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MAGDALENA WÓJCIAK, IRENEUSZ SOWA, WERONIKA WÓJCIAK, MAGDALENA ŻUK

FREEZE-DRIED RASPBERRY POMACE: A RICH RESERVOIR OF POLYPHENOLS WITH ANTIOXIDANT AND PROTECTIVE EFFECTS AS A PROMISING FUNCTIONAL FOOD ADDITIVE

S u m m a r y

Background. Raspberries (*Rubus idaeus* L.) hold significant importance in the food industry. Raspberry pomace, commonly regarded as a waste product during fruit processing, is a rich source of biologically active components. This study aimed to assess the detailed profile of polyphenols in fresh and freeze-dried raspberry pomace. Furthermore, we examined its potential to protect normal human gastric epithelial cells against oxidative stress in an H_2O_2-induced stress model. A qualitative and quantitative analysis of phenolic compounds was performed using the high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) method. Cell viability was assessed based on the integrity of lysosomal membranes (neutral red NR assay) and mitochondrial dehydrogenase activity (MTT). Additionally, the reducing potential (FRAP), the ability to scavenge free radicals in the DPPH$^{\bullet}$ test and studies on ABC-HP021X cells based on the H_2DCFDA test were evaluated.

Results and conclusion. It was found that the extract from freeze-dried raspberry pomace is a rich source of various polyphenolic compounds, including ellagitannins, epicatechin and anthocyanins. The extract was characterized by high antioxidant potential and exhibited a protective effect on human gastric epithelial cells against H_2O_2-induced cytotoxicity. Cell incubation with the extracts prior to oxidative stress induction attenuated H_2O_2-induced cytotoxicity in a concentration-dependent manner, and at a concentration of 250 μg/cm^3, it abolished the detrimental effects of H_2O_2. Additionally, the extract at this concentration restored the redox status in cells with induced oxidative stress and reduced ROS levels to values similar to the control. Thus, raspberry pomace extracts may be considered a valuable food supplement.

Keywords: raspberry, polyphenols, functional food, protective effect

Wprowadzenie

Rubus idaeus L., commonly known as raspberry, is a perennial fruiting plant from the Rosaceae family. Its woody stem reaches from 150 cm to 250 cm in height, and

Prof. dr hab. M. Wójciak ORCID: 0000-0003-0466-1343, prof. dr hab. I. Sowa ORCID: 0000-0001-9346-6325, W. Wójciak, M. Żuk, Zakład Chemii Analitycznej, Wydział Farmaceutyczny, Uniwersytet Medyczny w Lublinie. Ul. Witolda Chodźki 4a, 20-093 Lublin.
Kontakt: e-mail: magdalena.wojciak@umlub.pl

shoots are covered with thorns. Leaves are composed of 3, 5 or 7 leaflets, and fruits consist of many small red stone fruits, creating single aggregate fruit. The blooming period starts in May and ends in August when white, drooping flower clusters are formed. The raspberry fruits are a great resource of valuable components having positive influence on human body. In this context, their antioxidant, anti-inflammatory, antimicrobial and antiproliferative properties are worth noting [10, 11]. This fruit is rich in polyphenols, ellagitannins, ellagic acid, tocopherols, dietary fiber and vitamin C [3, 8]. Due to abundance in its composition, raspberry is fruit having multiple uses like confectionery, as a flavoring component, dietary supplement and for the cosmetic industry. However, it has the greatest significance in the food industry, for jam and juice production. Owing to such wide usage, there is a lot of post-production waste, which remains a challenge given the current trend of zero waste living. To keep up with new standards, scientists are researching new ways of utilizing food industry remains and many of them are proving to be useful and abundant sources of components with pro-health benefits [6, 15].

Raspberry pomace, despite being also considered a waste product, could be deemed a useful source of biological active components. For example, it has been shown that seeds found in waste contain oil rich in phytosterols, carotenoids, tocopherols, polyphenolic compounds and polyunsaturated fatty acids [7, 13, 14]. Furthermore, they are abundant in dietary fiber which provides multiple health benefits like lowering risk for developing coronary heart disease, stroke, diabetes, hypertension and obesity [1, 12]. Seeds also contain proteins and various plant secondary metabolites, with ellagitannins and flavan-3-ols being predominant. Residues possess strong antioxidant potential and are characterized by high value of total polyphenols [23]. As is commonly known, polyphenolic compounds are desirable components of daily diet because their regular consumption can help reduce the incidence of cardiovascular diseases, diabetes, obesity and colon cancer [18]. Therefore, raspberry pomace seems to be a valuable candidate as a food additive. The main objective of our study was to evaluate the detailed polyphenolic composition of this material and investigate its potential to protect normal human gastric epithelial cells against oxidative stress. Furthermore, the polyphenolic profile of fresh and freeze-dried residues was compared to assess the impact of sample processing on chemical composition.

