

Effect of soaking with *Lactiplantibacillus plantarum* LB-1 and heat treatment on the nutritional composition and microstructure of wholegrain oat (*Avena sativa* L.)

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ABSTRACT

The impact of soaking and thermal processing (boiling) on the chemical composition and microstructure of kilned wholegrain oats with their hull remains poorly characterised, despite its relevance for nutritional enhancement. This study examined the effects of soaking, both with and without *Lactiplantibacillus plantarum* LB-1 (LAB), followed by heat treatment on oat kernel composition, dietary fibre fractions, molecular weight, phytic acid, and total phenolics. Soaking and boiling induced a decline in resistant starch and an increase in non-resistant starch, suggesting partial gelatinisation and enzymatic breakdown. Phytic acid, an antinutrient that limits mineral absorption, decreased by 32–37% after soaking and cooking compared to its initial value in the raw material. The phenolic content decreased after pre-treatment, likely due to solubilisation and heat-related degradation. Microstructural analysis showed cell wall loosening, starch granule swelling, and nutrient dispersion, particularly in LAB-treated samples. During the soaking step the pH dropped to 4–5 over 12 h with LAB compared to pH 6 in water-soaked oats. These structural and chemical modifications indicate that soaking, notably when combined with LAB, could improve starch accessibility and reduce antinutritional factors. The findings offer mechanistic insight into how pre-treatment can improve the nutritional quality and functionality of oats.

1. Introduction

Oat (*Avena sativa* L.) is an annual cereal of the *Poaceae* family, widely recognised for its superior nutritional quality compared to other grains. In 2023, global oat production reached approximately 18.8 million tons (FAO, 2023), with Sweden contributing around 630,000 tons every year, comprising roughly 10% of its total grain output (Gustafsson, 2024). Oats cultivated in Sweden and Finland, are valued for their high and stable β -glucan content, balanced protein composition, favourable lipid profile, and low mycotoxin contamination, attributes that are reinforced by low-pesticide agricultural practices (Nikinmaa et al., 2023). These characteristics render Nordic oats as a highly desirable raw material for the development of healthy and sustainable foods.

Wholegrain or hulled oats (whole kernels with the outer hull intact) are distinguished by their substantial contents of starch (\approx 60% dry weight), protein (9–17%), lipids (\approx 6%), and dietary fibre (9–15%). The

soluble fibre fraction, predominantly β -glucan (1.8–5.5%) (Biel et al., 2009; Nikinmaa et al., 2023), confers functional and physiological benefits, including cholesterol reduction and effects associated with hypocholesterolaemia and glycaemic modulations (Jayachandran et al., 2018). Oat proteins primarily consist of globulins (avenalins) with high digestibility but limited lysine content (Ibrahim et al., 2020). The lipid fraction, rich in unsaturated fatty acids, contributes to nutritional quality whilst predisposing the grain to oxidative instability (Nikinmaa et al., 2023). To inactivate the lipase enzyme in oats, kilning, a heat treatment, is used to prevent rancidity and improve flavour. Phenolic constituents further enhance the antioxidant and anti-inflammatory capacity of oat foods (Ibrahim et al., 2020).

Despite their nutritional value, hulled oat grains contain antinutritional factors, primarily phytic acid (myo-inositol hexakisphosphate) that chelate divalent cations such as Fe^{2+} , Zn^{2+} , and Ca^{2+} , forming insoluble phytate–mineral complexes which are largely concentrated in

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the aleurone layer of cereal grains (H. Li et al., 2014). The hydrolysis of phytic acid is strongly influenced by hydration level, temperature, and pH, affecting endogenous cereal phytases and exogenous microbial enzymes. Oat phytase exhibits optimal activity at pH 4.0–4.5 and 38 °C, under conditions more acidic than the reported optima for barley (5.0), wheat (5.6), and rye (6.0) (Esmailipour et al., 2013).

Soaking and thermal processing, such as boiling, are well-established methods for activating grain metabolism and endogenous enzymes, including amylases, proteases, and phytases. Water uptake triggers starch gelatinisation and partial solubilisation of proteins and cell wall polysaccharides, providing the aqueous environment required for enzymatic hydrolysis of phytic acid. The pH of the soaking medium is critical, as it influences enzyme ionisation, structure, and catalytic efficiency (Esmailipour et al., 2013). The addition of *Lactiplantibacillus plantarum* (*L. plantarum*) during soaking gradually lowers the pH through lactic acid production, reaching levels that optimise phytase activity and promote sequential hydrolysis of phytate (Amritha and Venkateswaran, 2018). Despite the potential benefits of these treatments, the chemical and microstructural effects of soaking and boiling wholegrain oats remain underexplored.

Soaking with or without *L. plantarum* Harvest LB-1 followed by thermal treatment is hypothesised to modify kernel composition and microstructure, which would suggest changes in starch–protein–cell wall interactions. Thus, this study investigates how soaking wholegrain oats with *L. plantarum* LB-1, followed by boiling, affects kernel microstructure, nutritional profile, dietary fibre fractions, and phytic acid, to provide mechanistic insight for optimising pre-treatments to improve oat nutritional quality.

2. Materials and methods

2.1. Materials

Wholegrain oat (*Avena sativa* L.) possessing the hull which had undergone steam-treatment (kilning) was grown in Sweden and processed in 2021 by Lantmännen Oats AB (Kimstad, Sweden). For the soaking step during the pre-treatment process, *L. plantarum* (LAB) (Harvest LB-1, pure culture, freeze-dried) provided by Chr. Hansen (Bagsvaerd, Denmark) was used with a total cell count of $>10^{11}$ CFU/g, as specified by the manufacturer.

