Optimisation of conditions for extracting bioactive compounds exhibiting antioxidant properties from hawthorn fruit (Crataegus)

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Abstract. Hawthorn berries are traditionally used as a medicinal raw material in folk medicine. This plant contains important organic acids and flavonoids, such as carotene, pectin, ascorbic acid, saponins, starch and group B vitamins, which are believed to facilitate the restoration of the cardiovascular system. Medicinal raw materials are primarily used in the form of extracts. In this work, we use three extraction technologies: one conventional technique, i.e. infusion (37 °C, 2 hours), and innovative ones involving the use of microwave (800 W, 1 min) and ultrasonic irradiation (0.5 W, 2 hours). In order to determine the most optimal method for obtaining a complex of substances exhibiting antioxidant properties from hawthorn fruit extracts, we studied the content of dry substances, phenols and flavonoids. In addition, the antioxidant activity was determined by trapping free radicals and using the FRAP method (restoring force). On the basis of the obtained experimental results, the hawthorn fruit extraction conducted using ultrasonic irradiation is found to be the most effective of all the considered technologies, since it yields the highest values for all the studied parameters as compared to other extraction methods. Thus, the following levels were achieved: phenols – 723 mg of gallic acid per 100 g; flavonoids – 194 mg of catechin per 100g; dry substances – 1.68 %; anti-radical activity – 14.5 mg/cm³; restoring force – 14.5 mmol of Fe²⁺ per 1 kg. Our results show that the extraction using microwave irradiation cannot be recommended, since the values obtained using this method are lower than those obtained by ultrasonic irradiation and infusion.

Keywords: hawthorn fruit, extraction, ultrasonic, microwave, phenols, flavonoids, anti-radical activity, restoring force

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Оптимизация условий экстракции биологически активных соединений с антиоксидантными свойствами из плодов боярышника (Crataegus)

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Резюме: Плоды боярышника являются традиционным лекарственным сырьем в народной медицине. В них содержатся важные органические кислоты и флавоны, которые способны восстанавливать сердечно-сосудистую систему организма: каротин, пектин, аскорбиновая кислота, сапонины и крахмал, а также витамины группы В. Основной формой использования лекарственного сырья являются экстракты. В данной работе использованы три технологии экстрагирования: традиционная – настаивание (37 °C, 2 ч), и инновационные – с использованием микроволнового (800 Вт, 1 мин) и ультразвукового облучения (0.5 Вт, 2 ч). С целью определения наиболее оптимального метода экстрагирования комплекса веществ с антиоксидантными свойствами из экстрактов плодов боярышника было изучено...
INTRODUCTION
Hawthorn comprises a large group of shrubs and small trees, including more than two thousand species. They are trees of a small and medium size with umbrella-shaped clusters of white or pink flowers, glossy green leaves with serrated margins, as well as bright shiny red fruit [1]. Most of them grow in North America. The territory of Eurasia accounts for about eighty tree species. In Europe, hawthorns are cultivated for ornament, so they can often be found in parks and squares (e.g. as a hedge). The largest number of species grows in Turkey, whose hawthorn resources are quite substantial. Therefore, the production of pharmacological preparations based on hawthorn fruit is of great importance for the country's economy [2]. The most common hawthorn species are midland hawthorn, redhaw hawthorn, prickly hawthorn and one-seed hawthorn.

Hawthorns are well known for their healing properties, whose range of pharmacological action is rather wide. The beneficial properties of these plants are recognised by both folk and contemporary medicine. Their antioxidant properties, as well as the ability to stimulate collagen production have been known and used for a long time. Generally, the pharmacological properties of hawthorn are used to treat a wide range of inflammatory conditions. But first and foremost, it is used in treatment of a variety of cardiovascular diseases, such as hypertension, arrhythmia, coronary artery disease, etc. [3]. Hawthorn berries support the heart activity due to a high content of bioflavonoids increasing the body's ability to use oxygen and the heart's ability to use calcium [4].

The beneficial properties of hawthorn are explained by the chemical composition of its berries, which contain not only a wide range of vitamins (A, K, C, E, group B vitamins), but also a large number of bioactive substances, such as saponins and tannins, flavonoids, fructose, glycosides, cardiotonic amines, crataegolic acid, essential oils, organic acids, choline, sorbitol, pectin, etc. [5].

Research into various hawthorn genotypes is currently underway all over the globe, which can be evidenced by a large number of studies conducted by scientists in different countries. A particular focus is laid on the chemical composition and range of biological action of various hawthorn species.

Hawthorn is an important plant in the traditional Chinese medicine. There are 11 known species of hawthorn in the Chinese Pharmacopoeia, while the official pharmacopoeia includes only three: Chinese hawthorn (Crataegus Pinnatifida Bze.), Japanese hawthorn (Crataegus cuneata S. et. Z.) and prickly hawthorn (Crataegus oxyacantha).

