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Effects of frozen of marula fruits *(Sclerocarya birrea)* on chemical, antioxidant activities, and sensory properties of marula fruit juice

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ABSTRACT

This study determined the effects of frozen storage on physicochemical, phenolic content and antioxidant activities of fruit juice extracted from frozen marula fruits. The marula fruits were frozen for zero to eight weeks after which the frozen marula fruits were thawed between 10 and 12 h and the juice extracted manually. There was 36.35% decrease in vitamin C content of the juice, 36.70% decrease in TPC, 46.50% decrease in FRAP and 53.22% decrease in TFC. The colour of the marula fruit juice decreases with increase in frozen storage time and the marula fruit juice extracted from unfrozen marula fruit was score highest in all the sensory properties evaluated. Although, freezing is one of the best preservative methods of fruits, the type of freezer used for the freezing process determines the nutritional value of the fruits and the juice. A home freezer may not be good to store marula fruits.

1. Introduction

One of the most natural forms of preserving food is by freezing, which helps food stay fresh and last longer as it does not require any preservatives but rather a reduced temperature. Maintaining the quality of food by freezing is important as it slows down the enzyme activity that causes food spoilage and prevents microorganisms from growing [1,2&3]]. The effects of freezing as a mode of preserving fruits and fruit juices cannot be overemphasized. Fruits like marula have been kept by freezing due to their seasonal availability. Inasmuch as spoilage is delayed by freezing, freezing fruits may have a negative effect on the nutritional content of the fruit [2,3].

Marula fruit (*Sclerocarya birrea*) is a small, round-shaped fruit that is green in colour, and yellow when ripe. It has a distinct smell and tastes sour. This indigenous fruit tree is mostly found in African countries like Ethiopia and South Africa. They are found in KwaZulu Natal, Mpumalanga, but are more dominant in Phalaborwa, an area in Limpopo [4]. The marula fruit can be collected from the ground from January to March each year. Once it is collected, it is used for cosmetics and medicinal purposes, eaten raw, or used in the preparation of juice, beer, and wine. This fruit is rich in vitamin C, which is of good health benefit. The pulp of the marula fruit is said to have a higher vitamin C content than the pulp of other fruits like oranges, guava, and pineapple [5].

The locals collected this fruit to use as a source of income by using the juice to make local jam and beer. Since it is seasonal, the fruits and pulp are usually stored frozen for up to a year. This helps them produce more products (Local beer) when the fruit is out of

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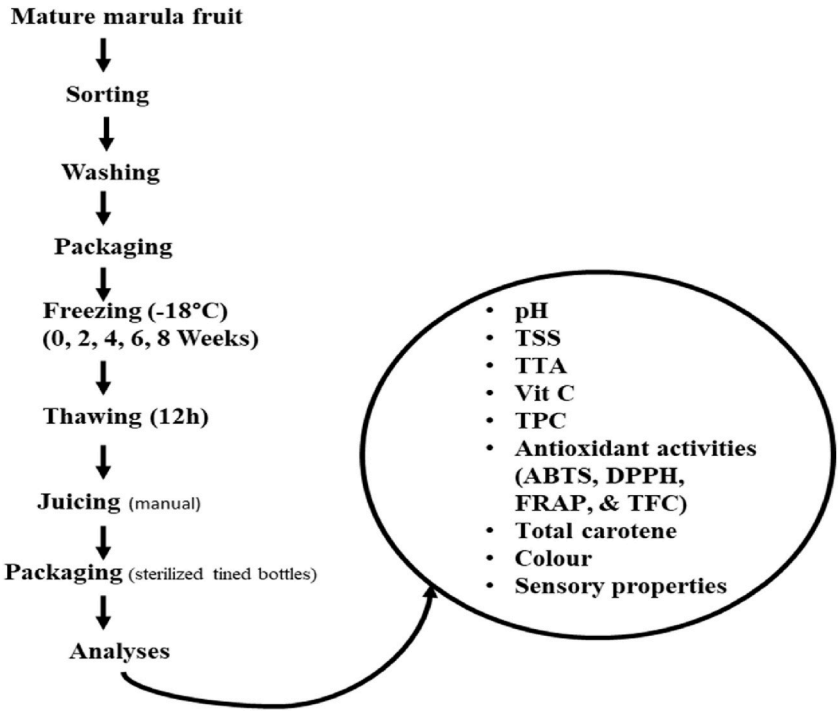

Fig. 1. Flow diagram showing the process of sample preparation and analyses.

season [5]. As much as freezing is a well-known preservation method, freezing fruit can affect its nutritional composition due to factors such as temperature, water activity, and acidity [1,5]. We hypothesized that the use of home freezer will have negative effects on the chemical, antioxidant activities and sensory properties of the juice extracted from frozen marula fruits. Freezing fruits may cause chemical decomposition, which can result in the loss of nutrients, flavour, and colour [3,6]. The freezing of marula fruits before juice extraction, necessitated by their limited availability throughout the year, holds the potential to influence the chemical composition, antioxidant activities, and sensory properties of marula fruit juice. However, despite this potential impact, there remains a lack of comprehensive information on the subject. Therefore, this study aims to investigate the effects of marula fruit freezing and freezing duration on the chemical composition, antioxidant, and sensory properties of marula fruit juice.

