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Effects of extraction, purification, and drying on the antioxidant capacity of wheat bran extracts

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ABSTRACT

Background: Antioxidants (AO) present in wheat bran (WB) contribute to the prevention of chronic diseases such as cancer and type 2 diabetes. The choice of extraction technology is critical for obtaining biologically active compounds from plant material. Depending on the processing conditions, extracts obtained from the same raw material may vary in compositions, which directly affect their antioxidant potential (AOP).

Objective: This study aimed to evaluate the effect of different extraction, purification, and freeze-drying methods on the antioxidant potential (total antioxidant capacity – TAC, total phenolic compounds – TPC, total flavonoid compounds – TFC), as well as on protein and reducing sugar contents of wheat bran extracts, in order to identify the most effective approach for producing antioxidant-rich dry extracts.

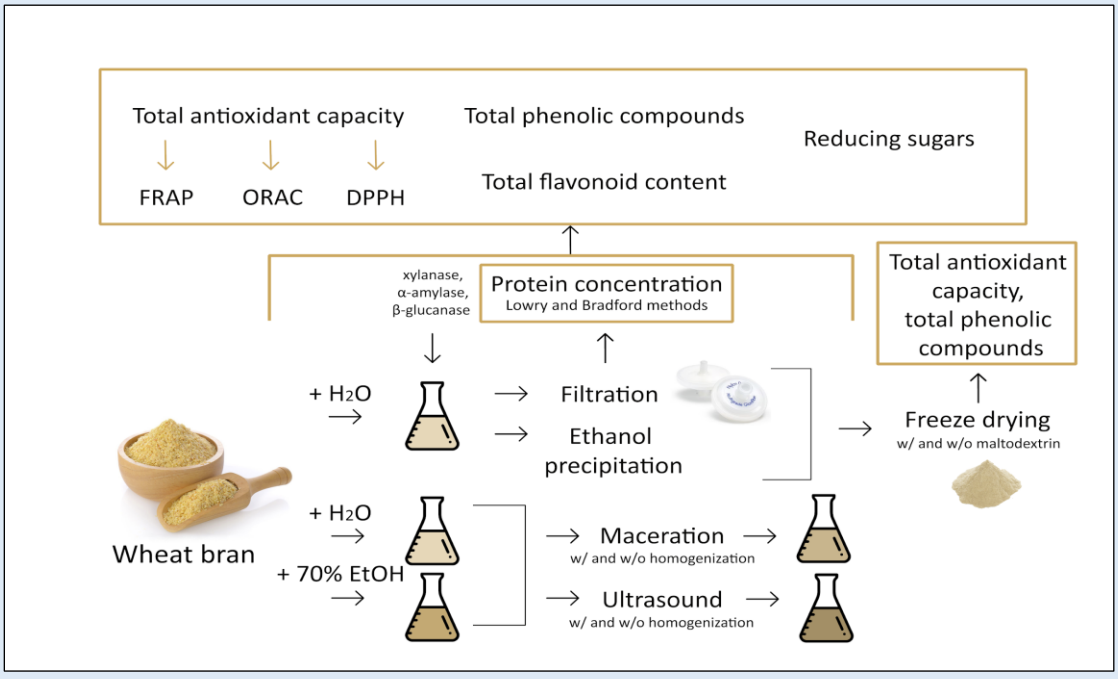
Methods: Wheat bran extracts were prepared via maceration (MM, aqueous and ethanol), ultrasound-assisted extraction (UAE, aqueous and ethanol), and enzymatic aqueous extraction (EAE). TAC was evaluated using FRAP, ORAC and DPPH assays; , TPC by the Folin-Ciocalteu method, and TFC by the aluminum chloride method. Extracts were purified

by either nylon-membrane filtration or ethanol (96%) precipitation and then freeze-dried with or without maltodextrin (MD). Reducing sugars and proteins were quantified using the sulfuric acid method and Lowry/Bradford assays, respectively. Statistical analysis was conducted with STATISTICA 10.0.

