Journal of Cereal Science 132 (2026) 104571

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Fermentation of enzyme-hydrolysed oats by *Lactobacillus delbrueckii*: Viability, EPS production and nutritional properties

Sharmeen Mustafa, Swarnima Agnihotri *, Patrik R. Lennartsson

Swedish Centre for Resource Recovery, University of Borås, Borås, 50190, Sweden

ARTICLE INFO

Keywords:
Oats
Lactobacillus
Exopolysaccharides
Fatty acids
Proteins
Fermentation

ABSTRACT

Oats (*Avena sativa*) are commonly cultivated in the temperate climate of the Nordic countries and are being increasingly used to produce dairy yoghurt replacements, similar to soy-based products common in East Asia. This study had two main aims: i) to achieve viable counts of the exopolysaccharide (EPS)-producing *Lactobacillus delbrueckii* subsp. *Bulgaricus*, a strain previously reported to possess probiotic characteristics, grown on enzyme hydrolysed oat substrate, and (ii) to assess the post-fermentation impact mainly on the macronutrient profile of the oat substrate compared with untreated oat flour. Bacterial growth was investigated on solid loadings of 3% and 5% (d.w.b.) at 37°C and 30°C, temperatures optimal for cell growth and EPS production, respectively. The highest cell concentration of 1.33×10^8 cells/mL was recorded at 18 h for 5% solid loading incubated at 37°C. EPS production was indicated by the presence of characteristic O–H, C–H, and C=O functional groups, corresponding to FTIR absorption bands at 3400 cm^{-1}, 2925 cm^{-1}, and 1640 cm^{-1}, respectively. A significant ($p < 0.05$) increase in the total lipid content of the fermented oats (19.92 ± 0.19% d.w.b.) was observed, including a marked increase in mono- and polyunsaturated fatty acids such as oleic (6.36 ± 0.08% d.w.b.) and linoleic acid (8.69 ± 0.08% d.w.b.). The crude protein content also increased to 49.23 ± 1.32% with a modest improvement in methionine content. Overall, these nutritional properties, together with polysaccharide production indicated that enzyme hydrolysed and fermented oats have strong potential as a nutritious dairy-free alternative.

1. Introduction

Lactic Acid Bacteria (LAB) have historically been used to extend the shelf life of both fresh and cooked foods through fermentation. LAB encompass several genera, including *Lactobacillus* and *Lactococcus*, and are named for their primary metabolite, lactic acid. The genus *Lactobacillus* is commonly found in milk and fermented milk products, with various species isolated from these dairy products. The bacterial strain of interest in this study, *Lactobacillus delbrueckii* subsp. *bulgaricus,* is one such example and was isolated from Bulgarian yoghurt in 1919 (Weiss et al., 1983).

Lactobacillus delbrueckii is a major LAB strain studied for its probiotic potential, attributed to properties such as bile resistance and hence tolerance to extreme pH, production of antimicrobial compounds, adhesion to the epithelial lining of the human gut, and colonization of the intestine (Gotcheva et al., 2002; Kumar and Salminen, 2016).

In addition to its probiotic properties, *Lactobacillus delbrueckii* produces exopolysaccharides (EPS), which have been reported to have some prebiotic properties in some studies(Tarique et al., 2023). EPS contribute to the formation of protective biofilms, cell adhesion, aggregation, and intercellular interaction. Beyond their prebiotic role, EPS have been reported to exhibit several bioactive properties, including anti-cancer, anti-viral, anti-gastritis, and anti-ulcer effects, as well as immunomodulatory, antioxidant, and cholesterol-lowering potential (Kaur and Dey, 2023). *Lactobacillus delbrueckii ssp bulgaricus* is known to secrete EPS_5, which, in addition to its well-studied potential therapeutic properties, may support periodontal regeneration, a promising yet underexplored area in clinical sciences (Khalil et al., 2022).

