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IMPACT OF ULTRASOUND PROCESSING ON PHYSICOCHEMICAL AND BIOACTIVE ATTRIBUTES OF GRAPE BASED OPTIMIZED FRUIT BEVERAGE

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Blends by mixing different fruit juices gained popularity due to nutritional value, flavor and health benefits. In this study, the compositions of grape, apple juice and sugar solution were optimized by D-optimal design to develop a functional beverage with acceptable sensory attributes. Best formulation with 80:17:3 (v/v) ratio was processed via ultrasound and pasteurization. Among studied parameters, pH (3.44) and TA (0.70%) were significantly increased after sonication but reduced after pasteurization, while for TSS (15.99 °Brix) increase was non-significant for both processing techniques. Phenolic profile including TPC (1503 µg GAE/ml) and TFC (488 µg CE/ml), radical scavenging activity including DPPH (72.8 %) and FRAP (651 mmol FE/ml) and organic acids including tartaric acid (2583 µg/ml), citric acid (254 µg/ml), malic acid (3671 µg/ml) and methylmelonic acid (MMA) (136.6 µg/ml) were increased after sonication treatment for 20 min but reduced in pasteurization. A principal component analysis (PCA) was also used to identify the pattern in the data and express in a way as to underscore the similarities and dissimilarities among the analyzed parameters. Finally, US treatment was recommended as process for retention of maximum antioxidants in the optimized beverage.
Keywords: Functional beverage, pasteurization, ultrasound, phenolic compounds, radical scavenging activity

INTRODUCTION

Nowadays fruit beverages are considered as nutritionally rich and active food class because of their ease and likelihood to meet the consumer requirements for ampule contents, appearance; ease of dissemination and enhanced shelf life along with great potential to incorporate bioactive compounds and desirable nutrients (Kausar *et al.*, 2012). In Pakistan, grapes and apples are mostly cultivated in Baluchistan and Khyber Pakhtunkhwa (GOP, 2010). Blends prepared by mixing different fruit juices are widely acceptable and gain popularity due to nutritional aspects, availability, better flavor and associated health benefits (Oludemi and Akanbi, 2013). Blending of different juice provide unique sensory attributes and a way of efficient use of the underutilized fruits. Some pure fruit juice is unacceptable by many consumers due to intense taste, cost and astringent flavor. Such limitations allow producer to develop mixed fruit juice. Different trials had been conducted by mixing different juices such as snake tomato and pineapple juice (Bamidele and Fasogbon, 2017) carrot, spinach and pineapple fruit juice (Dhaliwal and Hira, 2001) kinnow, aonla, pomegranate and ginger juice (Bhardwaj and Mukherjee, 2005), bael and papaya juice (Tandon *et al.*, 2007) and grape and carrot juice blends (Nadeem *et al.*, 2018).

Inactivation of microorganism and extension of the shelf life can successfully be achieved through use of thermal pasteurization. However, thermally treated juices for example orange, strawberry, and watermelon juices showed reduction in nutritional quality (Bhat *et al.*, 2011). Thermal treatment degrades the phenolics, organic acids, anthocyanins and other valuable compounds. Therefore, substitute food processing technologies are available nowadays showing minimal adverse effects, among which ultrasound is well known and extensively studied by the researchers (Bhat *et al.*, 2011). Sonication is also having the potential to achieve the US Food and Drug Administration (FDA) condition of a 5-log reduction of food borne pathogen s in fruit juices. Ultrasound processing provides the simplicity of effective mixing, reduced thermal and concentration gradients, quick energy and mass transfer rate, reduced temperature, minimize equipment size, selective extraction, faster response to process extraction control, amplified production, quicker start-up, and abolition of process steps (Chemat and Khan, 2011). Ultrasound treatment produces cavitation phenomena and mass transfer during food processing which ultimately provide the preservation effect (Luque-Garcıa and De Castro, 2003).

Blended fruit juices are the most efficient functional food category. Novel food processing technologies were included in the industries due to the consumers demand for nutritious and healthy food product. Therefore, the current research was designed to explore the consequence of conventional and novel processing on physicochemical attributes, functional characteristics, radical scavenging activity and organic acids profile of mixed fruit beverage.

