STUDIES ON PREPARATION OF READY TO SERVE MANDARIN (*Citrus reticulata*) DIET DRINK

Mubeen Ahmed[a], Asif Ahmad[b], Zia Ahmad Chatha[a] and Syed Muhammad Raihan Dilshad[c]
[a]National Institute of Food Science & Technology, University of Agriculture Faisalabad
[b]Department of Food Technology, PMAS, Arid Agriculture University Rawalpindi
[c]Department of Farm Machinery & Power, University of Agriculture, Faisalabad

Citrus fruits are considered to be the rich source of ascorbic acid, pectin, carotenes, citric acid, and minerals like calcium and phosphorous. Consumption of high sugar drinks lead to various diseases such as diabetes, obesity and dental caries. This study aimed at formulation of diet beverage with various combinations of intense sweeteners and was compared with control beverage containing sucrose. Physiochemical changes in beverage were also investigated at various storage intervals. A gradual increase in reducing sugar level was observed in all treated samples with the passage of time, while non-reducing sugars decreased gradually during storage studies. The declining trend in ascorbic acid contents of mandarin drink was increased as a function of storage. Sensory results showed that there was declining trend in the scores obtained for color. The overall results showed that combination of different sweeteners gave best results for taste than without combinations.
Keywords: Citrus, diet drink, storage, physiochemical properties

INTRODUCTION

Mandarin (*Citrus reticulata*) is one of the most popular citrus fruit having attractive bright colour, appealing taste and flavor. In Pakistan, approximately 60 per cent of total citrus production is comprised of mandarin being popularly known as 'Kinnow'. The composition of citrus fruit juice is beneficial with respect to its mineral and ascorbic acid contents (Fladae *et al.,* 2003). There is a great potential to use this fruit in value added products such as diet drinks. These types of citrus drinks are probably the most recognized and globally accepted fruit drinks (Nchez-moreno *et al.*, 2003; Gorinstein *et al.*, 2004).
Alternative sweeteners can avoid problems with dental decay and other health risks associated with the excessive consumption of caloric sweeteners, such as sucrose (Cardello and Damasio, 1997). In Pakistan use of artificial sweeteners is limited but being economical, it is helpful to control obesity and other potential diseases. A variety of artificial sweeteners are available in the market like, aspartame, cyclamate, sucralose, saccharin, and acesulfame-K (Pottasium salt of Acesulfame) etc. These are the non-nutritive sweeteners which are not metabolized by the body and do not contribute any energy or calories to the diet. The use of low-calorie sweeteners could improve dietary quality if consumers used energy savings for the consumption of nutrient dense foods (ADA, 2004). Leth *et al.,* (2007) reported that use of sweeteners like for acesulfame-K, aspartame and saccharin in Danish society is much lower than the acceptable daily intake (ADI). A recent analysis (Sigman-Grant and Hsieh, 2005) of data from national diet surveys indicates that American adults who use reduced-sugar products have better diets than those who use the full-sugar versions of the same foods and beverages. Extensive scientific research has demonstrated the safety of the low-calorie sweeteners and is currently approved for use in foods in the United States. (Kroger *et al.*, 2006). More recently safety of aspartame was reconfirmed by European Food Safety Authority in First European conference on aspartame (Renwick, and Nordmann, 2007)
In developed countries such sweeteners are being utilized for certain preparations to reduce the calories but in Pakistan their use in limited. Keeping in view all these facts, the project was designed to replace sugar with non-nutritive sweeteners completely to prepare ready to serve Mandarin diet drink. Thus, this study was aimed at selection of suitable sweetener for diet drink preparation that can replace sucrose partly or wholly. Furthermore, the influence of sweeteners alone and in blends was evaluated on the basis of sensory and physiochemical characteristics of diet drink.