The popularity of certain LAB strains, especially those that belong to the genera *Lactobacilli*, lies in the traditional consumption of fermented foods worldwide, especially fermented dairy products. However, the predominance of dairy-based probiotic foods poses challenges for lactose-intolerant and vegan consumers, creating a potential demand for non-dairy probiotic products. In Eastern Asia, soy-based fermented products and probiotics are already well-established and are popular, owing to the cultural and historical significance of soy in the diet. This

* Corresponding author.
E-mail address: swarnima.agnihotri@hb.se (S. Agnihotri).

https://doi.org/10.1016/j.jcs.2026.104571
Received 15 April 2026; Received in revised form 9 October 2026; Accepted 16 October 2026
Available online 17 October 2026
0733-5210/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

has facilitated a shift towards adapting non-dairy probiotic products in these regions (Xie and Gänzle, 2023).

Recent trends have focused on cultivating LAB on alternative substrates such as low-value agricultural residues, and industrial byproducts to produce higher value products. Byproducts from rice and cereal processing industries, such as rice and wheat flour, wheat bran and brewer's spent grain have been effectively utilised for the growth of LAB. The starch-rich fraction of such residues, often combined with amylolytic treatment, makes these a suitable substrate for cultivation of LAB. (Juodeikiene et al., 2016; Karetkin et al., 2017; Babini et al., 2020). Oats are a popular grain easily grown in the temperate climate of Nordic countries, renowned for their beneficial nutritional profile compared to other grains. Besides, being rich in starch, oats are particularly noted for their lipid profile, which features a relatively high percentage of unsaturated fatty acids relative to saturated fatty acids (Kouřimská et al., 2018) and their abundance of β-glucan, a major soluble dietary fibre (Wang et al., 2016). These characteristics, along with the presence of other beneficial compounds such as avenanthramides, vitamins, and flavonoids, contribute to reducing cholesterol, cardiovascular diseases and other non-communicable diseases (Djorgbenoo et al., 2023). Consequently, oats are an attractive choice for a local agro-based alternative for the cultivation of the probiotic strain *Lactobacillus delbrueckii* while simultaneously being a good model substrate for starchy byproducts.

The aim of this study is to determine the suitability of enzyme-treated oat flour as a substrate for cultivating *Lactobacillus delbrueckii*, with a focus on achieving viable bacterial counts, EPS production, and beneficial modifications to the oat lipid profile and protein content.

2. Material and methods

2.1. Material

Oat flour was received in two bags of 20 kg from AXA Lantmännen Cerelia (Malmö, Sweden) to be used as the substrate for all cultivations.

2.2. Enzymatic hydrolysis and substrate preparation

Oat suspensions at 3% and 5% w/v dry weight basis (d.w.b.) were prepared for enzymatic hydrolysis before cultivation. Both alpha amylase (heat stable alpha amylase from *Bacillus licheniformis*, 20000-60000 U/mL, A3306, Sigma Aldrich) and gluco-amylase (Amyloglucosidase from *Aspergillus niger*, 3260 U/mL, Megazyme, Ireland) were used simultaneously to completely hydrolyse the starch-rich oat suspension to extract a glucose rich fraction. A total volume of 10 μL and 24 μL of alpha amylase and 20 μL and 40 μL of glucoamylase was used to hydrolyse 100 mL of 3% and 5% oat suspensions respectively. The enzyme hydrolysis was carried out at 60°C for 48 h, where half of the total volume of each enzyme was added to the suspension at 0 h, followed by addition of 0.25× at 16 h and 24 h each.

The oat slurry after the enzyme hydrolysis was first passed through a sieve of pore size 63 μm and then centrifuged at 3000×g for 20 min to remove the remaining oat residues. The supernatant was collected and autoclaved for both inactivation of enzymes and for further use as the substrate for cultivation.

