

Factors Affecting Physico-Chemical, Sensory and Microbiological Quality of Kinnow Juice Blends

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Abstract

Different fruit juice blends were prepared as (Kinnow juice: Aonla juice: Ginger juice in 100: 0: 0, 95: 5: 0, 92: 5: 3 ratio and Kinnow juice: Pomegranate juice: Ginger juice in 90: 10: 0, 87: 10: 3 ratio) for improving flavour, palatability, nutritive and medicinal value. The juice blends were preserved by pasteurization (75°C or 85°C for 15 minutes) and by addition of potassium meta-bi-sulphite (500 or 750 ppm). These blends were stored in 200 ml colourless glass bottles at refrigerated condition ($4 \pm 1^\circ\text{C}$) for six months and tested at three months interval for physico-chemical, sensory evaluation and microbial population. The results revealed that total soluble solid, acidity, ascorbic acid, total sugars, limonin, non-enzymatic browning, flavour, colour, bitterness and microbial (Bacteria, fungi, yeast) population were affected significantly up to sixth month of storage. The individual effect of juice blending ratio, processing temperature and potassium meta-bi-sulphite treatment was found to be significant in prolonging storage duration and maintaining the acceptable quality of juice blends. The juice blend of 87:10:3 ratio followed by 92:5:3, processed at 75°C for 15 min with 750 ppm KMS was the most effective treatment for physico-chemical and sensory quality of the juice blend but minimum microbial population was recorded with juice processed at 85°C temperature with same treatment combination in both year of experimentation.

Keywords: Kinnow juice; Juice blends; Microbial quality; Physico-chemical properties; Ready-to-serve

Practical Applications

The study of the factors affecting physico-chemical, sensory and microbiological quality of kinnow juice blends is of utmost importance from beverage industrial applications point of view. Kinnow mandarin juice turns bitter after extraction due to conversion of a chemical compound. So, for improving the taste, aroma, palatability and nutritive value, Kinnow juice was blended with some other highly nutritive fruit juices and spices extract for preparation of nutritive Ready-To-Serve (RTS) beverages which is thought to be a convenient and economic alternative for utilization of kinnow juice. The juice blends are preserved by thermal processing and addition of preservative for prolonged storage period without any quality deterioration. This renders processed Kinnow juice blend availability round the year and at the places where the fruits are unavailable.

Introduction

The Kinnow is a variety of citrus fruit cultivated extensively in India and Pakistani Punjab Province. It is a hybrid of two citrus cultivars — “King” (*Citrus nobilis*) × “Willow Leaf” (*Citrus deliciosa*), first developed by H. B. Frost at the Citrus Research Centre of the University of California, Riverside, USA. Kinnow mandarin is quite important as it has a great variety of beverage, industrial and medicinal uses due to its attractive colour, distinctive flavour and being rich source of vitamin ‘C’, vitamin ‘B’, β -carotene, calcium and phosphorous [1]. The post-harvest shelf life of kinnow fruit at room temperature is very limited [2] and shelf life can be extended to a maximum period of up to 45 days under refrigerated storage conditions. In view of its limited shelf life, the fruit must be processed to extend its availability period and also to minimize the glut in the market in its peak season of production. Like all fresh products, the quality of kinnow mandarin juice changes with time. Several key parameters influence the rate of microbial spoilage, enzymatic degradation, chemical changes and deterioration in flavour or turn bitter after extraction. For improving the taste, aroma, palatability, nutritive value and reducing bitterness

kinnow juice was blended with some other highly nutritive fruit juices namely pomegranate and aonla juice with spice extracts like ginger. All these fruits are valued very much for their refreshing juice with nutritional, medicinal properties and ginger juice also have anti-bacterial and anti-fungal properties. Sandhu and Sindhu [3], Saxena et al. [4], Langthasa [5], Jain and Khurdiya [6], Bhardwaj and Mukherjee [7] have reported that two or more fruit juice/pulp may be blended in various proportions for the preparation of nectar, RTS beverages etc. Bhardwaj and Mukherjee [7], Atkins et al. [8], Ranote and Bains [9], Ghosh et al. [10], Mehta and Bajaj [11] have conducted few studies on the use of chemical preservatives and processing of the juice at high temperature which checks the growth of micro-organism and reduces quality losses. To popularise the kinnow mandarin, pomegranate, aonla and ginger juice and its blends among masses, it is necessary to seek meaningful information relating to the development of juice processing technologies to examine untested, old concept in various fields of juice processing, de-bittering and storage. Therefore, this study was aimed at standardizing processing temperature for the thermal processing, chemical preservatives and blending ratio of kinnow mandarin juice in relation to physico-chemical; sensory attributes evaluation and microbial counting of kinnow juice during storage.

