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Effects of Freeze-drying and Freezing on Vitamins and Sugars of Mango Pulp ('Apple' Cultivar): A Preliminary Comparison of Methods for Improving Sample Storage

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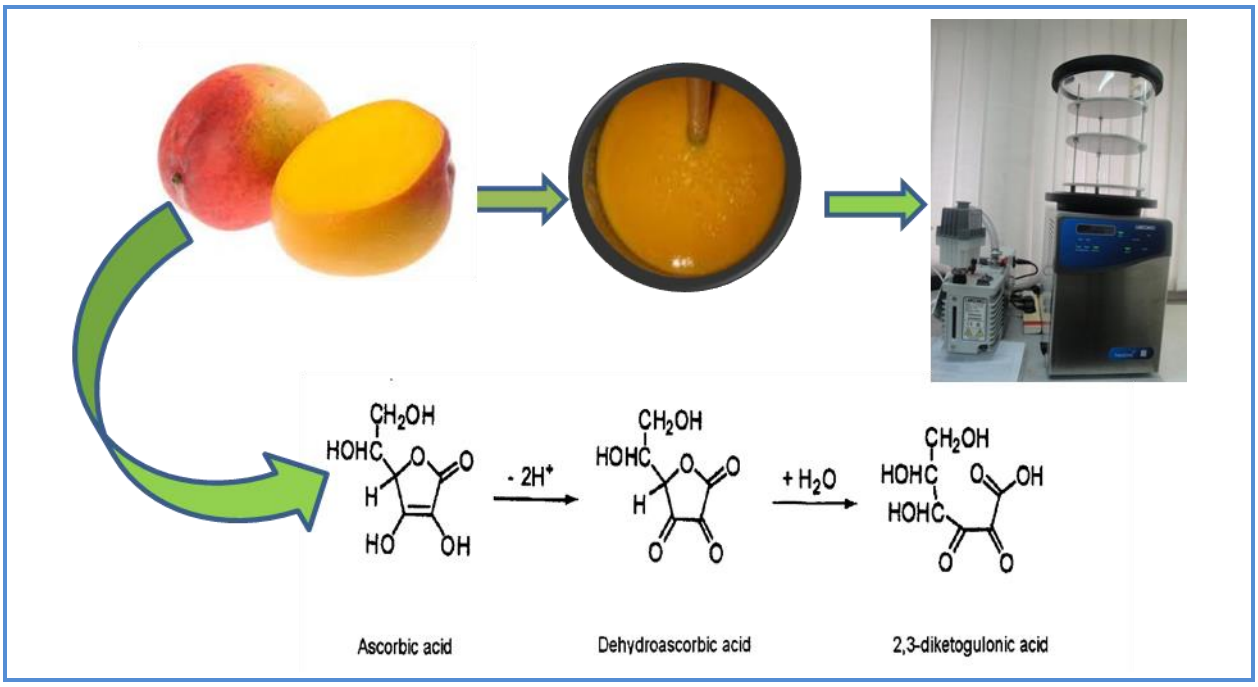
Bioactive substances
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ABSTRACT

Mango (*Mangifera indica L.*) fruits are highly perishable ones whose important nutrients such as vitamins and sugars quickly decrease under storage. In this study, we compared two methods of fruit pulp storage; freezing and freeze-drying in order to compare total carotenoids, *β*-carotene, ascorbic acid (AA), titratable acidity (TTA), total soluble solid (TSS) and pH. Mean total carotenoid, *β*-carotene and AA of frozen pulp were 7.08±0.15 µg/g, 1.47±0.09 µg/g and 92.77±17.49 mg/100 g respectively. While freeze-dried pulp had 8.06±0.03 µg/g, 2.35±0.03 µg/g and 113.02±0.07 mg/100 g for total carotenoid, *β*-carotene and AA respectively. The total carotenoid, *β*-carotene and AA of fresh pulp were; 8.15 µg/g, 2.44 µg/g and 119 mg/100 g respectively. TTA, TSS and pH were; 3.01±1.01%, 9.40±1.42 °Brix and 2.97±0.19 for frozen pulp and 2.97±0.01%, 11.70±0.08 °Brix and 2.99±0.01 for freeze dried pulp respectively. TTA, TSS and pH for fresh pulp were; 4.85%, 11.90 Brix% and 3.48 respectively. All measured parameters were significantly higher ($P<0.05$) in fresh sample than in the stored pulp (frozen or freeze-dried). Mean total carotenoids, *β* carotene, AA and TSS were significantly ($p<0.05$) higher for freeze-dried pulp than for frozen pulp. However, mean TTA and pH did not differ between freeze-dried and frozen pulp. Slow decrease of AA in the freeze-dried pulp further suggests the method as preferred for long term storage of mango pulps.

