THE QUANTITATIVE ESTIMATION OF SOME BIOCHEMICAL COMPOUNDS WITH ANTIOXIDANT PROPERTIES IN THE FRUITS OF THREE CULTIVARS OF *ELAEAGNUS UMBELLATA* THUNB. INTRODUCED IN THE REPUBLIC OF MOLDOVA

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Abstract

The article includes the results of the phytochemical evaluation in frozen and dried fruits of three taxa of Elaeagnus umbellata Thunb. ('Amoroso', 'Fortunella' and 'Sweet 'n' Sour'). The analysis of ascorbic acid revealed values between 85.97 and 99.69 mg/100 g in frozen fruits and 127.62 - 135.34 mg/100 g in dried fruits. Irrespective of the type of fruit preservation, the maximum amount of vitamin C was detected in the cultivar 'Amoroso'. The minimum amount of tannins was found to be characteristic of the cultivar 'Sweet' n' Sour' in both frozen (1.25%) and dried (1.88%) fruits. The total phenolic content recorded values from 1037.61 mg GAE/100 g d.m. to 1183.64 mg GAE/100 g d.m. in frozen fruits and from 1121.14 mg GAE/100 g d.m. to 1260.34 mg GAE/100 g d.m. in dried fruits. The comparative analysis of the obtained data allowed us to conclude that the fruits of analysed taxa are a promising source of natural antioxidants, which provide them with powerful antioxidant properties, and drying proved to be a more effective method of preserving the fruits than freezing, maintaining a higher amount of active principles.

Key words: Elaeagnus umbellata, autumn olive, biochemical parameters, ascorbic acid, tannins, phenolic compounds.

INTRODUCTION

Elaeagnus umbellata (Thunb.), commonly known as autumn olive, Japanese silverberry or spreading oleaster, is native to Southern Europe and Central Asia (Dirr, 1998).

The nutritional and therapeutic value of autumn olive fruits is due to their composition and nutraceutical diversity. Thus, the fruits of *Elaeagnus umbellata* are a rich source of vitamins (particularly vitamin A, C and E), minerals (phosphorus, potassium, calcium, magnesium and iron), flavonoids, essential fatty acids, alkaloids, terpenoids, saponins and other bioactive compounds (Matthews, 2001; Wu, Hu and Yang, 2011; Fordham et al., 2001; Fordham et al., 2002; Bhuvaneswari and Nagini, 2005; Perveen et al., 2015; Patel, 2015; Khattak, 2012).

Besides, the fruits contain the vitamins B_1 , B_2 , B_3 , B_6 , biotin, folic acid and vitamin K (Aziz et al., 2015). Carotenoids, such as lycopene, α -cryptoxanthin, β -carotene, lutein, phytoene, phytofluene, etc., are also valuable compounds. Autumn olive red fruits

contain 17 times more lycopene than fresh tomatoes (Fordham et al., 2001).

Due to the antioxidant properties of biologically active substances from fruits, seeds and leaves, the species *Elaeagnus umbellata* is attributed phytotherapeutic properties that are beneficial in the treatment of diseases involving oxidative stress: rheumatoid arthritis, fever, asthma (Niknam et al., 2016), type 2 diabetes (Nazir et al., 2018), cardiovascular diseases (Qayyum et al., 2019) and breast cancer (Jabeen et al., 2020).

Several studies were focused on the analysis of autumn olive as a rich source of lycopene, which is considered an important phytonutrient and is believed to protect against heart attack (Kohlmeier et al., 1997) and various forms of cancer (Clinton, 1998), including prostate cancer (Giovannucci et al., 1995). In recent years, the *E. umbellata* has also been researched as a species with antidiabetic potential (Nazir et al., 2018; Spínola et al., 2019), with an important role in inhibiting the progression of diabetes (Nazir et al., 2021). The potential of autumn olive fruits as an antiinflammatory, anti-nociceptive (Ahmad et al., 2009; Uddin and Rauf, 2012; Hamidpour et al., 2016; Özen et al., 2017) and antiproliferative agent (Wang, Bowman and Ding, 2007; Özen et al., 2017) has also been mentioned.

The therapeutic value of *Elaeagnus umbellata* against heart diseases and other health problems may be due to the presence of a large amount of oil in the fruits. Vegetable oil and phytosterols are known to have anticoagulant properties, which are recommended for lowering blood cholesterol and treating angina (Fordham, 2001). The seeds and flowers of Elaeagnus umbellata are used as a remedy in cardiovascular disorders such as hypertension and also as stimulants in coughs and bowel disorders (Chopra, Navar and Chopra, 1969; Hussain, 2011).