2. Materials and methods

Marula fruits (*Sclerocarya birrea* subsp. *Caffra*) fruit that was mature, fresh, and ripe was randomly selected from three distinct places in Mbombela City, Mpumalanga. From Merck Chemicals in Johannesburg, analytical chemicals were bought.

2.1. Juice preparation

The collected fruit (Marula) was brought to the laboratory in the University of Mpumalanga where it was sorted, cleaned (with distilled water), and then separated into five parts. The first part was juiced (after the peel was removed manually), and the remaining four batches were stored in zip-top bags in the freezer (−18 °C) for eight weeks, with samples being taken every two weeks. The frozen samples were allowed to thaw at room temperature for 12 h, after which the marula fruits were juiced- and the juice stored inside a sterilized tinted bottle. The fruit juice inside pasteurised translucent bottle was kept inside the fridge (4 °C) for further analysis. The flow diagram of the study is shown in Fig. 1.

2.2. Methods

2.2.1. Determination of pH, total soluble solids (^{0}Brix), and total titratable acidity (TTA)

A pH meter (Hanna Instrument, Poroa de Varzim, Portugal), previously calibrated with buffer solutions (4 and 7), was used to determine the pH of the marula fruit juice samples. Using a portable refractometer (Hanna Instruments, Italy) that had been previously calibrated to zero with distilled water, the total soluble solids of the marula fruit juice were measured in 0Brix. After each analysis, the refractometer's prism was cleaned with distilled water. The samples' titratable acidity was assessed using the AOAC [7] technique.

2.2.2. Ascorbic acid content (AA)start

The ascorbic acid concentration of marula fruit juice samples was assessed using the AOAC [7] technique. With oxalic acid (0.1 M), 20 mL (20 mL) of the marula fruit juice samples were increased to 50 mL (50 mL) and then filtered. The filtrate (5 ml) was pipetted into a beaker and then titrated with standardized 2,6-dichlorophenol indophenol dye. The coloured solution changed from orange to pink at the conclusion of the titration. The analysis was carried out three times. The results of the titre were converted into milligrams of AA per litre of the marula fruit juice sample.

2.2.3. Total phenols (TPC) determination

The spectrophotometric method using the Folin Ciocalteu procedure with minor modifications was used to measure the total phenolic content (TPC) of the samples [8]. Briefly, Folin Ciocalteu reagent (1:10 v/v dilution with water) (1.5 mL) was mixed with 0.5 mL of test sample extractor and allowed to react for 5 min. Then, 2 mL of 7.5 g/100 g sodium carbonate was added and incubated for 90 min in a dark place with occasional stirring. A spectrophotometer with a 96 well microplate (Hitachi Model 100-20) was used to read the absorbance value of the juice sample at 725 nm. The catechin standard was used to prepare the standard calibration curve. The results were expressed in mg of catechin equivalents per ml (mg CE/ml) of juice sample. All determinations were carried out in triplicate.

2.2.4. ABTS. + radical scavenging activity of the fruit juice

The ABTS + radical scavenging activity of the marula fruit juice was analysed using modified method of Awika et al. [9]. The ABTS + stock solution was made by mixing an equal amount of potassium persulfate (2.45 mM) aqueous solution (Merck, India) with ABTS (7 mM) aqueous solution (Sigma Aldrich, India). The combination was then let to stand for 12–16 h at room temperature in the dark. The stock solution of ABTS+ was diluted in methanol to produce the working solution, which had an absorbance of 0.70 0.02 at 734 nm. Then, 1 mL of the marula fruit juice was combined with 2.0 mL of the ABTS + solution. The combination was then incubated for exactly 30 min at room temperature and in the dark. A 2.0 mL solution of ABTS+ was combined with 1 mL of double-distilled water to create the control. At 734 nm, the absorbance was measured using a spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan).

2.2.5. DPPH radical scavenging activity of the fruit juice

The DPPH radical scavenging activity of the fruit juice (marula) was analysed by the method of Apea-Bah et al. [10]. The DPPH stock solution (A 0.609 mM) was prepared in 80% (v/v) aqueous methanol to generate a working solution of 0.102 mM. A 5 x dilution of the fruit juice (marula) mixture (10 μl) and 190 μl of the DPPH working solution put inside a 96-well plate then incubated for 1 h at 15 °C in the dark. The absorbance was determined at 570 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, Shanghai, China). Using trolox as the reference substance, results were represented as millimoles of trolox equivalent per milligram of sample (μmol TE/ml).

2.2.6. Ferric reducing antioxidant power (FRAP)

The FRAP assay utilizes the rapid decrease in ferric-tripyridyltriazine (Fe^{3+}-TPTZ) through the action of antioxidants found in the samples. This reaction forms ferrous-tripyridyltriazine (Fe2+-TPTZ), which appears as a blue-coloured product. A standard curve was established by introducing the FRAP reagent to various known concentrations of Fe^{2+} solutions. This standard curve enables the calculation of the Fe^{2+} concentration in the samples, thereby determining their antioxidant capacity. The FRAP method is based on the work of Benzie and Strain [11]. To create the standard solutions, a range of iron (II) sulfate heptahydrate concentrations, from 0.1 to 7.5 mM, was prepared. The FRAP reagent was formulated by combining 300 mmol/L acetate buffer, 10 mmol/L TPTZ/HCL solution, and 20 mmol/L ferric chloride. Each sample was treated with the FRAP reagent and incubated for 15 min in darkness. Absorbance was then directly measured at 600 nm.

