Klara ZGLIŃSKA* ORCID: 0000-0001-9287-7616, Scopus Author ID: 57221114574;
Anna RYGAŁO-GALEWSKA ORCID: 0000-0002-2770-6201, Scopus Author ID: 57221112830;
Tomasz NIEMIEC ORCID: 0000-0001-5838-042X

DOI: 10.15199/180.2022.1.4

Division of Animal Nutrition, Institute of Animal Science, Warsaw University of Life Science, Poland. Ciszewskiego 8, 02-786 Warsaw, Poland
* Corresponding author: klara_zglinska@sggw.edu.pl

ANTIOXIDATIVE PROPERTIES OF AUTUMN OLIVE (*ELAEAGNUS UMBELLATA*) FRUITS ACCORDING TO THE METHOD OF FRUIT PRESERVATION AND SOLVENT USED IN EXTRACTION

WŁAŚCIWOŚCI ANTYOKSYDACYJNE OWOCÓW OLIWNIKA BALDASZKOWATEGO (*ELAEAGNUS UMBELLATA*) W ZALEŻNOŚCI OD SPOSOBU UTRWALANIA OWOCÓW I ROZPUSZCZALNIKA UŻYTEGO DO EKSTRAKCJI

Summary: *Fruits of autumn olive (Elaeagnus umbellata) are the abundant source of antioxidative substances such as carotenoids, polyphenols, flavonoids, fat-soluble vitamins and Vitamin C. The conducted study was aimed at the selection of the optimum method for extraction of berries. To this end, two methods for preservation of the berries were compared: drying at 60°C and freeze-drying (lyophilisation) and two types of solvent, employed in the extraction: water and methanol. The results showed that the methanol extracts produced from lyophilized fruits were characterized by the highest antioxidative activity*

Keywords: *Autumn olive, plant extracts, antioxidative activity, antioxidant, functional food*

Streszczenie: *Owoce oliwnika baldaszkowatego (łac. Elaeagnus umbellata, ang. Autumn olive) są bogatym źródłem substancji antyoksydacyjnych, takich jak karotenoidy, polifenole, flawonoidy, witaminy rozpuszczalne w tłuszczach oraz witamina C. Przeprowadzone badanie miało na celu wybranie optymalnej metody ekstrakcji jagód. W tym celu porównano dwie metody utrwalania owoców: suszenie w 60°C i liofilizację oraz dwa rodzaje rozpuszczalników użytych do ekstrakcji: wodę i metanol. Wyniki wykazały, że najwyższą aktywnością antyoksydacyjną charakteryzowały się ekstrakty metanolowe wykonane z owoców liofilizowanych.*

Słowa kluczowe: *oliwnik baldaszkowaty, ekstrakty roślinne, właściwości antyoksydacyjne, antyoksydanty, żywność funkcjonalna*

Introduction

Genus *Elaeagnus* includes numerous species of bushes which produce fruits having the nutritive and decorative values. In Asia countries, the mentioned berries are employed in the traditional medicine and, also, serve as food. In Europe, they are mainly known as decorative plants. The discussed bushes are eagerly planted as hedge due to their easy adaptation to environmental conditions, compact structure, resistance to the high and low temperature and soil salinity [1].

During the recent years, there have appeared many reports confirming the health-promoting properties of the fruits and leaves of autumn olive which were earlier in the traditional medicine [2–8]. Due to the mentioned reason, the interest in autumn olive as a raw material for production of functional food and diet supplements has increased also in Europe. Such utilization requires, in most cases, preservation of berries, and removal of water in particular as its content contributes to quicker deterioration of the product. Besides it, it is necessary to concentrate the bioactive substances by their extraction from the initial material. As it is known, the method of the raw material preparation (especially the temperature of treatment) and the reagent used in the extraction have a significant effect on the content of bioactive compounds in the extract [9].

The choice of the appropriate methodology of preserving and extracting the plant raw material in connected, first of all, with the chemical properties of its biologically active compounds. Berries of autumn olive contain numerous antioxidants, differing in the sensitivity to temperature and in solubility. Dark-red colour of the berries is caused by the content of the high quality carotenoids, from which relatively-resistant-to-processing likopen is dominating [10–12]. Pro-vitamin A and vitamin E are other fat-soluble compounds which are more sensitive to temperature effect. *E. umbellata* berries are also abundant in water-soluble vitamin C [1, 13]. Its effect, consisting in elimination of free radicals, is very quick but the discussed vitamin is

extremely sensitive to temperature and effect of sunlight; when administrated in high doses, it reveals a pro-oxidative effect [14]. Phenol compounds, and, in particular, polyphenols are an interesting group, responsible for a considerable part of antioxidative effect of plant raw materials. Free phenol acids reveal the hydrophilic properties, free flavonoids – lipophilic ones, and their derivates (esters and glycosides) are partially hydro- and lipophilic. Due to their presence in water as well as in fat phase, they are considered as the most comprehensive antioxidants [15].

