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Effect of juice extraction methods, potassium metabisulphite concentration and storage temperature on the extent of degradation and reactivity of chemical constituents in mandarin (*Citrus reticulata* Blanco) juice

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Abstract

Fresh mandarin juice is perceived to be more wholesome than processed juice. Fresh clarified mandarin juice turned in brownish dark colour and ascorbic acid retention is also difficult during long storage. The experiment consisted of 18 treatment combinations with 3 concentrations of potassium metabisulphite, 3 storage temperatures and 2 types of juice extraction methods. Juice extracted with screw type juice extractor and preserved with 1000 ppm potassium metabisulphite and stored at 3°C maintained better qualitative characteristics *viz.,* total soluble solids, acidity, ascorbic acid, sugars, free amino acids, soluble proteins and non-enzymatic browning during 6 months storage. Mandarin juice extracted with screw type hand operated extractor can be stored for 6 months at 3°C storage temperatures with minimum biochemical changes.

Key words: Mandarin, juice, storage temperature, potassium metabisulphite, juice extraction method, non-enzymatic browning.

Introduction

Citriculture is the third largest fruit industry in India next only to mango and banana. It accounts for 12.60% of total fruit production and occupies 15.0% of total area under fruits in the country. In India, the area under citrus is 9.23 lakh ha which produces 86.08 lakh tonnes of fruits [1]. The mandarin having largest area and maximum production constitutes about 26.54% of total area under citrus[1]. About 95% of the fruits are sold fresh because after processing of the fruits, bitterness developed in the products which are not preferred by consumers. The shelf life of fruit is very short at room temperature [2]. In view of its limited shelf-life, it must be processed to assure availability of it's produce and also to minimize the glut in the market in its peak season of production[3]. Citrus juices are consumed in many countries. Orange juice accounts for 60% of all Western Europe consumption of fruit juices and juice based drinks. In the USA, orange juice is the most popular juice, being consumed for instance five more times than apple juice [4]. At present, its wide-spread use in citrus industry is handicapped because of a hindrance to the popularity and processing of the juice which is the development of non-enzymatic browning and bitterness [5, 6] .This has been recognized as a serious problem of citrus industry all over the world. Fruit juices and beverages must be handled carefully during processing and storage to control nutrient losses. Ascorbic acid is an important nutritional component of many juice products, and the label ascorbic acid content per serving must be valid throughout the product shelf life. Numerous complex factors, including juice extraction method, preservative treatment and storage temperatures, affect ascorbic loss in foods and the kinetics of degradation appears to be depended on the specific processing system[7] . Ascorbic acid degradation is also associated with non-enzymatic browning [8] .

As far as the methods of juice extraction are concerned, few methods have been in use for extraction of citrus juice. Among them screw type juice extractor is only used to extract the juice from mandarins [9]. For these reasons, the main objective of this study was to find out the effect of juice extraction methods, potassium metabisulphite concentration and storage temperature on chemical constituents and non-enzymatic browning of mandarin juice.

Materials and Methods

Location of experiment: The experiment was conducted in the Postharvest Technology Laboratory, Department of Horticulture, SKN College of Agriculture, Jobner, Rajasthan, India. Jobner is situated at 26°05´ N latitude and 75°20´ E longitude at an elevation of 427 m above mean sea level.

Treatment application: The fully matured, well-developed and uniform sized fruits of 'Nagpur' mandarin (*Citrus reticulata* Blanco) were purchased from fruit market, Jaipur, and brought to the Postharvest Technology Laboratory of the Department on the same day. Fruit were inspected thoroughly for any damage

and spoilage. Selected fruits were thoroughly washed in tap water to remove dirt, dust particles and insecticidal residues. Juice was extracted after manual peeling. Two juice extraction methods *viz.*, screw type hand operated juice extractor (J_1) and power operated commercial juice extractor (J_2) were used for the study. The peeled fruits were fed into J_1 and J_2, separately. In the extractor, the juice and the pomace were separated and both were collected separately. The juice was divided into three lots. It was filled in the pre-sterilised bottles. Potassium metabisulphite (KMS) @ 0.05 (K_1), 0.075 (K_2) and 0.1 (K_3)% were added to the juice filled bottles by dissolved in small quantity of water and sealed with crown corking machine. Bottles of each treatment combination were stored in three different storage temperatures *viz.*, room temperature (T_1), 10°C (T_2), and 3°C (T_3), respectively. Therefore, total 18 treatment combinations were used for the study. Stored juices were used for physico-chemical analysis at one month interval for six months.

