# THE OXIDATIVE STABILITY OF SEABUCKTHORN LIPOPHILIC EXTRACTS

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**Abstract.** There is an increased interest for sources of natural antioxidants in order to enrich oils towards reducing lipid oxidation. The seabuckthorn berries are natural concentrate of vitamins (C, P, B1, B2, E, K), carotenoids, folic acid, volatile oil, etc. Results obtained through analysis of different methods of research has found that lipophilic extract samples enriched with natural antioxidants are characterized by a higher antioxidant capacity compared to samples that were not enriched with natural antioxidants.

Keywords: antioxidants, lipophilic, extracts, seabuckthorn.

#### Introduction

Nowadays local manufacturers tend to replace the synthetic substances with natural ones. A safe and effective possibility would be to use biologically active compounds extracted from local natural resources.

There is an increased interest in berries because they are characterized by a large area of cultivation and they are rich in nutritionally important antioxidants, vitamins and minerals [1]. In this research, we studied mainly sea buckthorn (*Hippophae rhamnoides L.*).

Studies on sea buckthorn fruits and lipophilic extraction are increasing such as it becomes a potential ingredient rich in biologically active compounds for functional food products. The association of sea buckthorn fruits and the prevention of cardiovascular disease and cancer are justified by the rich content of antioxidants (carotenoids, vitamin C) and phytonutrients [2-4].

The aim of this study is to optimize the extraction process of lipophilic compounds and to obtain stable and high quality lipophilic extracts. For this purpose, it is intended to analyze in time the impact of biologically active compounds on the physico-chemical characteristics of lipophilic extracts and to investigate their oxidative stability.

#### **Materials and Methods**

Sea buckthorn berries (*Hippophae rhamnoides L.*) were harvested in the central area of Republic of Moldova, in 2016. Reagents Folin-Ciocâlteu, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Merck, Germany. The sea buckthorn berries were air dried, then grounded and sieved.

The extractions were carried out in deodorized refined sunflower oil (1 g vegetal material extracted in 12 ml of oil). The extractions were performed using stirring extraction technique at 45°C for 1,0h. Before decanting, the extracts were centrifuged at 8000 rpm for 10 min. The obtained extracts were kept in dark glass bottles at 4°C.

# 1. Determination of the total content of assimilating pigments (chlorophyll a, chlorophyll b, total carotenoids)

For the determination of the content of assimilating pigments, was measured the absorbance at wavelengths of 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for total carotenoids, to 10 ml of extract versus the deodorized refined oil (blank). The carotenoid content were determined by the following equations [5]:

$$C_a(mgL^{-1}) = (12,25 \times A_{663.2}) - (2,79 \times A_{646.8})$$
 (1)

$$C_h(mgL^{-1}) = (21.5 \times A_{646.8}) - (5.1 \times A_{663.2})$$
 (2)

$$C_{a+b}(mgL^{-1}) = \frac{(1000 \times A_{470} - 1.82 \times C_a - 85.02 \times C_b)}{198}$$
(3)

where:

 $A_{663.2}$  – solution absorbance at  $\lambda = 663.2$  nm;

 $A_{646,8}$  – solution absorbance at  $\lambda = 646.8$  nm;

 $A_{470}$  – solution absorbance at  $\lambda = 470$  nm;

## 2. Antioxidant activity determination using free radical DPPH

Determination of the antioxidant activity of the lipophilic extracts was performed using HACH LANGE DR-500 spectrophotometer and expressed as a % inhibition of DPPH using the following equation [6]:

$$AA\% = \frac{A_0 - A_t}{A_0} \times 100\% \tag{4}$$

where:

 $A_0$  – absorbance of the DPPH solution at t = 0 s;

A<sub>t</sub> – absorbance of the DPPH solution after 30 min;

A lower value of A<sub>t</sub> in the analyzed sample shows a higher antioxidant activity.

#### 3. Determination of Peroxide Value (PV)

Peroxide Value determination was performed by the volumetric method and the results obtained were calculated according to the following equation [7]:

$$PV = \frac{(S-B) \times N \times 1000}{\text{mass of sample. g}}, [\text{mEq O}_2/\text{kg}]$$
 (5)

where:

B – volume of titrant, [ml of blank],

S – volume of titrant, [ml of sample],

N – normality of sodium thiosulfate solution.

