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EFECTS OF SOME DRYING METHODS ON THE CONTENT IN BIOACTIVE COMPOUNDS IN SEA BUCKTHORN BY-PRODUCTS

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Abstract

Recent research has shown that the consumption of sea buckthorn (Hippophae rhamnoides) berries has many benefits for human health. Large amount of waste which result from sea buckthorn berries processing, yet containing significant quantities of bioactive substances, is being the subject of numerous latest research studies. Sea buckthorn by-products represent a valuable source of bioactive compounds that could be used for their favorable nutritional and functional properties in the pharmaceutical, cosmetic and food industries.
The aim of this work is to establish a method for drying process of sea buckthorn by-products in order to obtain a powder from dry sea buckthorn waste with minimum loss of the bioactive substances. For this purpose, sea buckthorn by-product (peel and seeds) was dried at different temperatures (30˚C, 40˚C, 50˚C and 60˚C) by conventional hot air drying versus lyophilization method and then the antioxidant activity (DPPH), the content of total polyphenols and ascorbic acid content were determined.

Key words: *bioactive compounds, by-products, conventional drying, lyophilization, sea buckthorn.*

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*) is a nutritionally valuable plant which is mostly consumed in the form of whole fruits. It contains a number of bioactive compounds such as polyphenols, flavonoids, organic acids, vitamin C, polysaccharides, unsaturated fatty acids and various natural amino-acids, thus it is used as a valuable food product but at the same time as medicine, outcomes which have been emphasized in the studies carried out until now (Krejkarova et al., 2015; Nilova and Malyutenkova, 2018; Sotler et al., 2019) The development of research and interest in this plant led to the extend cultivation of seabuckthorn from the spontaneous flora into crops. However, the expansion of sea buckthorn crops also influenced its processing. With easy adaptability and good profitability, the industrialization of sea buckthorn fruits presented great interest to the economic agents. Approximately 150 species, subspecies and varieties of sea buckthorn have been identified; they differ in shrub habitat, appearance of berries and their use-value (Ciesarova et al., 2020). Sea buckthorn berries are attractive due to their nutritional properties, being scientifically recognized for their content in vitamin C, carotenoids, flavonoids, sterols and tocopherols (Maheshwari et al., 2011; Olaru & Popa, 2019; Ciesarova et al., 2020). Sea buckthorn juice is the most consumed product obtained from sea buckthorn fruits processing. It has a long shelf life without needing any heat treatment, consequently there is no influence on its bioactive compounds content.
Effective valorisation of agri-food industrial wastes/by-products targets to contribute to an enhanced economy and also to minimize the negative impact on the environment, with positive effect on ensuring food security (Bhat, 2021; Kumar et al., 2021b).
Studying the nutritional aspects of sea buckthorn by-products could lead to new opportunities for obtaining nutraceuticals and natural functional food ingredients at low prices. The content of bioactive compounds in sea buckthorn by-products and their use in functional foods development have been studied in recent years. Most of the available literature has focused on evaluating the effect

of the extraction protocol or method used for plant extracts preparation. However, the method for post-extraction conditioning is also important for assessing the content in bioactive compounds, especially since recent studies have reported that the drying method used to process the pre-extraction of plant biomass affects its bioactivity and chemical composition (Altemimi et al., 2017; Fernandes et al., 2018; Pham et al., 2015). Lyophilization is the "gold standard" for drying plant extracts, playing an important role in preserving their quality and extending their shelf life (ElNaker et al., 2021). Lyophilization limits oxidative modifications of metabolites because the oxygen concentration is deficient in vacuum (Papageorgiou et al., 2008). However, compared to other methods of drying plant extracts, lyophilization is more expensive due to equipment, materials and operational costs (Soquetta et al., 2018).

The alternative to modern freezedrying is conventional drying, by hot air, in grate or screen dryers, which is frequently used due to the low cost and availability of the equipment.