Chinese hawthorn fruit (Crataegus pinnatifida) is used to improve digestion and increase appetite. The berries are also recommended for treating certain tumours and cardiovascular diseases associated with the formation of various radicals as a result of oxidative stress [6]. In this paper, the authors determined the chemical composition of hawthorn wines, which can be produced by fermentation of various yeast strains, for example, with the use of Saccharomyces cerevisiae (baker's yeast) – unicellular microscopic fungi belonging to the class of saccharomycetes. Chemical characteristics and antioxidant properties of hawthorn wines prepared by fermentation were studied. It was demonstrated that processing raw materials with cellulose could enhance and diversify the aromatic profile of wines, whereas processing with pectase improves wine transparency as well as accelerates methanol release. The authors believed that, in order to improve the quality of hawthorn wine, the fermentation process should be optimised.
Hawthorn is a complex genus of plants, including a large number of hybrids and apomictic populations. Thus, hawthorn samples (21 in total) studied in [7] were found to 6 different taxa. Among them were four Crataegus aronia, three Crataegus monogyna, three Crataegus orientalis var. orientalis, two Crataegus monogyna ssp monogyna, Crataegus meyeri, Crataegus aronia and Crataegus bornmuelleri, as well as one of Crataegus pseudhepheraphylla, Crataegus aronia var. Denate and Crataegus monogyna ssp. Azarellia. In this study, important differences between the berries of the aforementioned hawthorn genotypes were statistically determined.

The authors of [8] carried out a quantitative assessment of the total content of phenolic substances, flavonoids and vitamin C in 10 g of hawthorn fruit of each genotype, selected from the Germplasm Bank of the Chapingo Autonomous University (Mexico). The antioxidant activity of hawthorn berries was assessed using the DPPH method. The total content of phenolic substances, flavonoids and vitamin C was shown to be independent of the species. Some genotypes from the Chiapas state was established to be interesting in terms of commercial use and consumption due to their nutraceutical properties. The majority of the fruit of 20 hawthorn genotypes had a higher content of phenolic compounds than that of other berries (lychee, peach and strawberry). The nutraceutical properties of hawthorn fruit give an additional value to this plant.

Essential oils from azarole (Crataegus azarolus L.) and single-seeded hawthorn (Crataegus monogyna Jacq.), collected across the territory of Eastern Algeria, were analysed using gas chromatography and mass spectrometry [9]. It was found that the oil produced from Crataegus azarolus L. comprised such compounds as tetradecamethylocycloheptasiloxane (39.43%), mandelic acid (7.97%), 3,4-dihydroytetramethylsilyle (19.23%), dodecamethylocyclohexasiloxane (17.14%), decamethylcyclopentasiloxane (10.57%) and 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethyl-siloxyl) tetrasiloxane (5.66%). The seeds of Crataegus azarolus L. were established to be a good source of siloxanes widely used for various industrial and consumer purposes, such as detergents, shampoos, cosmetics, paper coatings and textiles.

Hawthorn flowers, leaves and fruit are traditionally used to treat such diseases as hypertension and atherosclerosis. The medicinal effect of this plant is associated with the phenolic compounds contained therein. However, hawthorn berries are a perishable raw material because of high water content. Hence, the berries are dried and stored for use during out-of-season periods. The main objective of the research conducted by the Brazilian scientists [10] was to investigate the effect of various drying methods on the phenolic compounds of hawthorn fruit. Berries were harvested from wild trees in Turkey. The study results showed that the drying of hawthorn fruit using a microwave-assisted freeze dryer caused significant changes in phenolic compounds, antioxidant activity and fruit colour. Freeze-drying resulted in a high-quality product both in terms of the content of total phenol and individual phenol groups, antioxidant activity, as well as colour values as compared to drying methods using ordinary or microwave ovens.

The aim of the study [11] was to assess the methanol extract of hawthorn as a prophylactic agent in treating alcohol poisoning. Prickly hawthorn (Crataegus oxyacantha) is a medicinal plant containing a wide variety of polyphenolic compounds with antioxidant and lipid-lowering effects. In this work, the preventive effect of hawthorn on liver damage caused by chronic ethanol consumption was demonstrated by the example of rats. The application of hawthorn extract produced an antioxidant effect, as well as decreased lipid peroxidation in the liver tissue. In addition, the glycochen level was regulated. In patients suffering from alcoholic liver damage, hawthorn acted as a therapeutic regulator of total cholesterol and serum lipids in the form of triglycerides, reducing their high level.

Crataegus monogyna Jacq. (one-seed hawthorn) is one of the most important edible plants traditionally used in folk medicine. Hawthorn is both a medicinal plant used in folk medicine and a common edible plant, which is widely used for preparing various foodstuffs. The article [12] showed that hawthorn has a number of pharmacological effects due to the presence of various bioactive natural compounds, such as flavanonoids and triterpene compounds. For example, hawthorn can be used to prevent and/or alleviate cardiovascular diseases. This plant is capable of reducing such cardiovascular risk factors as hypertension, thrombosis, etc. Moreover, hawthorn exerts a beneficial effect on cardiac functions.