Materials and Methods

The experiment was conducted during 2008-09 to study the factors affecting the physico-chemical, sensory and microbiological quality

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of kinnow juice blends in refrigerated storage condition. The fully matured, freshly harvested kinnow, pomegranate and aonla fruits and well-developed ginger rhizomes were procured from Lal kothi mandi, Jaipur and brought to the Post Harvest Technology Laboratory, S.K.N. College of Agriculture, Jobner.

Juice preparations

Fruits were washed with clean running water to remove dust particles and to reduce the microbial load on the surface of the fruits and ginger rhizomes. Peeled kinnow fruits were crushed in screw type juice extractor machine for extraction of juice. Pomegranate fruits were cut into pieces and arils were separated. These arils were passed through the juicer for extraction of juice. Aonla and ginger were sliced with the help of stainless steel knives and crushed with mixer cum juicer for the extraction of juice. The juices were kept for 24 hours in refrigerator ($4\pm 2^\circ\text{C}$) for sedimentation. Then the clear juice was siphoned off. The juice was filtered through muslin cloth and divided into 5 lots.

Prepare juice blends as per following blending ratio

S.No	Types of juice	Blanding Ratio	Treatment Symbol
1	Kinnow juice: Aonla juice: Ginger juice	100: 0: 0	K_1
2	Kinnow juice: Aonla juice: Ginger juice	95: 5: 0	K_2
3	Kinnow juice: Aonla juice: Ginger juice	92: 5: 3	K_3
4	Kinnow juice: Pomegranate juice: Ginger juice	90: 10: 0	K_4
5	Kinnow juice: Pomegranate juice: Ginger juice	87: 10: 3	K_5

Each lot was divided in to 2 sub lots and heated separately at 75°C or 85°C for 15 minutes, respectively in a double-jacketed stainless steel kettle. Again, each sub-lot was divided in to two lots. A calculated quantity of potassium meta-bi-sulphite (500 or 750 ppm) were dissolved in small quantity of water according to the treatments and well mixed in the blended juice with the help of stirrer. Treated juice blends were filled into pre-sterilized 200 ml capacity, 240 bottles {Treatment combination (20) \times Observations (3) \times Replication (3) = 180} as soon as possible and tightly closed using crown corking machine. These bottles containing juice were stored at refrigerated ($4 \pm 1^\circ\text{C}$) condition and analysed at 90 days interval for six months.

Methods of analysis

The physico-chemical parameters including Total Soluble Solids (TSS) of the fruit juice was determined by Zeiss Hand Juice Brix Refractometer, values corrected to 20°C and expressed as $^\circ\text{Brix}$. Acidity (as citric acid) was determined by using standard N/10 NaOH solution in the presence of phenolphthalein as an indicator, AOAC [12]. The vitamin 'C' (ascorbic acid) content of the juice was estimated by visual titration method with 2, 6-dichlorophenol-indonenol dye solution [12]. Total sugars in the juice were determined by the method of Lane and Eynon [13], and limonin of the juice was estimated using the modified Burulian reagent [14] method. The non-enzymatic browning in the juice was determined by alcohol extraction method [15]. Microbiological study was carried out by a series of dilution and spread plate method [16]. In order to find out the consumer preference juice blend ratio on the organoleptic evaluation of juice was done by a panel of ten semi-trained judges using 9 point hedonic scale [17]. All estimations were carried out in triplicate, determinations were made for each attribute and data pertaining to the physico-chemical, sensory quality and microbial population were statistically analyzed by using completely randomized design [18].

Results and Discussion

Effect on physico- chemical properties

Total Soluble Solids (TSS): Retention or minimum increase in total soluble solids content of juice during storage is desirable for preservation of good juice quality. The total soluble solids increased with gradual passage of storage time, which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. The results revealed that the total soluble solids were significantly affected as a result of juice blending ratio (K_3 and K_5), processing temperature (T_1) and potassium meta-bi-sulphite (P_2) during storage. The minimum increase (10.0% and 6.4%) in total soluble solids was recorded in K_5 treatment in both years of experimentation, which was statistically superior to other treatments (Table 1 and 2). Similar trend of increase in total soluble solids with advancement of storage period were observed in mandarin, sweet orange and lemon juice by Bhardwaj and Mukherjee [7], Mehta and Bajaj [11]. The minimum increase was recorded in total soluble solids of stored juice, when the juice was blended with ginger juice and preserved with potassium meta-bi sulphite (750 ppm) at 75°C processing temperature. It might be due to the fact that the ginger juice affected the microbial growth, which may be responsible for higher metabolic rate. Similar results were also reported by Deka and Sethi [19] in mango juice blends. The application of potassium meta-bi-sulphite also resulted in lowered rate of hydrolysis of polysaccharides, which ultimately reduced increase in total soluble solids. Similar observation was also reported by Maini et al. [20], Sethi [21] in stored litchi juice, Bhardwaj and Mukherjee [7] in Kinnow juice blend.