Material and methods

Sample preparation

The research material consisted of raspberry pomace obtained after the extraction of fruit juice from a local juice manufacturer. Part of the material was freeze-dried for 48 h in a Christ Alpha 2-4 LDplus apparatus (Martin Christ Ge-friertrocknungsanlagen,

GmbH, Germany). The fresh and freeze-dried materials were crushed in a mortar and accurately weighed. Subsequently, in an ultrasonic bath, approximately 200 mg of fresh samples and 30 mg of freeze-dried samples were sequentially extracted with methanol (Step I) and aqueous methanol solutions with increasing water content: 20 % (Step II) and 40 % (Step III). In each step, 1.5 cm^3 portions of the solvent were used, and the supernatant was decanted. Each step lasted 15 min. All the extracts were acidified with 0.1 mol/L HCl for anthocyanin preservation. The extracts were combined, adjusted to a final volume of 5 cm^3, and then filtered.

HPLC-DAD-MS analysis

The analyses were conducted following a previously published procedure [24]. Ultra-high performance liquid chromatograph (UHPLC) Infnity Series II with a DAD and MS detector (Agilent Technologies, Santa Clara, CA, USA) equipped with Titan column (10 cm length, 2.1 mm i.d., 1.9 μm particle size) (Supelco, Sigma-Aldrich, Burlington, MA, USA) was used. Water (A) and acetonitrile (B), both acidified with 0.05 % of formic acid at flow rate 0.2 mL/min, were used for elution in line with the following program: 0 ÷ 8 min from 98 % A to 93 % A, 8 ÷ 15 min from 93 % A to 88 % A, 15 ÷ 29 min from 88 % A to 85 % A, 29 ÷ 40 min from 85 % A to 80 % A, and 40 ÷ 60 min from 80 % A to 65 % A. All solvents were MS grade (Sigma-Aldrich). Temperature was set at 30 °C. MS conditions: ions were collected in a negative mode, drying gas temperature and flow rate were 325 °C and 8 L min^{-1}, respectively, nebulizer pressure was 30 psi, voltage on capillary, skimmer and fragmentator were 3500 V, 65 V and 220 V, respectively. Calibration curves obtained based on standard solutions of the identified compounds were used for quantification.

For a quantitative analysis, ellagitannins were hydrolyzed following the procedure elaborated by Klimczak et al. [9] and were calculated based on the content of ellagic acid obtained as a result of hydrolysis.

Antioxidant assay

The extract obtained from the freeze-dried sample was evaporated to dryness, and a stock solution at a concentration of 10 mg/cm^3 was prepared by dissolving residues in methanol. Subsequently, a series of dilutions were prepared for the DPPH and FRAP tests. The methodology for these assays was based on the literature [22].

Cell culture assay

Human Gastric Epithelial Cells (ABC-HP021X) were cultivated under condition recommended by the manufacturer (AcceGen Biotech, Fairfield, NJ,USA). Next, the cells were transferred to a 96-well plate and incubated for 24 h at 37 °C. The cell density was $1x10^5$ cells/well. The cytotoxicity of the samples was assessed after 24 h incubation, with investigated samples using MTT and NR methods. Stock solution at a

concentration of 10 mg/cm^3 was prepared using DMSO in water, and it was further diluted using a medium. The concentration of DMSO in working solution did not exceed 0.5 %.

MTT assay: A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma), with a concentration of 5 mg/cm^3, was added to the cells (25 µL/well). Following 3 h of incubation at 37 °C, a solution of 10 % sodium dodecyl sulfate (SDS) in 0.01 M HCl was added to solubilize the samples overnight. Subsequently, the absorbance was measured at λ = 570 nm using an E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA).

Neutral red (NR) assay: Neutral red dye (NR) at a concentration of 40 µg/cm^3 was added to the cells (100 µL/well). After 2-hour incubation at 37 °C, the NR was removed and the cells were washed with PBS, and then 200 µL of 0.5 % formalin in 1 % $CaCl_2$ follow by 150 µL of decolorizing buffer (1 % glacial acetic acid in 50 % ethanol) was added. The plates were shaken for 10 min, and absorbance was measured at λ = 540 nm using an E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA).

