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Studies on physico-chemical, sensory and microbiological quality of kinnow juice blends under refrigerated storage

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ABSTRACT

Various fruit juice blends were prepared as (i) Kinnow juice:*Aonla* juice:Ginger juice in 100:0:0; 95:5:0; 92:5:3 ratios and Kinnow juice:Pomegranate juice:Ginger juice in 90:10:0; 87:10:3 ratios for improving flavour, palatability, nutritive value and medicinal value. The juice blends were preserved by pasteurization at 75°C or 85°C for 15 minutes, and, by adding potassium meta-bisulphite (KMS) at 500 or 750 ppm. These blends were stored in 200ml colourless glass bottles under refrigerated conditions (4±1°C) for six months and tested at three month intervals for physico-chemical sensory quality and microbial population. Individual effect of juice blending ratio, processing temperature and KMS treatment was found to be significant for prolonging storage life and for maintaining an acceptable quality of the juice blends. The blend of Kinnow:Pomegranate:Ginger juice at 87:10:3 ratio, followed by Kinnow:Aonla:Ginger juice @ 92:5:3, processed at 75°C for 15 min with 750 ppm KMS, was the most effective for obtaining superior physico-chemical and sensory quality of the blend. However, minimum microbial population was recorded in the juice processed at 85°C (and not 75° C) with the same treatment combination.

Key words: Kinnow juice, juice blends, microbial quality, physico-chemical properties, ready-to-serve

INTRODUCTION

Kinnow mandarin is a fairly important crop as it has a great variety of beverage, industrial and medicinal uses due to its attractive colour, distinctive flavour and is a rich source of Vitamins B & C, β-carotene, calcium and phosphorus (Sogi and Singh, 2001). Post-harvest shelf-life of kinnow fruit at room temperature is very limited (Jawanda and Singh, 1973) and can be extended to a maximum of 45 days under refrigerated storage conditions. The fruit should be processed to extend its availability and to minimize glut in the market in peak season of production. Like in all fresh products, quality of kinnow mandarin juice changes with time. Several parameters influence the rate of microbial spoilage, enzymatic degradation, induce chemical change and deterioration in flavour, or turn bitter upon extraction. For improving taste, aroma, palatability and nutritive value, and for reducing the bitterness, kinnow juice was blended with other, highly nutritive fruit juices, namely pomegranate and *aonla*, with spice extracts like ginger. All these fruits are greatly valued for their refreshing juice with nutritional and medicinal properties. Ginger juice has anti-bacterial and anti-fungal properties. Jain and Khurdiya (2005) and Bhardwaj and Mukherjee (2011) reported that juice/pulp of two or more fruits may be blended in various proportions for preparation of nectar, RTS beverages, etc. Ranote and Bains (1982), Mehta and Bajaj (1983) and Bhardwaj and Mukherjee (2011) conducted studies on use of chemical preservatives and processing of juice at high temperature (this checks growth of micro-organisms and reduces loss of quality). To popularize kinnow mandarin, pomegranate, *aonla* and ginger juice and their blends among the masses, it is necessary to seek meaningful information on juice processing technologies to examine untested, old concepts in various fields of juice processing, de-bittering and storage. Therefore, this study was aimed at standardizing processing temperatures for thermal processing, chemical preservatives and blending ratio of kinnow mandarin juice in relation to physico-chemical parameters, sensory quality and microbial safely during storage.

MATERIAL AND METHODS

The experiment was conducted during 2008-09 to study factors affecting the above parameters. Fully mature, freshly harvested kinnow, pomegranate and *aonla* fruits, and well-developed ginger rhizomes, were procured for the purpose from Lal Kothi Mandi, Jaipur, and brought to Post-Harvest Technology Laboratory, S.K.N. College of Agriculture, Jobner.

Juice preparation

Fruits were washed in clean tap-water to remove dust particles and reduce microbial load on the surface of fruits and ginger rhizomes. Peeled kinnow fruits were crushed in a screw-type juice extractor machine for extraction of juice. Pomegranate fruits were cut into sectors arils separated and passed through a juicer. *Aonla* and ginger were sliced with stainless steel knives and crushed in a mixer-cum-juicer. The juices were placed for 24 hours under refrigeration (4±2°C) for sedimentation. The clear juice was then siphoned off. The juice was filtered through muslin cloth and divided into five lots.