The main purpose of this work was to compare both drying methods for the sea buckthorn by-products and to establish which is the optimal drying method with optimal working parameters in order to maintain the content on chemical and nutritional substances. Due to its properties, sea buckthorn by-products could be used as a valuable functional ingredient in the new product development process.

MATERIALS AND METHODS

The plant material used in the experiments

In this study three organic sea buckthorn varieties, respectively Mara, Clara and Sorana were analyzed for water activity, dry matter, total polyphenol content, antioxidant activity and ascorbic acid content in order to investigate the effects of drying methods on the bioactive compounds content of the by-products obtained after sea buckthorn processing.

Pretreatments applied to sea buckthorn by-products

A series of preliminary test were performed in order to establish a juice squeezing method for sea buckthorn berries, as to prepare the sample for lyophilization by preliminary freezing and optimizing the way that samples could be better arranged into the lyophilization chamber.

In order to squeeze the sea buckthorn fruits, tests were carried out in parallel with two constructive types of fruit juicer: a centrifugal juicer and an auger juicer.

The centrifugal juicer has a series of knives arranged circularly in the centre of a sieve in the shape of a truncated cone. The fruits are first cut into very small pieces and thrown by centrifugal force into the sieve. This process happens very quickly and involves exposure of the juice droplets to air (oxygen) and metal (sieve). The fruit squeezing process, in this case, is a fast one.

The squeezing method for the auger juicer is by mastication and pressing. The fruits are cut by the auger at the top of the spindle, then crushed in the middle of the auger spindle, and the actual pressing is done at the bottom. The fruit squeezing process in this case is a slow one.

The by-products resulted after the sea buckthorn processing was conditioned by lyophilization respectively conventional drying, at different temperatures 30^0C, 40^0C, 50^0C, 60^0C.

Setting up the conventional drying and freeze-drying methods

The conventional drying was carried out with a Biovita Deluxe-10 food dehydrator (Figure 1). Regarding the setting up of sea buckthorn by-products conventional drying, the working parameters were tested at different temperatures for certain time intervals as follows: 60°C for 9 hours, 50°C for 12 hours, 40°C for 15 hours, respectively 30°C for 19 hours.

The sea buckthorn by-products lyophilization was carried out with a Labconco - FreeZone lyophilizer (Figure 1), which has a capacity of 2.5 litres. It allows lyophilization of moderate volume samples at a temperature of -50°C, which is why, following optimization tests of the freezing process as a preliminary stage in the lyophilization process, it was established to store the samples at -35°C for 24 hours. It is preferable that the freezing temperature of the samples is as close as possible to the operating temperature of the collector of the lyophilizer.

The collector is made of stainless steel and can hold approximately 2.5 liters of ice before defrosting.

Food dryer	Labconco Freeze dryer

Figure 1. The equipment used for air drying/liophilization of sea buckthorn by-products

In order to establish the lyophilization technology of the sea buckthorn by-products, a series of parameters were tested, such as:
- collector temperature;
- the temperature of the shelf inside the lyophilization chamber;
- pressure, in order to obtain vacuum;
- the freeze-drying time.

The preliminary tests to establish the lyophilization parameters of sea buckthorn by-products were carried out on Mara variety.

- the temperature of the collector varied in the range -46°C-48°C;
- the temperature of the shelves in the freeze-drying chamber was kept constant at 60°C;
- the pressure varied between 0.200-0.800 mbar;

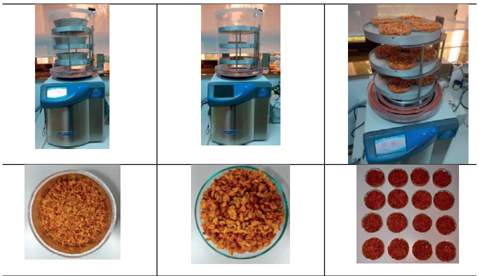
The lyophilization time tested was 24 hours, 48 hours, and 72 hours, respectively.
The by-products obtained were frozen, this being a mandatory preliminary stage in the lyophilization process. The low temperature allows the final product to be of higher quality. To optimize the freezing process, different freezing temperatures were tested. In the preliminary stage, the freezing of sea buckthorn by-products was tested in a Hotpoint refrigerator at freezing temperatures of -18°C, -20°C, and -24°C, respectively. Subsequently, the freezing of sea buckthorn by-products was tested in the Feutron climate chamber (Figure 2) which allowed freezing at -35°C. The freezing time of sea buckthorn by-products was also tested.