The work [13] presented interesting results on the effect of hawthorn extract on the growth, carcass size, the mass of internal organs, serum protein concentration and the incidence of pulmonary hypertension syndrome of broiler chickens. 225 chickens (aged 1 day) were divided into three equal groups (one control and two experimental). Hawthorn extract was added to the drinking water of the chickens from the experimental groups in the amount of 0.1 and 0.2 ml of extract per 1 l of water, respectively. As a result, an increase of 53.3 g/day and 54.6 g/day in the live weight of chickens from the experimental groups was observed compared to the control group (48.7 g/day). The results of this study showed that antioxidants from hawthorn improve the health and survivability of broiler chickens, whereas bioactive flavonoids protect them against oxidative stress and lipid peroxidation.
Researchers from the Ural State Academy [14] showed a CO₂ hawthorn extract to possess antimicrobial properties, which action was tested against gram-positive coxal and spore-forming microflora by diluting the extract in acetone in various ratios (1:10; 1:20; 1:30; 1:40). According to the results, gram-positive coxal microflora can be suppressed by a CO₂ hawthorn extract diluted in acetone in the ratio of 1:20, whereas a 1:40 dilution is needed against spore-forming microflora. The results showed that a CO₂-extract of hawthorn has a sufficient antimicrobial effect, making it an excellent antibacterial preparation for independent use or as a stabilising additive to drugs.

Salakhov and Garmonov found optimal conditions for the quantitative separate determination of vitexin-2-rhamnoside, rutin and hyperoside in a dry tableted extract of hawthorn fruit [15]. The researchers also developed a procedure for HPLC determination of flavonoids in hawthorn and optimised sample preparation of dry extract in the form of fast dissolving tablets. The proposed technique, which is characterized by a good sensitivity and reproducibility of analytical determinations, allows the aforementioned compounds in hawthorn-based dosage forms (tablets, tinctures) to be quantitatively determined.

Thus, numerous publications on the chemical composition and properties of hawthorn fruit confirm the potential of studies aimed at searching for an optimal technology for obtaining an extract of hawthorn fruit with the maximal content of bioactive substances. In the present article, the authors set out to study the effect of extraction methods on the antioxidant properties of hawthorn fruit.

**EXPERIMENTAL PART**

**Determination of dry substances in extracts.**

The mass fraction of dissolved dry substances in the extracts of hawthorn fruit was determined using a refractometric method according to GOST ISO 2173-2013 "Fruit and vegetable products. Refractometric method for determination of soluble solids content".

The temperature of the extract was raised up to 20 °C. Subsequently, 2–3 drops of the extract were applied onto a fixed refractometer prism, which was then covered with a movable prism. When the line separating the dark and light fields in the eyepiece was positioned exactly at the crosshair in the eyepiece window, data on the mass fraction of dry substances in the test solution was recorded. The measurements were performed three times, followed by the determination of the arithmetic mean value of the obtained results.

The extracts of the studied samples were prepared via infusion. A sample intake of crushed hawthorn fruit weighing 3 g (for the extract in a concentration of 0.1 g/cm³) was placed in a flask with a ground-in stopper. Afterwards, 30 ml of a 1:1 mixture of distilled water and ethanol 96% were added, followed by keeping the resulting mixture in a thermostat at 37 °C for 2 h under continuous stirring. Eventually, a clear layer of the extract was separated by centrifuging for 15 minutes at a speed of 3000 rpm.

**Extraction using ultrasonic (US) irradiation.** A sample intake of crushed hawthorn fruit weighing 3 g (for the extract in a concentration of 0.1 g/cm³) was placed in a flask with a ground-in stopper followed by addition of 30 ml of a 1:1 mixture of distilled water and ethanol 96% and holding of the resulting mixture at 0.5 W for 2 hours. Afterwards, a clear layer of the extract was separated by centrifuging for 15 minutes at a speed of 3000 rpm.

**Extraction using microwave (MW) irradiation.** A sample intake of crushed hawthorn fruit weighing 3 g (for the extract in a concentration of 0.1 g/cm³) was placed in a flask with a ground-in stopper followed by addition of 30 ml of a 1:1 mixture of distilled water and ethanol 96% and holding of the resulting mixture at 800 W for 1 minute. Afterwards, a clear layer of the extract was separated by centrifuging for 15 minutes at a speed of 3000 rpm.

**Preparation of concentrated extracts.** Sample intakes of crushed hawthorn flowers and berries weighing 200 g were placed in a flask with a ground-in stopper. Subsequently, aqueous ethanol (1:2) in the amount of 400 ml was added followed by holding of the resulting mixture at 0.5 W and at a temperature of 37 °C for 12 hours under continuous stirring. Afterwards, a clear layer of the extract was separated by centrifuging for 15 minutes at a speed of 3000 rpm. The resulting substance was concentrated at a temperature of 50–55 °C and a pressure of 6.6 ± 1.3 kPa until the content of dry substances was equal to 19.20%.

