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FULL LENGTH ARTICLE

Cryogenic freezing of fresh date fruits for quality preservation during frozen storage

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Abstract Fresh date fruits, especially *Barhi* cultivar, are favored and widely consumed at the Khalal maturity stage (first color edible stage). These fruits are seasonal and perishable and there is a need for extending their shelf life. This study evaluates two different freezing methods, namely cryogenic freezing using liquid nitrogen and conventional deep freezing on preserving the quality and stability of date fruits (cv. *Barhi*) at Khalal maturity stage. Fresh date fruits (cv. *Barhi*) at Khalal stage were frozen utilizing the two methods. The produced frozen dates were stored under frozen storage conditions for nine months (at −20 °C and −40 °C for the conventional and cryogenic freezing, respectively). Color values, textural properties (hardness, elasticity, chewiness and resilience), and nutrition attributes (enzymes and sugars) for fresh dates before freezing and for the frozen dates were measured every three months during the frozen storage. Color values of the frozen dates were affected by the freezing method and the frozen storage period. There are substantial differences in the quality of the frozen fruits in favor of cryogenic freezing compared to the conventional slow freezing. The results revealed a large disparity between the times of freezing of the two methods. The freezing time accounted to 10 min in the cryogenic freezing method, whereas it was 1800 min for the conventional slow freezing system.

1. Introduction

The date palm (*Phoenix dactylifera* L.) is a dominant tree in South West Asia and North Africa. Presently global production, consumption and industrial development of dates are constantly growing as date fruits are important source of energy and essential nutrients and possess some medicinal benefits (Al Farsi and Lee, 2008; Al-Abdoulhadi et al., 2011; Chandrasekaran and Bahkali, 2013). There is a necessity to

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utilize freezing and frozen storage for prolonging the shelf life of fresh date fruits, especially *Barhi* cultivar, which is favored and widely consumed at Khalal maturity stage (the first edible where the fruit is sweet, crispy and yellow in color).

Freezing and frozen storage can be utilized for the long-term preservation of some fruits and vegetables. Freezing decreases the water activity, inhibits microorganism growth and reduces enzymatic activity resulting in extending the shelf life of the product (Fellows, 2000; Heldman, 1992). Many published research works have confirmed the close relationship between quick freezing and high quality frozen products and the resulting increase shelf life with maximum preservation of initial quality (Sanz et al., 1999; Sun and Li, 2003; Zhang et al., 2004).

Color plays a fundamental part in the consumers' evaluation of the food quality. Color changes are considered as the major quality attribute that affects consumers' selection (Zhang et al., 2004). Enzymatic oxidation of phenolic substances is the main reason that induces color changing (browning). Ice crystals formed during freezing will enhance enzymatic oxidation due to the destruction of the cells and tissues of the product and therefore increased contact between phenolics, oxygen and enzymes (Ruenroengklin et al., 2008).

Textural parameters of frozen foods play an essential part in determining the acceptability of these products by consumers. Higher values of hardness, chewiness and resilience of the pulp indicate better quality products (Zhang et al., 2007; Krause et al., 2008; Kaushik et al., 2014). Several researchers have studied the effects of freezing on textural quality of fruits (Delgado and Rubiolo, 2005; Van Buggenhout et al., 2006; Sousa et al., 2007).

Enzymatic activity is responsible for the quality deterioration in most of the frozen fruits. The enzyme activity decreases as the temperature decrease; however as a result of freezing, the chemical reactions catalyzed by the enzymes occur due to the increase in concentration of salts (Maier et al., 1964; Whitaker, 1972; Marin and Cano, 1992).

Fruit sugars have a significant part in preserving fruit quality and determining its nutritional status (Akhatou and Angeles, 2013). Dates, irrespective of the cultivars, contain more than 75% sugars on a dry-weight basis (Kanner et al., 1978). Al-Mashhadi et al. (1993) found that the reducing sugars (fructose and glucose) in date fruits increased while the sucrose sugar decreased at the end of twelve months of frozen storage. In another study a decrease in the reducing sugars of date fruits was reported at the end of six months of frozen storage (Mikki and Al-Taisan, 1993).

This research work deals with the study of the utilization and comparison of three freezing methods *viz*., cryogenic freezing using liquid nitrogen, individual quick freezing and conventional deep freezing on the quality and stability of date fruits (*Barhi* cultivar) at Khalal stage by evaluating color attributes, textural parameters, sugar contents, enzymatic activity and freezing rates during nine months of frozen storage.

