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THE EFFECT OF EXTRACTION METHODS ON THE DETERMINATION OF THE CONTENT OF POLYPHENOLS AND ANTI-OXIDANT ACTIVITIES OF ENRICHED EGG POWDER

S u m m a r y

Background. The utilization of phenolic compounds is limited by many factors, such as low solubility, low permeability, lability, rapid release, vulnerability towards environmental impacts and low bioavailability. To overcome these constraints, polyphenols can be closed within lipid-based and protein-based nanoparticles. In this study, nanocapsules derived from egg powder, enriched with chokeberry pomace extracts were examined. The content of antioxidant compounds (polyphenols, anthocyanins, phenylpropanoids, total phenolic compounds) and antioxidative potential (with the participation of radicals: $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP assays) were determined to assess the influence of the extraction method (heat extraction, ultrasound assisted extraction, stirring extraction) and encapsulation process of the fruit extract.

Results and conclusions. In the present study, the highest amounts of most examined compounds (regardless of the powder type) were achieved by heat extraction. The lowest values were obtained by stirring the solution at room temperature. The highest antioxidative potential towards radicals was assessed when ultrasounds were used as the extraction factor. Furthermore, the closure of fruit extracts in the protein shell appears to act in a protective manner on the antioxidant compounds present in the extract. The results show that the extraction method applied in the phase of preparing samples to the analysis is of great importance for determining the antioxidant compound content and antioxidant capacity.

Keywords: fruit pomaces, enrichment, antioxidant properties, polyphenols, chokeberry

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Introduction

Food consumption is one of the main factors determining human health and life, which stems from the necessity for providing nutrients and energy essential for the proper functioning of our body. Over the years, changes have been observed in the model of food consumption and consumer behavior. The changes arise from the interaction of many factors of a socio-economic and environmental nature [5]. The growing consumer awareness of food quality, but also in terms of environmental protection, impels food producers to seek new technological solutions within their field.

Due to the high amount of vitamins, minerals or antioxidants, fruit constitutes a pivotal element of human diet. The growing popularity of fruit preserves, including juices, purées, smoothies or jams, yields an increase in the amount of waste products [12]. Reprocessing of unexploited feedstock components is a significant concern for the fruit industry. An example of a sparing solution of the problem is valorization, the process of creating valuable products from low-value waste and by-products of the food industry. Combining environmental and economic aspects, valorization choicely fits into the mold of sustainable growth and less waste conceptions. It allows for obtaining enriched products of good quality, which address the needs of modern-day customers, concurrently fulfilling the goals associated with environmental protection [14]. Additionally, it enables creating completely new products and semi-products, that are useful, for instance, in the food or cosmetics industries.

Pomaces, similarly to fruit they are derived from, are a very rich source of natural antioxidants, of the carotenoid and polyphenol classes – mostly anthocyanins and flavonoids. These compounds belong to the group of biologically active substances, which by the elimination of reactive oxygen species (ROS) can contribute to oxidative stress reduction and anti-aging effects [11], thereby factoring into proper homeostasis between antioxidant protection and ROS production in cells of every living organism. With the aim of avoiding nutritional deficiencies of antioxidants and preventing acute, chronic and degenerative diseases, it is essential to maintain their proper level in everyday diet.

Despite having many advantages, bioactive compounds, including polyphenols, are chemically unstable and easily undergo oxidative degradation. Due to the rapid release, low solubility and low bioavailability, the application of pure bioactive substances in food products and pharmaceutical drugs is severely limited [17, 25]. Encapsulation as a technique for bioactive substance immobilization, may insulate antioxidants from environmental stresses, improve the physicochemical properties, thereby enhancing/reinforcing their health-promoting effects [1, 4, 10, 19].

Encapsulation is a technique, by which one or more active materials (so called core) are coated or closed inside another substance or system (cover/carrier/envelope). The encapsulation process is composed of two stages: often, the first step is the emulsi-

fication of the core with dense solution of the shell material, the next phase is drying or cooling down the emulsion. The efficient utilization of the technique requires the comprehension of physicochemical mechanisms, which underly the encapsulation and release of given compounds [13]. Currently, the encapsulation process of sensitive substances is well-developed and willingly applied in the pharmaceutical, chemical, cosmetic, food, as well as printing industries. Substances undergoing the encapsulation process include fats, oils, fragrance compounds, oleoresins, vitamins, minerals, enzymes and colorants [8]. In the food industry, stable and permanent capsules may not only be employed in the production of functional foods, but also to enrich traditional foods, like mousses, yoghurts, kefirs or fruit juices in bioactive substances [21,22].