**Method for determining the total content of phenolic substances.** Determination of the total content of phenolic substances is based on their ability to bind with protein substances, to be precipitated with metal salts, to be oxidised and to give colour reactions. The studies were carried out according to the method proposed in [16]. The colorimetric method for determining the total content of phenolic substances is based on the use of Folin's phenol reagent (Folin-Ciocalteu reagent) prepared from sodium tungstate (Na₂WO₄) and sodium molybdate (Na Na₂MoO₄). Water (H₂O), orthophosphoric acid 85% (H₃PO₄), hydrogen chloride (HCl), lithium sulfate (Li₂SO₄) and bromine (Br₂). Folin's reaction and its variants are used to detect and photometrically determine phenols, thiols and disulfides (cystine, cysteine), purine bases (guanine, xanthine, 2-hydroxyadenine), uric acid, peptides and proteins containing tyrosine and tryptophan. In the presence of the above-mentioned compounds in an alkaline environment, Folin's reagent is reduced by oxidation of phenols to a mixture of blue oxides (WO₂ nWO₃ or MoO₂ nMoO₃). The resulting blue colouring is proportional to the amount of phenolic substances. The intensity of the
blue colour is measured using a spectrophotometer at a wavelength of 725 nm.

0.25 ml of the prepared extract of hawthorn fruit (in a concentration of 0.1 mg/cm³) placed in sterile test tubes was mixed with the following:

0.25 ml of an aqueous solution of Folin-Ciocalteu reagent (50%), 0.50 ml of a saturated solution of sodium carbonate and 4.00 ml of distilled water. Instead of the extract, 0.25 ml of distilled water was poured into the control sample. The mixture was held for 25 minutes at 25 °C under continuous stirring to complete the reaction. After that, a clear layer of the extract was separated by centrifuging for 10 minutes at a speed of 2000 rpm.

The content of phenolic substances in a clear solution of hawthorn-fruit extract was determined using a spectrophotometric method at a wavelength of 725 nm in a cuvette having a liquid layer of 10 mm. The control sample was placed in the comparison cuvette. Calculation of phenolic compounds (gallic acid in milligram per 100 g of the product) was carried out according to the calibration curve.

**Method for determining total flavonoids.** Studies on the content of flavonoids were carried out according to the method proposed in [17], with a modification for hawthorn fruit extracts and selection of the optimal ratios of the initial reagents, effective concentrations of the original extract. 0.50 ml of hawthorn fruit extract in a concentration of 0.1 mg/cm³, 2.50 ml of distilled water and 0.15 ml of sodium nitrite solution (5%) were placed in tubes and held for 5 min at 20–25 °C. Then, 0.30 ml of aluminium (III) chloride solution (10%) was added, followed by holding of the mixture at 20–25 °C for 5 minutes. After that 1.00 ml of 1 M sodium hydroxide and 5.00 ml of distilled water were added.

The content of flavonoids was determined spectrophotometrically at a wavelength of 510 nm in a cuvette having a liquid layer of 10 mm. Distilled water was placed in the comparison cell. Calculation of flavonoids (catechin in milligram per 100 g of the product) was carried out according to the calibration curve.

**Method for determining the radical-trapping capacity using a 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH method).** Free radical colorimetry is one of the ways to evaluate the antioxidant activity of various substances. This method is based on the stable synthetic radical DPPH (2,2-diphenyl-1-picrylhydrazyl), dissolved in ethanol, reacting with a sample of antioxidant contained in the extract [18]. As a result of reducing DPPH by antioxidants of functional products, the purple-blue colour of the reagent changes to yellow, since the free radical 2,2-diphenyl-1-picrylhydrazyl having a purple-blue colour is converted into a stable molecule having a yellow colour. There are two ways to conduct an experiment using this method: static and dynamic. The static way shows at what concentration of the extract the best inhibition of free radicals is observed, whereas the dynamic one characterizes the inhibition process over time and shows the time necessary to inhibit DPPH radicals by the antioxidants of the extract in a concentration, at which the best inhibition of free radicals is observed. For characterizing the antioxidant activity, there is also a parameter E_{50} – concentration of the extract, at which 50% inhibition of the DPPH radical by an antioxidant of the extract occurs. The lower this parameter is, the faster the inhibition of reactions involved in oxidative decomposition occurs and the higher the antioxidant activity level of the samples.

In the course of the study, 0.20 ml of hawthorn-fruit extract of different concentrations, 2.00 ml of distilled water, as well as 2.00 ml of an alcohol solution of 2,2-diphenyl-1-picrylhydrazyl were placed in tubes. Distilled water instead of 2,2-diphenyl-1-picrylhydrazyl solution was placed in the control extract sample. In addition, distilled water was poured in the control sample of 2,2-diphenyl-1-picrylhydrazyl solution instead of the extract. The mixture was held for 30 min at 20–25 °C in a place away from light. The colorimetry of free radicals of 2,2-diphenyl-1-picrylhydrazyl was performed using the spectrophotometric method at a wavelength of 517 nm in a cuvette having a liquid layer of 10 mm. Ethanol was placed in the comparison cell. Calculation of flavonoids (catechin in milligram per 100 g of the product) was carried out according to the calibration curve.