Results: Enzymatic aqueous extraction (EAE) produced the highest TACFRAP (1,64 ± 0,03 µmol-eq. Q/ g of bran, $p < 0.05$) and the highest TPC (1.39 ± 0.12 µmol-eq. G/g of bran). The ethanolic UAE exhibited the highest TACORAC and TACDPPH (8,12 ± 0,27 and 0,89 ± 0,001 µmol-eq. Q/g of bran, respectively, $p < 0.05$) but had lower TPC and TFC compared to other extracts. Ethanol precipitation reduced protein content by 84-92% and sugars by 40% ($p < 0.05$), while increasing TACFRAP and TACORAC by over 60% ($p < 0.05$). Freeze-drying preserved high TAC values in both control and filtered extracts, regardless of maltodextrin (MD) addition

Conclusion: The choice of extraction method strongly affects the recovery of antioxidants from wheat bran. Enzymatic extraction combined with ethanol precipitation and freeze-drying produces extracts with enhanced antioxidant potential, reduced impurities, and preserved bioactivity, representing an effective strategy for obtaining antioxidant-rich wheat bran extracts for functional food and nutraceutical applications. This study provides the first comprehensive evaluation of this combined approach, offering a practical method for producing high-quality, antioxidant-rich wheat bran extracts.

Keywords: plant antioxidants, phenolic compounds, ultrasonic extraction, enzymatic extraction, freeze-drying, ethanol precipitation, optimization, functional food, nutraceutical applications



Graphical Abstract: Effects of extraction, purification, and drying on the antioxidant capacity of wheat bran extracts

INTRODUCTION

The food industry has shown increasing interest in utilizing secondary resources to reduce anthropogenic impact and support the circular economy [1]. Cereal crops, particularly wheat, account for approximately 60% of arable land in Russia, covering up to 29 million hectares [2]. Wheat processing generates up to 25% by-products, including bran, of which 85% is used in animal feed, biofuels, and functional foods, while the remainder is typically discarded [3-4].

Wheat bran (WB), comprising the pericarp, seed coat, and aleurone layer (≈7% of grain mass), contains up to 53% dietary fiber—including xylans, lignin, and cellulose—along with up to 16% protein, B-group, E, and C vitamins, minerals, and phenolics [5-7]. The aleurone layer alone contains over 38% protein, 9-10% fat, 15% fiber, and water-soluble vitamins B_1, B_2, and PP [8-9]. Phenolic compounds, particularly ferulic acid (≈95% of total phenolics), are key contributors to WB's high antioxidant potential and are widely recognized as essential components for functional food development [10]. These phenolics exist in both free and bound forms, the latter esterified to arabinoxylans (AX), which constitute 60-70% of the 35-50% non-starch polysaccharides [11-12]. AX serves as a reservoir for phenolic compounds; however, their covalent bonds with lignin and AX hinder efficient extraction [13].

Phenolic compounds and polysaccharides have demonstrated the ability to inhibit α-glucosidase activity, potentially modulating postprandial blood glucose levels by slowing the digestion and absorption of dietary carbohydrates [14]. The complex structure of WB necessitates effective strategies for disrupting cell walls to release biologically active compounds (BAC), which comprise a diverse range of phytochemicals that provide health benefits beyond basic nutrition, potentially aiding in the prevention and management of chronic diseases [15]. Mechanical milling increases surface area and can double antioxidant extract capacity [16], though it may cause local heating and degradation of thermolabile components [17]. Traditional solid–liquid extraction and maceration are now complemented by ultrasonic, microwave-assisted, supercritical fluid, and enzymatic extraction techniques [18-20]. Enzymes such as xylanases, cellulases, and esterases hydrolyze polysaccharide bonds, enhancing phenolic availability [21]. Alkaline hydrolysis is particularly effective for releasing bound phenolics, whereas acid hydrolysis is less favorable due to degradation of sensitive components [22-24]. Extracts often contain impurities - such as sugars, proteins, and pigments - necessitating purification via filtration, centrifugation, precipitation, ultrafiltration, or adsorption [25]. In wheat bran fractionation, 96% ethanol is commonly used to precipitate AX, leaving phenolics in the supernatant [26]. To preserve bioactivity, freeze-drying is the preferred final step, as it maintains thermolabile phenolic acids [27]. Carriers such as maltodextrin, inulin, and gum arabic are used during drying to stabilize the extracts [28].

Thus, the efficiency of antioxidant recovery from wheat bran depends on an integrated approach to extraction, purification, and drying, considering both the structural features of the raw material and the sensitivity of biologically active compounds to processing conditions.

