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THE INFLUENCE OF SOME USUAL PRESERVATION METHODS ON THE CONTENT OF VITAMIN C, CHLOROPHYLLS AND CAROTENOIDS FROM BASIL (*OCIMUM BASILICUM*), LOVAGE (*LEVISTICUM OFFICINALE*) AND THYME (*THYMUS VULGARIS*) LEAVES

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Abstract

Basil (Ocimum basilicum), lovage (Levisticum officinale) and thyme (Thymus vulgaris) are aromatic plants highly valued and used by consumers around the world for the flavor they give to the foods in which are added. Because these plants are not always available fresh on the market, in order to be able to enjoy them longer, many consumers choose to either dry or freeze them at home. The purpose of this paper was to study the influence of traditional drying (for 7 days in dark room, at a temperature of 20-22°C) and freezing (at -18°C) on the content of vitamin C, chlorophylls and carotenoids from basil, lovage and thyme leaves. Vitamin C content was determined by iodometric method, and concentration of chlorophylls and carotenoids by a spectrophotometric assay. The determinations were performed on fresh plants, immediately after the drying process and at 2 months of storage in a dry state, respectively after 7days of freezing and after 2 months of storage in the freezer. In fresh samples, the highest concentration of vitamin C was found in lovage, followed by thyme. Fresh lovage also showed the highest content of chlorophyll and carotenoid. The experimental data showed that in the preserved plants the content of vitamin C, chlorophylls and carotenoids decreases, compared to the fresh ones, the losses being higher after the traditional air drying, than after freezing process. For both dried and frozen plants, a decrease in the concentration of the compounds mentioned above was found after 2 months of storage under specific conditions, but the losses were higher during the storage of dried plants.

Key words*: basil, carotenoids, chlorophylls, lovage, thyme, vitamin C.*

INTRODUCTION

Although aromatic herbs have been used for medicinal purposes for thousands of years and have also played a major role in cooking, especially due to their very pleasant aromas, they have only recently been studied in many scientific papers (Rosłon et al., 2013; Reda et al., 2007; Politeo et al., 2007). Basil (*Ocimum basilicum*) - *Lamiaceae* family- is native to areas from Asia and Africa and was brought to Europe from India in the sixteenth century, and later to America in the seventeenth century. Basil is one of the most important aromatic herbs in many cultures and cuisines, including the Mediterranean, Thai, Vietnamese (Stobart, 1982). In terms of chemical composition, it contains essential oils (0.10-0.20%), triterpene saponosides, tanoids, chlorophyll, vitamin C, carotenoids, a wide range of phenolic compounds, having different antioxidant activities, depending on the species and varieties of basil (Savu et al., 2002; Politeo et al., 2006; Nurzyńska-Wierdak, 2011). Lovage (*Levisticum officinale*) - *Apiaceae* family - perennial plant known and cultivated since antiquity worldwide as an aromatic plant, the leaves and petiole being used in the

