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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF FRESH AND FROZEN MIRABELLE PLUM TO ENHANCE ANTIOXIDANT POTENTIAL, POLYPHENOLS, PLANT PIGMENTS, AND PHENOLIC ACID CONTENT

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ABSTRACT

Background. The Mirabelle plum fruit is a known and valuable source of biologically active compounds, including antioxidants. This fruit is used in the food industry due to its savory taste as well. The leaves, despite their high content of active compounds, are used much less. This study aimed to optimize the extraction process of Mirabelle plum leaf and fruit. The effect of the freezing-thawing process on the antioxidant activity of extracts was evaluated, as was the content of selected phenolic compounds. The concentration of some plant pigments in fresh and frozen raw material and the content of a few phenolic acids in the selected leaf extracts were also determined.

Materials and methods. The extracts of Mirabelle plum leaf and fruit (peel and flesh separately) were prepared using ultrasound-assisted extraction. Evaluation of antioxidant activity was performed using *in vitro* techniques such as DPPH, ABTS, FIC, CUPRAC, and FRAP. The polyphenol, flavonoid, anthocyanin, chlorophyll, and carotenoid contents were also determined spectrophotometrically, as was the concentration of some phenolic acids in the selected leaf extracts by HPLC.

Results. The leaf extracts had higher antioxidant activity, polyphenols, and plant pigment contents than in the fruit. The lowest potential was observed for flesh extracts. The freezing-thawing process had rather an unfavorable effect on the activity of the leaf and peel extracts. The 120–180-minute extraction seemed to be more effective as compared to the 15–30-minute process. However, the prolongation of extraction up to 240 minutes led to a decrease in antioxidant potential and total polyphenol content. The leaf extracts had a relatively high concentration of phenolic acids, particularly chlorogenic acid (2.833 to 5.035 mg/100 mL of extract, on average).

Conclusion. To sum up, many factors, such as the plant part, extraction time, and the freezing-thawing process, could affect the antioxidant activity and content of biologically active agents in Mirabelle plum extracts. Due to the high content of active compounds including antioxidants in the Mirabelle leaves, their application in many areas of industry could be taken into consideration.

Keywords: antioxidant activity, freezing-thawing process, ultrasound-assisted extraction, plant pigments, polyphenols, *Prunus domestica* L.

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INTRODUCTION

Prunus domestica L. subsp. *Syriaca* Janch. (Mirabelle plum) belongs to the Rosacea family (*Prunus* genus) and is one of the popular plum species (Shahidi et al., 2013). It is known as a source of biologically active compounds, including antioxidants, with a beneficial effect on human health. The antioxidant potential of *P. domestica* is partly related to the content of polyphenols, including phenolic acids, flavonoids, and anthocyanins. These compounds could have many therapeutic effects, i.e., anti-inflammatory, antimicrobial, antiallergic, and antimutagenic. Due to these activities, this plum species could be effective in the prevention of many diseases, such as cardiovascular or neoplastic (Dehghannya et al., 2017).

Pigments are found in many plant parts, such as the leaves, fruit, stems, roots, and flowers and are responsible, among other things, for the antioxidant potential of plants. The most common plant pigments are fat-soluble chlorophylls and carotenoids and water-soluble anthocyanins. Carotenoids are responsible for red, orange, and yellow, chlorophylls for green, and anthocyanins, depending on their pH, for the red and blue color of plants (Boo et al., 2012).

Fruit belongs to a group of rather short-lived foods. Freezing or drying is commonly and frequently used to preserve these kinds of products. The processing of fruit could lead to changes in its physical and chemical parameters, including antioxidant contents in the raw material (Dehghannya et al., 2017; Paciulli et al., 2015). The peel and flesh of fruit are frequently characterized by a different profile of active compounds, including antioxidants. Moreover, fruit peels, as a rule, contain more active compounds than the flesh. Unfortunately, peels are often discarded, as they are considered to be difficult to digest and could be contaminated (Dabbou et al., 2017).

Conventional extraction techniques such as Soxhlet extraction and maceration are characterized by large (often toxic) solvent consumption, relatively long extraction times, higher costs of equipment, and higher consumption of energy. Moreover, these processes often require high temperatures, which can lead to the degradation of active substances. Therefore, modern, faster, more efficient, and environmentally friendly extraction processes were developed without the degradation of thermolabile compounds. Ultrasound-assisted extraction (UAE) seems to be a good alternative to conventional methods to obtain biologically active compounds from plant material, such as polyphenolic compounds (Savic Gajic et al., 2019; 2021; Savic and Savic Gajic, 2020).

