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STUDY ON WHEAT AND OAT BRAN EXTRACTS AND THEIR ANTIOXIDANT PROPERTIES

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Abstract. The article presents the results of a study on aqueous and aqueous-ethanolic extracts derived from the cell wall material of oat and wheat bran, focusing on their content of phenolic compounds, flavonoids, and amino acids. The total phenolic content was assessed spectrophotometrically using the Folin–Ciocalteu method, while the total flavonoid content was determined *via* an aluminum chloride colorimetric assay. Gallic acid, ferulic acid, and quercetin were also identified in the samples using thin-layer chromatography. The antioxidant activity of the extracts was evaluated using rat liver hepatocytes under *in vitro* conditions of free radical oxidation. The results indicated that the aqueous-ethanolic extracts of wheat and oat bran did not significantly reduce lipid peroxidation or oxidative protein modification. However, wheat bran extracts exhibited higher antioxidant activity than those from oat bran.

Keywords: bran extracts, bioactive compounds, biological activity, antioxidant activity, oxidative protein modifycations, thin-layer chromatography, malondialdehyde.

1. Introduction

Currently, in both the EU and the USA, special attention is given to the culture of consuming functional foods as potential sources of biologically active substances (BAS). These products contribute essential nutrients and energy to the body, help reduce the risk of chronic diseases, and support overall health.[1-3] Numerous scientific studies have investigated the antioxidant potential of extracts from rice, corn, oat, wheat, and barley bran. It is well known that phenolic compounds, tocopherols, carotenoids, ubiquinones, and vitamins C and K exhibit strong antioxidant properties.[4-9] Particular focus has been placed on the polyphenols present in cereal bran, especially wheat and oat bran, which constitute the cell wall material rich in valuable macronutrients – such as proteins, amino acids, polysaccharides, starch, and high levels of dietary fiber beneficial to human health – as well as a range of phytochemicals with antioxidant activity, including phenolic acids, bioflavonoids (*e.g.*, quercetin, rutin), polyphenols, alkylresorcinols, coumarins, *etc.*

Dietary fiber, chiefly consisting of cellulose, hemicellulose, and lignin, is known for its ability to bind ammonia and water, adsorb organic and bile acids, facilitate cation exchange, and eliminate toxins, nitrates, radionuclides, and other harmful substances from the body. Owing to these properties, bran holds great potential for incorporation into functional food products.[10-14]

Hydroxycinnamic acids (HCAs) – such as ferulic, caffeic, and chlorogenic acids – are phenolic compounds found in rice, wheat, oat, and corn bran, as well as in flaxseed, oranges, pineapples, apples, artichokes, pine nuts, and coffee. These substances exhibit a wide range of biological activities, including anti-inflammatory, antitumor, hepatoprotective, antihistamine, antitoxic, and antiviral effects. Owing to their ability to inhibit lipid peroxidation in cellular biomembranes, HCAs influence enzymatic activity and thereby suppress free radical reactions.[15]

Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid), in particular, has garnered significant attention due to its antimicrobial, anti-inflammatory, antiarrhythmic, antithrombotic, and antioxidant properties, making its identification in bran-derived raw materials especially important.[16,17]

According to the literature, the main analytical methods used for the study of FA and other HCAs include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), electrophoresis, and spectrophotometry.[18-24]

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Investigating the content of BAS in secondary grain processing products is of interest not only to researchers but also to manufacturers developing functional foods, medicinal and cosmetic products, BAS-based dietary supplements, restorative tinctures, sports nutrition, *etc.*[10,25-32]

In the comprehensive analysis of agricultural grain processing products, assessing the levels of phenolic compounds and flavonoids is of particular importance.[33,34] Given their practical significance, this study aimed to obtain extracts from oat and wheat bran and to evaluate them for their content of phenolic compounds, flavonoids, and amino acids, as well as their antioxidant activity.

2. Experimental

2.1. Materials

Oat bran (ELOVENA Nordic, Finland) and wheat bran (Organic Eco Product, Ukraine) were used as received. Solvents and chemicals, all analytical grade or better, were used without additional purification.

2.2. Methods

2.2.1. Preparation of Extracts

Oat and wheat bran extracts were prepared as follows: 5.0 g of wheat or oat bran were placed in a flask, and the appropriate extractant – either water or a 20%, 40%, or 70% aqueous-ethanolic solution – was added at a solid-to-liquid ratio of 1:20. The mixture was extracted in a boiling water bath using a reflux condenser for 30 minutes. The resulting extracts were then filtered and centrifuged at 6000 rpm for 10 minutes.[35-37]

2.2.2. Detection and Quantitative Study of Phenolic Compounds in Aqueous and Aqueous-Ethanolic Bran Extracts

2.2.2.1. Qualitative Analysis of Phenolic Compounds

The qualitative composition of phenolic compounds in the obtained aqueous-ethanolic extracts of oat and wheat bran was assessed using classical qualitative reactions and TLC. The following colorimetric reactions were performed: with iron(III) chloride solution; with a freshly prepared mixture of equal volumes of 1% iron(III) chloride and 1% potassium hexacyanoferrate(III) solutions; with bromothymol blue solution; with vanillin in concentrated hydrochloric acid; and with a mixture of iron(III) chloride, potassium hexacyanoferrate(III), and concentrated nitric acid.[38]

TLC analysis was conducted on 5×10 cm "Silica Gel 60" plates using a solvent system of chloroform: methanol: formic acid (85:15:1, v/v/v). Standard solutions of phenolic hydroxycinnamic acids, *i.e.*, ferulic acid, gallic acid, and quercetin, were used as references. After chromatographic separation, the samples were examined under UV light for fluorescence, both before and after treatment with an $AlCl_3$ reagent.[39,40]

