

Article

Bioprocessing of Wheat Bran by Combined Extrusion and Solid-State Fermentation: Impact on Biopolymer Physicochemical and Functional Properties

Daiva Zadeike , Svajune Norvilaite, Renata Zvirauskiene  and Dalia Cizeikiene 

Department of Food Science and Technology, Kaunas University of Technology, LT-44249 Kaunas, Lithuania

* Correspondence: daiva.zadeike@ktu.lt; Tel.: +370-37-300188

Featured Application

The bioprocessed wheat bran obtained via combined extrusion and microbial fermentation can be directly utilised as a functional, nutrient-dense ingredient in the food industry. Specifically, it can be incorporated into baked goods, such as high-fibre breads and biscuits, at substitution levels of 5–15% (*w/w*) for refined flour, or up to 20% in functional snacks and meat analogues. At these levels, the ingredient enhances the nutritional profile by increasing soluble dietary fibre content and improving the *in vitro* digestibility of the gluten protein fractions. Concurrently, it may optimise processing and techno-functional properties: the disrupted cellular structure and thermo-responsive thickening behaviour of the modified bran have been shown to enhance water-binding capacity and gas retention in doughs, thereby potentially mitigating the typical loaf volume reduction associated with raw bran, while supporting a stable viscosity matrix during thermal food processing.

Abstract

This study aimed to investigate the effects of thermo-mechanical treatment and solid-state fermentation (SSF) on the structural, physicochemical, and techno-functional characteristics of wheat bran (WB), with a particular emphasis on specific isolated protein fractions, starch, and dietary fibre. WB was subjected to extrusion at different temperatures (90, 115, and 130 °C) and screw speeds (16 and 25 rpm), followed by a 24-h SSF with *Liquorilactobacillus uvarum*. Changes in chemical composition, hydration properties (water absorption, solubility, swelling capacity), temperature-dependent (30–80 °C) extract viscosity, as well as protein extraction yields and *in vitro* protein digestibility of isolated protein fractions were systematically evaluated. Extrusion reduced insoluble dietary fibre (IDF) content by 15.7–33.9%, while increasing soluble dietary fibre (SDF) content by 59.1–94.2%, and SSF with *L. uvarum* additionally increased the SDF fraction by 6.5–7.9%. Regarding technological properties, extrusion increased WB water solubility (WS) up to 14.6%, and 24-h fermentation further enhanced WS, reaching up to 16.3%. Extrusion increased the degree of starch gelatinisation up to 49.1%, whereas fermentation reduced it to 40.7% and decreased the WB extract viscosity by 18.3–24.6%. Furthermore, 24-h SSF enhanced the *in vitro* digestibility of globulin, gliadin, and glutenin fractions from initial values of 71.3–78.8%, 70.6–75.2%, and 70.2–73.0% to 82.0–85.6%, 83.3–88.6%, and 79.4–89.0%, respectively. The observed thermo-induced thickening and enhanced digestibility suggest that modified wheat bran has potential as a functional bio-ingredient for future application in novel food formulations.

Keywords: wheat bran; extrusion; fermentation; protein fractionation; hydration properties; *in vitro* digestibility; beta-glucans; starch gelatinisation



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1. Introduction

Wheat bran (WB), a major by-product derived from flour milling, is an abundant raw material with an annual global yield of 100–150 million tonnes [1]. Due to its high energy value and substantial content of crude protein and trace elements, wheat bran is extensively utilised as a raw material for animal feed [2]. In feedstuff production, WB processing primarily involves hydrothermal and enzymatic treatments [3,4].

Extrusion is a high-temperature, short-time thermo-mechanical process widely employed for the modification and production of various food products. During this continuous process, the material is subjected to temperatures of 90–180 °C and high pressure for 30–300 s [5]. A range of chemical and structural transformations occurs during extrusion, such as starch gelatinisation, protein cross-linking, and flavour generation [6–8]. As a multi-stage, multi-functional technology, extrusion enables a wide array of food applications [5].

From a nutritional perspective, expanding the application of cereal processing by-products is crucial to moving beyond treating them merely as feed ingredients. To facilitate their broader integration into human food systems, a deeper understanding of processing technologies, such as fermentation, is required. Nevertheless, studies on WB extrusion-cooking have mainly focused on the hydration properties of the extruded material, alongside changes in the physicochemical properties, bioavailability, and functionality of soluble dietary fibre (SDF) [9,10]. For example, Li et al. investigated the extrusion modification of WB and its effect on the structural and rheological properties of wheat flour dough [11]. Similarly, Andersson et al. analysed the effect of different extrusion parameters on water-unextractable arabinoxylan (WU-AX), water-extractable arabinoxylan (WE-AX), and the release of β -glucan in wheat and rye bran [12].

Moreover, it was reported that WB fermented by *Lactocaseibacillus paracasei* and *Lactiplantibacillus plantarum* significantly increased the apparent digestibility of feed [13]. Additionally, fermentation can effectively reduce the content of insoluble dietary fibre (IDF) in rice bran and corn bran [14], while enzymes produced during fermentation may enhance mineral absorption [15,16]. Combining these treatments presents an effective strategy for the functionalisation of cereal-based by-products, with the potential to improve their safety, nutritional, biochemical, and technological properties. In our previous study, solid-state fermentation (SSF) with *Lactobacillus sakei* MI401 and *Pediococcus acidilactici* PA-2 strains applied to extruded corn-milling by-products significantly increased essential amino acid (EAA) content, improved the water absorption, foaming, and emulsifying capacities of albumins and globulins, and enhanced the in vitro digestibility and free-radical scavenging activity of prolamins [17]. In this context, *Liquorilactobacillus uvarum*, selected for the present study, represents a highly promising candidate for the bioprocessing of complex cereal-based matrices. This specific strain was chosen for its technological potential, characterised by rapid acidification and high lactic acid productivity. Furthermore, *L. uvarum* exhibits strong proteolytic and xylanolytic activities, which are crucial for breaking down cell wall structures, alongside a potential antifungal capacity that could enhance substrate safety [18].

Despite growing interest in utilising WB as a functional ingredient, its industrial application remains limited by its rigid cell wall matrix, which restricts the hydration and techno-functional performance of WB proteins and dietary fibre. Although extrusion and microbial fermentation have been independently investigated as methods to degrade this matrix, comprehensive studies evaluating the synergistic effects of combined extrusion and subsequent solid-state fermentation remain limited. While alternative SSF approaches, such as fungal-based systems, have recently been explored for crop by-product valorisation and the structural modification of plant-protein scaffolds [19,20], they often require prolonged processing times and can introduce sensory limitations. In contrast,

bacterial SSF using tailored lactic acid bacteria like *L. uvarum* offers rapid acidification and targeted enzymatic breakdown while maintaining high substrate safety. Fully understanding these fundamental macromolecular changes is essential for effectively utilising WB as a functional ingredient in clean-label food systems. This approach was designed on the hypothesis that thermo-mechanical extrusion helps to open the rigid WB matrix, thereby increasing its susceptibility to further enzymatic degradation by microbial enzymes during fermentation, which may subsequently improve both nutritional bio-accessibility and techno-functional performance.

Thus, this study aimed to investigate the combined effects of extrusion and solid-state fermentation with *Liquorilactobacillus uvarum* on the structural, physicochemical, and techno-functional characteristics of WB biopolymers. Emphasis was placed on evaluating extrusion- and fermentation-induced alterations in macromolecular structures, specifically starch gelatinisation, resistant starch (RS) dynamics, and β -glucan extractability. Furthermore, the study systematically evaluated changes in protein and WB hydration properties, temperature-dependent extract viscosity, and the in vitro digestibility of individual protein fractions.

2. Materials and Methods

2.1. Materials

The wheat bran (WB) samples were obtained from local mill (Pasvalys, Lithuania). Extruded WB samples and extrusion conditions are presented in Table 1.

Table 1. Wheat bran extrusion parameters and samples.

Extrusion Parameters	E90/16	E115/16	E130/25
Temperature (°C)	90	115	130
Screw speed (rpm)	16	16	25
Final moisture, g/100 g	7.06 ± 0.01	6.13 ± 0.01	5.20 ± 0.00

2.2. Chemical Analysis

Moisture (Method 44-01), crude protein (Method 46-13.01), and crude fat (Method 30-25.01) contents were determined according to standard AACCI/AOAC methods [21]. Soluble dietary fibre (SDF), insoluble dietary fibre (IDF), total starch, and β -glucan contents were quantified using commercial assay kits: Total Dietary Fibre Assay Kit (K-TDFR-200A), Total Starch Assay Kit (K-TSTA-50A), and Mixed-Linkage β -Glucan Assay Kit (Megazyme, Co., Ltd., Wicklow, Ireland), respectively, following the manufacturer's instructions. The reducing sugars were determined spectrophotometrically according to the method described by Miller [22].