For the chemical analyses, hydrochloric acid (HCl, 3.5% and 0.1 M), sodium hydroxide (NaOH), sodium azide (NaN_3), sodium nitrate (NaNO_3), anhydrous sodium sulphate (Na_2SO_4), hexane (C_6H_{14}), isopropanol (2-propanol, $\text{C}_3\text{H}_8\text{O}$), ethanol ($\text{C}_2\text{H}_5\text{OH}$, 78%), methanol (CH_3OH), sodium carbonate (Na_2CO_3), Folin–Ciocalteu reagent, gallic acid, sodium phytate, Wade reagent (0.03% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% sulfosalicylic acid), and AG1-X8 anion-exchange resin (chloride form, 100–200 mesh) were purchased from Sigma-Aldrich (USA) and Bio-Rad (Solna, Sweden). All chemicals were of analytical or High-Performance Liquid Chromatography (HPLC) grade, and deionised (Milli-Q) water was used for all analyses.

2.2. Sample preparation

Wholegrain or hulled oats (100 g, dry weight) were rinsed with tap water and subjected to one of two treatments: No LAB or LAB. For the No LAB treatment, wholegrain oats were soaked in 250 mL of tap water whereas for the LAB treatment, wholegrain oats were soaked in 250 mL of tap water containing *L. plantarum* (0.1 g) resulting in an initial LAB concentration of approximately 4×10^7 CFU/mL in the soaking solution as confirmed by plate counting on appropriate agar.

Soaking was performed in 600 mL beakers at 25 °C for 12 h. After soaking, the water was discarded, and the wholegrain oats were drained and boiled in tap water (1:4 w/v) for 8 min under atmospheric pressure. Detailed CFU data were not included, as microbial enumeration was not the primary focus of this study.

2.3. Chemical composition analysis

Raw wholegrain oats and pre-treated samples were freeze-dried and milled prior to analysis. Protein content was determined by the Kjeldahl method by Association of Official Analytical Chemists (AOAC) 978.04 using a nitrogen-to-protein conversion factor of 5.4. Digestion was performed in a DT 220 digestion system, and nitrogen was quantified with a Kjeltec™ 8200 distillation unit (FOSS Analytical, Hillerød, Denmark).

Total lipid content was determined gravimetrically according to AOAC 996.06, using a hexane:isopropanol (3:2, v/v) solvent system (Sigma-Aldrich, USA). Freeze-dried samples (2 g) were homogenised with 15 mL of solvent, treated with anhydrous sodium sulphate (6.7% w/v), and centrifuged. The upper lipid phase was collected, and the solvent was evaporated under a stream of nitrogen prior to gravimetric determination.

Resistant starch (RS) and non-resistant starch (NRS) contents were quantified using the Resistant Starch Assay Kit following the official analysis methods: AOAC Method 2002.02, American Association of Cereal Chemists (AACC) Method 32-40.01, Codex Alimentarius Type II Method (Megazyme, Bray, Ireland), based on enzymatic hydrolysis with pancreatic α -amylase and amyloglucosidase.

Raw wholegrain oats and pre-treated samples were analysed without freeze-drying to determine moisture and ash contents. Moisture content was determined according to AOAC 934.01 by oven drying at 105 °C to a constant weight (Model, 2000655, J.P. Selecta, Barcelona, Spain). Ash content was quantified following AOAC 942.05 by incineration in a muffle furnace at 550 °C for 12 h (Model 62700, Barnstead Thermolyne, Ramsey, USA). All results were expressed on a dry matter basis.

2.4. Determination of dietary fibre profiling

Samples of raw wholegrain oats and pre-treated materials (1 g, milled to 0.5 mm) were analysed in triplicate for total dietary fibre (DF), including RS and non-digestible oligosaccharides (NDO) degree of polymerisation ($\text{DP} \geq 3$). To this end, the Rapid Integrated Total Dietary Fiber Assay Kit (Megazyme, Bray, Ireland) was used following AOAC method 2017.16 which was modified to replace the filtering steps with centrifugation.

Enzymatic digestion with α -amylase, amyloglucosidase, and protease was performed to remove starch and proteins. Insoluble dietary fibre (IDF) and high-molecular-weight soluble dietary fibre (SDFP) were separated through centrifugation (10 min, $3220 \times g$), with SDFP precipitated in 78% ethanol. Low-molecular-weight soluble dietary fibre (SDFS), including NDO, remained in the supernatant. Both IDF and SDFP residues were dried at 105 °C and corrected for ash (550 °C, overnight) and protein content (DUMAS, AOAC 992.23).

SDFS was quantified by HPLC size exclusion chromatography (SEC) using an Agilent 1200 Series system with TSKgel® PWXL columns PW_{XL} (6.0 mm id x 4 cm; G2500PWXL) and two TSKgel® LC columns in series (7.8 mm id x 30; G2500PWXL (Tosoh Corporation, Japan)). Chromeleon v7.2.7 (Thermo Scientific, USA) and Microsoft Excel were used for data analysis (Microsoft, USA).

2.5. Weight average molecular weight (Mw)

The weight average molecular weight (Mw) of the soluble fibres was estimated by gel permeation/size exclusion chromatography (GPC/SEC). The SDFP fraction of the dietary fibre assay (section 2.4) was dissolved to a concentration of 3 mg/mL in 0.1 M NaNO_3 and 0.02% NaN_3 (SEC eluent) by heating the solutions for 1 h to 80 °C, 1 h to 60 °C, and finally overnight stirring at room temperature. Prior to analysis the solutions were re-heated to 60 °C for 2 h, centrifuged (15 min, $3220 \times g$), and filtered (0.45 μm , hydrophilic) into glass vials. The analysis was performed using a multi-detector system (OMNISEC, Malvern Panalytical Ltd., UK) with the reveal module consisting of a low-angle and

right-angle light scattering detector (LALS, 7°, RALS, 90°), a refractive index (RI) detector, and a viscometer. Autosampler was maintained at 60 °C, the columns and detectors at 35 °C, injection volume was 100 µL, and flow rate 0.7 mL/min (Nikinmaa et al., 2023). The calibration was performed and verified with PolyCAL™ standards (Malvern Panalytical Ltd, UK) 0.79 mg/mL pullulan (PUL-97K, Mw = 96'794 g/mol, Mn = 92'979 g/mol, Intrinsic Viscosity (IV) = 0.390 dL/g, refractive index increment dn/dc = 0.147 mL/g) and 2.279 mg/mL dextran (DEX-66K, Mw = 66'487 g/mol, Mn = 53'833 g/mol, IV = 0.245, dn/dc = 0.148 mL/g), respectively. For data acquisition and analysis, OMNISEC software (version 11.42) was used with a dn/dc of 0.145 mL/g applied for cereal β-glucan molecular weight estimations. Measurements were taken individually for three separately prepared SDFP extracts.