2.2.2.2. Quantitative Spectrophotometric Analysis of Total Phenolic Content Expressed as Gallic Acid Equivalent

The total phenolic content was determined spectrophotometrically using a modified Folin–Ciocalteu method. To 0.1 mL of the test solution (pre-diluted at a ratio of 1:10), 0.1 mL of Folin–Ciocalteu reagent, 1.5 mL of distilled water, and 0.3 mL of a concentrated Na_2CO_3 solution were added. The mixture was incubated in the dark for 150 minutes. The absorbance of the resulting solution was measured at 760 nm using a ULab 108UV spectrophotometer. The total phenolic content was calculated as gallic acid equivalent (GAE) based on a calibration curve constructed under identical conditions, using gallic acid as the standard. All measurements were performed in triplicate to ensure data reliability.[41]

2.2.3. Detection and Quantitative Study of Flavonoids in Aqueous and Aqueous-Ethanolic Bran Extracts

2.2.3.1. Qualitative Analysis of Flavonoids

The qualitative analysis of flavonoids in the obtained aqueous-ethanolic extracts of oat and wheat bran was assessed using classical reactions: cyanidin reaction, reaction with iron(III) chloride, reaction with alkali, and reaction with lead acetate. The formation of a precipitate was observed.[37]

2.2.3.2. Quantitative Spectrophotometric Analysis of Total Flavonoid Content Expressed as Quercetin Equivalent

The total flavonoid content was determined using a modified spectrophotometric method based on the complexation reaction between flavonoids and $AlCl_3$. For this purpose, a 5% sodium nitrite ($NaNO_2$) solution, a 0.1 M sodium hydroxide (NaOH) solution, and a 10% aluminum chloride ($AlCl_3$) solution were prepared.

An aliquot of 0.2 mL of the bran extract was placed in a test tube and mixed with 0.8 mL of ethanol. Then, 0.06 mL of the 5% $NaNO_2$ solution was added, and the mixture was allowed to stand for 5 minutes. Subsequently, 0.06 mL of the 10% $AlCl_3$ solution was added, and the reaction was allowed to proceed for another 5 minutes. Next, 0.4 mL of the 0.1 M NaOH solution and 0.480 mL of ethanol were

added. The resulting mixture was incubated in the dark for 5 minutes. The absorbance of the final solution was measured at 510 nm using a ULab 108UV spectrophotometer. The total flavonoid content was calculated as quercetin equivalent (QE) using a calibration curve prepared under identical conditions with quercetin as the standard. All measurements were performed in triplicate to ensure data accuracy and reproducibility.[42]

2.2.4. Detection and Quantification of Amino Acids in Aqueous and Aqueous-Ethanolic Bran Extracts

The quantitative determination of amino acids was performed using a spectrophotometric method based on the well-established reaction between amino acids and ninhydrin, which yields a colored complex.[43] The optical density (absorbance) of the resulting compound was measured at a wavelength of 570 nm. Simultaneously, the absorbance of the ninhydrin-alanine reaction product was measured and used as a reference, as alanine is the predominant amino acid in the bran samples under investigation.

The amino acid content in the bran extracts was calculated as a percentage (X%) using the following formula:

$$X(\%) = \frac{A_x \cdot m_0}{A_0 \cdot m} \cdot 100 \qquad (1),$$

where A_x is the absorbance of the sample solution, A_0 is the absorbance of the standard alanine solution, m_0 is the mass of the standard alanine sample (g), and m is the mass of the extract used for analysis (g).

2.2.5. Assessment of the Antioxidant Activity of Aqueous-Ethanolic Bran Extracts on Rat Liver Hepatocytes under *In Vitro* Conditions of Free Radical Oxidation

2.2.5.1. Preparation of Rat Liver Homogenate

To prepare the homogenate, 5 mL of potassium phosphate buffer was added to 0.5 g of thawed and minced rat liver tissue. A 0.3 mL aliquot of the studied bran extract was then added to 0.3 mL of the homogenate. Control samples received an equivalent volume of solvent without the extract. To initiate lipid peroxidation and protein oxidative modifications, 0.3 mL of 2.8% $FeSO_4$ solution was added, followed 10 minutes later by 0.3 mL of 4% H_2O_2. The mixture was incubated for 2 hours. The reaction was terminated by the addition of 1.2 mL of 40% trichloroacetic acid, which also precipitated proteins. Samples were then centrifuged for 10 minutes at 5000 rpm. Control samples contained the respective extractants without the active compounds. Oxidative stress was evaluated using a single sample: thiobarbituric acid-reactive substances (TBARS) were measured in the supernatant, and protein carbonyl content in the precipitate, according to the method of V.I. Lushchak.[44]

2.2.5.2. Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation was assessed by quantifying TBARS, based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA). Under high temperature and acidic conditions, MDA reacts with TBA to form a colored trimethine product with an absorption maximum at λ =532 nm. To 2 mL of the supernatant, 1.5 mL of 0.8% TBA solution in 0.1 M HCl (pH 2.5) was added. The mixture was incubated in a water bath at 95–100°C for 60 minutes, cooled on ice, and extracted with 3 mL of butanol. After centrifugation for 10 minutes at 5000 rpm, absorbance was measured in the upper butanol phase at λ =532 nm using a ULAB 108 UV spectrophotometer. Protein content in the samples was determined using the Lowry method.[45] TBARS concentration was calculated using the following formula:

$$[\boldsymbol{TBARS}] = \frac{E \cdot V_1 \cdot V_2}{\varepsilon \cdot V \cdot C}\ \mu mol\ /\ mg\ \text{protein}, \qquad (2)$$

where E is absorbance of the test sample, ε is millimolar extinction coefficient (ε = 156 $cm^2/\mu mol$), V_1 is volume of butanol (mL), V_2 is volume of the sample (mL), V is volume of the supernatant (mL), and C is protein concentration in the supernatant (mg/mL).