MATERIALS AND METHODS

Fully ripened, mature, fresh and sound mandarin fruit were purchased from the local market and the materials such as sucrose, cyclamate, aspartame and acesulfame-k (potassium salt of acesulfame i.e. an intense sweetener) were also purchased from local market. Fruit were washed in tap water and then were peeled and divided into halves. Fruit juice was extracted in a rose head citrus juice extractor. After juice extraction, raw juice was heated at 96^{o}C for two minute to inactivate enzymes. Following the heating process, the juice rapidly was cool down to room temperature, was filtered through 8-folded cheese cloth to eliminate particulates and then was blended in high speed blender along with other ingredients as shown in Table 1. Fifteen beverage treatments combination were formulated with sucrose, cyclamate, aspartame and acesulfame_k. The drink prepared with 100% sucrose was used as a control. The detail of treatment is depicted in Table 2. The prepared beverage was kept in 250 ml glass bottles. After bottling, all juice samples were again heated at 96^{o}C for 20 min. Then samples were cooled with tap water and were stored at 4°C. Treated drink samples were evaluated at 0, 10, 20, and 30 days of storage for physiochemical analysis and sensory evaluation.

Table 1. Formulation of diet citrus beverage

Ingredients	Concentration (w/w)
Juice/Pulp	15%
Water	80-85%
Stabilizer/CMC	0.4%
Citric acid	0.15%
Na-Benzoate	0.1%
Ascorbic acid	0.03%
Flavor	0.05%
Color	0.014%
Sweeteners	As mentioned in treatments table

Physiochemical Analysis

The total soluble solids in the samples were directly recorded by Abbee's stage refractrometer (Model RL No. 1373, USA) and the results were expressed as per cent soluble solids (°Brix) as describe by (Rangana, 1991). Titratble acidity was determined by AOAC (2000) methods and expressed in terms of percentage citric acid. Product pH was measured using a pH meter (Model HI 9020 Microprocessor, USA), by the method given by Ruck (1963). The pH meter was standardized by using buffers of pH 7.00 and 4.00 prior to recording pH of the samples.

Brix/Acid ratio was calculated by dividing the brix of the drink with the percent acidity. Reducing and non-reducing sugars in the drink were determined by Lane and Eynon method as described by Ruck (1963).The Ascorbic acid was determined by using spectrophotometer according to the method of Ruck (1963) by using 2, 6 dichlorophenol indophenol dye.

Sensory evaluation

Standard sensory evaluation procedures were followed to perform descriptive analysis; panelists were trained using repeated round table and individual evaluations of trial formulations of the control and diet beverages samples. Hedonic scale method as described by Larmond (1987) was used for the organoleptic evaluation of drink for color, flavor and taste by a panel of six judges at 0,15, 30, 45 and 60 days storage period .

Table 2. Combinations of treatments used in Juice formulation

Treatment code	Sweeteners combinations			
	Sugar (%)	Cyclamate (%)	Aspartame (%)	Acesulfame-k (%)
T0	100	0	0	0
T1	0	75	25	0
T2	0	50	50	0
T3	0	25	75	0
T4	0	75	0	25
T5	0	50	0	50
T6	0	25	0	75
T7	0	0	75	25
T8	0	0	50	50
T9	0	0	25	75
T10	0	100	0	0
T11	0	0	100	0
T12	0	0	0	100
T13	0	50	25	25
T14	0	25	50	25
T15	0	25	25	50

Statistical analysis

Statistical analyses of data were done by using ANOVA on all experimental groups with three replicates each. The experimental groups were then separated statistically by using Duncan's new multiple range tests, as described by Steel *et al.*, (1997).

RESULTS AND DISCUSSION

Total soluble solids (TSS)

The data on total soluble solids (TSS) for all treatments has been presented in Table 3. There was a marginal decrease in TSS was observed in all treatments as compared to control. Interaction between storage time and treatments was found non-significant. Maximum total soluble solids were found in control samples (T_0) that was formulated with sucrose (100%) whereas minimum TSS was found in T_7 that was formulated with combination of aspartame (75%) and acesulfame-K (25%). During storage a slight increase in TSS was observed in all samples. The increase in TSS might be due to the formation of pectic substances from protopectin and mono saccharides from disaccharides i.e. degradation of sucrose into glucose and fructose. Similar results have been reported by Sarolia and

Mukherjee (2002) in their studies on lime juice; These results are also in connection with previous studies of Kaunjoso and Luh (1967) while studying on the canning and storage of oranges and in canned peaches.

pH

The pH has great importance to maintain shelf stability; pH can also influence the flavor and processing requirements of the beverage. The data about pH (Table 3) indicated that there is a variation in control and treated samples. Highest pH was observed in T_7 while lowest was experienced in T_4 (cyclamate 75% and acesulfame-K 25%). Storage intervals also influenced the pH of the beverage. A decline in pH towards acidic region was noticed as the storage of beverage increased. However, this decline in pH was non significant at 45 day and 60 day interval. This decrease in pH was attributed to formation of acidic compounds by degradation of reducing sugars, as discussed by Zia (1987). Similar trend of decreasing pH was also reported by Saleem (1980).