2.6. Total phenolic content (TPC)

Freeze-dried raw and pre-treated wholegrain oat samples (0.5 g) were weighed into centrifuge tubes in triplicate. Phenolic compounds in methanol-soluble extracts were extracted with 5 mL of methanol/water (80:20, v/v) and acidified with 0.1% HCl (5 µL of 6 M HCl per 5 mL solvent). The mixture was vortexed for 10 s, sonicated in an ice bath for 1 min (two 30 s cycles with a 5 min interval), and subsequently stirred at room temperature for 1 h. The extracts were centrifuged at 9500 rpm for 12 min at 4 °C, and the supernatants were filtered through 0.45 µm membrane filters (Millipore) into clean tubes. Extracts were protected from light and stored at -20 °C until analysis (maximum one week).

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. In brief, 20 µL of extract was mixed with 1.58 mL of Milli-Q water and 100 µL of Folin-Ciocalteu reagent. After 8 min of reaction at room temperature, 300 µL of sodium carbonate solution was added, and the mixture was incubated at 40 °C for 30 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu PharmaSpec UV-1800). A calibration curve was constructed from gallic acid standards, and results were expressed as mg gallic acid equivalents (GAE) per 100 g dry matter.

2.7. Phytic acid

Phytic acid in raw and pre-treated wholegrain oats (No LAB and LAB) (freeze-dried and ground) was measured using a modified colorimetric method based on Latta and Eskin (1980). Phytate was extracted in deionised water with continuous stirring for 16 h at room temperature. After adjusting the pH to 0.75 using 3.5% HCl, samples were centrifuged to collect the supernatant. The extract was purified using AG1-X8 anion-exchange resin (Bio-Rad, Solna, Sweden) to separate phytate phosphorus and inorganic phosphorus. The phytate phosphorus fraction was adjusted to pH 3.0 with 0.1 M HCl. For analysis, 3 mL of extract sample was mixed with 1 mL of Wade reagent (Sigma-Aldrich, Stockholm, Sweden), and this was vortexed and centrifuged at 3000×g for 10 min. Absorbance was recorded at 500 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). A blank with deionised water and reagent corrected background, whilst a sodium phytate standard curve ensured accuracy. All measurements were performed in triplicate.

2.8. pH measurement

The pH of the soaking solutions (No LAB and LAB) was measured using a pH meter (PHM92, Radiometer Analytical A/S, Copenhagen, Denmark) at time zero, every 2 h for 12 h, and finally at 24 h. For each measurement, 30 mL of the soaking medium were withdrawn, the pH was determined, and the aliquot was returned to the solution to maintain the initial volume.

2.9. Physical and structural properties

2.9.1. Flour colour analysis

Raw wholegrain oats and oats pre-treated with No LAB and LAB were freeze-dried and ground into fine powder manually with a mortar and pestle. Following this, 30 g of a sample was placed in a round glass base and assessed in four replicates on various areas of its surface at room temperature using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Osaka, Japan). The CIE L* (lightness), a* (redness), and b* (yellowness) were calculated on each sample (CIE, 1986) (Supplementary Fig. 1).

2.9.2. Sample preparation and section

Frozen pre-treated wholegrain oats were divided into smaller fragments. Small fragment samples were embedded in OCT compound and direct frozen in a Leica CM1850 cryostat (Leica Microsystems, Wetzlar, Germany) at -25 °C chamber temperature. Sections (20 µm) were cut with a disposable steel blade. Sections were collected onto glass slides and air-dried for 30 min at room temperature for further light microscopy and CLSM analysis.

2.9.3. Light microscopy

Starch was visualised with Lugol's solution (0.05 g/L iodine in aqueous potassium iodide), and cell walls with toluidine blue. Bright-field and epifluorescence images were obtained using a Nikon Eclipse Ni-U microscope equipped with 10 × objective and captured with a Nikon Digital Sight DS-Fi2 camera (2560 × 1920 pixels, Nikon, Tokyo, Japan).

2.9.4. Confocal laser scanning microscopy (CLSM)

Micrographs were acquired using a confocal laser scanning microscope (CLSM; Zeiss LSM 780, Jena, Germany) equipped with an inverted Zeiss Axio Observer and a supersensitive GaAsP detector, using a 40 × oil immersion objective (N.A. 1.30). Samples were sequentially stained with calcofluor white (CW, 0.1% v/v in water) to visualise cell walls, Rhodamine B (RhB, 0.025% w/v in water) to label protein bodies, and fluorescein 5-isothiocyanate (FITC, 0.25% w/v in acetone) to selectively stain starch. The fluorophores were excited at 410 nm (CW), 568 nm (RhB), and 488 nm (FITC) using the corresponding filter sets. Under these conditions, cell walls appeared blue (CW), proteins red (RhB), and starch green (FITC). The pinhole diameter was set to 1 Airy unit. Laser power, detector gain, and offset were optimised to achieve strong signals without pixel saturation. Images were captured at a resolution of 1024 × 1024 pixels with line averaging to improve the signal-to-noise ratio. Image processing and analysis were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA; Fiji distribution, available at fiji.sc/Fiji), and the resulting micrographs were saved as TIFF files.

