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Development and Shelf-Life Study of an Antioxidant Rich Mix Fruits and Vegetable Juice

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A B S T R A C T

The present study aimed to develop a mix fruits and vegetable juice and assessment of sensory, physical, and nutritional quality of developed juice and shelf life. Various combinations of juices of three fruits and one vegetable were tried and sensory quality was assessed. Based on the sensory quality scores, one recipe was finalized. The finalized juice recipe was further studied for sensory, physical and nutritional quality- as assessed by antioxidant contents and antioxidant activity and microbial quality. The freshly prepared juice had mean pH 4.68, total soluble solids (TSS) 15.50 and total acidity 0.31. The raw ingredients and freshly prepared mix fruits and vegetable juice were assessed for different qualities. The freshly prepared mixed juice contained considerable amounts of vitamin C, vitamin E, β-carotene, flavonoids and showed antioxidant activity above 50%. Microbial quality of the juice indicated that the total bacterial counts were within the permissible limits.

***Key words*:** Antioxidant activity, Mix fruits, Vegetable juice, Sensory, Physical, Microbial, Nutritional quality

Fruit juices retain the organoleptic and physicochemical properties of the fruits, hence are important throughout the world [1]. The radical scavenging activity and protection of proteins, lipids, and DNA in body from oxidative damage are the major health benefits of dietary fruit juices [2]. Fruit juices contain vitamin C, phenolic compounds and carotenoids in major bioactive oxidants [3]. Dietary intake of the antioxidants reduces the cardiovascular, neurodegenerative and inflammatory diseases, prevention of some cancer, muscular degeneration etc. [3-6]. There is a great demand of fruit juices and fruit-based products in the market with their enhanced sensorial and nutritional attributes. This can be achieved by mixing (blending) of different fruit juices which can also help in overcoming the high cost of scarce fruits and only their seasonal availability. The mixing is also helpful in balancing of sourness, strong flavors, pungency, bitter taste, total soluble solids and color. The mixing may also improve the phytochemicals properties which offer to regulate sugar/acid ratios and reimburse detrimental juice uniformity [7]. Kinnow (*Citrus reticulata* cv. Kinnow Blanco), guava (*Psidium guajava* L.), aonla (*Phyllanthus emblica*) and carrot (*Daucus carota* L.) are available abundantly in Rajasthan. Therefore, nutrient dense mix fruits and vegetable juice can give out the maximum nutrients in a combined form at a time rather than having a single fruit or vegetable for a single vitamin.

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MATERIALS AND METHODS

The mix fruits and vegetable juice was prepared from the fruits (kinnow, guava, aonla) and a vegetable (carrot) available in the local markets of Jaipur city. Three recipes of mix fruits and vegetable juice were prepared using different proportions.

Preparation of product

Healthy fruits and carrots were selected and washed under running water for removal of dirt, microorganisms etc. present on their surface. After peeling of kinnow fruits, juice was extracted in screw type juice extractor. Juice of aonla fruits and carrots was extracted using a hard fruit juicer. Pulp of guava was prepared using a common blender.

The juices and guava pulp were strained through strainer and collected in separate jars. All the four juices and pulp were mixed in different ratios as well as different sugar levels well with the help of stirrer. All blends of juices were mixed and then packed in bottles.

Standardization of recipe

The raw ingredients were weighed and measured by weighing balance (Braun). To achieve uniformity, the amount of raw ingredients was kept constant for blending of each fruit juice in each recipe.

Selection of best recipe

Based on scores of various sensory attributes of all variations of the juice tried, the highest scored recipe was selected for further study.

Quality assessment of the developed juice

Sensory quality: The product was evaluated for subjective sensory evaluation using a panel of 10 experts selected through threshold method and trained using triangle method. Composite score test was used on a five-point scale where panel members were asked to assign scores out of five for each sensory attribute of prepared juice. The recipe having highest sensory scores was prepared in bulk and packed in sterilized bottles and stored at temperature below 0°C for further analyses.

Physical quality: Juice samples were thoroughly mixed and pH was determined by pH meter. The total soluble solids (TSS) of juice was measured by a digital refractometer (Atago, PR-I) and expressed in ºBrix. Total acidity of juice was determined titrametrically with 0.1N NaOH and results were recorded as ml 0.1N NaOH per 100 ml.