Due to the known antioxidant activity of Mirabelle plum, this study aimed to optimize the ultrasound-assisted extraction of the leaves, peels, and flesh of *P. domestica*, using 70% ethanol as an extractant. Moreover, the freezing-thawing effect of the raw material before extraction on the studied parameters was also evaluated. The antioxidant potential of the extracts was evaluated using a few *in vitro* methods, as were the polyphenol, flavonoid and anthocyanin contents. Moreover, in both the fresh and frozen raw material (without extraction with ethanol), the chlorophylls (a, b, and total), carotenoids, and anthocyanins were determined. Subsequently, the concentration of selected phenolic acids in the extracts of the leaves (both fresh and frozen) that had been extracted for 150 and 180 min was evaluated using high-performance liquid chromatography (HPLC). For this analysis, the extracts with the highest polyphenol content evaluated with the Folin-Ciocalteu (F-C) method were selected.

MATERIALS AND METHODS

Chemicals

Glacial acetic acid, acetone, aluminum chloride hexahydrate, copper(II) chloride dihydrate, ethanol, 36% hydrochloric acid, iron(II) chloride tetrahydrate, iron(III) chloride hexahydrate, methanol, potassium chloride, potassium persulfate, sodium acetate anhydrous, sodium carbonate anhydrous, sodium hydroxide, and sodium nitrite were purchased from Chempur, Poland; neocuproine was delivered by J&K Scientific, Germany; Folin-Ciocalteu reagent, iron(II) sulfate heptahydrate, and gallic acid by Merck, Germany; chlorogenic acid by Pol-Aura, Poland; rutin trihydrate by Roth, Germany; ferrozine by Serva Electrophoresis, Germany; 3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma-Aldrich, USA. All the chemicals were of analytical grade.

Plant material and extract preparation

The fresh and frozen leaves as well as the flesh (pulp) and peel of Mirabelle plum fruit were extracted at 40 kHz using a 250 W ultrasonic bath with a thermostat (Polsonic Sonic-2, Poland). The temperature was set at 40°C. The plant material was collected from the natural state (backyard garden) and identified by a specialist in the field of plant physiology (Anna Nowak, DSc.). The 5%(w/v) extracts of fresh and frozen raw material were prepared using 70% (v/v) ethanol for 5, 15, 30, 60, 120, 150, 180, and 240 min. Some of the fresh material consisting of leaves and also the flesh and peel of the fruit were frozen at –20°C and thawed at +20°C immediately before extraction. Moreover, some of the fresh and frozen material (without extraction) was used to evaluate the concentration of plant pigments – anthocyanins, chlorophylls, and carotenoids. All the extracts were stored in the refrigerator (+4°C) until analysis. Spectrophotometric measurements were performed using a Hitachi U-5100 (Japan) spectrophotometer in 1 cm cuvettes.

In vitro antioxidant activity and content of plant pigments

Evaluation of the DPPH, ABTS, FRAP, and Folin-Ciocalteu methods was performed as described by Muzykiewicz et al. (2019), while the CUPRAC technique was performed according to Apak et al. (2004). The results were expressed either as mg of reference substance/g fresh material or mg of reference substance/g frozen material. For the DPPH, ABTS, and CUPRAC techniques, the results were expressed as Trolox equivalents (TEAC) – mg Trolox/g RM (raw material), whereas in the FRAP method they were expressed as $FeSO_4$ equivalents – mg $FeSO_4$/g RM. The total polyphenol content, which was evaluated using the F-C method, was presented as gallic acid (GA) equivalents (GAE) – mg GA/g RM. The ferrous ions chelating ability (FIC) was determined according to the method of Amamra et al. (2018) with some modifications. Instead of methanol, distilled water was used to dissolve the ferrozine. The results were presented as chelating activity [%]. The content of flavonoid was evaluated as described by Saeed et al. (2012), whereas the anthocyanins were evaluated according to Lee et al. (2005). The results were expressed as rutin equivalents – mg rutin/g RM and as cyanidin-3-glucoside [mg/L of extract], respectively. Moreover, the anthocyanin content was also determined in the fresh and frozen raw material according to the methodology of Klimek (2011). The content of chlorophylls and carotenoids in the fresh and frozen raw material was measured using the method of Petkova et al. (2019), and the results were presented as µg/g fresh or frozen material.

HPLC analysis

The concentration of the phenolic acids in the leaf extracts (both fresh and frozen) obtained during 150-min and 180-min extraction was determined by HPLC (Knauer, Germany) using the method elaborated in our laboratory. Briefly, the phenolic acids were separated on a 125 × 4 mm column filled with Hyperisil ODS (C18), with a particle size of 5 µm. Aliquots of 20 µL of the samples were injected into the column. The flow rate of the mobile phase, consisting of 1% aqueous acetic acid and methanol 9:1 (v/v), was 1 mL/min. The spectrophotometric detector operated at 280 nm. The calibration curves were prepared for concentrations from 0 to 0.025 mg/mL (gallic acid), as well as from 0 to 0.1 mg/mL for other phenolic acids (chlorogenic; 3-hydroxybenzoic; 4-hydroxybenzoic; 3,4-dihydroxybenzoic acid; 2,5-dihydroxybenzoic acid). The results are presented as arithmetic means of three independent samples of each extract ±standard deviation (SD) and were calculated to mg/100 mL of extract.