Various juice blends and their ratios

Type of juice blends	Blending ratio	Treatment denoted as
Kinnow juice:*Aonla* juice:Ginger juice	100:0:0	K_1
Kinnow juice:*Aonla* juice:Ginger juice	95:5:0	K_2
Kinnow juice:*Aonla* juice:Ginger juice	92:5:3	K_3
Kinnow juice:Pomegranate juice:Ginger juice	90:10:0	K_4
Kinnow juice:Pomegranate juice:Ginger juice	87:10:3	K_5

Each lot was divided into two 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 into two lots. Known quantity of potassium meta-bi-sulphite (500 or 750 ppm) were dissolved in a small quantity of water as per treatments and mixed well with the blended juice. Treated juice blends were filled into pre-sterilized, 200ml capacity bottles as quickly as possible and shut tightly using a crown corking machine. These were stored at refrigerated (4±1°C) condition and analyzed at 90 day intervals for upto six months.

Methods of analysis used

Physico-chemical parameters including total soluble solids (TSS) of the fruit juice were determined by a refractometer (Zeiss make), values corrected to 20°C and expressed as °Brix. Acidity (in terms of citric acid) was determined using AOAC (1984). Vitamin 'C' (ascorbic acid) content of the juice was estimated by 2, 6-dichlorophenol-indonenol dye titration method (AOAC, 1984). Total sugars in the juice were determined by the method of Lane and Eynon (1923) and limonin in the juice was estimated using modified Burulian reagent (Vaks and Litshitz, 1981) method. Non-enzymatic browning (NEB) in the juice was determined by the alcohol extraction method (Klin and Nagy, 1988). Microbiological study was carried out by a series of dilution and spread plate methods (Ranganna, 1994). To assess consumer preference, organoleptic quality of the juice was tested by a panel of ten semi-trained judges, using the 9 point hedonic scale (Amerine *et al*, 1965). All estimations were carried out in triplicate, determinations were made for each attribute and data on physico-chemical, sensory quality and microbial population were statistically analyzed using Completely Randomized Design (Cochran and Cox, 1950).

RESULTS AND DISCUSSION

Effect on physico-chemical properties

Total Soluble Solids (TSS): Retention or slight increase in total soluble solids in the juice during storage is desirable for preserving juice quality. Total soluble solids of the juices increased with storage time, which might be due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides. Total soluble solids were significantly affected by blending ratio (K_3 and K_5), processing temperature (T_1) and potassium meta-bi-sulphite (P_2) during storage. Minimum increase (10.0% and 6.4%) in total soluble solids was recorded in K_5 treatment in both years of experimentation, respectively, and was statistically superior to other treatments. Similar increase in total soluble solids with increase in storage period was observed in juice blend of mandarin, sweet orange and lemon by Mehta and Bajaj (1983) and Bhardwaj and Mukherjee (2011). Increase in total soluble solids during storage was observed in our study in juice blended with ginger juice with added potassium meta-bi sulphite (750 ppm) at 75°C processing temperature. This may be due to ginger juice checking microbial growth which, otherwise, would have had higher TSS due to the higher metabolic rate. Similar results were also reported by Deka and Sethi (2001) in mango juice blends. Addition of potassium meta-bi-sulphite also resulted in lower rate of hydrolysis of polysaccharides, which ultimately reduced increase in total soluble solids. Similar result was also reported in stored litchi juice (Sethi, 1985) and in kinnow juice blends (Bhardwaj and Mukherjee, 2011).

Total sugars: Results revealed that total sugar content was significantly affected by blending with ginger juice, by the processing temperature and addition of potassium meta-bi-sulphite. Total sugar content in the juice increased during storage, which may have been due to the hydrolysis of polysaccharides into monosaccharides and oligosaccharides. Minimum increase (21.5% and 26.03%) in total sugar content was recorded in K_5 treatment in both years of experimentation, respectively. Perhaps the ginger juice checked microbial growth. Change in total sugar content of the beverage was almost negligible during storage for six months in bael:papaya (2:3) pulp blend (Tandon *et al*, 2007). Similar results were reported by Deka and Sethi (2001) in mango juice blends and by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Minimum increase in the level of total sugars in processed juice blends during storage could be due to inactivation of enzymes that are responsible for reducing acidity and for conversion of polysaccharides into simple sugars. The present findings are also in line with earlier reports by Ranote and Bains (1982) in kinnow juice. Potassium meta-bi-sulphite also reduced conversion of polysaccharides and acids into monosaccharides and oligosaccharides. These results are in close conformity with findings of Kalra and Tandon (1985) in mango pulp.