Refrigerator Hotpoint	**Climate chamber Feutron KPK 200**
Freezing temperatures tested: -18°C, -20°C, -24°C	Freezing temperatures tested: -30°C, -35°C

Figure 2. Equipment used for freezing sea buckthorn by-product samples that were subjected to freeze drying

Each shelf of the lyophilization chamber can have its own temperature probe for the most accurate temperature monitoring. The shelves of the lyophilization chamber can be removed from the chamber to be pre-frozen and also have the pre-freeze function with a cooling system of about 240 watts. In order to optimize the operation of the lyophilizer, but also to avoid losing the freezing temperature of the samples, it was decided to start the lyophilizer before introducing the samples into the lyophilization chamber, so that the collector reaches the operating temperature before placing the samples on the shelves of the lyophilization chamber.
Regarding the arrangement of the samples on the lyophilizer shelves, the distribution of 4 glass Petri dishes (with a diameter of 90 mm) on each of the 3 shelves of the lyophilization chamber was preliminarily tested. Thus, the sea buckthorn by-products were previously distributed in the glass Petri plates, which were subsequently subjected to freezing at -35°C for 24 hours. In this way, temperature losses could be minimized when handling the samples, which involves moving them from the climate chamber to the lyophilization chamber.
In order to make more efficient use of the space in the lyophilization chamber, we resorted to replacing the 90 mm diameter Petri dishes that did not fully cover the surface of the shelves with aluminum trays that have a diameter of 25 cm. These aluminum trays have a number of advantages, including:
- allow a quick heat transfer;

- ensures uniform distribution of the sample, in a thicker layer, thus allowing lyophilization of a larger amount of sample;
- ensures efficient use of the space on the shelves of the lyophilization chamber;
- reduce the risk of scattering the sample inside the lyophilization chamber, during handling or depressurization;
- ease of handling samples;
- simpler and more effective sanitation;
- multiple use.



A. Distribution of samples in aluminum trays | B. Distribution of samples into glass Petri dishes

Figure 3. The arrangement of the samples in the lyophilizer

In order to highlight the effects of the freeze-drying treatment versus the conventional drying treatment on sea buckthorn by-products, the following indicators were analysed: vitamin C content, antioxidant activity and polyphenol content.

Antioxidant activity

The effect of antioxidant activity on 1,1-dipheny l-2- picrylhydrazyl (DPPH) was estimated according to the procedure described by Villaño et al. (2007), with some modification presented further. Briefly, 10 g of sample was macerated in 50 ml ethanol (75%) for 48 h in the dark, at room temperature. For each measurement, 0.05 ml filtered extract solution was added to 1.95 ml DPPH ethanolic solution and thoroughly homogenized, and incubated in dark at room temperature for 30 min. Sample absorbance was measured at 515 nm. Results were expressed as quercetin equivalents (QE) per 100g D.W.

Ascorbic acid content

The content of ascorbic acid was determined by extracting 10 g of sample in 100 ml of 2% oxalic acid. The extract was filtered and 2 ml from the extract solution, 1 ml oxalic acid 2%, 5 ml buffer solution, 2 ml indophenol (2, 6-Dichlorophenol Indophenol) and 20 ml xylene, were placed in a centrifuge tube and centrifuged for 20 min at 5°C and 9000 rpm. The absorbance of the samples was measured 500 nm and the results were expressed as mg ascorbic acid/100 g D.W.

