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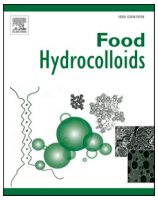

Oat sourdough fermentation enhanced β-glucan viscosity and extractability from wholegrain oat bread in an *in vitro* gastrointestinal model

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ABSTRACT

Sourdough fermentation has been demonstrated to enhance the texture, sensory and nutritional properties of wheat- and rye-based bread. However, its use in wholegrain oat bread is not well known, especially its impact on altering the physiological functionality of β-glucan. This study aimed to design fermentation conditions for oat sourdough to enhance the β-glucan functionality in wholegrain oat bread using two types of germinated oats as a source of enzymes, showing different β-glucanase activity. Two sourdoughs, fermented by an association of *Lactiplantibacillus plantarum* and *Fructilactobacillus sanfranciscensis* strains, have been obtained: one fermented with oat malt (OM) and the other with sprouted oat grains (SPO). Sourdough was added to the bread as 30% of the total dough weight (61% of flour weight). An *in vitro* gastrointestinal simulation model was used to assess the physiological functionality of β-glucan in the breads. The use of the two germinated materials induced a different degradation of β-glucan (from 1060.3×10^3 g/mol to 681.7×10^3 g/mol with SPO vs. 117.4×10^3 g/mol with OM). The addition of sourdough with sprouted oats made the bread softer and increased its volume. The extracts obtained after *in vitro* digestion from this bread showed high value of viscosity and the highest β-glucan extractability, indicating an increased physiological functionality of β-glucan. This study demonstrated the potential of sourdough fermentation to modulate β-glucan degradation and improve the physiological functionality of oat bread.

1. Introduction

The food industry is increasingly interested in using oats due to their several established health-beneficial effects (Rasane et al., 2015) and their suitability for celiac patients when not contaminated by other cereals (Aaltonen et al., 2017). Oats' (*Avena sativa*) nutritional profile is characterised by the presence of high amounts of soluble fibres, such as β-glucan, good quality protein, unsaturated fatty acids, bioactive compounds, phenolic compounds, vitamins, and minerals (Joyce et al., 2019; Rasane et al., 2015). Oat β-glucan is a soluble fibre shown to effectively reduce LDL-cholesterol and regulate postprandial glycaemic response. Due to strong scientific evidence supporting these effects, regulatory bodies such as EFSA and FDA have approved health claims highlighting the role of β-glucan in the promotion of human health (EFSA Panel on Dietetic Products, 2010, 2011; Food and Drug Administration (FDA), 1997).

β-glucan is a linear polysaccharide mainly present in the endosperm cell wall of oat kernels, and it is composed of D-glucose units with β-(1 → 4) and β-(1 → 3) linkages. The structure of oat β-glucan is characterised by β-(1 → 4)-linked cellotriosyl (DP3) and cellotetraosyl (DP4) units in a specific DP3:DP4 ratio, which depends on oat variety and growing conditions and affects its solubility and viscosity. Oat β-glucan usually has a DP3:DP4 ratio of 1.5–2.3 and a molecular weight of about 1000–2000 $\times 10^3$ g/mol, the highest among cereals (Lazaridou & Biliaderis, 2007; Wood, 2011). The physiological functionality of this polysaccharide has been mainly attributed to its ability to enhance the viscosity of aqueous solutions. However, this ability is influenced by different features of the β-glucan, such as extractability, therefore concentration in the solution, and molecular weight.

The viscosity of solutions containing polymers is primarily determined by their concentration, molecular size, and molecular weight distribution (Flory, 1953). For β-glucan, both molecular weight and concentration are key determinants of solution viscosity, which in turn

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ABBREVIATIONS

dw	dry weight
M_w	Weight-average molar mass
LAB	Lactic acid bacteria
OM	oat malt grains
SPO	sprouted oat grains
WOF	wholegrain oat flour
T0C-WOF	time 0 control (not fermented) containing only oat flour (no germinated ingredient added)
T0C-OM	time 0 control (not fermented) containing oat malt grains
T0C-SPO	time 0 control (not fermented) containing sprouted oat grains
SOM	sourdough containing oat malt grains
SSPO	sourdough containing sprouted oat grains
CB	control bread (no sourdough addition)
BSOM	bread added with the sourdough containing oat malt grains
BSSPO	bread added with the sourdough containing sprouted oat grains

mediates its physiological effects on postprandial glycaemic response. Postprandial blood glucose response and glycaemic index reduction are significantly greater when high molecular weight β-glucan is consumed, even at lower doses, a phenomenon that is attributed to increased viscosity, which slows glucose absorption and delays gastric emptying (Wood et al., 1994). However, a significant correlation between the changes in peak blood glucose response and concentration and molecular weight of β-glucan was demonstrated (Wood et al., 2000). The capacity of β-glucan to reduce the glycaemic response of oat foods is related to both molecular weight and solubility (extractability), hypothesising a correlation of these properties with the development of the luminal viscosity (Tosh et al., 2008). Highly viscous bolus containing β-glucan hinders the action of digestive enzymes on other available polysaccharides, such as starch, causing a reduced and slower glucose absorption (Regand et al., 2011). A recent study found that β-glucan's molecular weight was more important than the viscosity in lowering glycaemic response (Tan et al., 2024). Although several studies investigated the positive impact of β-glucan on the reduction of peak blood glucose, the optimal molecular weight range was not identified, since the dosage, extraction conditions, and possible depolymerisation also play a role (Henrion et al., 2019). β-glucan consumption can have an impact on the decrease of total and low-density lipoprotein (LDL) cholesterol levels (Whitehead et al., 2014). Evidence that the formation of a viscous layer in the small intestine hinders both cholesterol absorption and bile acid reuptake has been found (Othman et al., 2011; Wolever et al., 2010; Wood, 2011). β-glucan with medium (530×10^3 g/mol) and high (2210×10^3 g/mol) molecular weight significantly reduced LDL-cholesterol, while low molecular weight (210×10^3 g/mol) showed no impact (Wolever et al., 2010). However, medium molecular weight ($200–500 \times 10^3$ g/mol) still had similar benefits to high molecular weight β-glucan (Rosa-Sibakov et al., 2020).

The extractability of β-glucan significantly varies depending on the food matrix characteristics. For example, the properties of β-glucan after *in vitro* gastrointestinal (GI) simulation considerably differed among different oat products, such as breads and spoonable products (Mäkelä et al., 2020). Previous studies demonstrated that the physicochemical properties of β-glucan (e.g., molecular structure, solubility, viscosity, and physiological functionality) are influenced by physical, mechanical, and bioprocessing treatments (Jurkaninová et al., 2024; Tosh et al., 2010). It was shown that in baking, endogenous enzymes (β-glucanases) present in cereal flours induced β-glucan degradation during mixing, fermentation, and proofing in composite breads containing wheat, barley, rye, and oats, whereas heat-treatment or baking did not influence β-glucan molecular weight (A. A. M. Andersson et al., 2004; R. Andersson et al., 2009; Gamel et al., 2015; Maina et al., 2021; Mejía et al., 2020; Moriartey et al., 2010; Rieder et al., 2012, 2015). Fermentation has also been shown to improve bread texture in barley β-glucan-enriched composite bread (Rieder et al., 2012). In oat sourdough, a decrease in β-glucan molecular weight led to increased solubility (Lu et al., 2019). Despite these findings, the impact of fermentation on the content of cereal β-glucan remains unclear, with conflicting evidence across studies (Djorgbenoo et al., 2023; Jurkaninová et al., 2024; Maina et al., 2021). Furthermore, research focused on oat sourdough is still limited. Typically, kilning of oat ingredients, required for ensuring longer shelf-life of the ingredients, inactivates endogenous enzymes, including β-glucanases (Blontrock et al., 2025). Therefore, in a wholegrain oat recipe, β-glucan content and molecular structure should not be significantly affected by endogenous enzyme activity during processing. Recently, interest in understanding the influence of β-glucan having native or partly hydrolysed molar mass on the texture of gluten-free bread has increased (Bieniek & Buksa, 2024).