Acidity: There was a significant reduction in titratable acidity during storage. This might be due to conversion of acids into salts and sugars by enzymes, particularly, invertase (Kumar *et al*, 1992). Maximum acidity of 0.68 and 0.66 % was recorded in the kinnow juice blended with ginger juice and *aonla* juice (K_3) in both years of experimentation, respectively. Minimum decrease (17.10% and 18.91%) in acidity was observed in K_5 treatment in the two years, respectively, and could be due to the inhibitory effect of ginger juice on enzymes involved in conversion of acids into sugars and salts. Similar results were reported by Deka (2000) in lime- aonla, mango- pineapple and guava- mango blends by Tiwari (2000) in guava and papaya blended RTS; by Dhaliwal and Hira (2001) in carrot juice blends; and, by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Highest acidity (0.64% and 0.61%) was seen in low-temperature processing (75°C), perhaps due to inactivation of enzymes. But, high temperature treatment for longer duration decreased acidity sharply during processing. Similar results were also reported by Ghorai (1996) in heat-processed kinnow juice. 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 conformity with findings of Goyle and Ojha (1998) in orange juice.

Ascorbic acid: Ascorbic acid (Vitamin C) content of the juice decreased during storage, probably due to oxidation of ascorbic acid in the presence of oxygen by both enzymatic and non-enzymatic catalysts (Mapson, 1970). Among the juice blends, beverages prepared with *aonla* juice were superior in ascorbic acid content, but, the rate of decrease was very slow with ginger juice blend. Ginger juice may have decelerated the oxidation process. Maximum ascorbic acid (43.70 mg/100ml juice and 43.20 mg/100ml juice) was recorded in kinnow juice blended with *aonla* juice (5%) and ginger juice (3%) (K_3) in both years of experimentation, respectively. These findings are in conformity with studies of Jain and Khurdiya (2005) who reported that Indian gooseberry juice had the highest Vitamin C content (478.56 mg/100ml juice). Hence, Indian gooseberry juice was blended with other fruit juices for preparation of blended, ready-to-serve beverages with higher Vitamin C content. In the present investigation, maximum retention of ascorbic acid (28.4 mg/100ml juice and 28.0 mg/100ml juice) was observed in low-temperature (75°C) processing was done. This might be due to lower oxidation of ascorbic acid. Similar results were also obtained by Ranote and Bains (1982) in kinnow juice and by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Comparatively lower loss in ascorbic acid content was observed in juice samples preserved with higher concentration (750 ppm) of potassium meta-bi-sulphite, because, this reduced oxidation of ascorbic acid. Similar results were reported by Khurdiya (1979) in *phalsa* juice.

Limonin content: Gradual increase in limonin content in juice blends with increase in storage period may be due to conversion of a chemical compound, limonate-a-ring lactone (non-bitter), into limonin (bitter) (Premi *et al*, 1994). Among juice blends prepared with ginger, *aonla* and pomegranate juice and processed at low temperature (75°C), or preserved by high concentration potassium meta-bi-sulphite (750 ppm), exhibited significantly less limonin content compared to pure, unprocessed kinnow juice. This is because of blending of non-bitter juice with the bitter one in a proper ratio reduced formation of limonin during storage. Minimum limonin content (0.138 mg/ml juice and 0.181 mg/ml juice) was recorded at the end of the storage period in kinnow juice blended with pomegranate juice (10%) and ginger juice (3%). Guadagni *et al* (1993) reported that blending citrus juice with sugar in a proper ratio also reduced bitterness. Similar results were reported by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Beneficial results of juice processing at 75°C might be due to inhibition of oxidation of the D-ring lactone into limonin during storage. These results are well supported by Berry (2001) in citrus juice. Similarly, higher concentration of potassium meta-bi-sulphite effectively inhibited hydrolysis of D-ring lactone and lactone rings into limonin, during storage. Sethi *et al* (1980) reported bitterness (limonin) to be absent in canned kinnow juice preserved with 700 ppm sulphur dioxide.