Relevant studies carried out also include those referring to the phytotherapeutic role of the respective species on antibacterial (Sabir et al., 2007), antifungal, insecticidal and phytotoxic activity (Aziz et al., 2015). The investigations carried out by Sabir et al. (2007) demonstrated the antibacterial activity of aqueous extracts of E. umbellata fruits in inhibiting the growth of Staphylococcus aureus and Escherichia coli.

Taking into consideration the appreciable medicinal and nutritional value of autumn olive, particularly due to the organic substances involved in redox processes, we set out to quantitatively explore the amount of some biochemical compounds in the fruits of three taxa of E. umbellata Thunb.

MATERIALS AND METHODS

The biological material used for research consisted of dried and frozen fruits of three taxa of Elaeagnus umbellata Thunb. ('Amoroso', 'Fortunella' and 'Sweet 'n' Sour'). which have been introduced in the collection of "Alexandru Ciubotaru" National Botanical Garden (Institute) in 2018 and began to bear fruit abundantly three years later.

The taxa of *Elaeagnus umbellata* 'Amoroso', 'Fortunella' and 'Sweet 'n Sour' are melliferous, fruit-bearing, silvicultural and ornamental shrubs. They can tolerate drought and frost. The researched shrubs grow and bear fruit regularly, thriving in sunny places, drained and humus-rich soils, to obtain high productivity and organic, high-quality fruits, rich in bioactive compounds. The three researched taxa of *Elaeagnus umbellata* differ in the colour of the flowers, the number of flowers per flowering stalk, the number of flowering stalks per shoot developed in the previous year, the average weight of fruits, seeds, their size, mesocarp yield and percentage of fruit set. The cultivar 'Fortunella' differs significantly from the other two cultivars in the larger size and weight of fruits and seeds, higher density of flowers and fruits per 20-cmlong shoot, and 'Amoroso' - by the lowest indices of fruit diameter and fruit weight, respectively (Onica et al., 2021). The general aspect of the investigated taxa, in the fruit development stage, is shown in Figure 1.



Figure 1. The general aspect of Elaeagnus umbellata Thunb. plants in the fruit development stage (1 - 'Amoroso', 2 - 'Fortunella', 3 - 'Sweet 'n' Sour') (Onica, Rosca and Cutcovschi-Mustuc, 2021;

http://www.lubera.co.uk/plants/soft-fruit/superfood-berries/pointilla-sweet-n-sour)

The biochemical analyses were performed at the Institute of Genetics, Physiology and Plant Protection (Republic of Moldova, Chisinau) by using different biochemical methods.

The quantitative determination of vitamin C content. The determination of the ascorbic acid included spectrophotometric content quantification using potassium hexacvanoferrate. In acidic medium, ascorbic acid stoichiometrically reduces potassium hexacyanoferrate (Fe^{+3}) K₃[Fe(CN)₆] (a red salt) to potassium hexacyanoferrate (Fe⁺²) $K_4[Fe(CN)_6]$ (a yellow salt), which in the presence of ferric ions produces iron (III) hexacvanoferrate (II) ("Berlin blue") $Fe_4[Fe(CN)_6]_3.$

To determine the concentration of vitamin C in the plant extract, the calibration curve was used and the following formula was applied:

$$K = (49.967 \cdot \text{D opt}) - 11.938$$
 (1)

where: D opt - absorbance detection at 680 nm. To calculate the ascorbic acid content in the sample, the following formula was used:

$$C = \frac{K \cdot V}{m} \cdot 100 \tag{2}$$

where: C - ascorbic acid content, $\mu g/100$ g biological material; K - concentration of ascorbic acid per 1 ml de extract, calculated according to the calibration curve, $\mu g/ml$; V - total volume of the extract, ml; m - weight of the biological sample, g.

The determination of tannins in the researched biological material consisted in their quantification with potassium permanganate (0.1 N), according to the classical titrimetric method (GOST 19885-74) as a result of the process of oxidation of tannins.

The calculation of the percentage of tannin content was done using the formula:

$$C(\%) = \frac{(a-a_1) \cdot 0,004157 \cdot V \cdot 100}{V_1 \cdot m}$$
(3)

where: a - the quantity of potassium permanganate consumed to oxidize the tannins in the sample; a_1 - the quantity of potassium permanganate consumed to oxidize the tannins in the control (water and indigo carmine); V the total volume of the sample; V_1 - the volume of the sample used for quantification; m - the dry mass of the sample, g; 0,004157 - the quantity of tannins oxidized by 1 ml of potassium permanganate (0.1 N), g.