To date, no studies have investigated the changes induced by sourdough fermentation on the status of oat β-glucan in bread, or how this affects its physiological functionality. Here, we studied the effects of oat sourdough fermentation on β-glucan functionality in wholegrain oat bread. Two oat sourdoughs were designed, fermented by an association of two lactic acid bacteria (LAB) typical of sourdough, and containing two different types of germinated oat grains with different β-glucanase activity. The aim was to induce β-glucan breakdown to varying extents, obtaining two sourdoughs characterised by significantly different β-glucan molar masses. The molar mass of β-glucan extracted from the sourdoughs was determined before and after fermentation. Bread containing the above sourdoughs underwent an *in vitro* GI digestion simulation that mimicked upper human digestion. The β-glucan extractability and the viscosity of the extracts were observed in relation to the actual β-glucan concentration. Both extractability and viscosity served as indicators of the physiological functionality of β-glucan.

2. Material and methods

2.1. Raw materials

The raw material used for sourdough preparation was heat-treated wholegrain oat flour (WOF, Helsingin Mylly Oy, Järvenpää, Finland), added with sprouted oat grains (SPO, Sproutgrain oat, commonly used by the baking industry, Puratos Estonia OÜ, Peetri, Estonia; 48% water), or oat malt grains, commonly used by the brewing industry (OM, Moisture max. 7%; Viking malt Oy, Lahti, Finland). The nutritional composition of WOF per 100 g consisted of 13 g of protein, 6.9 g of fat, 59 g of carbohydrates, and 10 g of dietary fibre. The germinated oat ingredients (SPO, OM) were required as a source of enzymes and nutrients to boost the microbial starter fermentation performance. Furthermore, SPO and OM were selected for this study based on their different β-glucanase activity (**see test in 2.1.1**) to induce changes to the molecular weight of oat β-glucan. The raw materials used for bread making were whole grain oat flour (same as in the sourdough), gluten-free fine oat endosperm flour (Raisio Oyj, Raisio, Finland), tap water, fresh baker's yeast (Suomen Hiiva Oy, Lahti, Finland), sucrose (Suomen Sokeri Oy, Kantvik, Finland), and salt (Meira Oy, Helsinki, Finland).

2.1.1. β-glucanase test on germinated oat raw materials

Qualitative assessment of the β-glucanase activity was conducted on germinated oat ingredients by measuring the viscosity of a 1% β-glucan solution incubated for 24 h at 50 °C with the extracted supernatant from the SPO (sprouted oat grains, Puratos) and OM (oat malt, Viking). A substrate containing 1% β-glucan was prepared using high viscosity

β-glucan (495 kDa, purity >94%, from barley, Megazyme Ltd, Bray, Ireland). A 1% β-glucan solution was selected because this concentration could provide a measurable, reproducible viscosity in the control sample (incubated without germinated oat extracts), allowing reliable detection of β-glucanase activity through viscosity change in the samples incubated with extracts. Additionally, a 1% concentration was low enough to ensure complete solubilization of the β-glucan and to allow consistent measurement with the Haake Mars rheometer. Four hundred milligrams of β-glucan was weighed, moistened with 4 mL of 99.5% (v/v) ethanol and mixed with a magnetic stirrer at 600 rpm for 10 min. Sodium citrate buffer (0.05 M, pH 5) was added while constantly stirring the solution. The β-glucan solution was then heated to 80 °C (700 rpm). When the temperature of the substrate reached 80 °C, the weight of the solution was adjusted (Milli-Q water), and heated to 85 °C for 2 h (800 rpm). After the incubation time, the weight of the substrate was adjusted again and left to cool down. The substrate was kept at 4 °C until the day of the analysis.

Germinated oat material (1 g) was weighed in test tubes (SPO, OM, previously freeze-dried and finely homogenised with the use of a kitchen blender, Bamix ®, Switzerland). Five millilitres of sodium citrate buffer (0.05 M, pH 5) was added to each test tube containing the oat grains, then vigorously mixed. Two centrifugation steps at 10000 rpm for 15 min at 20 °C were performed to get a clear supernatant (extract). To test the presence of β-glucanase activity, 200 μL of the extract was added to 1.8 mL of 1% β-glucan substrate solution in duplicate. As a blank for viscosity measurement, three tubes containing 1.8 mL of the 1% β-glucan substrate were added with 200 μL of sodium citrate buffer (0.05 M, pH 5) (no addition of extract from germinated materials). Samples were vigorously mixed and incubated for 24 h at 50 °C at 1000 rpm in a ThermoShaker Mixing Block (MB-202, Hangzhou Bioer Technology Co., Ltd., Hangzhou, China) to mimic the fermentation time while maintaining the optimal temperature for β-glucanase activity. After the incubation, samples were boiled for 5 min to inactivate the enzymes and left to cool down. Viscosity was measured as described in **2.7.1**.

2.2. Microbial starters and sourdough fermentation

2.2.1. Microbial starters

Two LAB strains: *Lactiplantibacillus plantarum* SPC72-1 (KCTC 13314 BP) and *Fructilactobacillus sanfranciscensis* SPC-SNU 70–4 (KCTC 12779 BP) belonging to the Korean Collection for Type Cultures (KCTC, Korea, accession no., KCTC13314BP, KCTC 12779 BP) (Park et al., 2019) were used. The two LAB strains selected represent a species combination very commonly encountered in sourdoughs (De Vuyst et al., 2023) and were chosen as the best-performing to achieve proper sourdough acidification (see 2.1, Supplementary material). LAB were routinely cultivated in modified De Man, Rogosa, and Sharpe broth (MRS) at 30 °C for 24 h. Modified MRS broth preparation required 5.5% MRS Lactobacilli broth, 1% maltose monohydrate, and 0.5% yeast extract (Neogen®, Lansing, MI, USA) and the adjustment of pH to 5.6 by using 2.5 M HCl.

2.2.2. Sourdough preparation

Sourdoughs were prepared as 200 g batch. Two different recipes for sourdoughs were prepared, one containing the oat malt grains OM (named SOM) and one containing the sprouted oat grains SPO (named SSPO). The recipe of SOM sourdough consisted of 32.67% WOF, 0.67% OM, and 66.67% distilled water, while SSPO sourdough consisted of 30% WOF, 3.33% SPO, and 66.67% distilled water, with the same ratio of dry ingredients to distilled water (1:2). For the inoculum, microbial cells were cultivated at 30 °C for 24 h, centrifuged (10000 rpm, 10 min), and washed one time in sterile 0.9% (w/v) NaCl solution. The two microbial strains were inoculated together, aiming for an initial cell density of ca. 6 Log CFU/g each (0.2% of dough weight for *F. sanfranciscensis* 12779, 0.1% of dough weight for *L. plantarum* 13314). Fermentation was conducted at 28 °C for 24 h. Untreated oat doughs without starter inoculum were included (time 0 control dough having the same recipe of SOM = T0C-SOM and time 0 control dough having the same recipe of SSPO = T0C-SPO). An additional control consisting of only 33.33% WOF and 66.67% distilled water (T0C-WOF) was included in the study for the size exclusion chromatography analysis to investigate the molar mass of β-glucan, avoiding β-glucanase activity deriving from germinated ingredients.

2.2.3. Microbial enumeration, pH, total titratable acidity, organic acids and sugars

The microbial enumeration was performed on sourdoughs before and after fermentation (SOM, SSPO). Ten grams of sample were homogenised via a stomacher (Colworth, UK), with 90 mL of sterile 0.9% (w/v) NaCl and serially diluted for a proper plating in accordance with the plate count method followed. Plate count method in modified MRS agar having same recipe of mMRS broth with the addition of 1.5% no.1 bacteriological agar (Neogen®, Lansing, MI, USA) was used to confirm LAB presence on unfermented and not inoculated doughs at time 0 and on sourdoughs before and after fermentation. Total mesophilic bacteria have been counted on sourdoughs before and after fermentation using PCA agar (23.5 g in 1 L distilled water) (Neogen®, Lansing, MI, USA). The presence of other microbial groups was determined as explained in Cera et al. (2024). Before and after fermentation for 24 h, pH was measured using a Mettler Toledo 340 pH meter (Leicester, UK). The measurement of TTA was conducted using an automatic pH titrator (Easy Plus, Mettler Toledo, Columbus, OH, USA) by following the method described in Pöri et al. (2023) and reported as mL of NaOH 0.1 N required for getting 10 g of sourdough diluted with 85 mL of Milli-Q water (Merck Millipore, Darmstadt, Germany) and 5 mL of technical grade acetone to pH 8.5.