Statistical analysis

Statistical analysis was carried out using Statistica 12 software (Statsoft, Poland). Wilcoxon's signed-rank test (parameter z) was used to evaluate the statistical significance of differences between the antioxidant activities and content of the tested compounds. The Pearson's correlations (r) between the results determined with the individual methods were also evaluated. The significance level was assumed as $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity, total polyphenol, flavonoid, anthocyanin, and plant pigment content

The antioxidant activities of the extracts are presented in Figures 1–2, and the total polyphenol (assessed by the F-C technique), flavonoid, and anthocyanin contents in the extracts are shown in Figure 3. Regardless

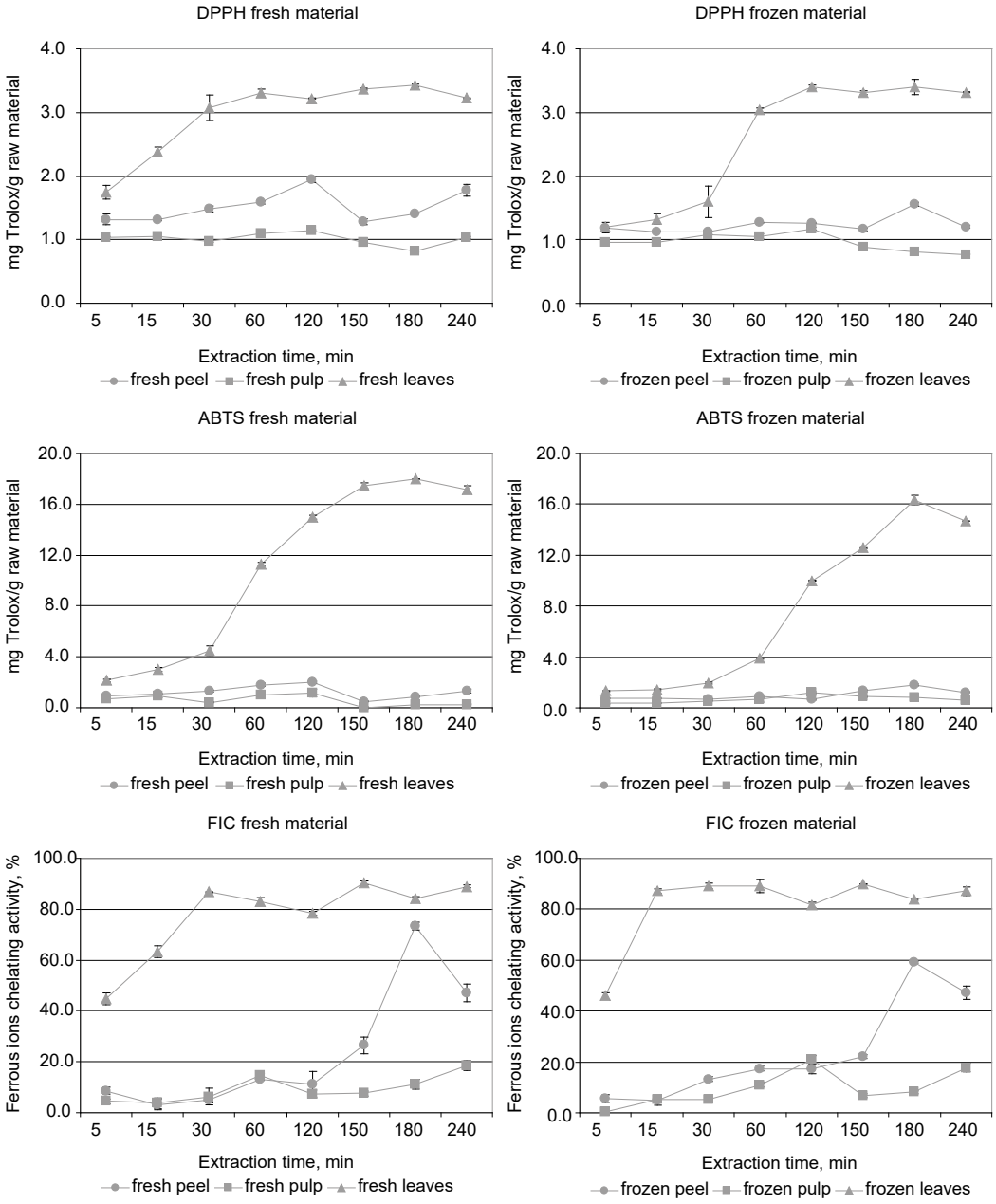

Fig. 1. The antioxidant activity of fresh and frozen Mirabelle plum extracts evaluated by the DPPH, ABTS, and FIC methods. Vertical lines represent standard deviations

of the applied method, the fresh leaf extracts had the highest antioxidant potential as well as the highest content of polyphenols, including flavonoids and anthocyanins. Muzykiewicz et al. (2018a; 2018b) also found the higher activity of quince and sea buckthorn leaf extracts as compared to fruit (both ripe and unripe) in a study on antioxidant potential. For this purpose, ultrasound-assisted extraction was applied, along with