2.10. Statistical analysis

One-way ANOVA was performed for each numeric variable to evaluate differences among samples. Significant differences were further assessed using Tukey's HSD test, and group means were annotated with letters to indicate statistically distinct groups using RStudio (Version 5033, RStudio Inc., MA, USA).

3. Results and discussion

3.1. Chemical composition

Soaking with *L. plantarum* (LAB) and heat treatment (boiling) as a pre-treatment markedly influenced the chemical composition of wholegrain oats (Table 1). The increase in moisture was similar for both soaked samples, indicating that LAB did not impact water uptake.

Raw wholegrain oats exhibited a protein content of 13.7% (Table 1), which is consistent with previous reports for wholegrain oat flour. For

Table 1

Chemical compositions of raw wholegrain oats, and pre-treated soaking with No LAB and LAB and subsequent heat treatment (boiling).

Parameter	Raw Oat	Oat -No LAB	Oat -LAB
Moisture (% wet matter)	9.1 ± 0.1 (b)	45.1 ± 1.1 (a)	46.2 ± 2.2 (a)
Ash (% DM)	1.7 ± 0.1 (a)	0.9 ± 0.1 (b)	0.9 ± 0.1 (b)
Protein (N × 5.4) (% DM)	13.7 ± 0.2 (a)	11.6 ± 0.5 (b)	10.5 ± 0.5 (c)
Fat (% DM)	5.4 ± 0.2 (a)	5.4 ± 0.1 (a)	5.2 ± 0.3 (a)
IDF (% DM)	6.1 ± 1.0 (a)	7.0 ± 0.6 (a)	6.3 ± 0.1 (a)
SDFP (% DM)	3.8 ± 0.6 (a)	4.6 ± 0.7 (a)	4.3 ± 0.7 (a)
SDFS (% DM)	0.7 ± 0.0 (a)	0.3 ± 0.1 (b)	0.3 ± 0.1 (b)
Total Dietary Fibre (% DM)	10.6 ± 0.4 (b)	12.0 ± 0.5 (a)	10.9 ± 0.7 (ab)
RS (% DM)	2.7 ± 0.3 (a)	0.7 ± 0.1 (b)	0.2 ± 0.1 (c)
NRS (% DM)	62.1 ± 0.9 (b)	67.2 ± 1.4 (a)	68.3 ± 3.0 (a)
Total Starch (% DM)	64.8 ± 0.7 (a)	67.9 ± 1.5 (a)	68.6 ± 3.0 (a)
L*	84.3 ± 0.6 (c)	85.9 ± 0.3 (b)	87.3 ± 0.7 (a)
a*	-8.5 ± 0.2 (a)	-9.4 ± 0.1 (b)	-9.7 ± 0.3 (b)
b*	40.0 ± 0.2 (a)	39.5 ± 0.1 (b)	40.1 ± 0.2 (a)

%DM = g/100 g of dry matter.

IDF: Insoluble dietary fibre, SDFP: soluble dietary fibre that precipitated in 78% ethanol, SDFS: Soluble dietary fibre soluble, RS: Resistant starch, NRS: Non-resistant starch.

L* implies a light, nearly white colour; negative a* suggests greenish colour; and positive b* indicates yellowness.

Data are presented as means ± standard deviation. Within each row, values followed by the same letter are not significantly different ($p < 0.05$). Different letters in the same column indicate significant differences determined by one-way ANOVA followed by Tukey's HSD post-hoc test.

instance, Russo et al. (2016) reported a 13.4% content. In contrast, studies on hulled oats from Poland have reported lower values of around 11.5% (Biel et al., 2009). It is important to note that these studies used the general nitrogen-to-protein conversion factor of $N \times 6.25$. Using a crop-specific factor of 5.4, which is more accurate for oats, would result in slightly lower protein estimates. The lower protein content observed in wholegrain oats compared to naked oats is largely due to the presence of the hull. Indeed, the hull represents 23–35% of the oat kernel and is high in cellulose but low in protein, thereby diluting the overall protein content of flours derived from hulled varieties (Aparicio-García et al., 2020).

Following soaking and boiling, protein content declined significantly in response to LAB treatment (10.5% DM) compared to both No LAB (11.6% DM) and raw wholegrain oats (13.70% DM) ($p < 0.05$). This additional reduction suggests that LAB mediated enzymatic hydrolysis cleaved proteins into smaller, water-soluble peptides, which were subsequently lost during soaking or partially degraded during thermal processing (Wang et al., 2023). Thus, the combined effects of enzymatic proteolysis and physical leaching contributed to the observed decrease in protein content.

The fat content of raw wholegrain oats was 5.5%, with no significant changes observed after soaking and boiling. In comparison, previous studies have reported fat contents ranging from 2.59% to 4.48% in wholegrain oats from four different cultivars (Kouřimská et al., 2021).

Regarding the dietary fibre profile, the total fibre content of wholegrain oats in this study ranged from 10.6 to 12.0% DM. In comparison, Biel et al. (2009) reported 12.1 to 16.4% DM of crude fibre across five cultivars of hulled oats, whereas Sterna et al. (2016) found a higher value of approximately 17.6% DM for hulled oats. The variation in fibre content across studies is likely due to differences in analytical methods and milling grade as well as the hull to groat ratio which is influenced by both environmental and genetic factors (Givens et al., 2004).

Biel et al. (2009) used the AOAC (1990) crude fibre method, which predominantly quantifies cellulose and lignin through acid and alkali digestion, and thus underestimates total dietary fibre by removing soluble fractions such as hemicelluloses, pectins, and β -glucans. Sterna et al. (2016) applied the Fibertex enzymatic-gravimetric method, which measures total, soluble, and insoluble fibre gravimetrically but may

slightly overestimate values due to retained non-fibre residues. In contrast, the Megazyme AOAC 2017.16 method used in the present study quantifies insoluble and soluble fibre, resistant starch, and oligo-saccharides, thereby providing a more comprehensive and accurate assessment of dietary fibre composition.