2.3. Lactic Acid Bacteria

Liquorilactobacillus uvarum (formerly *Lactobacillus uvarum*, strain 8) was selected for the fermentation experiments. Prior to use, the bacterial culture was propagated in MRS broth (Oxoid Ltd., Basingstoke, UK) at 35 °C for 48 h under anaerobic conditions. The resulting bacterial cell suspension (3.2×10^9 CFU/mL) was subsequently used for the fermentation experiments.

2.4. Fermentation Procedure

For the solid-state fermentation (SSF) experiments, the WB sample (5 ± 0.001 g) was mixed with distilled water at a 1:4 ratio (w/v) in 100 mL glass bottles with caps. The mixtures were then sterilised at 121 °C for 15 min. After cooling to 35 °C, the samples were inoculated with 2 mL of the *L. uvarum* culture suspension. All samples were incubated at 35 °C for 24 h. To monitor the fermentation process, samples were collected for analysis

at 0, 12, and 24 h of incubation. The fermentation process was terminated by freezing the samples at -20°C . The frozen samples were subsequently lyophilised using a freeze-dryer (Zirbus Technology, Bad Grund/Harz, Germany) at -40°C for 48 h prior to further testing. Each fermentation was performed in triplicate, and two sub-samples were taken from each replicate for analysis.

2.5. Extract Viscosity Analysis

Each WB sample (5 ± 0.001 g) was mixed with 30 mL of distilled water on a magnetic stirrer at 20°C for 60 min. After, samples were centrifugated ($6000\times g$; 20 min), and the viscosity of obtained extracts were measured using RHOETEST RN4.1 rheometer (RHEOTEST Medingen GmbH, Ottendorf-Okrilla, Germany). Apparent viscosity was determined as a function of temperature ($30\text{--}80^{\circ}\text{C}$) at a constant shear rate of 30 s^{-1} , using a linear heating rate of $2^{\circ}\text{C}/\text{min}$ and an equilibrium holding time of 1 min at each temperature point to ensure thermal stability before data acquisition. Each sample was analysed in triplicate.

2.6. Determination of the Degree of Starch Gelatinisation

The degree of starch gelatinisation was determined using the iodometric method [23]. Test samples were prepared by mixing 1 g of WB with 10 mL of 0.25 M KOH solution, whereas standard samples were prepared by mixing 1 g of the respective sample with 10 mL of 0.7 M KOH solution. The mixtures were centrifuged ($6000\times g$; 20 min), and 1 mL of the supernatant from both test and standard samples was collected and neutralised using 1 mL of 0.25 M and 0.7 M HCl, respectively. Following neutralisation, the solutions were diluted to 20 mL with distilled water. Subsequently, a 0.5 mL aliquot of each diluted sample was mixed with 4.5 mL of distilled water and 50 μL of iodine solution, and the absorbance was measured at 600 nm. Each sample was analysed in triplicate. The degree of starch gelatinisation (DSG) was calculated according to the equation:

$$\text{DSG (\%)} = (A - A_1)/A \times 100, \quad (1)$$

where A—the absorbance of standard, A_1 —the absorbance of test sample.

2.7. Protein Fractionation

Protein fractionation was performed according to the Osborne method [24], as adapted for the high-fibre wheat bran matrix. For albumin extraction, the WB sample (10 g) was extracted with distilled water at a ratio of 1:10 (w/v) using continuous agitation for 1 h. The mixture was then centrifuged ($8000\times g$; 20 min, 8°C) to obtain the albumin supernatant. For globulin extraction, the remaining residue was extracted with 100 mL of 0.5 M NaCl solution, following the same centrifugation procedure. For gliadin and glutenin extraction, the residues remaining after soluble protein extractions were mixed with 100 mL of 50% (v/v) 2-propanol or 50% (v/v) 2-propanol with 0.08 M Tris-HCl (pH 8.0), respectively. The extracted albumin, globulin, glutenin, and gliadin fractions were precipitated using 1 M HCl by adjusting the pH to the respective isoelectric points of each fraction (4.1, 4.3, 4.6, and 5.5, respectively) and held at 4°C overnight. The resulting protein precipitates were collected by centrifugation and washed twice with distilled water via re-centrifugation. The protein pellets were neutralised to pH 7.0 using a 0.1 M NaOH solution, lyophilised at -40°C for 48 h, and weighed. The protein extraction procedure was carried out in triplicate. The protein extraction yield was expressed as a percentage relative to the total protein content of the raw material.

2.8. Hydration Properties

Water absorption capacity (WAC), water solubility (WS), and swelling capacity (SC) were determined by the method of Singh and Singh [25]. Briefly, the sample of 1 g of WB or 0.25 g of protein (m_s) was mixed with 6 mL or 3 mL of distilled water, respectively, and incubated in a 30 °C water bath. After 30 min, the samples were centrifuged (6000× g; 20 min), supernatant was collected, and precipitate was weighed (m_{wet}). Supernatant was dried in an oven at 105 °C to a constant weight (m_d). Each sample was analysed in triplicate. WAC, WS, and SC were calculated according to the following equations:

$$WAC(\frac{g}{g \text{ d.w.}}) = \frac{m_{wet} - m_s}{m_s}, \quad (2)$$

$$WS \text{ (d.w.)} = \frac{m_d}{m_s} \times 100, \quad (3)$$

$$SC(\frac{g}{g \text{ d.w.}}) = \frac{m_{wet}}{m_s} \times (1 - \frac{WS}{100}), \quad (4)$$

where m_s —the weight of sample, g; m_{wet} —the weight of wet sample after incubation, g; m_d —the weight of dried supernatant, g.

2.9. Oil Absorption Capacity Determination

For the analysis, 0.25 g of the isolated protein powder was mixed with 3 mL of rapeseed oil and incubated in a water bath at 30 °C for 30 min. Subsequently, the mixture was centrifuged (6000× g; 20 min), the oil layer was carefully decanted, and the wet sediment was weighed (m_f). Each sample was analysed in triplicate. The oil absorption capacity (OAC) was expressed as grams of oil absorbed per gram of dry sample (g/g d.w.).

2.10. Protein In Vitro Digestibility Determination

The in vitro protein digestibility of the protein fractions isolated from unextruded, extruded, and 24-h fermented WB was determined according to the method described by Lqari et al. [26]. Trypsin from bovine pancreas (≥ 18 U/mg protein, Product No. T8003), α -chymotrypsin from bovine pancreas (≥ 40 U/mg protein), and protease (peptidase) from *Streptomyces griseus* (≥ 15 U/mg solid) were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). A lyophilised sample containing 62.5 mg of protein was suspended in 10 mL of distilled water. The pH of the suspension was adjusted to 8.0 using a 0.1 M NaOH solution, and the mixture was incubated in a water bath at 55 °C for 15 min. Following incubation, a multi-enzyme solution, consisting of 1.6 mg of trypsin, 3.1 mg of α -chymotrypsin, and 1.3 mg of peptidase, was added to the protein suspension at a ratio of 1:10 (v/v). The pH of the mixture was recorded exactly after 10 min digestion. All measurements were performed in triplicate. The in vitro protein digestibility (DG) was calculated according to the following equation:

$$DG (\%) = 210.46 - 18.103 \times \Delta pH_{10min}, \quad (5)$$

where ΔpH_{10min} is the decrease in pH of the substrate during the first 10 min of digestion.

2.11. Statistical Analysis

All experimental data are presented as the mean $\pm$ standard deviation (SD) of three independent replicates. Statistical evaluation was carried out using IBM SPSS Statistics software (version 28.0; SPSS Inc., Chicago, IL, USA). To evaluate the main effects of the processing parameters (extrusion conditions and fermentation time) as well as their interactive effect on the evaluated parameters, a two-way analysis of variance (ANOVA) was

performed. Additionally, one-way ANOVA followed by Fisher's Least Significant Difference (LSD) post-hoc test was used to determine significant differences between specific treatment groups. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. The Composition of Extruded Wheat Bran

Understanding the behaviour of macromolecular components during extrusion-cooking is essential to predict the functional characteristics of the final processed materials. The chemical composition of raw and extruded wheat bran (WB) is presented in Table 2. Under mild processing conditions (E90/16), extrusion had no significant effect ($p < 0.05$) on the relative protein content compared to the untreated material (16.38 g/100 g d.w.). However, significantly lower ($p < 0.05$) protein levels were observed in samples extruded at temperatures above 100 °C, showing a reduction of 6.6% for E115/16 and 11.9% for E130/25. Similarly, extrusion temperatures exceeding 100 °C led to a significant decrease ($p < 0.05$) in fat content, dropping from 2.63 g/100 g d.w. in the control sample to an average of (1.45 g/100 g d.w.) d.w. in both the E115/16 and E130/25 treatments. Conversely, starch content increased progressively with greater extrusion severity, reaching its highest level in sample E130/25. A comparison of total carbohydrates, dietary fibre, and starch contents suggested the generation of approximately 6–8 g/100 g d.w. of free reducing sugars during extrusion, corresponding to an estimated 50–100% increase compared to raw WB.

