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Comparison between Vitamin(C) Content in Some Fresh and Frozen Samples of Fruits

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Abstract

The concentrations of Vitamin C, also known as L-ascorbic acid, in eleven different types of fresh and frozen fruits were determined by titration method. The effects of some conditions such as time offreezing on the Vitamin C contents were studied. Samples were collected from farms close to Janzour overall seasons. Each sample or a part of it was squeezed to get juice and centrifuged; the volume and the weight of the supernatant liquid were taken. The estimation of vitamin C concentrations were done for fresh samples, after 12, 24, 48 and 72 hours and frozen samples after 1, 2, 3 and 4 weeks. From obtained results, the concentrations of vitamin C were decreased with increasing time of experiments for fresh and frozen samples with statistically significant deference. The highest concentration was found in blood oranges and lowest concentration noticed with apple for fresh and frozen samples. Meanwhile, the concentration of vitamin C was higher in frozen sample than fresh sample without significant differences. In conclusion, the freezing method for limited time (month for example) is the best way to maintain the concentration of vitamin C because it works to inhibit the activity of oxidative enzymes and break down the vitamin C.

Keywords: Vitamin C, Frozen fruits; Fresh fruits; Titrimetric method; L-ascorbic acid.

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مقارنة محتوى فيتامين C في بعض عينات الفواكه الطازجة والمجمدة

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الملخص:

تم تحديد تركيزات فيتامين C، المعروف أيضًا باسم حمض الأسكوربيك L، في أحد عشر نوعًا مختلفًا من الفواكه الطازجة والمجمدة بطريقة المعايرة. تمت دراسة تأثيرات بعض الحالات مثل وقت التجميد على محتويات فيتامين C. تم جمع العينات من المزارع القريبة من جنزور بموسم شامل تم عصر كل عينة أو جزء منها للحصول على العصير ومعاملتها بالطرد المركزي؛ تم أخذ حجم ووزن السائل الطاف. تم تقدير تراكيز فيتامين C للعينات الطازجة بعد 12 و 24 و 48 و 72 ساعة والعينات المجمدة بعد 1 و 2 و 3 و 4 أسابيع. من النتائج التي تم الحصول عليها، انخفض تركيز فيتامين C مع زيادة زمن التجارب للعينات الطازجة والمجمدة مع وجود فرق كبير إحصائيا. تم العثور على أعلى تركيز في البرتقال الدموي ولوحظ أقل تركيز في التفاح للعينات الطازجة والمجمدة. وفي الوقت نفسه، كان تركيز فيتامين C أعلى في العينة المجمدة منه في العينة الطازجة دون اختلافات معنوية. وفي الختام فإن طريقة التجميد لفترة محدودة (شهر مثلا) هي الطريقة الأفضل للحفاظ على تركيز فيتامين سي لأنها تعمل على تثبيط نشاط الإنزيمات المؤكسدة وتكسير فيتامين سي.

الكلمات المفتاحية: فيتامين ج، الفواكه المجمدة؛ الفواكه الطازجة طريقة المعايرة حمض الاسكوربيك.

Introduction:

Vitamin C was first isolated in 1928، and in 1932 it was proved to be the agent, which prevents scurvy. Vitamin C or L-ascorbic acid has the chemical formula $C_6H_8O_6$. It consists of two inter-