Protection against H_2O_2-induced oxidative stress: Prior to H_2O_2 treatment (250 µM), the cells were pre-treated with the tested samples for 6 h. After 30 min, H_2DCFDA was added to the cells, and they were incubated in the dark for 45 min. Subsequently, the fluorescence emitted by 2',7'-dichlorofluorescein (DCF) was measured using an excitation wavelength of λ = 485 nm and an emission wavelength of λ = 530 nm [24]. The measurements were performed using a microplate reader (FilterMax F5, Thermo Fisher Scientific, Waltham, MA, USA). As a positive control, ascorbic acid (AA) was used at a concentration of 50 µg/cm^3.

Statistical analysis

The extraction procedure was repeated twice, and three measurements were carried out for each sample. The results were analyzed using Statistica ver. 13.3 software. Data was presented as the mean ± standard deviation (SD). One-way ANOVA, followed by Dunnett's post hoc test, was used to assess the significance of differences. Differences were considered significant at a *p*-value of < 0.05.

Results and discussion

It was determined that the pomace contained approx. 16.8 ± 1.1 % of fresh fruit, with a seed-to-pulp ratio of approx. 69:31 by weight. The weight of the freeze-dried sample was approx. 3.8 times lower than that of the fresh pomace, indicating a water loss of 73.5 ± 0.2 %.

UHPLC-DAD-MS analysis revealed no significant qualitative differences in the polyphenolic profiles between the fresh and freeze-dried samples (Fig. 1), indicating that freeze-drying does not alter the polyphenolic composition.

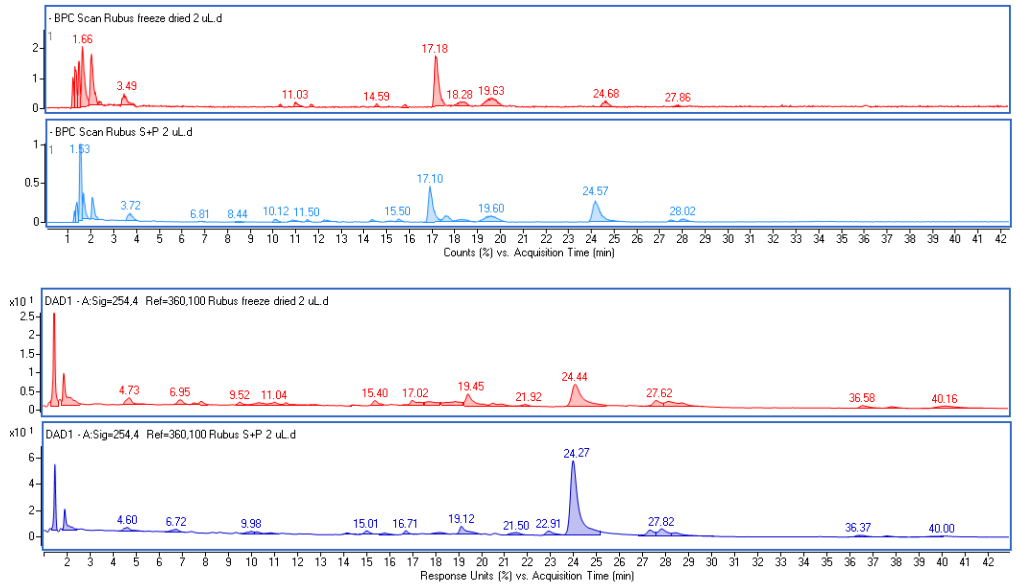

Fig. 1. Base peak chromatogram (BPC) and chromatogram recorded at a wavelength of 254 nm for extracts obtained from fresh and freeze-dried raspberry pomace.

Rys. 1. Chromatogram głównych pików (BPC) i chromatogram zarejestrowany przy długości fali 254 nm dla ekstraktów z wytłoków malinowych świeżych i liofilizowanych.