Total polyphenolic content

Total content of polyphenols (TP) was determined using the Folin-Ciocalteu method. Briefly, for each measurement, 1.58 ml distilled water, 20 µl filtered extract solution (10 g of fruit macerated in 50 ml ethanol (75%) for 48 h in the dark at room temperature), and 100 µl Folin-Ciocalteu reagent were mixed and then 300 µl Na_2CO_3 (20%) was added. The solutions were mixed and stored in the dark at room temperature for 2 hours. Sample absorbance was measured at 765 nm. Total polyphenol concentration was expressed as mg/L Gallic acid equivalents (GAE) per 100 g D.W.

Statistical analysis

All determination was performed in duplicate. The obtained data was statistically analysed using Microsoft Excel 2017. In all tests, it was considered the significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

In order to highlight the effects of the freeze-drying treatment versus the conventional drying treatment on sea buckthorn by-products, the following indicators were analysed: vitamin C content, antioxidant activity and polyphenol content. Following the preliminary tests made with Mara sea buckthorn variety (Table 1), freeze-drying operating parameters were established as follows:
- vacuum: 0.270 mbar;
- collector temperature: - 48°C;
- freeze-drying time: 24 hours;
- temperature of the shelf in the freeze-drying room: 60°C, 50°C, 40°C, respectively 30°C.

Afterwards by-products from all three sea buckthorn varieties tested (Mara, Clara, Sorana) were freeze-dried and the notations used are presented in the Table 2.

Table 1. Preliminary tests in order to establish the lyophilization parameters of sea buckthorn by-products

No. crt.	Sample	Pretreatments	Liophylization parameters				
			Collector temperature (^0C)	Vacuum (mbar)	Shelf temperature (^0C)	Freeze drying time (hours)	Final moisture content (%)
1.	MARA variety	Fruits squeezing, By-products preliminary freezing at -35^{0}C for 24 hours Ui = 53,285 %	- 46.7	0.794	60	24	8.755
2.			- 47	0.233	60	48	7.439
3.			- 47.6	0.237	60	24	10.963
4.			- 47.6	0.240	60	24	9.082
5.			- 46.9	0.297	60	48	8.491
6.			- 47.3	0.290	60	72	8.310

Table 2. The notation used in the presentation of results

No. crt.	Samples analyzed	Coding of the samples
1.	Sea buckthorn by-product from the Mara variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LM 30
2.	Sea buckthorn by-product from the Mara variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LM 40
3.	Sea buckthorn by-product from the Mara variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LM 50
4.	Sea buckthorn by-product from the Mara variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LM 60
5.	Sea buckthorn by-product from the Clara variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LC 30
6.	Sea buckthorn by-product from the Clara variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LC 40
7.	Sea buckthorn by-product from the Clara variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LC 50
8.	Sea buckthorn by-product from the Clara variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LC 60
9.	Sea buckthorn by-product from the Sorana variety lyophilized at 30^{0}C temperature on the shelf of the lyophilization chamber	LS 30
10.	Sea buckthorn by-product from the Sorana variety lyophilized at 40^{0}C temperature on the shelf of the lyophilization chamber	LS 40
11.	Sea buckthorn by-product from the Sorana variety lyophilized at 50^{0}C temperature on the shelf of the lyophilization chamber	LS 50
12.	Sea buckthorn by-product from the Sorana variety lyophilized at 60^{0}C temperature on the shelf of the lyophilization chamber	LS 60
13.	Sea buckthorn by-product from the Mara variety conventionally dried at 30^{0}C	CM 30
14.	Sea buckthorn by-product from the Mara variety conventionally dried at 40^{0}C	CM 40
15.	Sea buckthorn by-product from the Mara variety conventionally dried at 50^{0}C	CM 50
16.	Sea buckthorn by-product from the Mara variety conventionally dried at 60^{0}C	CM 60
17.	Sea buckthorn by-product from the Clara variety conventionally dried at 30^{0}C	CC 30
18.	Sea buckthorn by-product from the Clara variety conventionally dried at 40^{0}C	CC 40
19.	Sea buckthorn by-product from the Clara variety conventionally dried at 50^{0}C	CC 50
20.	Sea buckthorn by-product from the Clara variety conventionally dried at 60^{0}C	CC 60
21.	Sea buckthorn by-product from the Sorana variety conventionally dried at 30^{0}C	CS 30
22.	Sea buckthorn by-product from the Sorana variety conventionally dried at 40^{0}C	CS 40
23.	Sea buckthorn by-product from the Sorana variety conventionally dried at 50^{0}C	CS 50
24.	Sea buckthorn by-product from the Sorana variety conventionally dried at 60^{0}C	CS 60