For the quantification of lactic and acetic acid, 4 g of sample was weighed and diluted with distilled water 5-fold for unfermented doughs and 10-fold for fermented samples. Extraction was carried out as described in Xu et al. (2017) with some modifications reported in Cera et al. (2024). A Waters Alliance e2695 high-performance liquid chromatography (HPLC) system equipped with a photodiode array detector (PDA, Waters 996, Waters Corp., Milford, MA, USA), a refractive index detector (RI; Waters 2414, Waters Corp., Milford, MA, USA) and a Hi-Plex H column (300 × 6.5 mm; Agilent, CA, USA) maintained at a temperature of 65 °C was used. Sulphuric acid (0.01 mol/L) was used as mobile phase, with a flow rate of 0.6 mL/min. Organic acids quantified were reported as mg/g of sample.

2.2.4. Sugars and total starch content

Quantification of sugars was carried out on freeze-dried samples of both unfermented controls and sourdoughs, according to Xu et al. (2017) with modifications reported in Cera et al. (2024). One hundred milligrams of the sample was added with 5 mL of Milli-Q water, mixed for 5 min and boiled for 5 min. After cooling, the sample was centrifuged (10000×*g*, 10 min, 4 °C). The preparation of the sample to be injected and an HPLC analysis have been carried out, as reported in Cera et al. (2024). Sugars were reported as % of dry weight (dw).

The total starch content (% dw) was analysed in the freeze-dried sourdough samples SOM and SSPO, and in their respective unfermented controls (T0C-OM, T0C-SPO), by using the total starch assay kit (AA/AMG) (Megazyme Ltd, Bray, Ireland). Buffers and reagents used were prepared according to the provided protocol and reported in Supplementary material (Appendix A). Analysis was performed including two replicates of control maize starch (one as blank, one as reaction tube) and three analytical replicates for each biological replicate of samples (one as blank, two as reaction tubes). For each tube, 100 mg of starch or sample was weighed. The rapid total starch (RTS) method (A in the kit assay) was used for analysing the control maize starch, while other procedures (E, followed by D of the kit assay) were used for analysing the T0C and fermented samples to remove D-glucose and possible maltodextrins with alcohol washing and to also include the

resistant starch in the calculation. The detailed procedure followed was reported in Supplementary material (Appendix A).

2.3. Molar mass determination of β-glucan

2.3.1. Material preparation

In the extraction of β-glucan, thermostable microbial α-amylase (1,4-α-D-glucan glucanohydrolase, Termamyl® 300L) and pancreatin (from porcine pancreas) (Sigma-Aldrich, St. Louis, MO, USA) were used. Alpha-amylase was heated to 80 °C in a heating block and kept for 15 min to inactivate side activities coming from enzymes other than the thermostable amylase. Afterwards, the solution was left to cool down and centrifuged twice (5000 rpm, 10 min) to get a clear supernatant used in the extraction procedure. Protease solution was prepared by dissolving pancreatin in 150 mM $NaHCO_3$ (Merck, Darmstadt, Germany) to reach a concentration of 18.75 mg/mL. It was then heated to 37 °C for 20 min, after which the solution was centrifuged (4000 rpm, 10 min) and the supernatant was used in the extraction procedure.

2.3.2. Extraction and purification of β-glucan

The extraction of β-glucan was performed on sourdoughs after fermentation (SOM, SSPO) and an untreated control dough that did not contain germinated oat (T0C-WOF) by following the method described by Laitinen et al. (2023) with a few modifications. The untreated control dough mentioned in this analysis was prepared specifically for size exclusion chromatography (**for recipe see 2.2.2**). Sourdoughs and unfermented control were mixed as such, and 126 g were weighed and added with 600 mL of Milli-Q water (ratio 21 g sourdough/100 mL water). After vigorous mixing, samples were incubated for 1 h at 40 °C with constant shaking (250 rpm). Incubation was followed by a centrifugation (8300 rpm, 20 min). Supernatant of centrifuged samples was collected, added with inactivated α-amylase as 1% (v/v) of the solution and incubated for 90 min at 80 °C in a water bath with frequent mixing. After treatment with amylase, solutions were boiled for 10 min and left to cool. Solutions were centrifuged again as mentioned above, and the supernatant was filtered with Miracloth (Merck Millipore, Darmstadt, Germany). The protease treatment was applied by adding pancreatin solution to the supernatant obtained at 1.5% (v/v) and incubating the solutions for 10 min in a water bath at 40 °C. The enzymatic treatment was stopped by using a boiling water bath for 10 min from the time when the solution reached 80 °C. Samples were then cooled down and centrifuged (as above). Supernatant was collected into new bottles and left overnight at 4 °C. On the day after, precipitation of β-glucan in ethanol was conducted by dropwise addition of 2.5 vol of 95% (v/v) ethanol to the solutions with continuous stirring via a glass burette. Solutions were left overnight at 4 °C. The next day, supernatant was removed, and precipitate was washed four times with the addition of 2 vol of 99% (v/v) ethanol and centrifugations (4000 rpm, 10 min). Pellet was suspended in 99% (v/v) ethanol, mixed, and dried for 3 days at 60 °C. The purity of β-glucan was determined using the mixed-linkage β-glucan assay kit (Megazyme Ltd, Bray, Ireland) (**see 2.6.2**).

2.3.3. High-performance size exclusion chromatography

To ensure consistent results in the determination of β-glucan molar mass and to prevent aggregate formation, as previously shown by Mäkelä et al. (2015), an approach using an organic solvent and a salt (DMSO with LiBr) was chosen. The β-glucans were dissolved in DMSO containing 0.01 M LiBr (as 2 mg/mL) and incubated for 2 h at 85 °C with continuous stirring at 1000 rpm. After incubation, the β-glucans were left overnight at room temperature and filtered (0.45 μm, Acrodisc™ filters, Pall, USA) before injecting. The molar mass was determined using high-performance size exclusion chromatography (HPSEC) with a Viscotek triple detection system. A system with a combination of a light scattering and viscometric detector Viscotek 270, Malvern Panalytical, Malvern, UK) and two light scattering angles (7° and 90°, $\lambda 0 = 670$ nm), and a refractive index (RI) detector (VE 3580, Viscotek) was used. The system was equipped with two PLgel MIXED-A LS 20 μm analytical columns maintained at 40 °C (7.5 × 300 mm; Agilent Technologies, Santa Clara, CA, USA) and a PLgel MIXED 20 μm guard column (7.5 × 50 mm) that led to the separation by using 0.01 M LiBr in DMSO as mobile phase (filtered via 0.1 μm membrane) at a flow rate of 0.8 mL/min, with an injection volume of 100 μL. OmniSEC 4.6 software (Viscotek) calculated the weight-average molar mass values (M_w) using a dn/dc value of 0.062 mL/g (Qin et al., 2013).

2.4. Bread making

Bread recipes are reported in Table 1. A control bread (CB), not added with sourdough, was baked on the same day as the sourdough breads. Bread added with sourdough SOM was reported as BSOM, while bread with SSPO was reported as BSSPO. Breads were made according to the method described by Sammalisto et al. (2024). Sourdoughs were added as 30.3% of the total bread dough weight (ca. 61% of flour weight). The ingredients were combined in a Varimixer (Metos Oy, Kerava, Finland) with the sourdough (CB excluded) and mixed for 7 min. After resting at room temperature (15 min), the dough was hand-shaped into 500 g loaves and placed in oiled baking pans. The loaves were then proofed (Lillnord TopLine, Odder, Denmark) (35 °C, 100% humidity, 30 min) and baked in a convection oven (Sveba Dahlen, Fristad, Sweden) at 205 °C for 30 min, starting with 20 s of steaming. After baking, breads were left to cool down for 1 h, and loaves were stored at room temperature for hardness and volume measurements, while frozen for the *in vitro* gastrointestinal simulation model.

2.4.1. Volume and hardness measurements

The volume of bread was measured on day 1, using a VolScan Profiler laser scanner (Stable Micro Systems Ltd, Godalming, UK), on three loaves of each bread. Specific volume (SV) was calculated as loaf volume/loaf weight (mL/g). Hardness was measured on days 1 and 4 after baking using a two-bite compression test using a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). The method used was previously described by Sammalisto et al. (2021) with a few modifications reported in Cera et al., 2024). The analysis was performed using a 5 kg load cell paired with a P/36 R cylindrical probe. Bread crumb samples were compressed twice to 40% deformation at a speed of 2 mm/s, with a 3 s interval between compressions.