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Graphical Abstract



Introduction

Mango (*Mangifera indica L.*) is among the most important commercial fruit crops in Kenya with an estimated 34,371 ha under mango production with a net worth of Kshs 7.8 billion [1]. Mango fruits are potential sources of bioactive antioxidants such as *β*-carotene, ascorbic acid and phenolics [2]. These antioxidants play therapeutic and preventive roles against several diseases such as aging, inflammation and certain cancers [2, 3]. Being a climacteric fruit, the fruit is highly perishable due to, among other causes, the activity of degradative enzymes (such as polygalacturonase and cellulose) [3], hence a challenge in maintaining mango fruit quality over a long time is still at hand.

Developing efficient methods to reduce postharvest waste and maintain mango fruit quality throughout reasonably long periods is of utmost importance. To achieve this, appropriate storage methods are required. Post-harvest processes such as freezing, freeze-drying and packaging are some of the methods widely used. Freezing method is simple and inhibits the growth of microbes such as bacteria, mold, and the spoiler yeasts [4]. While freeze-drying method involves a process whereby water is removed by dehydration from the fruit, through sublimation of ice in the materials [5].

The solid state of water during freeze-drying protects the primary structure and minimizes changes in the shape of the product, with minimal reduction of volume [5]. It is a method recommended for drying

materials containing heat-sensitive antioxidant components such as tocopherols, ascorbic acid, carotenoids and plant phenolics [5].

Both the freezing and freeze-drying methods have been applied to fruit preservation [6-8]. The assumption has been that if significant portion of vitamin C content is retained after processing, other nutrients are also likely to be preserved [6]. This is because the ascorbic acid is extremely unstable to heat, oxygen, light, pH, moisture content, and heavy metallic ions (Cu^{2+}, Ag^{+}, Fe^{3+}) [6].

This makes ascorbic acid content of a fruit as a quality index of nutrients in fruit storage [6]. Other physiological parameters like soluble sugars (usually expressed as total soluble solids, TSS, as most of the solids are sugars) and titratable acidity (which can be expressed as the percentage of citric acid in the flesh) are also used.

In this study, we compare freezing and freeze-drying as methods of storage and evaluate the retention of total carotenoids; *β*-carotene, ascorbic acid, soluble sugars, titratable acidity and pH contents in mango pulp held over a period of time.

Experimental

Reagents, sample collection and preparation

Petroleum ether, ammonium sulphate, sodium hydroxide, sodium bicarbonate, anhydrous sodium sulphate, TCA and 2,6-dichloroindophenol (DCIP) were purchased from Fluka (Sigma Aldrich, Switzerland). All these chemicals and reagents were of analytical grade. TCA reagent was prepared by dissolving 10 g of TCA in 100 mL of distilled water and DCIP solution prepared by dissolving 0.250 g of 2,6-dichloroindophenol in 500 mL of water. Sodium bicarbonate (0.21 g) was then added and dissolved. The resulting solution was diluted to 1 L with distilled water to make approximate concentration of 250 mg DCIP/L.