The predominant phenolic constituent of the samples was a high molecular weight ellagitannin (m/z - H = 1869.15), which produced a mass spectrum with a pseudo-molecular ion corresponding to ellagic acid (m/z - H = 300.99). This compound was identified as sanguiin H-6, which is one of the main ellagitannins found in *R. idaeus* [16]. The ellagitannin content was assessed based on ellagic acid concentration found in the hydrolyzed samples. Epicatechin was another constituent of the pomace present at a high concentration. Furthermore, a slight amount of ellagic acid pentoside and ellagic acid were detected. Additionally, a few components with maximum absorbance in the visible region were identified in the samples. They belong to the anthocyanin group and all exhibited pseudo-molecular ions with m/z-H in the range of 285.0405-285.0413, corresponding to the estimated formula $C_{15}H_{10}O_6$, indicative of the aglycone cyanidin (Cy). Based on literature [16], they were described as Cy-3-O-glucosyl-rutinoside (m/z-H = 755), Cy-3-O-glucoside (m/z-H = 447) and Cy-3-O-rutinoside (m/z-H = 593). Interestingly, the distribution of specific anthocyanin derivatives in fresh and freeze-dried samples differed. The ratio of Cy-3-O-rutinoside to Cy-3-O-

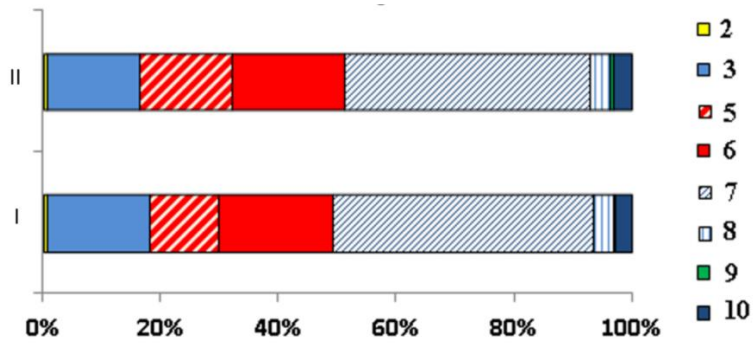

Fig. 2. Percentage distribution of phenolic constituents in the total content of polyphenols in fresh (I) and freeze-dried (II) raspberry pomace. Numbering of the components is given in Table 1.

Rys. 2. Procentowy udział składników fenolowych w ogólnej zawartości polifenoli w świeżych (I) i liofilizowanych (II) wytłokach malinowych. Numeracja składników podana jest w Tabeli 1.

Table 1. Chromatographic and mass data of the components identified in raspberry pomace and the results of quantification in fresh (calculated on a dry weight) and freeze-dried samples, expressed as micrograms per gram of plant material ± SD

Tabela 1. Dane chromatograficzne i masowe składników zidentyfikowanych w wytłokach malinowych oraz wyniki oznaczeń ilościowych w próbkach świeżych (w przeliczeniu na suchą masę) i liofilizowanych w przeliczeniu na g materiału roślinnego ± SD

No	Retention time / Czas retencji (min)	Observed ion mass / Obserwowany jon molekularny [M-H]-/(fragments)	Identified compound / Oznaczony składnik	Fresh material / Świeży materiał (µg/g d.w.)	Freeze-dried material / Liofilizowany materiał (µg/g d.w.)
1	2.06	191.01985	Citric acid	7895±207[a]	7256±37[b]
2	10.48	341.08799 (179, 161)	Caffeic acid glucoside	28.8±4.3	22.1±1.8
3	17.18	289.07196 (221,245)	Epicatechin	748.5±61.8[a]	576.2±21.3[b]
4	18.11	755.20402	Cyanidin -3-O-glucosyl-rutinoside	d	d
5	18.28	447.09474	Cyanidin-3-O-glucoside	494.7±51.1[a]	577.3±37.6[b]
6	19.63	593 (285)	Cyanidin-3-O-rutinoside	828.1±78.1[a]	671.8±44.2[b]
7	24.68	1869.15095 (934,301)	Sanguiin H-6*	1895 ± 97[a]	1521 ± 71[b]
8	27.76	433.04325 (301)	Ellagic acid pentoside	142.5±10.7	131.6±9.7
9	27.86	609.14682 (301,463)	Rutin	14.5±0.8	14.11±1.21
10	28.41	300.99931	Ellagic acid	124.7±9.6	119.6±10.3

Explanatory notes / Objaśnienia:

* calculated after hydrolysis to ellagic acid; d – detected; different letters indicate statistically significant differences between values in the same line
* obliczono po hydrolizie do kwasu elagowego; d – wykryto; różne litery wskazują statystycznie istotne różnice pomiędzy wartościami w tym samym wierszu

glucoside decreased after freeze-drying from 1.67 to 1.16, indicating partial hydrolysis of Cy-3-O-rutinoside. Percentage distribution of other phenolic constituents was similar in both analyzed materials (Fig. 2).