2.4.2. pH and total titratable acidity of breads

The TTA of the bread was determined using an automated pH titrator following the same procedure used for the sourdoughs. Ten grams of homogenised bread crumb were dissolved in 85 mL of Milli-Q water (Merck Millipore, Darmstadt, Germany) and 5 mL of acetone. The total titratable acidity (TTA) was quantified by the volume of 0.1 N NaOH required to reach pH 8.5, with the analyses performed on three

Table 1
Bread formulations reported as % on flour weight (% fw) and % on dough weight (% dw). CB = control bread (no sourdough addition), BSOM = bread added with sourdough containing oat malt grains (SOM), BSSPO = bread added with sourdough containing sprouted oat grains (SSPO).

Ingredients	CB		BSOM		BSSPO	
	% fw	% dw	% fw	% dw	% fw	% dw
Water	90.00	45.45	54.22	26.60	51.02	25.25
Wholegrain oat flour	50.00	25.25	29.62	14.53	30.61	15.15
Endosperm oat flour	50.00	25.25	50.21	24.63	51.02	25.25
Sourdough			**61.75**	**30.30**	**61.22**	**30.30**
Salt	2.00	1.01	2.01	0.99	2.04	1.01
Sugar	3.00	1.52	3.01	1.48	3.06	1.52
Yeast	3.00	1.52	3.01	1.48	3.06	1.52
Total	**198.00**	**100.00**	**203.84**	**100.00**	**202.04**	**100.00**

biological replicates. The value of pH of breads reported corresponds to the initial pH measured by the pH titrator.

2.5. *In vitro* gastrointestinal simulation model and related analyses

To model the human gastrointestinal (GI) digestion of β-glucan from oat bread, an *in vitro* GI simulation with separate oral, gastric, and small intestine phases was used in combination with viscosity measurements, as already carried out and described in a previous research paper (Mäkelä et al., 2020) (Fig. 1).

2.5.1. *Material preparation*

The preparation of the buffers was done according to the recipes and instructions from Mäkelä et al. (2020). A 20 mM Na_2HPO_4 (Merck, Darmstadt, Germany) solution was made in Milli-Q water, and the pH was adjusted to 6.9. When the final volume was reached, NaCl was added to reach a final concentration of 10 mM. Enzyme solutions were prepared to mimic human digestion *in vitro*. Alpha-amylase solution (Termamyl® 300 L) and pancreatin (from porcine pancreas) solutions were prepared as described for the extraction of β-glucan (**see 2.3.1**). Pepsin solution was prepared by mixing pepsin from porcine stomach mucosa (>2500 U/mg, Sigma-Aldrich, St. Louis, MO, USA) with 0.9% (w/v) NaCl solution (final concentration 0.5 mg/mL). Bile acid from bovine and ovine (Sigma-Aldrich, USA) was dissolved in 150 mM $NaHCO_3$ to reach a concentration of 150 mg/mL to make a solution that was heated to 37 °C for 30 min with constant shaking. Buffers and enzyme solutions were prepared on the day before the *in vitro* GI procedure and stored at 4 °C until use.

2.5.2. *Sample pre-treatment*

For each bread sample, two replicate extractions from three baking days were analysed. Breads were left to thaw overnight in plastic bags at room temperature. On the day of the analysis, bread was cut into small pieces and homogenised with a kitchen blender (Bamix, Switzerland) prior to weighing. Part of the same bread was used for moisture content analysis (**see 2.5.3**). Additionally, part of the air-dried sample from moisture content analysis was milled to a particle size of 0.5 mm with an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany) and used for β-glucan content analysis (**see 2.6.3**). Bread samples were weighed based on an estimated value, aiming for a β-glucan concentration in the range of 0.6–0.8% (including buffer and enzymes). This concentration range was selected to take into consideration the high variation in extractability that can occur among bread samples, which varied from 36% to 54%, as observed by Mäkelä et al. (2020). The actual β-glucan content of *in vitro* extracts was then measured and compared to the observed extractability.

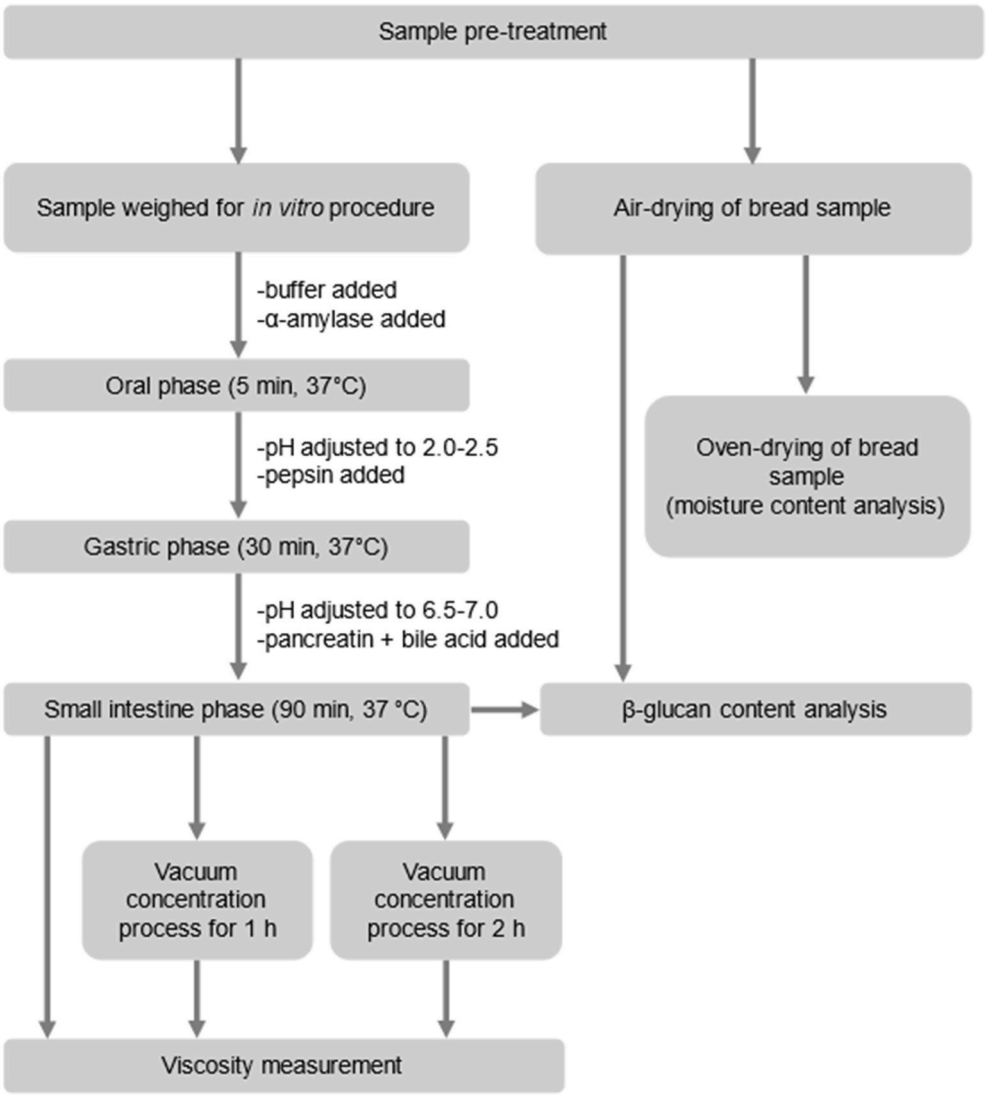

Fig. 1. Outline of the upper gastrointestinal model and analyses conducted on samples, adapted from (Mäkelä et al., 2020).

2.5.3. Moisture content analysis of breads

Total moisture content of all breads was measured according to the air-oven procedure AACC 44–15.02 (two-stage method). Bread samples pre-treated for *in vitro* (**see 2.5.2**) were accurately weighed (3 g) in crucibles and left to airdry at room temperature for 48 h. After air-drying, samples were weighed again to calculate the moisture content lost and incubated at 130 °C for 1 h for a second stage of drying. At the end, samples were weighed to calculate the total moisture content. Values of moisture content were used to calculate the β-glucan content in the original bread that underwent the *in vitro* digestion.