2.2.7. Total carotene determination (TC)

The concentration of TC in the samples was determined using the spectrophotometric technique, as modified by Goula and Adamopoulos [12]. The TC was extracted using hexane, acetonitrile, and ethanol in a ratio of 50:25:25 (v: v: v). Exactly 1 ml of the sample was extracted using about 50 ml of the solvent mixture. The liquid was stirred on a magnetic stirring plate for 15 min in the dark to extract the carotenoids. 3 cc of distilled water was added to the mixture and stirred for an additional 5 min in order to achieve phase separation. The absorbance of the filtered hydrophobic phase at 450 nm for total carotenoids was measured using hexane as a blank.

2.2.8. Total flavonoid content (TFC)

The Total flavonoid content (TFC) of the fruit juice samples was determined by modified method of Mahloko et al. [13]. In 50 ml test tube, 5 ml of the fruit juice was mixed with 0.3 mL $NaNO_2$ (5%). About 5 mL distilled water and 2 mL of NaOH (1 M) was added after 6 min and vortexed. The absorbance was read at 510 nm with spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan). The standard curve was plotted using quercetin and the results was reported as quercetin per 100 ml of marula fruit juice.

2.2.9. Colour determination of the fruit juice

The colour attributes (L*, a* and b*) of marula fruit juice was analysed using a Hunter Lab colourimeter (MiniScan XE Plus, Model CM-3500d, Hunter Associate laboratory, Reston, VA, USA) with a D65 light source. The colourimeter was calibrated before the analysis with distilled water. The values for L*, a* and b* expressing the colour readings were used to calculate the chroma (C) (Eqn i), hue angle (H°) (Eqn ii), and colour change (ΔE) (Eqn iii) using the following formulas:

Table 1
pH, TSS, TTA and Vit C content of fruit juice extracted from frozen (marula) fruits.

Sample	pH	TSS (°Brix)	TTA (% citric acid)	Vit C (mg/100)
MFJ (FMF 0 Week)	4.00^{a}±0.01	11.23^{a}±0.38	2.51^{b} ± 0.49	95.11^{e} ±0.3
MFJ (FMF 2 Weeks)	4.01^{a}±0.01	11.47^{b} ± 0.06	2.47^{b} ± 0.03	86.86^{d} ± 0.25
MFJ (FMF 4 Weeks)	4.31^{b} ± 0.01	11.73^{b} ± 0.15	2.35^{a}±0.05	82.11^{c}±0.32
MFJ (FMF 6 Weeks)	4.32^{b} ± 0.01	11.78^{b} ± 0.17	2.28^{a}±0.09	71.46^{b} ± 0.21
MFJ (FMF 8 Weeks)	4.41^{b} ± 0.01	11.79^{b} ± 0.12	2.16^{a}±0.04	60.51^{a}±0.10

Values are means ± standard deviations of replicate determinations (n = 3). Mean values with the same letter in the same column are not significantly ($p > 0.05$) different. MFJ is Marula fruit juice. FMF is Frozen marula fruit.

$$\text{Chroma, } (C*) = \sqrt{a^2 + b^2} \tag{i}$$

$$\text{Hue angle } (H*) = (b / a) \tag{ii}$$

$$\text{Total colour difference } (\Delta E*) = \sqrt{(L - Lc)^2 + (a - ac)^2 + (b - bc)^2} \tag{iii}$$

where L = lightness, a = redness, b = yellowness, Lc = lightness of control sample, ac = redness of control sample, bc = yellowness of control sample.

2.2.10. Sensory evaluation

The fruit juice sensory properties were determined with the help of 50 semi-trained panellists who were familiar with the fruit juice (Marula). They were asked to rate the fruit juice based on colour, taste, flavour, and overall acceptability. The Hedonic scale (9-point) was used to evaluate the sensory properties of the fruit juice. On the scale of 1–9, the panellist was asked to rate the juice where 9 represented like extremely (highest score) and 1 dislike extremely (lowest score). The fruit juice was presented with code to the panellist. Also, water was presented to the panellist to rinse their mouth after each tasting.

2.3. Statistical analysis

The IBM SPSS Version 26.0 software (SPSS Inc., Chicago, IL, USA) was used to statistically analyse the results obtained from the fruit juice. The mean values and standard deviations of the triplicates of the fruit juice (Marula) was reported. The results obtained was analysed using the one-way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to determine the significant differences between the mean values ($p < 0.05$).

3. Results and discussions

3.1. pH, TSS, TTA and vit C content of the fruit juice (marula)

Table 1 displays the physicochemical characteristics of the fruit juice (marula) obtained from the frozen fruit. Marula fruit juice that has been extracted from frozen marula fruit for 0 and 2 weeks has a pH that is comparable (4.00 & 4.01) with no discernible variation at $p < 0.05$. The pH of the remaining samples (marula fruit juice extracted from frozen marula fruits for 4, 6, and 8 weeks) fluctuated, but there was no discernible difference at $p < 0.05$ between any of them (4.31, 4.32, and 4.41). The fruit juice made from frozen marula fruits had a range of total soluble solids (TSS). The control sample (0 week) had the least value of 11.23 (°Brix), while the TSS of the remaining samples increases with the increase in the frozen week of the fruits (Marula fruits). Although, there are variation in their TSS values (11.47, 11.73, 11.78 & 11.79), there is no significant difference ($p < 0.05$) among the values.