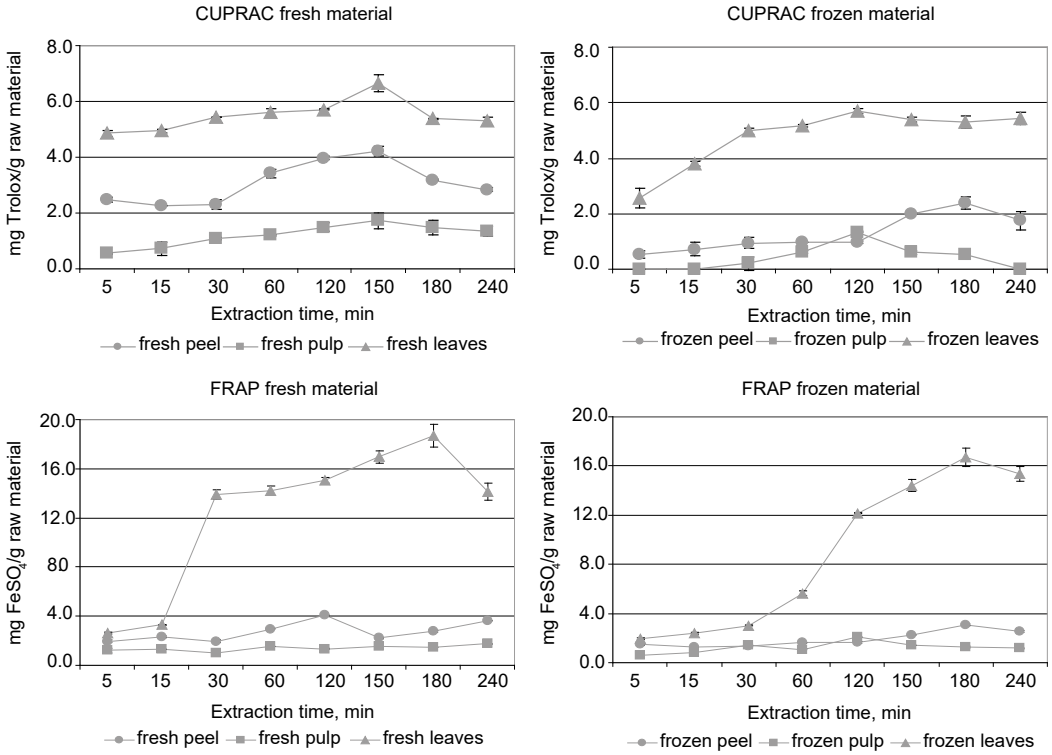

Fig. 2. The ability of fresh and frozen Mirabelle plum extracts to reduce the cupric and ferric ions, evaluated by the CUPRAC and FRAP techniques, respectively. Vertical lines represent standard deviations

the DPPH, FRAP, ABTS, and Folin-Ciocalteu methods. Orak et al. (2019) analyzed the antioxidant activity and total polyphenol content in the leaves, peel, flesh, and seed extracts of *Annona muricata* L., and found that the leaf samples had the highest polyphenol content, including flavonoids, and by the highest activity assessed by the FRAP, DPPH, and ABTS methods. The seed and flesh extracts also contained antioxidants, but the concentration was lower. The lowest potential was observed for the flesh extracts. Teleszko and Wojdyło (2015) compared the antioxidant potential and polyphenol profiles of the leaves and fruit of various plants, such as *Malus domestica*, *Cydonia oblonga*, *Chaenomeles japonica*, *Ribes nigrum*, *Aronia melanocarpa*, *Vaccinium macrocarpon*, and *Vaccinium myrtillus*. Based on the obtained results, they concluded that, similar to our results, the leaf extracts of these plants showed higher antioxidant potential than the fruit. They also noted that the antioxidant potential of the fruit was analyzed more often than that of the leaves, though, fruit is not only a valuable source of biologically active compounds to be used in the food, pharmaceutical, and cosmetic industries. In another study, Muzykiewicz et al. (2019) compared the antioxidant potential of flesh and peel extracts from different varieties of grapefruits (separately albedo and flavedo). They confirmed that the peel extracts, in particular from the white part (albedo), showed higher activity than the flesh extracts. In our study, Wilcoxon's signed-rank test analysis showed the statistically significant differences between the antioxidant activity of the extracts (evaluated by all applied methods, including polyphenols, anthocyanins, and flavonoids) prepared from all raw materials (leaf *vs.* peel, leaf *vs.* flesh, peel *vs.* flesh, both fresh and frozen): $z = 9.185$, $z = 9.185$ and $z = 7.905$, respectively. Moreover, the content of flavonoids and anthocyanins in the fresh and frozen raw material extracts also differed significantly ($z = 5.323$, $p < 0.001$). Statistically significant correlations were found between