2.5.4. In vitro GI digestion simulation model procedure

The *in vitro* GI digestion simulation model started with the oral phase that required the addition of 20 mL of extraction buffer 20 mM Na_2HPO_4 + 10 mM NaCl (pH 6.9) (heated to ca. 37 °C in a microwave prior to addition). Ultraturrax T25 (Janke & Kunkel, IKA, Germany) was used to homogenise the samples at a speed of 8000 min^{-1} for 5 s. After the addition of 1 mL of inactivated Termamyl (α-amylase solution), samples were vigorously mixed and incubated horizontally with constant shaking at 37 °C for 5 min.

Gastric phase started with the adjustment of the pH of the samples to 2–2.5 by using 2.5 M HCl. Two mL of pepsin solution was then added, and samples were vigorously mixed and incubated for 30 min at 37 °C in a shaking incubator. To continue to the small intestine phase, the pH of the samples was adjusted to 6.5–7 using 4 M NaOH. Addition of 2 mL of pancreatin solution and 1 mL of bile acid solution was carried out, and after vigorous mixing, the samples were incubated at 37 °C for 90 min (shaking incubator). The samples were centrifuged (10000 rpm, 10 min), and the supernatant obtained was collected. To mimic the water absorption from the chyme that occurs in the small intestine phase, supernatant was weighed in a test tube, which was placed in a vacuum concentrator (SpeedVac Plus, SC110A combined with Refrigerated Condensation Trap RT100, Savant, Savant Instruments Inc. U.S.). One replicate tube from each sample was concentrated for 1 h, while the second replicate tube from the same sample was concentrated for 2 h. The total β-glucan content of the extracts was measured (**see 2.6.4**), and based on those results, the contents in concentrated samples were calculated taking into account the amount of evaporated water. Viscosities were measured (**see 2.7.3**) from the original extracts (duplicate samples, measured twice), additionally from the 1 h and 2 h concentrated samples (one replicate of each, measured twice).

2.6. β-glucan content analysis

The total β-glucan content was measured in sourdoughs and respective unfermented controls, in their purified β-glucan extracts for molar mass determination, in breads and in liquid extracts obtained from *in vitro* digestion using the Mixed Linkage β-glucan Assay kit (Megazyme Ltd Bray, Ireland). Two replicates of control oat flour were always analysed along with samples by following the procedure "A" described in **2.6.1**. Buffers and reagents used were prepared according to the commercial kit assay and reported in Supplementary material (Appendix A).

2.6.1. β-glucan content of sourdoughs

For sourdoughs and unfermented control doughs, the procedure "A" of the commercial kit was followed, except for the incubation time with diluted lichenase (2 h instead of 1 h). Freeze-dried samples (80 mg) were weighed in a tube and added with 0.2 mL of 50% (v/v) ethanol. Four millilitres of sodium phosphate buffer (20 mM, pH 6.5) was added. Tubes were incubated in a boiling water bath for 1 min, and after vigorous mixing for another 2 min. Samples were incubated at 50 °C for 10 min. After the addition of diluted lichenase (0.2 mL), samples were incubated for 2 h at 50 °C, with mixing every 15 min. Five millilitres of sodium acetate buffer (200 mM, pH 4.0) was added. Tubes were left to equilibrate to room temperature for 10 min and centrifuged (1000×*g*, 10 min). Aliquots of 0.1 mL of supernatant were dispensed into three test tubes (one as a blank, two as reaction tubes). Diluted β-glucosidase (0.1 mL) was added to the reaction tubes, while acetate buffer (0.1 mL, 50 mM, pH 4.0) was added to the blank tube. Incubation at 50 °C for 10 min was then carried out. Four D-glucose standards were prepared with sodium acetate buffer (50 mM, pH 4), two with 100 μg and the other two with 50 μg. All tubes (standards included) were added with 3 mL of GOPOD reagent and incubated at 50 °C for 20 min. Absorbance at 510 nm against water was measured for all samples. β-glucan content was expressed as % of dw.

2.6.2. β-glucan content of purified extracts for molar mass determination

To assess the purity of extracts obtained from sourdoughs (SOM, SSPO) and T0C-WOF to determine the molar mass of β-glucan (**see 2.3**), the procedure "A" in the assay kit (**see 2.6.1**), was followed with a few modifications. One analytic replicate was weighed and used for each extract obtained (one extract from one biological replicate), due to the limited amount of sample. In addition, the sample weight was 20 mg. Finally, a 3-fold dilution using sodium acetate buffer (200 mM, pH 4) was applied before dispensing the aliquots for the β-glucosidase enzymatic treatment. β-glucan content was expressed as % dw.

2.6.3. β-glucan content of bread samples

For air-dried breads, the procedure "B" in the assay kit, which included preliminary steps of extraction with ethanol, was performed to ensure the removal of free sugars and to reduce the levels of fats and oils (AOAC Method 995.16, AACC Method 32–33 and IC Standard Method No. 166). Sample pre-treatment was described in **2.5.2**. Two hundred milligrams of dried and milled sample was weighed in a test tube to get three analytical replicates for each sample, and the procedure described in the protocol was followed. Samples were added with 50% (v/v) ethanol (5 mL), placed in a boiling water bath for 5 min, added with further 5 mL of 50% (v/v) ethanol, and centrifuged (1800×*g*, 10 min). Supernatant was discarded, and pellet was resuspended in 5 mL of 50% (v/v) ethanol, mixed, and an additional 5 mL of 50% (v/v) ethanol was added. Centrifugation was carried out as mentioned above, followed by the removal of supernatant. After washing steps with ethanol, samples were treated by following the procedure "A" of the assay kit (**see 2.6.1**) from the addition of the diluted lichenase. Procedure changed only for the addition of sodium acetate buffer (200 mM, pH 4), which was 2 mL, whereas for procedure "A" in the assay kit, it was 5 mL.

2.6.4. β-glucan content of in vitro liquid extracts and β-glucan extractability

For the *in vitro* extracts, the procedure "C" in the assay kit was followed, with the only modification of the weight of the extract (1 g). On the day of *in vitro* digestion, two replicates were prepared and frozen until the day of analysis for each extract. Samples were left to thaw and incubated in boiling water for 5 min. After cooling, 3 mL of 95% (v/v) ethanol was added, the samples were mixed, and an additional 5 mL of 95% (v/v) ethanol was added. Centrifugation (1800×*g*, 10 min) was performed, and the supernatant was discarded. Pellets were resuspended in 8 mL of 50% (v/v) ethanol. Samples were then mixed, centrifuged (1800×*g*, 10 min), and the supernatant was discarded. Pellets were resuspended in 4 mL of sodium phosphate buffer (20 mM, pH 6.5) and incubated at 50 °C for 10 min. After incubation, the procedure "A" in the assay kit (**2.6.1**) from the addition of the diluted lichenase was followed.

The extractability of β-glucan (%), as the proportion of β-glucan dissolved from the oat matrix to the aqueous extract during the *in vitro* extraction, was calculated as follows:

$$Extractability\ (\%) = c \times \frac{V}{m} \times 100$$

where c is the β-glucan concentration of the extract (mg/mL), V is the

final volume of the extract (mL), and *m* is the total amount of β-glucan in the *in vitro* sample (mg).

2.7. Viscosity measurements

2.7.1. Viscosity for β-glucanase test on raw materials

The viscosity of pure β-glucan substrate after incubation with extracts obtained from germinated oats was used as an indicator of β-glucan degradation. Measurement was carried out three times for each sample using the HAAKE MARS 40 rheometer (Thermo Scientific, Germany). A rotational measurement program was used with shear rate increasing stepwise from 1 s^{-1} to 100 s^{-1}. Mean values of viscosity (mPa × s) at a shear rate of 10 s^{-1} were used.

2.7.2. Viscosity of sourdoughs

The viscosity of the untreated control doughs and fermented samples was measured using a rotational rheometer (Rheolaab QC, Anton Paar GmbH, Graz, Austria) equipped with a temperature device set up at 23 °C (C-PTD 180/AIR/QC), an ST22.02–4 V probe, and a measuring cup C-CC27/QC-LTD. The rheometer measured from a shear rate of 2 s^{-1} to 100 s^{-1}. Mean values of viscosity (Pa × s) at a shear rate of 100 s^{-1} were used.