2. Materials and methods

2.1. Fresh dates

Fresh yellow dates (cv. *Barhi*) at Khalal stage of maturity were obtained from a commercial farm in Qassim, Saudi Arabia. Dates were sorted to discard the damaged fruits and immediately kept for less than 6 h in a cold store at 5 °C. Physical properties of the fresh date fruits (length and diameter, surface area, volume, mass, density), moisture content and water activity were measured. The color values, textural properties (hardness, elasticity, chewiness and resilience), and nutrition (enzymes, sugars) properties of the date fruits were evaluated for the fresh date fruits before freezing and for the frozen ones after thawing every three months during a period of nine months of frozen storage.

2.2. Moisture content and water activity determination

Moisture content was determined for the flesh of dates using Association of Official Analytical Chemists (AOAC) standard procedure (AOAC, 2005), where the samples were dried at 70 °C for 48 h under a vacuum of 200 mm of mercury (Vacutherm model VT 6025, Heraeus Instrument, D-63450. Hanauer, Germany). Water activity of the dates flesh was measured at room temperature using Aqua-lab (Model CX-2T, readability 1 mg, Decagon Devices Inc., Washington).

2.3. Color examination

The fruits' color values were expressed by the parameters (L^*, a^*, b^*) measured by a spectrophotometer device (Color Flex, Model No. 45/0, Hunter Associates Laboratory. Inc., VA, USA).

Here L^* indicates (whiteness or brightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness). In addition the color was expressed by the total color difference (ΔE), Chroma, hue angle and browning index (BI) as defined by the following equations (Maskan, 2001):

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (1)$$

where L_0^*, a_0^* and b_0^* are the color parameters of fresh fruits (before freezing).

$$\text{Chroma} = \left(a^{*2} + b^{*2}\right)^{0.5} \quad (2)$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

$$\text{BI} = \frac{[100(x - 0.31)]}{0.17} \quad (4)$$

where

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (5)$$

2.4. Texture profile analysis (TPA)

The texture profile analysis parameters were measured using a texture analyzer (TA-HDi, Model HD3128, Stable Micro Systems, Surrey, England). Fruit samples were compressed with a rod velocity of 1.5 mm/s to a depth of 5 mm. The compression was done twice to give two complete texture profile curves. The force–time deformation curves were obtained in which the following parameters were obtained: Hardness (the maximum force required to compress the sample), Resilience (the ability

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of the sample to recover its original form after deformation), Chewiness (the work needed to be done to make a solid food swallowable, which is numerically formulated by the product of gumminess and springiness). The data were processed using Texture Expert Exceed, version 2.05 (Stable Micro systems).

2.5. Extraction and estimation of invertase enzymes

The method described by Hasegawa and Smolensky (1970) was used to extract the invertase enzyme by mixing a sample (15 g) with NaCl solution (4%) containing 1 g polyvinyl polypyrrolidone for two min. at a temperature of 2 °C and then centrifuged at 2000 rpm for 30 min at 5 °C. The upper liquid layer containing the enzyme (supernatant) was collected and kept for enzyme assay. The previous steps were repeated again on the remaining solid material and then the supernatant was taken and added to the previous amount. This solution is soluble invertase. The insoluble invertase was obtained by water dialysis of the extraction residues at 2 °C till all sugars were removed.

Invertase activity in *Barhi* dates at Khalal stage and during frozen storage was measured by a method described by Kanner et al. (1978). The assay mixture included 0.5 M acetate buffer pH 4.5, 1.5 M sucrose and enzyme extract (1 mL) with total volume of 5 mL. This mixture was kept for 1 h at 37 °C and 1 mL sample was withdrawn at 10 min interval. One unit of invertase was defined as the amount of enzyme, which hydrolyzed 0.5 μM sucrose per min under the above conditions.

2.6. Extraction and estimation of peroxidase and polyphenol oxidase enzymes

Peroxidase and polyphenol oxidase enzymes were extracted and estimated according to the method used by Cano et al. (1995). Each enzyme was extracted by 0.2 M sodium phosphate buffer and 1 M sodium chloride at 5 °C and then filtered and centrifuged and the supernatant was collected. The amount of enzymes in the samples was measured using the polarization device (Polarimeter, Autopol IV Six Wavelength) manufactured by Rudolph Research, USA.