Table 2. Comparison of the chemical composition (g/100 g d.w.) of untreated wheat bran (WB) and wheat bran extruded under different conditions.

Sample	Protein	Fat	Starch	SDF	IDF	TC
WB	16.38 ± 0.17 ^a	2.63 ± 0.01 ^a	20.26 ± 0.31 ^d	2.25 ± 0.02 ^c	50.35 ± 0.20 ^a	76.86 ± 0.25 ^b
E90/16	15.99 ± 0.21 ^a	1.91 ± 0.02 ^b	25.98 ± 0.35 ^c	3.58 ± 0.03 ^b	42.46 ± 0.23 ^b	78.02 ± 0.27 ^b
E115/16	15.29 ± 0.13 ^{ab}	1.48 ± 0.01 ^c	28.23 ± 0.41 ^b	4.29 ± 0.04 ^a	38.85 ± 0.31 ^c	78.77 ± 0.41 ^a
E130/25	14.72 ± 0.12 ^b	1.42 ± 0.00 ^c	33.62 ± 0.27 ^a	4.37 ± 0.03 ^a	33.28 ± 0.21 ^d	79.27 ± 0.37 ^a

Data are presented as the means ± standard deviation ($n = 3$). Values in the same column with different superscript letters indicate statistically significant differences ($p < 0.05$). SDF—soluble dietary fibre, IDF—insoluble dietary fibre; TC—total carbohydrates.

Regarding the dietary fibre profile, extrusion significantly affected the distribution between soluble and insoluble fractions. The IDF content decreased significantly ($p < 0.05$) in all extruded samples compared with untreated WB, with the greatest reduction observed under the most intensive extrusion conditions (E130/25) (Table 2). In contrast, extrusion substantially increased the SDF fraction, with the highest values observed for the E115/16 and E130/25 treatments, corresponding to approximately a two-fold increase compared with untreated WB.

These compositional changes may be associated with thermo-mechanical effects occurring during extrusion. The reduction in protein content observed at higher extrusion temperatures may reflect processing-induced structural modifications and reduced extractability under severe conditions. In addition, elevated temperatures may promote non-enzymatic reactions, such as Maillard reactions between amino acids and reducing sugars generated during carbohydrate transformation, potentially contributing to the formation of less extractable protein–carbohydrate complexes [27]. According to Hülsebusch et al., reduced fat extractability may also be related to interactions between lipids and other bran components, including amylose or proteins, under intensive extrusion conditions [28].

Changes in dietary fibre composition may similarly be associated with thermo-mechanical modification of the wheat bran matrix. The combined effects of elevated temperature and mechanical shear may alter the structural organisation of non-starch

polysaccharides, including arabinoxylans and β -glucans, thereby increasing the accessibility and solubility of fibre components. These structural modifications could contribute to the observed shift from insoluble to soluble dietary fibre fractions [29]. The lack of further SDF increase at the highest extrusion temperature (130 °C) may indicate that more severe processing conditions promote additional transformations of solubilised fibre components, which could affect their recovery during analytical determination.

Overall, these findings suggest that the extent of fibre modification depends on the applied extrusion conditions [30,31], highlighting the potential of thermo-mechanical processing to tailor wheat bran functionality and improve nutrient accessibility while reducing antinutritional factors [32].

3.2. The Effect of Fermentation on Nutritional Profile of Extruded Wheat Bran

The combined effects of extrusion conditions and fermentation time on the chemical composition of WB are presented in Figure 1. Solid-state fermentation resulted in a gradual increase in the relative protein content of both unextruded and extruded WB samples compared with their respective unfermented baselines. In the unextruded control, fermentation for 12 and 24 h increased the relative protein proportion by 9.0% and 10.6%, respectively, compared with the initial value of 16.38 g/100 g d.w. A similar upward trend was observed for all extruded samples, with 24-h fermentation resulting in an additional increase of approximately 6.4–6.6% relative to their initial non-fermented values (Figure 1A).

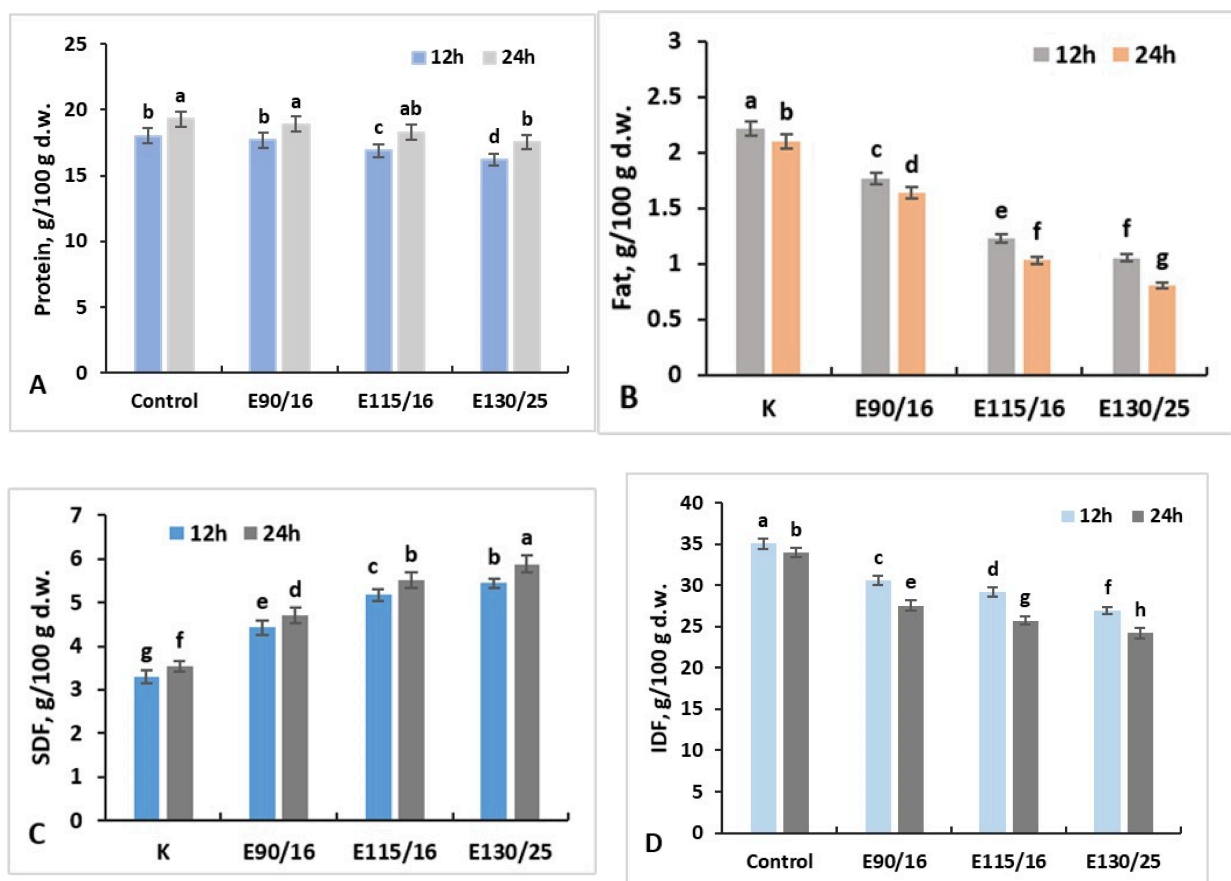


Figure 1. The effect of wheat bran (WB) fermentation on protein (A), fat (B), soluble dietary fibre (SDF) (C), and insoluble dietary fibre (IDF) (D) contents. Control—unextruded WB. Values with different lowercase letters (a–h) within each chart differ significantly ($p < 0.05$). Data are presented as the means $\pm$ standard deviation ($n = 3$).

This increase in relative protein proportion may primarily reflect dry matter losses during fermentation rather than actual protein synthesis. The utilisation of soluble carbohydrates by microbial metabolic activity may decrease the contribution of non-protein components, resulting in a concentration effect on the remaining macronutrients [33]. Therefore, the observed trend likely represents redistribution of dry matter components and an increased relative contribution of nitrogen-containing compounds within the residual matrix rather than *de novo* protein formation [33].

In general, the fat content remained relatively low across all WB samples, ranging from 1.42 to 2.63 g/100 g d.w. before fermentation and decreasing to 0.82–2.22 g/100 g d.w. after fermentation (Figure 1B, Table 2). Fermentation consistently reduced fat content in both unextruded and extruded WB samples. After 24 h of fermentation, the unextruded control exhibited a 20.2% reduction in fat content compared with its initial value, whereas the most intensively extruded sample (E130/25) showed a more pronounced decrease of 42.3%. Extending fermentation from 12 to 24 h resulted in a further reduction, particularly in the E130/25 sample, where fat content decreased by an additional 22.6%, compared with a 5.4% reduction in the control. These significant ($p < 0.05$) changes may reflect alterations in lipid extractability and microbial-mediated lipid transformations during SSF. Previous studies have indicated that lactic acid bacteria may contribute to lipid transformations, including triacylglycerol hydrolysis and the formation of free fatty acids and other lipid-derived metabolites during fermentation of plant-based substrates [34].