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convertible compounds: Lascorbic acid, which is a strong reducing agent, and its oxidized derivative, L-dehydroascorbic acid $C_6H_8O_6$(Carpenter, 1988; Whitney *et al.*, 2019). Vitamin C, the Lenantiomer of ascorbic acid, is a water-soluble vitamin used by the body for several purposes. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component (Whitney *et al.*, 2019; Yang *et al.*, 2009). As a participant in hydroxylation, vitamin C is needed for the production of collagen in the connective tissue. Some tissues have a greater percentage of collagen, including: skin, mucous membranes, teeth, bones (Whitney et al., 2019; Yang et al., 2009). Vitamin C is also required for synthesis of dopamine, noradrenaline and adrenaline in the nervous system or in the adrenal glands. It is a strong antioxidant (Guleria *et al.*, 2017; Moritz *et al.*, 2020), and has been shown to regenerate other antioxidants within the body, including alpha-tocopherol (Vitamin E) (Bendich *et al.*, 1986; Rock *et al.*, 1996). It isan antioxidant that protects body from free radical damage. It is used as therapeutic agent in many diseases and disorders. Vitamin C protects the immune system, reduces the severity of allergic reactions and helps to fight off infections. Meanwhile, the significance and beneficial effect of vitamin C in respect to human disease such as cancer, atherosclerosis, diabetes, neurodegenerative disease and metal toxicity(Chambial *et al.*, 2013). Fresh fruits and vegetables are the major sources of this vitamin, therefore increasing its concentration will have an important impact in human nutrition. Most fruits and vegetables especially leafy ones wererich in ascorbic acid (Uusiku *et al.*, 2010; Vincente *et al.*, 2014). Citrus fruits (lime، lemon, orange, grapefruit) and tomatoes are good common sources of vitamin C. Other foods that are good sources of vitamin C include papaya, broccoli, brussels sprouts، blackberries, strawberries, cauliflower, spinach, cantaloupe, and blueberries(Nagy, 1980; Yahia *et al.*, 2019). They are many factors affect the stability of Vitamin C including moisture, oxygen, pH and light (Ottaway, 2010). Vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage During the initial food processing (slicing, shredding، grinding)

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losses of vitamin C can reach 20%, and during thermal processing (cooking) even up to 50%. Losses of vitamin C are also observed when meals are heated or defrosted too quickly.Vitamin C is also easily destroyed under the influence of food preservation or exposure to air and light. The degradation of ascorbic acid is also affected by the presence of metal ions, e.g. iron, copper (Ottaway, 2010; White-Stevens, 1982). Commercial juices and home-made juices differ in terms of nutritional value, e.g. homemade juices have higher concentrations of biologically active compounds and higher antioxidant activity, compared to commercial juices (Abdulla *et al.*, 2022; Kujawińska *et al.*, 2022). Many analytical techniques are mentioning in the literature for the determination of vitamin C in different matrices, such as: titrimetric (Nyyssonen *et al.*, 2000), fluorimetric(Casella *et al.*, 1989), spectrophotometric (Güçlü *et al.*, 2005), high-performance liquid chromatography (Gazdik *et al.*, 2008) and enzymatic (Zhang *et al.*, 2018) techniques. This work was aimed to determine the Vitamin C content in some Fruits, and studying the correlation between the content of Vitamin C and the state of fruits fresh for (12 hours, 24 hours, 48 hours and 72 hours) and frozen for (week, 2 weeks, 3 weeks and 4 weeks) using titrimetric method.

Material and Methods

Materials

Titration method determines vitamin C in the form of ascorbic acid. Eleven types of fruits were studied individually. Fruits were lemon, different type of oranges(sweet, Abu Surra, Kenyan, blood, Bitter and sour oranges (Richie)), strawberry, cantaloupe, watermelon and an apple. Samples were collected from farms in Janzour City. The determination of vitamin C content in juices obtained from the fruits using a squeezer, juicer, and slow-release juicer was performed as follows: 12 hours, 24 hours, 48 hours, and 72 hours after squeezing. Other samples of juices were stored in a refrigerator at 6 °C and analyzed after week, 2 weeks, 3 weeks and 4 weeks of frozen. An iodine solution was prepared (with a concentration of 0.005 mol/l) and the starch guide atconcentration of 0.5% were used.

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Methods

Titration process to estimate the vitamin C concentration in sampleswere done as following: 20 ml of natural juice then 25 ml of distilled water and 2 ml of starch index were added. After that, the burette was filled with iodine solution. The vitamin was slowly titrated until the equivalence point appeared, which is (blue color). This process was repeated three times and the average volume of iodine was recorded. necessary for calibration.