With an increase in the number of frozen weeks of the marula fruits, the TTA (titratable acidity) of the fruit juice (marula) extracted from those fruits exhibited a drop in values. The control sample had the highest value (2.51% citric acid), followed by the fruit juice extracted from frozen marula fruits for 2 weeks (2.47% citric acid). The fruit juice extracted from frozen marula fruits for 4, 6, and 8 weeks had values of 2.35, 2.28, & 2.16% citric acid, respectively. There was an approximately 36% decrease in the vitamin C content of all the fruit juice. The fruit juice from frozen (marula) fruits at week 0 in this study had the highest vitamin C content (95.11 mg/100g). With more frozen weeks, the value declines. The lowest value, 60.51 mg/100g, with a drop of 36%, was found in the (marula) fruit juice that was extracted from frozen (marula) fruit that had been kept for 8 weeks. These findings are in line with the reported by Sahari et al. [14], who reported vit C decrease of frozen strawberry fruit juice. It is also in agreement with the study conducted by Chen et al. [15], which also reported a significant decrease in vitamin C content in green pepper juice after freezing.

The slight increase in pH of the (marula) fruit juice extracted from the frozen fruits may be attributed to the effects of type of freezing condition, time, and temperature on the fruit juice. During freezing, ice crystals are formed in the extracellular environment of the fruit due to the lower solute concentration and higher freezing point [16]. The formation of ice crystals in the plant cell resulted in a temporary supercooling of the cell and a difference in osmotic pressure in the fruits that pulled the liquid water out of the supercooled cell. This resulted in dehydration of the tissue, shrinkage, and collapse of the cell wall [17]. The loss of water from the intracellular components may have led to the slight increase in pH observed in this study and the slight increase in TSS, which occurred due to an

Table 2
Total phenolic content and the antioxidant capacities of the fruit juice extracted from frozen marula fruits.

Sample	TPC (mg CE/ml)	ABTS (μmol TE/ml)	DPPH (μmol TE/ml)	FRAP (Fe^{2+} E/ml
MFJ (FMF 0 Week)	196.42^{e}±1.9	158.75^{e}±1.6	150.16^{e}±1.8	210.12^{e}±1.2
MFJ (FMF 2 Weeks)	155.64^{d} ± 1.7	136.87^{d} ± 1.2	128.33^{d} ± 1.4	198.71^{d} ± 1.4
MFJ (FMF 4 Weeks)	142.41^{c}±3.4	112.66^{c}±1.4	117.18^{c}±1.9	153.22^{c}±1.5
MFJ (FMF 6 Weeks)	136.38^{b} ± 2.4	106.63^{b} ± 2.4	107.03^{b} ± 1.8	134.66^{b} ± 1.6
MFJ (FMF 8 Weeks)	124.16^{a}±2.4	98.12^{a}±2.4	96.16^{a}±1.7	112.41^{a}±1.8

Key: CE = catechin equivalents, TE = Trolox equivalents. Values are means of duplicates ± standard deviations. Means in a row with different superscripts are significantly different ($p > 0.05$) from each other using the least significant difference (LSD). MFJ is Marula fruit juice.

increase in the solute concentration of the fruit juice after extraction. The slight decrease in the total titratable acid of the fruit juice (marula) may be due to pH slight decrease in the juice due to an increase in the citric acid of the fruit juice. These results are consistent with the study conducted by Tan et al. [18], which reported that frozen storage had no significant effect on the pH and TTA of Musang King and D24 durian pulp. However, the results obtained in this study differed from those of Orellana-Palma et al. [19], who observed a gradual reduction in pH for cryoconcentrated calafate juice compared to fresh juice. The pH of the concentrated marula fruit juice did not show a significant difference when compared to the pH of the fresh juice. In the report of Orellana-Palma et al. [19], the pH decrease was attributed to the higher concentration of total soluble solids and organic acids resulting from the concentration process. However, in the current study, freezing the marula fruit before juice extraction did not significantly impact the total soluble solids content, as indicated in Table 1, leading to no significance difference in the pH between the fresh and frozen fruit-derived juice.

The loss in vit C content of the fruit juice extracted from frozen fruit (marula) may be attributed to drip loss caused by frozen storage and thawing patterns of the frozen fruits and freeze-concentration (i.e., the increase in concentration of solutes) that occurs in the unfrozen phase during freezing [20]. Drip loss occurs due to the formation of large ice-crystals during thawing. The mobility of the water molecule in the system during thawing increases, and partially bound water becomes free water, leading to damage of the cell tissue that may contain important nutrients (such as vitamin C) and then drip loss [21,22].