aromatization of culinary preparations, and as a medicinal plant in Europe, the seeds and root rich in active principles being used for therapeutic qualities (Złotek et al., 2020; Kemzūraitė et al., 2014). Lovage leaves are rich in essential oil, vitamin C, carotenoids, chlorophyll pigments, polyphenolic compounds, minerals (Złotek et al., 2019; Złotek et al., 2020; Miran et al., 2018). Thyme (*Thymus vulgaris*) –*Lamiaceae* family has its origins on the European shores of the Mediterranean Sea and is cultivated today throughout Europe (De Martino et al., 2009). The chemical composition of thyme leaves includes essential oil, flavonic derivatives, polyphenolcarboxylic acids, waxes and triterpenes, a bitter principle, dietary fiber, vitamin C, vitamin B6, chlorophylls and carotenoids, mineral elements (especially iron, calcium, magnesium, manganese) (Reda et al., 2007; El-Qudah, 2014). Vitamin C (also called ascorbic acid) is a water-soluble vitamin, with a strong antioxidant activity, particularly important for the life of living organisms, it intervenes in a multitude of biochemical and metabolic processes and is found in high concentrations in green plants and in various fruits and vegetables (Devaki & Raveendran, 2017). Chlorophylls are a class of natural green compounds, found in almost all green parts of plants, such as leaves and stems and are also present in some algae and cyanobacteria, these pigments being involved with carotenoids in the process of photosynthesis (Pareek et al., 2017). So far, six different types of chlorophyll have been discovered and studied: chlorophyll *a*, chlorophyll *b*, chlorophyll *c*, chlorophyll *d*, chlorophyll *e* and chlorophyll *f.* Chlorophyll *a* is the most common in green plant tissues, followed by chlorophyll *b* (Vernon and Seely, 1966; Eugene and Govindjee, 1969; Chen et al., 2010). The human body cannot synthesize chlorophylls but is able to deposit dietary chlorophyll. The importance of this pigments for human health is not to be neglected: interrupting diverse diseases such as cancer, cardiovascular, and other chronic diseases, also helps solve pancreatic problems (pancreatitis) (Sangeetha & Baskaran, 2010). Carotenoids are non-nitrogenous natural pigments with a polyisoprene structure that give yellow, orange or red color to the tissues in which they are found. More than 700 different types of carotenoids have been discovered so far. Carotenoid pigments are synthesized only by the plant kingdom. Animals, both vertebrates and invertebrates, as well as humans, do not have the ability to synthesize carotenoids, which must be brought into these organisms through food (Rodriguez Amaya & Kimura, 2004). Without carotenoids, photosynthesis in an oxygenated atmosphere would be impossible. Also, carotenoid compounds intervene in the process of vision (provitamins A), in growth and reproduction, protection against cancer and heart disease, as antioxidants and regulators of the immune system (Krinsky, 1993; Olson, 1999). As aromatic plants are not available fresh all over the world, throughout the year, different preservation techniques have been developed over time, aiming to ensure a better protection of their bioactive compounds and sensory properties (Petcu C.D et al., 2014; Bhatta et al., 2020; Calín-Sánchez et al., 2020). However, two traditional techniques of preserving aromatic plants in their own households are still used to a large extent among the population: traditional drying and freezing. The aim of this paper was to study the effects of traditional drying (for 7 days in dark rooms, at 20-22°C) and freezing (at -18°C) on the content of ascorbic acid, chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids in basil, lovage and thyme leaves.

MATERIALS AND METHODS

The raw materials were purchased fresh from the local market in Timișoara, Romania (supermarket), respectively leaves of: basil (*Ocimum basilicum*), lovage (*Levisticum officinale*) and thyme (*Thymus vulgaris*). Samples were taken from fresh leaves and were preserved by traditional drying for 7 days at 20-22°C, in a dark room as well as by freezing at -18°C. For the analysis of vitamin C, chlorophylls and carotenoids content, samples were taken both from fresh and preserved plants and also immediately after the drying process and after 2 months of dry storage (in the dark room, at 20-22°C), as well as after 7 days, respectively after 2 months of freezing. The fresh, dried and frozen samples

were weighed to determine the water loss relative to the fresh samples and to be able to express the results by reference to fresh weight (FW) of plant tissue. The vitamin C content of the samples was determined by the adapted iodometric method, using the same working methodology as the one presented by Dumbrava et al. (2016). Analysis of the of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids content was performed by a spectrophotometric method (Lichtenthaler, 1987; Porra et al., 1989). Weighed 1 g of each sample of fresh and processed aromatic plant and then crushed it in a mortar with a little quartz sand and acetone 80%. The obtained homogenate was then centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected in brown glass containers. The precipitate was taken up in solvent and centrifuged until the colourless. The combined supernatants were analyzed at 646 nm, 663 nm and 470 nm on a UV-VIS spectrophotometer (Analytic Jena Specord 205, Jena, Germany). The chlorophylls and carotenoids content was quantified according to the formulas of Lichtenthaler and Wellburn (1983):

Chl a=12.21·(A_{663})-2.81·(A_{646})

Chl b=20.13·(A_{646})-5.03·(A_{663})

Chl_{total} =17.32·(A_{646})+7.18·(A_{663})

Carotenoids=[(1000·A_{470})-(3,27·Chl a)

-(104· Chl b)]/229

where:

Chl a - chlorophyll *a*, in mg/l,

Chl b - chlorophyll *b*, in mg/l,

Chl_{total} - total chlorophyll content, in mg/l,

A_{663} - absorbance of the sample at 663 nm,

A_{646} - sample absorbance at 646 nm

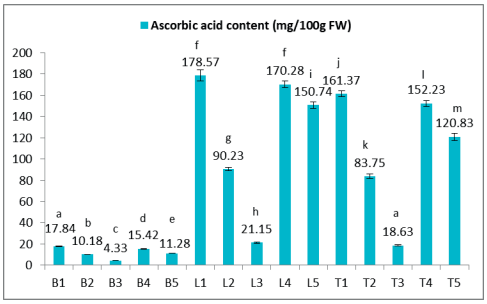
A_{470} - sample absorbance at 470 nm

All determinations were made in duplicate or triplicate and the results are reported as mean values ± standard deviation (SD). t-Test: Two-Sample Assuming Equal Variances were applied to evaluate the statistical significance ($p<0.05$). Statistical processing data was performed using the Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

In all experimental determinations we noted the samples with the following codes which will be used in the following figures and tables: B1-basil leaves fresh, B2-basil leaves immediately after traditional drying (7 days at room temperature in dark room), B3-basil leaves after 2 months of dried storage, B4-basil leaves after 7 days of freezing, B5-basil leaves after 2 months of freezing, L1-lovage leaves fresh, L2-lovage leaves immediately after traditional drying (7 days at room temperature in dark room), L3-lovage leaves after 2 months of dried storage, L4-lovage leaves after 7 days of freezing, L5-lovage leaves after 2 mounts of freezing, T1-thyme leaves fresh, T2-thyme leaves immediately after traditional drying (7 days at room temperature in dark room), T3-thyme leaves after 2 months of dried storage, T4-thyme leaves after 7 days of freezing, T5-thyme leaves after 2 months of freezing.

The ascorbic acid content of the analyzed samples is presented in Figure 1.



Each value was the mean of triplicate measurements; a-m Different letter indicate significant difference within samples ($p<0.05$)

Figure 1. Ascorbic acid content of fresh and preserved samples

Among the fresh plants, the highest content of ascorbic acid was found in the lovage leaves (178.57±5.43 mg/100 g FW), followed by the thyme leaves (161.37±3.06 mg/100 g FW), while the basil leaves were about 10 times poorer in vitamin C (17.84±0.83 mg/100 g FW) than those of lovage. As it can be seen from Figure 1, the preservation of these aromatic herbs by traditional drying has led to a more pronounced decrease in vitamin C content than preservation by freezing, especially after a period of 2 months of plant storage in the dry state, when values of 4.33±0.07 mg/100 g FW for dry basil, 21.15±0.41 mg/100 g FW for dry lovage and 18.63±0.48 mg/100 g FW for dry thyme were found. After one week of freezing, the ascorbic acid content of studied plants was only slightly lower than that of fresh plants

(15.42±0.33 mg/100 g FW - for basil, 170.28±3.05 mg/100 g FW - for lovage, 152.23±2.94 mg/100 g FW - for thyme), however, after 2 months of freezing there is a more significant reduction in this content (11.28±0.30 mg/100 g FW for basil, 150.74±3.12 mg/100 g FW for lovage, 120.83±3.44 mg/100 g FW for thyme).

In Figure 2 is presented the average losses (%) of vitamin C in the samples of preserved plants, compared to fresh plants

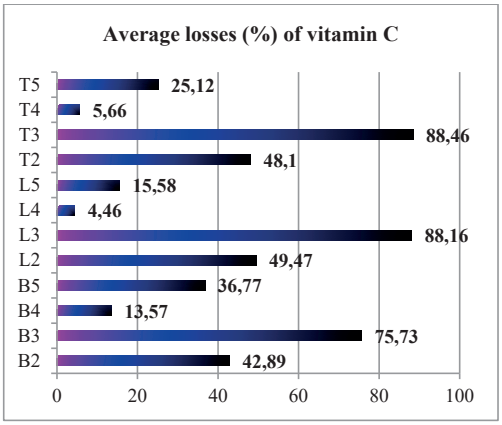


Figure 2. Average losses of vitamin C (%) in the samples of preserved aromatic plants