2.7.3. Viscosity of in vitro liquid extracts

Viscosity measurements of the extracts at three different concentration levels (original extracts and the concentrated ones) were performed with HAAKE MARS 40 rheometer (Thermo Scientific, Germany) by using a rotational measurement program with shear rate increased stepwise from 0.3 s^{-1} to 300 s^{-1}. The viscosities (mPa × s) at a shear rate of 25 s^{-1} were used to report the results of apparent viscosities as a function of the extracted β-glucan concentration.

2.8. Statistical analyses

For all analyses, data from three biological replicates (n = 3) were analysed, except for viscosity measurements and β-glucan content of *in vitro* liquid extracts (**see 2.5.4, 2.6.4**). One-way analysis of variance (ANOVA) was applied to assess differences among groups. When the ANOVA indicated significant effects ($p < 0.05$), Tukey's test was used for all pairwise comparisons, and Dunnett's test was used to compare each treatment group with the control. For β-glucanase activity in germinated oat grains, pairwise comparisons were performed using Bonferroni correction. All statistical analyses were conducted using IBM SPSS Statistics (Version 29, IBM®, Chicago, IL, USA).

3. Results

3.1. β-glucanase activity of germinated oat grains

The decrease in viscosity of β-glucan substrate after incubation with extract obtained from germinated oat grains indicated the presence of β-glucanase activity in the starting material. The viscosity of β-glucan substrate incubated with Na-citrate buffer (control), SPO extract, and OM extract was: 49.8 ± 0.6, 44.4 ± 1.2, and 2.2 ± 0.1 mPa × s, respectively. Significant differences were found between OM and the other samples (control and SPO).

3.2. Sourdough fermentation

3.2.1. Microbial enumeration

In the WOF, no LAB, yeasts, Enterobacteriaceae and *B. cereus* were detected. Before fermentation, SOM and SSPO had a LAB cell density of 6.6 ± 0.02 and 6.6 ± 0.1 log CFU/g, respectively. After 24 h of fermentation at 28 °C, LAB cell density was 9.5 ± 0.04 and 9.5 ± 0.2 log CFU/g in SOM and SSPO, respectively. Additional results regarding microbial enumeration are reported in Supplementary material

Table 2
pH, total titratable acidity (TTA) (mL of NaOH), lactic and acetic acid (as mg/g of sample), sugars (% dw), total starch (% dw) and total β-glucan content (% dw), and viscosity values (Pa × s) in unfermented controls (T0C-OM, T0C-SPO) and after 24 h of fermentation (SOM, SSPO). Data were reported as means of three biological replicates ± standard deviation.

Sample	pH	TTA (mL of NaOH)	Lactic Acid (mg/g)	Acetic Acid (mg/g)	Glucose % dw	Sucrose % dw	Fructose % dw	Maltose % dw	Total starch content (% dw)	Total β-glucan content (% dw)	Viscosity (Pa × s)
T0C-OM	6.1 ± 0.05^{c}	1.6 ± 0.04^{a}	nd^{a}	nd^{a}	nd^{a}	1.1 ± 0.02^{c}	nd	0.2 ± 0.2^{a}	47.3 ± 4.4^{a}	4.4 ± 0.2^{b}	7.6 ± 0.9^{b}
SOM	4.1 ± 0.02^{a}	9.7 ± 0.4^{c}	6.9 ± 0.2^{c}	0.4 ± 0.04^{b}	0.01 ± 0.02^{a}	0.7 ± 0.02^{b}	nd	1.3 ± 0.03^{b}	45.9 ± 2.6^{a}	4.3 ± 0.1^{b}	1.9 ± 0.4^{a}
T0C-SPO	6.1 ± 0.01^{c}	1.4 ± 0.02^{a}	0.1 ± 0.01^{a}	nd^{a}	0.03 ± 0.03^{a}	1.2 ± 0.2^{c}	nd	0.2 ± 0.04^{a}	51.7 ± 2.2^{a}	3.9 ± 0.2^{a}	8.2 ± 0.2^{b}
SSPO	4.3 ± 0.05^{b}	7.2 ± 0.2^{b}	5.6 ± 0.1^{b}	nd^{a}	nd^{a}	nd^{a}	nd	nd^{a}	48.3 ± 2.2^{a}	4.3 ± 0.1^{b}	11.1 ± 0.4^{c}

dw: dry weight; different superscript lowercase letters (a-c) in the same column indicate statistically significant (Tukey's, $p < 0.05$) differences (n = 3); nd = not detected.

(Appendix A).

3.2.2. pH, total titratable acidity (TTA), organic acids, sugars and total starch content

All sourdoughs reached a pH value of ca. 4. SOM was the most acidic sourdough with a TTA of 9.7 mL (of NaOH), while SSPO had 7.2 mL of NaOH (Table 2). Lactic acid produced ranged in the interval 5.6–6.9 mg/g of sample, whereas acetic acid was found only in SOM (0.4 mg/g) (Table 2). Glucose was detected in SOM and T0C-SPO in traces (0.01% and 0.03% dw). Maltose significantly increased in SOM from 0.2 to 1.3% dw, while no significant difference was found between SSPO and the unfermented counterpart. Sucrose significantly decreased from 1.1 to 0.7% dw in SOM, while in SSPO after fermentation, no sucrose was found. Fructose was not detected in any of the samples (Table 2). Total starch content slightly decreased after fermentation, but the difference was not statistically significant.

3.2.3. β-glucan content and viscosity measurements of sourdoughs

β-glucan content remained stable in SOM, while it significantly increased in SSPO when compared to the unfermented dough (Table 2). A significant decrease in viscosity occurred in the sourdough containing the oat malt (SOM) from 7.6 (of T0C-OM) to 1.9 Pa × s, whereas a significant increase was detected in the sourdough with sprouted oat grains (SSPO), reaching 11.1 Pa × s from a mean value of 8.2 Pa × s in T0C-SPO (Table 2).

3.2.4. β-glucan molar mass determination

The purity of β-glucan extracts obtained ranged from 60.8% dw to 65.5% dw, which was considered reasonable to proceed with molecular weight analysis. Molecular weight distribution of β-glucan was clearly different among samples (Fig. 2). The weight-average molar mass value

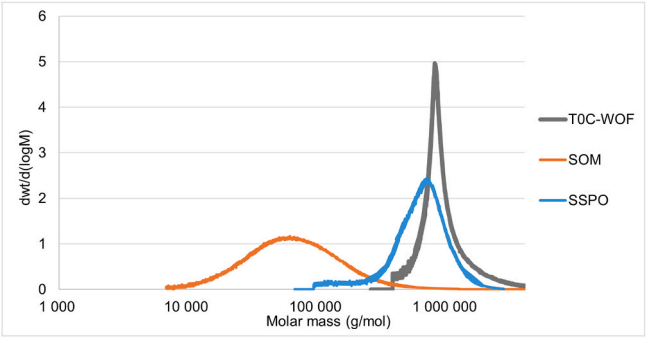

Fig. 2. The distribution of molar mass for three different samples (T0C-WOF, SOM, and SSPO) on a logarithmic scale with base 10. The molar mass (g/mol) on a logarithmic scale on the x-axis. The differential weight fraction (dwt/d (logM)) on the y-axis. T0C-WOF = purified extract of unfermented dough containing only wholegrain oat flour and water; SOM = purified extract from sourdough with oat malt grains OM; SSPO = purified extract from sourdough with sprouted oat grains SPO.

Table 3
Weight-average molar mass (M_w) of β-glucan in T0C-WOF (unfermented dough containing wholegrain oat flour and water), SOM (sourdough with oat malt grains, OM), and SSPO (sourdough with sprouted oat grains, SPO). Data are reported as mean values ± standard deviation (n = 3).

Purified β-glucan extract	× 10^3 g/mol M_w
T0C-WOF	1060.3 ± 83.6^c
SOM	117.4 ± 23.1^a
SSPO	681.7 ± 21.4^b

Different superscript lowercase letters (a-c) in the same column indicate statistically significant (Tukey's, $p < 0.05$) differences (n = 3).