2.2.5.3. Determination of Protein Carbonyl Groups (CG)

The degree of protein oxidative modifications was evaluated by measuring the formation of carbonyl groups on amino acid side chains. These were quantified *via* reaction with 2,4-dinitrophenylhydrazine (DNPH). To the protein pellets obtained after homogenate centrifugation, 1 mL of 1% DNPH in 2 M HCl was added. The mixture was vortexed and incubated at room temperature for 1 hour, followed by centrifugation for 10 minutes at 5000 rpm. The precipitates were washed three times with 1 mL of an ethanol–ethyl acetate (1:1) mixture and centrifuged each time under the same conditions. The washed pellets were then dissolved in 3 mL of 50% aqueous urea for 45 minutes. Any insoluble material was removed by centrifugation. Using a ULAB 108 UV spectrophotometer, absorbance of the supernatants was measured at a wavelength of 370 nm, which corresponds to 2,4-diphenylhydrazones formed. The content of protein carbonyl groups was calculated according to the following formula:

$$[CG] = \frac{\Delta D \cdot V_{samples}}{E_{370} \cdot C} nmol / mg\ protein, \quad (3)$$

where $\Delta D = D_{test} - D_{control}$ is the difference in absorbance between test and control samples, $V_{samples}$ is sample volume (3 mL), E_{370} is the molar extinction coefficient of DNPH (22,000 $M^{-1}cm^{-1}$), and C is total protein concentration (mg/mL).

2.2.5.4. Statistical Analysis

All data were expressed as mean ± standard error (M ± SE), with n = 5. Statistical significance of differences between experimental groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.[46]

3. Results and Discussion

To identify and analyze phenolic compounds in the bran extracts, qualitative testing was conducted. The results are summarized in Table 1.

The qualitative analysis confirmed the presence of phenolic compounds in all tested samples of both aqueous and aqueous-ethanolic bran extracts at varying concentrations.

Thin-layer chromatography (TLC) was employed to further identify specific phenolic compounds. By comparing the chromatographic profiles of the test samples with those of standard reference compounds, individual phenolic constituents were identified. The findings are presented in Table 2.

Table 1. Qualitative identification of phenolic compounds in aqueous and aqueous-ethanolic bran extracts

Qualitative reaction	Expected result	Studied extract					
with iron(III) chloride solution	red color	+	±	+	+	+	+
with a mixture of iron(III) chloride and potassium hexacyanoferrate(III) solutions	blue-green color	+	+	+	+	+	+
with bromothymol blue solution	yellow color	+	+	+	+	±	+
with vanillin in concentrated hydrochloric acid	red color	+	+	±	+	+	+
with a mixture of iron(III) chloride, potassium hexacyanoferrate(III), and concentrated nitric acid	dark brown color	+	±	+	+	+	+

Note: 1-W is 40% aqueous-ethanolic extract of wheat bran; 2-W is aqueous extract of wheat bran; 3-W is 70% aqueous-ethanolic extract of wheat bran; 1-O is 40% aqueous-ethanolic extract of oat bran; 2-O is aqueous extract of oat bran; 3-O is 70% aqueous-ethanolic extract of oat bran; + is positive qualitative reaction and appearance of characteristic color; ± is weak color of qualitative reaction.

Table 2. TLC results for phenolic compounds in aqueous and aqueous-ethanolic bran extracts

#	Bran extracts		Identified compound		
			Gallic acid	*Quercetin*	*Ferulic acid*
1	oat	aqueous	n/i	R_f 0.90	R_f 0.98
2	oat	40% aqueous-ethanolic	R_f 0.34	R_f 0.89	R_f 0.95
3	oat	70% aqueous-ethanolic	R_f 0.34	R_f 0.87	R_f 0.99
4	wheat	aqueous	n/i	R_f 0.89	R_f 0.99
5	wheat	40% aqueous-ethanolic	R_f 0.34	R_f 0.90	R_f 0.95
6	wheat	70% aqueous-ethanolic	R_f 0.34	R_f 0.87	R_f 0.97

Note: n/i is not identified

TLC analysis of the studied oat and wheat bran extracts revealed the presence of several phenolic compounds, which were identified based on their characteristic coloration under both visible and ultraviolet light:

1) gallic acid (yellow-green coloration) was detected in both the 40% aqueous-ethanolic and 70% aqueous-ethanolic extracts of oat and wheat bran, with a retention factor (R_f) of 0.34 in all cases;

2) quercetin (bright green-yellow coloration) was identified in all tested extracts, including the aqueous oat and 40% aqueous-ethanolic wheat bran extracts (R_f = 0.90), the 70% aqueous-ethanolic oat and wheat bran extracts (R_f = 0.87), as well as the 40% aqueous-ethanolic oat and aqueous wheat bran extracts (R_f = 0.89);

3) ferulic acid (blue coloration) was detected in the aqueous oat and wheat bran extracts (R_f = 0.98 and 0.99, respectively), in the 40% aqueous-ethanolic oat and wheat

bran extracts (R_f = 0.95), and in the 70% aqueous-ethanolic oat and wheat extracts (R_f = 0.99 and 0.97, respectively).

Qualitative data on the presence of flavonoids in the bran extracts, obtained from the experiments, are summarized in Table 3.

Analysis of the obtained qualitative data confirmed the presence of flavonoids in all experimental samples of the oat and wheat bran extracts. The total phenolic content was determined using a spectrophotometric method at a wavelength of 760 nm and expressed as gallic acid equivalents. To ensure data reliability, each measurement was performed in triplicate. Quantitative calculations were based on a standard calibration curve, described by the following equation: y = 0.4130x + 0.0253.