2.3. Cell culture and media

The strain *Lactobacillus delbrueckii sub species bulgaricus* DSM 20081 was obtained from Culture Collection University of Gothenburg (CCUG), Sweden, in a freeze-dried form. It was activated using sterile water and grown on de Man Rogosa Sharpe (MRS) (Merck) agar plates for 24 h at 37°C. An anaerobic atmosphere was maintained using Anaerocult ™ (Merck). Distinct colonies were then selected from the plate and grown in MRS broth to prepare stock cultures in 20% v/v glycerol, which were stored at −80°C. The stock cultures were propagated in fresh MRS broth twice before preparing working stock cultures in 20% v/v glycerol stored in −20°C. The working stock culture was transferred at 1% v/v into MRS broth and grown anaerobically at 37°C until the cell concentration was 1×10^8 CFU/mL. This was then used as the pre-inoculum for all cultivations in both synthetic media and oat flour suspension.

2.4. Growth kinetics

The pre-inoculum was added to freshly prepared MRS broth to achieve an initial cell concentration of 1×10^5 CFU/mL. The cultures were then incubated under anaerobic conditions (in 250 mL erlenmeyer flasks with silicone stopper) at both 30°C and 37°C. Cell concentration was determined spectrophotometrically every 2 h until stationary phase. The spectrophotometric values were then used to calculate the doubling time and duration of the exponential phase in synthetic media at both temperatures.

The oat flour suspensions were inoculated at 5% v/v (Mårtensson et al., 2002) and incubated at both 30°C and 37°C. Cell concentration was determined every 2 h by cell counting using a Neubauer's chamber until stationary phase. The cell concentration at each sampling point was used to calculate the doubling time and the length of exponential growth phase in the oat suspension.

2.5. Exopolysaccharide extractions and FTIR

The cultures were grown until exponential phase and then centrifuged at 3000×g for 20 min to remove bacteria, debris and medium components. The collected supernatant was diluted in a 1:1 ratio with 14% trichloroacetic acid to remove proteins. After centrifugation at 3000×g for 20 min, the clear supernatant was collected and concentrated to half the initial volume in a rotary evaporator at 60°C at a pressure of 10 mbar. The concentrated supernatant was precipitated in absolute ethanol, mixed in a 2:1 ratio and stored at 4°C for 24 h. The precipitate collected after centrifugation (3000×g, 20 min) was dialysed in deionised water for 24 h. The concentrate was then freeze-dried to a powdered form for FTIR (Nicolet iS50, Thermo Fisher Scientific, USA) analysis. The spectra were analysed for functional groups in the range 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} (Khalil, Sonbol et al. 2022).

2.6. Lipid profile

The cell culture and medium were centrifuged at 3000×g for 20 min to form a pellet consisting of both substrate and microbial biomass, which was then freeze-dried to a powdered form. A sample of 25 mg was taken from this powder. Lipid extraction was performed using toluene containing 0.05% dibutyl hydroxytoluene (BHT) and 1 mg/mL heptadecanoic acid (C17:0) as an internal standard. Transesterification was carried out for 2 h at 70°C by adding 10% acetyl chloride in methanol following the work of Cavonius et al. (2014). The transesterification was stopped by adding 0.2 mL water. A two-phase system was created by adding 5 mL of petroleum ether, forming the upper phase, from which 4 mL was transferred for evaporation by nitrogen flushing. The evaporated fractions were re-dissolved in isooctane of volume 2 mL and analysed using gas chromatography (GC) system (PerkinElmer Clarus 590) equipped with a flame ionisation detector (FID) and a capillary column (60 m × 0.250 mm, 0.15 μm thickness, 250°C, Agilent Technologies USA)(Cavonius et al., 2014). The fatty acid content of the fermented fractions was expressed relative to the dry weight of the original oat flour used for each treatment.