Non-enzymatic browning (NEB): Linear increase in non-enzymatic browning (NEB) was observed during six months of storage, irrespective of the juice blend. This may be due to non-enzymatic reaction of organic acid with sugars, or oxidation of phenols which leads to formation of brown pigments. Khurdiya and Anand (1981) also reported a gradual increase in browning and formulation of hydroxylmethylfurfural (dark pigment) in stored phalsa beverage. Minimum increase (25.88%) in non-enzymatic browning in juice blended with *aonla* juice (5%) and ginger juice (3%) could be attributed to suppression of polyphenol oxidase activity by ascorbic acid (Arogba *et al*, 1998), abundantly found in *aonla* juice. Similar results were reported by Jain *et al* (2003) in *aonla* juice and by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Pomegranate juice was also effective in reducing non-enzymatic browning due to its higher sugar content. Beneficial results of heat processing of the juice might be due to inhibition of the formulation of hydroxylmethylfurfural and other dark pigments. Similar findings were reported later by Kim *et al* (1993) in apple juice. Juice treated with potassium meta-bi-sulphite showed minimum non-enzymatic browning, due to inactivation of enzymes and the protective action of b-carotene.

Sensory evaluation

In the present study, results indicated that flavour, colour and organoleptic score (bitterness) of juice blends decreased with advancement in storage period (Tables 1 to 4). Colour, flavour, taste, appearance, as well as higher nutritional elements in the blends, were found to be superior compared to non-blended (pure) juices. 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) at the end of storage in both years of experimentation, respectively. The explanation for this is that ginger juice checks microbial and enzymatic activities in stored juice. (which produce off-flavour and change natural colour and taste). Tandon *et al* (2007) reported that blending papaya pulp with bael pulp was very effective in checking browning and in improving the appearance of the beverage. They also observed that beverage prepared from 2:3 blend of bael:papaya pulp scored maximum (7.4 out of 10.0) at six months of storage. This result is supported by Gowda (1995) in mango and papaya blend and by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Beneficial results of thermal processing might be due to inhibition of polyphenol oxidase and enzymes involved in discolouration development of off-flavour during storage. Similar results were reported by Kim *et al* (1993) in apple juice. Potassium meta- bi-sulphite was found effective for retaining good flavour, colour and organoleptic taste of the juice during the entire storage period. Mehta and Bajaj (1983) reported colour retention during storage to be better in citrus juice preserved with 700 ppm potassium meta-bi-sulphite. All the samples were found acceptable for upto six months of storage. Overall qualities including colour, flavour and organoleptic scores were better in juice blended in the ratio kinnow juice:pomegranate juice:ginger juice (87:10:3) processed at 75°C and fortified with 750ppm potassium meta-bi-sulphite.

Microbial population

Untreated fruit juices and pulp were highly contaminated with bacteria, yeast and mold. Data presented in Tables 1 to 5 show minimum increase in bacteria, yeast and mold population when the juice was blended with ginger juice and processed at 85° C temperature with addition of potassium meta-bi-sulphite (750 ppm). This result is supported by Attri *et al* (1998) who reported that 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* (1998) reported that heating mango juice to 55°C for 15 minutes and supplementing it with nutmeg (4% v/v) and ginger (4% v/v) markedly inhibited microbial growth. Similar results were also reported by Deka (2000) who notified negligible growth of molds and yeasts in lime-aonla and mango–pineapple spiced RTS beverages. Spoilage was further reduced during storage from the inhibitory effect on microorganisms and antioxidative properties of spices. Deka and Sethi (2001) observed no bacterial growth in spice mixed fruit juice RTS beverages. In microbiological analysis of stored juice samples, it all the samples were found contaminated with a variety of bacterial, fungal and mold species, but these were within the acceptable limit. Juice blended with 3% ginger juice (K_5) was lowest in bacterial ($2.1x10^3$ and $2.5x10^3$ TVC/ml juice), mold ($1.2x10^3$ and $1.3 x10^3$ cfu/ml juice) and yeast ($9.0x10^2$ and $9.9x10^2$ cfu/ml juice) population as recorded at the end of storage period (six months) in the two years of experimentation, respectively. Similar results were also reported by Bhardwaj and Mukherjee (2011) in kinnow juice blend. Processing juice at 85°C for 15 minutes holding time could be considered effective processing time and temperature, to minimize microbial growth in juice blends. Ghorai (1996) reported

Table 1. Physico-chemical, sensory and microbiological quality of freshly prepared juice and blends at the time of processing and storage

Parameter	Fresh fruit juice (year 2008)					Fresh fruit juice (year 2009)				
	K_1	K_2	K_3	K_4	K_5	K_1	K_2	K_3	K_4	K_5
TSS (ºBrix)	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, at 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 microbial count	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}
**Yeast	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}
**Mold	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}

K_1 = Kinnow juice (100%); K_2 = Kinnow juice (95%) + *Aonla* juice (5%); K_3 = Kinnow juice (92%) + *Aonla* juice (5%) + Ginger juice (3%), K_4 = Kinnow juice (90%) + Pomegranate juice (10 %); K_5 = Kinnow juice (87%) + Pomegranate juice (10%) + Ginger juice (3%)