Regarding the setting up of conventional drying parameters for sea buckthorn by-products, the drying options were tested at different temperatures for certain time intervals that are specified in the Table 3 for all three varieties of studied sea buckthorn (Mara, Clara and Sorana). In terms of the completion of the drying process, the final moisture content of samples was considered the most important indicator, the results obtained ranging between 7.121% and 9.003%.

Table 3. Conventional drying treatments applied to sea buckthorn by-products

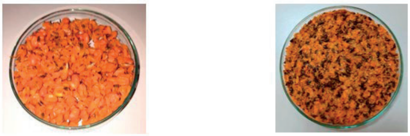
No. crt.	Sea buckthorn variety	Drying temperature (^0C)	Drying time (hours)	Initial moisture content of samples (%)	Final moisture content of samples (%)	Dry matter of samples (%)
1.	Mara	60	9	48.837	8.252	91.748
2.	Clara			50.276	7.712	92.288
3.	Sorana			51.551	8.006	91.994
4.	Mara	50	12	48.837	9.003	90.997
5.	Clara			50.276	8.155	91.845
6.	Sorana			51.551	8.352	91.648
7.	Mara	40	15	48.837	8.908	91.092
8.	Clara			50.276	7.149	92.851
9.	Sorana			51.551	7.899	92.101
10.	Mara	30	19	48.837	7.816	92.184
11.	Clara			50.276	7.548	92.452
12.	Sorana			51.551	7.121	92.879

Regarding the setting up of the sea buckthorn by-products lyophilization, freeze-drying options were tested at different temperatures for certain time intervals that are specified in Table 4 for all three varieties of sea buckthorn studied (Mara, Clara and Sorana). In terms of the completion of the freeze-drying process, the final moisture content of samples was considered the most important indicator, the results obtained ranging between 3.686% and 6.988%, lower values being recorded than in the case of conventional drying.

Table 4. Freeze-drying treatments applied to sea buckthorn by-products

No.	Sea buckthorn variety	Freeze-drying shelf temperature (^0C)	Freeze-drying time (hours)	Initial moisture content of samples (%)	Final moisture content of samples (%)	Dry matter of samples (%)
1.	Mara	60	24	48.837	4.587	95.413
2.	Clara			50.276	3.686	96.314
3.	Sorana			51.551	4.286	95.714
4.	Mara	50	24	48.837	5.711	94.289
5.	Clara			50.276	5.635	94.365
6.	Sorana			51.551	4.616	95.384
7.	Mara	40	24	48.837	6.063	93.937
8.	Clara			50.276	5.052	94.948
9.	Sorana			51.551	4.651	95.349
10.	Mara	30	24	48.837	6.988	93.012
11.	Clara			50.276	5.102	94.898
12.	Sorana			51.551	5.014	94.986

With respect to the appearance of the by-product obtained after squeezing, it was found that the one obtained with the auger juicer is devoid of juice or pulp, being entirely composed of very well pressed and crushed sea buckthorn skins and seeds (Figure 4B). As it can be seen in Figure 4A, which shows the appearance of the sea buckthorn by-products samples obtained after squeezing with a centrifugal juicer, it is insufficiently pressed, presenting pulp and juice remaining in the fruit.