The treatment of plant raw materials at a high temperature is related to a lower antioxidative capacity. The mentioned effect is a result of degradation of bioactive compounds, as affected by a high temperature and also, by the activation of oxidative processes. To preserve the antioxidative properties by the raw materials, the process of lyophilisation is recommended; it consists in sublimation of water from the samples with the application of a low temperature and vacuum [9, 16]. It is not, however, deprived of defects. One of them includes the necessity of initial freezing of the raw materials, higher interference into the cellular structure [9] and, also, high costs in relation to drying process.

It might seem that the lowest possible temperature should be employed but the results of the studies are not univocal. The analysis of blueberry fruit revealed that the samples subjected to lyophilisation process were characterized by lower antioxidative properties (after consideration of the process yield) as compared to the fresh, frozen, pasteurized berries as well as jams made from fresh berries [17]. Sometimes, drying at a high temperature but for a shorter period of time allows maintaining the higher amount of antioxidants. The studies carried out on the carrot root showed that the content of carotenoids had the highest value in the samples dried at the lowest temperature (40°C) even for a longer time. However, in the same study, the loss of the heat-labile vitamin C was lower in the case of higher temperature (60°C) but during the shorter drying period [18]. From among carotenoids, likopen deserves a special attention; it is one of the stronger plant antioxidants and the main dye of ripen berries of autumn olive [12]. Numerous studies indicate that processing with the application of high temperature did not have the unfavourable impact on its content in the product. Besides it, the treatment processes such as cooking, steaming or microwave heating affected the change of likopen structure into more assimilable by humans and monogastric animals [11, 19].

The choice of the appropriate temperature of berries' processing is also not univocal in the case of polyphenols, being considered as the main compounds, responsible for antioxidative properties of many plant species. The studies of the grape marc showed that it was possible to preserve even 90% of phenol compounds during the extraction at temperature up to 150°C if the heating did not last longer than 1 minute. Semi-liquid marc was more sensitive as compared to the filtrated liquid extract [20]. Such short time of heat treatment is, however, insufficient for preservation of the product.

The next challenge, which may have an impact on the antioxidative properties of the extract, is the appropriate choice of solvent. In the case of autumn olive, ethanol or methanol alcohol is the extractor being most frequently described in literature [2, 5, 6, 8]. However, polyphenols are, in majority, well soluble in water; owing to this fact, they act well in water systems such as biological fluids. Ishaq et al, showed that water extracts of autumn olive berries had the highest level of phenol compounds and of flavonoids and revealed the strongest effect, preventing from oxidation in brain and liver of mice as compared to methanol, acetone and hexane extracts [21]. The mentioned extraction is also safer for analyst and for the environment.

The aim of the present study was to develop the optimum method for handling with the autumn olive berries in aspect of obtaining the extracts with strong antioxidative properties. To this end, the extracts obtained from lyophilised (free-dried) berries and those ones dried and extracted with water of methanol, were compared.

Materials and methods

The autumn olive berries were collected after obtaining the stage of ripeness in the farm, situated at the territory of the Łódzkie voivodeship. A part of material was lyophilised and another part was dried in the traditional dryer at 60°C. The both mentioned processes were carried out in two repetitions. To calculate the yield of the process, the sampled were weighed before and after the treatment in analytical balance with accuracy of 0.01 mg (Radwag).

Then, for each type of raw material, extraction with deionised water or 96-% methanol of analytical grade was carried out. The sample was poured with solvent in ratio (1:20), shaken for one hour and subjected to ultrasounds (100W) for 5 minutes. Next, the upper layer was filtrated on the Whatman filter (18.5 cm). Four types of extracts were obtained.

Alcohol extracts were evaporated at 50°C in vacuum evaporator (Büchi R-210) and water extracts were lyophilised (Lyovac GT 2). The resulting powders were stored in dark until the time of analyses. The parameters were selected on the grounds of the procedures obligatory in the laboratory.