MATERIAL AND METHODS

***Reagents and raw material*:** Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan), Merck (Merck KGaA, Darmstadt, Germany) and Uni-Chem chemical reagents were the source of all chemical and reagents. Grape and apple fruits were collected from indigenous fruit market of Faisalabad. The fruits were washed, peeled and juice was extracted using household juicer (OT-MJ176A, Guangdong, China). Sugar solution (50%) was prepared using distilled water in the laboratory.

***Juice formulations*:** Juice was extracted by household juicer (OT-MJ176A, Guangdong, China). Fourteen (14) different formulations of functional beverages using grapes, apple juice and sugar solution were developed and optimized using D-optimal mixture design (Table 1). Best formulation based on sensory attributes was further processed through pasteurization and ultrasound application.

***Sensory evaluation*:** The sensory assessment was conducted by the panel of 20 semi-trained participants on a 7-point structured hedonic scale (where, 1= dislike extremely", "2= slightly dislike", 4=dislike, "4=Average", "5=Fair", "6=Good", "7=Extremely Good").

Optimization: Numerical optimization method was adopted to obtain the optimal values of sensory scores for each sensory parameter. This method was directed a highest desirability index (D), as calculated by Equation 1 given below. Here, d_i is the desirability index for *i*th parameter showing the relative importance of r_i.

$$D = (d_1^{r1} \times d_2^{r2} \times d_3^{r3} \times d_4^{r4} \times d_5^{r5} \times d_6^{r6} \times d_7^{r7})^{1/(r_1+r_2+r_3+r_4+r_5+r_6+r_7)} \quad (1)$$

In optimization study the desirability index (d_i) was established on its target value (highest or lowest) analogous to each constraint (both process parameter and responses). The value of D as well as d_i was within the range of 0 (minimum required) and 1 (maximum required). Each parameter adjusted possessing the significance of each sensory constraint and its relative significance (r_i) to attain good consumer acceptance. Maximization of sensory scores was the standard set for optimization of the mixed fruit beverage and it is highly significant constraint in any product development investigation. Maximum desirability indices were being used to the interpolated optimized composition of design.

***Ultrasound processing*:** The optimized formulated beverage (100 mL) was processed by sonication apparatus (VCX750, Sonics & Materials, Inc. Newtown, CT, USA) at 750W power and 20 kHz frequency. The ultrasound probe was inserted in the depth of 25 mm in the beverage to constantly sonicate at 100% amplitudes for three different time durations i.e. 20 min (US_1), 30 min (US_2) & 40 min (US_3) (Alighourchi *et al.*, 2013).

***Thermal pasteurization processing*:** A 100 mL juice sample was heated using hot plate (Corning 6798-420D) at 90°C in a beaker for 10-15 seconds and then places the sample in water bath for 30 min to cooled down to room temperature (Rabie *et al.*, 2015).

***Measurement of pH, total soluble solids (TSS) and titratable acidity (TA)*:** The pH was determined through benchtop pH/mv meter (PHS-25 CW, Zhejiang, China). The TSS content was measured by using a refractometer (RHW-80wATC, Fujian, China). For the determination of the TA, 20 mL juice sample with 80 mL distilled water was taken in a beaker. This solution was then titrated against standardized 0.1 N NaOH and the phenolphthalein (pH 8.2±0.1) was used

Table 1. Response of the sensory evaluation of mixed fruit juice formulations.