2.7. Elemental analysis

The analysis was performed using Elemental Analyser (FlashSmart, Thermo Fisher Scientific, Germany) with a multi-valve control (MVC) and nitrogen (N_2), and helium (He) as the carrier gases and oxygen (O_2)

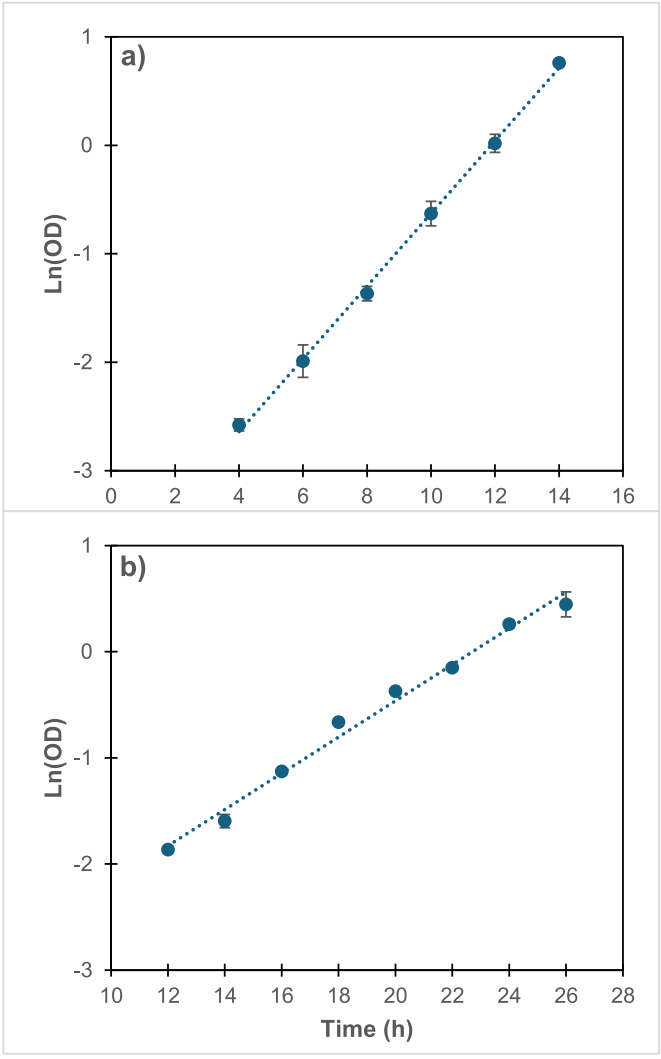

Fig. 1. The exponential phase of *Lactobacillus delbrueckii* grown on MRS media at **a.** 37°C with doubling time of 2 h **b.** 30°C with doubling time of 4 h. Error bars show standard deviation of n = 2. (Regression equation and coefficient included in supplementary).

as the combustion gas. The right furnace was operated at 950°C and the oven containing the gas chromatographic column was set at 65°C. A sample between 3 and 4 mg was taken from each of the powdered freeze-dried pellets used in the lipid analysis and the freeze-dried pellets of the extracted EPS. The samples were analysed, against methionine as the standard, for the elements Carbon (C), Nitrogen (N), Hydrogen (H) and Sulphur (S). The elemental contents of the fermented fractions were expressed relative to the dry weight of the original oat flour used for each treatment.

2.8. Amino acid profile

Samples for the fermented fractions at 37°C of both 3% and 5% solid loading were sent to Eurofins Food & Feed Testing Sweden AB for analysing the amino acid profile according to the method ISO 13903:2005.

2.9. Statistical analysis

All experiments were performed in triplicate for each condition and control with the exception of the growth curve analyses on MRS media which were done in duplicates and the amino acid analysis. A single replicate for the fermented fractions at 37°C of both 3% and 5% solid loading were used to analyse the amino acid profile.

One-way ANOVA test on Minitab 22 was used to determine statistical significance for the results of fatty acid profile and total fat content, EPS concentration, and the protein content. The results of the fermented fractions for fatty acid and total fat content, and the protein content were compared among the four combinations (3% solid loading fermented at 37°C, 3% solid loading fermented at 30°C, 5% solid loading fermented at 37°C, and 5% solid loading fermented at 30°C) of the fermentation conditions and against oat flour as the control. The results of EPS concentration from the four fermented fractions were compared amongst the different fermentation conditions. Tukey's test was used for post hoc analysis where equal variances could be assumed. However, Games-Howell pairwise comparison was done as equal variances could not be assumed in the case of protein content.