MATERIALS AND METHODS

Xylanase and β-glucanase (enzyme activity: 10000 U/g, food-grade preparation) was purchased from Biopreparat (Russia), α-amylase from Aspergillus oryzae

(enzyme activity: 30000 U/g, ≥ 95%) was purchased from Sigma Company (Switzerland). Quercetin (≥ 95%, HPLC), gallic acid (≥ 99%, HPLC) and glucose (≥ 99%, HPLC) was purchased from Sigma Company (India), bovine serum albumin (BSA, ≥ 99%) was purchased from Solarbio (China). Aluminium chloride hexahydrate (pharmpur) was purchased from ScharLab (Spain). Coomassie brilliant blue G-250 (≥ 95%) was purchased from Helicon (Russia). Phenol (≥99%) was purchased from Dia-m (Russia). Copper (II) sulfate · $5H_2O$ was purchased from Component-Reaktiv (Russia). Sulphuric acid (≥ 98%) was purchased from Acros Organics (Belgium). Fluorescein sodium salt (≥ 97%, USA), 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH, purity ≥ 97%, USA), quercetin (≥ 95%, India), and iron (III) chloride hexahydrate (≥ 99%, Germany), were purchased from Sigma-Aldrich. Sodium carbonate (≥ 99.5%), sodium hydroxide (≥ 98%), Folin-Ciocalteu, sodium acetate anhydrous (≥ 99%), were purchased from PanReac AppliChem (Spain). Dipotassium hydrogen phosphate anhydrous (≥ 98%), potassium dihydrogen phosphate (≥ 98%), hydrochloric acid (≥37%), were purchased from PanReac AppliChem (Germany). Furthermore, 2,4,4-tris(2-pyridyl)-1,3,5-triazine (TPTZ, purity ≥ 96%) was purchased from BLDpharm (Shanghai, China). Acetic acid (≥ 99.8%) was purchased from Component-Reaktiv (Moscow, Russia).

Wheat bran was obtained from class 1 grain grown in Tula region in Russia in 2023.

Preparation of Plant Extracts: Wheat bran was mixed with either distilled water or 70% aqueous-ethanol at a 1:5 (g:mL) ratio.

Water and ethanol extracts were obtained via maceration (MM), with or without prior homogenization. For homogenized samples, the mixture was processed for 2 min at 8000 rpm using a handheld homogenizer S10 (Stegler, China), followed by macerating for 24 h at 22±2 °C with periodic stirring. The mixture was then centrifuged at 3600 rpm for 10 min (LISTON C2204, Russia).

Ultrasonic-assisted extraction (UAE) was performed on both homogenized and non-homogenized samples (water and ethanol extracts). Samples were sonicated for 30 min at 60°C and 35 kHz in an ultrasonic bath (Sapphire, Russia) and subsequently centrifuged under the same conditions as above.

For enzymatic extraction (EAE), wheat bran was mixed with water (1:5, g/mL), followed by the addition of 2% (w/w) xylanase, 0.001% (w/w) α-amylase from *Aspergillus oryzae*, and 0.001% (w/w) β-glucanase. The mixture was homogenized and incubated in a shaker (Immunochem-2200, High Technology Inc., USA) at 60°C and 500 rpm for 3 h, then centrifuged at 3600 rpm for 10 min.

Samples were homogenized for 2 min at 8000 rpm using a handheld homogenizer (S10, Stegler, China) to disperse the bran–solvent mixture, increase the contact surface, and facilitate mass transfer during extraction.

For all extracts, after centrifugation the supernatants were collected and stored at −40 °C for up to two weeks until analysis. On the day of analysis, the extracts were thawed at 4 °C, gently mixed until complete dissolution, and centrifuged again at 4 °C for 8 min at 10,000 rpm (Eppendorf 5427 R, Germany) prior to subsequent assays.

The enzymatic extract (control) was further purified by filtration and precipitation. TFC, reducing sugars, and protein. Filtration was performed using a 0.2 µm, 25 mm nylon syringe filter (Teknokroma, Spain). For precipitation, the control extract was mixed with 96% ethanol (1:2, v/v), shaken, and centrifuged for 15 min at 3600 rpm (LISTON C2204); the supernatant was

collected. Purification efficiency was evaluated by measuring TAC_{FRAP}, TAC_{DPPH}, TAC_{ORAC}, TPC, TFC, reducing sugars, and protein.

Native (control) and purified extracts were freeze-dried. Portion of the native and filtration-purified extracts was mixed with maltodextrin (MD) (Zdorovaya semya ot A do Ya, Russia) to reach a total solids content of 20%, while other portions were dried without MD. The ethanol-precipitated extract was freeze-dried without MD due to its ethanol content. Freeze-drying was carried out using an Iney-6 unit (Russia) for 24 h at −40°C and 3.3 kPa.