To calculate the yield of the process, the raw materials and the resulting extracts were weighed in two repetitions in analytical balance with accuracy of 0.01 g (Radwag).

DPPH Assay

The ability to inhibit the oxygen, radical DPPH (DPPH, eng. 2,2-diphenyl-1-picryl hydrazyl radical) was measured in the same way as in the earlier work of the team [8, 10]. 280 µl of 0.1 mM solution DPPH was combined with the extracts of concentration equal to 3 mg/ml. For each type of extract, six repetitions were performed. After 20-min incubation in dark, the absorbance at 517 nm wave in plate spectrophotometer Nano-200 (Tecan, Switzerland) was read out. The results were submitted as the percentage of neutralized radicals and were calculated from the following formula:

% of inhibition DPPH = (A0 – A1) /A0 x 100

where:
A0 - is the initial value of absorbance of radical DPPH
A1 – is the absorbance of radical after reaction with the sample

FRAP Assay (eng. Ferric reducing antioxidant power)

Capacity to reduce ferric ions Fe (III) to Fe (II) was tested according to Matusiewicz et al. [22]. Extracts were mixed in ratio 1:1 with 0.2 M sodium-potassium buffer (PBS) having pH 6.6 and 1-% potassium ferricyanide. After incubation in water bath (50°C, 20 min), 10% TCA was added to the samples. After centrifugation, they were diluted twice in deionised water. Next, 0.1% ferric chloride was added and the absorbance at 700 nm wave was measured (Infiniti NANO 200, Tecan, Switzerland). For each type of extract, six repetitions were performed. The results were presented as TROLOX equivalent (synthetic analogue of vitamin E) on the grounds of the standard curve, developed in the range 0-80μM.

Laboratory is functioning in accordance with standard ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories".

Results and discussion

Yield of drying and lyophilisation processes has been given in Tab.1.

Tab.1. Weight loss of E. umbellata berries after drying at 60°C and lyophilisation. N=2°

Method of berries' preservation	Mean weight loss (%)	Time (hours)
Drying at 60°C	80.02	4
Lyophilisation	78.89	24

Drying at 60°C and lyophilisation were characterized by a similar weight loss, with a small domination of the first mentioned process. When taking into consideration the time which in the case of lyophilisation was twelve times longer, it may be stated that drying seems to be more optimum method for preservation of autumn olive berries.

The yield of extraction process (Tab. 2) was higher when methanol was employed as solvent, in comparison to water as it was earlier evaluated by the team from Turkey [21]. The yield recorded in the present study was considerably higher as in the case of earlier assays what probably results from the application of more intensive shaking and ultrasounds. Again, the lyophilised berries had a higher yield of extraction than their dried equivalents (Tab. 2). More intensive interference into the cellular structure during lyophilisation was already earlier related to higher sensitivity of the processed raw material to the contact with solvent [9].

Tab.2. Yield of extraction of E. umbellata berries, with the consideration of the method for preservation of the raw material and of the employed solvent. N=2

Type of extraction	Yield (%)
Alcohol extract from dried berries	33.49%
Alcohol extract from lyophilised berries	37.31%
Water extract from dried berries	27.81%
Water extract from lyophilised berries	29.95%

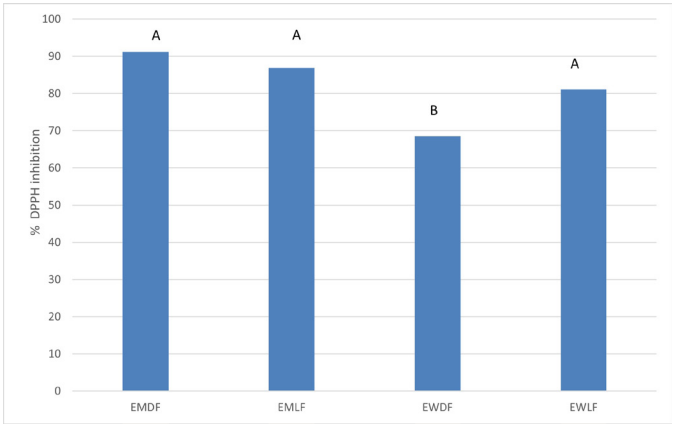

Fig.1. Ability to inhibit radical DPPH by autumn olive berries subjected to different extraction processes: EMLF – methanol extracts from lyophilised berries; EMDF – methanol extracts from dried berries; EWDF – water extracts from dried berries; EWLF – water extract from lyophilised berries. N=6. Different letters mean that the groups differ statistically at p<0.05