Significant ($p < 0.05$) changes were also observed in dietary fibre fractions (Figure 1C,D). Fermentation promoted an increase in SDF accompanied by a reduction in IDF across all treatments compared with the initial unfermented samples. Statistical analysis confirmed that extrusion intensity, fermentation time, and their interaction significantly affected dietary fibre redistribution. The highest SDF content and the lowest IDF level were observed after combined severe extrusion (E130/25) and 24-h fermentation (Figure 1).

Fermentation significantly affected the redistribution of dietary fibre fractions (Figure 1C,D). The highest SDF levels (5.18–5.89 g/100 g d.w.) were observed in the E115/16 and E130/25 samples after 12–24 h of fermentation, representing an increase of approximately 21–35% compared with their initial unfermented values (4.29–4.37 g/100 g d.w.) (Table 2). In comparison, the unextruded control exhibited a more pronounced relative increase, with SDF content rising by 46.2% after 12 h of fermentation compared with the initial value (2.25 g/100 g d.w.). Extending fermentation to 24 h resulted in a further increase in SDF content, reaching 8.6% in the control and approximately 6.5–7.9% in extruded samples depending on the applied extrusion conditions.

Conversely, IDF content decreased during fermentation across all treatments. After 12 h, IDF reduction reached 30.4% in the unextruded control and 19.3–27.9% in the extruded samples. Further fermentation to 24 h caused an additional decrease, particularly in extruded WB samples, where IDF reduction approached 10%. This progressive shift from insoluble to soluble fibre fractions may indicate enhanced fibre solubilisation during fermentation. The observed changes could be associated with the combined effects of extrusion-induced structural modification and microbial enzymatic activity, which may increase the accessibility of structural polysaccharides and promote their partial transformation into smaller, more soluble fractions [35].

In summary, the combination of extrusion-cooking and solid-state fermentation induced significant compositional and structural changes in the WB matrix. Thermo-mechanical processing may contribute to protein denaturation and increased accessibility of the cell-wall structure, while subsequent fermentation further modifies carbohydrate fractions and may enhance the formation of soluble dietary fibre components. These combined effects contribute to the improved nutritional characteristics of processed wheat bran [36].

3.3. The Effect of Fermentation on Starch Gelatinisation and Wheat Bran Extract Viscosity

The effect of solid-state fermentation on the degree of starch gelatinisation (DSG) in raw and extruded WB is presented in Figure 2. Extruded WB samples exhibited substantially higher DSG values compared with the unextruded control, showing an approximately 1.8- to 3.6-fold increase (25.16, 39.07, and 49.11% d.w. for E90/16, E115/16, and E130/25 samples, respectively, compared with 13.64% d.w. for untreated WB). The extent of starch gelatinisation increased with increasing extrusion severity, with the highest DSG value observed for the E130/25 treatment. These results suggest that higher temperature and shear intensity during extrusion may enhance structural modifications of starch granules, increasing their accessibility and promoting molecular changes associated with gelatinisation [37].

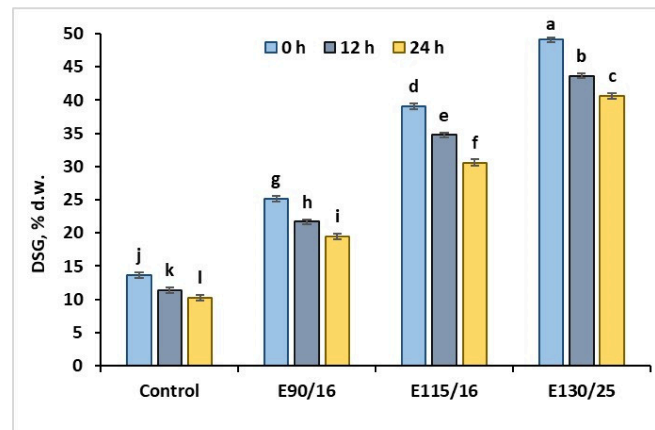


Figure 2. The degree of starch gelatinisation (DSG) of unextruded and extruded (E) at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm) wheat bran (WB) before and after 12–24 h of fermentation with *L. uvarum*. Control—unextruded WB. Values with different lowercase letters (a–l) differ significantly ($p < 0.05$). Data are presented as the means $\pm$ standard deviation ($n = 3$).

Fermentation with *L. uvarum* significantly ($p < 0.05$) decreased DSG across all treated samples (Figure 2). After 24 h of fermentation, DSG values of the extruded samples decreased by 19.5%, 30.6%, and 40.7% for E90/16, E115/16, and E130/25, respectively, compared with their corresponding unfermented baselines. This reduction may reflect changes in the proportion and structural organisation of gelatinised starch fractions during fermentation. Such changes could be associated with the combined effects of microbial metabolism and enzymatic activity under the acidic conditions developed during fermentation [38].

Regarding rheological properties, extrusion-cooking substantially increased the apparent viscosity of WB extracts (Figure 3A). Compared with the unextruded control (0.30 mPa·s at 30 °C), the initial viscosity of E90/16, E115/16, and E130/25 extracts increased by 25, 29, and 33%, respectively. This increase may be related to enhanced extraction of water-soluble components, including soluble arabinoxylans and other polysaccharide fractions generated or released during thermo-mechanical processing [7].

As shown in Figure 3A, all extruded samples exhibited higher extract viscosity than the untreated control across the investigated temperature range. Viscosity increased progressively with increasing temperature, suggesting temperature-dependent structural rearrangements of dissolved macromolecules. The untreated control showed the lowest viscosity throughout the measurement range (30–80 °C). At temperatures above 60 °C, the E115/16 sample exhibited slightly higher viscosity than the E130/25 treatment. The difference may be associated with the greater severity of thermo-mechanical processing applied in E130/25, which could promote additional structural modifications of high-molecular-weight biopolymers and alter their hydration and viscosity-forming capacity [11,39].

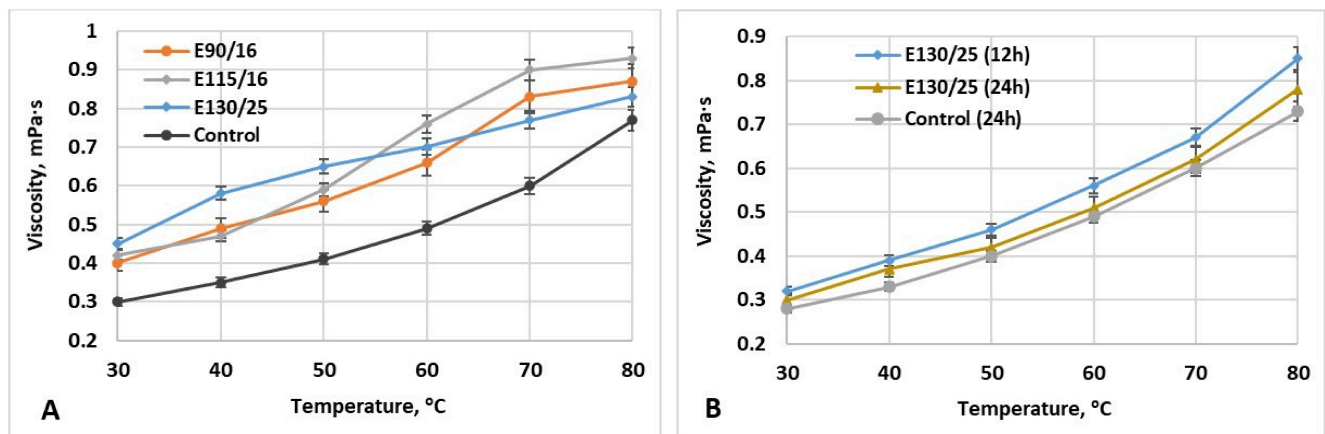


Figure 3. Effect of extrusion (A) and combined extrusion and solid-state fermentation (12 and 24 h) (B) on viscosity of wheat bran (WB) extracts. Control—unextruded WB; E90/16, E115/16, E130/25—extruded WB at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm). Data are presented as the means $\pm$ standard deviation ($n = 3$).

Fermentation with *L. uvarum* effectively reduced the extract viscosity (Figure 3B). Compared with the unfermented baselines, the average viscosity of the intensively extruded E130/25 sample decreased by 18.3% after 12 h and by 24.6% after 24 h of fermentation, whereas the unextruded control showed only a minor reduction (5.8%) after 24 h. This decrease may reflect fermentation-induced modifications of soluble biopolymers, potentially reducing their ability to form highly viscous molecular networks [40].