Determination of Antioxidant Potential: Dry extracts were diluted in distilled water at the original mass-to-volume ratio.

Total antioxidant capacity by DPPH radical method was determined using an SF-2000 spectrophotometer (OCB Spectr, Russia) according to the procedure described in [29]. TAC_{DPPH} values were calculated from a quercetin calibration curve (100-250 µM, $R^2 > 0.99$) and expressed as µmol-eq. Q/g of bran or powder. Total antioxidant capacity by FRAP method was also assessed using the SF-2000 spectrophotometer following [30]. TAC_{FRAP} values were based on a quercetin calibration curve (140-300 µM, $R^2 > 0.99$) and expressed as µmol-eq. Q/g of bran or powder.

Total antioxidant capacity by ORAC method was determined using a Fluoroskan Ascent FL reader (Thermo Labsystems, Finland) with black 96-well plates according to [29]. TAC_{ORAC} was calculated using a quercetin calibration curve (1-14 µM, $R^2 > 0.99$) and expressed as µmol-eq. Q/g of bran or powder. Total phenolic compounds were measured using the Folin–Ciocalteu method on the SF-2000 spectrophotometer following [31]. TPC values were calculated from a gallic acid (G) calibration curve ($R^2 > 0.99$) and expressed as µmol-eq. G/g of bran or powder.

Total flavonoid compounds were determined using the aluminum chloride method [32]. A quercetin calibration curve (50-500 µM, $R^2 > 0.99$) was used, with results expressed as µmol-eq. Q/g of bran or powder.

Determination of Reducing Sugars and Protein Concentrations: Reducing sugars (RS) were measured according to [33] using the SF-2000 spectrophotometer. A glucose (Gl) calibration curve (10-400 µg/mL, $R^2 > 0.99$) was used to calculate concentrations, which were expressed as mg-eq. Gl/g of bran or powder.

Protein concentration was determined using two methods: Lowry method according to [34], with a calibration curve using albumin (0-100 µg/mL, $R^2 > 0.99$), and Bradford method according to [34], with a BSA calibration curve (0-50 µg/mL, $R^2 > 0.99$; Solarbio, China). Results were expressed as mg-eq. BSA/g of bran or powder.

Statistical Analysis: Data were analyzed using STATISTICA 10.0. Results were presented as the mean ± standard deviation (Mean±SD). One-way analysis of variance (ANOVA) followed by Tukey's test was used to assess the significance of differences between means. A p-value of 0.05 was considered statistically significant.

RESULTS

The antioxidant potential (AOP) of wheat bran extracts obtained using different extraction methods was evaluated by measuring TAC (Table 1), total phenolic compounds (TPC) and total flavonoid compounds (TFC) (Table 2), and reducing sugar (RS) concentration (Table 3).

Table 1. TAC in wheat bran extracts obtained using different extraction methods.

Extraction method	Solvent	Homogenization	TAC, µmol-eq. Q/g of bran		
			TAC_{FRAP}	TAC_{DPPH}	TAC_{ORAC}
Maceration	Water	Yes	1,22±0,01[a]	0,79±0,001[a]	6,95±0,34[a]
		No	1,40±0,02[b,c]	1,00±0,01[b,c]	6,91±0,60[c]
	Ethanol	Yes	1,27±0,02[d,e]	0,69±0,01[b,d,e]	9,49±10,63[b,d,e]
		No	1,36±0,02[b,f,g]	0,73±0,001[b,d,f,g]	7,25±0,27[f,g]
Ultrasound	Water	Yes	1,16±0,02[b,d,f,h,j]	0,56±,01[b,d,f,h,j]	7,58±0,07[f,j]
		No	1,22±0,01[d,h,k,l]	0,68±0,001[b,d,h,k,l]	5,38±0,19[b,d,f,h,k,l]
	Ethanol	Yes	1,37±0,02[b,f,k,m,n]	0,86±0,01[b,d,f,h,k,m,n]	8,12±0,27[b,d,f,m]
		No	1,56±0,03[b,d,f,h,k,m,o,p]	0,89±0,001[b,d,f,h,k,m,o,p]	8,11±0,15[b,d,f,m]
Enzymatic	Water	Yes	1,64±0,03[b,d,f,h,k,m,o,q]	0,62±0,01[b,d,f,h,k,m,o,q]	7,46±0,38[f,m]

a-b, c-d, e-f, g-h, j-k, l-m, n-o, p-q – significant differences in TAC between aqueous and ethanolic extracts, with or without homogenization, obtained by different extraction methods were revealed by ANOVA followed by Tukey's test ($p<0.05$).