3.2. Total phenolic content and the antioxidant capacities of the marula fruit juice

The total phenolic content (TPC) and antioxidant activities (ABTS, DPPH, & FRAP) of the fruit juice extracted from frozen (marula) fruits are shown in Table 2. The TPC of the samples decreased with the increase in frozen weeks of the marula fruit. The control sample (fruit juice obtained from fresh (unfrozen) marula fruit) had the highest value of 196.42 mg CE/ml, while the marula fruit extracted from 8 weeks of frozen marula fruit had the lowest TPC value (of 124.16 mg CE/ml**).** The detrimental effects of freezing on the marula fruit cells, which may result in the degradation of some phenolic compounds, may be the reason for the decrease in TPC. According to Khattab et al. [23], freezing results in fruit cell breakdown, which permits enzymatic reactions to take place and phenolic chemicals to oxidize and degrade. Anthocyanins and ellagic acid are among the phenolic compounds present in fruit juice. Frozen storage has been reported to decrease anthocyanins and ellagic acid in fruit juices [24]. Cyanidin 3-glucoside, cyanidin 3, 5-diglucoside.

3.2.1. FMF is frozen marula fruit

pelargonidin 3-glucoside, and delphinidin 3-glucoside were reported to decrease at different percentages in pomegranate juice stored at −25 °C [24]. Although there are other anthocyanins that are stable, the degradation of the mentioned anthocyanins in (marula) fruit juice extracted from frozen marula fruit may be responsible for the decrease in the TPC of the fruit juice (marula) samples.

The outcomes of this study align with the findings of previous reports by Mirsaeedghazi et al. [24], Khattab et al. [23], and Orellana-Palma et al. [19]. Mirsaeedghazi et al. [24] observed a decrease in total phenolic content (TPC) in pomegranate juice, while Khattab et al. [23] reported a similar reduction in TPC for haskap berry. Furthermore, Orellana-Palma et al. [19] found a significant decrease in TPC of calafate juice due to cryo-concentration, although an initial increase was observed. This study's results are consistent with these reports, providing further support for the impact of various processing methods on the TPC of different fruit juices.

The degradation in TPC may be responsible for the decrease observed in the free radicals scavenging powers of the juice. Another contributing factor could be the polymerization of phenolic compounds with other components, such as proteins [19]. The ABTS results of the samples showed a decrease with an increase in the frozen time of the marula fruit. Marula fruit stored for zero day had the highest value (158.75 μmol TE/ml), while the marula fruit stored for 8 weeks had the least value (98.12 μmol TE/ml). Similar results were observed for the DPPH results of the fruit juice samples. The fruit juice extracted from the fresh fruit (marula) had the highest scavenging power (150.16 μmol TE/ml), while fruit juice extracted from the fruit stored for 8 weeks had the least scavenging power (96.16 μmol TE/ml). The degradation of the phenolic compounds in the juice caused by freezing and thawing may be responsible for the decrease in the scavenging power of the juice. There was a positive correlation between TPC and ABTS (0.9724), TPC and DPPH (0.9799), and TPC and FRAP (0.8979). These results are similar to the report of Mirsaeedghazi et al. [24], who reported a decrease in antioxidant activities of frozen pomegranate juice and Khattab et al. [23], who reported a decrease in antioxidant activities of frozen of haskap berry.

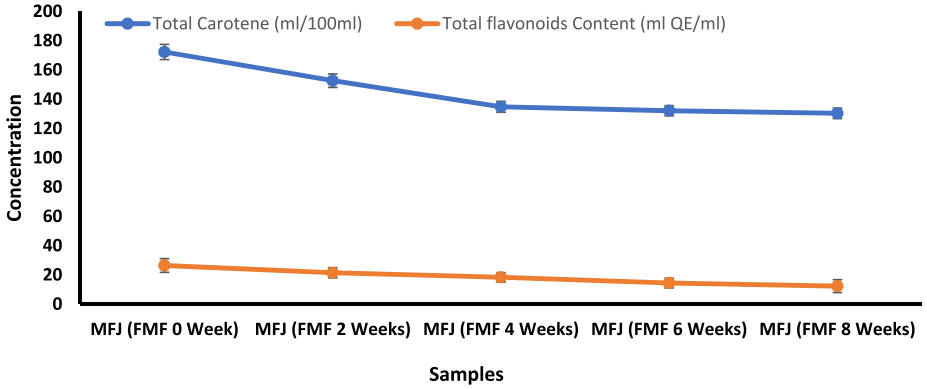

Fig. 2. The effects of frozen storage of marula fruits on total carotene and total flavonoids of marula fruit juices.

Table 3
Total carotene and total flavonoid of fruit juice extracted from frozen marula fruits.

Samples	Total Carotene (ml/100 ml)	Total flavonoids Content (ml QE/ml)
MFJ (FMF 0 Week)	$172.24^{d} \pm 1.2$	$26.42^{e} \pm 0.7$
MFJ (FMF 2 Weeks)	$152.65^{c} \pm 1.6$	$21.43^{d} \pm 0.5$
MFJ (FMF 4 Weeks)	$134.77^{b} \pm 1.7$	$18.37^{c} \pm 0.2$
MFJ (FMF 6 Weeks)	$132.12^{a} \pm 1.5$	$14.46^{b} \pm 0.3$
MFJ (FMF 8 Weeks)	$130.34^{a} \pm 1.6$	$12.36^{a} \pm 0.4$

Key: QE = Quercetin Values are means of duplicates ± standard deviations. Means in a row with different superscripts are significantly different ($p > 0.05$) from each other using the least significant difference (LSD). MFJ is Marula fruit juice. FMF is Frozen marula fruit.

Table 4
Colour of fruit juice extracted from frozen marula fruits.