A. obtained by squeezing with a centrifugal juicer (U = 53,285 %)

B. obtained by squeezing with the auger juicer (U = 43,063 %)

Figure 4. The appearance of sea buckthorn by-products samples from the Mara variety

Regarding the antioxidant activity of the studied samples (Figure 5 and Figure 6), higher values were obtained for the conventionally dried samples compared to the lyophilized samples. In the case of freeze-dried samples, the highest values were obtained for the following temperature regimes: 40°C for the Mara and Sorana varieties and 50°C for the Clara variety. For conventionally dried samples, the highest antioxidant activity values were obtained for samples dried at 50°C for the Mara and Sorana varieties and 30°C for the Clara variety.

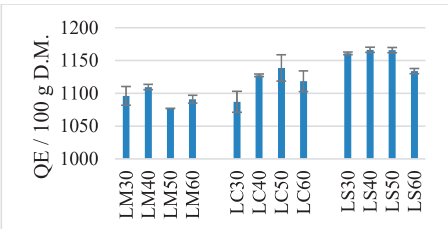


Figure 5. Antioxidant activity of freeze-dried sea buckthorn by-products

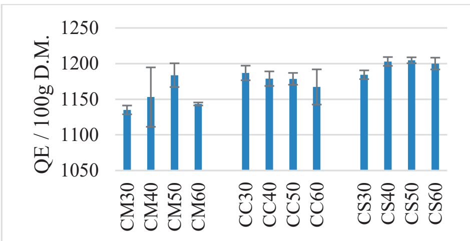


Figure 6. Antioxidant activity of conventionally dried sea buckthorn by-products

According to the results obtained from determination of the ascorbic acid content for the dehydrated sea buckthorn by-products, it was observed that the values obtained following the application of the studied drying regimes did not vary significantly, the ascorbic acid content showing close values.

Related to the working regimes studied, the highest content of ascorbic acid was obtained following the application of the temperature of 40°C in the lyophilization process, respectively conventional drying for the Mara variety, 50°C for the Clara variety and 60°C for the Sorana variety (Figure 7 and Figure 8).

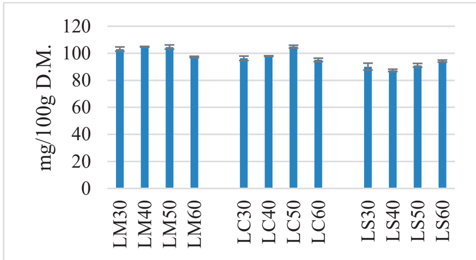


Figure 7. Ascorbic acid content of freeze-dried sea buckthorn by-products

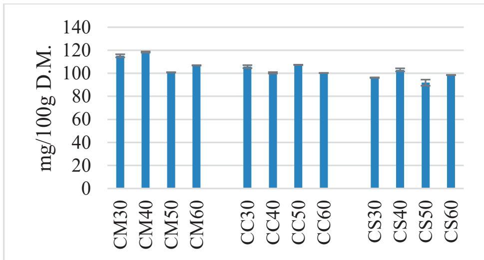


Figure 8. Ascorbic acid content of conventionally dried sea buckthorn by-products

Following the analysis of the results obtained in the case of the total polyphenol content determination (Figure 9 and Figure 10), no significant variations were observed between the two drying regimes, the values obtained for this parameter being similar.

In the case of freeze-dried samples, the highest values were obtained for the samples to which the following temperature regimes were applied: 30°C for the Mara variety, 40°C for the Clara variety and 60°C for the Sorana variety. Regarding conventionally dried samples, the highest polyphenol content was obtained for samples dried at 50°C for the Mara variety and 40°C for the Clara and Sorana varieties.