Table 3. Qualitative identification of flavonoids in aqueous and aqueous-ethanolic bran extracts

Qualitative reaction	Expected result	Studied extract					
		1-W	2-W	3-W	1-O	2-O	3-O
with 10% iron(III) chloride solution	brown color	+	±	+	+	+	+
with lead acetate	precipitate	+	+	+	+	+	+
with alkali	yellow color	+	+	+	±	+	+
cyanidin reaction	change from yellow to red color	±	+	+	+	±	+

Note: 1-W is 40% aqueous-ethanolic extract of wheat bran; 2-W is aqueous extract of wheat bran; 3-W is 70% aqueous-ethanolic extract of wheat bran; 1-O is 40% aqueous-ethanolic extract of oat bran; 2-O is aqueous extract of oat bran; 3-O is 70% aqueous-ethanolic extract of oat bran; + is positive qualitative reaction and appearance of characteristic color; ± is weak color of qualitative reaction.

The total flavonoid content in the bran extracts was determined using a modified spectrophotometric method at a wavelength of 510 nm, based on the formation of a complex between flavonoids and aluminum chloride. Quercetin was used as the reference standard. Measurements were performed in triplicate for statistical validity. The flavonoid content was expressed as milligrams of quercetin equivalent per gram (mg/g) of extract. Quantification was based on a standard calibration curve for freshly prepared quercetin solutions, described by the following equation: y = 0.3142x + 0.1925.

The results for the total phenolic and flavonoid contents are presented in Table 4.

Table 4. Total phenolic content expressed as gallic acid equivalent and total flavonoid content expressed as quercetin equivalent in aqueous and aqueous-ethanolic bran extracts

Bran extracts		Total phenolic content, mg/g $\bar{x} \pm \Delta\bar{x}$, n=3	Total flavonoid content, mg/g $\bar{x} \pm \Delta\bar{x}$, n=3
Oat	aqueous	0.11 ± 0.01	0.98 ± 0.02
	20% aqueous-ethanolic	0.09 ± 0.004	0.66 ± 0.01
	40% aqueous-ethanolic	0.91 ± 0.002	0.53 ± 0.03
	70% aqueous-ethanolic	0.33 ± 0.001	0.79 ± 0.01
Wheat	aqueous	0.16 ± 0.01	0.47 ± 0.04
	20% aqueous-ethanolic	0.51 ± 0.003	1.97 ± 0.03
	40% aqueous-ethanolic	0.06 ± 0.001	0.53 ± 0.01
	70% aqueous-ethanolic	0.06 ± 0.002	0.79 ± 0.05

Quantitative analysis further confirmed the presence of flavonoids in all tested bran extracts. The highest flavonoid content was recorded in the 20% aqueous-ethanolic extract of wheat bran (1.97 ± 0.03 mg/g) and the aqueous extract of oat bran (0.98 ± 0.02 mg/g).

For the detection and quantification of amino acids, aqueous and 70% aqueous-ethanolic extracts were selected. The subsequent analysis verified the presence of amino acids in the selected bran extracts.

The amino acid content in the bran extracts, determined spectrophotometrically *via* the ninhydrin reaction and expressed as a percentage (X%) of alanine equivalent, was as follows: 1.48 ± 0.01% and 12.28 ± 0.01% for the aqueous extracts of oat and wheat bran, respectively, and 3.51 ± 0.01% and 7.09 ± 0.02% for the 70% aqueous-ethanolic extracts of oat and wheat bran, respectively.

The antioxidant activity of the bran extracts was evaluated using rat liver hepatocytes under conditions of *in vitro* free radical oxidation initiation. Based on the results of the lipid peroxidation assays (Fig. 1), no clear correlation was observed between the ethanol concentration in the

extraction solvents and the antioxidant activity of the bran extracts. Compared to the control, MDA levels in the water-based and aqueous-ethanolic (40% and 70%) extracts were as follows: 116 ± 2.1%, 109.5 ± 7.8%, and 124.9 ± 14.3% for oat bran, and 116.2 ± 7.5%, 128.4 ± 9.4%, and 106 ± 5.4% for wheat bran, respectively.

Table 5. Total amino acid content expressed as alanine equivalent in aqueous and aqueous-ethanolic bran extracts (n = 3)

Extracts of oat and wheat bran	**Total amino acid content expressed as alanine equivalent, %**			
	Aqueous wheat	Aqueous oat	70% aqueous-ethanolic wheat	70% aqueous-ethanolic oat
	2.28 ± 0.01	1.48 ± 0.01	7.09 ± 0.02	3.51 ± 0.01

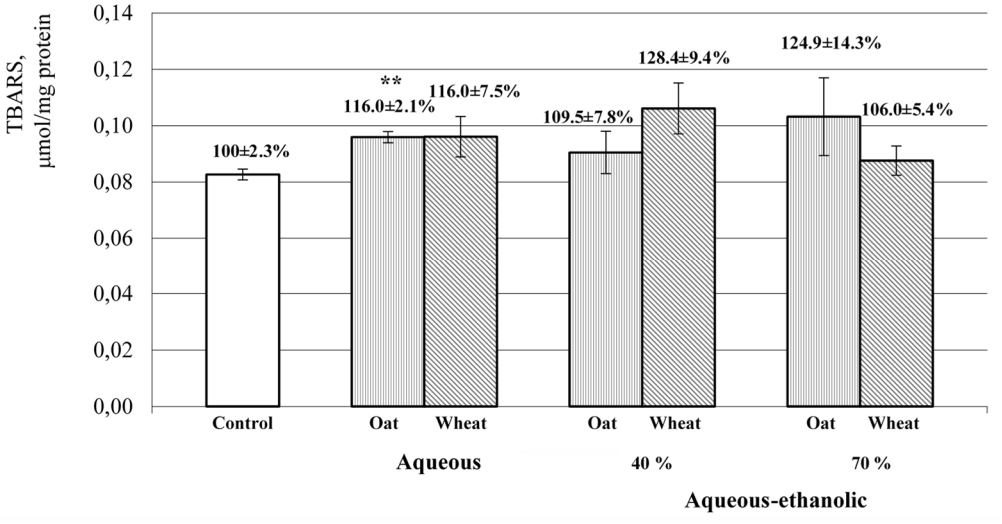

Fig. 1. TBARS content in rat liver homogenate under the action of aqueous and aqueous-ethanolic bran extracts (**- p≤0.01; M ± SE; n = 5)