3. Results and discussion

The lactic acid bacterium *Lactobacillus delbrueckii* was used to ferment enzyme hydrolysed oat substrate at 30°C and 37°C resulting in significant changes across the nutritional profile when compared to untreated oat flour.

3.1. Enzymatic hydrolysis

To enable efficient starch conversion, enzymatic hydrolysis using alpha amylase and glucoamylase was performed of 3% and 5% oat suspensions. The entire hydrolysed fraction was used for fermentation by *Lactobacillus delbrueckii* to analyse the nutritional profile, whereas for kinetic studies, the solid fraction was removed prior to fermentation to minimise interference of undissolved particulates during cell counting. The selected substrate concentrations (3-5%) are lower than industrial formulations of oat milk consisting of 10-20% d.w. (Mårtensson et al., 2002). Unlike the intense industrial measures to remove solid particles that ensure a milk-like product, the manner of preparing the oat suspensions in this study, where solid particles were retained, could lead to gelation and pasting during hydrolysis at 60°C or autoclaving at 121°C, and that would further prevent proper inoculation of the bacteria for fermentations (Loponen and Katariina, 2020). Therefore, enzymatic hydrolysis was performed to achieve a smoother suspension compatible for fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus* which lacks significant amylolytic activity (Schober et al., 2025). Thus, the 3% and 5% oat flour suspensions resulted in glucose concentrations of 19.45 ± 0.70 and 33.82 ± 1.18 g/L corresponding to 84 and 87% of the theoretical yields for the 3% and 5% suspensions, respectively.

3.2. Growth kinetics

The growth kinetics were evaluated to determine the growth rate of *Lactobacillus delbrueckii* on the hydrolysed oat substrate and to identify the exact time where the highest cell concentration was achieved, depending on the substrate concentration and temperature. This was based on first mapping out the growth curve on MRS media. The optimal growth temperature for *Lactobacillus delbrueckii* in MRS media is 37°C where it was found to have a doubling time of 2 h, consistent with previous studies (Chervaux et al., 2000) (Fig. 1a). Studies have shown that optimal growth temperatures are not ideal for production of EPS as it can be consumed by the bacterial cells (Cerning et al., 1992; Gancel and Novel, 1994, Minari et al., 2024). Hence, to prioritise EPS production on oat, a temperature of 30°C was considered appropriate at the expense of a compromised growth rate (Mårtensson, 2002, Minari et al., 2024). At 30°C, the doubling time increased to 4h, reflecting slower metabolic activity. Such temperature-dependent declines in growth rate are well documented, as lower fermentation temperatures reduce enzymatic activity and lower cell division, resulting in extended fermentation times (Wu et al., 2025).