Total sugars: The results revealed that the total sugars content was significantly affected as a result of juice blending with ginger juice, processing temperature and adding of potassium meta-bi-sulphite. The total sugars content in the juice increased apparently during storage, which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. The minimum increase (21.5% and 26.03%) in total sugar content was recorded in K_5 treatment in both years of experimentation, respectively (Table 1 and 2). It might be due to fact that the ginger juice checked the microbial growth, which may be responsible for higher metabolic rate of juice. The change in total sugars content of beverage was almost negligible during storage for 6 months in bael: papaya (2:3) pulps blend [22]. Earlier, similar results were also reported by Deka and Sethi [19] in mango juice blends, Bhardwaj and Mukherjee [7] in Kinnow juice blend. The minimum increase in total sugars level in processed juice blends during storage might be due to the inactivation of enzymes, which are responsible for decreasing acidity and conversion of polysaccharides into simple sugars. The present findings were also in support with the works conducted by Ranote and Bains [9] in kinnow juice. The potassium meta-bi-sulphite also reduced the conversion of polysaccharides and acids into monosaccharide and oligosaccharides. These results are in close confirmation with the finding of Kalra and Tandon [23] in mango pulp.

Acidity: There was a significant decrease in titratable acidity content during storage. This might be due to conversion of acids into salts and sugars by enzymes particularly invertase [24]. Maximum acidity of 0.68% and 0.66% were recorded in the kinnow juice blended with ginger juice and aonla juice (K_3) in both years of experimentation, respectively (Table 1 and 2). The minimum decrease (17.10% and 18.91%) in acidity was shown in K_5 treatment which might be due to inhibitory effect of ginger juice on conversion of acids into sugars and salts by enzymes (Table 1 and 2). Similar results were found by Deka [25] in lime- aonla, mango- pineapple, guava- mango blends, Tiwari

Treatments	TSS, °Brix		Total Sugar (%)		Acidity (%)		Ascorbic Acid (mg/100ml)		Limonin (mg/ml)		NEB, 640nm	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Juice blend												
K ₁	12.6	13.0	8.17	8.67	0.65	0.62	20.3	20.0	0.226	0.263	0.091	0.078
K ₂	12.0	12.3	8.12	8.62	0.71	0.69	45.3	44.8	0.170	0.207	0.096	0.082
K ₃	11.8	12.2	7.99	8.49	0.72	0.69	45.2	44.8	0.158	0.195	0.091	0.078
K ₄	13.5	13.8	8.90	9.40	0.63	0.60	18.88	18.3	0.135	0.172	0.076	0.062
K ₅	12.7	13.0	8.72	9.22	0.66	0.63	18.45	17.9	0.122	0.159	0.071	0.057
S Em. ±	0.107	0.112	0.071	0.057	0.004	0.004	0.263	0.257	0.002	0.002	0.001	0.001
CD at 5%	0.306	0.320	0.204	0.163	0.010	0.010	0.752	0.734	0.005	0.007	0.002	0.003
Temperature												
T ₁	12.2	12.6	8.47	8.97	0.69	0.66	30.3	29.9	0.146	0.183	0.090	0.076
T ₂	12.8	13.1	8.29	8.79	0.66	0.63	28.9	28.4	0.180	0.217	0.080	0.066
S Em. ±	0.068	0.071	0.045	0.057	0.002	0.002	0.166	0.162	0.001	0.001	0.001	0.001
CD at 5%	0.193	0.202	0.129	0.163	0.007	0.006	0.476	0.464	0.003	0.004	0.002	0.002
KMS												
P ₁	12.6	13.0	8.46	8.96	0.67	0.64	29.4	28.9	0.167	0.204	0.086	0.073
P ₂	12.4	12.7	8.30	8.80	0.68	0.64	29.9	29.4	0.159	0.196	0.084	0.078
S Em. ±	0.06	0.071	0.045	0.057	0.002	0.002	0.166	0.162	0.001	0.001	0.001	0.001
CD at 5%	0.193	0.202	0.129	0.163	0.007	0.006	0.476	0.464	0.003	0.004	0.002	0.002

* = Score out of 9 marks, **= cfu/ml juice, K₁ = Kinnow juice (100%), K₂ = Kinnow juice (95%) + Aonla (5%), K₃ = Kinnow juice (92%) + Aonla (5%) + Ginger (3%), K₄ = Kinnow juice (90%) + Pomegranate (10 %), K₅ = Kinnow juice (87%) + Pomegranate (10%) + Ginger (3%), T₁ = Processing at 75°C for 15 minute, T₂ = Processing at 85°C for 15 minute, P₁ = KMS 500 ppm, P₂ = KMS 750 ppm
Each value is a mean of 3 replication (n=3)

Table 1: Effect of blending ratio, processing temperature and potassium meta-bi-sulphite on physico-chemical quality of juice after 3 months of storage at refrigerated storage condition (4±1°C).