Vitamin C was estimated in the natural juices of (11 samples) from different types of fruits through the oxidation of ascorbic acid, i.e. vitamin C, with an oxidizing agent; iodine according to the molar ratios based on the following equation:

$$I_2 + I^- \longrightarrow I_3^-$$

$$C_6H_8O_6 + I_3^- \longrightarrow C_6H_6O_6 + 3I^- + 2H^+$$

At the equivalence point, (number of millimoles of iodine = number of millimoles of ascorbic acid).

$$\mathbf{M\ iodine \times V\ iodine = M\ juice \times V\ juice}$$

The concentration of vitamin C was converted from mole/L to mg/100ml using the following relationship:

$$\mathbf{C\ (mg/100ml) = C\ (mole/l) \times Am \times 100}$$

Am: The molecular mass of ascorbic acid = g/mol 176.12

Statistical analysis

Statistical analysis was carried out in Minitab software 17; statistical significance was assessed using one WAY ANOVA with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

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Results

Titration method determines vitamin C in the form ofascorbic acid. The amount of vitamin C isexpressed in mg/100g for each type of fruit. The concentrations of vitamin C in the fruit samples over season (fresh and frozen) were illustrated in Tables 1-3. Statistical significance between fruit types was tested.

Over all the concentrations of vitamin C were decreased with dependent manner at time of experiments at 12, 24, 48 and 72 hours, respectively with statistically significant deference. The highest concentration was found in blood oranges, that ranged from (77.87 ± 1.079 -60.13 ± 0.252 mg/100ml), followed by Boussara oranges (43.17 ± 1.137- 29.17 ± 1.021 mg/100ml), then Sour oranges, sweet, Bitter orange, strawberry, kenyan, cantaloupe and watermelon respectively. The lowest concentration was found in apple, that ranged from 3.20 ± 0.361 to 0.567 ± 0.1528 mg/100ml) (Table 1).

Table 1: Concentration of Vitamin C in fresh samples among three days of experiment (Mean± Standard division)

Parameters	12 hours	24 hours	48 hours	72 hours
Lemon	34.60 ± 0.656 A	31.50 ± 0.917 B	23.63 ± 0.473 C	20.60 ± 0.624 D
Abu-sarra	43.17 ± 1.137 A	41.47 ± 1.358 A	37.47 ± 0.945 B	29.17 ± 1.021 C
Kenyan	29.63 ± 0.833 A	28.10 ± 0.900 A	25.60 v 1.054 B	18.47 ± 0.551 C
Blood oranges	77.87 ± 1.079 A	74.40 ± 1.300 B	70.40 ± 0.500 C	60.13 ± 0.252 D
Sweet	42.30 ± 1.453 A	40.47 ± 0.839 AB	38.33 ± 0.651 B	30.40 ± 0.700 C
Bitter orange	36.40 ± 1.375 A	35.43 ± 1.168 A	32.60 ± 0.755 B	27.37 ± 0.709 C
Sour oranges	42.53 ± 0.666 A	41.23 ± 1.102 A	38.37 ± 0.603 B	25.37 ± 0.603 C
Strawberry	34.40 ± 0.755 A	32.70 ± 1.114 A	28.57 ± 0.451 B	21.43 ± 0.666 C
Cantaloupe	21.97 ± 0.950 A	21.50 ± 1.200 A	17.10 ± 0.800 B	13.40 ± 0.700 C
Watermelon	12.43 ± 1.007 A	11.33 ± 0.666 A	8.40 ± 0.781 B	4.73 ± 0.379 C
Apples	3.20 ± 0.361 A	2.47 ± 0.321 A	1.48 ± 0.404 B	0.567 ± 0.1528 C

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Values are expressed as means ± SD; n = 3 for each sample. Mean values within a column not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.