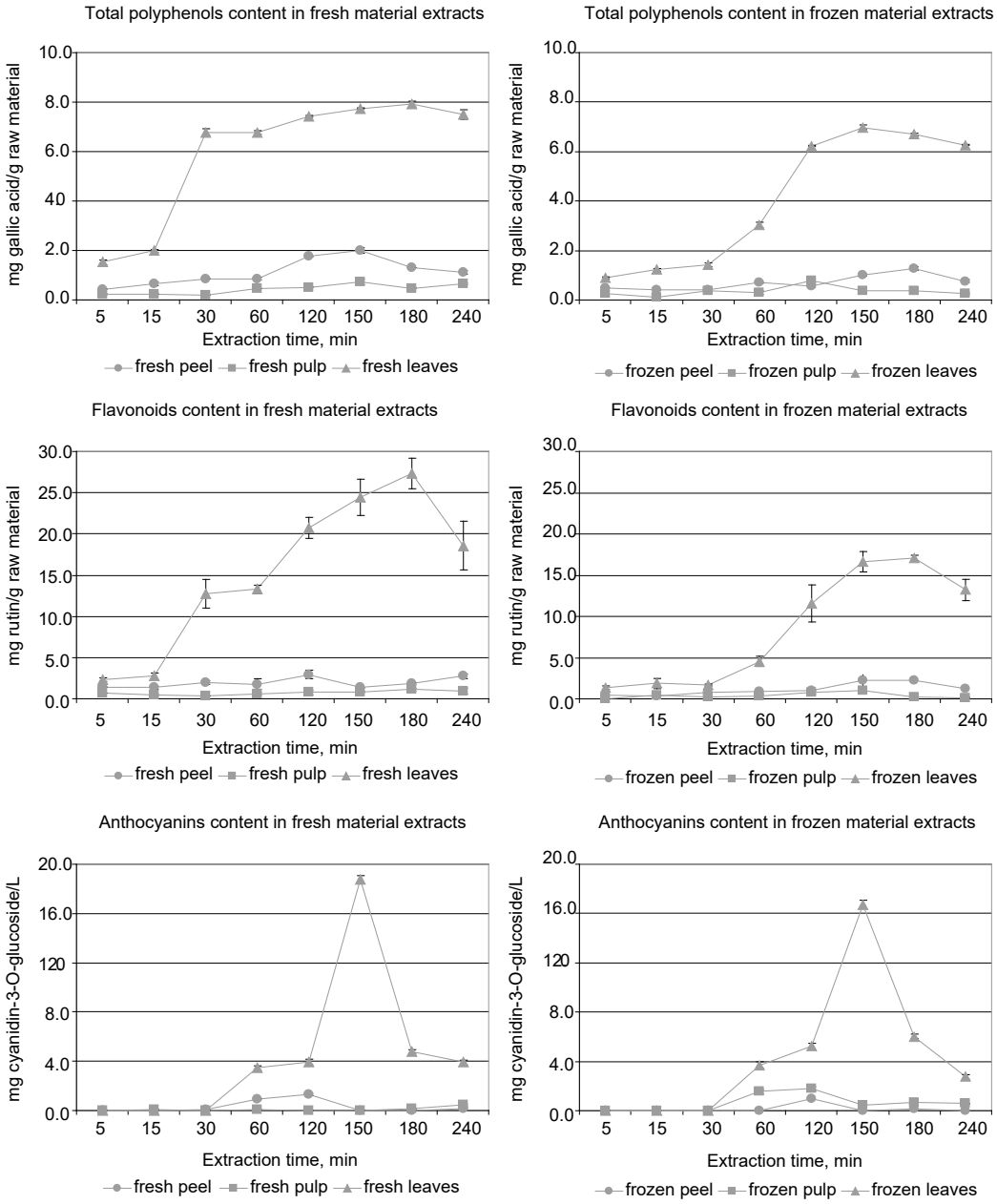

Fig. 3. The mean total polyphenol, flavonoid, and anthocyanin content in fresh and frozen Mirabelle plum extracts. Vertical lines represent standard deviations

the activity of the extracts evaluated using the DPPH, CUPRAC, ABTS, FRAP, and FIC techniques, and between the polyphenol, anthocyanin and flavonoid contents (Table 1). Maisuthisakul et al. (2008) compared the antioxidant potential and chemical composition of some plants. Similar to our results, they found statistically significant correlations between the antioxidant activity of plant extracts evaluated using the DPPH technique and the total polyphenol and flavonoid contents. They emphasized that the antioxidant potential

Table 1. The correlation coefficients (r) and their statistical significance (p) between the results obtained with different *in vitro* methods

	CUPRAC	ABTS	FRAP	Polyphenols	FIC	Flavonoids	Anthocyanins
DPPH	0.895*	0.901*	0.947*	0.957*	0.842*	0.898*	0.664*
CUPRAC	1.000*	0.755*	0.810*	0.844*	0.876*	0.767*	0.561*
ABTS		1.000*	0.962*	0.949*	0.736*	0.969*	0.728*
FRAP			1.000*	0.989*	0.788*	0.973*	0.701*
Polyphenols				1.000*	0.814*	0.965*	0.699*
FIC					1.000*	0.739*	0.537*
Flavonoids						1.000*	0.737*
Anthocyanins							1.000*

*$p < 0.001$.
DPPH – 2,2-diphenyl-1-picrylhydrazyl method, CUPRAC – cupric reducing antioxidant capacity method, ABTS – 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method, FRAP – ferric reducing and antioxidant power method, FIC – ferrous ions chelating ability.

of the extracts depended on many factors, including the content of a certain group of substances, phenolic compounds in particular. Their results were confirmed in our study on Mirabelle plum.