# 4. Determination of acid value (AV)

Determination of AV was performed by the volumetric method and the results obtained were calculated according to the following equation [8]:

$$AV = \frac{V_{KOH} \cdot N_{KOH} \cdot 5.611}{m}, [mg KOH/g]$$
 (6)

where:

V<sub>KOH</sub> – volume of the potassium hydroxide, [ml];

 $N_{KOH}$  – concentration of the potassium hydroxide, [mol/dm<sup>3</sup>];

m - mass of the sample, g.

## **Results and discussions**

Carotenoids are compounds which have a special role in slowing the photo-oxidation process and can provide oxidative stability to food products. The variation of carotenoids content extracted in oil is largely influenced by the methods used and the extraction conditions. According to bibliographic sources, the carotenoids content may vary between 1 and 20 mg/l, but usually does not exceed 10 mg/l [5].

Results with the content of chlorophyll a, b, and total carotenoids obtained by stirring are shown in Figure 1.

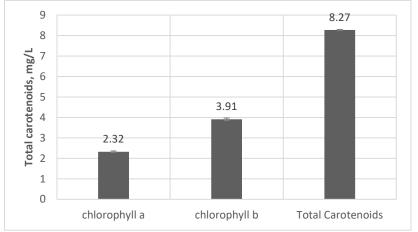


Figure 1. Total carotenoid content, mg/L.

The results obtained in the present study argue the obtaining of the lipophilic extracts which provide a high extraction degree in the case of lipophilic compounds.

The analysis of sea buckthorn extract with DPPH free radicals allows the evaluation of the antioxidant capacity of the biologically active compounds. The sea buckthorn extracts have a distinct antioxidant capacity compared to sunflower oil blank sample, the antioxidant capacity of the sea buckthorn extracts being about  $73,52 \pm 2.10\%$  (Figure 2).

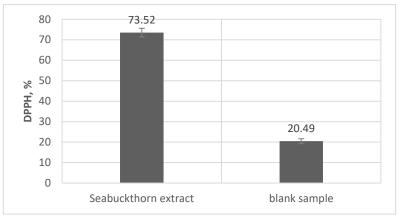


Figure 2. Antioxidant capacity, DPPH %.

The increased antioxidant activity of the sea buckthorn extract is due to the physicochemical composition, rich in carotenoids, vitamin C and phenolic compounds that have the ability to capture the radicals.

The basic quality parameters (AV, PV) were determined for the sea buckthorn extracts obtained by stirring technique, at temperature of 45°C and 1.0 h extraction time. The determination was performed on the selected obtained extracts because the results of physico-chemical determinations have attested the highest values. Quality parameters were determined presented in Table 1.

Table 1. The quality parameters of the sea buckthorn ( $Hippophae\ rhamnoides\ L$ .) extract and sunflower oil.

Quality indices	Seabuckthorn extract	Sunflower oil
AV, mg KOH/1g fat material	0.19±0.02	0.080±0.018
PV, mEq O₂/kg	1.31±0.04	2.23±0.07

According to normative documents, the AV value should not exceed 0.6 mg KOH/g. The AV in the investigated samples ranges from  $0.080 \pm 0.018$  ml KOH/g to  $0.19 \pm 0.02$  ml KOH/g. The sea buckthorn samples showed the highest value of AV, which is explained by the increased free fatty acid content.

The PV index expresses the degree of lipid oxidation of the extract and should not exceed 10 mEq  $O_2/kg$ . In our study, PV values of the sea buckthorn extracts ranged from  $1.31 \pm 0.04$  to  $2.23 \pm 0.07$  mEq  $O_2/kg$ .

#### **Conclusions**

The quality parameters of the investigated samples are within the maximum permissible limits according to the regulations and protocols for sunflower oils. The comparative analysis of sea buckthorn extracts and sunflower oil samples showed significant differences for several quality parameters studied. Sea buckthorn extracts are characterized by lower PV values  $(1.31 \pm 0.04 \text{ mEq} O_2/\text{kg})$  and AV  $(0.19 \pm 0.02 \text{ mg KOH/1g})$  compared to the values obtained for the sunflower oil samples. This is explained by the antioxidant action of the biologically active compounds from the sea buckthorn that contribute to slow down the oxidative process.

This research demonstrates the possibility to use the sea buckthorn lipophilic extracts in the production of food products. A particular interest is the opportunity to substitute synthetic antioxidants with natural ones obtained from local horticultural sources in order to provide consumers stable and safe food products for consumption.

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