Determination of phenolic compounds. The method of determining phenolic compounds in

the investigated biological material (Folin-Ciocalteu, 1927, with the modifications proposed by Singleton, Rossi, 1965) was based on the reaction of phenols with the Folin-Ciocalteu reagent, which is reduced in alkaline medium through the interaction with phenolic compounds, thus producing blue complexes. The reaction products were determined spectrophotometrically at a wavelength of 765 nm.

The content of phenolic compounds in one gram of fresh mass, extracted with ethanol (80%) was expressed in terms of gallic acid equivalents (GAE), which ensures the same optical density of the reaction (determined based on the calibration curve). To construct the calibration curve, gallic acid was used as a standard substance, and to calculate the content of phenolic compounds, the following formula was applied:

$$F = \frac{\Gamma(C \cdot V)}{m \cdot 1000} \tag{4}$$

where: F - the content of phenolic compounds, mg GAE /100 g d.m.; C - concentration of phenolic compounds determined on the basis of the calibration curve, mg GAE/l; V - total volume of the sample; m - the weight of the sample, g; 1000 - coefficient of converting litre to millilitres.

Statistical processing. The research results were analysed using the Microsoft Excel program. The average was calculated for each parameter and the data were expressed as the average of the repetitions.

RESULTS AND DISCUSSIONS

Ascorbic acid. The importance of ascorbic acid lies in the fact that it acts as an enzyme cofactor, contributes to the decomposition of free radicals and is an acceptor / donor in the transport of electrons in plasma membranes and in chloroplasts (Davey et al., 2000). In addition to its main role as an antioxidant and cofactor in redox reactions, recent studies have suggested an important role of ascorbic acid in the activation of epigenetic mechanisms, which control cell differentiation, the dysfunction of which can lead to the development of certain types of cancer (Fenech et al., 2019). Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in various horticultural crops and has several biological functions in the human body (Lee and Kader, 2000).

The quantitative determination of ascorbic acid in the frozen and dried fruits of the three cultivars of *Elaeagnus umbellata* subjected to research revealed values between 85.97 and 99.69 mg/100 g in the frozen fruits and 127.62-135.34 mg/100 g - in the dried ones. In the fruits of both types of preservation, the maximum amount of vitamin C was detected in the cultivar 'Amoroso', and the minimum - in the cultivar 'Sweet 'n' Sour' (Figure 2).

The values recorded in our research are much higher than those obtained by other researchers. Thus, the data obtained by Khattak (2012), in Pakistan, are significantly lower, demonstrating an ascorbic acid content of 27.2 mg/100 g in *Elaeagnus umbellata* fruits. Gamba et al.

(2020) quantified 29.12 mg/100 g fresh mass of vitamin C in autumn olive fruits in northeastern Italy. Ahmad et al. (2005) estimated even lower vitamin C content (14.1-14.3 mg/100 g). These variations could be conditioned not only by the geographical area and climatic conditions, but also by the cultivar and the stage of ripening at the time of harvesting the fruits.

The comparative study on the content of ascorbic acid in our research demonstrated a closer correlation between its amount and the type of fruit storage than the specific characteristics of the taxa. Thus, the difference in the average content of vitamin C between frozen and dried fruits was about 40%, while the difference between its minimum and maximum value in dried fruits was about 6%, and in frozen fruits - a maximum of 16%.



Figure 2. The ascorbic acid content (mg/100 g) in the fruits of different cultivars of *Elaeagnus umbellata* (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet 'n' Sour')

The obtained results allow us to conclude that, in terms of preserving the amount of ascorbic acid, drying is a more advantageous method of storing autumn olive fruits than freezing.

Tannins. Tannins are polyphenols, which have therapeutic properties and act as antioxidants. The interest in tannins as bioactive components has increased due to their wide range of biological activities, especially pharmacological properties, such as antitoxic, anticancer, antiallergic and anti-inflammatory, anthelmintic, antimicrobial, antiviral, antidysenteric etc. (King-Thom Chung et al., 1998; Ghosh, 2015; Sharmaa et al., 2019). Tannins also possess antiseptic properties due to the action of protein coagulation, preventing infection by inhibiting bacterial growth. Naturally, tannins are present in leaves, seeds, bark, roots, fruits and vegetables (Hassanpour et al., 2011; Ghosh, 2015; Sharmaa et al., 2019).

The determination of the tannin content by the titrimetric method showed minimum values characteristic of the cultivar 'Sweet 'n' Sour' in both frozen (1.25%) and dried (1.88%) fruits.

The cultivars 'Amoroso' and 'Fortunella' recorded the same content (1.66%) of tannins in frozen fruits, but not in the case of dried fruits, where the maximum value was achieved by the cultivar 'Fortunella' (2.49%), and the cultivar 'Amoroso' contained about 16.6% less tannins (Figure 3).