Samples of mango (*Mangifera indica L.*) fruits, apple cultivar, was collected from local market around JKUAT University in Juja region, Kenya. Fully mature ripe fruits (n=30) which did not have/show any visual signs of bruises, cuts, blackening or infestation were selected for the study. Mango fruit samples were transported to food biochemistry department laboratory, Jomo Kenyatta university of agriculture and technology for further processing. The mangoes were manually washed with tap water, peeled, seeds removed and pulp ground in a home type blender. All the pulps were homogenized together then sub-samples in small portion in zip-lock bags. Different fruit pulp parameters were determined the same day and remaining samples kept at -20 °C and other portions freeze-dried. Successive analysis at intervals of one week from the day of first analysis of the fruits was done. Frozen samples in the falcon tubes wrapped

with aluminum foil were freeze dried (700 Pa) in a laboratory freeze-dryer for 3 days; product was packed in falcon tubes and stored at -20 °C until use. All freeze-drying experiments were performed in triplicate.

Extraction and determination of ascorbic acid

Five grams of frozen mango fruit pulp was accurately weighed and ground in a mortar. The ground pulp sample was mixed with 100 mL of the extractant 5% TCA solution. The mixture was homogenized in 100 mL conical flask wrapped with aluminum foil. 10 mL of the sample was taken in a beaker. The amount of vitamin C in a sample was determined by redox titration using the reaction between ascorbic acid in the sample and 2,6-dichloroindophenol (DCIP) titration method according to AOAC [9] methods as described by *Ranganna* [10]. The ascorbic acid content was calculated using the dye factor, determined by the titration of the standard ascorbic acid solution with DCPIP dye. Several precautions were taken in order to perform all the operations under reduced light. Vitamin C was calculated using the balanced equation for the oxidation-reduction reaction between ascorbic acid and DCIP as below;

$$\text{Vitamin C content (mg/100g)} = (A - B) \times C \times V/v \times \frac{100}{w}$$

Where:

A = Volume in mL of the Indophenol solution used for sample titration

B = Volume in mL of the indophenol solution used for sample blank titration

C = Mass in mg of ascorbic acid equivalent to 1.0 mL of indophenol standard solution

V = Volume of the sample after toping up (100 mL)

v = Volume of the sample taken for titration (10 mL)

w = Weight in g of sample taken for sample preparation

Extraction of *β*-carotene and total carotenoids

Total carotenoids and *β*-carotene were extracted using acetone-petroleum ether as a solvent by the Rodriguez-Amaya and Kimura method [11]. Approximately 2–5 g of frozen homogeneous mango fruit pulp was weighed accurately then placed in a motor and about 3 g of Hyflo supercel (celite) was added. This mixture was ground thoroughly with 50 mL of cold acetone (acetone refrigerated for about 2 hours prior to analysis). The extraction with acetone was repeated until the residue no longer gives color to acetone. The mixture was filtered with suction through a sintered glass funnel (or Buchner funnel with filter paper). During the filtration; mortar, pestle, funnel, and the residue were washed with small amounts of acetone, receiving the washings in the suction flask through the funnel. The residue or washings must be devoid of color. If not, the extraction was repeated. Petroleum ether (PE) (~40 mL) phase containing carotenoids was poured in a 500 mL separating funnel with Teflon stop-cock and acetone added. Slowly

distilled water (~300 mL) was added letting it flow along the walls of the funnel and avoiding formation of an emulsion by not shaking. However, in the event of emulsion formation, it was broken by adding saturated sodium chloride solution. The two phases were allowed to separate and the lower aqueous phase discarded. Washing was repeated 3–4 times with distilled water (~200 mL each time) to remove residual acetone. Petroleum ether phase was collected in a 50 mL volumetric flask, making the solution pass through a small funnel containing anhydrous sodium sulfate (~15 g) to remove residual water. (Cotton wool plug was used to hold the sodium sulfate).

Spectrophotometric determination of *β*-carotene

Five solutions of standard pure *β*-carotene (Sigma chemical Co.) with concentrations between 0.5 µg/g and 2.5 µg/g were prepared from a stock solution containing 2.5 µg/g. The absorbance values of the solution were determined at 450 nm using UV-vis spectrophotometer (UV 1601PC model, Shimadzu Corp., Kyoto, Japan) and plotted against their corresponding concentration to give a standard curve [9]. The absorbance of the unknown samples were determined at 450 nm after proper calibration of the instrument with standard solution of pure *β*-carotene. A standard curve made from *β*-carotene standard was used to estimate the quantity of *β*-carotene content in the mango samples.