Quality evaluation: The total soluble solids (TSS) content of the fruit juice was determined by using 'Zeiss-Hand' refractometer of 0-32% range. The values obtained were corrected at 20°C with the help of temperature correction chart and expressed as ºBrix of fruit juices [10]. For change in acidity, a known volume of clean juice was diluted with distilled water and titrated against 0.1 N NaOH using phenolphthalein as an indicator. The ascorbic acid content was determined by diluting known volume of juice with 3% metaphosphoric acid as buffer and titrating it against 2,6-dichlorophenol indophenol dye solution until the stable faint pink color was obtained [10]. The results were expressed as mg ascorbic acid/100 ml of fruit juice. Total soluble sugars content was determined by using anthrone reagent method [11]. To 1 ml of diluted fruit juice (100 times), 4 ml of anthrone reagent was added and heated for 10 min in a water bath, cooled to room temperature (28.4 ºC) and absorbance was measured at 630 nm on spectrophotometer (GS 5700A, Electronics Corporation of India Limited, Hyderabad, India). The amount of sugars present in the juice was plotted against standard curve prepared from glucose. The content was expressed on per cent basis. Reducing sugar content was measured by following Nelson's modification of Somogyi method[12]. Non-enzymatic browning in the juice was determined by alcohol extraction method [13]. Free amino acids of the juice were estimated colorimetrically by a modified ninhydrin method [14, 15]. The amount of amino acid was calculated from a standard curve prepared with L-leucine and expressed as percentage of amino acids present in the juice. Soluble proteins of the juice were estimated colorimetrically by Lowry's method. The amount of proteins was calculated from a standard curve prepared with Bovine Serum Albumin (BAS) and expressed as per cent of proteins present in the juice [16].

Statistical analysis: The experiment was a factorial with a completely randomized design (CRD). Total number of treatment combinations was 18 with three replications and each treatment combination has six units. To test the significance of variation in the data, analysis of variance technique was adopted [17]. Significance of the difference in the treatment effect was tested through 'F' test.

Results

Effect of juice extraction methods: The power operated juice extraction method (J_2) recorded significantly higher TSS than the screw type juice extraction (J_1) from 1st to 6th month of storage. The higher TSS was recorded in J_2 treatment as compared to J_1 treatment at the end of storage (Fig. 1). J_2 recorded significantly lower acidity than the J_1 during entire storage. The acidity showed a decreasing trend from 0.96% in the 1st month to 0.42% after 6th month of storage in the juice due to screw type juice extraction (Fig. 2).