All obtained extracts revealed antioxidative properties. The lowest ability of inhibiting the synthetic oxygen radical DPPH was recorded for the water extracts from dried berries (Fig. 1). Any statistically significant differences between the remaining groups were not found although there was a visible tendency to stronger effect of alcohol extracts. It is supported by the results obtained for the berries coming from Pakistan. Khattak revealed that the extracts from autumn olive, produced with the participation of methyl alcohol and dried at 45°C, had stronger antioxidative effect as compared to the acetone and water extracts [13]. Another team of scientists showed that extracts with the application of methyl alcohol had the lowest value of EC50 (the concentration which is able to inhibit a half of radicals DPPH) in comparison to acetone, hexane and ethyl acetate [23].

Assay of the ability to inhibit radical DPPH is the most frequently used test for evaluation of antioxidative properties. It is, however, not deprived of certain defects. The basic problem is the presence of ions of transitory metals in the fruit extracts (especially of iron and cooper) which participate actively in generation of free radicals (reactions of Fenton and Haber-Weiss). It was found that the presence of ions of many metals (inter alia, ferric, cooper, calcium and potassium ions) had a negative effect on the run of DPPH reaction with antioxidants [24]. Therefore, it is recommended to apply the additional confirming methods when testing the natural products.

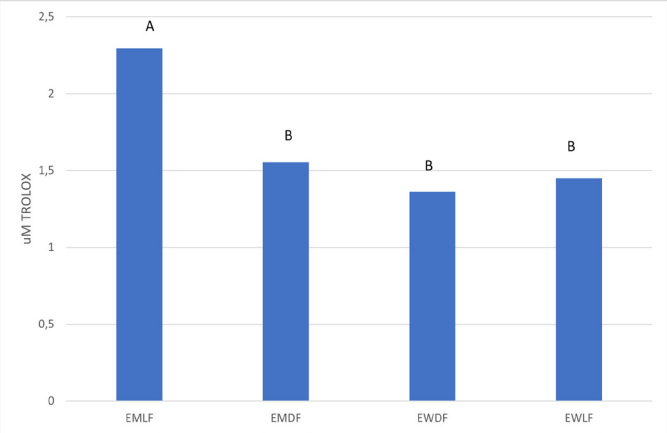

Fig.2. Ability to reduce ferric ions by autumn olive berries subjected to different extraction processes: EMLF – methanol extracts from lyophilised berries; EMDF – methanol extracts from dried berries; EWDF – water extracts from dried berries; EWLF – water extract from lyophilised berries. N=6. Different letters mean that the groups differ statistically at p<0.05

More information may be obtained from the test, examining the ability of antioxidants to reduce ferric ions Fe (III) to Fe (II). Alcohol extract from lyophilised berries was characterized by the highest donor activity. The statistical analysis showed that the application of alcohol as a solvent increased significantly the force of recuing ferric ions in relation to water extraction, irrespectively of the method of dehydration of the raw material ($p<0.05$). Similarly, the lyophilised berries were characterized by stronger antioxidant activity as compared to dried fruits, irrespectively of the employed reagent.

Also, Ozen et al. [3] showed stronger activity of alcohol extracts in comparison to water extracts in FRAP test and total antioxidant status (TAS) in the case of dried berries of autumn olive. Contrary to it, in the assay, testing the reduction capacities (DPPH), the team from Turkey demonstrated higher antioxidative properties of water extracts. Ishaq et al. revealed that in spite of a lack of differences in ferric iones' reduction between the alcohol and water extracts, the water extracts had stronger antioxidative effect in brain and liver of mice [21].

According to the knowledge of the authors, the comparative studies concerning the antioxidant properties of the dried and lyophilised *E. umbellata* berries have not been carried out until now. Similarly as in the present study, the lyophilised berries protected more strongly from the oxidative processes in comparison to the dried fruits in the case of wild rose (*Rosa rugosa*), strawberry tree (*Arbutus unedo*), [26], raspberry (*Rubus ideaus L.*), strawberry (*Fragaria ananassa*) and blueberry (*Vaccinum myrtillus*) [27].