Apart from phenolic compounds, citric acid was also found in the fresh and freeze-dried samples. Chromatographic and mass data, along with the results of quantitative analysis of the identified polyphenolic compounds, are summarized in Table 1. For the fresh material, the results were expressed per dry weight.

The high content of polyphenolic compounds, which belong to strong antioxidants, suggested significant potential for scavenging free radicals. Therefore, the ability of the freeze-dried raspberry pomace to neutralize ROS was evaluated using DPPH and FRAP assays and expressed as trolox and ascorbic acid concentration equivalents, respectively (Tab. 2).

Table 2. The results of radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) obtained for extract from freeze-dried raspberry pomace. Values are the means (n=3) ± SD.

Tabela 2. Wyniki testu zdolności wychwytywania wolnych rodników (DPPH) i potencjału redukującego żelazo (FRAP) otrzymane dla ekstraktu z liofilizowanych wytłoków malinowych. Wartości są średnimi (n=3) ± SD.

Concentration / stężenie [µg/cm^3]	DPPH (equivalent of trolox concentration / ekwiwalnet stężenia troloksu)	FRAP (equivalent of ascorbic acid concentration / ekwiwalnet stężenia kwasu askorbinowego)
50	6.852 ± 0.711	5.882 ± 0.413
100	15.414 ± 0.698	19.014 ± 0.741
200	23.878 ± 0.721	36.991 ± 0.528
250	30.798± 0.568	44.621 ± 0.704

The values obtained showed the significant antioxidant potential of raspberry pomace, which is in line with data reported by Vulić et al. [23].

The antioxidant and protective effects of the extract were further verified in in vitro cell assay using H_2O_2 stimulation as a model of oxidative stress. Neutral red (NR) and MTT assays were used to evaluate the effect of the extract on cell viability and cell metabolism, respectively. The results are shown in Figure 3.

It was found that H_2O_2 stimulation had a negative impact on cell viability, reducing the percentage of viable cells to 56 % and 58 % for MTT and NR assays, respec-

tively, compared to the control. On the other hand, pre-treating the cells with the extracts attenuated H_2O_2-induced cytotoxicity in a concentration-dependent manner. When the cells were pre-incubated with the extract at a concentration of 200 μg/cm^3 before H_2O_2 treatment, cell viability decreased only slightly, by approximately 12 % (NR) and 10 % (MTT) compared to the control.

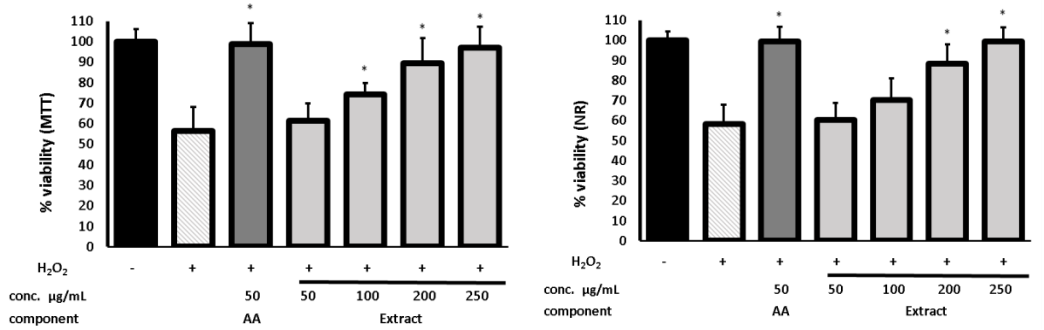

Fig. 3. Cell viability determined by the MTT and NR assays (n = 3) expressed as a percentage of the control (0.5 % DMSO). Cells were pre-treated with extract from freeze-dried raspberry pomace prior to the H_2O_2 exposure.

Rys.3. Żywotność komórek oznaczona testem MTT i NR (n = 3) wyrażona jako procent kontroli (0,5% DMSO). Komórki wstępnie traktowano ekstraktem z liofilizowanych wytłoków malinowych przed ekspozycją na H_2O_2.