Samples	L	a	b	Chroma	ΔE
MFJ (FMF 0 Week)	$54.17^{e} \pm 0.9$	$5.87^{c} \pm 0.3$	$20.09^{d} \pm 0.3$	$20.93^{d} \pm 0.5$	$46.39^{a} \pm 0.3$
MFJ (FMF 2 Weeks)	$49.69^{d} \pm 0.8$	$4.78^{b} \pm 0.3$	$20.30^{d} \pm 0.3$	$20.86^{d} \pm 0.4$	$50.39^{b} \pm 0.3$
MFJ (FMF 4 Weeks)	$48.68^{c} \pm 0.8$	$3.18^{a} \pm 0.2$	$15.13^{c} \pm 0.5$	$15.46^{c} \pm 0.4$	$49.92^{b} \pm 0.3$
MFJ (FMF 6 Weeks)	$44.61^{b} \pm 0.6$	$3.18^{a} \pm 0.5$	$12.05^{b} \pm 0.4$	$12.46^{b} \pm 0.3$	$53.01^{c} \pm 0.5$
MFJ (FMF 8 Weeks)	$42.46^{a} \pm 0.5$	$3.11^{a} \pm 0.4$	$8.07^{a} \pm 0.3$	$8.65^{a} \pm 0.3$	$54.56^{d} \pm 0.3$

Values are means ± standard deviations of replicate determinations (n = 3). Mean values with the same letter in the same column are not significantly ($p > 0.05$) different. MFJ is Marula fruit juice. FMF is Frozen marula fruit.

3.3. Total carotene and total flavonoid

The total carotene and total flavonoid results of the fruit juice extracted from frozen (marula) fruits are shown in Fig. 2. Both the total carotene and total flavonoid contents of the fruit juice decreased with an increase in freezing time. There was a 24.32% decrease in the total carotene content of the fruit juice and a 57.81% decrease in the total flavonoid content. These results align with the study conducted by Tan et al. [18], which demonstrated a significant reduction in total carotenoids and β-carotenoids content of Musang King and D24 durian pulp due to frozen storage. The degradation of carotene content of the (marula) fruit juice may be to the photo-oxidation, which may be catalysed by enzymatic oxidation. The process of thawing the frozen marula fruit takes 10–12 h (This is to follow the typical way the frozen marula fruits are allowed to thaw by the locals) before juicing. After thawing, the juicing process may encourage enzymatic oxidation since the peel of the thawed marula fruits was firstly pared and the juice extracted manually. During the juicing period, it is possible for the carotene to isomerise, leading to a diradical of carotene, which can be easily attacked by oxygen and thereby get degraded. Mordi et al. [25] reported that carotenoids in isolated form get degraded in the presence of oxygen and light, and the degradation may be from their systems of conjugated double bonds, which may render them open to oxidation.

Since the juice undergoes the same process, the degradation of flavonoids may also occur during juicing (Table 3). The degradation may be attributed to enzymatic reactions caused by polyphenol oxidase, which may be catalysed by both light and oxygen. Chaaban et al. [26] reported that the stability of flavonoids during storage can be affected by either light or oxygen, and both factors (Light and oxygen) may have synergistic effects. They reported the degradation of six different types of flavonoids based on the effects of either light or oxygen. Chaaban et al. [26] reported that no matter the amount of oxygen in a medium, mesquitol is completely degraded after 20 h in the presence of oxygen, while naringin may remain in the sample. Mesquitol and other similar flavonoids may be said to be present to be present in large quantities in marula fruit juice, which may be responsible for the decrease in total flavonoids recorded in

Table 5
Sensory attributes of fruit juice extracted from frozen fruits (marula).

Sample	Colour	Taste	Flavour	Overall acceptability
MFJ (FMF 0 Week)	$7.89^{d} \pm 0.4$	$7.46^{c} \pm 0.4$	$7.79^{d} \pm 0.4$	$7.87^{e} \pm 0.4$
MFJ (FMF 2 Weeks)	$7.34^{c} \pm 0.4$	$7.11^{c} \pm 0.4$	$7.12^{c} \pm 0.4$	$7.08^{d} \pm 0.4$
MFJ (FMF 4 Weeks)	$7.22^{c} \pm 0.4$	$6.56^{c} \pm 0.4$	$5.78^{b} \pm 0.4$	$6.77^{c} \pm 0.4$
MFJ (FMF 6 Weeks)	$6.19^{b} \pm 0.4$	$6.12^{b} \pm 0.4$	$5.55^{b} \pm 0.4$	$6.12^{b} \pm 0.4$
MFJ (FMF 8 Weeks)	$5.22^{a} \pm 0.4$	$5.34^{a} \pm 0.4$	$4.92^{a} \pm 0.4$	$5.45^{a} \pm 0.4$

Values are means ± standard deviations of replicate determinations (n = 20). Mean values with the same letter in the same column are not significantly ($p > 0.05$) different. MFJ is Marula fruit juice. FMF is Frozen marula fruit.

this study. Further analysis may be needed to confirm the types of flavonoids present in marula fruit juice.