Treatments	TSS, °Brix		Total Sugar, %		Acidity, %		Ascorbic Acid, mg/100ml		Limonin, mg/ml		NEB, 640nm	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Juice blend												
K ₁	13.5	13.6	9.13	9.54	0.59	0.57	17.8	17.6	0.254	0.297	0.108	0.093
K ₂	12.8	13.0	9.05	9.47	0.67	0.64	43.4	42.9	0.194	0.237	0.111	0.097
K ₃	12.5	12.6	8.82	9.24	0.68	0.66	43.7	43.2	0.179	0.222	0.107	0.093
K ₄	14.3	14.5	9.85	10.27	0.58	0.55	17.2	16.7	0.153	0.196	0.087	0.073
K ₅	13.2	13.3	9.60	10.02	0.63	0.60	17.1	16.7	0.138	0.181	0.081	0.066
S Em. ±	0.104	0.100	0.108	0.093	0.005	0.004	0.432	0.421	0.002	0.002	0.001	0.001
CD at 5%	0.297	0.287	0.308	0.267	0.015	0.010	1.234	1.205	0.005	0.006	0.003	0.003
Temperature												
T ₁	12.8	13.0	9.43	9.85	0.64	0.61	28.4	28.0	0.165	0.208	0.105	0.090
T ₂	13.6	13.8	9.15	9.57	0.62	0.60	27.2	26.8	0.203	0.246	0.093	0.079
S Em. ±	0.066	0.063	0.068	0.059	0.003	0.004	0.273	0.267	0.001	0.001	0.001	0.001
CD at 5%	0.188	0.181	0.195	0.169	0.010	0.010	0.780	0.762	0.003	0.004	0.002	0.002
KMS												
P ₁	13.38	13.5	9.39	9.81	0.63	0.60	27.4	27.0	0.191	0.234	0.102	0.087
P ₂	13.18	13.3	9.19	9.61	0.64	0.61	28.2	27.8	0.177	0.220	0.096	0.081
S Em. ±	0.066	0.063	0.068	0.059	0.003	0.004	0.273	0.267	0.001	0.001	0.001	0.001
CD at 5%	0.188	0.181	0.195	0.169	0.010	0.010	0.780	0.762	0.003	0.004	0.002	0.002

* = Score out of 9 marks, **= cfu/ml juice, K₁ = Kinnow juice (100%), K₂ = Kinnow juice (95%) + Aonla (5%), K₃ = Kinnow juice (92%) + Aonla (5%) + Ginger (3%), K₄ = Kinnow juice (90%) + Pomegranate (10 %), K₅ = Kinnow juice (87%) + Pomegranate (10%) + Ginger (3%), T₁ = Processing at 75°C for 15 minute, T₂ = Processing at 85°C for 15 minute, P₁ = KMS 500 ppm, P₂ = KMS 750 ppm
Each value is a mean of 3 replication (n=3)

Table 2: Effect of blending ratio, processing temperature and potassium meta-bi-sulphite on physico-chemical quality of juice after 6 months of storage at refrigerated storage condition (4±1°C).

[26] in guava and papaya blended RTS, and Dhaliwal and Hira [27] in carrot juice blends, Bhardwaj and Mukherjee [7] in Kinnow juice blend. The highest acidity level (0.64% and 0.61%) was maintained by the low temperature processing (75°C), which might be due to the inactivation of enzymes and other reactions responsible for decrease in acidity. But due to high temperature treatment for longer time the acidity decreased sharply during processing. Similar results were also reported by Ghorai

[28] in heat processed kinnow juice. The juice treated with potassium meta-bi-sulphite showed higher retention of acidity (0.63% and 0.60%) during storage in both years of experimentation, respectively. This could be due to alteration in metabolism and enzymatic activity. These findings are in close confirmation with the finding of Goyle and Ojha [29] in orange juice.