Specifically, soluble dietary fibre (SDFS) decreased significantly after soaking, from 0.7% DM in raw oats to 0.3% DM, in both LAB and No LAB treatments ($p < 0.05$), indicating that LAB utilisation of soluble sugars further reduced this fraction (Usman et al., 2022). Correspondingly, total dietary fibre in the LAB sample was slightly lower than in No LAB (10.9 vs 12.0% DM), mainly because of the reduction in soluble fractions.

RS declined from 0.7% in No LAB to 0.2% in LAB ($p < 0.05$), whilst NRS increased from 67.2% to 68.3%. This shift reflects pH-driven alterations in starch structure during soaking with *L. plantarum*. Acidification to around pH 4.0 promoted partial gelatinisation and disrupted RS2 crystallinity, resultingly enhancing solubility and enzymatic susceptibility (Wang et al., 2023). Although *L. plantarum* lacks amylolytic enzymes, its metabolic acid production likely increased starch hydration and accessibility, thereby enabling the conversion of resistant fractions into digestible ones (Wang et al., 2023). The total starch content remained stable, confirming reorganisation as opposed to loss of material.

Compared with No LAB soaking, LAB treatment selectively reduced protein, soluble sugars, total dietary fibre, and RS, whilst increasing NRS and the colour lightness. These changes reflect LAB-mediated enzymatic activity during soaking, with potential implications for digestibility, texture, functional properties, and future studies could evaluate starch hydrolysis and nutrient bioaccessibility to directly link these compositional and structural modifications with digestive outcomes.

3.2. Weight average molecular weight (Mw)

The soaking of wholegrain oats with and without LAB followed by a heat treatment (boiling) significantly impacted the size of the soluble polysaccharides contained in the SDFP fraction, predominantly cereal β -glucan. The overall Mw (integration from 11 to 17.7 mL) was in the range of 300 – 400 kDa, with the Mw of the pre-treated samples being significantly lower than the raw oats, and the addition of LAB provoking a further significant reduction compared to no LAB (Table 2). Literature values of oat β -glucan molecular weight estimates vary over a broad range of 35 – 3100 kDa because they are influenced by factors such as cultivar, raw material fraction, particle size, extraction, solubilisation, and analysis methods (Lazaridou and Biliaderis, 2007).

In the RINTDF method the main extraction step is performed at physiological 37 °C, which reportedly leads to the extraction of lower molecular weight molecules compared to extractions at higher temperatures (Izydorczyk and Biliaderis, 2000). Moreover, the bimodal distribution in all 3 samples (Fig. 1) points toward an enzymatic hydrolysis which may have already occurred in the raw materials, induced by remaining endogenous β -glucanase and continued during the soaking step (Channell et al., 2018).

Table 2

Weight average molecular weight (Mw) in kDa ±SD (n = 3) of the SDFP fraction. (SDFP: soluble dietary fibre that precipitated in 78% ethanol, obtained from the Rapid Integrated Dietary Fiber Assay).

	Mw overall	Mw population 1	Mw population 2
Elution volume (mL)	11 - 17.7	11 - 14	14 - 17.5
Raw Oat	416 ± 11 (a)	1242 ± 23 (a)	191 ± 2 (a)
Oat - No LAB	344 ± 7 (b)	1211 ± 36 (a)	184 ± 2 (a)
Oat - LAB	313 ± 8 (c)	1252 ± 82 (a)	82 ± 6 (b)

Data are presented as means ± standard deviation. Within each column, values followed by the same letter are not significantly different ($p < 0.05$). Different letters in the same column indicate significant differences determined by one-way ANOVA followed by Tukey's HSD post-hoc test.

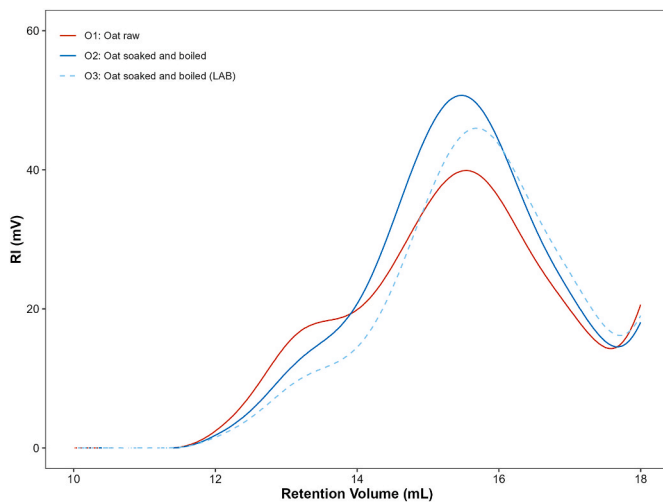


Fig. 1. GPC/SEC chromatogram (Refractive Index (RI) Signal) of one replicate of the SDFP fraction of O1: raw wholegrain oats, O2: Soaked and heat-treated wholegrain oats, O3: Soaked with LAB and heat-treated wholegrain oats. (SDFP: soluble dietary fibres that precipitated in 78% ethanol, obtained from the Rapid Integrated Dietary Fiber Assay).

The lowering of the pH in the samples where *L. plantarum* was added further supports the endo- β -glucanase activity, as its pH optimum in oat matrices was determined to be 5.5 and the dietary fibre degrading enzymes were shown to remain active even under more acidic conditions (pH 4.0) (Blontrock et al., 2025). Aside from enzymatic degradation, the boiling step also appeared to contribute to the reduction of the molar mass through oxidation and subsequent cleavage of β -glucan. Kivelä et al. (2012) observed a reduction of oat β -glucan Mw from 1600 to 1430 and 600 kDa after heating samples in an aqueous solution for 30 min at 95 and 120 °C, respectively.