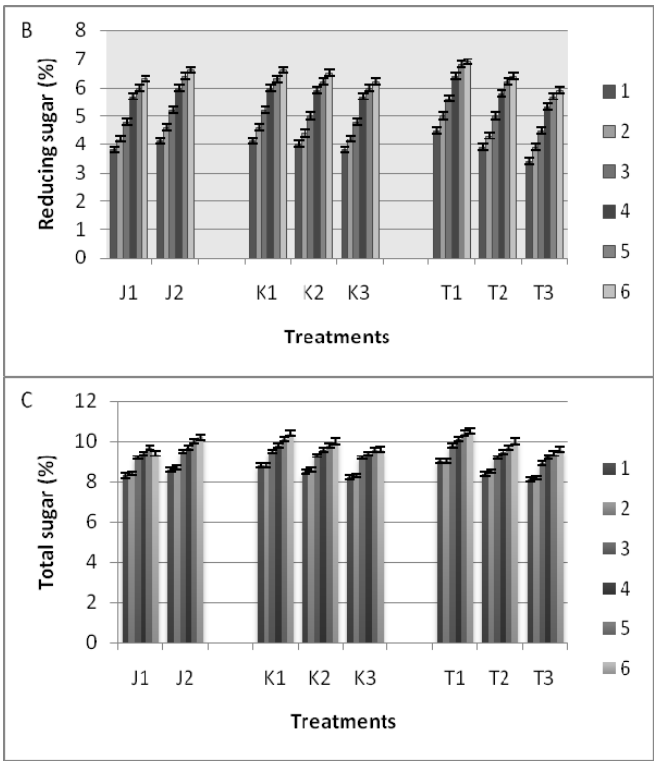

Figure 1. Effect of juice extraction methods, potassium metabisulphite and storage temperatures on (A) total soluble solids (B) reducing sugar and (C) total sugar content of mandarin juice. Data are means of 3 replications per treatments and vertical bars represent standard error. For abbreviations see material and methods.

The ascorbic acid content significantly decreased in J_2 compared with J_1 at all the stages of storage (Fig. 2). However, the ascorbic acid content progressively decreased under both extraction methods from 1st month to 6th month of storage. Reducing sugar was significantly higher throughout the storage period in the juice extracted from power operated extractor (J_2) in comparison to juice obtained from screw type extractor (J_1). At the end of storage it was higher by 4.77% over J_1 treatment. Data further revealed that reducing sugar increased with the increase in storage period and at the last month of storage 65.26% increase was noticed in reducing sugar over the 1st month of storage in J_1 treatment whereas it was 60.49% in J_2 treatment. Total sugars content of juice was significantly affected by juice extraction methods during entire storage. The rate of increase in total sugar content was almost similar in juice obtained from screw type juice extractor (18.24%) and power operated juice extractor (17.61%) at the end of storage over first month values. However, total sugar content was slightly higher in J_2 treatment as compared to J_1 treatments (Fig. 1).

The non-enzymatic browning was non-significantly affected by juice extraction methods on all the months of storage. However,

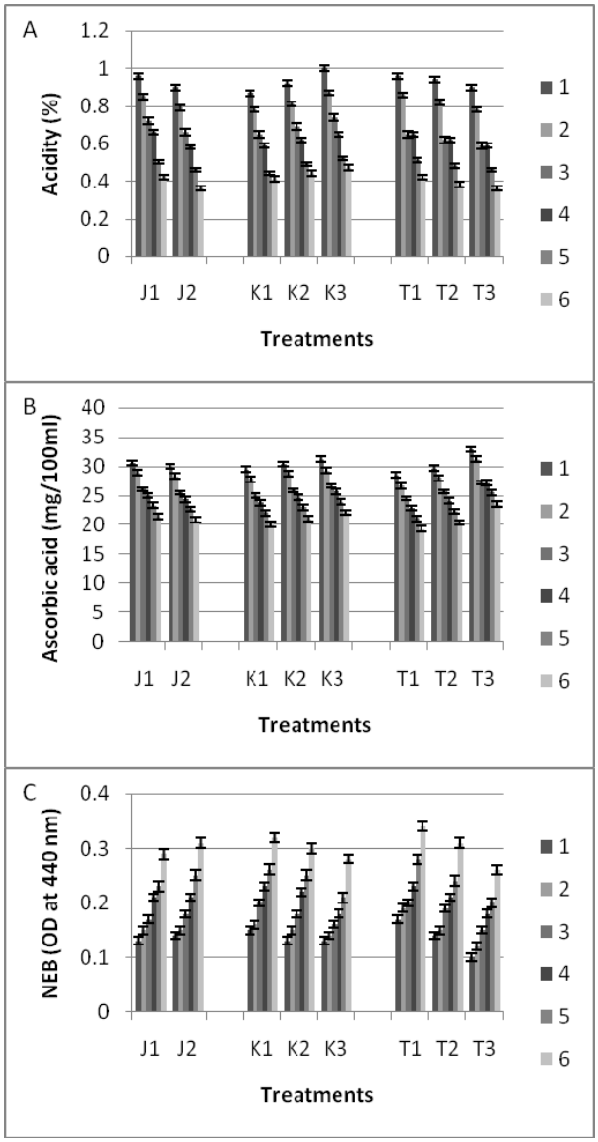

Figure 2. Effect of juice extraction methods, potassium metabisulphite and storage temperatures on (A) acidity (B) ascorbic acid and (C) non-enzymatic browning of mandarin juice. Data are means of 3 replications per treatments and vertical bars represent standard error. For abbreviations see material and methods.