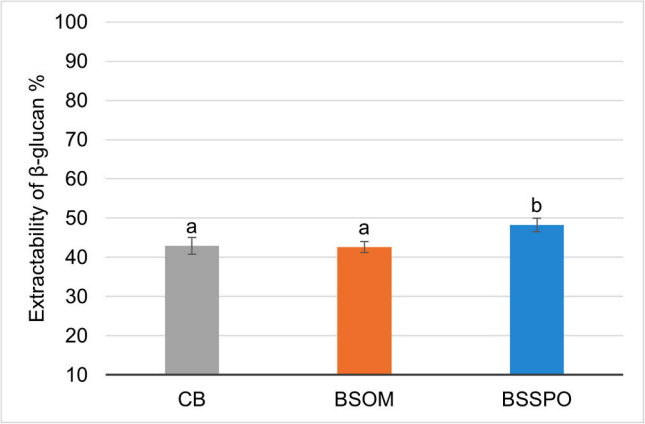

Fig. 3. Extractability of β-glucan reported as the percentage of β-glucan that was dissolved in the extract after *in vitro* GI simulation. CB = control bread (no sourdough addition), BSOM = bread added with sourdough containing oat malt grains (SOM), BSSPO = bread added with sourdough containing sprouted oat grains (SSPO). Different lowercase letters (a–b) indicate statistically significant (Tukey's, $p < 0.05$) differences (n = 3).

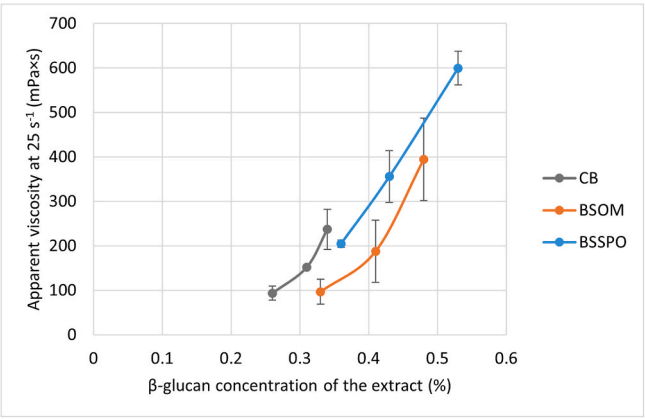

Fig. 4. Apparent viscosities at 25 s^{-1} of the *in vitro* extracts plotted against their β-glucan concentration. CB = control bread (no sourdough addition), BSOM = bread added with sourdough containing oat malt grains (SOM), BSSPO = bread added with sourdough containing sprouted oat grains (SSPO).

(M_w) of the extract obtained from the unfermented T0C-WOF was 1060.3 × 10^3 g/mol (Table 3). A statistically significant degradation of β-glucan was detected in SSPO with M_w 681.7 × 10^3 g/mol. However, the lowest value of M_w was observed in SOM (117.4 × 10^3 g/mol), which was significantly different from SSPO and T0C-WOF.

3.3. Bread: acidity, hardness and volume measurements

BSOM had a significantly higher acidity than BSSPO and CB. However, among sourdough breads, BSSPO had a significantly higher volume and softer crumb than CB after one day of baking, whereas BSOM was not statistically different from CB. More details about the results of hardness and volume measurements, and acidity of breads (pH and TTA) can be found in Supplementary material (Appendix A, Table A1).

3.4. Extractability of β-glucan and viscosity of extracts from in vitro GI simulation model

The extractability of β-glucan was calculated as the proportion of β-glucan dissolved from the cereal matrix into the aqueous extract during the *in vitro* digestion (Fig. 3). A statistically significant difference was found between BSSPO and other breads, with a value of 48.3%,

while CB and BSOM had 42.9% and 42.6% respectively.

The viscosity of extracts was measured right after *in vitro* digestion, and after concentration steps (1 h, 2 h), therefore, three concentration values were obtained. Viscosity values were reported as a function of β-glucan concentration (Fig. 4). In the samples analysed, after a 2 h concentration step, β-glucan content in the extracts ranged from 0.34% to 0.53%, whereas viscosity increased from 237 mPa × s to ca. 600 mPa × s at increasing concentration of β-glucan. CB had a viscosity of 237 mPa × s when the concentration was 0.34%. BSSPO had a viscosity of 205 mPa × s with a concentration of 0.36% and sharply increased to 599.6 mPa × s, corresponding to a concentration of 0.53%. Regarding the *in vitro* extract of BSOM, at concentrations comparable to BSSPO (0.33% and 0.41% for BSOM vs. 0.36% and 0.43% for BSSPO), viscosities were lower with 96.8 mPa × s and 187.7 mPa × s for BSOM compared to 205 mPa × s and 355.7 mPa × s for BSSPO.

4. Discussion

Oats have high lipase activity and high lipid content and thus are commonly heat-treated to inactivate lipid-degrading enzymes and ensure a longer shelf-life and better flavour in food (Jaksics et al., 2023; Lehtinen et al., 2003). However, heat treatment also deactivates other enzymes, such as proteases and amylases, which are essential for modifying the cereal substrate during fermentation to support LAB metabolism (Blontrock et al., 2025; Hammes et al., 2005). Preliminary tests with the raw materials in this study showed that the use of wholegrain oat flour alone was not enough to achieve the desired fermentation performances. Thus, in this study, oat flour fermentation was carried out with the addition of germinated ingredients as a source of enzymes (SOM and SSPO).

LAB viability reached at the end of fermentation is typical for sourdough, i.e., 9.5 log CFU/g after 24 h (Arora et al., 2021). The detection of different microbial groups in the germinated ingredients (LAB, Enterobacteriaceae, *Bacillus spp.*, yeasts, and moulds) and oat malt was most likely due to the germination process itself and the absence of severe heat treatment on these materials. The use of germinated oat in sourdough is a well-known practice in bakeries to achieve a higher nutritional profile and better flavour in bread (Benincasa et al., 2019; Mäkinen et al., 2013). Acidification of sourdoughs, both in terms of pH and TTA, was in line with previous findings, even though different raw materials and fermentation methods were used (Grgić et al., 2024; Hüttner et al., 2010). Lactic acid was the most prevalent acid produced during fermentation, whereas acetic acid was detected in traces only in the sourdough containing the oat malt (SOM). These results are in accordance with the metabolism of *L. plantarum* and *F. sanfranciscensis* as sourdough starters (De Vuyst et al., 2023; Felis & Dellaglio, 2007; Punia Bangar et al., 2022). Mono- and disaccharides detected in the unfermented control doughs were extensively consumed by LAB. The only exceptions were sucrose, which slightly decreased, and maltose, which significantly increased in SOM, most probably due to the presence of α-amylase activity from oat malt or microorganisms in this sample, resulting in mild starch degradation and the release of maltose (Khaswal et al., 2024; Mäkinen et al., 2013; Tiwari et al., 2015). However, the total starch content in this study did not change significantly during fermentation in either sourdough; therefore, hydrolysis was not detected.

In both sourdoughs, β-glucan degradation significantly differed, and this difference can be mainly linked to the type of germinated oat used in fermentation, since the same association of LAB (*L. plantarum* 13314 and *F. sanfranciscensis* 12779) and inoculum were used in both SOM and SSPO. Fermentation induced clear changes in macromolecules, which had an impact on the extractability of β-glucan and the viscosity after *in vitro* GI digestion simulation model. Oat malt (OM) had higher β-glucanase activity than sprouted oats (SPO) (**3.1.1**). The β-glucanase activity observed in these materials was most likely due to the endogenous enzymes of the grains activated during malting and sprouting processes, respectively, but possibly also to the grain microbiota, such as *Bacillus* spp., yeasts, and moulds (Jin et al., 2023). The M_w of β-glucan in the native wholegrain oat flour and without the addition of germinated oats (T0C-WOF) was 1060.3 × 10^3 g/mol (Fig. 2, Table 3), in accordance with (Laitinen et al., 2023) and other previous findings (Ajithkumar et al., 2005) reported oat β-glucan M_w as ca. 1200 × 10^3 g/mol and between 1250–1780 × 10^3 g/mol, respectively. The use of oat malt (SOM) led to a drastic decrease in the M_w of β-glucan, to 117.4 × 10^3 g/mol, with a decrease of 75% in sourdough viscosity (after 24 h of fermentation), while total β-glucan content did not change. In contrast, the M_w of β-glucan in the sourdough containing sprouted oats (SSPO) was only half of the native raw material (681.7 × 10^3 g/mol). Additionally, the molar mass distribution of β-glucan from T0C-WOF was mildly dispersed with a narrower peak (Fig. 2), especially when compared to the β-glucan from SOM and SSPO, which became more and more dispersed as a consequence of molecular degradation. In an earlier study, *L. plantarum* induced a significant decrease in the total content of β-glucan, but only a moderate degradation of its molecular weight after 8 h of oat fermentation at 30 °C (Lu et al., 2019). In our study, a high molecular weight of β-glucan was retained up to 24 h of oat fermentation, containing sprouted oats (SSPO). Even though *L. plantarum* and *F. sanfranciscensis* species may possess β-glucosidase enzymes capable of breaking down β-1→4 linkages from the non-reducing end of small glucosides deriving from β-glucan structure, the fermentation process used in our study did not decrease β-glucan content. In earlier studies conducted on oat substrates, the decrease in β-glucan was either significant or not, depending on the microbial strain used (Bernat et al., 2015; Djorgbenoo et al., 2023; Gupta et al., 2010; Mårtensson et al., 2002). However, although the M_w of β-glucan of SSPO was lower than that of the unfermented control, this decrease did not lower the viscosity. On the contrary, an increase in viscosity from 8.2 to 11.1 Pa × s and in total β-glucan content (from 3.9 to 4.3 % dw) was observed in SSPO. This is in accordance with the observation that in the presence of lower molecular weight β-glucan, a higher concentration of this polysaccharide is required to achieve a target viscosity (Wood, 2010).