The content of plant pigments in the fresh and frozen raw materials without previous extraction was also evaluated in our study (Fig. 4, 5). Similar to the extracts, the highest content of anthocyanins, chlorophylls, and

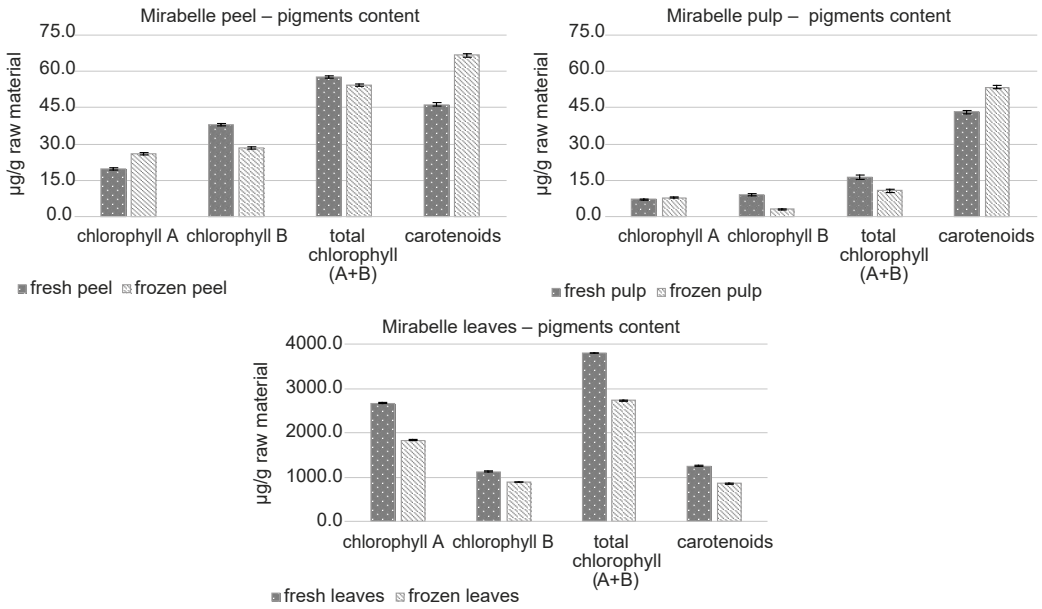

Fig. 4. The chlorophyll and carotenoid content in fresh and frozen different parts of Mirabelle plum. Vertical lines represent standard deviations

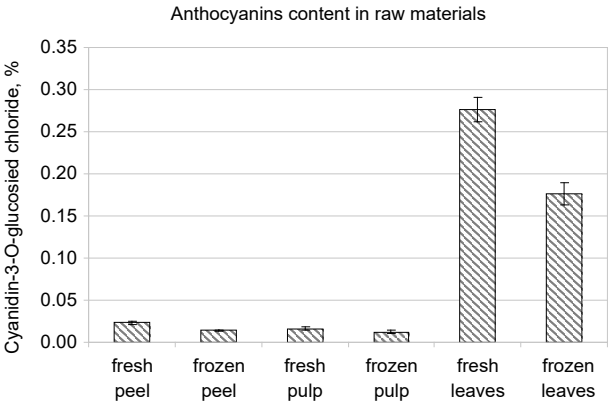

Fig. 5. The percentage of anthocyanin content in different fresh and frozen parts of Mirabelle plum. Vertical lines represent standard deviations