Formulation	Grape juice (%)	Apple juice (%)	Sugar sol. (%)	Aroma	Taste	Mouth feel	Texture	Color	Aftertaste	Overall acceptability
F_1	60	37	3	5.4	5.4	5.6	5.6	5.7	4.9	4.2
F_2	70	27	3	6.0	5.9	5.9	6.1	6.2	5.4	6.0
F_3	80	18	2	5.7	5.4	5.9	6.7	7.0	5.8	6.1
F_4	80	17	3	5.8	5.4	5.5	5.9	6.2	5.7	5.4
F_5	75	23.5	1.5	5.6	4.5	4.9	5.1	5.9	4.8	5.0
F_6	65	33.5	1.5	5.7	5.3	5.4	5.7	5.9	5.3	5.6
F_7	60	38	2	5.3	6.1	6.2	6.5	6.4	4.9	5.9
F_8	70	28	2	5.2	4.9	5.1	5.6	5.8	5.4	5.3
F_9	80	17	3	5.8	5.4	5.5	5.9	6.2	5.7	5.4
F_{10}	60	37	3	4.8	4.9	4.9	5.4	5.7	5.1	5.4
F_{11}	80	19	1	6.2	6.4	6.4	6.2	6.8	5.8	6.7
F_{12}	60	39	1	5.2	5.2	4.9	5.5	5.9	5.1	5.4
F_{13}	60	39	1	5.4	4.9	4.7	4.9	6.1	5.1	5.1
F_{14}	70	29	1	5.3	5.2	4.9	5.7	6.2	5.3	5.6

as indicator. The volume of NaOH was converted to citric acid (g)/100mL of juice (Alighourchi and Barzegar, 2009) using the equation given below:

$$TA (\%) = V \times 0.1\ N\ NaOH \times 0.067 \times 100/m$$

where V is volume of NaOH, and m is mass of fruit beverage (g)

Phenolic profile *total phenolic contents (TPC)*: TPC were calculated through Folin–Ciocalteu reagent (Tezcan *et al.*, 2009). One mL of Folin–Ciocalteu reagent (1 N) was mixed to 0.5 mL of filtered juice sample. Mixture was incubated for 6 min. Then, 2 mL of Na_2CO_3 (20%) was incorporated in the mixture. Mixture retained for 60 min of reaction at 30°C, then absorbance measured at 765 nm through spectrophotometer (Specord 200/plus, Germany). TPC were showed as µg gallic acid equivalents per ml of juice sample (µg GAE/mL).

***Total flavonoid contents (TFC)*:** TFC were calculated using the method described by Kim *et al.* (2003). Briefly, 1.25 mL of deionized water and 75 µL $NaNO_2$ solution (5%) were incorporated in 0.25 mL of the filtered juice sample. Incubated the mixture for 15 min and then, 150 µL $AlCl_3$ solution (10%) was incorporated. Mixture retained for 5 min and then, addition of 0.5 ml NaOH (1 M) was made. Distil water was added up to 2.5 mL and mixed well. Absorbance was taken at 415 nm. TFC were expressed µg catechin equivalents per ml of juice sample (µg CE/mL).

Radical scavenging activity:

***DPPH assay*:** DPPH assay was performed by using the method described by Khan (2010). One mL of DPPH (20 ppm) was added into 25 µL of filtered juice sample. The mixture was incubated for 30 min in dark. Absorbance was taken at 517 nm. The DPPH % was calculated by the following equation.

$$DPPH\ \% = [A_c - A_s]/ A_c] \times 100$$

Where, Ac was the absorbance of control sample and As was the absorbance of test sample.

***FRAP assay*:** FRAP assay was performed by using the method described by (Rupasinghe and Clegg, 2007). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 1 mM TPTZ solution and 20 mM ferric chloride in the ratio 10:1:1 and heated up to 37°C. Mixed fruit juice sample (6 µL) was added in the mixture. Retain the sample for 6 min. The absorbance was measured at 593 nm. Value of FRAP assay was expressed in mmol FE/mL of juice.

***Quantification of organic acids*:** Quantification of organic acids was done by using HPLC according to the method discussed by Tembo *et al.* (2017). A 20 mL filtered juice sample was incorporated in meta-phosphoric acid (0.3 g/L, 40 mL), mixed for 20 seconds and centrifuged (4000 rpm, 10 min, 4°C). The solution was passed through a Millipore 0.45 µm PTFE filter into amber glass HPLC vials. Quantification was conducted with HPLC (RF-10 AxL, Shimadzu, Japan) equipped with UV-visibledetector and Gemini C18 column (25 cm × 4.6 mm, 5 µm). Isolation was performed out under isocratic conditions (0.5 mL/min) using 0.1% mL H_3PO_4 (pH 2.6) as the mobile phase. Chromatograms of organic acids [citric acid (CA), malic acid (MA), tartaric acid (TA), methylmalonic acid (MeA)] were recorded at 215 nm and results were expressed in µ/mL.