Table 2. Total phenolic compounds and total flavonoid compounds in wheat bran extracts obtained using different extraction methods

Extraction method	Solvent	Homogenization	TPC, µmol-eq. G/g of bran	TFC, µmol-eq. Q/g of bran
Maceration	Water	Yes	10,85±0,19[a]	<0,01
		No	9,25±0,09[b, c]	0,11±0,06[a]
	Ethanol	Yes	8,51±0,13[b, d, e]	0,31±0,00[b, c]
		No	6,40±0,06[b, d, f, g]	0,28±0,00[b, e]
Ultrasound	Water	Yes	8,95±0,08[b, d, f, h, j]	0,04±0,05[d, f, g]
		No	7,82±0,00[b, d, f, h, k, l]	<0,01
	Ethanol	Yes	9,40±0,18[b, f, h, k, m, n]	0,09±0,01[d, f, j]
		No	8,34±0,09[b, d, h, k, m, o, p]	0,24±0,00[b, d, h, k, l]
Enzymatic	Water	Yes	11,39±0,12[b,d, f, h, k, m, o, q]	0,06±0,00[b, d, f, m]

a-b, c-d, e-f, g-h, j-k, l-m, n-o, p-q – significant differences in the values of TPC and TFC between aqueous and ethanolic extracts, with or without homogenization, obtained by different extraction methods, ANOVA, Tukey's test ($p<0.05$)

All extracts exhibited significant AOP, with the highest phenolic yield observed in the enzymatic extract and the aqueous extract subjected to homogenization before maceration. The aqueous extract withhomogenization before maceration showed the lowest TACFRAP, while its TACDPPH and TACORAC values were 21.0% and 26.77% lower than the respective maxima, and TFC was below the detection limit. EAE produced the highest TACFRAP, whereas the aqueous extract with homogenization before maceration showed the highest TACDPPH. Overall, homogenization tended to reduce TACFRAP and TACDPPH. Ethanol-based extracts demonstrated higher TACORAC and more efficient TFC extraction compared to aqueous extracts.

Table 3. Reducing sugar concentration in WB extracts obtained using different extraction methods.

Extraction method	Solvent	Homogenization	Reducing sugar, mg-eq. Gl/g of bran
Maceration	Water	Yes	136,84±1,03[a]
		No	137,09±2,36[c]
	Ethanol	Yes	48,65±1,36[b, d, e]
		No	40,77±0,23[b, d, f, g]
Ultrasound	Water	Yes	269,02±1,07[b, d, f, h, j]
		No	285,78±4,92[b, d, f, h, k, l]
	Ethanol	Yes	49,89±0,70[b, d, h, k, m, n]
		No	50,61±0,45[b, d, h, k, m, p]
Enzymatic	Water	Yes	212,77±0,47[b, d, f, h, k, m, o, q]

a-b, c-d, e-f, g-h, j-k, l-m, n-o, p-q – significant differences in the values of reducing sugar between aqueous and ethanolic extracts, with or without homogenization, obtained by different extraction methods, ANOVA, Tukey's test ($p<0.05$)

The highest reducing sugar concentration was observed in aqueous extracts obtained via ultrasonic treatment. However, homogenization before UAE led to a 5.86% decrease ($p < 0.05$), likely due to increased viscosity, which hinders diffusion and limits the extraction of low-molecular-weight compounds. Despite effective polysaccharide release, TAC values in UAE extracts were relatively low, suggesting that TPC remained bound within the polysaccharide matrix, particularly arabinoxylan, thus limiting antioxidant properties. In contrast, enzymatic treatment enabled efficient release of both RS and phenolics, resulting in the highest TAC_{FRAP} and strong values in the other assays. Enzymatic extraction was selected as the optimal method for antioxidant isolation. The resulting control extract was further purified by two methods: filtration through a nylon filter and precipitation with 96% ethanol. AOP parameters (Table 4, Figures 1 and 2) and reducing sugar concentrations (Figures 3 and 4) were determined for both native and purified extracts.

Table 4. Antioxidant potential parameter in WB extracts before and after purification.