For the samples stored in the dry state for 2 months, the highest losses of ascorbic acid were reported: 88.46% in the samples of dried thyme, 88.16% for dried lovage and 75.73% for dried basil. Much less vitamin C was lost during freezing than during traditional drying and dry storage, with the lowest losses in the case of lovage leaves (4.46% after 7 days of freezing and 15.58% after 2 months) and the highest in the case of basil (13.57% after 7 days of freezing and 36.77% after 2 months). In the literature there are very different data for the content of vitamin C in the leaves of basil, lovage and thyme, which vary greatly depending on the plant variety, growing conditions, geographical area, method of processing etc. Cătunescu et al. (2017) reported a vitamin C content for fresh lovage leaves of 173.49±3.37 mg/100 g FW, and for those stored at cold (4°C) for 8 days: 171.33±5.48 mg/100 g FW. Złotek et al. (2020) found a smaller content in the fresh lovage leaves, of only 49.13±0.63 mg/100 g FW, and after traditional drying: 4.84±0.11 mg/100 g FW. For fresh basil Holland et al. (1991) reported 26 mg ascorbic acid/100 g and for fresh thyme, Dauqan & Abdullah (2017) found 160.1 mg ascorbic acid/100 g.

In Table 1 are presented the concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll in the fresh and preserved aromatic plants. In all samples, both fresh and processed, chlorophyll *a* was much higher concentration than chlorophyll *b.* Among the raw materials, the highest chlorophylls content was determined in fresh lovage leaves (chlorophyll *a*: 945.10±14.24 µg/g FW. chlorophyll *b*: 315.21±11.22 µg/g FW, total chlorophylls: 1260.31±25.46 µg/g FW) and the lowest in basil leaves (chlorophyll *a*: 287.42±8.32 µg/g FW, chlorophyll *b*: 94.70±2.04 µg/g FW, total chlorophylls: 382.12±10.35 µg/g FW). As in the case of vitamin C, it was found that in samples preserved by drying the concentration of chlorophyll pigments is lower than in samples of plants preserved by freezing.

Table 1. Chlorophyll a, chlorophyll b and total chlorophyll content of the fresh and preserved aromatic plants

Sample	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl total (µg/g FW)
B1	287.42±8.32^{a}	94.70±2.04^{a}	382.12±10.35^{a}
B2	134.72±4.99^{b}	40.28±0.99^{b}	175±5.98^{b}
B3	88.92±1.98^{c}	25.41±0.50^{c}	114.33±2.47^{c}
B4	270.24±7.17^{a,d}	91.25±2.12^{a,d}	361.49±9.29^{a,d}
B5	250.61±6.96^{d}	82.32±2.97^{d}	332.93±9.92^{c,d}
L1	945.10±14.24^{e}	315.21±11.22^{e}	1260.31±25.46^{e}
L2	402.44±10.49^{f}	145.92±2.26^{f}	548.36±8.23^{f}
L3	215.36±6.35^{g}	80.41±2.84^{d}	296.77±9.19^{g}
L4	928.64±15.08^{e}	306.98±7.10^{e}	1235.62±22.17^{e}
L5	860.45±12.19^{h}	296.41±6.70^{e}	1156.86±5.49^{h}
T1	420.45±7.65^{f,i}	151.30±3.90^{f}	571.75±11.55^{f,i}
T2	207.63±3.37^{g}	75.72±2.25^{d}	283.35±5.61^{g}
T3	101.23±2.63^{k}	42.64±2.14^{b}	143.87±4.77^{k}
T4	411,78±7.55^{f,i}	147.33±2.90^{f}	559.11±10.45^{f}
T5	382.21±5.05^{f,j}	130.14±2.94^{g}	512.35±7.99^{f,j}

Each value was the mean of triplicate measurements; a-j Different letter indicate significant difference within samples ($p<0.05$)

Figure 3 shows the average chlorophyll losses from the samples of preserved aromatic plants, compared to the fresh ones. As it can be seen, traditional drying causes significant losses of chlorophyll pigments in all studied herbs, even after the end of the drying process (7 days) losses exceeding 50% (for basil: 53.13%, 57.46% and 54.20%; for lovage: 57.42%, 53.71%, and 54.49%; for thyme: 50.61%,

59.95% and 50.44% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively) and after 2 months of dry storage reaching over 70% (for basil: 69.06%, 73.16% and 70.08%; for lovage: 78.03%, 74.49%, and 76.45%; for thyme: 75.92%, 71.82% and 74.84% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively). Instead, freezing at -18 C of these plants causes much lower losses of chlorophyll, which are very small after 7 days of freezing and increasing slightly after two 2 months of freezing. Lovage leaves had the lowest chlorophyll losses during freezing (after 7 days: 1.74%, 2.61% and 1.96% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively; after 2 months: 8.96%, 5.96% and 8.21% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively).