Acidity

Acidity is also an important attribute because tartness is a major factor in the acceptability of kinnow drink. Acid gives the characteristic sourness to the product. Citric acid is the major acid in kinnow juice that enhance the characteristic flavor of kinnow drink. The data regarding acidity in different treatments of ready to serve kinnow diet drink is presented in Table 3. Data showed that treated sample differ from control for the parameter of acidity. Highest acidity was recorded in T_{11}(aspartame 100%) while lowest was observed in T_7. Highest acidity in aspartame treated sample was due to acidic nature of aspartame. There was gradual increase in acidity in all treatments during storage upto 60 days. However non significant variation in acidity was observed at storage interval of 45 and 60 day. This increase in acidity was attributed to the degradation of sugars into carboxyl acids. Similar trend was also observed by the Saito *et al.* (1974) during studies on fruit juices.

Brix acid ratio

Brix acid ratio is a best indicator for measuring relative sweeteners or tartness of the product. The higher the brix in relation to acid contents of the drink then it means higher will be the ratio of sugars and sweeter the taste. Similarly lower the brix in relation to acidity of the drink, the lower the ratio of sugars and product will be sour in taste. Statistical analysis indicated that the results are highly significant for storage and treatment and their interactions are non-significant. Higher brix acid ratio was observed in control samples due to presence of sucrose that gives higher brix to drink. Brix acid ratio was found nonsignificant for treatment T_1 to $T_{6.}$ Lowest brix acid ratio was observed in $T_{11.}$ This showed a little bit more sourness in samples treated with aspartame.

Reducing sugars

For reducing sugars there appeared a significant variation between control and treated samples (Table 3). However, all treated samples varied nonsignificantly with each other. Reducing sugar increased with increase in storage time and significant variation exist at all storage intervals (Table 4). This significant increase in reducing sugars during storage intervals might be due to inversion process of sucrose to glucose and fructose by the acid of the diet drink. Similar observations were also reported by Babsky *et al.* (1986), and Pruthi *et al.* (1984) that non reducing sugars of drinks is converted in to reducing sugars during storage.

Nonreducing sugars

The data regarding non-reducing sugars (Table3) revealed that treated samples differed significantly from control samples. However, for this parameter all treated samples differed nonsignificantly with each other. As concerned with storage, a slight decline in reducing sugar was experienced at all storage intervals. However this decline in non reducing sugar was slow in initial storage and found non significant at 0 day and 15 day storage. For the rest of intervals these changes were significant with each other. Such decline in non-reducing sugars was attributed to its conversion into reducing sugars during storage. A similar decline in non reducing sugar was observed by Sandi *et al.*, (2004), during storage of drinks.

Ascorbic acid

Marginal differences in ascorbic acid contents were observed in various treatments. Treated samples also differed from control samples with respect to ascorbic acid contents (Table 3). Statistical Analysis showed that the results are highly significant for storage period. Ascorbic acid contents decreased significantly at all storage intervals. These losses of ascorbic acid were attributed to the effect of processing, storage time and exposure to light. The degradation of ascorbic acid in juice or drink may follow aerobic and an-aerobic pathways (Moshonas and shaw, 1989). Similar decreasing trend for ascorbic acid contents in different fruit beverages were also reported by the Ranote and Bains (1982), Robertson and Samaniego (1986).