Ascorbic acid: The ascorbic acid (vitamin 'C') content of the juice

decreased during storage with the advancement of storage period, which was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst [30]. Among the beverages prepared with aonla juice were better in ascorbic acid content but rate of decrease was very slow with ginger juice blend because ginger juice might have reduced the oxidation process. Maximum ascorbic acid (43.70 mg/100 ml juice and 43.20 mg/100 ml juice) was recorded in kinnow juice blended with aonla juice i.e. 5 per cent and ginger juice i.e. 3% (K_3) in both years of experimentation, respectively (Table 1 and 2). These findings are in conformity with the studies of Jain and Khurdiya [31] in Indian gooseberry juice blends. In present investigation, the maximum retention of ascorbic acid (28.4 mg/100ml juice and 28.0 mg/100ml juice) was observed where low temperature (75°C), processing was done (Table 1 and 2). This might be due to more oxidation of ascorbic acid at high temperature. Similar results were also observed by Ranote and Bains [9] in kinnow juice, Bhardwaj and Mukherjee [7] in Kinnow juice blend. Comparatively lower losses of ascorbic acid were observed in juice samples preserved with higher concentration (750 ppm) of potassium meta-bi-sulphite because higher concentration of potassium meta-bi-sulphite reduced oxidation of ascorbic acid during storage for longer time. Similar results were also reported by Khurdiya [32] in phalsa juice.

Limonin: A gradual increase in limonin in juice blends with increase in storage period might be due to conversion of a chemical compound limonate-a-ring lactone (non-bitter) into limonin (bitter) in the juice [33]. Among the juice blend prepared with ginger, aonla and pomegranate juice and processing at low temperature (75°C), or preserve by high concentration potassium meta-bi-sulphite (750 ppm) exhibited significantly lesser limonin as compared to pure unprocessed kinnow juice because blending of non-bitter juice with bitter one in proper ratio reduced the formation of limonin during storage. Minimum limonin (0.138 mg/ml juice and 0.181 mg/ml juice) was recorded when the juice blended with pomegranate juice (10%) and ginger juice (3%) at the end of storage in both years of experimentation, respectively (Table 1 and 2). Guadagni et al. [34] reported that blending of citrus juice with sugar in proper ratio also reduced bitterness. Similar results were also reported by Bhardwaj and Mukherjee [7] in Kinnow juice blend. The beneficial results of juice processing at 75°C temperature might be due to inhibition of oxidation of D-ring lactone and lactones ring into limonin during storage. The results are well supported by Berry [35]

in citrus juice. Similarly the higher concentration of potassium meta-bi-sulphite effectively inhibited the hydrolysis of D-ring lactone and lactones rings into limonin during storage. Sethi et al. [36] reported that the bitterness (limonin) was absent in the canned kinnow juice, preserved with 700 ppm of sulphur dioxide.

Non-enzymatic browning: A linear increase in non-enzymatic browning was observed during 6 months of storage irrespective of juice blend. The increase in non-enzymatic browning during storage might be due to non-enzymatic reaction of organic acid with sugars or oxidation of phenols, which leads to the formation of brown pigments. Khurdiya and Anand [37] also reported a gradual increase in browning and formulation of hydroxymethylfurfural (dark pigment) in stored phalsa beverage. The minimum increase (25.88%) in non-enzymatic browning in the juice blended with aonla juice (5%) and ginger juice (3%) might be due to suppression of polyphenol oxide activity by ascorbic acid [38], which is abundantly found in the aonla juice (Table 1 and 2). Similar results were reported by Jain et al. [31] in aonla juice, Bhardwaj and Mukherjee [7] in Kinnow juice blend. The pomegranate juice was also effective in the reduction of non-enzymatic browning due to higher sugar content. The beneficial results of heat processing of juice might be due to inhibition of formulation of hydroxymethylfurfural and other dark pigments. Similar findings were reported later by Kim et al. [39] in apple juice. The juice treated with potassium meta-bi-sulphite showed minimum non-enzymatic browning due to inactivation of enzymes and protective action of β -carotene. Pathak [40] reported that 100 ppm of potassium meta-bi-sulphite reduced the browning in stored aonla pulp.

Sensory evaluation

In the present study, results indicated that flavour, colour and organoleptic (bitterness) score of juice blends, decreased with advancement of storage period (Table 3, 4 and 5). The colour, flavour, tastes and appearance as well as higher nutrient elements of the blends was found to be superior as compared to the juices prepared from individual fruits. The juice blend of kinnow juice (87%) + pomegranate juice (10%) + ginger juice (3%) recorded higher score for colour (7.33 and 7.23), flavour (7.53 and 7.73) and organoleptic taste (7.74 and 7.94) as compared to other blends at the end of storage in both years of experimentation, respectively (Table 5). This may be explained as ginger juice checks microbial and enzymatic activities in stored juice, which produce off flavour and change in natural colour and taste.