carotenoids was found in the fresh leaves. The flesh contained less of these compounds than the peels. The concentration of anthocyanins in the extracts of frozen peels and flesh was the exception (Fig. 3). In the group of frozen raw material extracts, more anthocyanins were found in the flesh sample than in the peel extracts (1.77 ±0.18 and 1.00 ±0.07 mg cyaninidin-3-O-glucoside/L, respectively). Kaulmann et al. (2014) also evaluated the antioxidant activity of extracts of whole Mirabelle fruit evaluated using the FRAP and ABTS methods, as well as the polyphenol content (estimated by the F-C method). In their study, the concentration of flavonoids was about 65.3 mg catechin/100 g fresh material, whereas anthocyanins were 7.39 mg cyanidin-3-glucoside/100 g fresh material. In our study, the flavonoid content, depending on extraction time and plant part to be analyzed, ranged from 0 (frozen pulp extract) to 2.94 (fresh peel extract) mg rutin/g raw material. However, in our study, the antioxidants were evaluated separately in the Mirabelle peels and flesh, and this could be a reason for the observed differences between the obtained results. Gil et al. (2002) estimated the content of antioxidants, such as phenolic compounds and carotenoids, in the peels and flesh of different fruit, including plums. They confirmed that plums with different skin colors could be a valuable source of phenolic compounds. Similar to our results, they found higher total polyphenol content in the fruit peels than in the flesh. Also, a higher carotenoid content, ranging from 83 to 231 µg/100 g of fruit, was observed in the plum peels. In the present study, the carotenoid concentration depended on the form of raw material to be evaluated – fresh or frozen, and varied from 43.15 to 53.49 and from 46.28 to 66.64 µg/g fresh or frozen raw material for the flesh and peels, respectively. Moreover, the differences between the content of anthocyanins, chlorophylls a and b, and carotenoids in the raw material before extraction were statistically significant ($z = 2.201$, $p < 0.05$). On the contrary, the differences between the amount of chlorophyll a and b, chlorophyll a and carotenoids, and chlorophyll b and carotenoids were statistically insignificant ($p > 0.05$).

One of the aims of our study was to optimize the extraction time for various Mirabelle plum raw materials to obtain extracts with the highest antioxidant activity. The analysis performed using all of the applied methods suggested that, in the majority of cases, the most optimal extraction time varied between 120–180 min, and the least efficient was 5–30 min. It should be noted that, in most cases, prolongation of the extraction time up to 240 min led to a decrease in the antioxidant potential of the extracts. Savic and Savic Gajic (2021) optimized the time of UAE of plum seeds. The applied extraction time in their study was 10 to 40 min. Based on the statistical model (the Box-Behnken design) they determined the optimal extraction time for the extraction of antioxidants from plum seeds to be 21 minutes. De Mello et al. (2017) optimized the time of ultrasound-assisted extraction to obtain chia seed oil. They extracted plant material for 20 to 60 min and concluded that the most effective was the 40 min process. Xu et al. (2017) applied the ultrasound-assisted extraction time of *Limonium sinuatum* flowers between 0 and 30 min and found the highest antioxidant activity of the samples extracted to be less than 10 min. However, after the prolongation of the extraction time above 15 min, degradation of the antioxidants contained in this raw material might occur. Based on our results, it could be expected that the decrease in the antioxidant activity of the extracts prepared for longer than 120–180 min is associated with the degradation of the antioxidants in the Mirabelle plum raw materials. However, a process that is too short may be insufficient for satisfactory antioxidant extraction. Many authors have suggested optimizing not only the extraction time but also some other parameters, particularly the type and

concentration of solvent to be applied for individual plant raw material (Azmir et al., 2013; Azwanida, 2015).

In the present study, the effect of freezing the raw material before extraction on the antioxidant activity of the extracts was also evaluated. In the case of the leaves and peels, higher activity and polyphenol content, including flavonoids and anthocyanins, was found in the extracts prepared from fresh raw materials. Among all of the analyzed parts of Mirabelle plum, the pigment content was also higher in the fresh than the frozen raw materials. The exception was the flesh extracts – a higher activity (evaluated by the FIC, F-C, FRAP, ABTS, and DPPH methods) was observed in the frozen material. The highest concentration of anthocyanins was also found in the frozen material extracts. Moreover, the frozen Mirabelle plum peels had a higher carotenoid content than the fresh ones. Based on the obtained results, it could be noted that freezing-thawing before extraction generally increased the antioxidant potential of the flesh extracts, though it decreased the efficiency of the leaf and peel extraction. However, it should be emphasized that the antioxidant potential and content of the individual compounds in the flesh extracts were relatively low. Lohachoompol et al. (2004) analyzed the effects of freezing, drying, and storage on the antioxidant activity and anthocyanin content of *Vaccinium corymbosum* berries. They showed that freezing had no effect on the tested properties. Çubukçu et al. (2019) evaluated the impact of freezing the various vegetable extracts on the DPPH radical scavenging activity and total polyphenol content. In the case of broccoli, the frozen material exhibited a higher antioxidant potential and polyphenol content. The freezing of garlic also increased the antioxidant activity, but no effect on the polyphenol content was found. This process had an unfavorable impact on the tested properties of onion. The freezing of cauliflower decreased the antioxidant activity but did not affect its total phenolic content. They also suggested that in the case of some vegetables, freezing could decrease the antioxidant content, but not as much as polyphenols. Al-Sanabani et al. (2016) evaluated the impact of freezing on the antioxidant potential (estimated using the DPPH and ABTS techniques) and total polyphenol, anthocyanin, and flavonoid content in pomegranate seeds. Freezing of the seeds had an unfavorable effect on the tested properties. The authors suggested that the decrease in the total anthocyanins could be due to the activity of oxidative enzymes. They found that anthocyanins could be released from the cellular matrix by freezing. It can be concluded that analysis of the released compounds may render the nutrient more detectable, although there is no evidence that these compounds will be available biologically. Based on the literature data, Al-Sanabani et al. (2016) suggested that the ability of extracts to scavenge the DPPH and $ABTS^{\bullet+}$ radical indicated that some components of these samples were electron donors, and they could react with free radicals to terminate radical chain reactions and, consequently, were able to boost the natural antioxidant defense mechanism.