*= Score out of 9 marks, **= cfu/ml juice
Each value is a mean of 3 replications (n=3)

Table 2. Effect of blending ratio, processing temperature and potassium meta-bi-sulphite (KMS) on physico-chemical quality of juice after three months of refrigerated storage (4±1ºc)

Treatmentss	TSS (ºBrix)		Total sugars (%)		Acidity (%)		Ascorbic acid (mg/100ml)		Limonin (mg/ml)		†NEB at 640nm	
Year	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 (*P=0.05*)	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 (*P=0.05*)	0.193	0.202	0.129	0.163	0.007	0.006	0.476	0.464	0.003	0.004	0.002	0.002
Potassium meta-bi sulphite (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 (*P=0.05*)	0.193	0.202	0.129	0.163	0.007	0.006	0.476	0.464	0.003	0.004	0.002	0.002

*, **, K_1 - K_5, TVC: As in Table 1; T_1= Processing at 75º for 15 min; T_2= Processing at 85º for 15 min; P_1 = KMS 500 ppm; P_2= KMS 750 ppm
†NEB = Non-Enzymatic Browning
Each value is a mean of 3 replications (n=3)

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

Treatment	*Flavour		*Colour		*Bitterness		TVC		Yeast		Mold	
Year	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 (*P=0.05*)	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 (*P=0.05*)	0.086	0.887	0.159	0.150	0.091	0.101	17.990	16.475	19.73	17.915	26.102	24.487
Potassium meta-bi sulphite (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 (*P=0.05*)	0.086	0.887	0.159	0.150	0.091	0.101	17.990	16.475	19.730	17.915	26.102	24.487

*, **, TVC, K_1-K_5, T_1, T_2, P_1, P_2: As in Tables 1and 2 (n=3)
Each value is a mean of 3 replication (n=3)

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

Treatment	TSS (°Brix)		Total sugars (%)		Acidity (%)		Ascorbic Acid (mg/100ml)		Limonin (mg/ml)		†NEB at 640nm	
Year	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 (*P=0.05*)	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 (*P=0.05*)	0.188	0.181	0.195	0.169	0.010	0.010	0.780	0.762	0.003	0.004	0.002	0.002
Potassium meta-bi sulphite (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 (*P=0.05*)	0.188	0.181	0.195	0.169	0.010	0.010	0.780	0.762	0.003	0.004	0.002	0.002

*, **, TVC, K_1-K_5, T_1, T_2, P_1, P_2: As in Tables 1and 2 (n=3)
†NEB = Non-Enzymatic Browning
Each value is a mean of 3 replications (n=3)

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

Treatment	*Flavour		*Colour		*Bitterness		TVC		Yeast		Mold	
Year	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 (*P=0.05*)	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 (*P=0.05*)	0.098	0.102	0.143	0.154	0.098	0.072	26.710	25.484	22.49	20.628	38.398	33.876
Potassium meta-bi sulphite (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 (*P=0.05*)	0.098	0.102	0.143	0.154	0.098	0.072	26.710	25.484	22.49	20.628	38.398	33.876

*, **, TVC, K_1-K_5, T_1, T_2, P_1, P_2: As in Tables 1and 2 (n=3)
Each value is a mean of 3 replications (n=3)

considerable reduction of microbial population in kinnow juice in heat-processing at 90^{0}C for 10 minutes. Potassium meta-bi- sulphite produces sulphur dioxide which, is turn, acts as a preservative and checks oxidation of juice constituents and growth of micro- organisms. Sethi *et al* (1980) reported that kinnow juice preserved with 700ppm of sulphur dioxide remained unspoilt upto six months.

It may be concluded that formulations of mixed (blend) fruit juice beverage can be developed to satisfy consumer taste and preference. These juice blends can be stored effectively for a period of six months. 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^{o}C for 15 minutes with 750 ppm potassium meta-bi-sulphite proved to be the most effective treatment for superior physico-chemical and sensory score of the juice blends. Minimum microbial population (Bacteria, 2.0 x 10^3 and 2.5 x 10^3 TVC/ml juice; yeast, 9.0 x 10^2 and 9.9 x 10^2 cfu/ml juice; mold, 1.2 x 10^3 and 1.3 x 10^3 cfu/ml juice) was recorded in juice processing at 85^0 C temperature with same treatment combination.

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