	Fresh fruit juice (2008)					Fresh fruit juice (2009)				
	K_1	K_2	K_3	K_4	K_5	K_1	K_2	K_3	K_4	K_5
TSS, (°Brix)	11.5	11.0	11.0	12.5	12.0	12.0	11.5	11.0	12.0	12.5
Acidity, %	0.79	0.84	0.83	0.76	0.76	0.77	0.82	0.81	0.74	0.74
Ascorbic acid, mg/100 ml juice	26.4	50.9	50.3	24.0	23.3	23.6	48.1	47.6	21.4	20.5
Total Sugars %	7.20	7.20	7.20	8.00	7.90	7.35	7.36	7.30	8.10	7.95
Limonin, mg/ml	0.156	0.109	0.098	0.083	0.080	0.226	0.189	0.107	0.090	0.092
Non-enzymatic browning, 640nm	0.065	0.071	0.068	0.055	0.050	0.060	0.066	0.065	0.052	0.048
*Flavour	8.5	8.0	8.0	8.8	8.9	8.4	7.9	7.9	8.7	8.8
*Colour	8.5	8.4	8.3	8.8	8.8	8.5	8.4	8.3	8.9	8.9
*Bitterness	8.2	8.4	8.5	8.8	8.9	8.2	8.4	8.5	8.7	8.9
Total viable counts**	9.5x10 ³	8.7x10 ³	7.5x10 ³	8.9x10 ³	7.2x10 ³	1.0x10 ⁴	9.0x10 ³	8.0x10 ³	9.0x10 ³	7.8x10 ³
Yeasts**	4.0x10 ³	3.6x10 ³	3.3x10 ³	3.7x10 ³	2.3x10 ³	5.0x10 ³	4.2x10 ³	3.6x10 ³	4.0x10 ³	2.6x10 ³
Mould**	4.9x10 ³	4.4x10 ³	4.0x10 ³	4.5x10 ³	4.1x10 ³	5.1x10 ³	4.7x10 ³	4.2x10 ³	4.7x10 ³	4.4x10 ³

* = Score out of 9 marks, ** = cfu/ml juice, K_1 = Kinnow juice (100%), K_2 = Kinnow juice (95%) + Aonla (5%), K_3 = Kinnow juice (92%) + Aonla (5%) + Ginger (3%), K_4 = Kinnow juice (90%) + Pomegranate (10%), K_5 = Kinnow juice (87%) + Pomegranate (10%) + Ginger (3%)
Each value is a mean of 3 replication (n=3)

Table 3: Physico - chemical, sensory and microbiological quality of freshly prepared juice at the time of processing and storage.

Treatments	*Flavour		*Colour		*Bitterness		TVC		Yeasts		Mould	
Years	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Juice blend												
K ₁	7.25	7.39	7.36	7.45	6.56	6.70	1.8x10 ³	1.8x10 ³	9.8x10 ²	1.0x10 ³	1.2x10 ³	1.3x10 ³
K ₂	6.74	6.88	7.29	7.38	6.97	7.11	1.5 x10 ³	1.5 x10 ³	7.8 x10 ²	8.6 x10 ²	1.0 x10 ³	1.1 x10 ³
K ₃	6.81	6.95	7.33	7.42	7.23	7.37	1.1 x10 ³	1.1 x10 ³	6.4 x10 ²	7.1 x10 ²	8.5 x10 ²	8.9 x10 ²
K ₄	8.00	8.18	7.93	8.00	7.77	7.91	1.6 x10 ³	1.7 x10 ³	8.4 x10 ²	9.1 x10 ²	1.1 x10 ³	1.1 x10 ³
K ₅	8.39	8.53	8.04	8.12	8.11	8.25	1.1 x10 ³	1.2 x10 ³	6.4 x10 ²	7.1 x10 ²	8.5 x10 ²	8.9 x10 ²
S Em. ±	0.047	0.048	0.088	0.083	0.050	0.056	9.950	9.114	10.910	9.911	14.440	13.546
CD at 5%	0.135	0.137	0.252	0.237	0.144	0.160	28.447	26.049	31.190	28.326	41.271	38.718
Temperature												
T ₁	7.62	7.76	7.74	7.83	7.28	7.41	2.3x10 ³	2.3 x10 ³	1.2 x10 ³	1.3x10 ³	1.6x10 ³	1.7x10 ³
T ₂	7.27	7.41	7.44	7.53	7.38	7.52	6.4 x10 ²	6.6 x10 ²	3.1 x10 ²	3.8 x10 ²	4.0 x10 ²	4.5 x10 ²
S Em. ±	0.030	0.030	0.056	0.052	0.032	0.035	6.295	5.764	6.90	6.268	9.133	8.568
CD at 5%	0.086	0.887	0.159	0.150	0.091	0.101	17.990	16.475	19.73	17.915	26.102	24.487
KMS												
P ₁	7.40	7.54	7.51	7.59	7.08	7.31	1.8 x10 ³	1.8 x10 ³	9.4 x10 ²	1.0 x10 ³	1.2x10 ³	1.3x10 ³
P ₂	7.49	7.63	7.67	7.76	7.17	7.42	1.1 x10 ³	1.2 x10 ³	6.1 x10 ²	6.7 x10 ²	8.0x10 ²	8.4 x10 ²
S Em. ±	0.030	0.030	0.056	0.052	0.032	0.035	6.295	5.764	6.900	6.268	9.133	8.568
CD at 5%	0.086	0.887	0.159	0.150	0.091	0.101	17.990	16.475	19.730	17.915	26.102	24.487