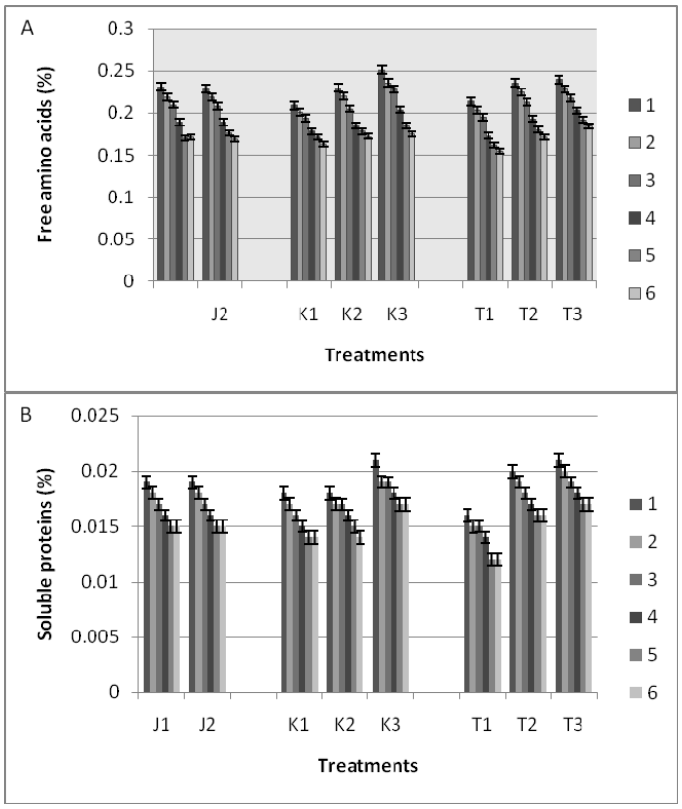

Figure 3. Effect of juice extraction methods, potassium metabisulphite and storage temperatures on (A) free amino acids and (B) soluble proteins of mandarin juice. Data are means of 3 replications per treatments and vertical bars represent standard error. For abbreviations see material and methods.

non-enzymatic browning was found to be progressively increased with the advancement of storage period (Fig. 2). Free amino acids of mandarin juice under storage was not affected significantly due to both types of juice extraction methods. However, it was noticed that free amino acids were progressively decreased during the storage from 1st to 6th month (Fig. 3). Soluble proteins in mandarin juice under storage was not affected significantly due to juice extraction methods. However, it was noticed that soluble proteins were progressively decreased during the storage from 1st to 6th month (Fig. 3).

Effect of potassium metabisulphite: TSS of juice decreased significantly with the increase in concentration of KMS up to 0.1%. The addition of 0.1% KMS (K_3) decreased the TSS content of juice by 4.88 and 2.59% at the end of storage over 0.05 and 0.075% KMS (K_1 and K_2), respectively (Fig. 1). Acidity decreased under all the three concentrations of KMS with the advancement of storage period, whereas, it showed an increasing trend with the increase in KMS concentration. Further reference to data indicated that acidity percentage of juice increased significantly with the increase in the concentration of KMS up to 0.1% and recorded a significant increase of 14.63 and 6.82% over 0.05 and 0.075% KMS, respectively, at the end of storage. The K_2 treatment also exhibited significant increase in acidity on 6th month of storage by 7.32% over K_1 treatment (Fig. 2).