The changes in viscosity may also be related to the observed reduction in DSG. During fermentation, enzymatic modification of bran components and the production of organic acids may alter the interactions between soluble polysaccharides and starch, thereby influencing water availability and starch hydration behaviour [41]. The limited difference between 12- and 24-h fermented samples suggests that prolonged fermentation may have reduced the availability of fermentable substrates required for further formation of soluble viscosity-contributing components.

Interestingly, all extracts exhibited temperature-dependent viscosity increases between 40 and 80 °C (Figure 3B), which differs from the behaviour commonly observed for many polymer solutions, where viscosity decreases with increasing temperature due to enhanced molecular mobility [42]. In the present study, this thermo-thickening behaviour may be associated with extrusion-induced modifications of wheat bran components, including increased solubility of arabinoxylans, partial starch gelatinisation, and changes in protein structure. The combined thermal response of soluble proteins and polysaccharide fractions may contribute to increased hydration and formation of transient macromolecular networks during heating [43].

Overall, these findings indicate that extrusion is an effective pre-treatment for modifying the solubility and techno-functional properties of WB components [7,44]. The observed thermo-responsive viscosity behaviour highlights the potential of processed WB extracts as hydrocolloid-like ingredients or natural thickening agents in liquid and semi-solid food systems subjected to thermal processing. Combining extrusion with solid-state fermentation provides a promising strategy for tailoring the rheological properties of wheat bran-derived functional ingredients.

3.4. Hydration Properties of Extruded and Fermented Wheat Bran

The changes in water absorption capacity (WAC), swelling capacity (SC), and water solubility (WS) of unextruded and extruded WB following solid-state fermentation with *L. uvarum* are presented in Figure 4.

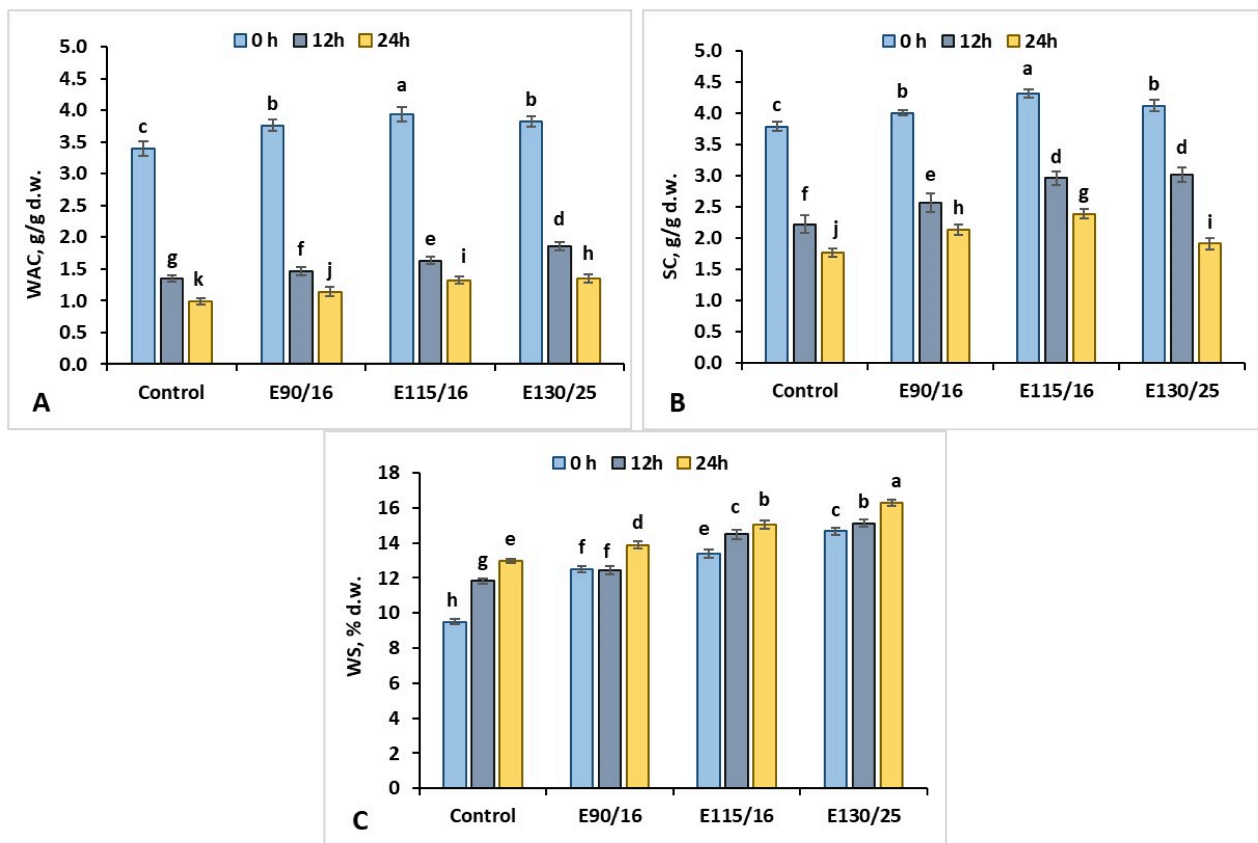


Figure 4. Water absorption capacity (WAC) (A), swelling capacity (SC) (B), and water solubility (WS) (C) of unextruded (Control) and wheat bran (WB) extruded at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm) before (0 h) and after 12–24 h of fermentation with *L. uvarum*. Control—unextruded WB. Values with different lowercase letters (a–k) within each chart differ significantly ($p < 0.05$). Data are presented as the mean $\pm$ standard deviation ($n = 3$).

Extrusion pre-treatment generally improved both WAC and SC relative to the untreated control (3.40 and 3.79 g/g d.w., respectively) (Figure 4). Among the extruded samples prior to fermentation (0 h), the highest WAC and SC values were observed for the E115/16 treatment (3.94 and 4.32 g/g d.w., respectively), whereas more severe processing conditions (E130/25) led to a slight reduction to 3.82 and 4.12 g/g, respectively. The increase in hydration capacity following extrusion may be associated with thermo-mechanical modification of the wheat bran matrix, which could improve the accessibility of hydrophilic components, such as soluble dietary fibres and pentosans, and enhance their interactions with water molecules [45].

The water solubility (WS) of untreated and extruded WB samples progressively increased with increasing extrusion temperature and screw speed, from 9.5% in the unextruded control to 14.7% in the E130/25 sample (Figure 4C). Water solubility is strongly influenced by extrusion-induced modifications of starch and other bran polymers. The applied thermo-mechanical treatment may promote starch gelatinisation and structural rearrangement, potentially increasing the accessibility and solubility of starch-derived components [46]. The higher WS values observed under more intensive extrusion conditions may be associated with greater thermo-mechanical modification of the WB matrix, where increased shear and thermal energy could alter the organisation of macromolecular components.

Conversely, microbial fermentation significantly ($p < 0.05$) decreased both WAC and SC of unextruded WB (Figure 4A,B). A similar tendency was observed for extruded samples, where extending fermentation from 12 to 24 h gradually reduced both parameters.

This behaviour may reflect fermentation-induced modifications of water-interacting macromolecules, potentially decreasing their ability to retain water within the matrix network [47]. In addition, organic acids produced by lactic acid bacteria reduced substrate pH from 5.98–6.00 to 3.58–3.85 in extruded samples and from 6.32 to 3.45 in the control after 24 h (Appendix A, Figure A1). This acidification may influence protein hydration behaviour by reducing protein surface charge as the pH approaches the isoelectric region, thereby potentially decreasing water-binding capacity [48].

Despite this reduction, E115/16 and E130/25 samples maintained the highest hydration capacities throughout fermentation (Figure 4A,B). This behaviour may be related to the structural modifications introduced during the initial thermo-mechanical treatment. Previous studies have shown that extrusion can alter the organisation and accessibility of bran macromolecules, including fibre components, thereby affecting hydration properties [11]. Such modifications may contribute to the preservation of higher water-binding capacity during subsequent solid-state fermentation [49].

In contrast, fermentation progressively increased the WS of bran components (Figure 4C). In unextruded WB, WS increased from 9.5% before fermentation to 11.8% and 13.0% after 12 and 24 h, respectively. A similar tendency was observed in extruded samples; for example, E130/25 increased from 14.7% before fermentation to 16.3% after 24 h. This increase may reflect progressive biochemical modification of bran components during fermentation, where microbial and endogenous enzymes could contribute to the solubilisation of fibre and protein fractions and increase the proportion of water-soluble compounds [50].

3.5. The Effect of Combined Processing on Extraction Yield and Hydration Properties of Protein

3.5.1. The Extraction Yield and In Vitro Digestibility

The results of protein extraction yields indicated that extrusion and fermentation significantly ($p < 0.05$) influenced the distribution and in vitro digestibility of individual protein fractions (Figure 5).