Nutritional quality (assessed as antioxidant contents and antioxidant activity)

Raw materials as well as prepared juice were analysed for vitamin E [8], vitamin C [9] and β- carotene [10] using high performance liquid chromatography (HPLC) method. The flavonoid content of the samples was assessed in the methanolic extracts by UV spectrophotometric method [11]. The antioxidant activity in methanolic extraction of samples in terms of percent inhibition of free radicals was estimated using 2, 2 Dipheny-1-picrylhydrazyl (DPPH) method [12].

Microbial quality: Total bacterial and total fungal counts were assessed [1] and expressed as CFU/g.

Shelf-life analysis: Total amount of juice was portioned as 100 ml juice in plastic bottles which were labeled and stored in freezer of household refrigerator i.e., at the temperature below 0ºC. The juice samples were assessed for sensory, nutritional quality including antioxidants and antioxidant activity and microbial quality at 1st, 7th, 15th and 30th day of storage duration.

RESULTS AND DISCUSSION

Sensory quality of various combinations of mix fruits and vegetable juice

In the present study mix fruits and vegetable juice was prepared by mixing of separate juices and pulp of selected fruits (kinnow, aonla and guava) and vegetable (carrot) in various proportions. The fruits of kinnow, guava, aonla, and vegetable carrots are most abundantly available in the local market during winter season, and they are rich source of nutrients. Kinnow provided bright color, appealing taste while consistency and flavor were given by guava pulp. Aonla has been added as it is rich in nutritional content and therapeutic values. Carrot contributed to good color (pinkish red) and masking the bitter test of aonla therefore, mixing of fruits and carrot juices was thought to be selected for preparation of new mix fruits and vegetable juice.

Table 1 Sensory quality of all variations of mix fruits and vegetable juice

Juice variations	Color	Consistency	Appearance	Taste	Flavor	Overall acceptability
Recipe-1 (R1)	3.03±0.26	3.25±0.21	3.13±0.33	3.50±0.26	3.26±0.12	3.40±0.14
Recipe-2 (R2)	3.60±0.40	3.43±1.09	3.64±0.18	4.06±0.44	3.86±0.33	3.77±0.24
Recipe-3 (R3)	4.87±0.14	4.63±0.36	4.76±0.19	4.94±0.10	4.54±0.24	4.86±0.16
CD5%	0.266	0.622	0.228	0.280	0.227	0.173

The scores are out of 5 point hedonic scale test
Values are the mean of three replicates

Nutritional quality as assessed antioxidant contents and antioxidant activity in raw ingredients and mix fruits and vegetable juice

The kinnow juice used for development of mix fruit juice contained 0.56±0.025 mg/100g vitamin E, 41.53±0.08mg/100g vitamin C, 3.33±0.13mg/100g β-carotene and 47.55±0.07 mg QE/100g flavonoids and showed 50.08±0.02% antioxidant activity (Table 2). Carrot juice had 0.88±0.04 mg/100g vitamin E, 0.47±0.03 mg/100g vitamin C, 6.92±0.05 mg/100g β-carotene and 6.58±0.08 mg QE/100g total flavonoids. Carrot juice showed 25.17±0.03% antioxidant activity. Earlier Leahu *et al*. [13] reported 5.54 mg/100 ml vitamin C and 5.98 mg GAE/100ml total phenol in fresh carrot juice. Miean and Mohamed [14] reported 232.5 mg/kg dry weight flavonoid in carrots. Carrot juice is an important rich source of carotenoids namely alpha and β-carotene, zeacarotene, lutein and lycopene [15]. Carrot juice reduces the bitterness of 'Kinnow' mandarin juice when blended with carrot juice [16]. Guava pulp contained 0.62±0.04mg/100g vitamin E, 186.35±0.06mg/100g vitamin C, 0.032±0.00 mg/100g carotene and 213.27±0.63mg QE/100g total flavonoids. Guava pulp showed 58.76±0.09% antioxidant activity. Kadam et al. [17] reported 41.5 mg/100g vitamin C in guava pulp. Tanwar et al. [18] reported 246 mg/100 g vitamin C and 0.01mg/100g β-carotene in fresh guava pulp. Choudhary *et al*. [19] reported 64.2 mg/100g total carotenoids, 460 mg GAE/100g total phenol and 56% antioxidant activity in off seasonal 'Allahabad Safeda' guava fruits. Miean and Mohamed [14] reported 1128.5 mg/kg dry weight flavonoid in guava fruit. In the present study, fresh aonla juice contained 63.76±0.15mg/100g vitamin E, 215.38±2.9 mg/100g vitamin C, 0.020±0.00mg/100g β-carotene and 661.38±1.07 mgQE/100g total flavonoids. Agte *et al*. [20] reported high contents of vitamin C (804.4±0.8mg/100g) and polyphenols (1300.3±99.6mg/100g) in fully ripen aonla. The antioxidant activity in terms of DPPH scavenging activity (IC50 values) was reported to be 21.9±0.024.