**Method for determining the iron-binding activity of extracts (FRAP method).** Studies on the re-storing force were carried out according to the method proposed in [19], with a modification for hawthorn fruit extracts and selection of the optimal ratios of the initial reagents, effective concentrations of the original extract. In order to prepare the FRAP reagent, a tube was filled with the following ingredients: 10.00 ml of acetate buffer (pH 3.6), 1.00 ml of 20 mM iron (III) chloride solution, 1.00 ml of 2,4,6-tri-(2-pyridyl)-1,3,5-triazine reagent (TPTZ). The mixture was held in a thermostat for 10 minutes at a temperature of 37 °C with occasional stirring.

1.00 ml of the FRAP reagent, 3.00 ml of distilled water, and 0.10 ml of the prepared extract of hawthorn fruit in a concentration of 0.1 mg/cm³ were added to the tubes. Instead of the extract, 0.10 ml of distilled water was poured into the control sample. The mixture was held for 4 minutes at a temperature of 37 °C under occasional stirring.

Iron-binding activity was determined spectrophotometrically at a wavelength of 593 nm in a cuvette having a liquid layer of 10 mm. Distilled water was poured into the comparison cuvette. The determination of iron-binding activity was carried out according to the calibration curve in mmol of Fe²⁺ per 1 kg of raw material.
RESULTS AND DISCUSSION

Mass fractions of dissolved dry substances of hawthorn fruit are presented in Fig. 1. Three types of extracts contain approximately the same amount of dissolved dry substances: MW – 1.62%, infusion – 1.63%, US – 1.68%. The maximum values were obtained for a concentrated US extract of hawthorn fruit (23.3%).

Most researchers studying the antioxidant activity of raw animal and plant materials consider the direct correlation between chemical composition and the level of antioxidant activity to be an undisputable fact [20]. Therefore, the next stage of our research consisted in determining total phenols and flavonoids. In terms of the total phenols, all the obtained extracts of hawthorn fruit can be ranked in a descending order. The highest results were obtained for an US extract (723 mg of gallic acid per 100 g), whereas the maceration method showed average results (707 mg of gallic acid per 100 g). The lowest results were obtained for an extract prepared using microwave-assisted extraction (630 mg of gallic acid per 100 g), which is shown in Fig. 2.

The obtained values of phenol content in the concentrated US extract of hawthorn fruit were close in their level to those of the extract prepared using the method of infusion, namely 736 mg of gallic acid per 100 g. It is likely that a large percentage of phenolic substances decompose at a sufficiently high temperature during concentration.

The class of flavonoids is characterised by a different biological activity at different levels [21]. In terms of total flavonoids, the extracts can be arranged in a descending order: concentrated extract (223 mg of catechin per 100 g); US extract (194 mg of catechin per 100 g); extract obtained using the method of infusion (172 mg of catechin per 100 g); microwave-assisted extract (168 mg of catechin per 100 g). The data on total flavonoids is shown in Fig. 3.

The ability to trap free radicals is an important property of antioxidants [22]. That is why the determination of antioxidant activity includes the determination of antiradical activity. In this research, we used a procedure for studying antiradical activity in terms of capturing the free radical 2,2’-diphenyl-1-picrylhydrazyl. The analytical results for the \( E_{50} \) level are presented in Fig. 4.
The lowest antiradical activity was detected in the microwave-assisted extract (29.5 mg/cm³), as well as in that obtained via infusion (29.5 mg/cm³). Quite a high level of antiradical activity was observed in the US extract (14.5 mg/cm³). The highest activity was noted for the concentrated extract of hawthorn fruit (8 mg/cm³).

Restoring force characterises the ability of antioxidants to inhibit the catalysing effect of metal ions in oxidation reactions [23]. All extracts of hawthorn fruit have high values of restoring force ranging from 12.06 to 14.76 mmol of Fe²⁺ per 1 kg (Fig. 5).

Thus, all the studied objects can be divided into two groups in terms of the restoring force: the concentrated extract of hawthorn fruit having the highest value (14.76 mmol of Fe²⁺ per 1 kg) and the remaining three extracts (infusion, US-assisted extraction and microwave-assisted extraction) differing slightly from each other (12.06–12.92 mmol of Fe²⁺ per 1 kg).

In the second group, US extract has quite a high value of restoring force (12.92 mmol of Fe²⁺ per 1 kg), whereas the highest result is observed for the concentrated US extract (14.76 mmol of Fe²⁺ per 1 kg).
S.V. Trapeznikova studied the content of ascorbic acid, organic acids and sugars in hawthorn fruit in order to compare various methods of extracting bioactive substances from them [24]. In this connection, the extraction was carried out via infusion under different heating conditions:
1) in a thermostat at a temperature of 60 °C for 1.5 hours;
2) in a microwave oven at 300 W for 5 minutes;
3) in a thermostat with simultaneous US treatment at a temperature of 60 °C for 1.5 hours.

As a result, the possibility of extracting components of hawthorn fruit using ethanol 70% via infusion in a thermostat and in a microwave extractor was shown. The US extract did not demonstrate the expected effect; therefore, US treatment was not recommended for extracting bioactive substances from fruit.