The discrepancies between the results of different methods, testing the antioxidant properties are frequent. To understand them, we should consider the assumptions of the tests. The basic defect of FRAP method results from the fact that the ability to reduce complex Fe (III) with (2,4,6-tris(2-pirydylo)-1,3,5-triazyne (TPTZ) is revealed also by other compounds which are not classified as antioxidants. So, it may give the too high results. On the other hand, not all antioxidative compounds show the capability to reduce ferric ions. The endogenous protein antioxidants such as, inter alia, glutathione, act as strong antioxidants, however their activity will be not considered in FRAP method. Autumn olive berries contain relatively low (ca.5%) level of total protein in dry solids [13]. Adulteration will be higher in the case of high-protein products.

The comparison of the studies, examining the extracts, is also hampered due to the possible differences in running the extraction process and earlier preparation of the extract. It is known that even subtle changes in production of extracts (including, inter alia, degree of the raw material disintegration, temperature and application of ultrasounds) [28] may affect their properties. Moreover, each team prepares the extracts according to own method and the protocols are often insufficiently precise as to reproduce such process in the ideal way.

Podsumowanie

The conducted study showed that in order to obtain extracts from autumn olive berries with antioxidative properties, the choice of methyl alcohol as extractor was more favourable than in the case of water. FRAP assay (reduction of ferric ions) indicates also that the extracts obtained from lyophilised berries have a greater antioxidative effect as compared to the fruits dried at 60°C. The further research work is necessary which would show the effect of different drying temperatures on the antioxidative properties of *E. umbellata*.

Bibliography

[1] Gamba G, Donno D, Mellano MG, Riondato I, Biaggi M De, Randriamampionona D, et al. Phytochemical Characterization and Bioactivity Evaluation of Autumn Olive (Elaeagnus umbellata Thunb.) Pseudodrupes as Potential Sources of Health-Promoting Compounds. Appl Sci 2020, Vol 10, Page 4354. 2020;10(12):4354.

[2] Lee J, Sung JM, Cho HJ, Woo SH, Kang MC, Yong HI. Natural Extracts as Inhibitors of Microorganisms and Lipid Oxidationin Emulsion Sausage during Storage. Food Sci Anim Resour. 2021;41(6):1060.

[3] Ozen T, Yenigun S, Altun M, Demirtas I. Phytochemical Constituents, ChEs and Urease Inhibitions, Antiproliferative and Antioxidant Properties of Elaeagnus umbellata Thunb. Comb Chem High Throughput Screen. 2017;20(6).

[4] Qayyum R, Qamar HMUD, Salma U, Khan S, Khan T, Shah AJ. Insight into the cardiovascular activities of elaeagnus umbellata. Farmacia. 2019;67(1):133–9.

[5] Kim JI, Baek HJ, Han DW, Yun JA. Autumn olive (Elaeagnus umbellata Thunb.) berry reduces fasting and postprandial glucose levels in mice. Nutr Res Pract. 2019;13(1):11–6.

[6] Nazir N, Zahoor M, Nisar M, Karim N, Latif A, Ahmad S, et al. Evaluation of neuroprotective and antiamnesic effects of elaeagnus umbellate thunb. On scopolamine-induced memory impairment in mice. BMC Complement Med Ther. 2020;20(1):1–17.

[7] Gamba G, Donno D, Mellano MG, Riondato I, De Biaggi M, Randriamampionona D, et al. Phytochemical Characterization and Bioactivity Evaluation of Autumn Olive (Elaeagnus umbellata Thunb.) Pseudodrupes as Potential Sources of Health-Promoting Compounds. Appl Sci 2020, Vol 10, Page 4354 [Internet]. 2020 Jun 25 [cited 2022 Mar 23];10(12):4354.

[8] Zglińska K, Niemiec T, Łozicki A, Matusiewicz M, Szczepaniak J, Puppel K, et al. Effect of Elaeagnus umbellata (Thunb.) fruit extract on H_2O_2-