Table 2 Nutritional quality as assessed by antioxidant contents and antioxidant activity in raw ingredient and mix fruits and vegetable juice (R3)

Juice	Vitamin E mg/100g	Vitamin C mg/100g	β-carotene mg/100g	Flavonoids mgQE/100g	Antioxidant activity (% inhibition)
Kinnow	0.56±0.025	41.53±0.08	3.33±0.13	47.55±0.07	50.08±0.02
Carrot	0.88±0.04	0.47±0.03	6.92±0.05	6.58±0.08	25.17±0.03
Guava	0.62±0.04	186.35±0.06	0.032±0.00	213.27±0.63	58.76±0.09
Aonla	63.76±0.15	215.38±2.9	0.020±0.00	661.38±1.07	68.86±0.10
Mix fruit and vegetable juice (R3)	3.58±0.040	46.14 ± 0.07	9.7 ± 0.067	79.00 ± 5.00	58.84±0.11

Values are the mean of three replicates

The developed mix fruits and vegetable juice was found rich in antioxidants. The major contributory is kinnow which contains flavonoids and phenols. Aonla is an excellent source of vitamin C. Carrots are important source of β-carotene. Guava contains abundant vitamin C and flavonoid compounds therefore all together mixing of the selected fruits and carrot turned into an excellent antioxidant rich mix fruit juice. Bhardwaj and Mukherjee [21], Gao and Rupasinghe [22], Jan and Masih [23], Juhaimi and Ghafoor [24], Leahu *et al*. [13], Balaji and Prasad [7], Mohamed *et al*. [25], Ullah *et al*. [1] also developed different types of ready to serve drinks through blending of different fruit juices in different ratios and found rich in antioxidant contents such as total phenols, flavonoids, vitamin C, β-carotene and antioxidant activity.

Microbial quality of freshly prepared mix fruits and vegetable juice

The freshly prepared mix fruits and vegetable juice was assessed for total bacterial and total fungal counts to assess the microbial quality. The mean total bacterial count ($0.2118x10^4 \pm 6.50$ cfu/g) and total fungal count ($0.86x10^2 \pm 7.637$ cfu/g) revealed that freshly prepared juice was within the permissible limits and safe from microbial contamination.

Shelf-life analysis

Effect of storage on sensory (subjective) quality

The results revealed that there was a significant decrease in the mean scores for all the sensory attributes of the prepared juice when stored for 30 days in freezer at temperature below 0°C. The consistency of the product was decreased non-significantly up to seven days, but after seven days it decreased significantly. However, the product scored overall acceptability more than 3 out of 5 points for all sensory attributes (Table 3) which indicates good quality of the product and by the end of storage period, the mix fruits and vegetable juice was acceptable. Similar decrease in the scores for flavor, color, consistency, appearance, taste and overall acceptability of juice blends decreased with the advancement of storage period were reported by Ndife *et al*. [26] and Balaji and Prasad [7]. The gradual loss in taste and flavor of mix fruit juice was reported due to change in volatile compounds throughout storage [27]. Similarly, loss in taste of juice was observed by Ullah *et al*. [1] during their study on physicochemical and sensory properties of orange juice drink. In the present study, the appearance was based on how the color appealed to the panelist. Loss in color may be due to Maillard reaction occurring due to reaction between reducing sugars and amino acids. Decrease in scores for consistency of the blended mix fruits and vegetable juice might be due to addition of sugar and due to sublimation at low temperature.