Purification method	TAC, µmol-eq. Q/g of bran			TPC, µmol-eq. G/g of bran	TFC, µmol-eq. Q/g of bran
	TAC_{FRAP}	TAC_{DPPH}	TAC_{ORAC}		
Control extract	2,74±0,02[a, E]	0,92±0,01[a, F, G]	3,77±0,42[a, F, H]	13,29±0,19[a,]	<0,01
Filtration	2,70±0,02 [c, E]	0,88±0,01[b, F, G]	4,41±0,33[c, F, H]	12,91±0,19[b, c]	<0,01
Precipitation	4,39±0,05[b, d, E]	0,89±0,02[b, F, G]	6,81±0,41[b, d, F, H]	10,57±0,10[b, c]	0,66±0,01

a-b, c-d – significant differences in the values of TAC_{FRAP} or TAC_{DPPH} or TAC_{ORAC} of the control and purified extracts, ANOVA, Tukey's test ($p<0.05$); E-F, G-H – significant differences in the values of TAC_{FRAP}, TAC_{DPPH} and TAC_{ORAC} for one sample, ANOVA, Tukey's test ($p < 0.05$); q-r, s-t – significant differences in the concentration of reducing sugars or protein in bran extracts before and after purification, ANOVA, Tukey's test ($p<0.05$).

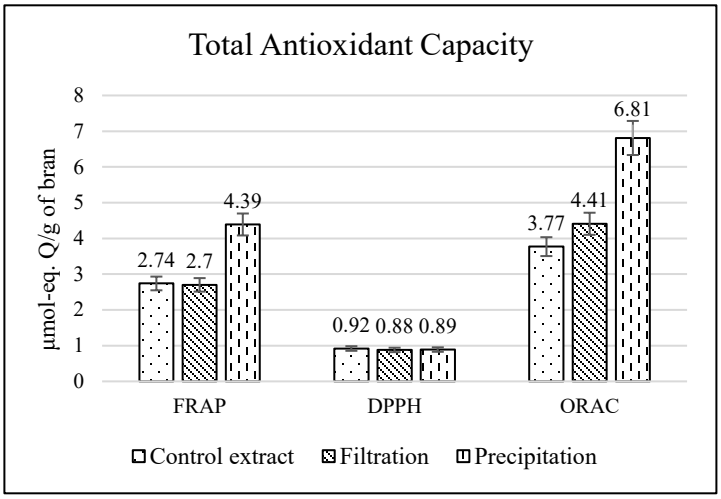


Figure 1. The TAC in WB extracts before and after purification

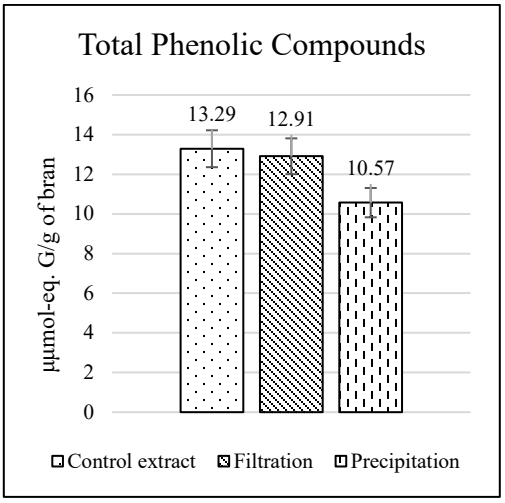


Figure 2. The TPC in WB extracts before and after purification

Filtration had a moderate impact on the extract composition. TAC_{FRAP} remained unchanged ($p > 0.05$), TAC_{DPPH} decreased by 3% ($p < 0.05$) but was not significantly different from post-precipitation values ($p > 0.05$), and TAC_{ORAC} increased by 17%, also without statistical significance ($p > 0.05$). In contrast, precipitation with 96% ethanol resulted in a significant 1.6-fold increase in TAC_{FRAP} and a 1.8-fold increase in TAC_{ORAC} ($p < 0.05$ for both). Flavonoid compounds increased, whereas TPC slightly decreased, likely due to the removal of polymeric impurities and the concentration of low-molecular-weight compounds.