The bimodal Mw distribution was further explored by integrating two populations, namely from 11 to 14 mL and from 14 to 17.7 mL. The pre-treatment steps did not influence the size of these larger polysaccharides in population 1 (1200 kDa). However, the intensity of the RI signal (Fig. 1) suggests a concentration reduction of these larger molecules caused by the pre-treatment steps with the addition of LAB generating a more pronounced effect.

The Mw of population 2 (190 kDa), on the other hand, was reduced through soaking and boiling, but only if LAB was present (Table 2). The concentration of these smaller fibres seemed to increase in response to soaking and boiling, but less so when *L. plantarum* was added (Fig. 1). A possible explanation could be that *L. plantarum* uses shorter soluble

fibres as substrate, which would be in line with the reduced content of SDFP and SDFS in the samples soaked with LAB compared to samples soaked without LAB. Based on the analytical recovery calculated by the OMNISEC system, the amount of molecules in population 2 is approximately 5 x higher than in population 1, which is also evident from the differences of the overall Mw values versus Mw of population 1 and 2. In summary, the pre-treatment steps and the addition of LAB appear to impact the size of the soluble fibres and induce a redistribution within the bimodal Mw distribution.

3.3. Total phenolic content (TPC) and phytic acid

In this study, raw wholegrain oats were found to contain 92.59 mg GAE/100 g DM of TPC, whilst soaked and boiled wholegrain oats contained 52–58 mg GAE/100 g DM. The pre-treated samples demonstrated slightly lower values than those observed for raw wholegrain oats and values reported in other studies (Fig. 2). For instance, a study that analysed 39 oats in China reported 64.49–153.03 mg GA/100 g DM of TPC, however, all samples were initially dehulled (X. Li et al., 2017). Another study that examined five oat cultivars from Sargodha, Pakistan, reported a TPC ranging from 36.07 to 101.56 mg GAE/100 g (Ibrahim et al., 2020). The observed variations in TPC across studies may be due to genotypic differences, environmental factors such as temperature and cultivation conditions, and differences in analytical protocols, especially the extraction methods.

Phenolic compounds in cereals act as antioxidants, reducing oxidative stress and the risk of chronic diseases. Oats are notable for their frequent consumption as wholegrains and their high phenolic content (Jensen et al., 2004). Thus, optimising pre-treatments to lower anti-nutritional factors such as phytic acid whilst preserving dietary fibre, protein, and phenolics is essential to improve the nutritional quality of cereal-based foods.

In this study, raw wholegrain oats contained 563.5 mg/100 g DM of phytic acid (Fig. 2B). Soaking and boiling significantly reduced phytic acid to 382.75 mg/100 g DM in the No LAB treatment and 350 mg/100 g DM in the LAB pre-treatment, corresponding to reductions of 32% and 37.7%, respectively. Wholegrain oats are expected to possess a high phytic acid content, as it is primarily concentrated in the aleurone layer. Processing steps such as dehulling and milling reduce phytic acid, as well as other nutrients including dietary fibre and phenolic compounds, with the greatest losses typically occurring during milling (H. Li et al., 2014). Other studies have reported phytic acid values of 630 to 1040 mg/100 g DM in three oat cultivars from China (H. Li et al., 2014). In a separate study by Cai et al. (2014), oats containing 420 mg/100 g DM of phytic acid were fermented with *Aspergillus oryzae* and *Rhizopus oryzae*. No significant reduction in phytic acid was observed after 24 h of fermentation, whereas a notable decrease occurred after 72 h. However,

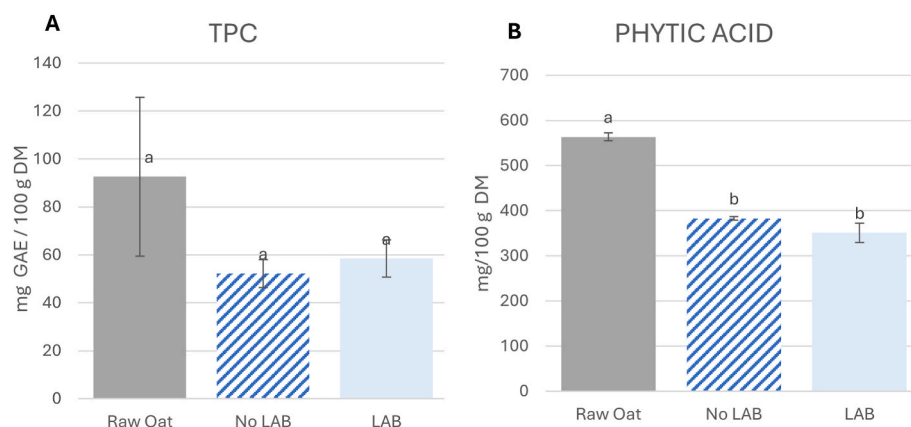


Fig. 2. A) Total phenolic content and B) Phytic acid in raw and pre-treated (soaking + boiling) wholegrain oats. Means with different superscript letters in a column are significantly different at $P \leq 0.05$ (Tukey's range test). DM, dry weight.

prolonged fermentation may have had sensory implications that were not evaluated.

In this study, phytic acid was reduced by 32 % and 37.7 % following pre-treatment with No LAB and LAB, respectively. These reductions can be attributed to partial activation of endogenous phytases in oats during soaking, which hydrolyse phytic acid into lower inositol phosphates. However, as oats are a non-Triticeae cereal they exhibit low mature grain phytase activity (~84 FTU/kg) compared to rye (~5147 FTU/kg) (Brinch-Pedersen et al., 2014), and phytase activity is strongly dependent on pH, with an optimum at around 4–5. At this pH, the catalytic site is correctly ionised, facilitating effective hydrolysis of phytic acid (Oatway et al., 2001). Soaking in near-neutral pH in water limits endogenous phytase activity, whereas LAB pre-treatment can gradually acidify the medium to pH 4–5 and provide exogenous phytase. Nevertheless, no significant difference was observed between No LAB and LAB pre-treatments, indicating that under the applied conditions, phytic acid degradation was limited.