2.7. Sugar analysis

Sugar analysis (fructose, glucose, and sucrose) of dates was determined by the AOAC standard procedure (AOAC, 2005) using high-performance liquid chromatography system (HPLC), LC-10 AD, Shimadzu Corporation, Kyoto, Japan. The separation column is 250 × 4.6 mm. The liquid phase consists of (20% water and 80% acetonitrile HPLC grade) pumped by a pump (model LC-10-AZ, Shimadzu) at a rate of 2.5 ml/min. 5 μL sample is injected into the system with an injector (model SIL-10A, Shimadzu). The results and the curves for the sugars under study were received by an integrator (Model C-R7A, Shimadzu Chromatopac data processor) where they were compared to the standard solutions for sugars (Sigma Chemical Co., St. Louis, Mo.).

2.8. Freezing methods

Two different freezing methods were used in freezing the fresh date fruits namely cryogenic freezing using liquid nitrogen and conventional slow freezing using traditional deep freezers.

2.8.1. Liquid Nitrogen Cryogenic Freezing (LNCF) method

Continuous cryogenic freezer using liquid nitrogen (Cryogenic Freezing Tunnel, CQF 2076, Packo Inox NV, Torhout Sesteenweg, Zedelgem, Belgium) was utilized to freeze the fresh dates in a 4 m long tunnel. After the freezer reached steady state, fresh fruits were fed inside the tunnel at a rate of 5 kg/min. The temperature of the fruits pulp (near the pit) and the surrounding air inside the freezer tunnel were measured every 5 s using type K thermocouples connected with data Loggers. The freezer was set at −120 °C inside the tunnel. The frozen fruits were collected at the end of the freezing tunnel and packaged in rigid polyethylene boxes (ø kg capacity) and directly stored in ultra-freezers at −40 °C.

2.8.2. Conventional Slow Freezing using deep freezers (CSF) method

A traditional deep freezer (Chest Freezer, Sanyo Elec. Co., Ora/Gun Japan) was used for the conventional freezing of fresh fruits. The fruits were filled in rigid polyethylene plastic boxes (½ kg capacity). Thermocouples were placed to measure the temperature of the fruits pulp (near the pit) and the temperature inside the freezer every five minutes. Freezers' temperature ranged between −18 and −24 °C at steady state. However, it was assumed that the average freezer temperature is −20 °C.

2.9. Thawing processes

The frozen fruits were removed from the storage freezers and thawed by leaving them at room temperature for two hours.

2.10. Sensory evaluation

Sensory evaluation of the fresh and frozen fruits tests has been used by arbitrators. The most important attributes tested were as follows: color, taste, texture, and finally the general appearance and acceptance. The taste tests were conducted at equal time intervals starting from zero time (after three days of frozen storage) and compared to subsequent storage periods (3, 6.9 months). Each arbitrator gives the result ranging from one (worse degree) to nine (best degree).

2.11. Statistical analysis

All needed statistical analyses were performed using the IBM SPSS software package (IBM SPSS version 22), and data are represented as mean ± SD. Experimental data were analyzed by means of analysis of variance (ANOVA).

3. Results and discussion

3.1. Physical properties, moisture content and water activity

The data obtained on the physical properties of the fresh *Barhi* fruits are given in Table 1. The average mass of the fruits of fresh *Barhi* fruits is 14.8 g. The average density values of the pulp of the fresh fruits, which is important for the calculation of temperature distribution within the fruit, were higher than the density of water as shown in Table 2.

Table 2 also shows the experimental data on the moisture content (wet basis) and water activity of the fresh *Barhi* fruits.

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The fresh *Barhi* fruits are characterized with high moisture content (68.6%) and water activity (0.91) values. This indicates that *Barhi* fruits were highly perishable.

3.2. Color of fresh and frozen fruits

The experimental data on the basic color parameters (L^*, a^* and b^*) of the *Barhi* fruits frozen by two freezing methods, i.e. LNCF and CSF as a function of time are displayed in Fig. 1.