In our study, it can be assumed that the degradation of β-glucan might have led to an increased solubility and extractability, probably assisted also by a longer exposure to water (24 h) compared to the unfermented control. Indeed, moderate β-glucan breakdown by endogenous enzymes from sprouted oats, in combination with changes induced by fermentation in protein or starch fractions, might have played a role in enhancing β-glucan extractability, increasing the solubilised β-glucan concentration, and thereby viscosity. Although protein-polysaccharide interactions or their effects on β-glucan behaviour were beyond the aim of this study, it can be hypothesised that in a bread matrix, protein and polysaccharide, such as starch, can theoretically modulate the extractability of dietary fibre (Ziobro et al., 2016) and, therefore, also the solubility of β-glucan by altering matrix structure or competing for water during digestion. However, literature on the interaction between oat β-glucan and food matrix in a gluten-free system is currently very limited (Bieniek & Buksa, 2024; Karp et al., 2020; Sammalisto et al., 2024; Sciarini et al., 2017). An increase in soluble β-glucan was previously observed after 8 h of fermentation by *L. plantarum*, possibly due to the degradation of the insoluble fraction (Lu et al., 2019). Acidification of breads was comparable to previous findings on wholegrain oat breads (Cera et al., 2024) and others conducted on composite wheat-oat breads (Flander et al., 2011; Grgić et al., 2024). Bread added with the sourdough containing sprouted oats (BSSPO) had significantly higher specific volume (SV) (1.7 mL/g) and softness than the other sourdough and control bread. This is in line with previous findings on sourdough addition to oat bread (Cera et al., 2024; Hüttner et al., 2010). A possible explanation for the differences in bread quality between sourdough breads could be attributed mainly to the varied β-glucan degradation. Indeed, a recent study showed that the addition of oat β-glucan having high M_w (1714 × 10^3 g/mol) was the most effective to increase the volume, reduce hardness, and increase moisture content of gluten-free

bread (Bieniek & Buksa, 2024). More hydrolysed β-glucan (24×10^3 and 85×10^3 g/mol) was also able to reduce crumb hardness. In contrast, in an earlier study, the addition of hydrolysed oat β-glucan did not affect the volume of bread; however, it helped to reduce the hardness of the crumb (Pastuszka et al., 2012). It can be assumed that the different results obtained depend on the interaction of β-glucan with other macromolecules present in the matrix. Furthermore, the addition of oat malt <1% on dry flour basis was beneficial for increasing the volume and improving the crumb in bread due to the enzymatic activities (Mäkinen et al., 2013).

In this study, the degradation of β-glucan in the sourdoughs affected its *in vitro* extractability from the bread, even though the sourdough represented "only" 30.3% of the total dough weight. Extractability of β-glucan from breads, defined as the proportion of β-glucan dissolved from the oat matrix to the aqueous extract during the *in vitro* extraction, ranged from 42.6% to 48.3%, consistently with previous results on oat breads (Mäkelä et al., 2020). Although freezing might reduce the extractability of β-glucan (Beer et al., 1997), all samples were kept frozen for a similar time and underwent *in vitro* digestion after a single thaw to minimise variability. Extractability, molecular weight, and concentration of β-glucan have all been considered critical for its ability to increase the viscosity of the digesta (Rieder et al., 2017; Wood, 2010). Several factors, including extraction temperature, moisture content, and particle size of the food matrix, can affect the solubility and extractability of β-glucan (Mälkki & Virtanen, 2001). In this study, the extraction temperature was kept constant in the *in vitro* digestion, and all bread samples had a similar moisture content, with dough recipes differing only in proportion and type of germinated oats. Thus, differences in the extractability of β-glucan between BSSPO and other bread samples were likely due to variations in β-glucan M_w and molar mass distribution in the sourdough. However, other enzymatic activities from the germinated material might also induce matrix changes during mixing and proofing time that were not assessed. In our study, bread containing sourdough with sprouted oats showed the highest extractability of β-glucan, whereas it was similar in control bread and bread with sourdough added with oat malt. Bread with the sourdough containing oat malt (BSOM) showed similar extractability of β-glucan to the control bread. In this case, a more extensive degradation of β-glucan to low molecular weight structures probably led to the formation of unextractable aggregates, as probably occurred during fermentation in a wheat-rye bread (Trogh et al., 2004). As a result, although sourdough was added to the recipe, β-glucan extractability did not increase.

The increased viscosity of β-glucan extracts after *in vitro* GI digestion has been positively correlated with reduced starch digestibility, suggesting potentially improved *in vivo* functionality (Kim & White, 2013). High-M_w β-glucan provided the greatest viscosity and effectively lowered the rate of starch digestion, and consequently the estimated GI values after *in vitro* digestion, although it exhibited lower solubility than medium- and low-M_w β-glucan (Kim & White, 2013). *In vivo*, higher molecular weight oat β-glucan requires lower doses to reduce blood peak glucose compared with lower molecular weight fractions in healthy non-diabetic individuals (Noronha et al., 2023). Nevertheless, it should be noted that the impact of β-glucan extracts is always modulated by their physicochemical properties (solubility, M_w, and viscosity), considered key factors in modulating starch digestibility and in predicting glycaemic response in *in vitro* models (Kock et al., 2018). Our *in vitro* extracts showed high viscosity values, especially when compared to other oat-based products analysed in a previous study (Mäkelä et al., 2020). Extracts from BSSPO exhibited higher extractability and higher viscosity at comparable β-glucan concentrations (both at 0 h and after 1 h of the concentration step) to the other sourdough bread studied (BSOM) (Figs. 3 and 4). The higher viscosity of the extract from BSSPO, when compared to BSOM, showed a rheological behaviour that might lead to an increased *in vivo* physiological functionality since it has been previously linked with the ability of oat β-glucan to increase luminal viscosity (Wolever et al., 2010). Breads did not contain other components that influenced viscosity, such as hydrocolloids, and LAB used did not show the ability to produce exopolysaccharides (data not shown). However, it was not possible to directly compare the viscosity of extracts from control and sourdough breads due to differences in β-glucan concentrations and this should be considered in future studies.

5. conclusion

The findings showed that oat fermentation process can be designed to achieve moderate β-glucan degradation and an increase in β-glucan extractability, leading to improved bread texture and possibly physiological functionality of β-glucan. This study contributes to the development of oat-based food with enhanced nutritional properties that can benefit public health. However, to validate the observed health benefits, *in vivo* studies would also be needed.

CRediT authorship contribution statement

Silvia Cera: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Maija Ojanen:** Investigation. **Emmi Santapakka:** Investigation. **Miikka Laitinen:** Writing – review & editing, Methodology. **Seongbong Song:** Writing – review & editing, Conceptualization. **Sungwon Hur:** Writing – review & editing, Conceptualization. **Kati Katina:** Writing – review & editing, Conceptualization. **Ndegwa H. Maina:** Supervision, Methodology. **Noora Mäkelä-Salmi:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rossana Coda:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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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.foodhyd.2026.112522.

Data availability

Data will be made available on request.

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