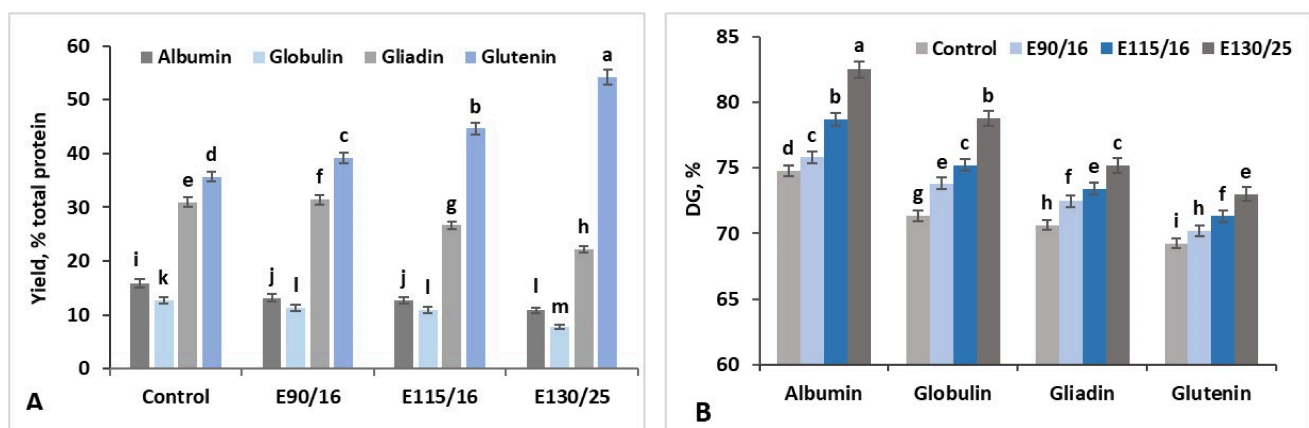


Figure 5. Yields (A) and in vitro digestibility (DG) (B) of protein fractions isolated before and after extrusion of wheat bran (WB). Control—untreated WB; E90/16, E115/16, E130/25—extruded WB at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm). Values with different lowercase letters (a–m) differ significantly ($p < 0.05$). Data are presented as the mean $\pm$ standard deviation ($n = 3$).

Increasing extrusion temperature from 90 to 115 °C progressively reduced the extraction yields of albumin and globulin fractions. Compared with the unextruded control, albumin extraction decreased by 16.9–19.5%, while globulin extraction decreased by 10.4–13.8% (Figure 5A). In contrast, gliadin extractability remained relatively unchanged under mild extrusion conditions (E90/16), showing only a minor non-significant increase compared

with the control (30.8%), but decreased at higher processing intensity. Conversely, glutenin extraction increased by 9.9% and 25.0% at 90 and 115 °C, respectively, compared with the untreated WB.

The most intensive extrusion treatment (E130/25) resulted in the highest glutenin extraction yield (54.1%), whereas albumin and globulin extraction decreased by 31.5% and 39.0%, respectively, relative to the unextruded control. These changes suggest that extrusion conditions influenced the solubility and extractability of individual protein fractions. Albumin and globulin, which are relatively soluble metabolic and structural proteins, may be more susceptible to thermo-mechanical modification. The combined effects of elevated temperature and shear stress could promote protein unfolding and intermolecular interactions, potentially leading to the formation of less soluble protein aggregates and reduced extraction efficiency in aqueous and salt media [51,52].

High extrusion temperatures (115–130 °C) and increased screw speed resulted in a marked reduction in gliadin extraction yield. Compared with the unextruded control, gliadin recovery decreased by 13.9% in E115/16 and by 28.2% in E130/25 (Figure 5A). In contrast, glutenin extraction increased under intensive processing conditions, reaching 54.27% in the E130/25 sample, which represented a 52.3% increase compared with the control.

These changes in protein extractability may be associated with thermo-mechanical modifications of protein structure during extrusion [52]. Under mild conditions, partial disruption of non-covalent interactions may increase the accessibility of some protein fractions. However, more intensive processing may promote protein unfolding and intermolecular interactions, potentially favouring the formation of less soluble protein structures and reducing gliadin recovery during ethanol extraction [53,54]. Similarly, the increased glutenin fraction observed after severe extrusion may reflect changes in the gluten protein network, where thermally and mechanically induced structural modifications could alter the distribution between extractable and less soluble protein fractions [52–54].

The effect of extrusion on *in vitro* digestibility (DG) of WB protein fractions is presented in Figure 5B. The DG values ranged from 69.2% to 82.5%, depending on protein fraction and extrusion conditions. Compared with the untreated samples, extrusion increased digestibility of albumin, globulin, gliadin, and glutenin fractions, with the highest values generally observed for the most intensive treatment (E130/25).

Among the isolated fractions, glutenin showed the lowest digestibility (69.2–73.0%), which may be related to its high-molecular-weight polymeric structure and extensive disulphide cross-linking that can limit enzyme accessibility [55]. The simultaneous decrease in protein extraction yield and increase in digestibility suggests that extrusion-induced structural modifications may have different effects on protein solubility and enzymatic susceptibility. While thermo-mechanical treatment may reduce extractability through aggregation or structural rearrangement, it may also expose previously less accessible peptide regions, facilitating enzymatic hydrolysis [56]. Additionally, high extrusion temperatures may reduce thermolabile anti-nutritional factors, such as trypsin inhibitors, thereby potentially contributing to improved protein digestibility [56].

The results demonstrated that 24-h solid-state fermentation (SSF) with *L. uvarum* significantly ($p < 0.05$) increased the *in vitro* protein digestibility (DG) of both untreated and extruded WB samples compared with their unfermented counterparts (Figure 6). The most pronounced improvements were observed in globulin, gliadin, and glutenin fractions, particularly in the E115/16 and E130/25 treatments. Following fermentation, DG values of pre-extruded samples reached 82.0–85.6% for globulin, 83.3–88.6% for gliadin, and 79.4–89.0% for glutenin, indicating improved enzymatic accessibility of these protein fractions after combined extrusion and fermentation processing.

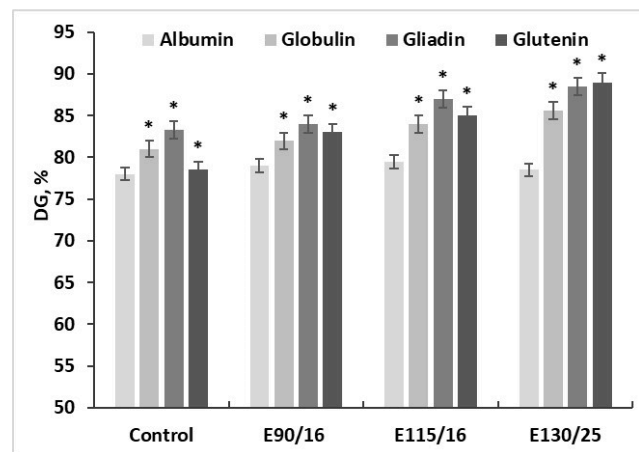


Figure 6. In vitro digestibility (DG) of protein fractions isolated from untreated (control) and extruded wheat bran (WB) after 24-h fermentation with *L. uvarum*. E90/16, E115/16, E130/25—extruded WB at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm). (*) indicates significant difference ($p < 0.05$) compared to the respective unfermented control fraction. Data are presented as the mean $\pm$ standard deviation ($n = 3$).

The observed increase in DG may be associated with the combined effects of extrusion-induced protein modification and microbial activity during SSF. Extrusion may alter protein conformation and accessibility, while subsequent fermentation could further modify protein fractions through microbial enzymatic activity, potentially increasing their susceptibility to digestive enzymes [13,15,41].

The greater improvement observed for gliadin may reflect changes in the gluten protein matrix during fermentation, which could facilitate enzyme access to previously less available protein regions. In contrast, albumin digestibility increased only slightly (approximately 0.7–3.2%), which may be related to its high intrinsic solubility and relatively high initial digestibility, leaving limited potential for further improvement through fermentation (Figure 6).

Simultaneously, fermentation may induce further structural modifications of the protein–polysaccharide matrix. Microbial degradation of non-starch polysaccharides, such as arabinoxylans, may alter the carbohydrate network surrounding bran proteins, potentially reducing physical barriers that limit enzyme accessibility. These structural changes may facilitate the interaction of digestive enzymes with both hydrophilic and hydrophobic protein regions, thereby contributing to improved protein digestibility [43].

Overall, the combination of high-temperature extrusion and subsequent fermentation may provide complementary effects by modifying the structural organisation of WB components and enhancing the nutritional accessibility of protein fractions. These findings suggest that wheat bran extruded at 115–130 °C and fermented with *L. uvarum* for 24 h has potential as a more digestible and functionally improved food ingredient.

3.5.2. The Hydration Properties of Protein

The water absorption capacity (WAC), oil absorption capacity (OAC), and swelling capacity (SC) of albumin, globulin, gliadin, and glutenin fractions isolated from unextruded and extruded WB are presented in Figure 7.