Similar pattern of results were also observed with frozen samples. over all the concentrations of vitamin C were decreased with dependent manner at time of frozen at one week, two weeks, three weeks and four weeks respectively with statistically significant deference. The highest concentration was found in blood oranges, that ranged from one week to four weeks (76.20 ± 0.964 - 70.93 ± 1.290 mg/100ml), followed by Abu Sarra orange (43.37 ± 0.643 - 40.57 ± 0.802 mg/100ml), then sour oranges, sweet, bitter orange, strawberry, kenyan, cantaloupe and watermelon respectively. The lowest concentration was found in apple, that ranged from 2.67 ± 0.493 to 2.53 ± 0.404 mg/100ml) (Table 2).

Table 2: Concentration of Vitamin C in frozen samples among four weeks of experiment (Mean± Standard division)

	1 Week	2 Weeks	3 Weeks	4 Weeks
Lemon	33.53± 0.907 A	32.07 ± 1.150 A	31.37 ± 1.429 A	31.33± 0.611 A
Abu Sarra	43.37 ± 0.643 A	42.03 ± 0.702 AB	41.37 ± 1.210 AB	40.57 ± 0.802 B
Kenyan	29.23± 0.611 A	28.30 ± 0.721 AB	26.63 ± 0.666 BC	25.57 ± 0.757 C
Blood Oranges	76.20 ± 0.964 A	75.13 ± 0.802 AB	73.33 ± 1.069 BC	70.93 ± 1.290 C
Sweet	41.10 ± 0.917 A	40.36 ± 0.737 A	38.87 ± 1.060 AB	37.50 ± 1.453 B
Bitter Orange	35.50 ± 0.656 A	34.50 ± 0.819 AB	33.46 ± 1.201 AB	32.53 ± 1.350 B
Sour Oranges	42.07 ± 0.907 A	41.53 v 0.586 A	40.30 ± 0.624 AB	38.87 ± 1.060 B
Strawberr y	33.33 ± 1.069 A	33.43 ± 0.929 A	31.567 ± 0.611 AB	30.33 ± 0.651 B
Cantaloup e	21.33 ± 0.681 A	20.47 ± 1.206 A	19.47 ± 0.666 AB	18.10 ± 0.900 B
Watermelo n	11.53 ± 0.709 A	10.60 ± 0.755 AB	9.43 ± 0.666 BC	8.50 ± 0.436 C
Apples	2.67 ± 0.493 A	2.33 ± 0.252 A	2.50 ± 0.1000 A	2.53 ± 0.404 A

Values are expressed as means ± SD; n = 3 for each Sample. Mean values within a column not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.

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In term of comparative between concentration of Vitamin C in fresh and frozen samples were also studied. Over all samples, the concentration of vitamin C was higher in frozen sample than fresh sample without significant differences. Concentration of vitamin C was increased in frozen sample of lemon and Abu Sarrawith significant differences as shown in Table 3.

Table 3: Comparison between fresh and frozen samples (Mean± Standard division)

	Fresh	Frozen	P. Value
Lemon	27.58 ± 5.96	32.07 ± 1.30	0.025*
Abu Sarra	37.82 ± 5.73	41.83 ± 1.30	0.035*
Kenyan	25.45 ± 4.53	27.43 ± 1.60	0.176
Blood Oranges	70.70 ± 6.99	73.90 ± 2.27	0.155
Sweet	37.88 ± 4.81	39.46 ± 1.71	0.302
Bitter Orange	32.95 ± 3.77	34.00 ± 1.46	0.384
Sour Oranges	36.88 ± 7.15	40.69 ± 1.47	0.097
Strawberry	29.28 ± 5.26	32.17 ± 1.53	0.093
Cantaloupe	18.49 ± 3.74	19.84 v 1.47	0.264
Watermelon	9.22 ± 3.18	10.02 ± 1.32	0.439
Apples	1.92 ± 1.08	2.508 ± 0.320	0.097

Values are expressed as means ± SD; n = 12 (four different time) for each Sample. Mean values within raw were significantly different, $p<0.05$ upon two sample T Test.