3.4. Colour of the fruit juice

Table 4 shows the results of colour measurement of the fruit juice extracted from frozen marula fruits. The "L", "a", and "b" values of the marula fruit juice decreased. The “L” means the lightness of the fruit juice while “a” mean reddish/greenish of the fruit juice. The fruit juice (marula) that had been frozen for two weeks came in second to the control samples in terms of lightness (54.17), followed by fruit juice that had been extracted from unfrozen fruit for two weeks. The (marula) fruit juice that was produced from the fruit that had been frozen for eight (8) weeks had the least amount of lightness (42.46). The lightness (L) of the marula fruit juice samples varied significantly ($p < 0.05$), with a 21.62% drop in lightness value in this study. The lightness of the fruit juice decreased with an increase in the storage time of the fruit (marula). These results are consistent with the findings of Tan et al. [18], who reported a significant decrease in the "L" value of Musang King and D24 durian pulp during frozen storage The “a” value, which is the measure of reddish/greenishness of the food sample, showed that the fruit juice contains a very small amount of red/green colour. Every sample’s "a" value showed a significant difference at $p < 0.05$. The greatest "a" value was found in marula fruit juice that has been extracted from unfrozen/zero week marula fruit (5.87), followed by marula fruit juice produced from frozen marula fruits for two weeks (4.78). Juice from marula fruit that had been frozen for eight weeks had the lowest value, 3.11. The "a" value of the extracted juice dropped by 47.01%. This finding contradicts the earlier report by Orellana-Palma et al. [19], who observed a significant increase in the value of "a" as a result of cryoconcentration. The authors attributed this increase to a substantial rise in total soluble solids (TSS) resulting from the separation of more concentrated solutes from the ice crystal. However, in the current study, freezing did not lead to a significant increase in TSS, which may explain the differing results.

The colour of the fruit juice sample designated "b" for the fruit juice showed a significant difference ($p < 0.05$) depending on their yellowness or blueness. Juice from marula fruit displayed the same pattern of yellowness as the "L" and "a" value. The juice made from marula fruit (unfrozen marula fruit) had the highest "b" value (20.30), followed by juice made from marula fruit frozen for two weeks (20.09). The remaining samples also saw a decline in "b" values, with the fruit juice made from marula fruit that was frozen for eight weeks having the lowest value (8.07). The fruit juice extracted from fresh (marula) fruit had the greatest value (20.93), followed by fruit juice extracted from frozen (marula) fruit for two weeks (20.86). The fruit juice extracted from (marula) fruit that was frozen for eight weeks had the lowest chroma value ever measured.

The decrease in the colour parameters measured in this study may be linked to the effects of type of freezer, freezing time and thawing the marula fruit before juicing. The degradation of total carotene due to photo-oxidation and the flavonoid content of the juice through enzymatic action caused by polyphenol oxidase, may also be responsible for the decrease in colour parameters. The unfrozen state of the fruit prior to juice extraction may be responsible for the maximum intensity seen for the (marula) fruit juice obtained from fresh marula fruit. The marula fruits were fresh, and the juice was extracted freshly without degradation of the fruit cells, no freezing or thawing effects, and no drip loss. The ΔE (total colour difference) showed that all the marula fruit juice samples’ colours were visible to the naked eyes.

Although, freezing as a mode of preservation of fruits and vegetables has been reported to have negative effects on the nutritional composition of the products [27], the types of freezers used may have either positive or negative effects on the stored products. Formation of small ice-crystals during the freezing process has been said to protect the nutritional composition of the stored products, while formation of large ice-crystal (slow freezing rate) is believed to reduce the nutritional composition of the stored food materials [28]. Other processes like defrosting/thawing, and re-freezing may be detrimental to the nutritional composition of fruits and vegetables. Drip loss, enzymatic oxidation, hydrolysis, and browning (enzymatic) may contribute to the degradation of nutritional components in such fruits and vegetables [29].

3.5. Sensory attributes of the marula fruit juice

The sensory attributes of the fruit juice extracted from fresh and frozen fruits (marula) are shown in Table 5. The fruit juice was evaluated for its general acceptability, flavour, taste, and colour. The (marula) fruit juice used as the control sample, which was taken from fresh marula fruit, received the highest scores across all criteria (colour: 7.89, taste: 7.46, flavour: 7.79, and overall acceptability: 7.87). The marula fruit juice that was extracted from marula fruit that had been frozen for two (2) weeks was rated similarly to the control sample in terms of colour, taste, and overall acceptability (scores of 7.34 for colour, 7.11 for taste, and 7.12 for flavour). Out of