The objective of this study was to determine the antioxidant capacity and the polyphenol content of nanocapsules (spherical micellar nanostructures) containing chokeberry pomaces extracts within their core. Additionally, the aim of the study was to assess the extraction methods applied to the features (antioxidant properties and polyphenol content) of the examined structures.

Materials and research methods

Research material

The research material consisted of egg powder (BakePlus, Stalowa Wola, Poland) enriched with encapsulated chokeberry pomace (HORTINO ZPOW Leżajsk Sp. z o.o., Leżajsk, Poland) extracts (encapsulated pomace EP) and egg powder enriched with non-encapsulated extracts (non-encapsulated pomace NEP).

Preparation of extracts from chokeberry pomace

A sample of lyophilized (Gamma 1-16, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), ground (A 10, IKA®-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) and sieved (Laboratory shaker LPzE-2e, Multiserv-Morek Jan Morek, Poland) chokeberry pomace was extracted with 80 % (v/v) ethanol (Chempur, Poland) (extracting solution) for 30 minutes in an ultrasonic bath (Sonic-6D, Polsonic Palczyński Sp. J., Warsaw, Poland) at water temperature of 25 °C, followed by 5-minute centrifuging at 3,500 rpm (MPW-223e, MPW Med. Instruments, Warsaw, Poland). The supernatant was transferred to a vessel, while the precipitate was extracted and centrifuged twice, as described above. The ratio of the sample to extracting solution was 1:10 (m/v). The collected extracts were combined and concentrated in a vacuum evaporator (RVO 200 A, INGOS s.r.o., Czech Republic) at 40 °C. The residue was dissolved in 80 % (v/v) ethanol and made up to a volume equal to the volume of extracting solution used in each extraction step.

Obtaining egg powder enriched with chokeberry extract

Method of producing egg powder enriched with chokeberry extract not encapsulated in nanostructures (NEP)

An aqueous suspension of egg yolk powder (BakePlus, Stalowa Wola, Poland) (8 g powder in 16 g H_2O) was mixed with an aqueous suspension of egg white powder (BakePlus, Stalowa Wola, Poland) (3 g powder in 27 g H_2O) using a magnetic stirrer (Heidolph RZR 2020, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and then 10 g virgin rapeseed oil (Kujawski, Bunge Polska sp. z. o.o.) was added. To the resulting mixture, 14.5 g of ethanolic extract of chokeberry pomace was gradually added. The resulting emulsion was lyophilized. A NEP sample was taken.

Method of producing egg powder enriched with chokeberry extract encapsulated in nanostructures (EP)

Firstly, 14.5 g of ethanol extract from chokeberry pomace was mixed with 10 g of virgin rapeseed oil (Kujawski, Bunge Polska sp. z. o.o.) and sonified using an ultrasonic homogenizer (20 kHz, Sonopuls HD 4200, Bandelin, Berlin, Germany) until a stable emulsion was obtained. Next, an aqueous suspension of egg yolk powder (8 g powder in 16 g H2O) was added and homogenized (Polytron PT 2500 E, Kinematica AG, Malters, Switzerland). After obtaining a homogeneous emulsion, a suspension of egg white powder (3 g powder in 27 g H_2O) was added. The homogenization continued until a stable emulsion was achieved. The resulting emulsion was then lyophilized, resulting in a EP sample.

Preparation of extracts from NEP and EP samples for determination of polyphenol content and antioxidant activity

The extraction of enriched egg powders for the quantitative assessment of polyphenol content and antioxidant activity was conducted in three variants. For each method, 1 g of raw material was weighed with an accuracy of 0.001 g in a flat-bottom-flask and 20 cm^3 of methanol (80 % v/v) (Chempur, Poland) was added to the weighed amount. For the first extraction method, the prepared samples were boiled (65 ^oC) for 30 minutes with vapor recovery (heat extraction HE). Ultrasound assisted extraction (UE) was carried out by setting the methanol-soaked samples in an ultrasonic cleaner (SONIC 6, Polsonic, Poland) and incubating them for 30 minutes at room temperature and frequency of 50 kHz. In the third extraction approach, the samples of enriched egg powder were shaked (shaking extraction SE) (shaking water bath JWE 357, JWElectronic, Poland) for 24 hours at room temperature. Following the extraction, the mixtures were infiltrated to a volumetric flask (50 cm^3), which was subsequentlyfilled with 80 % (v/v) methanol solution. Thereafter, the solutions were centrifuged at 1,500 rpm for 10 minutes (centrifuge MPW - 350R, MPW Med. Instruments, Poland). After-

wards, the supernatants were collected for the antioxidant activity and polyphenol content quantitative assays. The prepared extracts were stored for about one week at a temperature of -20 ^{o}C until the time of the experiments.