Table 3. Effect of treatments on physiochemical properties of diet beverage

Treatment code	Physiochemical parameters						
	TSS	**pH**	**Acidity**	**B/A**	**RS**	**NRS**	**AA**
T_0	18.8 a	3.40 def	0.72 bc	26.81 a	2.72 a	14.88 a	26.2 ab
T_1	2.00 bc	3.33 fgh	0.66 cd	3.04 cd	0.75 b	0.75 b	25.0 ab
T_2	2.10 b	3.32 gh	0.68 bcd	3.09 cd	0.77 b	0.75 b	26.2 ab
T_3	2.00 bc	3.37 efg	0.64 de	3.15 cd	0.83 b	0.76 b	27.4 a
T_4	2.17 b	3.29 h	0.73 b	3.01 cd	0.81 b	0.75 b	25.8 ab
T_5	1.97 bc	3.47 bc	0.57 fgh	3.46 cd	0.83 b	0.75 b	24.4 b
T_6	1.98 bc	3.36 fgh	0.65 de	3.08 cd	0.81 b	0.75 b	24.8 b
T_7	1.80 c	3.65 a	0.28 j	6.64 b	0.85 b	0.74 b	25.4 ab
T_8	2.12 b	3.49 bc	0.51 hi	4.23c	0.87 b	0.75 b	27.4 a
T_9	2.08 b	3.50 b	0.49 i	4.26 c	0.85 b	0.74 b	27.4 a
T_{10}	2.04 bc	3.46 bcd	0.54 ghi	4.18 c	0.83 b	0.70 b	26.2 ab
T_{11}	2.12 b	3.32 gh	0.92 a	2.31 d	0.81 b	0.73 b	25.0 ab
T_{12}	2.09 b	3.43 cde	0.59 efg	3.54 cd	0.72 b	0.73 b	24.2 b
T_{13}	2.00 bc	3.47 bc	0.52 hi	4.06 c	0.74 b	0.75 b	24.8 b
T_{14}	1.94 bc	3.32 gh	0.69 bcd	2.84 cd	0.85 b	0.77 b	25.0 ab
T_{15}	2.04 bc	3.36 efg	0.63 def	3.25 cd	0.89 b	0.73 b	26.0 ab

TSS (Total soluble solids); B/A (Brix acid ratio); RS (Reducing sugars); NRS (Non reducing sugars); AA (Ascorbic acid)
Means carrying similar letters in a column do not differ significantly ($p<0.05$)

Table 4. Effect of storage on physiochemical properties of diet beverage

Parameters	Storage Intervals				
	0 day	**15 day**	**30 day**	**45 day**	**60 day**
TSS	2.93 c	3.02 bc	3.07 b	3.14 ab	3.22 a
pH	3.49 a	3.46 b	3.42 c	3.35 d	3.32 d
Acidity	0.51 d	0.56 c	0.61 b	0.68 a	0.71 a
Brix acid ratio	5.96 a	5.56 ab	4.87 bc	4.50 c	4.42 c
RS	0.69 e	0.80 d	0.94 c	1.04 b	1.20 a
NRS	1.77 a	1.71 a	1.64 ab	1.57 bc	1.46 c
AA	40.3 a	29.8 b	25.4 c	19.3 d	13.8 e

TSS (Total soluble solids); RS (Reducing sugars); NRS (Non reducing sugars); AA (Ascorbic acid)
Means carrying similar letters in a column do not differ significantly ($p<0.05$)

Color

The effect of treatments on color of diet drink is depicted in Figure 1. T_1 was ranked highest for color score this was followed by T_9 and T_{10}. These treatments shared nonsignificant difference with control samples. T_{14} was ranked lowest as regard to its color characteristics. Storage had a significant effect on color perception of diet drink. The maximum scores for color were observed when it was freshly prepared. As the storage period increased, a slight decline in color score was experienced (Figure 2). The gradual loss in color over the entire storage period was due to action of acid present in the drink. Previous studies by Muhammad *et al.*, (1987) also reported similar loss in color during storage of beverage samples.

Flavor

Flavor of the diet drink was affected significantly by treatments (Figure 1). T_{14} got the maximum score for flavor. While lower flavor perception was recorded in T_7 in which aspartame and acesulfame_k was added in 3:1. T_1, T_4, T_5, T_8 shared non significant difference with control. A significant variation was observed in flavor perception of diet beverage at various storage levels. The maximum scores for flavor were observed when it was freshly prepared. As the storage period increased, a slight decline in flavor score was experienced (Figure 2). The gradual loss in flavor scores over the entire storage period was due to changes in volatile compounds of the drink. Flavor deterioration in beverage products was also reported by Jain *et al.* (2003), Bezman (2001).