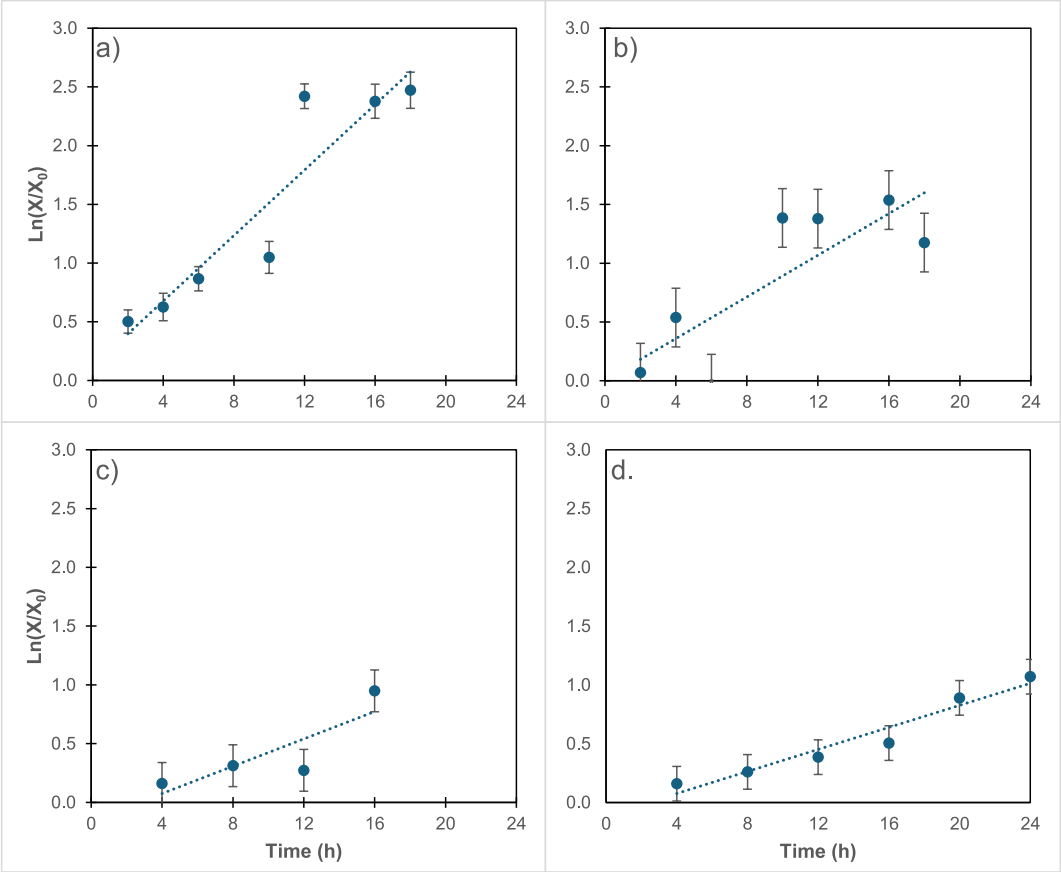

Fig. 2. The exponential phase of *Lactobacillus delbrueckii* grown on enzyme treated oat substrate at **a.** 5% solid loading at 37°C **b.** 3% solid loading at 37°C **c.** 5% solid loading at 30°C **d.** 3% solid loading at 30°C. Error bars show standard deviation of triplicates (n = 3) for each condition. (Regression equation and coefficient included in supplementary).

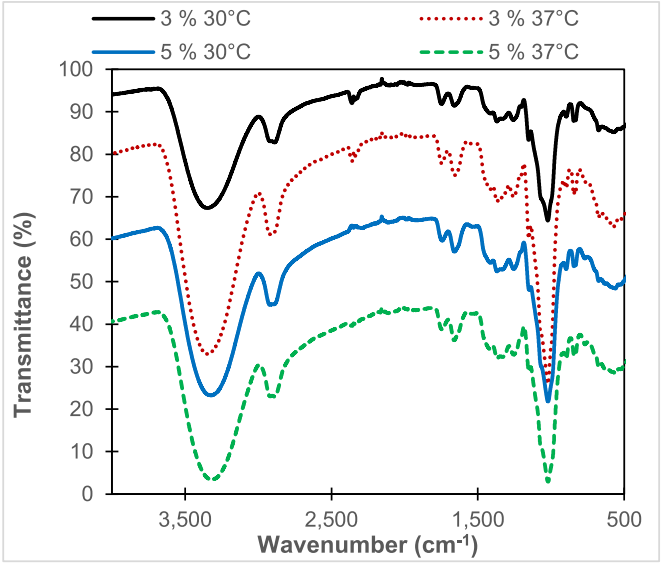

Fig. 3. The FTIR spectra of EPS extracted from each fermentation condition (n = 3). The peaks at 3400 cm^{-1}, 2925 cm^{-1}, and 1640 cm^{-1} correspond to O-H, C-H, and C=O groups respectively, characteristically present in bacterial exopolysaccharides. For readability, the spectra of 3% 37°C, 5% 30°C, and 5% 37°C were shifted down by 10, 30 and 50 units, respectively.

