 0.15^{abcde}	66.43 ± 1.45^{abcde}	65.17 ± 1.10^{abcd}
US_{80}	$3.88 \pm 0.02^{n.s.}$	$10.21 \pm 0.12^{n.s.}$	$0.38 \pm 0.07^{n.s.}$	57.33 ± 1.42^{abcd}	13.22 ± 0.12^{abc}	43.20 ± 1.73^{abcde}	50.29 ± 1.00^{abc}

C = control; *P* = pasteurization; US_{20} = sonication treatment at 20% amplitude; US_{40} = sonication treatment at 40% amplitude; US_{60} = sonication treatment at 60% amplitude; US_{80} = sonication treatment at 80% amplitude. Statistically significant differences indicated by various letters. n.s.: not significant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments; abcde: one treatment is significantly different from other five treatments.

sugar. Actually, stevia leaves are several times sweeter principally due to steviol glycosides than table sugar but have no calories [25]. Besides its sweetening properties, stevia is a good source of antioxidant compounds, phenolic compounds, and flavonoids [26].

3.2. Optimization. The numerical optimization gave 14 different formulations having different desirability indices. The formulation with the maximum desirability index of 0.887 was chosen based on the set parameters and constraints. It could be interpreted as "good" to "very good," from the consumer response regarding acceptance [27]. The maxima obtained in the plot was considered as the optimum formulation (apple juice: carrot juice: stevia extract (70 : 27 : 3 expressed as % v/v)). Moreover, the sensory scores of predicted compositions showed less than 5% deviation from their actual counterpart in terms of sensory scores, indicating the validity of the optimized blend formulation. Therefore, blend with composition of 70% apple juice, 27% carrot juice, and 3% of stevia extract was selected as optimum, based on sensory scores and the highest desirability index.

3.3. Effect of Sonication on Physicochemical Profile (pH, TSS, and TA). Results regarding the impact of pasteurization and ultrasound processing on titratable acidity, pH, and total soluble solids are shown in Table 2. It is clear from the data that there were not any significant ($p > 0.05$) changes among different treatments regarding pH (3.82 ± 0.02 to 3.88 ± 0.03), titratable acidity (0.29 ± 0.03 to 0.41 ± 0.05 %), and total soluble solids (10.07 ± 0.09 to 10.23 ± 0.10 °Brix) of juices after processing as compared with unprocessed juices. Processing did not induce any change in the pH, titratable acidity, and total soluble solids of mixed fruit juices; rather, these parameters remained stable even under different treatments. Trends of our results regarding titratable acidity, pH, and total soluble solids are in accordance with observations of sonicated apple juice [13]. It has been reported

that sonication treatment causes a significant decrease in °Brix of kasturi lime juice [28]; however, we found non-significant effect of sonication on the °Brix of processed juice which is in agreement with observation of ultrasound-treated apple and carrot juice blends [29].

3.4. Effect of Sonication on Phenolic Profile (TPC and TFC). The effect of pasteurization and sonication treatments on the total phenolic contents and total flavonoid contents of processed mixed fruit juices is shown in Table 2. It can be seen that there was a significant ($P < 0.05$) increase in TPC (57.33 ± 1.42 to 78.65 ± 1.37 mg GAE/100 mL) and TFC (13.22 ± 0.12 to 23.53 ± 0.15 mg CE/100 mL) in all the sonicated juice samples as compared to pasteurized and control unprocessed sample. Treatment US_{60} (processed at 60% amplitude level) showed maximum values for TPC and TFC among ultrasound-processed juice samples. Previous study conducted by Santhirasegaram et al. [30] on mango juice reported that total phenolic contents of juice increased from 97.83 ± 1.76 to 132.20 ± 1.96 mg GAE/100 mL after sonication treatment. Similarly, in another study, the sonicated juice samples of kasturi lime exhibited significant increase of TPC and TFC from 263.8 to 336.0 mg GAE/g and 0.26 ± 0.0 to 0.37 ± 0.1 mg CE/g, respectively, as compared to unprocessed sample [28]. The possible reason for significant increases in these phenolic phytonutrients might be attributed to efficient discharge of bound polyphenolic phytonutrients from the cell wall as the sonication caused disruption of cells. Inactivation of certain enzymes, for example, polyphenol oxidase degradation, also linked with this increment. In fact, sonication process produces cavitation pressure which helps cell wall to rupture and releases bound form of phenolic contents. Moreover, the insertion of hydroxyl group to the aromatic ring compounds caused by the sonication is also linked with higher phenolic contents after sonication [12].

3.5. Effect of Sonication on Antioxidant Potential (DPPH and FRAP). Results of our study showed that the sonication process significantly ($p < 0.05$) improved the antioxidant potential including DPPH and FRAP assay of mixed fruit juice (Table 2). All ultrasonic processed treatments showed significant change for DPPH and FRAP ranging between 43.20 ± 1.73 to $66.43 \pm 1.45\%$ and 50.29 ± 1.00 to 65.17 ± 1.10 mmol Trolox/100 mL, respectively, as compared to pasteurized and control unprocessed sample. Values of both parameters significantly increased in ultrasound-treated samples as compared to thermally pasteurized and control ones. Among ultrasound-processed samples, treatment US_{60} (processed at 60% amplitude level) showed maximum values for DPPH and FRAP assay. The observation was in agreement with that of sonicated grapefruit juice and apple-based beverage, and sonication treatment applied for 90 min showed significant increase in DPPH value (46.94 ± 0.86 %) (Abid et al. [13]). Similarly, in another study, maoberry juice sonicated at 40% amplitude exhibited increase of FRAP value (26.08 ± 3.49 mM $FeSO_4$/100 mL) as compared to the control sample [31]. The higher antioxidant activity in sonicated juice samples might be due to the increment in the concentration of ascorbic acids and other antioxidants such as polyphenolic compounds. This might happen due to the production of cavitation during ultrasound processing which enhances the rate of extraction and availability of bound components. Therefore, it can be concluded that sonication positively improves the activity of DPPH and FRAP by increasing concentration of organic acids and phenolic compounds [32].

4. Conclusion

To improve the sensory attributes and nutritional composition of beverages, blending of different fruit juices is very efficient method. According to D-optimal mixture design, a formulation composed of 70% apple, 27% carrot, and 3% stevia extract showed highest score for sensory attributes. Further, formulated processed apple-based mixed fruit juices were characterized for physicochemical analysis, phenolic profile, and radical scavenging activity. It is observed that blending causes the rise in the phenolic profile and radical scavenging activity of formulated apple-based mixed fruit beverage. In addition to that, best results of all assessed parameters were found for ultrasound treatment processed at 60% amplitude for 15 min. Moreover, use of stevia as natural sweetener to develop functional apple-based beverage may improve nutritional status of and health benefits for the consumers.

Data Availability

The actual data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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