The absorbance's of the samples were determined at 450 nm, and then total carotenoids calculated using equation below;

$$\text{Total carotenoids } (\mu\text{g/g}) = \frac{A \times Volume(mL) \times 10^4}{A_{1cm}^{1\%} \times Sample\ weight(g)}$$

A= absorbance; volume = total extract volume; $A_{1cm}^{1\%}$ = absorption coefficient of *β*−carotene in petroleum ether (2592)

Titratable acidity, total soluble solids, PH and data analysis

Total titratable acidity was determined by weighing 5 g of frozen ground mango fruit pulp. The ground pulp sample was mixed with 100 mL of distilled water. The mixture was homogenized in 100 mL conical flask wrapped with aluminum foil. 10 mL of the sample was taken in a beaker and titrated against 0.1 N sodium hydroxide with phenolphthalein as an indicator. The acidity was calculated as indicated in equation below and expressed as citric acid equivalent; 0.006404=equivalent weight of citric acid.

$$\text{Titratable acidity } (\%) = \frac{Titre\ volume \times 0.006404 \times 1000}{Sample\ weight}$$

Total soluble solids content was determined using a hand refractometer (1–32 °Brix) (Atago, Japan) and the results expressed as °Brix, while pH was determined with a glass–electrode pH meter. Data were expressed as mean value ± standard deviation. Independent t-test was applied to determine the significant difference at the level of $p<0.05$. R Statistical Package version 3.13 was used to analyze the data.

Results and discussion

Total carotenoid and *β*-carotene content

A total of 30 mango fruits of 'apple' cultivar were used. Total carotenoids and *β*-carotene contents of fresh, freeze-dried and frozen mango fruit pulps are shown in Table 1. No visible microbial growth was observed during the eight weeks of pulp monitoring. After eight weeks freezing at -20 °C, the total carotenoids and *β*-carotene content had mean of 7.14±0.22 µg/g and 1.48±0.08 µg/g respectively. While, for freeze-dried pulp, mean total carotenoids and *β*-carotene contents were 8.06±0.03 µg/g and 2.35±0.03 µg/g respectively. Linearity of the titration method was performed in a range of 0-2.5 µg/mL, the method showed good linearity with a regression of $y=0.2543 \times -0.0018$ ($r^2=0.9984$), where x and y were *β*-carotene concentration and UV absorption at 450 nm, respectively. According to Rodriguez-Amaya and Kimura [11], *β*-carotene standards must contain a 90% purity level, thus our result was comparable and satisfactory. There was a general decrease trend in beta carotene content after eight weeks of storage (Figure 1). It is recommended to freshly prepare the standard and extract carotenoids prior to UV measurements to prevent degradation of *β*-carotene and improve the method precision.