Depending on the cultivar, the content of tannins had a maximum difference of 32.9% in dried fruits and 33.4% in frozen fruits. The

dependence of tannin content on the method of fruit preservation proved to be more significant, and this difference exceeded 40%.

The research on *Elaeagnus umbellata* fruits carried out in Pakistan (Khattak, 2012) demonstrated a very high content of tannins as compared with our data, namely 126.5 mg/g.

In general, there is very little data on the evaluation of tannin content in autumn olive fruits.



Figure 3. The percentage of tannins in the fruits of different *Elaeagnus umbellata* cultivars (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet 'n' Sour')

Phenolic compounds are secondary plant metabolites and constitute the largest group of phytochemicals, with more than 8000 phenolic structures currently known (Harbone and Williams, 2000), which beneficially influence health due to their bioactive properties. Being important antioxidants, these compounds exert antihyperglycemic, antiviral, anticancer, antiinflammatory, anti-allergic and antimicrobial activities (Moyer et al., 2002; Manach et al., 2004; Bagchi et al., 2004; Seeram et al., 2006; Badjakov et al., 2008). Berries are rich sources phenolic compounds, of including anthocyanins, phenolic acids, flavonoids, tannins, etc. (Lee et al., 2020). The most common phenolic substances in the human diet are phenolic acids, flavonoids and tannins (King and Young, 1999).

The total content of phenolic compounds, gallic acid equivalent, evaluated in our study ranged from 1037.61 mg GAE/100 g d.m. ('Amoroso' cultivar) up to 1183.64 mg GAE/ 100 g d.m. ('Fortunella' cultivar) in frozen fruits and from 1121.14 mg GAE/100 g d.m. ('Amoroso' cultivar) to 1260.34 mg GAE/100 g d.m. ('Fortunella' cultivar) in dried fruits. Thus, regardless of the type of storage, the fruits of the cultivar 'Fortunella' contain the maximum amount of phenolic compounds, and the cultivar 'Amoroso' - the minimum amount (Figure 4). The fruits of the cultivar 'Sweet 'n' Sour' contain an average amount of phenolic compounds (1176.82 mg GAE/ 100 g d.m. in frozen fruits and 1197.27 GAE/ 100 g d.m. in dried fruits), the value being closer to those of the cultivar 'Fortunella'.



Figure 4. The content of phenolic compounds (mg GAE/100 g d.m.) in frozen and dried fruits of various cultivars of *Elaeagnus umbellata* (1 - 'Amoroso'; 2 - 'Fortunella'; 3 - 'Sweet 'n' Sour')

Similarly, to the other investigated parameters, the amount of phenolic compounds in dried fruits exceeds the respective values in frozen fruits, however this difference is not so great and constitutes only 5.3%. Instead, the biosynthesis and accumulation capacity of these organic substances depends to a greater extent on the genotype, the difference between cultivars reaching a maximum of 14.07% in frozen fruits.

In a study conducted by Surmanidze et al. (2021) on the content of phenols in fruits collected from *Elaeagnus umbellata* plants in different regions of Georgia, it was mentioned that the amount of these compounds in fruits varied greatly, being within the limits of 117.98 mg/100 g and 989.42 mg/100 g, thus demonstrating a very high dependence of the content of phenolic substances on the geographical area. The respective authors also concluded that the impact of climatic factors on this biochemical parameter is very significant. Even lower results have been reported in other papers (Perkins-Veazie et al., 2005; Wang and Fordham. 2007; Gamba et al., 2020). Significantly higher data, however, were presented in an investigation conducted in Pakistan (Khattak, 2012). Thus, a value more than twice higher as compared with the data obtained by us was identified in the fruits of Elaeagnus umbellata, namely 2332 mg GAE/100 g. A recent investigation, carried out by a group of researchers in Poland, revealed values of the total phenolic content equal to 1749 mg/100 g d.m. (Zglińska et al., 2021).

CONCLUSIONS

The quantitative determination of ascorbic acid showed relatively close values among the cultivars of the species *Elaeagnus umbellata*, but very different depending on the way the fruits were stored, this difference being about 40%.

The quantification of tannins showed significantly higher amounts in dried fruits in all the investigated cultivars, and in the case of dried fruits, the intraspecific dependence is also very high.

The spectrophotometric determination of phenolic compounds revealed a minimum content in the fruits of the cultivar 'Amoroso' and a maximum in those of the cultivar 'Fortunella', regardless of the type of fruit preservation. The comparative analysis of the obtained data allowed us to conclude that autumn olive fruits are a promising source of phytocompounds and natural antioxidants, which provide them with powerful antioxidant properties, and drying proved to be a more effective method of preserving the fruits than freezing, maintaining a higher amount of active principles.

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