Methods

Total polyphenols (TP) and phenols profile

The total polyphenol content in methanol extracts was examined using Folin-Ciocalteau, following the procedure described by Singelton et al. [20]. A certain amount of extract (0.5 cm^3) was combined with Folin-Ciocalteau reagent (0.125 cm^3) (Chempur, Poland) and 25 % solution of sodium carbonate (0.25 cm^3) (Eurochem, Poland). After mixing and one-hour incubation at room temperature, in darkness, the absorbance at 760 nm was measured. The total polyphenols were calculated using a standard curve, which was prepared for (+)-Catechin. The measurement was conducted in four repetitions, the result was present in mg/g dry content of the material tested.

The content of main phenolic compound groups, like phenylpropanoids, phenyls, flavonoids and anthocyanins, were assessed using the approach presented by Fukumoto and Mazza [7]. The sample for the test was prepared by combining 0.25 cm^3 of a methanol extract with 0.25 cm^3 of a 0.1 % HCl solution (Stanlab, Poland) (dissolved in a 96 % ethanol solution) and 4.5 cm^3 of a 2 % HCl solution (dissolved in water). For the purpose of assessing the content of phenols, flavonoids, phenylpropanoids and anthocyanins, the absorbance of the prepared solutions was measured at 280 nm, 320 nm, 360 nm and 520 nm, respectively. The results were converted by employing the molar absorbance (ε) of respective reference solutions: caffeic acid (CF 0.887 $M^{-1}cm^{-1}$) for phenylpropanoids, chlorogenic acid (CA 0.264 $M^{-1}cm^{-1}$) for phenols, quercetin (Q 0.513 $M^{-1}cm^{-1}$) for flavonoids and cyanidin (C 0.645 $M^{-1}cm^{-1}$) for anthocyanins. The measurements were performed four times for each sample type. The result was expressed in mg/100 g of dry mass of test material (mg/100 g d.m.).

Antioxidant activity by DPPH$^{\bullet}$, ABTS$^{\bullet+}$ free radical methods and FRAP assay

The antioxidant activity of enriched egg powder methanol extracts was assessed using ABTS$^{\bullet+}$ (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich, USA) radical cation and DPPH$^{\bullet}$ (diphenylpicryl-hydrazyl) (Sigma-Aldrich, USA) free radical following the procedure described by Miller et al. [15] and Brand-Williams [16]. In the case of measuring the antioxidant capacity of the research material towards DPPH$^{\bullet}$ radical, the absorbance at 516 nm wavelength was measured 10 minutes after adding the solution of radical (3 cm^3 0.1 M DPPH$^{\bullet}$) to 1 cm^3 of the extract. When using the second type of radical, the absorbance was measured at 734 nm wavelength, 10 minutes after combining 1 cm^3 of the extract and 2 cm^3 of ABTS$^{\bullet+}$. Where necessary, the sample solutions were diluted. Each measurement was taken four times for each

sample. The antioxidant activity of enriched egg powder was expressed in μM of Trolox per gram of dry mass of research material (μM trolox/g d.m.).

The assessment of antioxidant activity by FRAP (Sigma-Aldrich, USA) method was conducted following Benzie and Strain procedure [2]. A 3.6 cm^3 FRAP reagent, which was comprised of acetate buffer (Chempur, Poland), iron(III) chloride $FeCl_3$ (Lach-Ner, Czech Republic), TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine solution) (Sigma-Aldrich, Switzerland) in 10:1:1 volume ratio was added to 0.4 cm^3 of a methanol extract. The samples were incubated for 10 minutes at a temperature of 37 °C. Afterwards, the samples were cooled down, centrifuged and their absorbance was measured at 595 nm wavelength. The assay was repeated three times. The results were expressed in mmol Fe^{2+} per g of dry mass of the material.