The mean values of the basic color parameters L^*, a^* and b^* of the fresh *Barhi* fruits were 68.35, 10.87 and 48.44, respectively. These values show that *Barhi* fresh fruits at Khalal stage of maturity are characterized with their bright yellow color. From Fig. 1 it is clear that the L^*, a^* and b^* values had changed for frozen fruits with the period of frozen storage which extended for nine months. The L^* values of the frozen fruits decreased at the end of the frozen storage to 53.9 and 43.7 in LNCF and CSF, respectively. On the other hand, a^* values increased during the same period to 10.7 and 15.4 in LNCF and CSF, respectively. The b^* values followed the same behavior of L^* and decreased to 42.2 and 33.9 in LNCF and CSF, respectively. These results indicate that there were notable changes in the values of the basic color parameters of the fresh *Barhi* fruits after freezing and frozen storage in both freezing methods. Nevertheless, LNCF method is superior in preserving the fresh *Barhi* fruits basic color parameters compared to CSF method.

The data on the color derivative parameters i.e. color difference (ΔE), Chroma, hue angle and browning index (BI) of the fruits frozen by LNCF and CSF methods are plotted and depicted in Fig. 2.

The color derivative parameter values of the fruits frozen by both methods varied depending on the period of the frozen storage. For LNCF method the color difference increased from zero for fresh fruits to 9.94 after three months to reach a maximum value of 15.74 after nine months of frozen storage. Chroma values decreased from 49.64 for fresh fruits to 46.90 after three months to reach a value of 43.54 after nine months of frozen storage. The hue angle value decreased from 77.32 for fresh fruits to 75.74, whereas, the browning index value increased from 123.68 to 146.51 for the frozen fruits stored for a period of nine months. The results of CSF method indicate a large decrease in the values of L^* and b^* after 9 months of frozen storage. This has led to a significant decrease in Chroma and the hue angle values and an increase in the color difference and browning index compared to the liquid nitrogen cryogenic freezing. These results were similar to those observed by other researchers (Duan et al., 2007; Neog and Saikia, 2010; Ruenroengklin et al., 2008).

3.3. Textural parameters

The textural profile analysis (TPA) parameters for the fresh fruits and frozen fruits (after thawing) during nine months of frozen storage are shown in Fig. 3. The results indicate that fresh *Barhi* fruits are distinguished with their higher mechanical properties. The results also depict that fresh *Barhi* fruit is firm as revealed by its high average hardness value (119.48 N).

The TPA parameters of the frozen fruits were highly influenced by freezing method and frozen storage time. In general, all tested TPA parameters values of the frozen fruits were

Table 2 Mean values of pulp density, moisture content and pulp water activity for the fresh *Barhi* fruits at Khalal stage of maturity.

	Thickness of pulp (mm)	Density of pulp (cm^3)	Moisture content (w.b.) (%)	Water activity
Mean No. of samples	8.1 ± 1.4 9	1.2 ± 0.1 9	68.6 ± 5.2 9	0.911 ± 0.066 9

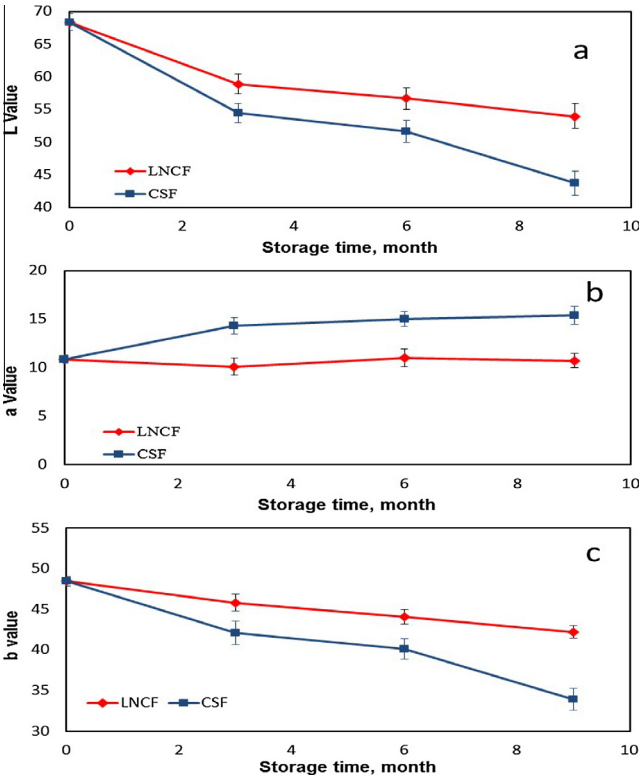

Figure 1 Changes in basic color parameters values of *Barhi* fruits frozen by LNCF and CSF at different storage times. (a) L^*, (b) a^* and (c) b^* (mean values ± standard deviation of ten replicate measurements are shown).