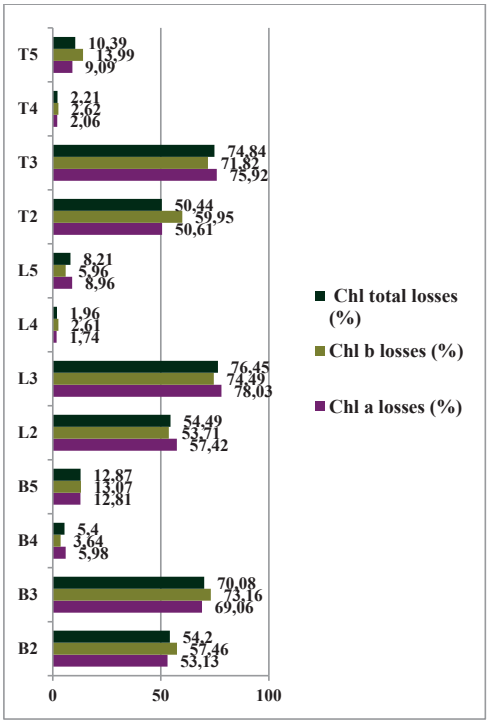
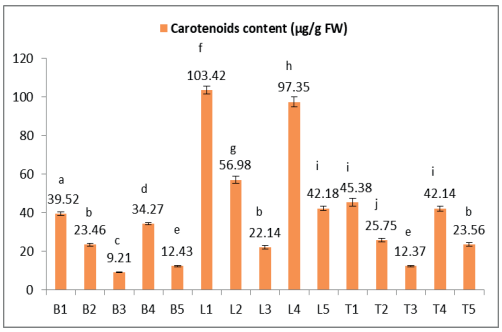


Figure 3. Average losses of chlorophylls (%) in the samples of preserved aromatic plants

Because the amount of chlorophylls accumulated in plant leaves matrix depends on a multitude of factors, including plant variety, pedoclimatic conditions, the degree of plant development (Vernon & Seely, 1966; Limantara et al., 2015), literature data for basil, lovage and thyme report quite different values of the chlorophylls concentration both in the fresh and processed plant material. Thus, Arunrangsi et al. (2013) reported for fresh basil leaves values of chlorophyll content quite close to what we found (chlorophyll *a*: 263.0±0.6 µg/g, chlorophyll *b*: 94.7±0.8 µg/g), but, also for basil Taie et al. (2010) report a higher chlorophyll content (chlorophyll *a*: 680±0.55 µg/g, chlorophyll *b*: 58±0.007 µg/g). Sledz and Witrowa-Rajchert (2012) studied the effect of microwave-convective drying on the chlorophyll content of several aromatic plants, including basil and lovage. They observed that the chlorophyll *a* content decreased after drying, compared to fresh plants, from 15.20 mg/g DW to 14.20 mg/g DW for lovage, respectively from 14.10 mg/g DW to 13.04 mg/g DW for basil. El-Qudah (2014) found an average contents of chlorophyll *a*, chlorophyll *b* and total chlorophylls in fresh thyme leaves of 2.82, 1.31 and 4.13 (mg/g DW), respectively.