A significant variation was observed in taste of diet beverage at various storage levels. The maximum scores for taste were observed when it was freshly prepared. As the storage period increased, a slight decline in taste score was experienced (Figure 2). The gradual loss in flavor scores over the entire storage period was due to changes in volatile compounds of the drink (Marcy *et al.*, 1984). The taste difference and loss might be due to time and temperature, and duration of storage. Similar findings were also reported by Jain *et al.*, (2003).

Fig. 1. Effect of treatments on organoleptic evaluation of diet beerage

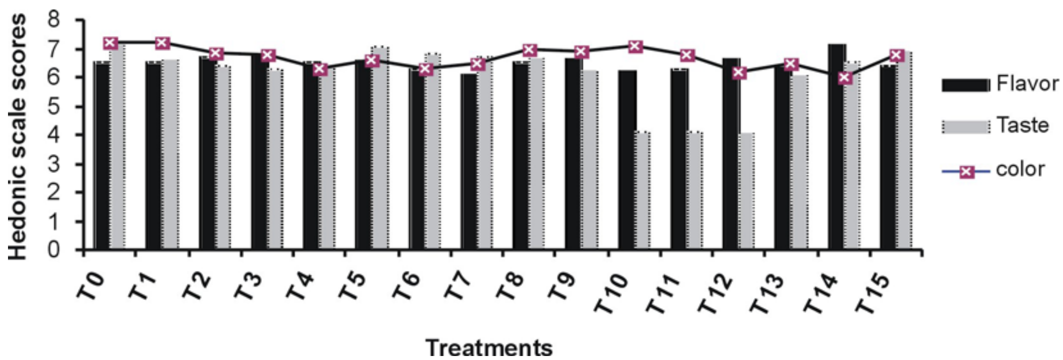
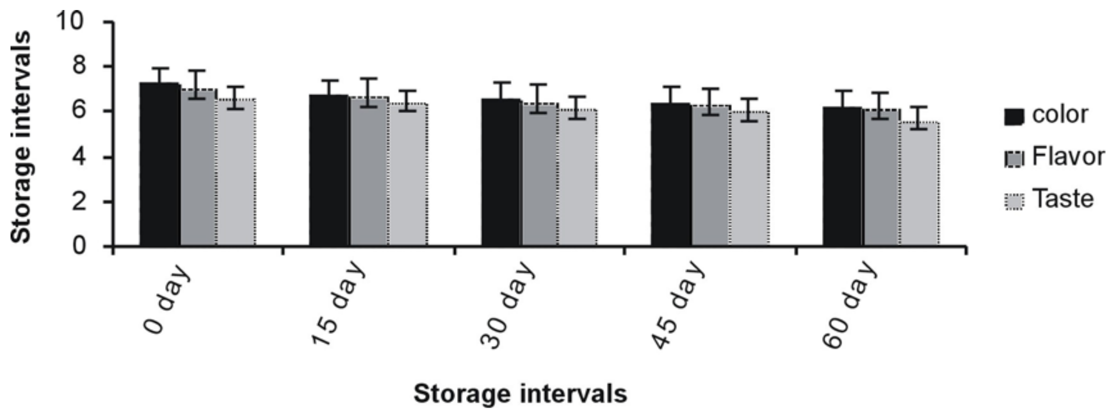


Fig. 2. Effect of storage on organoleptic characteristics of diet beverage



Taste

In organoleptic evaluation taste is very important factor after color and flavor. Statistical Analysis revealed a significant affect of treatment and storage on taste kinnow diet drink. Control sample got higher scores of taste as compared to treated samples (Figure 1). It was noted that diet drink produces with single sweetener got less scores as compared to drinks samples in which combination of sweeteners were used. Single sweetener, might added some bitter after taste while, in combinations/blends taste sensation was acceptable because bitterness of the single sweetener was masked by the blends of different types of sweeteners.

CONCLUSION

It can be concluded from this study that, the non-nutritive sweeteners can be effectively used as alternative source of sweetness in mandarin drink. Some changes in physiochemical characteristics were observed but these changes did not affect the product considerably. All of the sensory parameters decline slightly during storage but remain in acceptable region even after 60 days of storage.

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