The data presented in Fig. 2 demonstrate a clear, almost linear increase in the number of carbonyl groups relative to the control samples with increasing ethanol concentration in the extraction solvents. Specifically, the levels of carbonyl groups in the aqueous and aqueous-ethanolic extracts of oat bran were 93.4 ± 1.8%, 105.3 ± 5.6%, and 114.7 ± 8.6%, while for wheat bran they were 79.8 ± 8.8%, 89.3 ± 19.1%, and 108.2 ± 4.0%, respectively.

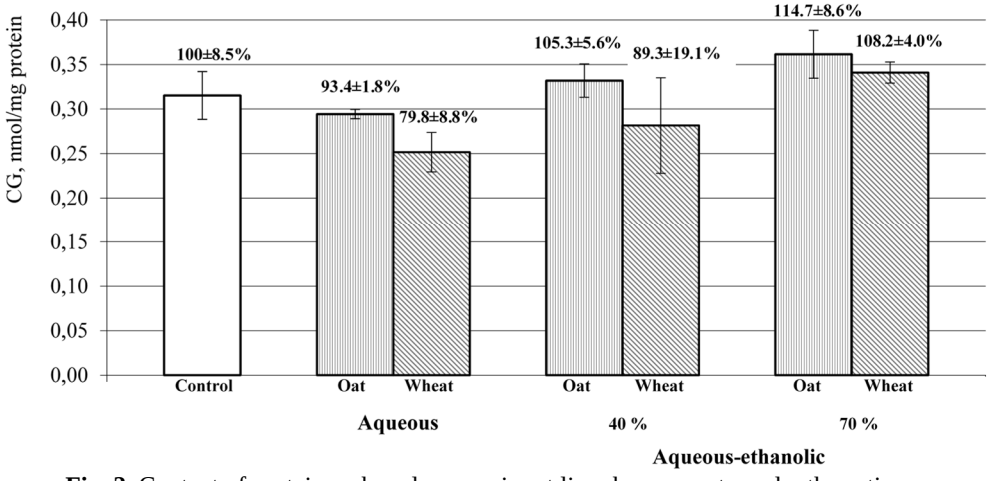

Fig. 2. Content of protein carbonyl groups in rat liver homogenate under the action of aqueous and aqueous-ethanolic bran extracts (M ± SE; n = 5)

As shown in Figs. 1 and 2, the tested bran extracts did not reduce malondialdehyde concentrations. Among the aqueous-ethanolic extracts, wheat bran demonstrated the highest effectiveness in modulating oxidative stress indicators. Although not statistically significant, the aqueous and 40% aqueous-ethanolic extracts of wheat bran showed the greatest reduction in protein oxidative modifications.

In summary, the available data indicate that, at the non-enzymatic level, the extracts did not exert a statistically significant and interdependent effect on lipid peroxidation and protein oxidative modification processes in rat liver cells. These findings suggest that further investigation into the enzymatic mechanisms underlying these processes may be warranted.

4. Conclusions

According to the results of qualitative and quantitative analysis, the presence of **biologically active substances** (BAS), including phenolic compounds, flavonoids, and amino acids, was established in aqueous and aqueous-ethanolic extracts of bran obtained from the outer layers of oat and wheat grains. Using thin-layer chromatography (TLC), gallic acid, ferulic acid, and quercetin were identified in the samples, confirming the antioxidant potential of the studied raw materials.

Despite this, the results of *in vitro* studies of antioxidant activity using a rat hepatocyte model under conditions of induced free radical oxidation revealed no statistically significant reduction in the levels of lipid peroxidation and oxidative protein modifications under the influence of bran extracts. Although increasing the ethanol concentration in the extractants did not demonstrate a reliable correlation with the degree of oxidative protein damage, graphical data indicated a tendency toward direct proportionality. Among the studied samples, wheat extracts exhibited higher antioxidant activity compared to oat extracts, which is likely due to a higher content of phenolic compounds, primarily ferulic acid, localized in the outer layers of the grain.

In addition to their direct antioxidant potential, wheat bran also exerts an indirect influence on the body's antioxidant status by modulating the composition of the gut microbiota. It demonstrates prebiotic activity, promoting the growth of beneficial microorganisms capable of fermenting fiber and releasing phenolic metabolites from their bound forms.

It was established that the concentration of the identified **BAS** is not the sole factor determining the antioxidant efficacy of bran extracts. Therefore, further investigation into the mechanisms of antioxidant action at the level of enzymatic cell defense, as well as an expansion of the range of identified **BAS**, is warranted. Given the presence of valuable biologically active substances, oat and wheat bran possess significant potential for use in functional foods, cosmetic products, and dietary supplements. Their inclusion in the diet may serve as an effective approach to enhancing the body's endogenous antioxidant defense, opening up prospects for the prevention of oxidative stress-related pathologies and substantiating the relevance of continued research in this area.