Statistical analysis

To determine statistical differences between the means, the data was subjected to a one-factor analysis of variance and the Duncan's Multiple Range test at a significance level of 0.05. The influence of the analyzed factors (the extraction method, the type of egg powder) was assessed with the use of two-way ANOVA. The normality of distributions was checked using the Shapiro-Wilk test, and the homogeneity of variances was checked using Levene's test. All calculations were done with a statistical software package Statistica 13.3 (StatSoft Inc., Tulsa, OK, USA).

Results and their discussion

Total polyphenols and phenols profile

Phenolic compounds are widely distributed in nature, especially in plants, which produce high amounts of these compounds. Due to the presence of hydroxylic groups, they exhibit antioxidant properties. These compounds easily decompose (e.g. through oxidation or hydrolysis). They can also form covalent and non-covalent complexes with different compounds [6]. Furthermore, it has been reported that phenolic compounds play a vital role in the lipid peroxidation stabilization. [24]. Hence, it is relevant to assess the amount of these compounds in the prepared samples of the extracted chokeberry pomace enriched with an egg powder, as it relates to the health-promoting activity of the product.

The contents of the total polyphenols (TP) assessed in both egg powders - with encapsulated extract (EP) and with non-encapsulated extract (NEP) - are presented in table 1. TP values range from 244.12 to 303.19 mg/100 g d.m., depending on the powder type and applied extraction method. Statistically, no difference was shown, except for the lowest result, obtained for the EP egg powder, subjected to stirring extraction at room temperature (SE).

Table 1. Content of polyphenols and anti-oxidant activities of enriched egg powder
Tabela 1 Zawartość polifenoli i działanie przeciwutleniające wzbogaconego proszku jajecznego

Type of egg powder / Rodzaj proszku jajecznego	Extraction method / Metoda ekstrakcji	Total polyphenols / Suma polifenoli mg/100 g d.m.	Anthocyanins / Antocyjany mg/100 g d.m.	Flavonoids / Flawonoidy mg/100 g d.m.	Phenylpro-panoids mg/100 g d.m.	Phenols / Fenole mg/100 g d.m.	DPPH$^{\bullet}$ µM trolox/g d.m.	ABTS$^{\bullet+}$ µM trolox/g d.m.	FRAP µM Fe^{2+}/g d.m.
EP	HE	297.11± 7.79^{b}	191.37±3.14^{ab}	190.37±15.50^{c}	175.99±12.53^{b}	1154.50±56.87^{b}	142.93±7.28^{a}	268.03±10.67^{bc}	48.69±1.28
	UE	301.54± 3.02^{b}	197.98±9.59^{bc}	205.34±17.54^{c}	186.25±13.14^{b}	1205.32±65.42^{b}	170.24±37.41^{b}	272.65±14.69^{c}	48.22±1.37
	SE	244.12± 10.43^{a}	200.65±18.73^{bc}	106.35±16.90^{b}	123.02±14.40^{a}	966.05±70.85^{a}	144.42±5.01^{a}	256.26±10.50^{b}	48.93±3.29
NEP	HE	303.19± 16.56^{b}	212.67±14.87^{c}	235.02±24.92^{d}	210.94±22.16^{c}	1332.37±124.26^{c}	135.73±2.58^{a}	263.17±1.75^{bc}	46.20±2.42
	UE	296.81± 4.17^{b}	194.35±8.61^{abc}	195.42±8.32^{c}	178.36±7.48^{b}	1160.91±43.81^{b}	146.62±5.94^{ab}	268.64±10.90^{bc}	46.63±0.73
	SE	293.75± 10.03^{b}	177.81±8.98^{a}	78.60±8.12^{a}	105.12±7.44^{a}	863.82±39.13^{a}	129.26±2.05^{a}	236.53±4.76^{a}	46.23±1.90
One-way ANOVA - *p*		< 0.001	0.016	< 0.001	< 0.001	< 0.001	0.035	0.001	0.216

Two-way ANOVA - *p*								
Factor A (Type of egg powder / Rodzaj proszku jajaecznego)	< 0.001	0.723	0.731	0.594	0.729	0.030	0.029	0.013
Factor B (Extraction method / Meoda ekstrakcji)	< 0.001	0.122	< 0.001	< 0.001	< 0.001	0.028	< 0.001	0.986
Factor A x Factor B	< 0.001	0.005	0.001	0.003	0.003	0.597	0.229	0.843

Explanatory notes / Objaśnienia:

The mean value of four replications ± standard deviation. The mean values designated with the same letters in particular columns do not differ significantly at 0.05 level of confidence. EP – egg powder with encapsulated extract; NEP - egg powder with non-encapsulated extract; HE – heat extraction; UE – ultrasonic extraction; SE – 24-hour extraction at room temperature with shaking. Średnia wartość z czterech powtórzeń ± odchylenie standardowe. Wartości średnie oznaczone tymi samymi literami w poszczególnych kolumnach nie różnią się istotnie przy poziomie ufności 0,05. EP – proszek jajeczny z ekstraktem kapsułkowanym; NEP – proszek jajeczny z ekstraktem niekapsułkowanym; HE – ekstrakcja ciepła; UE – ekstrakcja ultradźwiękowa; SE – ekstrakcja 24 godzinna w temperaturze pokojowej z wytrząsaniem.

The two-factor analysis indicated that the first factor (the type of egg powder EP/NEP), as well as the second one (the type of extraction - HE/UE/SE) had an impact on the content of polyphenols in the studied material. Additionally, an interaction between these two factors also affected the amount of assessed polyphenols. However, no statistically significant differences in extraction efficiency of TP were observed between the applied extraction methods UE and HE for both powder types. The efficiency of SE was comparable with the other methods for the non-encapsulated extracts. Although, this method was significantly less efficient for the encapsulated extracts (the TP content was approximately 19% lower for SE, in comparison with the highest value that was obtained from EP, specifically, when UE was employed). This result implies that the structure of nanocapsules form a barrier that hampers polyphenol elution and the application of ultrasounds or high temperature appears to be sufficient to violate the structure of the capsules. It might be attributable to high affinity of polyphenols towards proteins and peptides, by non-covalent interactions, like electrostatic interactions, hydrophobic interactions, hydrogen bonding and π-π stacking [9].

Diverse chemical, physical properties and omni-directional biological activity of polyphenolic compounds are caused by the diversity of their chemical structures. The several groups of phenolic compound contents (anthocyanins, flavonoids, phenylpropanoids, phenols) in the examined egg powders are shown in table 1.

In the case of anthocyanins, the content oscillated between 177.81 and 212.67 mg/100 g d.m., depending on the powder type and the extraction method. The lowest and the highest values were both obtained for the egg powder enriched with the non-encapsulated chokeberry extract (NEP). The EP powders in turn were marked by intermediate values, regardless of the extraction type.

The two-factor analysis indicated that both the type of egg powder (EP/NEP) and the extraction type (HE/UE/SE) had no influence on the content of anthocyanins in the sampled material. However, the interaction of these two factors exhibited an influence, which suggests that the selection of an appropriate extraction method, depending on the analyzed powder type, is vital. For the encapsulated preparations, the highest amount of anthocyanins was obtained by UE and SE, while the lowest content was observed in the samples isolated by heat extraction. For the non-encapsulated preparations, the relationship between anthocyanins content and extraction method was opposite - the most efficient was HE, whereas UE and SE, respectively, proved to be lesseffective.

Considering the changes of flavonoid, phenylpropanoid and phenols content in the enriched egg powders, depending on the preparation type and extraction method employed, the trend appears to be very alike. The lowest contents of these phenolic compounds were assessed in the NEP powder, when SE was employed, whereas the highest amounts characterized the same preparation, when HE was applied. As demon-

strated in the two-factor analysis, the obtained results were influenced by the extraction type and the interaction of the sample type and the extraction type. However, it was showed that the egg powder type did not affect the content.

In the case of the encapsulated preparations, the HE and UE methods proved to be comparable, whereas the lowest values were obtained using SE (the percentage decrease of content for flavonoids, phenylpropanoids and phenols relative to the HE was approximately 44 %, 30 % and 16 %, respectively). For the non-encapsulated preparations, the extraction efficacy was as follows: HE > UE > SE. The content of flavonoids, phenylpropanoids and phenols was significantly lower in the case of SE, approximately by 66 %, 50 % and 35 % respectively, compared to HE. When SE was applied to the EP and NEP preparations, the content of respective phenolic compounds declined in the following order: flavonoids > phenylpropanoids > phenols, whereby a higher decrease was observed for the powders with non-encapsulated chokeberry pomace extract.