Effect of storage on objective sensory attributes

pH: The mean pH of the freshly prepared juice was 5.79±0.19 which did not change till 7 days of storage but thereafter the pH of juice decreased significantly with increase in storage duration. The pH is important in maintaining shelf stability, the flavor and processing of the mix fruit juice. In the present study, there was statistically non-significant decrease in pH of mix fruits and vegetable juice. With the increase in duration of storage, a decline in pH was observed in beverage [7]. Like pattern of declined pH has been reported in ginger-kinnow squash [28]. The decline in pH may be due to degradation of reducing sugar and production of acidic compounds [1].

Total Soluble Solids (TSS)

The mean TSS of fresh mix fruits and vegetable juice was 15.59±0.63 °Brix which increased non-significantly with increase in storage period. The increase in TSS by 3.14% after one month of storage might have resulted into increase in consistency. In lime-aonla and mango-pineapple spiced ready to serve beverage, increase in total soluble solids during storage at ambient and low temperature has been reported [29]. The increase in TSS may also be attributed to conversion of polysaccharides into sugars (monosaccharide and oligosaccharides) and reduction in moisture content during storage [23]. Balaji and Prasad [7] also found an increase in TSS during storage in their study on kinnow - aonla blend RTS beverage.

Table 3 Sensory quality of mix fruits and vegetable juice at different storage period on 5 point hedonic scale

Storage period	Composite scores of sensory quality					
	Color	Consistency	Appearance	Taste	Flavour	Overall acceptability
Day 1	4.87±0.14	4.63±0.36	4.76±0.19	4.94±0.10	4.54±0.24	4.86±0.16
Day 7	4.69±0.17	4.48±0.27	4.27±0.11	4.63±0.17	4.29±0.14	4.48±0.15
Day 15	4.38±0.17	4.04±0.26	4.25±0.08	4.46±0.14	4.08±0.12	4.17±0.08
Day 30	3.64±0.20	3.62±0.21	3.51±0.12	3.47±0.18	3.37±0.19	3.46±0.17
CD5%	0.157	0.259	0.124	0.141	0.164	0.133

Values are the mean of three replicates

Total acidity: Total acidity of fresh mixed fruits and vegetable juice was 0.31 ± 0.07 (Table 4). The total acidity of prepared juice reduced during storage for 30 days at temperature 0°C or below but the difference was non-significant during the entire storage period. Acidity is a major factor in the acceptability of mix fruit juice. Citric acid is mainly found in kinnow and aonla juice that produces characteristic taste of kinnow - aonla RTS [7]. In present study, total acidity of blended mix fruits and vegetable juice was non-significantly increased during the storage period. This may be because juice was stored at temperature below 0°C which might have not allowed degradation of sugar which was a reason for increase in acidity as reported earlier [7].

Table 4 Physical quality as assessed by pH, TSS and total acidity of the mix fruits and vegetable juice on storage

Storage period	pH	Total Soluble Solid (TSS) (°Brix)	Total acidity ml 0.1N NaOH/100 ml
Day 1	5.79 ± 0.19	15.59 ± 0.63	0.31 ± 0.07
Day 7	5.74 ± 0.06	15.92 ± 0.05	0.32 ± 0.05
Day 15	4.68 ± 0.09	15.97 ± 0.79	0.34 ± 0.03
Day 30	4.48 ± 0.04	16.08 ± 0.03	0.36 ± 0.02
CD5%	0.223	0.951	0.091

Values are the mean of three replicates

Nutritional quality (assessed as antioxidant contents and antioxidant activity)