In the present research, three extraction technologies were used: conventional (infusion at 37 °C for 2 hours) and innovative (microwave-assisted extraction at 800 W for 1 minute and US-assisted extraction at 0.5 W for 2 hours). Two procedures were used for studying the content of dry substances, phenols, flavonoids, antioxidant activity: free radical scavenging and restoring force method. It was found that ultrasound treatment demonstrates the expected effect – an increase in antioxidant activity.

**CONCLUSIONS**
In order to determine the most optimal method for obtaining a complex of substances having antioxidant properties from hawthorn fruit extracts, we have studied the content of phenols, flavonoids, dry substances, as well as the antioxidant activity of extracts using two procedures, i.e. free radical scavenging and restoring force method. It should be noted that these indicators in hawthorn fruit are higher compared to other previously studied substances, such as fruit, vegetables, dairy products, wine, etc.

On the basis of the experimental results, it can be concluded that US extraction constitutes the most effective of all the considered technologies for hawthorn fruit, since the best results, as compared to the other extraction methods, were obtained for the concentrated extract prepared from the US extract using vacuum concentration at lower temperatures.

Thus, our results show that hawthorn fruit extracts obtained via the US-assisted extraction process can be used in pharmaceutical products, bioactive substances, as well as in functional products having a prophylactic effect.

**REFERENCES**

1. Kashyap C.P., Arya V., Thakur N. Ethnomedical and phytopharmacological potential of *Crataegus oxyacantha* Linn. – A review. Asian Pacific Journal of Tropical Biomedicine. 2012, vol. 2, Issue 2, pp. 1194–1199. DOI: 10.1016/S2221-1691(12)60383-9
2. Ercisli S., Yanar M., Sengul M., Yildiz H., Topdas E.F., Taskin T., Zengin Y., Yilmaz K.U. Physico-chemical and biological activity of hawthorn (*Crataegus spp.* L.) fruits in Turkey. Acta scientiarum Polonorum. Hortorum cultus. 2015, vol. 14, no. 1, pp. 83–93.
3. Lakshmi T., Geetha R.V., Anitha Roy. *Crataegus oxyacantha* Linn. commonly known as Hawthorn – a Scientific Review. Int. J. of Pharm. Tech. Res. 2012, vol. 4, no. 1, pp. 458–465.
4. Verma S.K., Jain V., Verma D., Khamesra R. *Crataegus oxyacantha* – a cardioprotective herb. Journal of Herbal Medicine and Toxicology. 2007, vol. 1, no. 1, pp. 65–71.
5. Salmanian S., Sadeghi Mahoonak A.R., Alami M., Ghorbani M. Phenolic content, antiradical, antioxidant, and antibacterial properties of hawthorn (*Crataegus elbusensis*) seed and pulp extract. J. Agr. Sci. Tech. 2014, vol. 16, pp. 343–354.
6. Wenye Z., Lei C., Yan W., Lei Z., Chinping X. Quality comparison of hawthorn wines fermented by saccharomyces cerevisiae with and without pulp contact and pectase treatment. Journal of Chemistry. 2017, vol. 1, no. 1, pp. 1–7. https://doi.org/10.1155/2017/6431818
7. Yanar M., Ercisli S., Yilmaz K., Sahiner H., Taskin T., Zengin Y., Akgul I., Celik F. Morphological and chemical diversity among hawthorn (*Crataegus spp.*) genotypes from Turkey. Scientific Research and Essays. 2011, vol. 6, no. 1, pp. 35–38. DOI: 10.5897/SRE10.192
8. Garcia-Mateos R., Ibarra-Estrada E., Nieto-Angel R. Antioxidant compounds in hawthorn fruits (*Crataegus spp.*) of Mexico. Revista Mexicana de Biodiversidad. 2013, vol. 84, no. 4, pp. 1298–1304. DOI: 10.7550/rmb.35675
9. Bechki S., Berrehal D., Semra Z., Bachari K., Kabouche A., Kabouche Z. Composition and biological activities of seeds oils of two *Crataegus* species growing in Algeria. Journal of Materials and Environmental Sciences. 2017, vol. 8, Issue 5, pp. 1526–1530.
10. Coklar H., Akbulut M., Kilinc S., Yildirim A., Alhassani I. Effect of freeze, oven and microwave pre-treated oven drying on color, browning index, phenolic compounds and antioxidant activity of hawthorn (*Crataegus orientalis*) fruit. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2018, vol. 46, no. 2, pp. 449–456. DOI: https://doi.org/10.15835/nbha46211027
11. Martinez-Rodriguez J.L., Reyes-Estrada C.A., Gutierrez-Hernandez R., Lopez J.A. Antioxidant, hypolipidemic and preventive effect of Hawthorn (*Crataegus oxyacantha*) on alcoholic liver damage in rats. Acad. J. 2016, vol. 28, no. 11, pp. 193–202.
12. Nabavi S.F., Habtemariam S., Touqeer A., Sureda A., Doglia M., Sobarzo-Sanchez E., Nabavi SM. Polyphenolic composition of *Crataegus monogyna* jack. from chemistry to medical...
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Effects of different drying methods on the antioxidant activities of leaves and berries of Carthamus tinctorius ssp. annuus. Sains Malaya. 2015, vol. 44, no. 2, pp. 275–280.