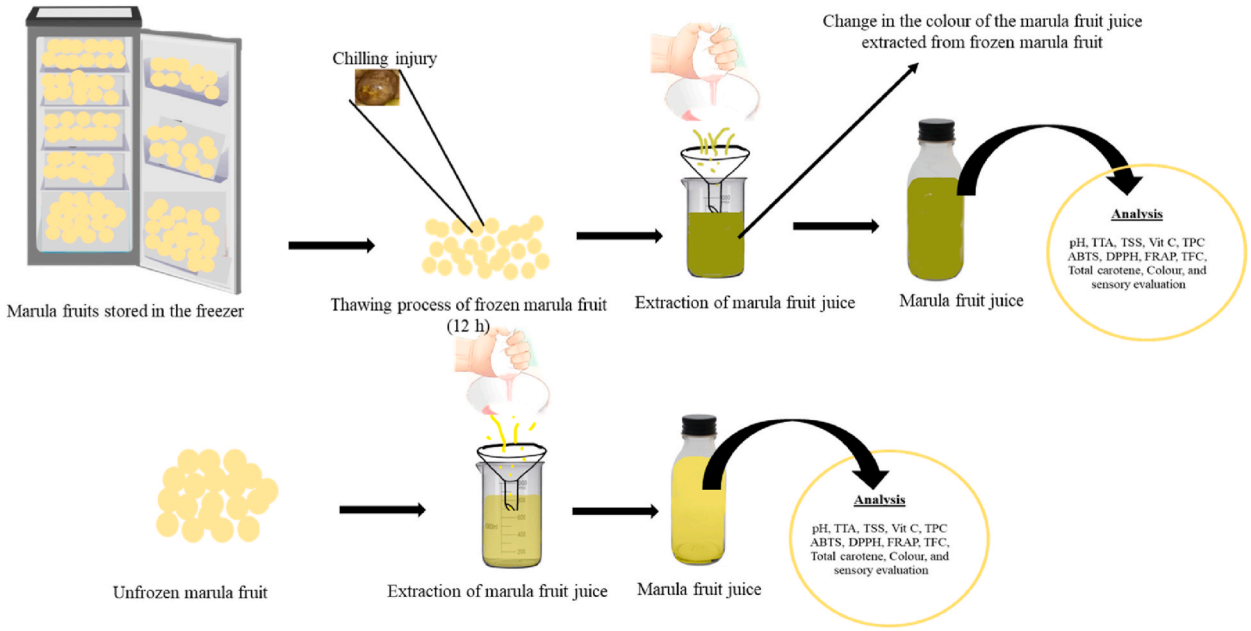

Fig. 3. Schematic diagram showing the effects freezing on colour of properties of marula fruit juice.

all the samples (marula fruit juice) examined, the (marula) fruit juice that was extracted from the fruit (marula) that had been frozen for eight (8) weeks had the lowest rating (colour = 5.22, taste = 5.34, flavour = 4.92, and overall acceptability = 5.45).

Even though the panellists were chosen on their familiarity with locally extracted juice made from regional fruits, their evaluation revealed that fresh (unfrozen) marula fruit juice had superior aroma, taste, and acceptability overall. The results we obtained for the sample of marula fruit juice's colour determination (Table 4) were supported by this score. According to the findings, fresh fruit juice had the highest values for lightness (L) and chroma (intensity).

The assigned values given to each extracted fruit juice may be because of freezing and freezing time on the fruit before juicing and the juice after juicing. The locals that engaged in freezing marula fruits since the fruits are seasonal normally used the juice for local beer production, so the effects of freezing on the nutritional composition of the juice may not have been understood, since the juice undergoes fermentation immediately.

The decrease in all the sensory attribute tested in the fruit juice may be due to the freezing method, freezing time, and thawing. The thawing of ice crystals that formed during the freezing of fruits could cause the rupturing of the cells, which may lead to degradation of colour pigments and have a negative impact on the taste, flavour, and overall acceptability of the juice. The report by Wang et al. [30] showed a decrease in sensory parameters of sweet corn frozen using refrigerator freezing. The authors reported a decrease in sensory attributes of sweet corn stored in a refrigerator freezer. In contrast, Orellana-Palma et al. [19] conducted a study where, on day 0, panelists were unable to discern any differences between the fresh juice and the cryoconcentrated sample in all the sensory attributes tested, including odour, aroma, flavour, and overall acceptability. However, as time passed, the cryoconcentrated samples received higher ratings than the refrigerated juice, suggesting that cryoconcentration was more effective in preserving the sensory qualities of the juice compared to refrigerated storage. Home freezers freezing rates are slow when compared with other types of freezers. The slow freezing rate may lead to the formation of ice-crystals which may lead to a loss of nutrients due to damage of the cells and enzymatic degradation.

The schematic diagram (Fig. 3) represents our findings in this study. The frozen of (marula) fruit was carried out using home freezer which may be responsible for all the decreases in the physicochemical, antioxidant properties, colour, and sensory parameters of the marula fruit juice. Since normal home freezers have a slow rate of freezing, that may lead to the formation of large ice-crystals in the outer cell of the fruit. This may lead to the intracellular movement of water out of the cell due to osmotic pressure. The phenomenon has been reported to cause mechanical damage to the cell wall during thawing and finally drip loss [31] which leads to a decrease in the ascorbic acid content of fruit juice. The ice-crystals formed may also injure delicate organelles and cell membranes in the fruit during freezing, initiating enzymatic reactions that are responsible for carotene degradation and that of flavonoid catalysed by light and oxygen.

4. Conclusion

The study shows that freezing of marula fruits before juice extraction has negative effects on the chemical properties and antioxidant activities of the fruit juices. The reduction in vitamin C value, total phenolic content, antioxidant activities, carotene, and flavonoid content is a confirmation of the negative effects of frozen storage on the marula fruit. The use of another type of freezer apart

from the normal home freezer may give a better result than the one obtained in this study. Freezing can be used to preserve marula fruit since it is a seasonal fruit, but a home freezer may not be the right type of freezer to use.

Author contribution statement

Lungile Kkany Nthabiseng: Performed the experiments; Contributed reagents, materials, analysis tools or data. Adeyemi Ayotundede Adeyanju: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Oluwaseun Peter Bamidele: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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