-induced oxidative and inflammatory responses in normal fibroblast cells. PeerJ. 2021.
[9] Kamiloglu S, Toydemir G, Boyacioglu D, Beekwilder J, Hall RD, Capanoglu E. A Review on the Effect of Drying on Antioxidant Potential of Fruits and Vegetables. Crit Rev Food Sci Nutr. 2016 Jul 29 [cited 2022 Mar 21];56 Suppl 1:S110–29.
[10] Zglińska K, Rygało-Galewska A, Bryś J, Koczoń P, Borek K, Roguski M, et al. Elaeagnus umbellata fruit - chemical composition, bioactive compounds, and kinetic of DPPH inhibition compared to standard antioxidants. Emirates J Food Agric. 2021. 1;33(8):639–46.
[11] Skiepko N, Chwastowska-Siwiecka I, Kondratowicz J. Właściwości likopenu i jego wykorzystanie do produkcji żywności funkcjonalnej. Żywność Nauk Technol Jakość. 2015;22(6):20–32.
[12] Fordham IM, Clevidence BA, Wiley ER, Zimmerman RH. Fruit of Autumn Olive: A Rich Source of Lycopene. HortScience. 2001;36(6):1136–7.
[13] Khattak KF. Free radical scavenging activity, phytochemical composition and nutrient analysis of Elaeagnus umbellata berry. J Med Plants Res [Internet]. 201;6(39):5196–203.
[14] Niemiec T, Sawosz E, Chwalibog A. Effect of high doses of L-ascorbic acid on theantioxidative/oxidative state in the rats. J Anim Feed Sci. 2005 Jun 26;14(Suppl. 1):553–6.
[15] Materska M. Wpływ warunków ekstrakcji na zawartość 0-glikozydów kwercetyny w preparatach z owoców papryki [Internet]. Żywność: nauka - technologia - jakość. 6. 91. 2013:131–50.
[16] Shofian NM, Hamid AA, Osman A, Saari N, Anwar F, Dek MSP, et al. Effect of Freeze-Drying on the Antioxidant Compounds and Antioxidant Activity of Selected Tropical Fruits. Int J Mol Sci 2011, Vol 12, Pages 4678-4692. 2011;12(7):4678–92.
[17] Kluszczynska D, Sowinska W. Wpływ procesów technologicznych na zawartość substancji bioaktywnych w owocach borówki czernicy. Żywność Nauk Technol Jakość. 2014 May 2;21(4):30–42.
[18] Mohamed S, Hussein R. Effect of low temperature blanching, cysteine--hci, N-acetyl-L-cysteine, Na metabisulphite and drying temperatures on the firmness and nutrient content of dried carrots. J Food Process Preserv. 1994:18(4):343–8.
[19] Gryszczyńska A, Gryszczyńska B, Opala B. Postępy Fitoterapii 2/2011.
[20] Sólyom K, Solá R, Cocero MJ, Mato RB. Thermal degradation of grape marc polyphenols. Food Chem. 2014 Sep 15;159:361–6.
[21] Ishaq S, Habib A, Rathore SM. Antioxidant Properties of Elaeagnus umbellata Berry Solvent Extracts against Lipid Peroxidation in Mice Brain and Liver Tissues. Food Sci Biotechnol. 2015;24(2):1–7.
[22] Matusiewicz M, Bączek KB, Kosieradzka I, Niemiec T, Grodzik M, Szczepaniak J, et al. Effect of Juice and Extracts from Saposhnikovia divaricata Root on the Colon Cancer Cells Caco-2. Int J Mol Sci 2019, Vol 20, Page 4526
[23] Uddin G, Rauf A. Phytochemical screening and biological activity of the aerial parts of Elaeagnus umbellata. Sci Res Essays. 2012;7(43):3690–4.
[24] Dawidowicz AL, Olszowy M. Mechanism change in estimating of antioxidant activity of phenolic compounds. Talanta [Internet]. 2012;97:312–7.
[25] Rutkowska J, Adamska A, Pielat M, Białek M. Porównanie składu i właściwości owoców dzikiej róży (Rosa rugosa) utrwalanych metodami liofilizacji i suszenia konwencjonalnego - Żywność Nauka Technologia Jakość. 2012. Tom 19, Numer 4.
[26] Orak HH, Aktas T, Yagar H, Isbilir SS, Ekinci N, Sahin FH. Effects of hot air and freeze drying methods on antioxidant activity, colour and some nutritional characteristics of strawberry tree (Arbutus unedo L) fruit. Food Sci Technol Int. 2012;18(4):391–402.
[27] Michalczyk M, MacUra R, Matuszak I. The Effect Of Air-Drying, Freeze--Drying And Storage On The Quality And Antioxidant Activity Of Some Selected Berries. J Food Process Preserv. 2009. 1;33(1):11–21.
[28] Wyrostek J, Kowalski R. Wpływ ultradźwięków i rozdrobnienia surowca na ekstrakcję związków fenolowych i flawonoidów z liści mięty pieprzowej i herbaty czarnej. Przem Chem. 2022; T. 101, nr 2.

Article reviewed
Received: 28.03.2022 r./Accepted: 08.04.2022 r.