The highest doubling time for the bacterium grown on 5% oat base at 37°C was approximately 5 h where the highest cell concentration was achieved at 1.33 × 10^8 cells/mL after 18 h of fermentation (Fig. 2). As observed on MRS media, the doubling time reduced at 30°C for both concentrations of enzyme hydrolysed oat bases (Fig. 2), indicating that the fermentation condition of 5% hydrolysed fraction at 37°C would be the most favourable to reach viable counts of *Lactobacillus delbrueckii*.

3.3. Exopolysaccharides

Lactobacillus delbrueckii is a well-known producer of exopolysaccharides (EPS) in milk that gives Bulgarian yoghurt its characteristic viscous texture (Mårtensson, 2002, Schober et al., 2025). The EPS produced by *Lactobacillus delbrueckii* in this study were qualitatively analysed using Fourier Transform Infrared (FTIR) spectroscopy (Fig. 3). Presence of functional groups in the EPS produced by *Lactobacillus delbrueckii* was detected in the FTIR spectra (Fig. 3). The broad absorption spectra around 3400 cm^{-1} indicate the presence of hydroxyl (O-H) bonds and thus indicate the presence of functional groups related to sugars. The peaks that appear around 2925 cm^{-1} and 1640 cm^{-1} correspond to aliphatic C-H groups and carbonyl bonds (C=O) respectively. The absorption in the 1100 – 1000 cm^{-1} region could be attributed to the presence of C-O bond. The absorbance bands in the stretch from 1000 to 800 cm^{-1} are usually representative of the fingerprint region and commonly used to structurally distinguish between different EPS.

These spectral features align with those reported for EPS produced by other lactic acid bacteria cultivated in defined or synthetic media (Khalil et al., 2022), further confirming the polysaccharide nature of the material detected. The presence of these characteristic functional groups in the spectra (Fig. 3) demonstrates that *Lactobacillus delbrueckii* was

