

EFFECT OF FREEZING AND STORAGE ON THE RETENTION OF VITAMIN C, β -CAROTEN AND PECTIC SUBSTANCES FROM THE SEA BUCKTHORN FRUITS (*HIPPOPHAË RHAMNOIDES L.*)

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Abstract. *Sea buckthorn (*Hippophaë rhamnoides* Lis) is a good source of nutrients and bioactive substances, such as vitamins, carotenoids, organic acids, pectic substances and other elemental components. Preservation by freezing is a modern and easy method to increase the shelf life of the fruit. The effects of freezing and storage on nutritional value and bioactive compounds have not been fully elucidated and the results are often contradictory. The purpose of this paper is to evaluate the effect of freezing and storage on the retention of vitamin C, β -carotene and pectic substances. The sea buckthorn fruits were frozen at -18, -20⁰ C and stored for 7, 14, 21, 28, 35, 42, and 56 days. After 56 days of storage, a vitamin C retention of 73.13%, a β -carotene retention of 86.52% and a pectic substances (expressed as calcium pectate) retention of 79.58% of was obtained.*

Keywords: *sea buckthorn , vitamin C, β -caroten, pectic substances, frozen.*

1. INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.), also known as sea-buckthorn river is a branched and spiny shrub which grows in Romania starting from the sand on the seaside to the mountainous regions, constituting quite large lawns and bushes.

Sea buckthorn fruits are used in the food industry, in forestry, in pharmacy but also as an ornamental plant. It is consumed as such or processed in the form of jam, juice, oil, yellow / orange pigments, jellies or preserved by various methods (freezing, dehydration) [1].

Fruits have a unique composition, containing a cocktail of bioactive compounds. Their composition varies depending on the species, the growth region and the degree of maturity. They have a yellow-orange color and are rich in nutrients and bioactive substances: vitamins (C, E, K, folic acid), carotenoids (β -carotene, lycopene, zeaxanthin and lutein), organic acids (malic acid, oxalic acid), carbohydrates (glucose and fructose), flavonoids, pectin, essential polyunsaturated fatty acids and essential amino acids, micro and macronutrients [2, 3]. Because of these compounds, the seabuckthorn fruits have a nutritional value and a high antioxidant capacity, becoming an important source of nutritional and medicinal products. It has cytoprotective, radioprotective, hepatoprotective, anti-tumor, anti-microbial, anti-stress, tissue regeneration and anti-bacterial role [2, 4]. Although the sea buckthorn trees grow in extreme cold and drought conditions, fruits have a very short storage life because they can suffer physical damage or fungal attacks. Thus, during transport, storage and processing,

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texture changes occur, the loss of nutrient-rich juice and the loss of aroma compounds [5]. Due to these considerations, the preservation of these fruits is necessary in order to allow them to be available for a longer period of time and on distant markets. Thermal conservation, conventional drying, vacuum drying, high pressure processing and preserving the pulp with different substances (potassium meta-bisulphite, potassium sorbate and sodium benzoate) have been studied by many researchers over time [5-7]. From previous research, performed on fruits and vegetables, it has been found that freezing is a good method of preserving nutritional value, antioxidant substances, texture and quality. The freezing process turns water from cellular vacuoles into ice crystals, so it is no longer available as a reagent, solvent or microorganism growth agent. This preservation method can result in a change in fruit texture due to depolymerization and solubilization of pectic substances, which ultimately lead to tissue degradation and softening of the fruit [8].

The objectives of this study were to study the effects of freezing and storage on the retention of vitamin C (ascorbic acid), β -carotene, pectic substances (expressed as calcium pectates) and variation soluble dry substance, acidity (expressed as malic acid) and dry matter of the sea buckthorn.

2. MATERIALS AND METHODS

2.1. MATERIALS

To make this study, the matured hippopotamus (*Hippophaë rhamnoides* L.) was purchased from the local market in September. Fruits were sorted, selected at approximately the same size and packed in plastic bags, weighing 100 g. The bags were frozen at -18 to -20° C in a classic refrigerator. The freezing effect was evaluated by analyzing the fruit after 1 day of storage at freezing temperature. Frozen fruits were stored for 7, 14, 21, 28 and 56 days and analyzed. Before performing the analyzes, the frozen samples were thawed at 4° C for 1 hours.