Vitamin C content of the mix fruits and vegetable juice showed a significant decreasing pattern with increase in storage duration. The decrease in vitamin C content from 46.14 mg to 43.27 mg (Table 5) was observed. It may be due to oxidation of ascorbic acid present in the juice. Jan and Masih [23] also found maximum ascorbic acid (33.09 mg/100 ml) in blended juice consisting of 80% pineapple juice, 10% carrot juice and 10% orange juice and decrease in vitamin C content during its storage. Maximum ascorbic acid (46.14 ± 0.07mg/100g) was recorded in fresh juice analyzed on same day of its preparation. Lowering content of ascorbic acid has been reported in seven fruit juices available in local market after storage for 10 days in refrigerated conditions [30] and carrot - kinnow blend [1]. β-carotene being lipid soluble is not vanished to leaching into water-soluble mediums during processing and storage. The degree of degradation depends upon temperature, light, acidity, an amount of existing oxygen thus, prone to loss through oxidation during processing.

The β-carotene content of the mix fruits and vegetable juice slightly decreased during storage period in the present study. The vitamin E content of the mix fruit juice decreased significantly after 7 days of storage. The total loss of the vitamin E was 23.46% during 30 days of storage period (Table 5). In foods, vitamin E is destroyed by oxygen while freezing causes degradation of the compound. It was observed that the flavonoid content of the mix fruit juice was non-significantly decreased till 15 days and significantly after 30 days of storage. Sarkar [30] also reported decline in flavonoid content of seven fruit juices procured from local market and stored for 10 days in refrigerated conditions. The antioxidant activity of the mix fruit juice was slightly decreased, and the difference was statistically non-significant.

Table 5 Antioxidant contents and antioxidant activity of mix fruits and vegetable juice on storage

Period of storage	Antioxidants				Antioxidant activity (% Inhibition)
	Vitamin E mg/100 g	Vitamin C mg/100 g	β-carotene mg/100 g	Flavonoids mgQE/100 g	
Day 1	3.58 ± 0.04	46.14 ± 0.07	9.7 ± 0.07	79.00 ± 5.00	58.84±0.11
Day 7	3.31 ± 0.52	44.71 ± 0.74	9.13 ± 0.59	81.00 ± 6.00	60.17±1.18
Day 15	3.03 ± 0.06	44.63 ± 0.61	9.07 ± 0.11	73 ± 4.65	58.93 ± 0.72
Day 30	2.74 ± 0.07	43.27 ± 0.39	8.90 ± 0.06	69.31 ± 0.24	59.08 ± 0.02
CD 5%	0.514	0.973	0.565	8.559	1.303

Values are the mean of three replicates

Table 6 Microbial quality of mix fruits and vegetable juice on storage

Period of storage	Total bacterial counts (cfu/g)	Total fungal counts (cfu/g)
Day 1	$0.2118 \times 10^4 \pm 6.51$	$0.866 \times 10^2 \pm 12.58$
Day 7	$0.968 \times 10^3 \pm 6.03$	$0.863 \times 10^2 \pm 7.64$
Day 15	$0.795 \times 10^3 \pm 9.45$	$0.503 \times 10^2 \pm 8.62$
Day 30	$0.514 \times 10^3 \pm 9.08$	$0.463 \times 10^2 \pm 7.51$
CD 5%	14.895	17.545

Values are the mean of three replicates

Effect of storage on microbial quality

Results of the study indicate that the total fungal and total bacterial counts present in the fresh product were in the acceptable limit. During storage both the counts were decreased drastically, might be due to storage of the juice below the temperature 0°C which adversely affected the microbial growth. The data regarding storage on microbial quality is depicted in (Table 6).

CONCLUSION

The fruits (kinnow, guava and aonla) and vegetable (carrots) used for preparation of mix fruits and vegetable juice were appropriate for preparation of mix fruits and vegetable juice. Each one has contributed in enhancing the attributes of sensory quality. The prepared juice had excellent sensory quality. Mix fruits and vegetable juice was rich in vitamin C, β-carotene, vitamin E, flavonoids and had antioxidant activity more than 50%. Storage of juice in freezer of domestic refrigerator for one month caused significant reduction in the scores of sensory quality but the scores remained more than 3 out of 5 indicating good quality. There was only slight decrease in antioxidant contents and antioxidant activity even after 15 days of storage. Considering the sensory and nutritional quality, the developed mixed fruits and vegetable juice can be explored further for mass production with proper processing and packaging measures.

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