14. Bashkurova I.B., Golikov V.F., Larionov L.P., Chemezov S.A., Gaisina E.F., Kolomiets O.V. Antioxidant activity, total phenolic contents, and cytotoxicity evaluation of Crataegus oxyacantha L. fruits in Turkey // Scientific Research // Int. J. of Pharm. Sci. Tech. 2014, vol. 16, no. 1, pp. 31–42.

15. Bier M.G., Nunes S., Dandlen S.A., Cava-co A.M., Antunes M.D. Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal. Food Science and Technology, 2014, vol. 34, no. 1, pp. 16–23.

16. Figueroa L.A., Navarro L.B., Vera M.P., Petricevich V.L. Antioxidant activity, total phenolic and flavonoid contents, and cytotoxicity evaluation of Bougainvillea xbuttiana. International Journal of Pharmacy and Pharmaceutical Sciences, 2014, vol. 6, no. 5, pp. 497–502.

17. Bechkehi S., Berrehal D., Semra Z., Bachari K., Bechkri S., Yanar M., Ercisli S., Vi̇ ldi̇ z H., Saúde R.A., Youssef M.M. Methods for determining the antioxidant activity: a review. Alex. J. Fd. Sci. Technol. 2014, vol. 11, no. 1, pp. 31–42.

18. Salakhov I.A., Garmonov S.Yu. Determination of hawthorn flavonoids in dosage forms using high performance liquid chromatography. Vestnik Kazanskogo teknologicheskogo universiteta. 2007, no. 6, pp. 22–27. (In Russian)

19. Momani A.H., Youssef M.M. Free radicals, oxidative stress and importance of antioxidants in human health. Journal of Medical and Allied Sciences. 2011, vol. 1, no. 2, pp. 53–60.

20. Ercisli S., Yanar M., Sengul M., Yi̇ ldi̇ z H. Methods for biologically active substances extraction from hawthorn fruit. Nauchno-metodicheskii elektronnyi zhurnal «Kontsept». 2016, vol. 11, pp. 3261–3265. Available at: http://e-koncept.ru/2016/86688.htm (accessed 15.11.2018).

21. Bashkurova I.B., Golikov V.F., Larionov L.P., Chemezov S.A., Gaisina E.F., Kolomiets O.V. Antioxidant activity, total phenolic contents, and cytotoxicity evaluation of Bougainvillea xbuttiana. International Journal of Pharmacy and Pharmaceutical Sciences, 2014, vol. 6, no. 5, pp. 497–502.

22. Salakhov I.A., Garmonov S.Yu. Determination of hawthorn flavonoids in dosage forms using high performance liquid chromatography. Vestnik Kazanskogo teknologicheskogo universiteta. 2007, no. 6, pp. 22–27. (In Russian)

23. Ohashi S., Oguni K., Sugiyama R. Study on the levitation and restoring force characteristics of the improved HTS-permanent magnet hybrid magnetic bearing. Physics Procedia. 2014, vol. 58, no. 1, pp. 282–285.

24. Trapeznikova S.V. Comparison of methods of biologically active substances extraction from hawthorn fruit. Nauchno-metodicheskii elektronnyi zhurnal «Kontsept». 2016, vol. 11, pp. 3261–3265. Available at: http://e-koncept.ru/2016/86688.htm (accessed 15.11.2018).

BIBLIOGRAPHIC SPISOK

1. Kashyap C.P., Arya V., Thakur N. Ethnomedicinal and phytopharmacological potential of Crataegus oxyacantha Linn. – A review // Asian Pacific Journal of Tropical Biomedicine. 2012. Vol. 2. Issue 2. P. 1194–1199. DOI: 10.1016/S2221-1691(12) 60383-9.

2. Ercisli S., Yanar M., Sengul M., Yildiz H., Topdas E.F., Taskin T., Zengin Y., Yilmaz K.U. Physico-chemical and biological activity of hawthorn (Crataegus spp. L.) bfruits in Turkey // Acta scientiarum Polonorum. Hortorum cultus. 2015. Vol. 14. No. 1. P. 83–93.

3. Lakshmi T., Geetha R.V., Anitha Roy. Crataegus oxyacantha Linn. commonly known as Hawthorn – a Scientific Review // Int. J. of Pharm. Tech. Res. 2012. Vol. 4. No. 1. P. 458–465.

4. Verma S.K., Jain V., Verma D., Khamesra R. Crataegus oxyacantha – a cardioprotective herb // Journal of Herbal Medicine and Toxicology. 2007. Vol. 1. No. 1. P. 65–71.