Abbreviations

BAS – biologically active substances
DNPH – 2,4-dinitrophenylhydrazine
FA – ferulic acid
GAE – gallic acid equivalent
HCAs – hydroxycinnamic acids
HPLC – high-performance liquid chromatography
M – mean
MDA – malondialdehyde
QE – quercetin equivalent
SE – standard error
TBA – thiobarbituric acid
TBARS – thiobarbituric acid reactive substances
TLC – thin-layer chromatography

References

[1] Praphasawat, R; Palipoch, S; Suwannalert, P; Payuhakrit, W; Kunsorn, P; Laovitthayanggoon, S; Thakaew, S; Munkong, N; Klajing, W. Red Rice Bran Extract Suppresses Colon Cancer Cells *via* Apoptosis Induction/Cell Cycle Arrest and Exerts Antimutagenic Activity. *Exp. Oncol.* **2023**, *45*, 220–230. https://doi.org/10.15407/exp-oncology.2023.02.220

[2] Reddy, S.S.; Krishnan, C. Production of Prebiotics and Antioxidants as Health Food Supplements from Lignocellulosic Materials Using Multienzymatic Hydrolysis. *Int. J. Chem. Sci.* **2010**, *8*, S535–S549.

[3] Kapreliants, L.; Zhurlova, O. Technology of Wheat and Rye Bran Biotransformation into Functional Ingredients. *Int. Food Res. J.* **2017**, *24*, 1975–1979. http://www.ifrj.upm.edu.my/volume-24-2017.html

[4] Grande, S.; Bogani, P.; de Saizieu A.; Schueler, G.; Galli, C.; Visioli, F. Vasomodulating Potential of Mediterranean Wild Plant Extracts. *J. Agric. Food Chem.* **2004**, *52*, 5021–5026. https://doi.org/10.1021/jf049436e

[5] Yu, L.; Perret, J.; Harris, M.; Wilson, J.; Haley, S. Antioxidant Properties of Bran Extracts from "Akron" Wheat Grown at Different Locations. *J. Agric. Food Chem.* **2003**, *51*, 1566–1570. https://doi.org/10.1021/jf020950z

[6] Georgé, S.; Brat, P.; Alter, P.; Amiot, M.J. Rapid Determination of Polyphenols and Vitamin C in Plant-Derived Products. *J. Agric. Food Chem.* **2005**, *53*, 1370–1373. https://doi.org/10.1021/jf048396b

[7] Elhaty, I.A.; Zeyoudi, S.A. A. Comparative Study of the Phenolic and Flavonoids Contents, and Antioxidant Activity of *Ziziphus Mauritiana*'s Leaves, Ripe and Unripe Fruit Extracts