***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 mixed fruit juices. The data obtained from ultrasound treatment and principal component analysis (PCA) were further subjected to statistical analysis using SPSS 21 Statistical Software and expressed as means ± standard deviations. Results were analyzed for variance at significance level of 5% and evaluated by LSD post hoc test.

RESULTS AND DISCUSSION

***Sensory analysis*:** All the 14 combinations of mixed juice formulations were quite satisfactory for the panelists (Table 1). However, eleven diverse combinations showed higher (> 5.0) results for overall sensory scores among all formulations. It was clearly revealed that the sensory quality of final product was significantly affected by the percentage of ingredients. All sensory attributes were determined using mixture experimental design. The outcomes for the D-optimal mixture design were suitable to response surface model. In current study, the highest sensory score for aroma, taste, mouth-feel and overall acceptability was obtained for formulation F_{11} composed of 80% grape juice, 19% apple juice and 1% sugar solution (Table 1). While for texture and color highest value was obtained for formulation F_3 composed of 80% grape juice, 18% apple juice and 2% sugar solution. For after-taste both formulations (F_3 and F_{11}) shared maximum values. 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.

***Optimization*:** Different concentrations of grape, apple and sugar was added. Sugar solution was mainly added to counter the bitter taste of fruit juices. Different desirability indices were observed on 14 different compositions produced by the numerical optimization (Table 2). The combination with the maximum desirability index of 0.587 was selected based upon set attributes and constraints. The optimum value in the plot was considered as the optimum combination (grape juice: apple juice: sugar solution= 80:19:1 expressed as % v/v). Incorporation of maximum grape juice in blend gave the highest sensory score. It could be deduced as 'good' to 'very good', from the consumer response regarding acceptance (Peryam and Pilgrim, 1957). Nevertheless, the sensory scores of expected compositions exhibited deviance from their actual counterpart in terms of sensory scores, showing the validity of the optimized blend formulation. Therefore, blend having