Table 1 Mean values of the physical properties of fresh *Barhi* fruits at Khalal stage of maturity.

Property	Mass (g)	Volume (cm^3)	Density (g cm^{-3})	Area (cm^2)	Length (mm)	Larger diameter (mm)	Diameter neck fruit (mm)	Diameter tip fruit (mm)
Mean No. of samples	14.8 ± 2.6 60	14.5 ± 2.9 60	1.02 ± 0.05 60	27.9 ± 2.1 30	34.3 ± 2.2 60	27.5 ± 2.4 60	21.7 ± 1.3 60	19.2 ± 1.3 60

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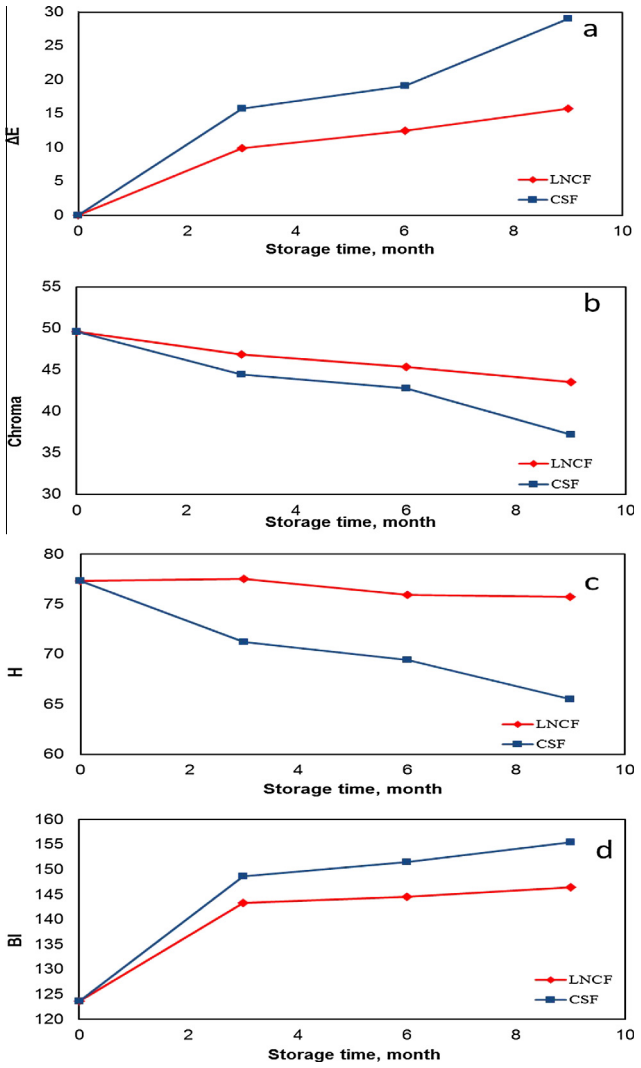

Figure 2 Changes in color derivative parameters of *Barhi* fruits frozen by LNCF and CSF at different storage times. (a) Color deference (ΔE), (b) chroma, (c) hue angle and (d) BI.

lower compared to the fresh ones. This is apparent in the high changes of texture after storage of three months. However, it tends to be more stable after six and nine months of frozen storage.

As displayed in Fig. 3(a) the reduction in hardness values of the frozen fruits during the first three months of storage is 59.8% for LNCF method, while it is 74.17% for CSF method. It is also noted that the hardness of the fruits frozen via LNCF method decreases by 12.1% during storage period of three to nine months, while the decline was much larger in the case of CSF method where it decreases by 32%.

The reduction in the elasticity values for the fruits frozen by both LNCF and CSF methods is illustrated in Fig. 3(b). It is clear that the decline is at a higher rate in CSF compared to LNCF method. The resilience property of the frozen fruits decreased during frozen storage for both freezing methods.

Fig. 3(c) displays that the resilience of fresh fruits decreased by 36.8% for LNCF and 41.69% for CSF methods during the first three months of storage. The chewiness values of fresh fruits decreased during the same period of storage by 75.2% for LNCF and 81.39 for CSF as given in Fig. 3(d). In addition, the elasticity of fresh fruits decreased during the first three months of storage by 28.78% for LNCF and 32.0% for CSF as shown in Fig. 3(b).