HPLC analysis

In the present study, we identified and quantitated the selected phenolic acids in Mirabelle plum leaf (both fresh and frozen) extracts. For this purpose, the extracts with the highest total polyphenol contents were analyzed using HPLC. In the case of fresh and frozen leaf extracts obtained after 150- and 180-min extraction, the concentrations of selected phenolic acids were evaluated. The results are presented in Table 2. Figure 6 shows an HPLC chromatogram of a fresh leaf sample obtained after 180-min extraction. In the tested leaf extracts, the highest concentration of chlorogenic acid was found. Stierlin et al. (2018) analyzed the content of phenol derivatives in the leaves of different varieties of Mirabelle plum. Regardless of the variety, they detected chlorogenic acid in these raw materials. Lenchyk (2015) also evaluated the chemical composition of Mirabelle leaves using the HPLC method and confirmed that chlorogenic acid was the major compound found in the highest concentration. In our study, gallic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic, 2,5-dihydroxybenzoic acid, and 3,4-dihydroxybenzoic acid were also detected in the leaf extracts. The highest content of these compounds, except for 3-hydroxybenzoic acid, was found in the fresh leaf extracts. The HPLC analysis confirmed the results obtained by other *in vitro* methods, that the leaves, particularly fresh ones, could be a valuable source of natural antioxidants. Moreover, the chromatographic analysis confirmed the assumption that the total antioxidant

Table 2. The phenolic acid concentration in Mirabelle plum leaf extracts (mean ±SD)

Phenolic acid	Concentration of phenolic acid, mg/100 mL of extract			
	fresh leaf extracts		frozen leaf extracts	
	150 min	180 min	150 min	180 min
Chlorogenic acid	5.035 ±0.105	5.802 ±0.091	2.833 ±0.073	3.299 ±0.045
Gallic acid	0.318 ±0.024	0.410 ±0.019	0.395 ±0.016	0.345 ±0.008
4-hydroxybenzoic acid	0.098 ±0.021	0.299 ±0.026	0.097 ±0.013	0.118 ±0.021
3,4-dihydroxybenzoic acid	0.646 ±0.028	0.378 ±0.009	0.271 ±0.022	0.255 ±0.015
3-hydroxybenzoic acid	0.710 ±0.053	0.761 ±0.063	0.584 ±0.038	0.852 ±0.078
2,5-dihydroxybenzoic acid	0.459 ±0.050	0.659 ±0.026	0.211 ±0.046	0.224 ±0.048

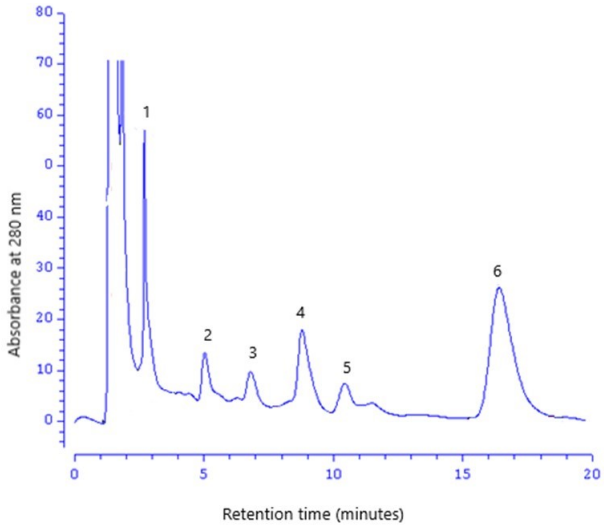

Fig. 6. HPLC chromatogram of phenolic acids in fresh leaf extracts obtained during 180-minute extraction: 1 – gallic acid, 2 – 3,4-dihydroxybenzoic acid, 3 – 2,5-dihydroxybenzoic acid, 4 – 4-hydroxybenzoic acid, 5 – 3-hydroxybenzoic acid, 6 – chlorogenic acid

potential of the extracts may be due to the polyphenol content, including phenolic acids.

CONCLUSION

The Mirabelle plum leaves could be a valuable source of antioxidants, such as chlorogenic acid. The leaf extracts have the highest antioxidant activity and plant pigment content, evaluated using *in vitro* methods, followed by the extracts from the peels and flesh of the fruit. The lowest potential, in most cases, was observed for Mirabelle flesh extracts. The freezing-thawing process of raw material before extraction had an unfavorable effect on the tested properties of the leaf and peel extracts. The evaluation of the extraction time showed that the most optimal was the ultrasound-assisted extraction which lasted 120–180 min, while the least efficient process lasted 15–30 min. However, the extending of the extraction time up to 240 min, in most cases, decreased the antioxidant potential and total polyphenol content in the Mirabelle plum extracts.

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