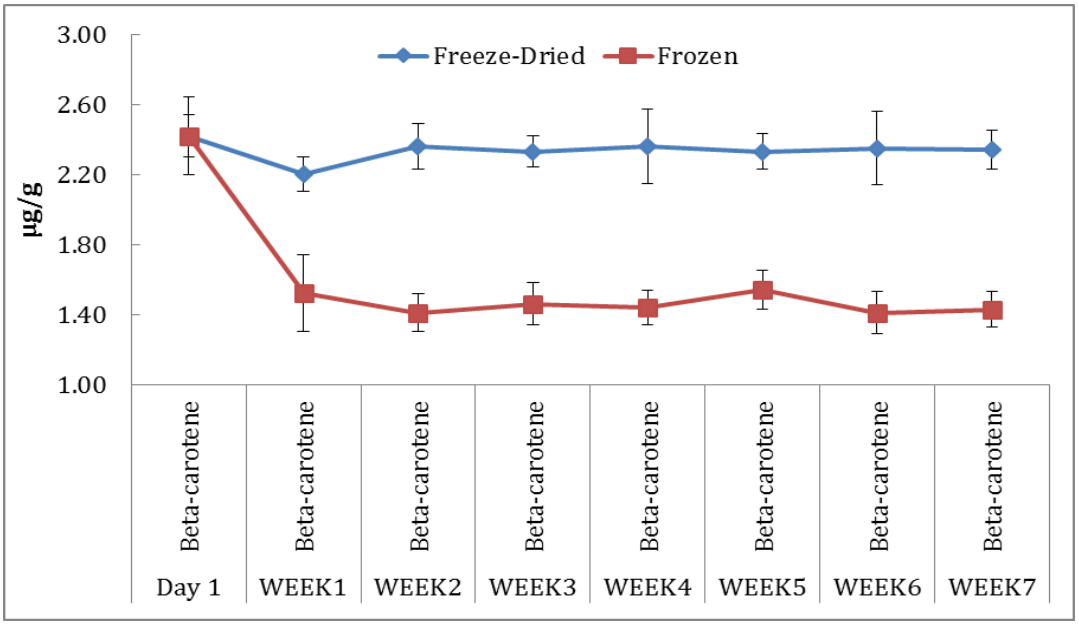


Figure 1. General trend in decrease of *β*-carotene content of mango pulp after seven weeks of storage

The mean vitamin contents of freeze-dried mango pulps were found to be significantly ($p<0.05$) higher than total carotenoid (8.06 µg/g) and *β*-carotene (2.35 µg/g) contents compared to those of frozen pulps which were total carotenoid (7.14 µg/g) and *β*-carotene (1.48 mg/100 µg/g) (Table 1). The mean values of freeze-dried mango pulp samples were similar to those found by *Muoki* et al., [12] in ripe 'Apple' mango cultivar, which ranged from 4.0-6.3 µg/g (mature green fruits), 8.0-14.8 µg/g (partially ripe fruits) and 11.3-32.0 µg/g (ripe fruits) collected from Kisumu, Mombasa and Machakos. *Okoth* et al., [13] reported *β*-carotene range of 4.61 µg/g of apple cultivar in Kitui and 35.27 µg/g in Machakos.

Table 1. Descriptive Statistics of freeze dried and frozen mango pulp after eight weeks of storage

Pulp Treatment		Total carotenoid (µg/g)	*β*-carotene (µg/g)	Vit. C (mg/100 g)	TTA (%)	pH	TSS (°Brix)
Frozen	Mean	7.14	1.48	93.42	3.22	3.03	9.68
	Std. dev.	0.22	0.08	21.81	1.12	0.24	1.57
	Min.	6.82	1.41	59.47	1.79	2.71	7.50
	Max.	7.61	1.66	119.00	4.85	3.48	11.90
	Range	0.79	0.25	59.53	3.06	0.77	4.40
Freeze-Dried	Mean	8.06	2.35	113.02	2.97	2.99	11.70
	Std. dev.	0.03	0.03	0.07	0.01	0.01	0.08
	Min.	8.03	2.33	112.92	2.96	2.97	11.63
	Max.	8.11	2.42	113.12	2.98	3.00	11.89
	Range	0.08	0.09	0.20	0.02	0.03	0.26

TSS=Total soluble solids, **TTA=**Titratable acidity. Where significant difference was seen within a row ($P\leq0.05$; Mann-Whitney test was used), values are lettered in descending order of size. n=number of weeks

Carotenoids in their natural form are esterified to fatty acids. Thus, mechanisms for carotenoid oxidation would follow that of lipid oxidation. Initiation of lipid oxidation results when hydrogen is abstracted, by action of light or trace metals, from an olefinic compound and a radical is formed [14]. Oxidation begins with the introduction of oxygen into the carotenoid molecule, forming carotenoid epoxides, followed by cleavage. Successive fragmentations result in small volatile compounds. Mango pulp was exposed to light due to storage in clear vials and dissolved oxygen in the pulp. These conditions would allow for the initiation and propagation of oxidation and esterified carotenoids resulting in degradation reactions leading to reduced carotenoid concentrations in stored mango pulp [14]. The activity of the other carotenoids decreases according to their chain length and number of conjugated double bonds [14].