Addition of 0.1% KMS (K_3) significantly increased ascorbic acid content in juice over 0.05 and 0.075% KMS. At the end of storage K_3 treatment registered an increase of 10.32 and 5.28% over K_1 and K_2 treatment, respectively. Addition of 0.075% KMS also increased the ascorbic acid content of juice at the end of storage by 4.79% over 0.05% KMS. Ascorbic acid was gradually decreased during the storage from 1st to 6th month. The minimum loss at the end of storage was noticed in the juice added with 0.1% KMS (K_3) over 1st month of storage (Fig. 2). Progressive increase in KMS concentration significantly decreased the reducing sugar content subsequently over previous concentration. Addition of 0.05% KMS (K_1) increased the reducing sugar to the extent of 2.48 and 6.09% over 0.075 and 0.1% KMS (K_2), respectively, at the end of storage period. The K_2 treatment also exhibited significant increase in reducing sugar on last month of storage by 3.53% over K_3 treatment. Succeeding increase in KMS concentrations significantly decreased the total sugar content over preceding treatment throughout the storage period. Total sugar increased with the advancement of storage from 1st

month to 6th month. The increase was highest in the juice added with 0.05% KMS on 6th month of storage over 1st month by 18.22%. Increasing concentrations of KMS significantly decreased the non-enzymatic browning in juice over the preceding levels in all the storage months. Addition of 0.1% KMS produced minimum non-enzymatic browning. Addition of 0.075% KMS (K_2) also decreased non-enzymatic browning on 6th month of storage by 17.24% over 0.05% KMS (K_1). Non-enzymatic browning increased during the storage from 1st to 6th month. The minimum per cent increase (107.69) at the end of storage was noticed in the juice added with 1000 ppm KMS (K_3) over 1st month of storage while maximum non-enzymatic browning (126.67%) increased in the juice added with 0.05% KMS at the end of storage over 1st month (Fig. 2).

The successive increase in KMS concentration added to juice increased the free amino acids under all the months of storage. Addition of 0.1% KMS (K_3) produced significantly higher free amino acids as compared to all other concentrations of KMS during all the storage months. Similarly, 0.075% KMS also produced significantly higher free amino acids as compared to 0.05% KMS in 6 months storage. Addition of 0.1% KMS retained the free amino acids by 2.33% and 1.74% over 0.05% and 0.075% KMS, respectively, at the end of storage. Addition of 0.075% KMS also showed higher free amino acids by 5.52% over 0.05% KMS on the 6th month of storage. Data further revealed that free amino acids content decreased in all the treatments as the storage period advanced (Fig. 3). The successive increase in KMS concentration added to juice increased the soluble proteins under all the months of storage (Fig. 3). Addition of 0.1% KMS produced significantly higher soluble proteins as compared to all other concentrations of KMS during all the storage months.

Effect of storage temperature: A significant decrease of TSS content was found in juice due to successive decrease in storage temperature. Lowest TSS at the end of storage was obtained in T_3 treatment, which was lower by 6.63 and 6.99% as compared to T_1 and T_2 treatment, respectively. Juice stored at 10ºC exhibited a significant decrease in TSS content over juice stored at room temperature by 2.68% at the end of storage period.

The acidity per cent significantly decreased in all the storage temperature treatments with the advancement of storage period (Fig. 2). Acidity also decreased with the decrease in storage temperature from room temperature (T_1) to 3ºC storage temperature (T_3). At the end of 6th month, storage temperature 3ºC recorded minimum acidity 0.36% which was lesser by 5.55 and 14.28% compared to T_2 and T_1 treatment, respectively. Ascorbic acid content of mandarin juice increased significantly with the decrease in storage temperature in all the months of storage. At the end of storage period the increase in ascorbic acid content under T_3 treatment was 21.96 and 12.17% higher over T_1 and T_2, respectively. The minimum per cent loss in ascorbic acid content during storage observed in the juice stored at lower temperatures of 3 ºC (Fig. 2). Reducing and total sugar content progressively increased with the successive increase in storage period from 1st to 6th month in all the three treatments. However, the absolute values were lowest in the juice stored at lower temperatures of 3ºC, but per cent increase was found to be highest at 6th month of storage over 1st month (Fig. 1).