* = Score out of 9 marks, **= cfu/ml juice, K₁ = Kinnow juice (100%), K₂ = Kinnow juice (95%) + Aonla (5%), K₃ = Kinnow juice (92%) + Aonla (5%) + Ginger (3%), K₄ = Kinnow juice (90%) + Pomegranate (10 %), K₅ = Kinnow juice (87%) + Pomegranate (10%) + Ginger (3%), T₁ = Processing at 75°C for 15 minute, T₂ = Processing at 85°C for 15 minute, P₁ = KMS 500 ppm, P₂ = KMS 750 ppm
Each value is a mean of 3 replication (n=3)

Table 4: Effect of blending ratio, processing temperature and potassium meta-bi-sulphite on sensory and microbiological quality of juice after 3 months of storage at refrigerated storage condition (4 ± 1°C).

Treatments	*Flavour		*Colour		*Bitterness		TVC		Yeasts		Mould	
Years	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Juice blend												
K ₁	6.25	6.45	6.11	6.00	5.77	5.97	2.8x10 ³	3.4x10 ³	1.3x10 ³	1.4x10 ³	1.8x10 ³	1.9 x10 ³
K ₂	5.60	5.80	6.14	6.05	6.24	6.44	2.5 x10 ³	2.9 x10 ³	1.1 x10 ³	1.2 x10 ³	1.5 x10 ³	1.6 x10 ³
K ₃	5.71	5.91	6.20	6.10	6.52	6.72	2.1 x10 ³	2.5 x10 ³	9.4 x10 ²	1.0 x10 ³	1.3 x10 ³	1.4 x10 ³
K ₄	7.09	7.29	7.12	7.00	7.27	7.47	2.5 x10 ³	3.0 x10 ³	1.1 x10 ³	1.2 x10 ³	1.6 x10 ³	1.7 x10 ³
K ₅	7.53	7.73	7.33	7.23	7.74	7.94	2.0 x10 ³	2.5 x10 ³	9.0 x10 ²	9.9 x10 ²	1.2 x10 ³	1.3 x10 ³
S Em. ±	0.054	0.056	0.079	0.085	0.054	0.040	14.776	14.098	12.44	11.412	21.242	18.741
CD at 5%	0.154	0.161	0.277	0.244	0.156	0.113	42.232	40.293	35.57	32.616	60.712	53.563
Temperature												
T ₁	6.70	6.90	6.72	6.62	6.65	6.85	3.4 x10 ³	3.4 x10 ³	1.8 x10 ³	1.9 x10 ³	2.5 x10 ³	2.6 x10 ³
T ₂	6.18	6.38	6.44	6.34	6.76	6.96	1.3 x10 ³	1.4 x10 ³	3.4 x10 ²	4.3 x10 ²	4.7 x10 ²	5.3 x10 ²
S Em. ±	0.034	0.036	0.050	0.054	0.034	0.025	9.345	8.916	7.87	7.217	13.435	11.853
CD at 5%	0.098	0.102	0.143	0.154	0.098	0.072	26.710	25.484	22.49	20.628	38.398	33.876
KMS												
P ₁	6.38	6.58	6.45	6.35	6.55	6.70	2.9x10 ³	2.9 x10 ³	1.3 x10 ³	1.4 x10 ³	1.7 x10 ³	1.8 x10 ³
P ₂	6.49	6.69	6.71	6.61	6.70	6.82	1.8 x10 ³	1.9 x10 ³	9.0 x10 ²	9.8 x10 ²	1.2 x10 ³	1.5 x10 ³
S Em. ±	0.034	0.036	0.050	0.054	0.034	0.025	9.345	8.916	7.87	7.217	13.435	11.853
CD at 5%	0.098	0.102	0.143	0.154	0.098	0.072	26.710	25.484	22.49	20.628	38.398	33.876

* = Score out of 9 marks, **= cfu/ml juice, K₁ = Kinnow juice (100%), K₂ = Kinnow juice (95%) + Aonla (5%), K₃ = Kinnow juice (92%) + Aonla (5%) + Ginger (3%), K₄ = Kinnow juice (90%) + Pomegranate (10 %), K₅ = Kinnow juice (87%) + Pomegranate (10%) + Ginger (3%), T₁ = Processing at 75°C for 15 minute, T₂ = Processing at 85°C for 15 minute, P₁ = KMS 500 ppm, P₂ = KMS 750 ppm
Each value is a mean of 3 replication (n=3)

Table 5: Effect of blending ratio, processing temperature and potassium meta-bi-sulphite on sensory and microbiological quality of juice after 6 months of storage at refrigerated storage condition (4 ± 1° c).