from UAE. *Chem. Chem. Technol.* **2024**, *18*, 363–371. https://doi.org/10.23939/chcht18.03.363
[8] Asma, F.; Salah Eddine, H.; Yassmine, C.; Hanane, Z. Phytochemical Screening, Antibacterial and Antioxidant Activities of *Ocimum basilicum* L. Cultivated in Biskra, Algeria. *Chem. Chem. Technol*. **2023**, *17*, 397–406. https://doi.org/10.23939/chcht17.02.397
[9] Wijesooriya, S.S.; Pandithavidana, D.R. Investigation and Comparison of Antioxidant Potential of Catechins Present in Green Tea: DFT Study. *Chem. Chem. Technol*. **2022**, *16*, 591–599. https://doi.org/10.23939/chcht16.04.591
[10] Rebolleda, S.; González-San José, M.L.; Sanz, M.T.; Beltrán, S.; Solaesa, Á.G. Bioactive Compounds of a Wheat Bran Oily Extract Obtained with Supercritical Carbon Dioxide. *Foods* **2020**, *9*, 625. https://doi.org/10.3390/foods9050625
[11] *Prebiotics: Development and Application.* Gibson, G.R., Rastall, R.A., Eds.; John Wiley & Sons, Ltd., 2006. https://doi.org/10.1002/9780470023150
[12] Dwivedi, S.; Sahrawat, K.; Puppala, N.; Ortiz, R. Plant Prebiotics and Human Health: Biotechnology to Breed Prebioticrich Nutritious Food Crops. *Electron. J. Biotechnol.* **2014**, *17*, 238–245. https://doi.org/10.1016/j.ejbt.2014.07.004.
[13] Singh, R.D.; Banerjee, J.; Arora, A. Prebiotic Potential of Oligosaccharides: A Focus on Xylan Derived Oligosaccharides. *Bioact. Carbohydr. Dietary Fibre* **2015**, *5*, 19–30. https://doi.org/10.1016/j.bcdf.2014.11.003
[14] Collins, H.M.; Burton, R.A.; Topping, D.L.; Liao, M.-L.; Bacic, A.; Fincher, G.B. Review: Variability in Fine Structures of Noncellulosic Cell Wall Polysaccharides from Cereal Grains: Potential Importance in Human Health and Nutrition. *Cereal Chem.* **2010**, *87*, 272–282. https://doi.org/10.1094/CCHEM-87-4-0272
[15] Chlopicka, J.; Pasko, P.; Gorinstein, S.; Jedryas, A.; Zagrodzki, P. Total Phenolic and Total Flavonoid Content, Antioxidant Activity and Sensory Evaluation of Pseudocereal Breads. *LWT Food Sci. Technol.* **2012**, *46*, 548–555. https://doi.org/10.1016/j.lwt.2011.11.009
[16] Balasubashini, M.S.; Rukkumani, R.; Viswanathan, P.; Menon, V.P. Ferulic Acid Alleviates Lipid Peroxidation in Diabetic Rats. *Phytother. Res.* **2004**, *18*, 310–314. https://doi.org/10.1002/ptr.1440
[17] Andersson, A.A.M.; Lampi A.-M.; Nyström, L.; Piironen, V.; Li, L.; Ward, J.L.; Gebruers, K.; Courtin, C.M.; Delcour, J.A.; Boros, D.; Fraś, A.; *et al.* Phytochemical and Dietary Fiber Components in Barley Varieties in the HEALTHGRAIN Diversity Screen. *J. Agric. Food Chem.* **2008**, *56*, 9767–9776. https://doi.org/10.1021/jf802037f
[18] Martinez, A.W.; Phillips, S.T.; Butte, M.J.; Whitesides, G.M. Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays. *Angew. Chem. Int. Ed.* **2007**, *46*, 1318–1320. https://doi.org/10.1002/anie.200603817
[19] Sharma, O.P.; Bhat, T.K.; Singh, B. Thin-Layer Chromatography of Gallic Acid, Methyl Gallate, Pyrogallol, Phloroglucinol, Catechol, Resorcinol, Hydroquinone, Catechin, Epicatechin, Cinnamic Acid, *p*-Coumaric Acid, Ferulic Acid and Tannic Acid. *J. Chromatogr. A* **1998**, *822*, 167–171. https://doi.org/10.1016/S0021-9673(98)00490-7
[20] Borges, M.F.M.; Pinto, M.A.M.M. Separation of the Diastereoisomers of Ethyl Esters of Caffeic, Ferulic, and Isoferulic Acids by Thin-Layer and High Performance Liquid Chromatography. *J. Liq. Chromatogr.* **1994**, *17*, 1125–1139. https://doi.org/10.1080/10826079408013390
[21] Sheng, Y.X.; Li, L.; Wang, Q.; Guo, H.Z.; Guo, D.A. Simultaneous Determination of Gallic Acid, Albiflorin, Paeoniflorin, Ferulic Acid and Benzoic Acid in Si-Wu Decoction by High-Performance Liquid Chromatography DAD Method. *J. Pharm. Biomed. Anal.* **2005**, *37*, 805–810. https://doi.org/10.1016/j.jpba.2004.11.002
[22] Luo, L.; Wang, X.; Li, Q.; Ding, Y.; Jia, J.; Deng, D. Voltammetric Determination of Ferulic Acid by Didodecyldimethyl-Ammonium Bromide/Nafion Composite Film-Modified Carbon Paste Electrode. *Anal. Sci.* **2010**, *26*, 907–911. https://doi.org/10.2116/analsci.26.907
[23] Li, L.-J.; Yu, L.-B.; Chen, Q.-F.; Cheng, H.; Wu, F.-M.; Wu, J.-L.; Kong, H.-X. Determination of Ferulic Acid Based on *L*-Cysteine Self-Assembled Modified Gold Electrode Coupling Irreversible Biamperometry. *Chin. J. Anal. Chem.* **2007**, *35*, 933–937. https://doi.org/10.1016/S1872-2040(07)60060-7
[24] Hamada, V.; Krvavych, A.; Konechna, R.; Mylyanych, A.; Buchkevych, I.; Holubieva, T.; Lubenets, V. Development of Technology for Obtaining Herbal Extracts of Adonis Vernalis. *Lett. Appl. NanoBioSci.* **2023**, *12*, 78. https://doi.org/10.33263/LIANBS123.078
[25] Mullen, W.; Stewart, A.J.; Lean, M.E.J.; Gardner, P.; Duthie, G.G.; Crozier, A. Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant Capacity of Red Raspberries. *J. Agric. Food Chem.* **2002**, *50*, 5197–5201. https://doi.org/10.1021/jf020141f
[26] Mullen, W.; McGinn, J.; Lean, M.E.J.; MacLean, M.R.; Gardner, P.; Duthie, G.G.; Yokota, T.; Crozier, A. Ellagitannins, Flavonoids, and Other Phenolics in Red Raspberries and Their Contribution to Antioxidant Capacity and Vasorelaxation Properties. *J. Agric. Food Chem.* **2002**, *50*, 5191–5196. https://doi.org/10.1021/jf020140n
[27] Kaur, R.; Ahluwalia, P.; Sachdev, P.A.; Kaur, A. Development of Gluten-Free Cereal Bar for Gluten Intolerant Population by Using Quinoa as Major Ingredient. *J. Food Sci. Technol.* **2018**, *55*, 3584–3591. https://doi.org/10.1007/s13197-018-3284-x.
[28] Kaprelyants, L.; Zhurlova, O. Biotechnological Approaches for the Production of Functional Foods and Supplements from Cereal Raw Materials. *Food Sci. Technol.* **2014**, *2*, 15–19. https://doi.org/10.15673/2073-8684.27/2014.29697
[29] Dias, F.S.; David, J.M.; David, J.P. Determination of Phenolic Acids and Quercetin in Brazilian Red Wines from Vale do São Francisco Region Using Liquid-Liquid Ultrasound-Assisted Extraction and HPLC-DAD-MS. *J. Braz. Chem. Soc.* **2016**, *27*, 1055–1059. https://doi.org/10.5935/0103-5053.20150363
[30] Kaprelyants, L.; Yegorova, A.; Trufkati, L.; Pozhitkova, L. Functional Foods: Prospectots in Ukraine. *Food Sci. Technol.* **2019**, *13*, 15–23. https://doi.org/10.15673/fst.v13i2.1382
[31] Alves, G.H.; Ferreira, C.D.; Vivian, P.G.; Monks, J.L.F.; Elias, M.C.; Vanier, N.L.; de Oliveira, M. The Revisited Levels of Free and Bound Phenolics in Rice: Effects of the Extraction Procedure. *Food Chem.* **2016**, *208*, 116–123. https://doi.org/10.1016/j.foodchem.2016.03.107
[32] Perales-Sánchez, J.X.K.; Reyes-Moreno, C.; Gómez-Favela, M.A.; Milán-Carrillo, J.; Cuevas-Rodríguez, E.O.; Valdez-Ortiz, A.; Gutiérrez-Dorado, R. Increasing the Antioxidant Activity, Total Phenolic and Flavonoid Contents by Optimizing the Germination Conditions of Amaranth Seeds. *Plant Foods Hum. Nutr.* **2014**, *69*, 196–202. https://doi.org/10.1007/s11130-014-0430-0