Non-enzymatic browning of mandarin juice decreased significantly with the subsequent decrease in storage temperature in all the months of storage. At the end of storage minimum non-enzymatic browning was noticed in the juice stored at lower storage temperatures and the per cent decrease in non-enzymatic browning under T_3 was to the extent of 40.91 and 72.72% over juice stored at 10ºC and at ambient temperatures, respectively. Juice stored at 10ºC also showed lower non-enzymatic browning on 6th month of storage by 22.58% over juice stored at ambient temperature. Progressive increase in storage months increased the non-enzymatic browning under all three storage temperatures (Fig. 2).

Free amino acids of mandarin juice increased significantly with the decrease in storage temperature in all the months of storage. At the end of storage period the free amino acids content under T_3 was 18.71 and 7.60% higher over T_1 and T_2, respectively. The minimum per cent loss was observed in free amino acids content during storage in the juice stored at 3ºC (Fig. 3). Soluble proteins of mandarin juice increased significantly with the decrease in storage temperature in all the months of storage. At the end of storage period, the soluble proteins content under T_3 was 38.84 and 7.01% higher over T_1 and T_2, respectively. Soluble proteins of juice decreased with the advancement of storage period. The minimum per cent loss in free amino acids content during storage observed in the juice stored at T_3 while maximum soluble proteins was lost in the juice stored at T_1 (Fig. 3).

Discussion

The addition of KMS resulted into lowered rate of hydrolysis of polysaccharides, which ultimately reduced enhancement in TSS. Similar observation was also reported by Masoodi *et al.*[18] in grape juice. The minimum increase in TSS under lower storage temperature might be due to lower reduction in hydrolysis of polysaccharides and acids. Prasad and Mali [19] also reported similar findings in Kinnow juice. The TSS in juice increased apparently during storage period from 1st to 6th month in all the treatments studied, which might be due to hydrolysis of polysaccharides (starch) into monosaccharides (sugars), increase in concentration of juice due to dehydration and degradation of pectic substances of juice in soluble solids.

The juice treated with KMS showed higher retention of acidity during storage and acidity was found to be increased with increase in KMS concentration. This could be due to alteration in metabolism and enzymatic activity. The acidity decreased with the decrease in storage temperature from ambient storage (T_1) to 3ºC storage temperature (T_3). Low temperature and high humidity declined the process of conversion of acids into sugars and salts by enzymes, particularly, invertase [20]. A slight decrease in titratable acidity was observed in kinnow juice during 74 days of storage[21] and in mandarin juice during 180 days of storage [22]. The ascorbic acid content of the juice during storage reduced with the advancement of storage period because the ascorbic acid is very sensitive to oxidation. This loss of ascorbic acid might be due to the presence of air at the headspace of glass bottles during storage. Besides that, enzymes *viz.*, cytochrome oxidase, ascorbic acid oxidase, and peroxidase are also responsible for oxidation of ascorbic acid and subsequent loss of vitamin C potency [23]. Further, Nagy[23] reported that loss of ascorbic acid potency in processed products is due to aerobic and anaerobic reaction of non-enzymatic nature also. The incorporation of air into the juice during extraction, finishing and bottle filling have long been recognized by Farnworth

et al.[24] as causing ascorbic acid loss. Beltran *et al*.[7] also reported the loss of ascorbic acid in stored orange juices with the storage period. After 6 months of storage 74% loss in vitamin C was observed in cucumber-litchi-lemon blended juice [25]. Comparatively lower losses of ascorbic acid was observed in juice samples preserved with higher concentration of KMS than those preserved with lower concentrations (K_1 and K_2). This might be due to the higher concentration of KMS reduced the oxidation of ascorbic acid during storage for long time. The retention of ascorbic acid was more at lower storage temperature as compared to storage temperature of 10ºC and ambient storage. This might be attributed to low temperature and high relative humidity in storage, which inhibited the rate of oxidation and metabolic activities. Sulphitation (0.035% SO_2) of Indian gooseberry juice coupled with storage at low temperature minimized the loss of vitamin C and prevented non-enzymatic browning even after 6 months of storage [26]. Ascorbic acid also decreased in the Indian gooseberry juice under storage treated with different pasteurization treatments and SO_2 concentrations [27].