The application of a physical factor such as ultrasounds or high temperature appears to be relevant for the efficient extraction of flavonoids, phenols and phenylpropanoids, regardless of the preparation form (capsulated/non-encapsulated). For the non-encapsulated preparations, employing the high temperature yielded in the relatively highest extraction efficiency. In the case of the encapsulated preparations, the assessed amounts of the studied compounds for the ultrasonic method and heat method, were not statistically different, however, there was a tendency towards higher values for the ultrasonic method, which indicates that the capsules might be more prone to ultrasound than heat degradation.

During the assessment of individual polyphenol groups in a tested material, one must be mindful of the variable efficacy of solvents used for polyphenol extractions. As reported by Covan [3], ethanol is the most commonly used solvent for flavonoids and tannins extraction, whereas the application of methanol allows for the isolation of anthocyanins, tannins, flavons and different polyphenol compounds. Acetone in turn is utilized for flavonoid extraction.

Antioxidant activity by DPPH$^{\bullet}$, ABTS$^{\bullet+}$ free radical methods and FRAP assay

Oxidative metabolism is essential for cell survival. As an effect of this mechanism, reactive oxygen species (ROS) are formed, which in oxidative stress conditions, might induce oxidative alterations adverse for our body (i.e. DNA, lipid and protein oxidation) [18]. The human body is endowed with endogenous defensive mechanisms, including enzymes such as superoxide dismutase, glutathione perooxidase and catalase [23]. Apart from these mechanisms, it is essential to consume antioxidants with one's diet. The antioxidant activity cannot be accurately assessed with one antioxidant test

without considering a few variables (i.e. the sample preparation method, reagents concentration, reaction time, pH), which influence the results.

For the objective assessment of polyphenol antioxidant activity, the employment of different tests is required. On this account, for the analysis of the examined extracts from egg preparations, three tests were carried out, two of which used radicals ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$), while one (FRAP) was based on the reduction of iron(III) ion. The obtained results are presented in the table 1.

The antioxidative potential values of the examined preparations, measured relative to $DPPH^{\bullet}$ radical, ranged between 129.26 μM Trolox/g d.m. to 170.24 μM Trolox/g d.m., which corresponds to the percent of the extinguished radical (% RDS) from 34.5 % to 46.5 %. The obtained results are not statistically different, except for the highest value, which was observed for the encapsulated preparation, after extraction using ultrasounds. The two-way ANOVA indicated no interaction between the sample type and extraction method in terms of impacting the results obtained. However, the influence of each factor alone was confirmed. In the course of the analysis performed for the individual preparations (EP, NEP), the highest values in both cases were obtained by employing UE, whereby the value acquired for NEP does not differ considerably from the results for HE and SE, which in turn do not differ statistically. A similar tendency was noted for the total polyphenol content.

The antioxidative potential of the analyzed materials regarding $ABTS^{\bullet+}$ radical cation changed in a wider range than towards $DPPH^{\bullet}$. Although, like for $DPPH^{\bullet}$, the lowest value was obtained for the NEP egg powder, subjected to SE and the highest for EP, after UE. The two-way ANOVA is in line with $DPPH^{\bullet}$ results. It did not confirm the impact of factors interaction, only the impacts of each of them. The antioxidative activity of the encapsulated preparations towards $ABTS^{\bullet+}$ was in the following sequence: UE>HE>SE. For the non-encapsulated preparations, the highest activity was achieved for UE and HE. Despite no statistical differences between these samples, the mean values indicate the same tendency as for encapsulated preparations (UE>HE>SE).

The mean values of antioxidative capacity obtained by the FRAP method did not show statistical differences, nevertheless the values obtained for the powders, where the chokeberry extract was not encapsulated (NEP), were lower than for the powders with encapsulated extract (EP). The differences ranged from 1.59 to 2.70 M Fe^{2+}/g d.m., depending on the extraction method. The two-way ANOVA indicated statistically significant influence only of the powder type. The influence of the extraction, as well as of the interaction of extraction method and powder type was not shown.