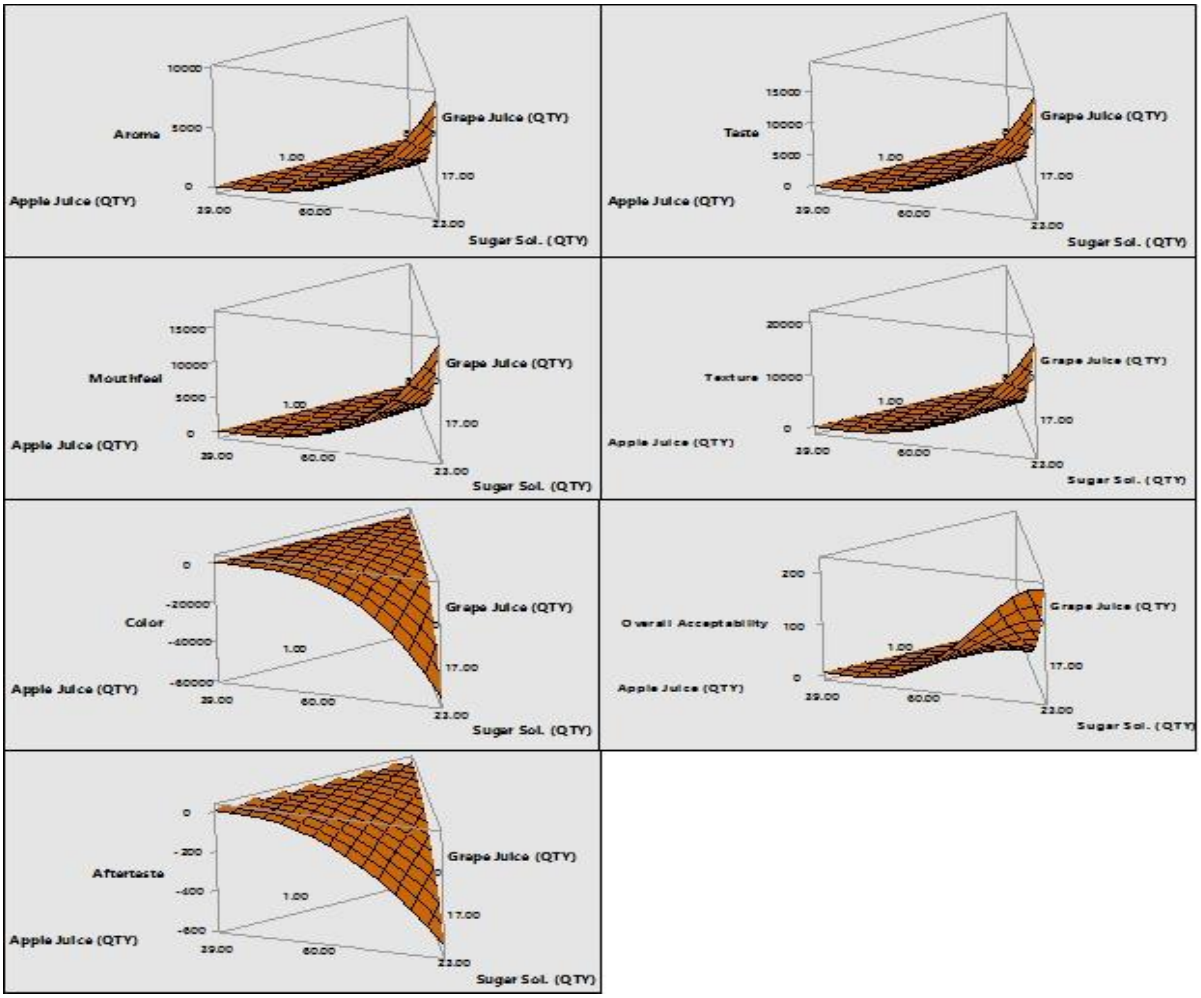

Figure 1. Mixed surface plot representation of sensory attributes (component amounts).

80% grape juice, 17% apple juice and 3% sugar solution was selected as optimum, based on sensory scores and the maximum desirability index. Optimum juice formulation was further treated with heat and different level of sonication.

Table 2. Constraints for optimization and validation of the optimized formulation for mixed fruit juice.

Responses	Optimized condition Predicted at D-0.5877	Desirability	Goal	Importance
Aroma	6.329	0.695	Max.	1
Taste	6.101	0.640	Max.	1
Mouthfeel	6.035	0.581	Max.	1
Texture	6.017	0.532	Max.	1
Color	6.145	0.204	Max.	1
Aftertaste	6.313	1.000	Max.	1
Overall acceptability	6.622	0.865	Max.	1

***Effect on pH, TSS & TA*:** Effect of pasteurization and ultrasound processing on pH, total soluble solids (TSS) & titratable acidity (TA) of juices were studied in comparison with control samples (Table 3). All processed treatments showed significant change for pH and total soluble solids except titratable acidity having non-significant change ($P > 0.05$). The unprocessed sample had a pH of 3.47±0.03, while it varied from 3.39 to 3.43 for processed samples. pH values significantly decreased in comparison to control. Pasteurized juice sample showed maximum value of TSS 16.07±0.03 °Brix while treatment US_3 gave highest value of TA 0.70±0.04 %.

The current investigation agrees with some of previous conducted studies in different juices. pH, TA and TSS of grape-apple juice blends were slightly different due to different processing techniques. Pomelo juice treated with conventional pasteurization also showed similar trends (Kumar *et al.*, 2017). Hydrolysis of sucrose and production of lactic acid might be associated with reduction in pH value.

Table 3. pH, TSS & TA, phenolic profile and radical scavenging activity for processed mixed fruit juice.