[33] Hole, A.S.; Grimmer, S.; Jensen, M.R.; Sahistrøm, S. Synergistic and Suppressive Effects of Dietary Phenolic Acids and Other Phytochemicals from Cereal Extracts on Nuclear Factor Kappa B Activity. *Food Chem.* **2012**, *133*, 969–977. https://doi.org/10.1016/j.foodchem.2012.02.017
[34] Bazavluk, Y.; Vanko, R.; Konechnyi, Y.; Konechna, R. Obtaining and Research of Callus Biomass of Some Plants of the Family Ranunculacae. *Herba Polonica* **2023**, *69*, 45–57. https://doi.org/10.5604/01.3001.0053.9652
[35] Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G.; Kaur, H. Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia* **2011**, *1*, 98-106.
[36] Blyznyuk, N.; Prokopenko, Yu.; Georgiyants, V. Development of Methods for Determination of Phenolic Acids and Flavonoids in Capsules Containing *Corylus avellana* L. Dry Extract. *ScienceRise: Pharmaceutical Science* **2016**, *2*, 18–22. https://doi.org/10.15587/2313-8416.2016.61495
[37] Vitalini, S.; Beretta, G.; Iriti, M.; Orsenigo, S.; Basilico, N.; Dall'Acqua, S.; Iorizzi, M.; Fico, G. Phenolic Compounds from *Achillea millefolium* L. and Their Bioactivity. *Acta Biochim. Pol.* **2011**, *58*, 203–219. https://doi.org/10.18388/abp.2011_2266
[38] Karpiuk, V.R. Research on the Development of a Complex Extract Based on Plants of the Ranunculaceae Family. *Chemistry, Technology and Application of Substances* **2022**, *5*, 94–99. https://doi.org/10.23939/ctas2022.02.094
[39] Tee-ngam, P.; Nunant, N.; Rattanarat, P.; Siangproh, W.; Chailapakul, O. Simple and Rapid Determination of Ferulic Acid Levels in Food and Cosmetic Samples Using Paper-Based Platforms. *Sensors* **2013**, *13*, 13039–13053. https://doi.org/10.3390/s131013039
[40] Stanek, N.; Jasicka-Misiak, I. HPTLC Phenolic Profiles as Useful Tools for the Authentication of Honey. *Food Anal. Methods* **2018**, *11*, 2979–2989. https://doi.org/10.1007/s12161-018-1281-3
[41] Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S.; Ju, Y.-H. Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of Limnophila aromatica. *J. Food Drug Anal.* **2014**, *22*, 296–302. https://doi.org/10.1016/j.jfda.2013.11.001
[42] Karpiuk, V.; Konechnyi, Y.; Yaremkevych, O.; Karpiuk, I.; Mylyanych, A.; Krvavych, A.; Konechna, R. Study of the Content of Phenolic Compounds, Antimicrobial and Antioxidant Properties of the Herb Caltha palustris. *Res. J. Pharm. Technol.* **2024**, *17*, 5673-5679. https://doi.org/10.52711/0974-360X.2024.00864
[43] Zarivna, N.O.; Horlachuk, N.V. Vyznachennia kilkisnoho vmistu aminokyslot u ridkomu ekstrakti chebretsiu povzuchoho, vybir kryteriiv pryjniatnosti. *Medychna ta klinichna khimia* **2022**, *1*, 77–80. https://doi.org/10.11603/mcch.2410-681X.2022.i1.13041
[44] Lushchak, V.I.; Bahniukova, T.V.; Luzhna, L.I. Pokaznyky oksydatyvnoho stresu. 2. Peroksydy lipidiv. *Ukr. Biokhim. Zh.* **2006**, *78*, 113–119. (in Ukrainian)
[45] Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6
[46] Morgan, G.A.; Leech, N.L.; Gloeckner, G.W.; Barrett, K.C. *IBM SPSS for Introductory Statistics. Use and Interpretation*, 4th ed.; Taylor & Francis Group, 2012.

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ДОСЛІДЖЕННЯ ЕКСТРАКТІВ ПШЕНИЧНИХ І ВІВСЯНИХ ВИСІВОК І ЇХНІХ АНТИОКСИДАНТНИХ ВЛАСТИВОСТЕЙ

Анотація. *У статті наведено результати дослідження водних і водно-етанольних екстрактів, отриманих з клітинної стінки вівсяних і пшеничних висівок, на наявність у них фенольних сполук, флавоноїдів і амінокислот. Загальний вміст фенолів оцінювали спектрофотометрично за методом Фоліна-Чокальтеу, а загальний вміст флавоноїдів визначали колориметричним аналізом з хлоридом алюмінію. У зразках методом тонкошарової хроматографії ідентифіковано галову кислоту, ферулову кислоту та кверцетин. Антиоксидантну активність екстрактів оцінювали з використанням гепатоцитів печінки щурів в умовах вільнорадикального окислення* in vitro. *Результати показали, що водно-етанольні екстракти пшеничних і вівсяних висівок істотно не знижують перекисне окислення ліпідів та окисну модифікацію протеїнів. Однак екстракти пшеничних висівок показали вищу антиоксидантну активність, ніж екстракти вівсяних висівок.*

Ключові слова: *висівкові екстракти, біологічно активні сполуки, біологічна активність, антиоксидантна активність, окисна модифікація протеїнів, тонкошарова хроматографія, малоновий діальдегід.*