Explanatory notes / Objaśnienia:

*means statistically significant difference at $p < 0.05$ vs H_2O_2-treated cells assessed using one-way ANOVA followed by Dunnett's multiple comparison post hoc test was used. AA – ascorbic acid /

oznacza statystycznie istotną różnicę przy $p < 0,05$ w porównaniu z komórkami traktowanymi H_2O_2 ocenianą za pomocą jednoczynnikowej analizy ANOVA, a następnie testem post hoc wielokrotnych porównań Dunnetta. AA – kwas askorbinowy.

The subsequent assay aimed to investigate the impact of the extract on the disturbance in oxidative balance caused by H_2O_2. The influence on the intracellular production of reactive oxygen species was determined using the H_2DCFDA test. The result is shown in Figure 4.

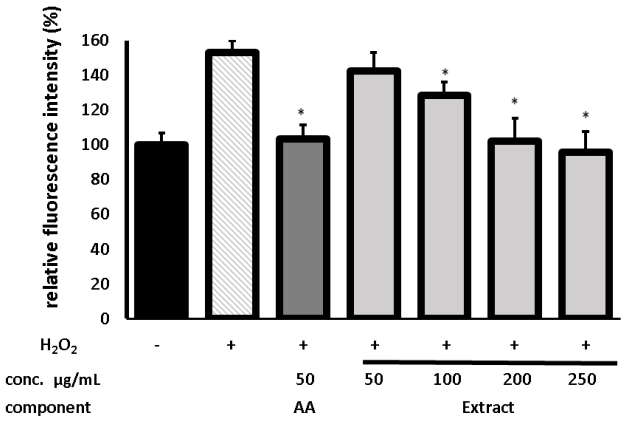

Fig. 4. Relative fluorescence intensity (n = 3) calculated as a percentage in comparison with untreated control cells. Cells were pre-treated with extract from freeze-dried raspberry pomace prior to the H_2O_2 exposure.

Rys. 4. Względna intensywność fluorescencji (n = 3) obliczona jako procent w porównaniu z nietraktowanymi komórkami kontrolnymi. Komórki wstępnie traktowano ekstraktem z liofilizowanych wytłoków malinowych przed ekspozycją na H_2O_2.

Explanatory notes / Objaśnienia:

*indicates a statistically significant difference ($p < 0.05$) vs H_2O_2 stimulated cells. One-way ANOVA followed by Dunnett's multiple comparison post hoc test was used. AA – ascorbic acid.

*wskazuje statystycznie istotną różnicę ($p < 0,05$) w porównaniu z komórkami stymulowanymi H_2O_2. Użyto jednoczynnikową analizę ANOVA, z testem post hoc wielokrotnych porównań Dunnetta. AA – kwas askorbinowy.

As expected, H_2O_2 caused oxidative imbalance and increased the ROS level up to 153 % compared to the control (100 %). Pre-treatment with the extract lowered ROS levels in a concentration-dependent manner. At a concentration of 200 µg/cm^3, the amount of ROS was nearly restored to the level of the untreated control.

Our study demonstrated that raspberry pomace is a rich source of components with high antioxidant potential and protective activity against damage caused by free radicals. It could be a result of the impact on cellular enzymatic antioxidant systems involved in the regulation of oxidative stress. It was evidenced that polyphenols could induce SOD, CAT and GPx, important enzymes involved in maintaining redox balance [19]. The human body is constantly exposed to adverse external factors such as reactive oxygen species (ROS). The excessive level of ROS in cells can lead to DNA damage, degradation of proteins and lipids peroxidation, which impairs the function of tissues. Consequently, this promotes the development of various disorders, including neurodegenerative, cardiovascular and age-related degenerative diseases [17]. In this context, plant materials rich in antioxidants are valuable components of the human diet.