The reducing and total sugar contents of juices were increased with the advancement of storage period in all the treatments. The increase in total sugars might be due to the hydrolysis of polysaccharides like pectin, cellulose, starch etc. and its conversion into simple sugars. An increase in reducing sugar with the increasing period of storage in all the treatments could be attributed to gradual inversion of non-reducing sugar and acids into reducing sugars in acidic medium. Reducing and total sugars found to be decreased with the increase in KMS concentration in juice, however, it was increased with increase in storage period. This might be due to the fact that KMS reduced the conversion of polysaccharides and acids into monosaccharides. The cause of higher increase in reducing and total sugar content in juice stored at ambient conditions, might be due to higher rate of solubilization. Another possible explanation of increasing reducing and total sugar may be the conversion of acids into sugars, which is also evident from the fact that the acidity content decreased during the storage. Thus, the storage at lower temperature reduced conversion of acid into sugars. Garg *et al*.[28] also observed the increase in reducing and total sugar content during storage in blended Indian gooseberry juices. TSS, acidity, ascorbic acid and sugars were most acceptable level in Indian gooseberry juice after 120 days of storage treated with 0.075% KMS [29].

A gradual increase in browning in juice with increase in storage period might be due to the enzymatic and non-enzymatic reactions in the juice. Storage temperature also influenced the browning intensity in mandarin juice. The effect of lower storage temperature (3ºC) on the non-enzymatic browning of juice could be attributed to low temperature and high humidity, which declined the enzymatic and non-enzymatic reactions in the juice during storage. Khurdiya and Anand [30] also reported a gradual increase in browning and formulation of hydroxy methyl furfural was noted in *Phalsa* beverage with increasing storage period and it was more pronounced at room temperature than at low temperature. The juice treated with 0.1% KMS showed minimum non-enzymatic browning during storage. The possible reasons might be due to inactivation of enzymes by KMS and protective action of β-carotene against non-enzymatic browning. Jain and Khurdia[26] reported that 0.035% SO_2 reduced the browning in stored Indian gooseberry juice. Handwerk and Coleman [31] showed that non-enzymatic browning in citrus juices is a complex phenomenon and affected with many factors. Sugar breakdown through the Maillard reaction is initiated by formation of hexose amines from amino acids and sugars present in juice and produce deoxy amino hexoses. These deoxy compounds go through a series of dehydrations, deaminations, and enolizations to produce either 1-deoxy-2,3-dicarbonyls or 3-deoxy-1,2-dicarbonyls. The pathway depends upon a relationship between the pH of the juice and the basicity of the amino hexoses. The 1-deoxy compounds further react to produce furanones and pyrones, whereas the 3-deoxy compounds produce furfurals and pyrroles. Both pathways are operable in mandarin juice. Further, Kacem *et al*.[32] proved that ascorbic and dehydroascorbic acids may enter into the browning scheme as highly reactive α-dicarbonyls. They also observed that the effect of amino acids on browning of single strength orange juice was linear with concentration and found to be more pronounced in the presence of high levels of ascorbic acid. The present findings are matched with these results. Pareek *et al*.[22] also reported the increase in non-enzymatic browning in mandarin juice under storage. Total phenolic contents and oxidation reactions were increased in apple juice stored for 30 days which was responsible for non-enzymatic browning.[33]

Free amino acids and soluble proteins are important compounds in juice from viewpoint of nutrition and chemical reactions occurred during processing and storage. These were maximum in the juice preserved with 0.1% KMS and stored at 3ºC, minimum in juice preserved with 0.05% KMS and stored at ambient temperatures. The decrease in the level of free amino acids and soluble proteins could be related to the involvement in the formation of non-enzymatic browning by interacting with organic acids and sugars.

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