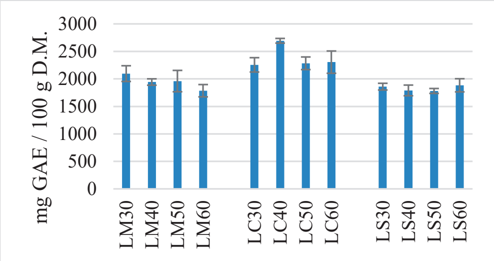


Figure 9. Polyphenol content of freeze-dried sea buckthorn by-products

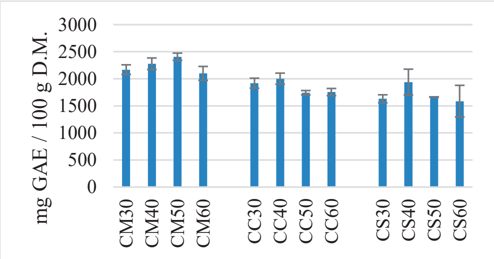


Figure 10. Polyphenol content for conventionally dried sea buckthorn by-products

CONCLUSIONS

Concerns related to the investigation of the most suitable conditioning method for sea buckthorn by-products continue to be a current topic, in order to keep the bioactive ingredient content so the important substances will not be degraded, for their further use in the food industry as potential functional ingredients. The effects of freeze-drying treatment were highlighted by comparison with conventional drying treatment.

Taking into account the results obtained, the best lyophilization regime, respectively drying, can be considered the regime with a working temperature of 40°C for 24 hours, generally obtaining high values in terms of the total polyphenols content, ascorbic acid content and the antioxidant activity for the samples subjected to this working regime compared to the other regimes tested for all the studied samples. Also, following preliminary tests, the following operating parameters were established for samples lyophilization:

- vacuum: 0.270 mbar;
- collector temperature: - 48°C;
- freeze-drying time: 24 hours.

From the point of view of the efficiency of sea buckthorn fruits juicing, it was found that the losses are lower when using the centrifugal juicer compared to the auger juicer, but in terms of the moisture and the appearance of the by-products, is recommended the use of the auger juicer.

Freezing at -35°C, in the climate chamber, is recommended as the preliminary stage of the lyophilization treatment, for the dehydration of sea buckthorn by-products.

After determining the antioxidant activity for the studied samples, higher values are observed for the conventionally dried samples compared to the lyophilized samples, for all varieties. The Sorana variety presented the highest values: CS30 - 1184 QE/100 g D.M., CS40 - 1203 QE/100 g D.M., CS50 - 1204 QE/100 g D.M., CS60 - 1200 QE/100 g D.M., the lowest values being recorded for the Mara variety, the samples subjected to the lyophilization operation: LM30 - 1096 QE/100 g D.M., LM40 - 1110 QE/100 g D.M., LM50 - 1077 QE/100 g D.M., LM 60 - 1091 QE/ 100 g D.M.

The results obtained from the determination of the ascorbic acid content were close, they are framed around the values of 100 mg/100g D.M., for the lyophilized and conventionally dried samples. A higher content of ascorbic acid is presented by the Mara variety, which conventionally dried at 30°C and 40°C shows values of 115.2 mg/100 g D.M., respectively 118.5 mg/100 g D.M.

The total content of polyphenols was influenced both by the variety of the samples and the process through which they were subjected. For lyophilization, the total content of polyphenols was assessed for the Clara variety samples, recording the highest values for LC30 - 2255 mg GAE/100 g D.M., LC40 - 2693 mg GAE/100 g D.M., LC50 - 2284 mg GAE/100 g D.M., LC60 - 2305 mg GAE/100 g D.M. Samples from the Clara variety subjected to conventional drying recorded the highest values: CM30 - 2169 mg GAE/100 g D.M., CM40 - 2278 mg GAE/100 g D.M., CM50 - 2407 mg GAE/100 g D.M., CM60 - 2100 mg GAE/100 g D.M. It is observed in both processes, as the temperature increases, the values decrease, the optimal temperatures being 30°C, 40°C, 50°C.

Laboratory research has shown that the application of the lyophilization process is suitable to preserve the bioactive properties of sea buckthorn by-products.

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