Treatments	pH	TSS (Brix°)	Titratable acidity (%)	Phenolic profile		Radical scavenging activity	
				TPC (µg GAE/mL)	TFC (µg CE/mL)	DPPH (%)	FRAP (mmol FE/mL)
C	3.47±0.03[a]	15.89±0.03[abc]	0.69±0.02[ns]	1431±2[a-d]	373±3[a-d]	61.6±2.3[a-d]	531±2[a-d]
P	3.43±0.03[ab]	16.07±0.03[a-d]	0.68±0.05[ns]	1404±1[a-d]	323±3[a-d]	57.4±1.8[a-d]	475±3[a-d]
US_1	3.44±0.03[abc]	15.93±0.03[abc]	0.68±0.02[ns]	1487±1[a-d]	469±5[a-d]	70.4±1.4[abc]	622±4[a-d]
US_2	3.41±0.04[ab]	15.97±0.05[abc]	0.69±0.01[ns]	1503±2[a-d]	488±4[a-d]	72.8±1.8[abc]	651±4[a-d]
US_3	3.39±0.03[ab]	15.99±0.01[abc]	0.70±0.04[ns]	1463±1[a-d]	423±3[a-d]	68.5±1.9[a-d]	612±1[a-d]

Statistically significant differences indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatments; 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.

The increase in TSS might be linked with the evaporation process due to heat processing which reduced the water content to some extent or may be due to citric acid surge the TSS (Kumar *et al.*, 2017). The increase in titratable acidity is due to oxidation of reducing sugars contributing to increase in the acidity of fruits. These results are also in agreement with those of apple-carrot juice blend treated with ultrasound also showed similar trends (Gao and Rupasinghe, 2012). Generation of new chemical compounds in the juice media after sonication might be linked with the changes in pH after sonication (Martínez-Flores *et al.*, 2015).

Phenolic profile (TPC & TFC)**:** Mean values regarding TPC and TFC of different treatments of processed juice blends are presented in Table 3. All processed treatments showed significant change for TPC and TFC ($P < 0.05$). Values of both parameters significantly increased in US treated samples as compared to thermally pasteurized and control ones. Among processed samples, treatment US_2 (processed for 20 min) showed higher results of TPC (1503±2 µg GAE/mL) and TFC (488±4 µg CE/mL) as compared to thermally pasteurized and control sample.

Alike outcomes were shared by Santhirasegaram *et al.* (2013) during the sonication of mango juice reported higher flavonoids contents. These compounds efficiently discharged from the cell wall as the sonication caused disruption of cells. Inactivation of certain enzymes, for example polyphenol oxidase caused degradation, also linked with the increment of flavonoids after sonication (Santhirasegaram *et al.*, 2013). Similarly, sonicated juice samples of Kasturi lime exhibited significantly higher total phenolic contents as compared to unprocessed sample. This surge was noted from 263.8 up to 336.0 mg GAE/g (Bhat *et al.*, 2011). This surge in TPC was due to breakage of cell wall as a result of cavitation pressure by sonication which released bound form of phenolic contents. Insertion of hydroxyl group to the aromatic ring compounds caused by the sonication also linked with higher phenolic contents after sonication (Aadil *et al.*, 2013).

Radical scavenging activity (FRAP & DPPH)**:** Mean values regarding DPPH and FRAP of processed fruit juice blends are shown in Table 3. All processed treatments showed significant change for DPPH and FRAP ($P < 0.05$). The maximum value of DPPH and FRAP of processed blended juice samples was 72.8±1.8% and 651±4 mmol FE/mL, respectively, observed in treatment US_2 (processed for 20 min). Values of both parameters significantly increased in US treated samples as compared to thermal pasteurized and control.

Similar findings were showed by Abid *et al.* (2013) who efficiently processed grape juice and apple-based beverage through sonication treatment for 20, 60 and 90 min. Significantly higher values of antioxidant activity were observed after US treatment. The higher outcomes in the form of antioxidant activity might be linked with increase in the concentration of ascorbic acids and other antioxidants (polyphenolic compounds). This might happen due to the creation of cavitation during US treatment which enhances the rate of extraction and availability of these mentioned compounds. Therefore, it can be concluded that sonication positively improve the activity of DPPH and FRAP by increasing concentration of organic acids and phenolic compounds (Mraihi *et al.*, 2013). Cavitation process also disrupts the cells, thus caused release of internal contents including some phenolics present in the bound form with cell wall contents. Compounds having antioxidant activity are extremely sensitive to heat thus become degraded through conventional pasteurization resulted in reduced DPPH and FRAP activity. At higher temperature, they are susceptible to oxidation as given in orange juice (Scalzo *et al.*, 2004).