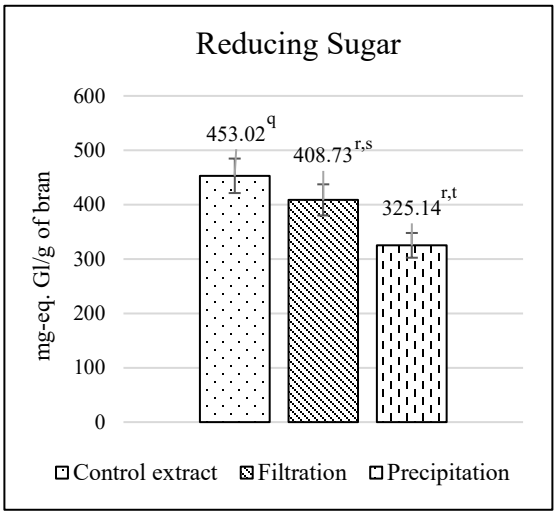


Figure 3. Reducing sugar concentrations in WB extracts before and after purification

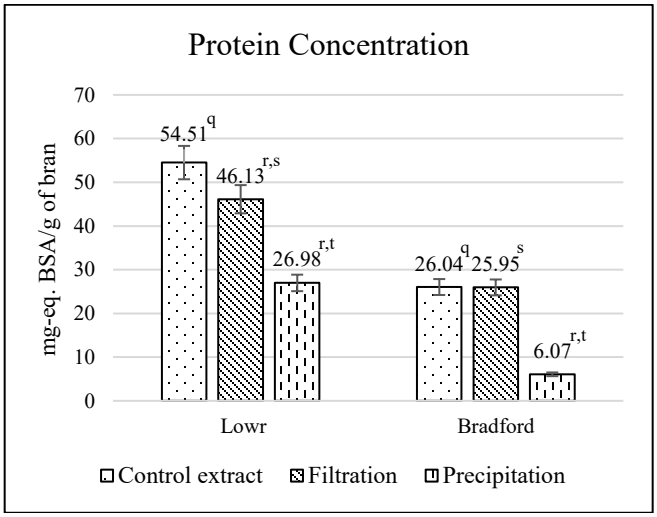


Figure 4. Protein concentrations in WB extracts before and after purification

q-r, s-t –significant differences in the values of reducing sugar or protein concentrations in wheat bran extracts before and after purification, ANOVA, Tukey's test ($p<0.05$)

Filtration reduced sugar content by 11% ($p < 0.05$), whereas precipitation resulted in a 40% decrease ($p < 0.05$). Protein content dropped by 84% (Lowry) and 92% (Bradford) after ethanol precipitation ($p<0.05$), indicating effective removal of high-molecular-weight impurities like arabinoxylan and protein aggregates.

The control extract and the purified samples were lyophilized with or without maltodextrin. The antioxidant potential of the lyophilized samples is presented in Table 5.

Table 5. Antioxidant potential values of lyophilized WB extracts.

Sample name	TAC, µmol-eq. Q/g of powder			TPC, µmol-eq. G / g of powder
	TAC_{FRAP}	TAC_{DPPH}	TAC_{ORAC}	
Control extract	$5,86±0,08^{a, J}$	$2,04±0,02^{a, K, L}$	$7,64±1,07^{a, K, M}$	$29,74±0,59^{a}$
Control extract+MD	$1,56±0,02^{b, c, J}$	$0,55±0,01^{b, c, K, L}$	$2,44±0,06^{b, c, K, M}$	$7,77±0,14^{b, c}$
Filtration	$5,83±0,11^{d, e, J}$	$2,00±0,04^{d, e, K, L}$	$8,99±0,84^{d, e, K, M}$	$28,09±0,53^{b, d, e}$
Filtration+MD	$1,56±0,03^{b, f, g, J}$	$0,52±0,01^{b, f, g, K, L}$	$2,47±0,09^{b, f, g, K, M}$	$7,53±0,11^{b, f, g}$
Precipitation	$4,98±0,07^{b, d, f, h, J}$	$1,82±0,03^{b, d, f, h, K, L}$	$8,51±0,30^{d, h, K, M}$	$20,70±0,18^{b, d, f, h}$

a-b, c-d, e-f, g-h – statistically significant differences in TAC_{FRAP}, TAC_{DPPH} or TAC_{ORAC} values between lyophilized extracts (ANOVA, Tukey's test, $p<0.05$); J-K, L-M- statistically significant differences between TAC_{FRAP}, TAC_{DPPH} and TAC_{ORAC} values within the same sample (Tukey's test, $p<0.05$).

The TAC values of lyophilized control and filtered samples did not differ significantly ($p > 0.05$), regardless of MD addition. However, samples without MD exhibited slightly higher TAC, likely due to the diluting effect of the carrier. The ethanol-precipitated extract showed lower AOP values, possibly resulting from partial loss of antioxidants during ethanol removal under vacuum.