5. Salemanian S., Sadeghi Mahoonaak A.R., Alami M., Ghorbani M. Phenolic content, antioxidant, and antibacterial properties of hawthorn (Crataegus elbusrens) seed and pulp extract // J. Agr. Sci. Tech. 2014. Vol. 16. P. 343–354.
pretreated oven drying on color, browning index, phenolic compounds and antioxidant activity of hawthorn (Crataegus orientalis) fruit // Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2018. Vol. 46. No. 2. P. 449–456. DOI: https://doi.org/10.15835/ nbha46211027.

11. Martínez-Rodriguez JL, Reyes-Estrada C.A., Gutierrez-Hernandez R., Lopez J.A. Antioxidant, hypolipidemic and preventative effect of Hawthorn (Crataegus oxyacantha) on alcoholic liver damage in rats // Acad. J. 2016. Vol. 28. No. 11. P. 193–202.

12. Nabavi S.F., Hambaramian S., Touqueer A., Sureda A., Dalgia M., Sobarzo-Sanchez E., Nabavi SM., Polyphenolic composition of Crataegus monogyna Jacq.: from chemistry to medical applications // Nutrients. 2015. Vol. 7. Issue 9. P. 7708–7728. https://doi.org/10.3390/nu7095361.

13. Ahmadipour B., Kalantar M., Hosseini S.M., Yang L.G., Kalantar M.H., Raza S.H.A., Schreurs N.M. Hawthorn (Crataegus oxyacantha) extract in the drinking water of broilers on growth and incidence of pulmonary hypertension syndrome (PHS) // Brazilian Journal of Poultry Sciences. 2017. Vol. 19. No. 4. P. 639–644. DOI: 10.1590/1806-9061-2017-0558.

14. Bashkirova I.B., Golikov V.F., Parinov N.P., Chemezov S.A., Gaysina E.F., Kolesiac O.V. Антиоксидантные свойства CO2-экстракта боярышника [Электронный ресурс] // Электронный научно-образовательный вестник «Здоровье и образование в XXI веке». 2007. T. 9. N 3. C. 91–92. URL: https://cyberleninka.ru/article/v/antimikrobyesvystva-so-2-ekstrakta-boyaryshnika (15.11.2018).

15. Sapolkov I.A., Garmenov S.Yu. Определение флавонидов боярышника в лекарственных формах методом высокоэффективной жидкостной хроматографии // Вестник Казанского технологического университета. 2007. N 6. C. 22–27.

16. Miguel M.G., Nunes S., Dandlen S.A., Cavaco A.M., Antunes M.D. Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal // Food Science and Technology. 2014. Vol. 34. No. 1. P. 16–23.

17. Figueroa L.A., Navarro L.B., Vera M.P., Petricevic V.L. Antioxidant activity, total phenolic and flavonoid contents, and cytotoxicity evaluation of Bougainvillea xbuttiana // International Journal of Pharmacy and Pharmaceutical Sciences. 2014. Vol. 6. No. 5. P. 497–502.

18. Rabeta M.S., Lin S.P. Effects of different drying methods on the antioxidant activities of leaves and berries of Caryatia trifolia // Sains Malaysiana. 2015. Vol. 44. No. 2. P. 275–280.

19. Freedes C., Montenegro G., Zoffoli J.P., Gómez M., Robert P. Polyphenol content and antioxidant activity of Maqui (Aristotelia chilensis [Molina] stuntz) during fruit development and maturation in central Chile // Chilean Journal of Agricultural Research. 2012. Vol. 72. No. 4. P. 582–589.

20. Moharram H.A., Youssef M.M. Methods for determining the antioxidant activity: a review // Alex. J. Fd. Sci. & Technol. 2014. Vol. 11. No. 1. P. 31–42.

21. Mierziak J., Kostyn K., Kulma A. Flavonoids as important molecules of plant interactions with the environment // Molecules. 2014. Vol. 19. Issue 10. P. 16240–16265. https://doi.org/10.3390/ molecules191016240.

22. Kunwar A., Priyadarshini K.I. Free radicals, oxidative stress and importance of antioxidants in human health // Journal of Medical and Allied Sciences. 2011. Vol. 1. No. 2. P. 53–60.

23. Ohashi S., Oguni K., Sugiyama R. Study on the levitation and restoring force characteristics of the improved HTS-permanent magnet hybrid magnetic bearing // Physics Procedia. 2014. Vol. 58. No. 1. P. 282–285.

24. Трапезников С.В. Сравнение методов экстракционного извлечения биологически активных веществ из плодов боярышника [Электронный ресурс] // Научно-методический электронный журнал «Концепт». 2016. Т. 11. С. 3261–3265. URL: http://e-koncept.ru/2016/86688.htm (15.11.2018).

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Aigul R. Valeeva, Nadezhda V. Makarova, Dinara F. Valiulina carried out the experimental work, on the basis of the results summarized the material and wrote the manuscript. Aigul R. Valeeva, Nadezhda V. Makarova, Dinara F. Valiulina have equal author's rights and bear equal responsibility for plagiarism.

**Conflict of interests**

The authors declare no conflict of interests regarding the publication of this article.
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