Organic acids profile**:** Mean values regarding organic acids (tartaric, malic, citric and MMA) of different processed fruit juice blends are presented in Table 4. All processed treatments showed a significant change ($P < 0.05$) in organic acids profile with maximum values of tartaric, malic, citric and MEA at 2583±1 µg/mL, 3671±1 µg/mL, 254±1 µg/mL and 136.5±0.9 µg/mL, respectively, in treatment US_2 (treated for 20 min). Processing of juices through sonication improved the values of organic acids as compared to control sample.

Other studies conducted on sonication of apple juice also produce the similar findings. Up to 75.5 % retention of ascorbic acid was observed in sample sonicated for 10 min (Tiwari *et al.*, 2009). Similar findings were shared by Cheng *et al.* (2007) who reported higher organic acid in sonicated

Table 4. Organic acids (µg/mL) for processed mixed fruit juice formulation.

Treatments	Tartaric acid	Citric acid	Malic acid	Methylmelonic acid
C	2507±2^{a-d}	196±1^{a-d}	3643±1^{abc}	80.1±2.3^{abc}
P	2488±2^{a-d}	175±1^{a-d}	3598±4^{a-d}	63.6±2.0^{abc}
US_1	2567±1^{a-d}	240±2^{a-d}	3655±2^{a-d}	121.9±1.7^{a-d}
US_2	2583±1^{a-d}	254±1^{a-d}	3671±1^{a-d}	136.6±0.9^{a-d}
US_3	2558±1^{a-d}	232±2^{a-d}	3650±2^{abc}	109.5±1.0^{a-d}

C: control; P: pasteurized; US_1, US_2, US_3: ultrasound processed; US_1: (10 min); US_2: (20 min); US_3: (30 min). Statistically significant differences indicated various letters. abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

Table 5. Total variance explained in PCA for ultrasonic processed and pasteurized juice samples.

Component	Initial Eigenvalues (US)			Initial Eigenvalues (P)		
	Eigenvalues	% of Variance	Cumulative %	Eigenvalues	% of Variance	Cumulative %
1	8.016	72.874	72.874	8.950	81.363	81.363
2	1.362	12.381	85.255	1.182	10.742	92.105
3	1.018	9.252	94.507	0.691	6.278	98.383
4	0.470	4.273	98.780	0.161	1.463	99.846
5	0.061	0.553	99.333	0.017	0.154	100.000
6	0.051	0.466	99.799	0.000	0.000	100.000

P: pasteurized; US: ultrasound processed

guava juice. . This trend after sonication could be directly related to the releases of diffused oxygen in juice due to cavitation phenomena created by the ultrasound waves. Hence, sonication is effective treatment to improve the juice quality by increasing the concentration of desirable acids. On the other hand, significant reduction in organic acids after conventional heat treatment (pasteurization) was observed, which is associated with hydrolysis of organic acids as these compounds are very much heat sensitive (Igual *et al.*, 2010).

***Principal component analysis (PCA)*:** It identifies the pattern in the data and express in a way as to underscore their similarities and dissimilarities (Shin *et al.*, 2010). It groups the data and retains the information of original data set. The integrity of data does not compromise. In case of ultrasound processed and pasteurized samples, Table 5 showed the total variance elaborated in PCA procedure. Regarding ultrasound samples highest eigenvalue i.e. 8.016 was reported for first principle component (PC1), considered for 72.874% of the variability while second (PC2) and the third (PC3) had eigenvalues of 1.362 and 1.018, considered for 12.381 and 9.252% of the variance, respectively. While for pasteurized samples, first principal component (PC1) had the highest eigenvalue of 8.950, accounted for 81.363% of the variability and second (PC2) had eigenvalues of 1.182 accounted for 10.742% of the variance. Since eigenvalues values smaller than 1.0 are not of utmost importance, only three (ultrasound) and two (pasteurization) PCs were used for further study.