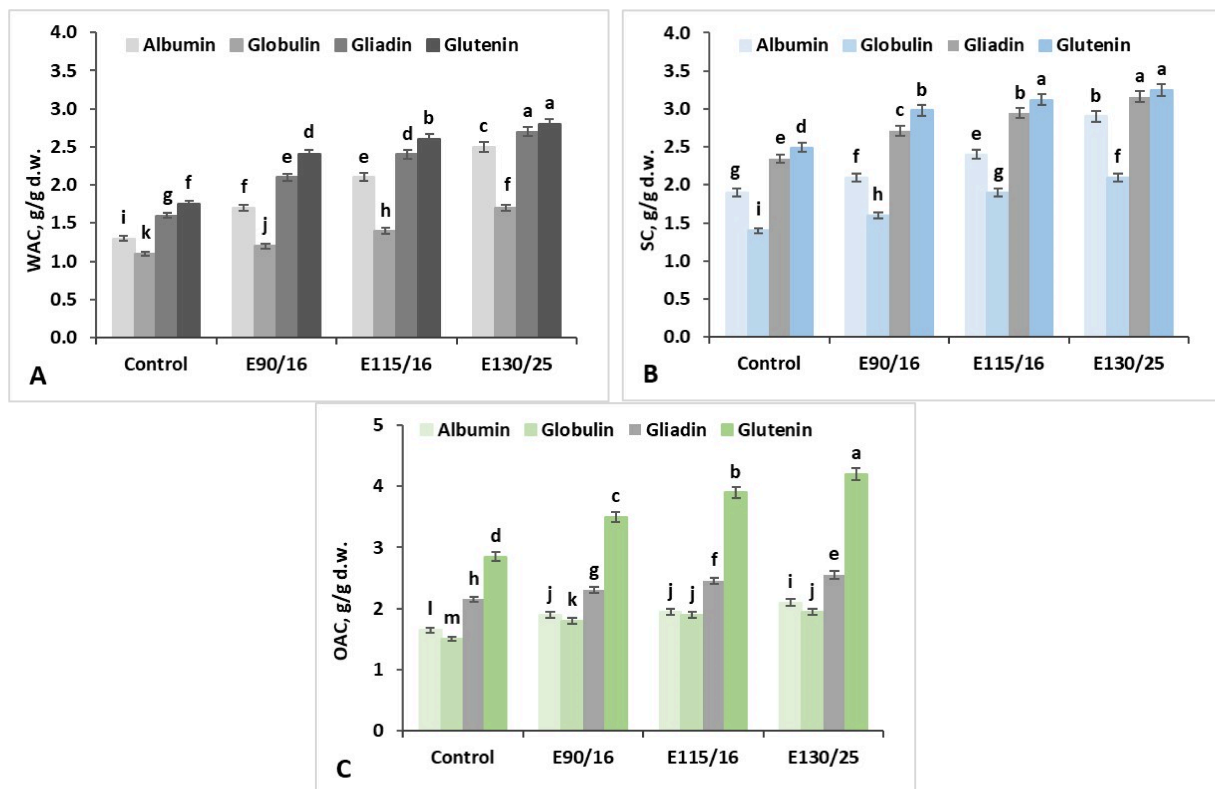


Figure 7. Effect of extrusion on the technological properties of wheat bran (WB) protein fractions: water absorption capacity (WAC) (A), swelling capacity (SC) (B), and oil absorption capacity (OAC) (C). Control—untreated WB; E90/16, E115/16, E130/25—extruded WB at different temperatures (90–130 °C) and screw speeds (16 and 25 rpm). Values with different lowercase letters (a–m) within each chart differ significantly ($p < 0.05$). Data are presented as the mean $\pm$ standard deviation ($n = 3$).

The extrusion process significantly ($p < 0.05$) enhanced WAC, OAC, and SC of all investigated protein fractions, with the magnitude of changes depending on extrusion temperature and screw speed (Figure 7).

In untreated WB, albumin showed higher baseline WAC and SC values than globulin (18.2 and 35.7% higher, respectively). Following the most intensive extrusion treatment (E130/25), albumin WAC increased by 92.3%, while globulin WAC increased by 54.5% compared with their unextruded baselines. Among all fractions, glutenin exhibited the highest hydration capacity, with WAC increasing from 1.75 to 2.80 g/g d.w. and SC from 2.49 to 3.25 g/g d.w. under E130/25 conditions, corresponding to increases of 60.0% and 30.5%, respectively. Similarly, gliadin showed increased hydration properties, with WAC and SC rising by 68.8% and 34.5%, respectively, compared with the untreated fraction (Figure 7A,B).

The enhanced hydration properties of extruded protein fractions may be associated with thermo-mechanical modification of protein structures during processing. Extrusion may alter protein conformation and expose additional polar regions, potentially increasing interactions between proteins and water molecules and improving hydration capacity [57].

Extrusion also increased the oil absorption capacity (OAC) of all protein fractions (Figure 7C). Glutenin exhibited the highest OAC, increasing from 2.85 g/g d.w. in the untreated control to 4.20 g/g d.w. after E130/25 treatment (47.4% increase). Gliadin showed an 18.6% increase compared with its initial value (2.15 g/g d.w.). Albumin and globulin also exhibited improved OAC, increasing by 27.3% and 29.6%, respectively, compared with untreated samples. The increased OAC may be related to extrusion-induced protein

rearrangement, which could increase the accessibility of hydrophobic regions and enhance interactions with lipid molecules [58].

In contrast, extrusion reduced the WS of metabolic protein fractions, with albumin and globulin solubility decreasing from 74.8 to 57.0% and from 79.6 to 61.1%, respectively (Appendix B). This reduction may reflect decreased protein extractability due to extrusion-induced aggregation or formation of less soluble protein structures. Conversely, gliadin and glutenin, which exhibited inherently low initial WS values (1.92 and 0.88%, respectively), showed a slight increase after intensive extrusion (4.75 and 2.67%, respectively). This minor increase may indicate limited structural modification or fragmentation of these protein fractions, resulting in slightly improved solubility [59].

3.6. Effect of Combined Treatment on the Changes in β -Glucan and Reducing Sugars

The effect of extrusion and subsequent fermentation on extractable β -glucan content in unextruded and extruded WB samples is presented in Figure 8A. Extrusion alone resulted in a reduction in extractable β -glucan levels, with the lowest value observed for the most intensive treatment (E130/25; 0.69 g/100 g d.w.) compared with untreated WB (1.63 g/100 g d.w.). Samples processed under milder extrusion conditions retained slightly higher β -glucan contents, reaching 0.89 and 0.94 g/100 g d.w. for E90/16 and E115/16, respectively.

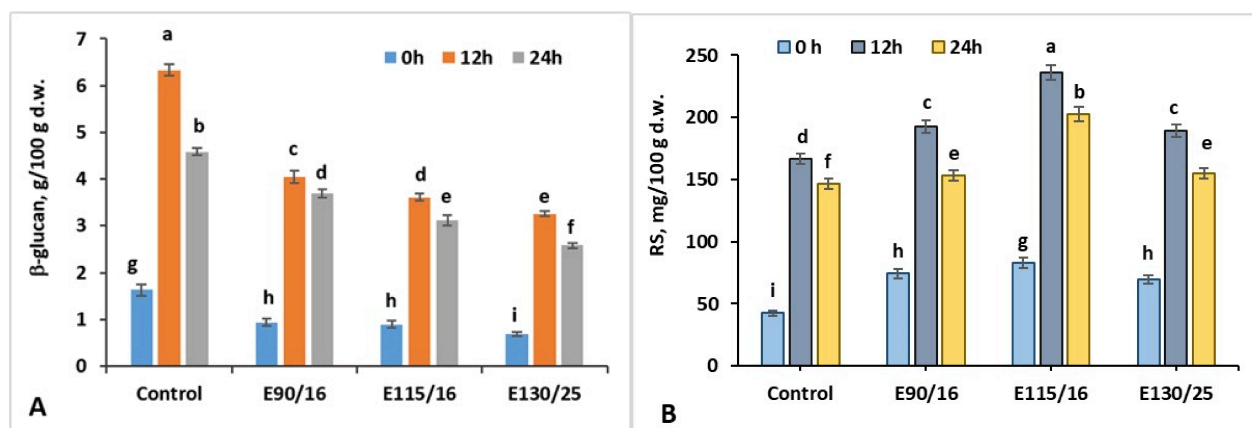


Figure 8. Contents of β -glucan (A) and reducing sugars (RS) (B) in unextruded (control) and extruded (E) wheat bran (WB) before (0 h) and after 12–24 h of fermentation with *L. uvarum*. Control—untreated WB; E90/16, E115/16, E130/25—WB extruded under different temperatures (90–130 °C) and screw speeds (16 and 25 rpm). Values with different lowercase letters (a–i) within each chart differ significantly ($p < 0.05$). Data are presented as the means $\pm$ standard deviation ($n = 3$).