Conclusions

1. Currently, customer interest in natural, plant antioxidants is increasing due to their medicinal properties and a safer nature compared to synthetic preparations. However, the use of these compounds is limited by many factors. It was shown that nanocarriers (including nanoparticles based on lipids and proteins) are excellent materials for encapsulating phenolic compounds because they improve their bioavailability and prevent degradation induced by the external environment.
2. The encapsulation of fruit extracts in a protein shell appears to act in a protective manner on the antioxidant compounds present in the extract. In the case of the heat extraction, it is possible that a protein shell denatures and hence the elution of the assessed compounds outwards might be restrained. This in turn lowers the assessed content for the egg powder, where the extract was encapsulated. The two other extraction methods yielded in higher values for the EP powder.
3. As for the antioxidant analysis, it can be stated that the results obtained were dependent not only on the preparation type, but also on the extraction method, as well as on the interaction of these two variables and the influence varies for the individual compound groups (total polyphenols, anthocyanins, flavonoids, phenylpropanoids, phenols). In the case of the TP content, all factors were shown to have an impact and the results obtained did not differ statistically (except for the EP powder after the SE extraction). The content of anthocyanins was impacted only by the interaction of the factors considered. On the other hand, the other analyzed groups of antioxidants depended on both the interaction, as well as the extraction method. For every assessed group, the lowest amount was assessed for the NEP egg powder after the SE extraction. For EP powders after the same extraction method, low amounts of phenylpropanoids and phenols were also noticed. The highest values for individual compound groups were obtained for NEP powder after the HE extraction.
4. The antioxidative potential measured by three separate methods was affected by the powder type. The result of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical assays was impacted by the extraction method. The FRAP test showed no statistical difference. The $DPPH^{\bullet}$ antioxidative potential was not very statistically diversified. The EP powder constituted an exception, where ultrasound assisted extraction yielded in the highest values than in the other cases. The lowest antioxidative capacity measured towards $ABTS^{\bullet+}$ radical was achieved for the NEP powder, extracted by SE, while the highest results were associated to the EP powder extracted with UE.
5. To conclude, in the present study, the highest amounts of most examined compounds (regardless of the powder type) were achieved by heat extraction (HE). The lowest values were obtained by stirring the solution at room temperature. The

highest antioxidative potential towards radicals was assessed when ultrasounds were used as the extraction factor.

6. The featured results of the study support the claim that the extraction method applied in the phase of preparing samples for the quantitative analysis of antioxidants is of great importance for determining the antioxidant compound content and antioxidant capacity. Hence, it is indispensable to precisely describe the method, as well as the material preparation conditions, when presenting the results from this field realm.

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WPŁYW METOD EKSTRAKCJI NA OZNACZANIE ZAWARTOŚCI POLIFENOLI I DZIAŁANIA PRZECIWUTLENIAJĄCEGO WZBOGACANEGO PROSZKU JAJECZNEGO

S t r e s z c z e n i e

Wprowadzenie. Wykorzystanie związków fenolowych jest ograniczone wieloma czynnikami, takimi jak niska rozpuszczalność, słaba przepuszczalność, labilność, szybkie uwalnianie, podatność na wpływy środowiska i niska biodostępność. Aby pokonać te ograniczenia, polifenole można zamknąć w lipidowych i białkowych nanocząsteczkach. W pracy zbadano nanokapsułki otrzymane z proszku jajecznego, wzbogacone ekstraktami z wytłoków aronii. W celu określenia wpływu metody ekstrakcji (ekstrakcja w podwyższonej temperaturze, ekstrakcja wspomagana ultradźwiękami, ekstrakcja z mieszaniem) i procesu kapsułkowania ekstraktu owocowego określono zawartość związków przeciwutleniających (polifenole, antocyjany, fenylopropanoidy, związki fenolowe ogółem) oraz potencjał przeciwutleniający (z udziałem rodników $DPPH^{\bullet}$, $ABTS^{\bullet+}$ i metodą FRAP) otrzymanych proszków.

Wyniki i wnioski. W niniejszym badaniu największe ilości większości badanych związków (niezależnie od rodzaju proszku) uzyskano poprzez ekstrakcję cieplną. Najniższe wartości uzyskano mieszając roztwór w temperaturze pokojowej. Największy potencjał antyoksydacyjny wobec badanych rodników

stwierdzono, gdy jako czynnik ekstrakcyjny zastosowano ultradźwięki. Ponadto wydaje się, że zamknięcie ekstraktów owocowych w otoczce białkowej działa ochronnie na związki przeciwutleniające obecne w ekstrakcie. Wyniki potwierdzają, że metoda ekstrakcji zastosowana na etapie przygotowania próbek do analizy ma ogromne znaczenie w określeniu zawartości związków przeciwutleniających i zdolności antyoksydacyjnej badanych materiałów.

Słowa kluczowe: wytłoki owocowe, wzbogacanie, właściwości przeciwutleniające, polifenole, aronia