It is important to note that the results of phytic acid reduction are highly dependent on both the microbial strain and the raw material used. Therefore, as a future consideration, it is recommended to verify the phytase activity of the strains to better understand their potential contribution under different conditions.

3.4. pH measurement

In Fig. 3 the pH of the soaking solutions for both the No LAB and LAB treatments was monitored over time to evaluate the effects of naturally occurring (endogenous) microorganisms and the added (exogenous) *L. plantarum* on wholegrain oats. Measurements were taken at 2 h intervals for 12 h, with an additional reading at 24 h. In the solution inoculated with *L. plantarum*, the pH gradually decreased to approximately 4.4 over 12 h and further declined to 4.1 after 24 h. In contrast, the water-only treatment demonstrated a slower pH decline, from an initial 6.8 to 6.1 after 12 h to pH 5.42 at 24 h. At this point, the No LAB soaking solution exhibited a viscous appearance and an off-odour, whereas the LAB-treated solution maintained a normal appearance and odour.

When *L. plantarum* was added to the soaking medium of wholegrain oats, the pH gradually decreased to approximately 4.4 after 12 h. This reduction is attributed to the metabolic activity of *L. plantarum*, which ferments soluble carbohydrates released from the oats and produces lactic acid as the main metabolite (He et al., 2022; Rajendran et al., 2023). In contrast, oats soaked in water without added *L. plantarum* demonstrated only a slight reduction in pH, likely reflecting the activity of naturally occurring microorganisms and the mild action of endogenous enzymes. This is consistent with previous observations that epiphytic lactic acid bacteria and plant enzymes on oats can produce limited amounts of organic acids during the initial stages of fermentation or soaking, even in the absence of an external inoculant (Wang

et al., 2023). To summarise, the lower pH observed in the LAB treatment may favour partial phytate degradation and increased mineral solubility.

3.5. Physical and structural properties

Colour changes were significant following LAB treatment (Table 1 & Supplementary Fig. 1). Indeed, L^* values increased (87.31 in LAB vs 85.91 in No LAB, $p < 0.05$) and a^* values slightly decreased, ultimately producing an overall lighter colour. These changes likely resulted from enzymatic or metabolic activity of LAB during soaking, which can modify minor pigments. Interestingly, despite their high fibre and TPC, the flours remained naturally light in colour with a potential use in food applications such as baked goods and snacks. LAB treatment may therefore offer a means to further enhance the visual appeal of these nutrient-dense ingredients without compromising their functional properties.

The microstructure of the pre-treated samples was analysed using light and confocal microscopy. Fig. 4 illustrates the microstructural organisation of wholegrain oats samples subjected to soaking (12 h at 25 °C) and boiling, either without (A, B & C) or with (D, E & F) *L. plantarum* pre-treatment.

None of the LAB pre-treated samples (A–C) displayed a compact and highly organised cellular structure. The light micrograph (Fig. 4A and B) revealed intact cell walls and tightly packed cells containing well-defined, densely stained starch granules, indicative of ungelatinised or only partially gelatinised starch. Toluidine blue-stained fibrous regions, delineated cell boundaries, and revealed a continuous parenchymal network with limited intercellular space; however no visual significant differences were found between micrograph B and E.

More detailed changes could be seen through CLSM observations (Fig. 4C and F), with strong Calcofluor blue fluorescence outlining the cell walls and a concentrated green colour signal within cells, representing starch entrapped by intact cellular matrices. The Rhodamine B signal was mainly localised around the periphery of starch granules, consistent with proteins bodies surrounding or embedded in the matrix. Together, these observations indicate that soaking and boiling did not fully disrupt the cellular integrity of hulled oat tissues, resulting in starch and protein encapsulation within cell walls. Such structural encapsulation is known to restrict enzymatic access and reduce the rate of starch hydrolysis during digestion (Alminger et al., 2012).

Oats pre-treated with *L. plantarum* (D–F) exhibited a more heterogeneous and disordered microstructure. The light micrograph (Fig. 4D) showed disrupted cell contours and diffuse Lugol's iodine staining, suggesting partial starch solubilisation and leaching, which correlate with the starch content mentioned above (Table 1). In CLSM images (Fig. 4F), the Calcofluor signal along some cell boundaries appeared slightly weakened or uneven, and isolated regions showed partial loss of wall definition. The starch granules were marginally more irregular in shape and size, and FITC emission appeared more diffused in certain cells, suggesting mild swelling or partial solubilisation. However, most of the tissue maintained its cellular integrity and compactness.

The results indicate that *L. plantarum* pre-treatment caused only minor, localised modifications in cell-wall architecture and starch organisation. These subtle changes may slightly increase surface permeability and matrix porosity but are unlikely to significantly affect starch encapsulation or macronutrient accessibility. This is consistent with previous studies which have shown that lactic acid fermentation of whole or minimally processed cereals can induce modest microstructural changes; however, substantial modifications generally require extended fermentation or prior mechanical disruption, which increase enzyme accessibility and promote partial hydrolysis of starch (You et al., 2025).

Future work should explore how soaked and boiled wholegrain oats perform as ingredients in foods such as porridges, gels, or substrates for solid-state fermentation, to understand their impact on texture, flavour,

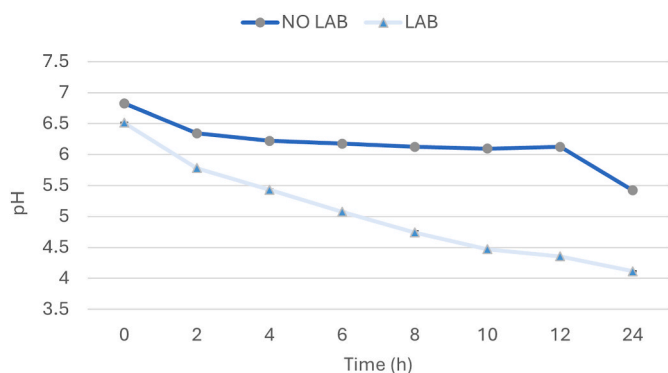


Fig. 3. pH values in the soaking media used as a pre-treatment for wholegrain oats with and without *L. plantarum* (LAB). Measurements were taken every 2 h for the first 12 h, with a final measurement at 24 h.