First three dimensions (ultrasound) explained 94.507% of the variances of all measured parameters (Table 5) while first two dimensions (pasteurization) explained 92.105% of the variances of all measured parameters. The loading variables of PCA in the first three PCs for ultrasound processed samples (Fig. 2) while in the first two PCs for pasteurized samples (Fig. 3), representing the correlation between tested parameters. Thus, for ultrasound processed samples, PC1 and PC3 were inversely related to pH and TSS, and directly related to remaining parameters, while PC2 was directly related to pH, TA, TPC, TFC and malic acid. Similarly, in pasteurization processing, PC1 was inversely related to TSS, and PC2 was inversely related to pH, TSS and DPPH. As exhibited in Figure 2 & 3 the whole data was confined to three and two isolated sets for sonicated and pasteurized juices respectively All this information obtained from Figure 2 & 3 well considered for the actual data. These findings were in accordance with the results summarized in in previous tables of mean values.

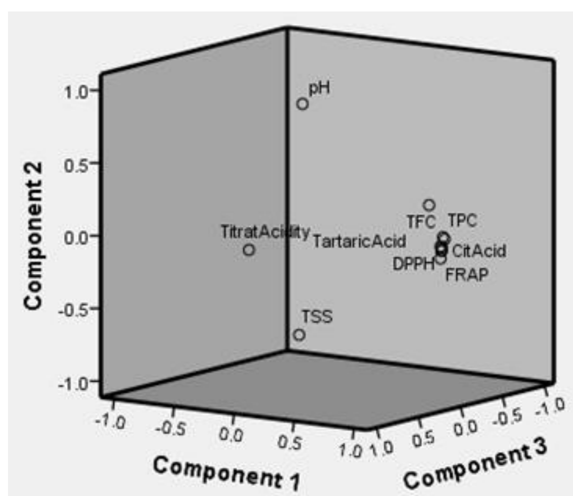

Figure 2. PCA score plot of ultrasound processed juices.

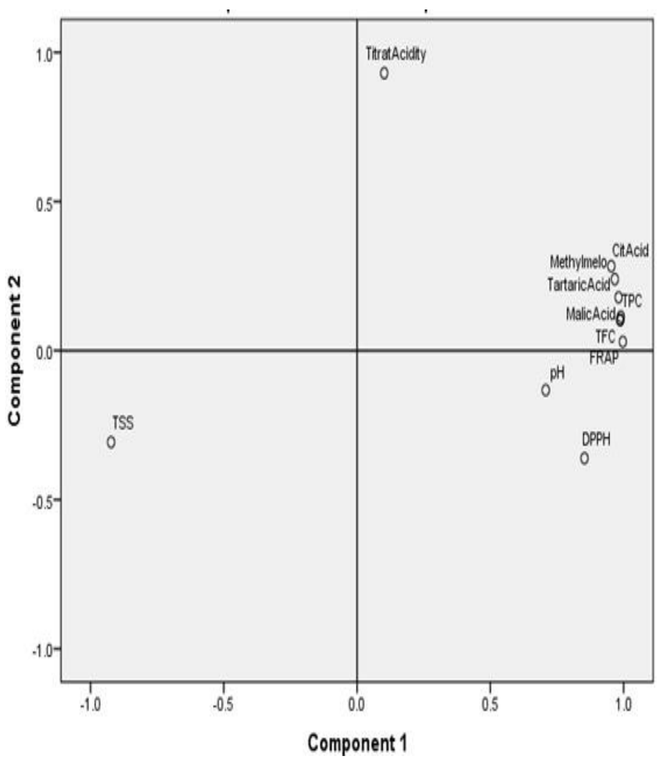

Figure 3. PCA score plot of pasteurized juices.

***Conclusion*:** A mixed fruit grape based functional beverage (grape: 80; apple 17; and sugar solution 3 v/v) was optimized, which had highest sensory score with 0.587 desirability. The physicochemical and functional components of the optimized beverage were characterized, according to the findings. The integrated effect of mixing of juices showed increment in the content of organic acids and bioactive compounds. This investigation expands the knowledge in area of effect of sonication in comparison with thermal pasteurization. It was observed from the outcomes ultrasonic processing technology is green and non-thermal process which increase the functional characteristics of optimized blend. Surge was observed in phenolic profile (TPC and TFC), radical scavenging activity (DPPH and FRAP) and organic acids (tartaric acid, citric acid, malic acid and MMA) after sonication as compared to thermal pasteurization. Therefore, we should look for novel processing techniques which minimally influence their composition.

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