Tandon et al. [22] reported that addition of papaya pulp with bael pulp was found to be very effective in checking the browning and improving the appearance of the beverage. They also observed that the beverage prepared from 2:3 blend of bael:papaya pulp scored maximum (7.4 out of 10.0) after six months of storage. The result is well supported by Murari

and Verma [41] in case of guava nectar, Gowda [42] in case of mango and papaya blend, Bhardwaj and Mukherjee [7] in Kinnow juice blend. The beneficial results of thermal processing might be due to inhibition of polyphenol oxidase and the enzyme involved in discolouration and developing of off flavour during storage. Later, the opinion was

supported by works of Kim et al. [39] in apple juice. The potassium meta-bi-sulphite was found to be effective in retention of good flavour, colour and organoleptic taste of fruit juice during the entire storage period. Mehta and Bajaj [11] reported that colour retention during storage was better in citrus juice preserved with 700 ppm potassium meta-bi-sulphite. All the samples were found acceptable up to 6 months of storage. Overall qualities including colour, flavour and organoleptic scores were better in the juice which was blended in the ratio of kinnow juice: pomegranate juice: ginger juice (87: 10: 3) with processing at 75°C temperature and addition of 750 ppm potassium meta-bi-sulphite.

Microbial population

It has been observed that the untreated fruit juices and pulp were highly contaminated with bacteria, yeast and mould. The data presented in Table 3, 4 and 5 showed the minimum increase in bacteria, yeast and mould population, when juice was blended with ginger juice, processed at 85°C temperature with addition of potassium meta-bi-sulphite (750 ppm). This result was supported by Attri et al. [43] who reported that the blends of sand pear juice with apple, apricot and plum could be stored at room temperature for six months without any spoilage. Ejechi et al. [44] reported that heating mango juice to 55°C for 15 minutes and supplementing with nutmeg (4% v/v) and ginger (4% v/v) markedly inhibited microbial growth. The similar results were also reported by Deka [25] with negligible growth of moulds and yeasts in lime - aonla and mango - pineapple spiced RTS beverages, which got further reduced during storage due to inhibitory effect and antioxidative properties of spices. This might be due to the inhibitory effect of these treatments on micro-organisms. Deka and Sethi [19] reported that no bacterial growth was observed in the spiced mixed fruit juice RTS beverages. In view of the microbiological analysis of the stored juice samples it was observed that all the samples were contaminated with a large variety of bacterial, fungal and mould species but within the acceptable limit. The juice blended with 3% ginger juice (K_3) was lowest in bacterial (2.1×10^3 and 2.5×10^3 TVC/ml juice), mould (1.2×10^3 and 1.3×10^3 cfu/ml juice) and yeasts (9.0×10^2 and 9.9×10^2 cfu/ml juice) population as recorded at the end of storage period (six months) in both years of experimentation, respectively (Table 5). Similar results were also reported by Bhardwaj and Mukherjee [7] in Kinnow juice blend. The processing of juice at 85°C for 15 minutes of holding time could be considered as effective processing time and temperature to minimize microbial growth in the juice blends. Ghorai [28] reported the considerable reduction of microbial population in kinnow juice by the heat processing at 90°C for 10 minutes. Potassium meta-bi-sulphite produces sulphur dioxide, which in turn acts as a preservative and check the oxidation of juice constituents and growth of micro-organism. Sethi et al. [36] reported that kinnow juice preserved with 700 ppm of sulphur dioxide was not spoiled up to six months.

Conclusion

On the basis of the results of this study it may be concluded that formulation of mixed (blend) fruit juice beverage is possible to satisfy consumer taste and preferences. These juice blends can be stored effectively for a period of 6 months. Their total soluble solids, total sugars, limonin and non-enzymatic browning increased with increased period of storage but acidity, ascorbic acid and sensory quality score was reduced with advancement of storage. The juice blend (Kinnow juice 87%+Pomegranate juice 10% +Ginger juice 3%) and followed by (Kinnow juice 92% +Aonla juice 5% + Ginger juice 3%), processed at 75°C for 15 minutes with 750 ppm potassium meta-bi-sulphite proved to be the most effective treatment for physico-chemical and sensory

scores of the juice blends. The maximum B: C. ratio (1.5:1) was obtained in K_3 treatment of formulating squash from the juice blend (kinnow juice 87: pomegranate juice 10: ginger juice 3) + processed at 75°C + KMS (750 ppm) and stored under refrigerated condition.

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