Ascorbic acid content

Freeze-dried mango pulp was found to have a significantly ($p<0.05$) higher ascorbic acid content (113.02 mg/100 g) compared with those of frozen mango pulp (93.42 mg/100 g FW) (Table 1 and Figure 2). There was a general trend in decrease in vitamin C as weeks of storage increased (Figure

2). There are two degradation pathways for ascorbic acid earlier identified; the non-oxidative and the oxidative pathways [15].

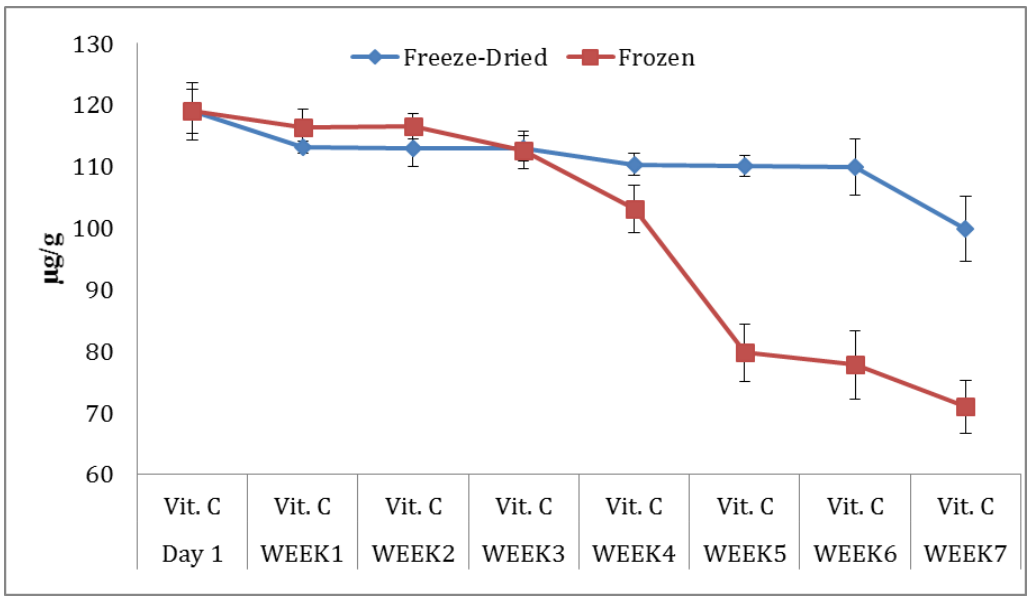
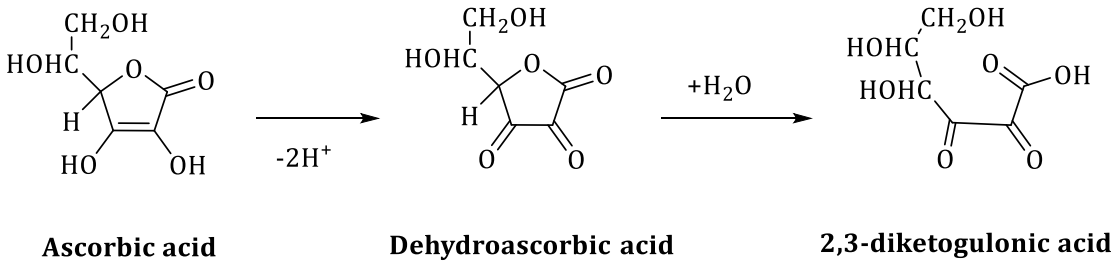


Figure 2. General trend in decrease of vitamin C content of freeze-dried and frozen mango-fruit pulp stored for seven weeks

Degradation of ascorbic acid involves oxidation to dehydroascorbic acid, followed by hydrolysis to 2,3-diketogulonic acid and further oxidation, dehydration and polymerization, forming a wide array of nutritionally inactive products (Scheme 1). Both ascorbic acid and dehydroascorbic acid have vitamin C activity. Loss of this activity occurs when dehydroascorbic acid is hydrolyzed with ring opening, forming of 2,3-diketogulonic acid (Figure 3). The dehydroascorbic acid is most stable at pH 2.5-5.5; its stability decreases as pH increases.