The above mentioned results showed that LNCF method is preferable compared to CSF method since the deterioration in the texture of the fruits frozen by the first method is less and the fruits' texture is closer to the natural texture of the fresh *Barhi* fruits. This is probably due to decrease in the injurious effects of crystallization and recrystallization on the microstructure of fruits tissues during such quick freezing method. These results proved the reported significant role of the freezing rate in maintaining the texture of frozen foods (Van Buggenhout et al., 2006; Delgado and Rubiolo, 2005; Sanz et al., 1999; Sousa et al., 2007; Sun and Li, 2003; Zhang et al., 2004).

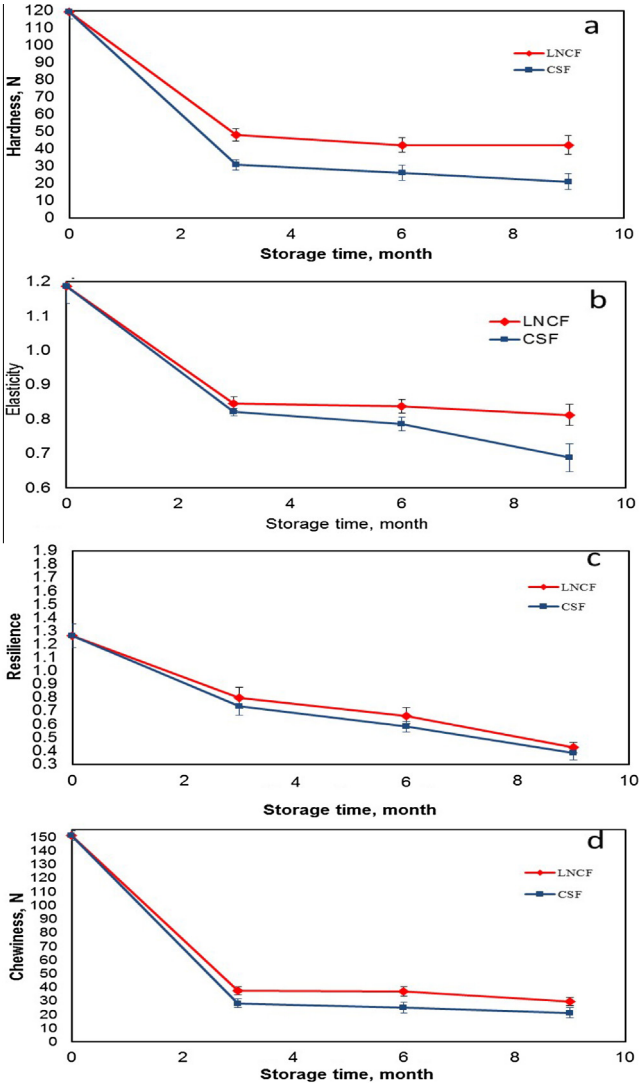

Figure 3 Effects of freezing methods in the TPA parameters of *Barhi* fruits at different storage times. (a) Hardness, (b) elasticity, (c) resilience and (d) chewiness (mean values ± standard deviation of ten replicate measurements are shown).

3.4. Effects of freezing methods on the enzymatic activity of Barhi fruits

Invertase enzyme activity was not detected in Brahi fruits (Khalal stage). This may be due to the temperature at which the dialysis process of the extract was done. Another possibility is that the invertase enzyme exists at minute quantities in the cultivar under study to the extent that it was out of the detection limits.

The enzymatic activity of both polyphenols oxidase (PPO) and peroxidase (POD) in *Barhi* fruits throughout frozen storage period (nine months) is shown in Fig. 4. It is evident from this figure there is an increase in the activity of both enzymes in two freezing methods, but the increase in CSF is greater. This may be due to the enzymatic activity in the fruits as a result of no use of blanching treatment. Moreover, it is noted that the activity of both enzymes increased at a slower rate in the first three months. Then the activity increased dramatically during the next three months, while it was almost constant during the last three months. As for LNCF method the activity of the two enzymes increased, but at very small rates during the frozen storage for a period of nine months. These enzymatic activities might be the cause of deterioration of fruit texture and color during storage (Whitaker, 1972; Marin and Cano, 1992). Moreover, the enzymatic activities are higher in case of CSF method compared to LNCF which has led to better retention of fruit properties with LNCF method.