2.2. METHODS

Determination of vitamin C (ascorbic acid). The method of quantitative determination of ascorbic acid is based on the oxidation of ascorbic acid to dehydroascorbic acid with 2,6 dichlorophenolindophenol [9].

Ascorbic acid content were calculated using the formulas:

$$\text{ascorbic acid (mg/g)} = \frac{V \times t}{m} d \times 100 \quad (1)$$

Where: V– the volume of the 2-6- diclorphenolindophenol solution used for titration, in cm³; t – the titer of the 2-6-diclorphenolindophenol solution, in mg/ cm³; m– sample weight, in g; d – the dilution factor.

Determination of β -carotene Weigh 1 gram of product that mixes with small portions of petroleum ether. At certain time intervals, the solvent in which the carotene was extracted is introduced into a 50 ml volumetric flask. The operation is repeated until the fruit is completely discolored. Make up to the mark with petroleum ether and read absorbance of the sample at $\lambda = 450$ nm using a spectrophotometer UV-VIS-NIR V-600, JASCO.

The amount of β -carotene is calculated using the formula [10].

$$\beta - \text{caroten } (\mu\text{g/g}) = \frac{A \times V \times 10^6}{A_{1\text{cm}}^{1\%} \times 100} \quad (2)$$

Where: A - absorbance; V - total extract volume, $A_{1\text{cm}}^{1\%} = 2592$, the absorption coefficient of the carotenoid in the petroleum ether.

Determination of dry matter. The dry substance (D.M.) is determined by drying the sample at the oven at a temperature of 105°C to the constant mass.

Determination of titratable acidity (%). Acidity is determined by titration of sample with 0.1 N NaOH in the presence of phenolphthalein as indicator. Titratable acidity was expressed in malic acid using a conversion factor of 0.067 [11].

Determination of soluble solids content ($^{\circ}\text{Brix}$). Soluble solids content were determined by using Abbe refractometer at room temperature.

Determination of the total content of pectic substances. The total content of pectic substances is determined as calcium pectate. For this purpose, the protopectin is hydrolyzed by boiling for a long time in a dilute acid solution and by the addition of sodium hydroxide, the pectin passes into the pectic acid sodium salt. For this purpose, protopectin is hydrolyzed by boiling for a long time in a dilute acid solution, and by the addition of sodium hydroxide, the pectin passes into the sodium salt of pectic acid. In the presence of excess acetic acid, the pectinic acid is released. Pectinic acid precipitates with calcium chloride in the form of calcium pectin is released. The calcium pectate precipitate dries and weighs.

The amount of calcium pectate is calculated using the formula:

$$(\%) \text{calcium pectate} = \frac{m_1 \times 10 \times 100}{m}$$

Where: m_1 – the amount of calcium pectin retained on the filter, g; m – the weight of the sample taken, g; 10 – the dilution factor [12].

3. RESULTS AND DISCUSSION

The effect of freezing and storage on the retention of vitamin C (ascorbic acid)

Several studies have shown the positive influence of bioactive compounds on the human body. Because the human body is unable to synthesize these substances, they need to be brought through regular consumption of fruits and vegetables [13]. Instead, these products are seasonal and suffer rapid changes in composition if stored at ambient temperature. They are therefore processed by various methods to extend their period of validity. Freezing is one of the most important preservation methods that ensure the preservation of color, flavor, nutrients and bioactive compounds [13,14]. In this context the effects of freezing preservation at $-18, -20^{\circ}\text{C}$ (the samples were analyzed after 1 day) and

storage for 7, 14, 21, 28, 35, 42, and 56 days, on retention of vitamin C, β -carotene, pectic substances (expressed as calcium pectate) and variation the amount of soluble dry substance, acidity (expressed as acidity malic) and dry matter of the sea buckthorn fruits were evaluated.