The carotenoids content of the analyzed samples of fresh and preserved aromatic plants is presented in Figure 4. Among the fresh plants analyzed, lovage had a much higher concentration of carotenoids (103.42±1.95 µg/g FW) than thyme (45.38±1.90 µg/g FW) and basil (39.52±1.00 µg/g FW). It was also found that all samples subjected to preservation suffered losses of this compounds, those preserved by traditional drying had lower concentrations of carotenoidic pigments than samples preserved by freezing. Thus, the lowest concentrations of carotenoids were determined in the samples of dried basil, then in those of dried thyme, after two months of dry storage (9.21±0.14 µg/g FW, respectively 12.37±0.37 µg/g FW). The highest carotenoid content of all samples preserved by drying was found in lovage samples (56.98±1.77 µg/g FW after completing the traditional drying process and respectively 22.14±0.81 µg/g FW after 2 months of dry storage). Also, among the samples of frozen plants, lovage had the highest content of carotenoid compounds (97.35±2.45 µg/g FW after 7 days of freezing and 42.18±1.17 µg/g FW after 2 months of freezing



Each value was the mean of triplicate measurements; a-j Different letter indicate significant difference within samples ($p<0.05$)

Figure 4. Carotenoids content of the fresh and preserved aromatic plants

Figure 5 shows the losses in carotenoids in the preserved samples of aromatic plants compared to the fresh plants.

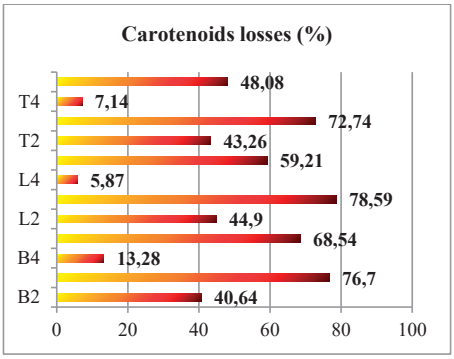


Figure 5. Average losses of carotenoids (%) in the samples of preserved aromatic plants

In the samples of aromatic plants preserved by drying, the greatest losses of carotenoids were reported for lovage (44,9% after completing the traditional drying process and 78.59% after 2 months of dry storage). However, lovage recorded the lowest carotenoids losses after 7 days of freezing (5.84%) and thyme had the lowest carotenoids losses both after 2 months of freezing (48.08%) and after 2 months of dry storage (72.74%). After completing the drying process (7 days) the lowest carotenoids losses were reported in basil (40.64%).

As for carotenoid compounds, the concentrations determined in plants vary depending on the growing conditions, geographical area, variety, extraction method, etc., the literature indicates quite different values for the concentrations of these compounds in fresh basil, lovage and thyme leaves. For these plants subjected to freezing or to traditional drying, there are relatively few data. Nazin et al. (2019) found for fresh basil grown in different lighting conditions, values of carotenoids concentration between 171.42±8.1 and 316.67±9.1 µg/g DW; also, Taie et al. (2010) reported very high carotenoid values for fresh basil between 184±0.014 and 516±0.02 µg/g FW. For fresh lovage leaves, Złotek et al. (2020) determined very low values for carotenoids: 1.37±0.28 µg/g FW and after the traditional drying process they found 0.22±0.01 µg/g FW (meaning losses of 83.94% carotenoids following the traditional drying process, higher than those obtained by us). Sharafzadeh & Alizadeh, (2011) studying the carotenoid compounds in thyme leaves, determined values between 560 and 920 µg/g DW (depending on the type of fertilizer used).

CONCLUSIONS

The application of classical preservation techniques (freezing at -18°C and respectively traditional drying at 20-22°C for 7 days) of aromatic plants in households causes different losses of vitamin C, chlorophylls and carotenoids. Of the two preservation methods studied, traditional drying has been shown to produce much greater losses than freezing. Thus, if after 7 days of freezing the losses of ascorbic acid, chlorophylls and carotenoids were relatively small for lovage and thyme and slightly higher in the case of basil, in the case of traditional drying, immediately after the completion of the process there were large losses of vitamin C (over 40% in all plants), chlorophylls (over 50% in all plants) and carotenoids (over 40% in all plants). For plants stored in the dry state for 2 months in specific conditions (20-22°C in the dark room) the losses of vitamin C and carotenoids in all plants were about twice as high and the losses of total chlorophylls reached over 70%. Also, the freezing for 2 months of the plants determined a more pronounced decrease of the concentration of the analyzed active principles, but the losses were still significantly lower than those found in the case of dried plants stored for 2 months. Thus, the present study shows that freezing, because it protects better the

content of vitamin C, chlorophylls and carotenoids in the studied plants, than traditional drying, is the most recommended classic method for preserving them in households.

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