3.5. Effects of freezing methods on Barhi fruits' sugars

Fig. 5 displays the effects of LNCF and CSF methods and frozen storage period on the fresh *Barhi* fruits sugars. For the fruits frozen by CSF method and stored at −20 °C, the proportion of glucose and fructose has increased substantially during the first three months of frozen storage. This proportion decreased considerably during the following three months and continues to decrease at a lower rate until the end of the frozen storage period. On the other hand, sucrose proportion greatly declined by about 81% of its initial value at the end of the first three months. The sucrose has gradually faded or disappeared completely until the end of the storage period. The disappearance of or decomposition of sucrose is probably due to enzymatic activity in the fruit.

For the fruits frozen by LNCF method and stored at −40 °C, the glucose and fructose percentages increased at slow and regular rate until the end of the sixth months of storage and then decreased gradually until the end of the ninth months. The sucrose percentage decreased gradually till the end of the frozen storage period.

The increase in the reducing sugars (fructose and glucose) perceived during the first period of frozen storage for both studied freezing methods was previously observed for dates fruits (Al-Mashhadi et al., 1993; Zhao et al., 2015). The reduction in the reducing sugars that was detected during the last period of storage was also recognized by Mikki and Al-Taisan (1993).

From the above results it is evident that the changes in sugars were much lower in case of fruits frozen by LNCF method and stored at −40 °C compared to those frozen by CSF method and stored at −20 °C.

3.6. Fruits freezing curves

Freezing curves of fresh *Barhi* fruits during LNCF are shown in Fig. 6. The curves represent the change of the fruit center and its surrounding air temperature with time. From the figure it is perceived that the temperatures dropped during the first five minutes from initial temperature of 21 °C to −22.1 °C and −91.8 °C at the fruit center and its surroundings air, respectively. From the freezing curves, it is shown that the temperature of the fruit center dropped to −26.4 °C after six minutes in the freezer and about two minutes later it fell to −74.7 °C. At the end of ten minutes from the beginning of the freezing process the temperatures dropped to −95 °C and −107.8 °C at fruit center and its surroundings air, respectively.

The results of CSF for fresh *Barhi* fruits are illustrated in Fig. 7. The freezing curves in this figure show the decrease in the temperature of the fruit center from 21.6 °C to zero degree during 4.41 h. After ten hours the temperature of the fruit center dropped to −9.5 °C and later to −18.1 °C after 24 h to

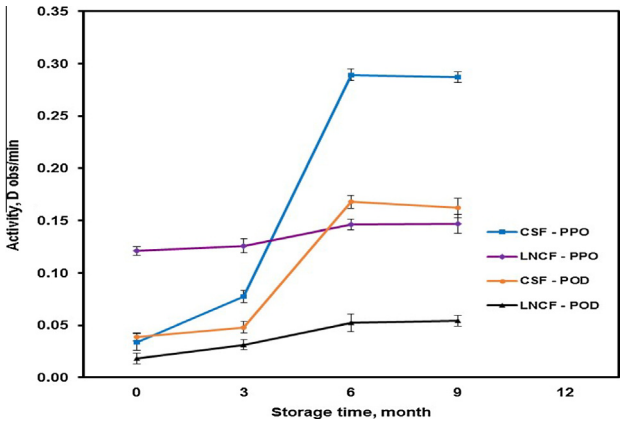

Figure 4 Effects of freezing methods in the enzymes activity of *Barhi* fruits at different storage times (mean values ± standard deviation of ten replicate measurements are shown).

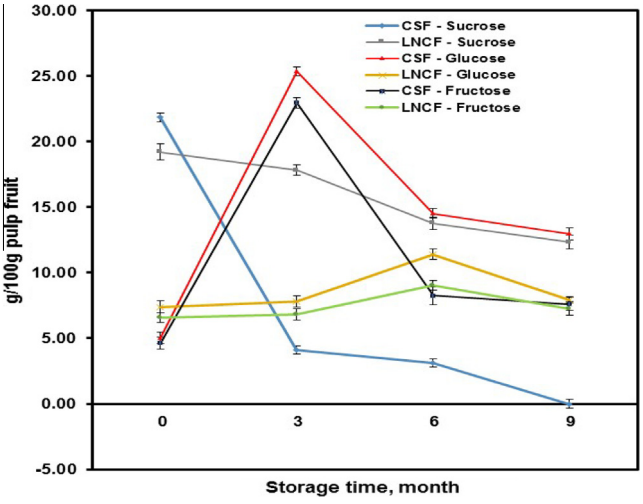

Figure 5 Effects of freezing methods on *Barhi* fruits' sugars at different storage times (mean values ± standard deviation of ten replicate measurements are shown).