Vitamin C is considered a "marker" that allows direct comparison of the quality of fresh fruits and vegetables with those preserved by various methods [16]. Vitamin C stability during freezing and storage is one of the most important parameters analyzed by many researchers because the presence of this vitamin influences the nutritional value and antioxidant capacity of frozen fruits [14,17].

The effects of freezing and storage on vitamin C retention are shown in Fig. 1. The freezing of the sea buckthorn fruits in a classic freezer leads to a 14.55% decrease in vitamin C. This value is lower than the one obtained by Favell in the freezing of the peas (30%), the vegetable with a comparable surface to the sea buckthorn fruits [17]. The difference may be due to the blanching operation applied to the peas before freezing. Storage of sea buckthorn fruits leads to small variations of vitamin C, so fruits stored for 56 days have 12.31% less ascorbic acid than the frozen ones 1 day.

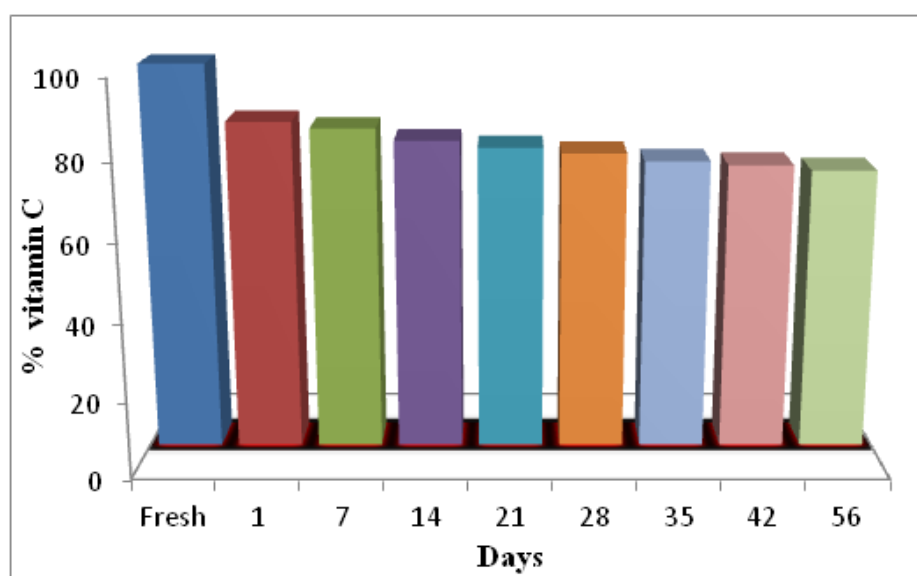


Figure. 1 The effect of freezing and storage on vitamin C retention in sea buckthorn fruits.

Slow loss of vitamin C in freezing vegetables were also obtained by Favell, who concluded that vegetables with a smaller surface are less vulnerable during freezing. Generally, the losses due to the freezing process preceded by blanching may vary from 10 to 80%, with an average of 50%. These losses are lower than other conservation methods where average values are 60% [17]. The decrease in the amount of vitamin C in fruits during freezing is most likely caused by enzyme-induced oxidation [16]. Instead, there is no explanation for increasing the degradation of vitamin C during the storage of frozen fruits and vegetables [14, 17].

Vitamin C retention in frozen products varies greatly, being positively influenced by several factors: low product surface, high freezing speed, freezing temperatures below -20°C [18].

Effect of freezing and storage on β -carotene retention

β -carotene is found in orange and red fruits and vegetables incorporated in oil droplets, and in the green ones incorporated in the carotenoid–protein–complexes in the chloroplasts. The bioavailability of β -carotene depends on the matrix in which it is incorporated, and technological processes can lead to increased bioavailability by breaking cell walls and release of protein complexes, or can lead to carotene isomerisation and degradation [19].