Scheme 1. Irreversible oxidation of ascorbic acid to diketogulonic acid (no vitamin C activity)

Titratable acidity, pH and total soluble solids

Changes in titratable acidity (TTA), pH and total soluble solids (TSS) values for freeze-dried and frozen mango pulp are presented in Table 1 and the general trend illustrated in Figure 4. After eight weeks the mean TTA, TSS and pH were 3.22 ±1.12%, 9.68±1.57 °Brix and 3.03±0.24 respectively for the frozen pulp and 2.97±0.01%, 11.70±0.08 °Brix and 2.99±0.01 respectively for the freeze dried mango pulp. However, after 0-3 weeks of storage, freeze dried mango pulp had higher acidity values than frozen mango pulp. Equally, continued storage resulted in the decrease of titratable acidity of the mango pulp preserved using either of the methods. The mean TSS of freeze dried pulp and frozen pulp were significantly different ($P<0.05$), while the mean values for TTA and pH did not significantly differ between freeze-dried and frozen pulp samples ($P>0.05$). A higher value of total soluble solids/total titratable acidity (TSS/TTA) relationship indicates an agreeable taste due to the excellent combination of sugar and acid, while lower values correlate to acidity and bitter taste [11]. The change in pH is associated with number of reasons; it might be due to the effect of treatment on the biochemical condition of the fruit and slower rate of respiration and metabolic activity [16]. In addition, it may be to the fact that after freezing, many solutes may be supersaturated in the unfrozen phase. Gradually, this may crystallize or precipitate to change the relative amounts of solutes and their initial concentrations [17]. Therefore, the ionic strength and the pH can change as a result of changing ratios of buffer components [17].

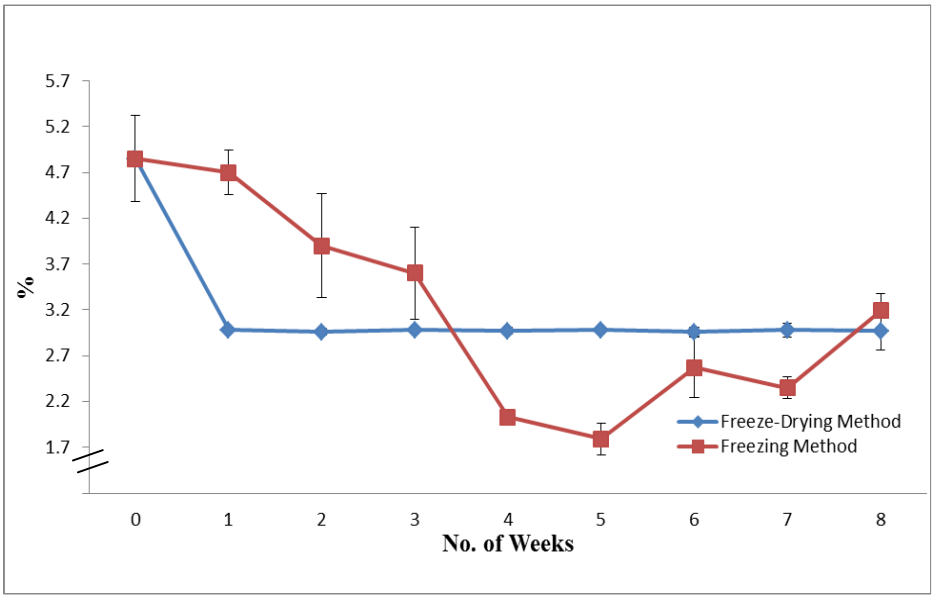


Figure 2. General trend in decrease of total soluble solids content of freeze-dried and frozen mango-fruit pulp stored for seven weeks

Total acid content of stored mango pulp and pH are indicators of fruit acidy and fruit quality. Higher total acid contents signify higher fruit acidity, which in turn results to low pH. pH in the fruit pulp plays an important role in flavor promotion as well as a preservation [18]. Increase in total soluble solids has been associated with sucrose, since sucrose has been reported to be the major sugar in mango [19]. This is due to transformation of starch into soluble sugars as the carbohydrates in the fruit pulp are broken down under the action of phosphorylase enzyme into simple sugars [20].