Discussion

Vitamin C concentration is affected by several factors such as light, heat, storage process, agricultural and harvesting conditions. On this basis, the concentration of the vitamin may vary from one study to another and from one analytical method to another. Therefore, there are no reference values for vitamin C in fruits and vegetables. Our purpose is to determine the Vitamin C content in some fruits, and studying the correlation between the content of vitamin C and the state of fresh (fresh for (12 hours, 24 hours, 48 hours and 72 hours) and freezing fruits for (week, 2 weeks, 3 weeks and 4 weeks). In this study, the highest concentrations of vitamin C were found in different type of orange. Orange juices are a rich source of vitamin C, which acts as an antioxidant in juices.Vitamin C concentration is an important indicator of orange juice quality and can serve as an

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indicator of product quality (Klimczak *et al.*, 2007). Comparing current results with the studies conducted previously, it was shown that the content of vitamin C differs depending on the kind of fruit (Fenech *et al.*, 2019; Nagy, 1980). Regardless of the fresh or frozen juice and kind of fruit, the content of vitamin C decreased with the increasing of time. The same relation is observed in other studies (Kaya *et al.*, 2010; Kujawińska *et al.*, 2022; Sablani *et al.*, 2006). Similar results to current study, in every studied material the amount of this vitamin decreased after 24 hours (Liu *et al.*, 2022; Nagy, 1980; Phillips *et al.*, 2010). The difference between the amount of this vitamin in fresh samples and frozen samples may be due to vitamin C degradation that can occur over the time. As confirmed by the another study, in which the amount of vitamin C decreased by an average of 37.8% after 24 hours of storage (Chanson-Rolle *et al.*, 2016). Fresh or processed fruits cover about 15% of human energy needs. Meanwhile, freezing is a method of fruit preservation in order to preserve the organoleptic and physic-chemical qualities for a longer period of time. Freezing increases the shelf life of food products by 5-50 times compared to refrigeration. In current study. Over all kind of fruits, the concentration of vitamin C was higher in frozen samples within (1. 2. 3 and 4 weeks) than fresh samples without significant differences. In contrast to the concentrations found at room temperature within (12, 24, 48 and 72) hours, it was observed that the concentration value of the vitamin decreased quickly, especially within 72 hours. This may due to the rapid loss of the vitamin C at room temperature, and the loss increases as the temperature increases due to the ease of oxidation and ascorbic acid breakdown. Additionally, it was necessary to determine if vitamin C content of archive samples stored for longer periods would still be representative of the original sample. The same relation is observed previous study that found vitamin C in frozen homogenates of representative raw fruits and vegetables is stable for some period of time with the typical processing and storage methods used for National Food and Nutrient Analysis Program (Phillips *et al.*, 2010). Recent study found that during the first 4 months of storage significant decrease was observed in vitamin C content in

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strawberry cultivars (Skupien & Jakubowska, 2023). In another study found that, the analysis of vitamin C content in melon revealed that the fresh sample had the highest vitamin C content and its amount significantly decreased after undergoing freezing processing for 90-day. it also appears that the mechanically frozen sample performed better at 7 days of storage, but as the storage duration increased, the cryogenically frozen sample exhibited better performance (Jha, *et al.*, 2024). Thus, this work concludes that the freezing method for limited time (month for example) is the best way to maintain the concentration of vitamin C because it works to inhibit the activity of oxidative enzymes and break down the vitamin C.

Conclusion

The freezing method is considered the best way to store fruit, but not for more than (4 weeks) (the present work was done only for 4 weeks using one mothed), i.e. a month because vitamin C is a water-soluble vitamin and therefore, we will lose it, and the freezing method is considered effective for fat-soluble vitamins.

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