This reduction may be associated with thermo-mechanical modifications of the wheat bran cell wall matrix during extrusion. The combined effects of heat and shear forces could alter the structural organisation of non-starch polysaccharides, potentially affecting β -glucan extractability and its distribution between soluble and insoluble fractions [7].

A distinct dynamic pattern of β -glucan changes was observed during SSF of WB with *L. uvarum* (Figure 8A). After 12 h of fermentation, extractable β -glucan content increased in all samples. The highest increase was observed in the unextruded control, where β -glucan levels rose from 1.63 to 6.33 g/100 g d.w. ($p < 0.05$), while extruded samples exhibited approximately 4–5-fold increases compared with their post-extrusion values. This initial increase may be associated with fermentation-induced modifications of the bran matrix, which could enhance the extractability of previously less accessible β -glucan fractions [35].

However, prolonged fermentation to 24 h resulted in a significant ($p < 0.05$) reduction in detectable β -glucan content, reaching 4.58 g/100 g d.w. in the control and 2.58–3.69 g/

100 g d.w. in extruded samples. This decline may indicate further transformation of soluble β -glucan fractions during fermentation, potentially resulting in lower-molecular-weight products that are not quantified by the applied analytical method. The lowest residual β -glucan content was observed for the most intensively extruded sample (E130/25; 2.58 g/100 g d.w.), suggesting that extrusion intensity may influence subsequent polysaccharide accessibility and transformation during SSF.

These results are consistent with previous studies showing that β -glucan extractability may increase during early fermentation stages, followed by a decline during prolonged incubation due to continued enzymatic modification of soluble polysaccharide fractions [35,60]. The observed changes were also accompanied by alterations in the reducing sugar (RS) profile (Figure 8B), where the decrease in RS between 12 and 24 h reflected microbial utilisation of available carbohydrates. Similar effects of processing-induced structural modifications on β -glucan solubility and microbial transformation have been previously reported [61].

Extrusion also affected starch-related carbohydrate fractions. Compared with untreated WB, extruded samples showed higher initial RS contents, with the highest values observed for E90/16 and E115/16 (43.4 mg/100 g d.w.; 1.7- and 1.9-fold increases, respectively). This increase may be associated with thermo-mechanical modification of starch structures, which could improve carbohydrate accessibility during subsequent hydrolytic processes [7]. The slightly lower RS content observed for E130/25 compared with E115/16 may be related to additional thermal reactions, such as Maillard reactions or caramelisation, which could affect reducing sugar availability [62].

SSF significantly influenced RS dynamics. After 12 h of fermentation, RS content increased in all samples, reaching the highest value in E115/16 (236.7 mg/100 g d.w.), followed by E90/16 (193.3 mg/100 g d.w.), E130/25 (190.1 mg/100 g d.w.), and the control (167.3 mg/100 g d.w.). After 24 h, RS levels decreased by 12% in the control and by 15–21% in extruded samples.

The initial increase in RS may reflect enhanced solubilisation of carbohydrate fractions and release of fermentable sugars from the bran matrix [63]. The subsequent decrease between 12 and 24 h suggests that microbial consumption of available sugars exceeded their release during this fermentation stage. Nevertheless, fermented extruded samples retained higher RS levels than untreated WB, indicating that extrusion may improve carbohydrate accessibility and support sustained release of fermentable substrates during fermentation [63,64].

Overall, extrusion at temperatures above 100 °C combined with 24-h fermentation using *L. uvarum* represents a promising strategy for converting wheat bran into functional food ingredients.

4. Conclusions

This study demonstrates the potential of integrating extrusion cooking and microbial solid-state fermentation as a combined bioprocessing strategy to simultaneously modify multiple wheat bran (WB) components rather than targeting a single biopolymer fraction. The results reveal that extrusion pretreatment created a more accessible WB matrix, which enhanced the effectiveness of subsequent fermentation with *Liquorilactobacillus uvarum*. This integrated approach enabled significant modifications of dietary fibre, starch, and protein fractions, resulting in improved nutritional and techno-functional characteristics of WB ingredients. Extrusion at 115–130 °C with screw speeds of 16–25 rpm provided the most pronounced improvements in hydration-related properties, including water absorption and swelling capacities. These changes indicate that thermo-mechanical modification can enhance the functional performance of WB by increasing the accessibility of water-interacting components. Such modified brans show potential as natural texturising, water-retention,

and viscosity-modifying ingredients in solid and semi-solid food formulations, including bakery products, structured foods, and plant-based meat alternatives. Subsequent 24-h fermentation with *L. uvarum* further modified the WB matrix by promoting the transformation of carbohydrate and protein fractions. The fermented extruded WB exhibited increased protein solubility and improved in vitro protein digestibility, demonstrating that microbial processing can complement extrusion by enhancing the nutritional accessibility of bran components.

These characteristics suggest potential applications of fermented WB ingredients in liquid and semi-solid food systems, such as functional beverages, fermented dairy products, and other formulations where improved dispersion, reduced particulate perception, and enhanced nutritional value are required. Furthermore, the observed temperature-dependent viscosity behaviour of WB extracts highlights the possibility of tailoring the rheological properties of cereal by-product ingredients through controlled processing. This finding provides new insights into how combined thermo-mechanical and microbial treatments can be used to design WB-based functional ingredients with adjustable technological properties.

Overall, extrusion-assisted fermentation represents a promising circular bioeconomy approach for upgrading wheat bran into value-added functional ingredients with enhanced nutritional quality and tailored technological functionality. Further validation in real food matrices and pilot-scale processing conditions is required to confirm their industrial applicability and facilitate their integration into sustainable food product development.

Author Contributions: Conceptualization, data curation, writing—original draft, supervision, writing—review and editing, D.Z.; Methodology, formal analysis, S.N.; Validation, writing—original draft, R.Z.; Resource management, writing—review and editing, D.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

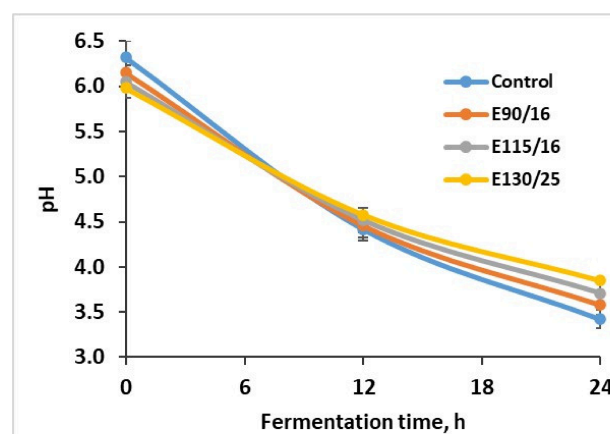


Figure A1. pH changes in untreated and extruded (E) wheat bran during wheat bran (WB) fermentation with *L. uvarum*. Control—untreated WB; E90/16, E115/16, E130/25—extruded WB under different temperatures (90–130 °C) and screw speeds (16 and 25 rpm).

Appendix B

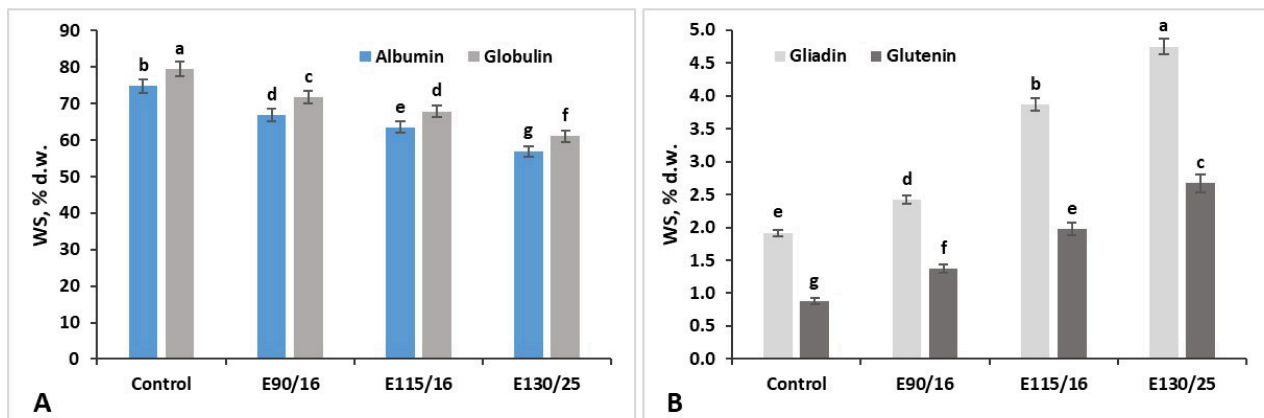


Figure A2. Effect of extrusion and fermentation with *L. uvarum* on water solubility (WS) of wheat bran protein fractions. Control—untreated WB; (A)—WB albumin and globulin; (B)—WB gliadin and glutenin. Values with different lowercase letters (a–g) within each chart differ significantly ($p < 0.05$). Data are presented as the means $\pm$ standard deviation ($n = 3$).

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