The decrease in titratable acidity during storage of mangoes has also been reported by [21] and [22]. *Medlicott* and *Thompson* [23] found that the decrease in acidity was due initially to the high rate of loss of citric acid with only small losses of malic acid. While *Aina* [24] suggests that the decline in acidity could be due to susceptibility of citric acid to oxidative destruction as impacted by the ripening environment.

Conclusions

The present study reveals that freeze-drying method can be used to retain considerable amounts of ascorbic acid and carotenoids. Freeze-drying method exerts minimal effect on the deterioration of water/fat soluble vitamins. Therefore, the method can be adopted as a reference method, when comparing vitamins retention in mango fruits, as well as comparing fruit preservation methods.

Conflict of Interest

We have no conflicts of interest to disclose.

References

[1] HCDA. Annual Horticultural Data report. Hortic. Crop. Dev. Auth. 2010; p 120

[2] Marques L.G., Ferreira M.C., Freire J.T. *Chem. Eng. Process.,* 2007, **46**:451

[3] Marques L.G., Silveira A.M., Freire J.T. *Dry. Technol.,* 2006, **24**:457

[4] Mulyawanti I., Dewandari K. T. *Indones. J. Agric*., 2010, **3**:32

[5] Shofian N.M., Hamid A.A., Osman A., Saari N., Anwar F., Dek M.S.P., Hairuddin M.R. *Int. J. Mol. Sci.,* 2011, **12**:4678

[6] Santos P.H.S., Silva M.A. *Dry. Technol.,* 2008, **26**:1421

[7] Yang C.S.T., Atallah W.A. *J. Food Sci.*, 1985, **50**:1233

[8] Chang C.H., Lin H.Y., Chang C.Y., Liu Y.C. *J. Food Eng.,* 2006, **77**:478

[9] AOAC. Official methods of analysis of the association of the analytical chemists. Washington DC, USA: Inc. Virginia. 2000; p 107

[10] Ranganna S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. New Delhi, India: Tata McGraw-Hill.2007; p 12–17

[11] Rodriguez-Amaya D.B., Kimura M. Harvestplus Handbook for Carotenoid Analysis vol 2, Washington, DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT). 2004; p 87

[12] Muoki P.N., Makokha A.O., Onyango C.A., Ojijo N.K. *Ecol. Food Nutr*., 2009, **48**:482

[13] Okoth E.M., Sila D.N., Onyango C.A., Owino W.O., Musyimi S.M., Mathooko F.M. *J. Anim. Plant Sci*., 2013, **17**:2619

[14] Mordi R. *Biochem. J.,* 1993, **292**:310

[15] Kurata T., Sakurai Y. *Agric. Biol. Chem.,* 1967, **31**:170

[16] Jitareerat P., Paumchai S., Kanlayanarat S., Sangchote S. *New Zeal. J. Crop Hortic. Sci.,* 2007, **35**:211

[17] Roos Y.H. *J. Food Sci.,* 1987, **52**:146

[18] Akhtar S., Mahmood S., Naz S., Nasir M., Saultan M.T. *Pakistan J. Bot.,* 2009, **41**:2821

[19] Gil A.M., Duarte I.F., Delgadillo I., Colquhoun I.J., Casuscelli F., Humpfer E., Spraul M. *J. Agric. Food Chem.,* 2000, **48**:1524

[20] Vazquez-Salinas C., Lakshminarayana S. *J. Food Sci.,* 1985, **50**:1646

[21] Ceballos A.M., Giraldo G.I., Orrego C.E. *J. Food Eng*., 2012, **111**:360

[22] Mamiro P.S., Fweja L.W.T., Chove B.E., Kinabo J., George V., Mtebe K. *African J. Biotechnol.,* 2007, 6:2477

[23] Medlicott A.P., Thompson A.K. *J. Sci. Food Agric.,* 1985, **36**:561

[24] Aina J.O. *Food Chem.,* 1990, **36**:205

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