Fig. 2 shows β -carotene retention during freezing and storage of sea buckthorn fruits. The amount of β -carotene decreased during the freezing process from 27,924 mg/100g D.M, the amount that fresh fruits have, to 25,044 mg/100g D.M. the amount that fruits after 1 day of freezing have. These values are comparable to those reported by Bernhardt and his collaborators who have obtained variation of β -carotene when freezing sweet pepper from 27.9 to 20.3 mg/100g [19]. Instead, Scott and his collaborators on corn freezing obtained significant increases in β -carotene from 0.82 to 2.37 mg/100 g fresh weight for White Shoepeg and from 15.69 to 16.68 mg/100 g fresh weight for the Golden Whole Kernel variety. These increases are attributed to the dehydration produced during the blanching operation [20].

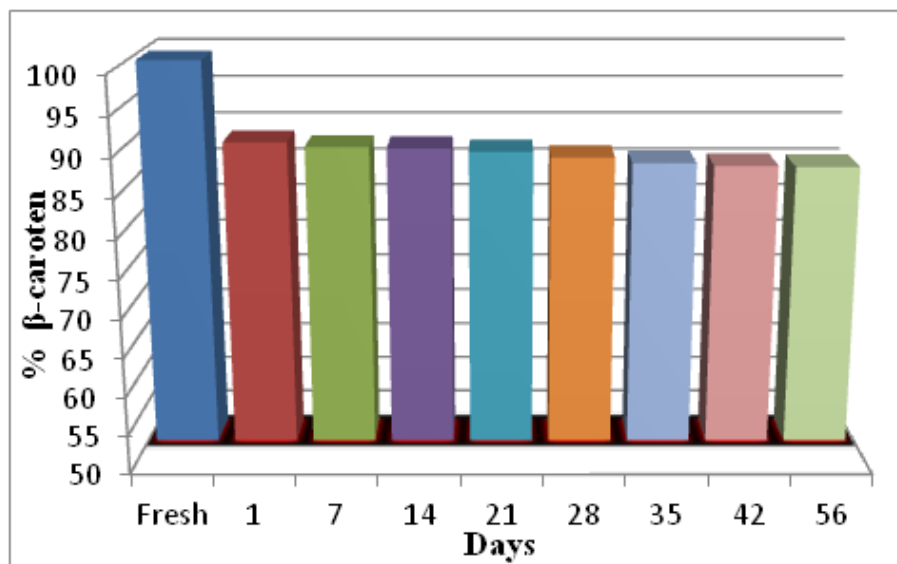


Figure. 2 Effect of freezing and storage on β -carotene retention in sea buckthorn fruits.

Storage of frozen buckthorn fruits resulted in very low changes in the amount of β -carotene. These contain after 56 days of storage 24,162 mg β -carotene per 100 g D.M., which represents a retention of 86.52%. Close values (90%) were also obtained by Wu and his collaborators when storing green beans at the freezing temperature [21].

Variation of dry matter, acidity (expressed as malic acid) and soluble dry matter ($^{\circ}$ Brix) during freezing and storage.

Table 1 shows the values of dry substance, acidity and soluble dry substance of fresh sea buckthorn fruits and the ones frozen and stored for 1, 7, 14, 21, 28, 35, 42 and 56 days.

Table 1. Dry substance, titratable acidity and soluble dry substance of fresh, frozen and stored sea buckthorn fruits.

Freezing and storing (in days)	Dry matter (g/100g)	Titratable acidity (malic acid, %)	Soluble solids (⁰ Brix)
Fresh	21.4648±0.05	1.165±0.01	13.257±0.5
1	22.2764±0.05	1.066±0.01	12.835±0.5
7	22.3877±0.05	0.943±0.01	12.632±0.5
14	23.0901±0.05	0.889±0.01	12.625±0.5
21	23.2817±0.05	0.859±0.01	12.628±0.5
28	23.3729±0.05	0.838±0.01	12.621±0.5
35	23.6529±0.05	0.774 ±0.01	12.624±0.5
42	23.5475±0.05	0.757±0.01	12.625±0.5
56	23.4631±0.05	0.737±0.01	12.627±0.5

The quantity of water of the fresh fruits ranges from 73.6-85.3%, the interval proposed by Beveridge and his collaborators [14]. During freezing the dry substance increased by 3.78%, and after 56 days of storage the fruit had a dry substance of 9.3% higher. The titratable acidity of the fresh fruit is 1.165% malic acid. This value is smaller than the one found by Zeb respectively 1.97% malic acid. [15]. During freezing and storage, the amount of malic acid decreases, after 56 days of freezing reaching a value of 36.73% lower.