Furthermore, as reported in the literature, particular components of the raspberry pomace have a positive impact on the gastrointestinal tract. Ellagitannins (ETs), which are the main constituents of pomace, belong to a group of non-flavonoid oligomeric polyphenols, composed of ellagic acid (EA), gallic acid, and a glycoside moiety. It was evidenced that the consumption of ETs beneficially affects human health and may prevent or alleviate various disorders, including cardiovascular issues, neurodegenerative conditions and cancer [2]. Although their bioavailability is limited, ETs are a source of urolithins, which are active metabolites formed by gut microbiota. The absorption of urolithins is much more efficient, and it is believed that the observed beneficial effects following from the consumption of ETs result from the activity of urolithins [4]. In vitro and in vivo studies revealed that urolithins possess anti-inflammatory, anticancer, antioxidant, cardioprotective, and neuroprotective properties [4]. Additionally, numerous research papers reported the positive effects of ellagic acid (EA) on the gastrointestinal tract, including hepatoprotective, gastroprotective and cancer preventive effects [20]. The activity of ellagitannins and ellagic acid is attributed to their strong antioxidant activity. Their ability to scavenge reactive oxygen species (ROS) and thereby reduce oxidative stress is a key factor in the prevention of oxidative stress-related diseases [2, 4]. Anthocyanins, which are other abundant constituents of raspberry pomace, also exert beneficial effects on human health, including their potential contributions to improving cardiovascular health, enhancing cognitive function and preventing cancer [5]. Citric acid, on the other hand, plays a role in maintaining redox homeostasis in the cells. It reduces oxidative damage in cells and protects against lipid peroxidation. As evidenced in numerous in vivo studies, citric acid exhibits antioxidant and anti-inflammatory properties. It can enhance the immune system and has protective effects on the liver. Furthermore, it may improve the bioavailability of essential mineral elements, such as iron [21]. Taking into account the above considerations, it can be assumed that pomace could be a valuable food additive.

Conclusions

1. Our study revealed that the extract derived from freeze-dried raspberry pomace is a rich source of various polyphenolic compounds, including ellagitannins, epicatechin and anthocyanins.
2. This extract demonstrated a robust antioxidant effect, as evidenced by DPPH and FRAP tests, as well as in an in vitro cell assay using an H_2O_2-induced stress model. It also exhibited a protective effect on human gastric epithelial cells against H_2O_2-induced cytotoxicity.
3. Based on these findings, it can be assumed that raspberry pomace has the potential to be a valuable addition to food products as a natural food additive with antioxidant properties.

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LIOFILIZOWANE WYTŁOKI Z MALIN: BOGATE ŹRÓDŁO POLIFENOLI O DZIAŁANIU PRZECIWUTLENIAJĄCYM I OCHRONNYM JAKO POTENCJALNY DODATEK DO ŻYWNOŚCI FUNKCJONALNEJ

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

Wprowadzenie. Malina (*Rubus idaeus* L.) ma duże znaczenie w przemyśle spożywczym. Wytłoki z malin, powszechnie uważane za odpad podczas przetwarzania owoców, są bogatym źródłem biologicznie aktywnych składników. Niniejsze badanie miało na celu ocenę szczegółowego profilu polifenoli w świeżych i liofilizowanych odpadach malin. Ponadto, zbadano potencjał tych pozostałości do ochrony normalnych ludzkich komórek nabłonka żołądka przed stresem oksydacyjnym wywołanym H_2O_2. Analizę jakościową i ilościową związków fenolowych przeprowadzono metodą wysokosprawnej chromatografii cieczowej sprzężoną ze spektrometrią mas (HPLC-MS). Oceniano żywotność komórek w oparciu o integralność błon lizosomów komórkowych (test czerwieni obojętnej NR) i aktywność dehydrogenazy mitochondrialnej (MTT). Oceniano również potencjał redukcyjny (FRAP) oraz zdolność do zmiatania wolnych rodników w teście DPPH i badaniach na komórkach ABC-HP021X na podstawie testu H_2DCFDA.

Wyniki i wnioski. Stwierdzono, że ekstrakt z liofilizowanych wytłoków malinowych jest bogatym źródłem różnych związków polifenolowych, w tym elagotanin, epikatechiny i antocyjanów. Ekstrakt charakteryzował się wysokim potencjałem antyoksydacyjnym i wykazywał działanie ochronne na komórki nabłonka ludzkiego żołądka przed cytotoksycznością indukowaną H_2O_2. Inkubacja komórek ekstraktami przed wywołaniem stresu oksydacyjnego osłabiała cytotoksyczność indukowaną H_2O_2 w sposób zależny

od stężenia a przy stężeniu 250 μg/cm^3 znosiła niekorzystne działanie H_2O_2. Dodatkowo ekstrakt w tym stężeniu przywracał status redoks w komórkach z indukowanym stresem oksydacyjnym i obniżał poziom ROS do wartości zbliżonych do kontroli. Można więc przypuszczać, że wytłoki malinowe mogą być wartościowym dodatkiem do żywności.

Słowa kluczowe: malina, polifenole, żywność funkcjonalna, działanie protekcyjne