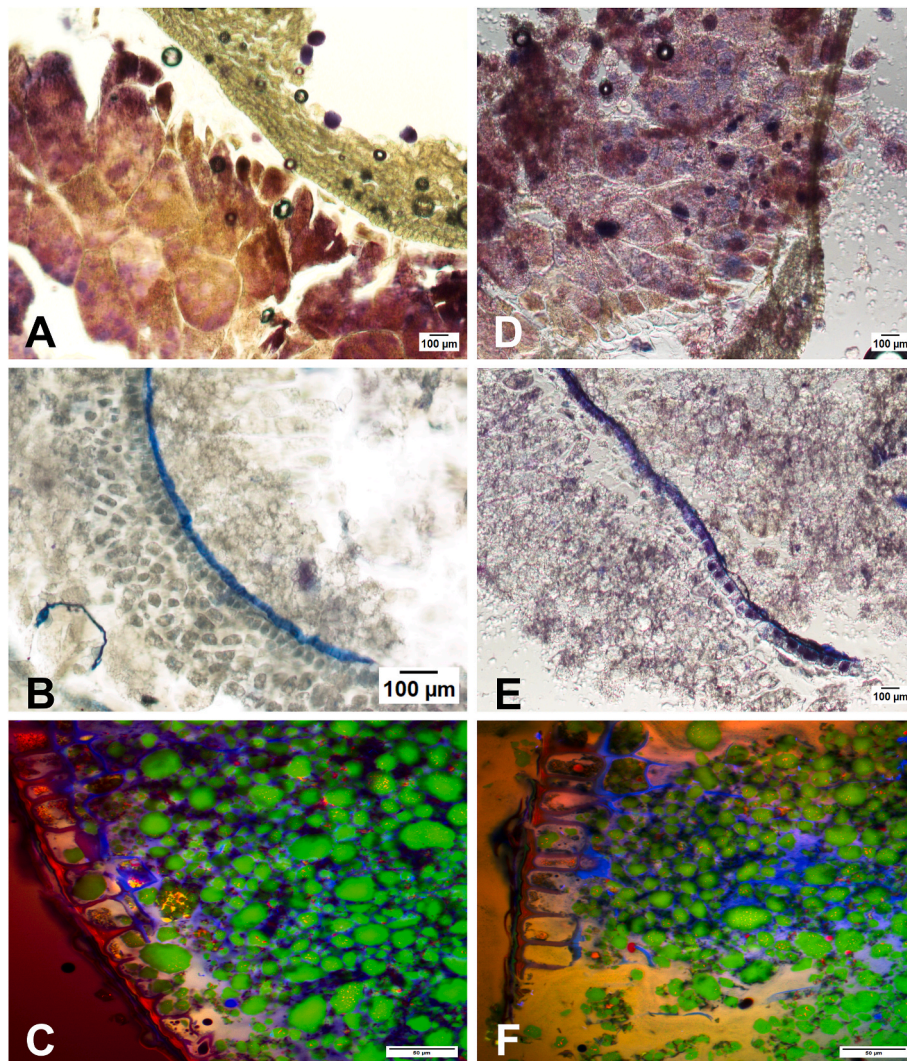


Fig. 4. Wholegrain oat pre-treated samples (soaking + boiling) micrographs A, B, D, and E were obtained using a light microscope with a 10 × objective and a 100 μm scale bar. Micrographs C and F were obtained using confocal laser scanning microscopy with a 40 × objective and a 50 μm scale bar. Sample A) without LAB and D) with LAB pre-treatment, stained with Lugol's iodine to visualise starch. Sample B) without LAB and E) with LAB pre-treatment, stained with toluidine blue to visualise fibrous regions/cell walls. Sample C) without LAB and F) with LAB pre-treatment, stained with Calcofluor White to show fibres in blue, Rhodamine B to show proteins in red, and FITC (fluorescein isothiocyanate) is to show starch in green.

and functional properties relevant to real-world applications.

4. Conclusions

Soaking wholegrain oats with *Lactiplantibacillus plantarum* (0.1 g/100 g dry oats) and subsequent boiling produced modest yet measurable changes in composition and structure. LAB metabolism acidified the soaking medium (pH ≈ 4.4 after 12 h), but enzymatic activity was limited, possibly due to the low inoculum and intact grain matrix. Protein and resistant starch declined, indicating partial hydrolysis and starch reorganisation, whilst total starch and fat remained stable. Phytic acid and total phenolics decreased relative to raw oats, but reductions were comparable to non-inoculated controls (No LAB). Microscopy revealed only localised cell wall weakening and mild starch swelling, with overall tissue integrity preserved. Furthermore, LAB treatment increased brightness (L^*) without compromising functionality as total dietary fibre was conserved, whilst lowering the Mw of the soluble fibre fraction from 416 kDa to 313 kDa. These findings demonstrate that short, mild LAB soaking can subtly improve the nutritional properties of wholegrain oats; however, extending the fermentation period could potentially further enhance these effects. Controlling pH is crucial, as

prolonged soaking at room temperature without LAB increases the risk of spoilage by undesirable microorganisms.

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original draft, Writing – review & editing. **Oksana Kravchenko:** Investigation. **Jing Lu:** Formal analysis, Writing – review & editing. **Laura Nyström:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Maud Langton:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Galia Zamaratskaia:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcs.2026.104434>.

Data availability

Data will be made available on request.

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