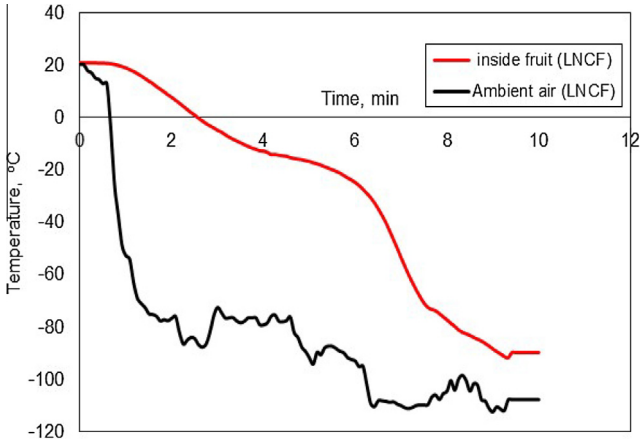

Figure 6 Temperature change with time during LNCF of *Barhi* fruits.

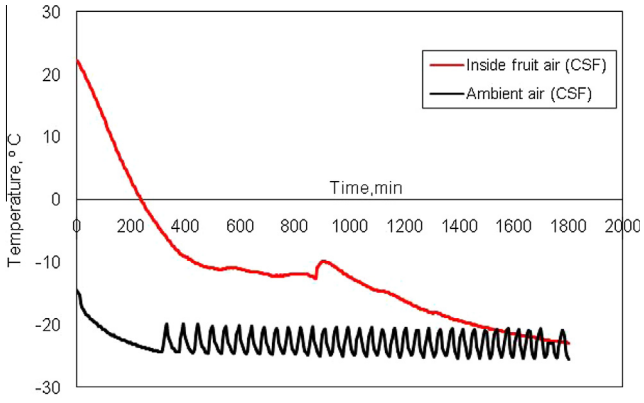

Figure 7 Temperature change with time during CSF of *Barhi* fruits.

reach −21.2 °C after 30 h of freezing. The air temperature inside the closed pack containing the fruits approaches the temperature of the fruit surface, while the temperature inside the freezer was fluctuating after reaching steady state where it varied in the range of −20.9 to −24.3 °C.

It can be noted that LNCF method is superior in fruit quality preservation and this may be due to the shorter freezing time. The freezing time taken to reach the fruit center accounted to 10 min for LNCF method, while it was 1800 min for CSF method.

Table 3 Effect of freezing method on the sensory attributes of fresh *Barhi* fruits.

Freezing method	Color	Taste	Texture	Final acceptance
Fresh	6.80 ± 2.11	7.40 ± 2.28	7.90 ± 1.29	7.10 ± 2.49
CSF	5.35 ± 2.23	5.40 ± 2.04	5.25 ± 1.94	5.15 ± 1.93
LNCF	5.75 ± 1.83	6.40 ± 2.01	5.85 ± 1.63	6.20 ± 1.77

3.7. Effect of freezing methods on the sensory evaluation of Barhi fruits

Table 3 displays the sensory attributes of the fresh *Barhi* fruits and those frozen by CSF and LNCF. From Table 3 it is clear that LNCF had given better results than CSF in terms of color and taste.

4. Conclusions

Freezing and frozen storage showed a clear effect on basic color values (L^*, a^* and b^*) and their derivative parameters of fresh *Barhi* fruit. Freezing and freezing methods greatly influenced the textural properties of the fruits. In general, textural parameters decreased with storage time and were dependent on freezing method. Analysis of basic sugars (fructose, glucose, and sucrose) in the fruits showed a sharp increase in fructose and glucose and decrease in sucrose for fruits until the end of the frozen storage period. The increase in enzymatic activities of Poly Phenol Oxidase and peroxidase led to a more deterioration of fruit quality with storage time especially with the conventional slow freezing method. Temperature distribution curves showed a high variation of freezing times of the three examined systems. There was a large difference between the times of freezing of the two methods in favor of cryogenic freezing.

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