DISCUSSION

This study assessed the impact of different extraction, purification, and drying methods on the antioxidant potential of wheat bran extracts, as well as protein and reducing sugar content. The results demonstrate that the extraction efficiency of biologically active compounds, primarily phenolics, is strongly influenced by the method used, due to the complex structure of wheat bran, which includes non-starch polysaccharides such as arabinoxylans and covalent bonds between phenolics and polysaccharides.

Enzymatic extraction yielded the highest levels of total phenolic compounds, surpassing maceration by at least 5% ($p < 0.05$) and ultrasound-assisted extraction by 21% ($p < 0.05$). A similar trend was observed for TAC_{FRAP}: EAE exceeded maceration by 17% ($p < 0.05$) and ultrasound by 5% ($p < 0.05$). These findings confirm the high efficiency of enzymatic cell wall disruption and hydrolysis of ester bonds anchoring phenolics - particularly ferulic acid - within arabinoxylans. As reported by Zhuang M. et al [21], xylanases, cellulases, and esterases enable selective breakdown of polymeric structures, releasing bound phenolics and improving extractability. Unlike physical methods, enzymatic extraction is performed under mild conditions (40-50°C, neutral pH), minimizing degradation of thermolabile compounds and preserving bioactivity.

Purification methods exhibited significant differences in impurity removal and retention of antioxidant potential. Filtration through a nylon membrane reduced sugar content by 9.77% ($p < 0.05$)

and protein content by 15% (Lowry, $p < 0.05$). The Bradford method showed only a 0.3% protein decrease ($p > 0.05$), indicating limited filtration efficiency for protein removal, possibly due to aggregation or polysaccharide interactions. Thus, filtration provides only partial purification and does not substantially concentrate target compounds.

In contrast, precipitation with 96% ethanol was effective, reducing sugars by 28% ($p < 0.05$) and proteins by 50.5% and 76.6% ($p < 0.05$, Lowry and Bradford, respectively). TAC_{DPPH} decreased by no more than 3% ($p < 0.05$), suggesting high phenolic stability under ethanol treatment. Moreover, TAC_{FRAP} and TAC_{ORAC} increased by 60% and 80% ($p < 0.05$), respectively, likely due to phenolic enrichment in the supernatant following removal of proteins and polysaccharides - consistent with Anderson C. et al. [26], who used ethanol for phenolic fractionation from bran. During drying, lyophilization preserved AOP most effectively. The resulting dry extracts retained high and stable TAC values, comparable to those measured prior to drying. This preservation is attributed to the low-temperature vacuum conditions, which prevent degradation of thermolabile compounds such as phenolic acids, flavonoids, and vitamins - the key antioxidants in wheat bran. Lyophilization maintains the structure and function of these compounds, as reported by Li H. et al [27], who demonstrated that freeze-drying preserves the integrity and bioactivity of heat-sensitive phenolics predominant in wheat bran.

CONCLUSION

This study demonstrates that enzymatic extraction (EAE) is the most effective method for isolating antioxidants from wheat bran, consistently yielding the highest levels of total phenolic compounds (TPC) and total antioxidant capacity (TAC) compared with maceration and ultrasound-assisted extraction. Purification via 96% ethanol precipitation markedly reduced protein content (50.5-76.6%, $p < 0.05$) and sugar content (28%, $p < 0.05$), while enhancing TAC_{FRAP} and TAC_{ORAC} by 60% and 80%, respectively ($p < 0.05$), confirming its efficiency. In contrast, membrane filtration was less effective, particularly in removing protein. Freeze-drying effectively preserved antioxidant potential, producing dry extracts with stable and retained bioactivity. Unlike previous studies that examined individual processing steps in isolation, this work provides the first integrated comparison of extraction, purification, and drying methods for wheat bran. It shows that combining enzymatic aqueous extraction with ethanol precipitation and freeze-drying yields antioxidant-rich extracts with reduced impurities and preserved bioactivity. These findings offer evidence-based guidance for optimizing wheat bran processing and support the development of functional food and nutraceutical products enriched in natural antioxidants.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: MV discussed the influence of various factors on liquid and dry wheat bran extracts, as well as participated in measuring indicators and analyzing the results. NV edited and finalized the manuscript for submission, participated in the development of the study, and led the project. MD participated in writing the abstract and introduction.

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