Fresh sea buckthorn fruits have a dry matter content of 13.25⁰Brix. This value fits into the results proposed by other researchers. Thus, Bal proposes for the soluble dry substance of the sea buckthorn fruits the interval 10.19-22.74⁰Brix, while Green the interval 8.9-12.5⁰Brix [22, 23]. The freezing process led to a 4.66% drop in the soluble dry matter, while it remained at almost constant value during the storage.

The effect of freezing and storage on pectic substances retention

Pectic substances are heteropolysaccharides found in the cell wall of fruits and vegetables that participate in cell membrane welding, thus being responsible for the mechanical resistance of the cell [8]. They also play a role in regulating cellular permeability by influencing osmotic processes and gas exchange. Pectic substances are part of the fiber category that helps improve intestinal transit.

The degree of retention of pectic substances during freezing and storage of sea buckthorn fruits is shown in Fig. 3.

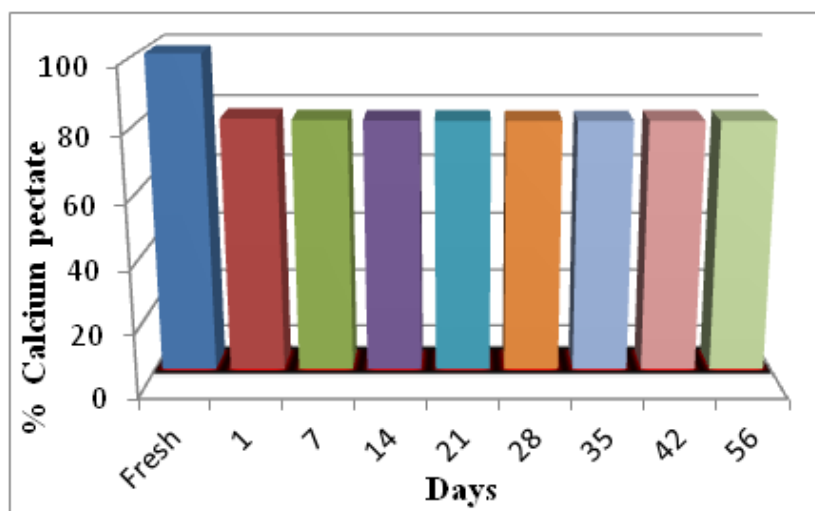


Figure. 3 The effect of freezing and storage on the retention of pectic substances (expressed as calcium pectate) from sea buckthorn fruits.

During freezing, formation of ice crystals leads to alteration of the cell wall structure. Changes in the structure of pectin polymers might have occurred during freezing and thawing, due to depolymerization and solubilization of pectic substances. The amount of calcium pectin decreases during freezing and storage from 16.08 g to 15.94 g/100g D.M. The average molecular weight of pectic substances decreases mainly in slow-frozen fruits, and the amount of soluble pectins increases. These changes are due to pectolytic enzymes that degrade pectin polymers during the defrosting process [8, 25].

4. CONCLUSIONS

Preservation by freezing led to a decrease in the amount of vitamin C during freezing and storage of sea buckthorn fruits from 322.3 mg ascorbic acid/100 g D.M. to 243.13 mg of ascorbic acid/100 g D.M. The amount of β -carotene dropped from 27.924 mg/100 g D.M to 24.162 mg/100 g D.M., and pectic substances expressed as calcium pectate from 20.032% to 15.943%. The obtained data can be useful for optimizing the freezing process in order to obtain frozen fruits with high nutritional value (e.g. β -carotene, vitamin C, dietary fiber).

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