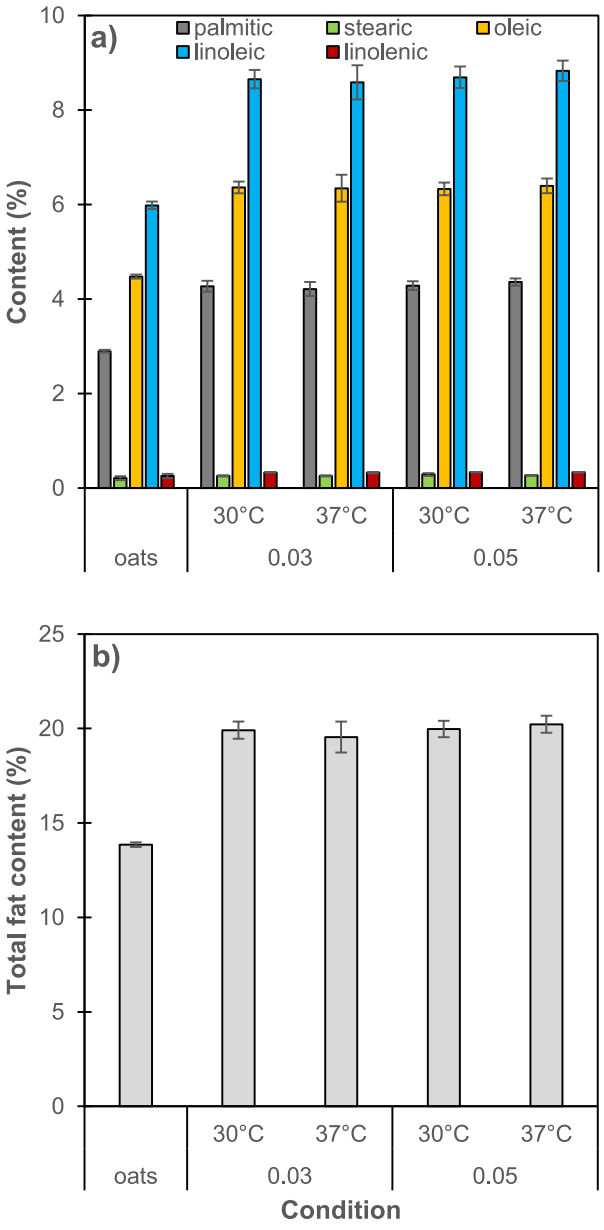

Fig. 4. Fatty acid composition and total lipid content of oat flour and fermented oat fractions in untreated oat flour and fermented oat fractions of 3% and 5% solid loading at 30°C and 37°C. **a.** The fatty acid profile consisting of palmitic, stearic, oleic, linoleic and linolenic acids. **b.** The total fatty acid content (%) across the different fermentation conditions against oat flour. Error bars show standard deviation of triplicates
(n = 3) for each condition.

successfully able to produce EPS during fermentation of oats at both 30°C and 37°C. At 3% solid loading of oats, *Lactobacillus delbrueckii* was able to produce EPS of concentrations 1.06 ± 0.20 mg/mL and 0.93 ± 0.08 mg/mL at 30°C and 37°C respectively. Similarly, EPS of concentrations 1.22 ± 0.33 mg/mL and 1.05 ± 0.12 mg/mL were produced at 30°C and 37°C respectively for 5% solid loading of oats. In the study by Galle et al. (2010), 0.8 to 8 g/kg EPS was produced in wheat and sorghum sourdough fermentation. In other grains, the presence of EPS as a fermentation by-product has enhanced the taste, texture and rheology of the fermented breads or yoghurts (Mårtensson et al., 2002; Torino et al., 2015, Daba et al., 2021). However, as most EPS produced in LAB fermented foods and beverages are hetero-polymeric structures,

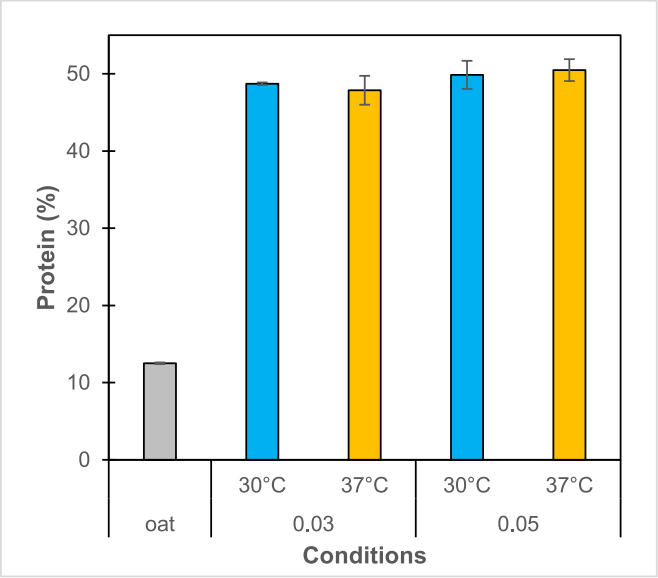

Fig. 5. Crude protein content in oat flour and fermented oat fractions of 3% and 5% solid loading at 30°C and 37°C. Error bars show standard deviation of triplicates (n = 3) for each condition.

likely as a result of the different sugars available in the cereal substrate for uptake during fermentation, there are no standardised extraction protocols or identification methods yet. Thus, the identified EPS concentrations in such fermented foods differ anywhere between 20 and 600 mg/mL (Torino et al., 2015). The notable increase in EPS concentrations at 30°C for both 3% and 5% solid loadings align with findings in studies where it was shown that non-optimal growth temperatures are ideal for production of EPS (Minari et al., 2024; Murugu and Narayanan, 2024). However, statistically, the concentrations between 30°C and 37°C are not significantly ($p = 0.434$) different for either 3% or 5% solid loading of oats.

3.4. Fatty acid content

The high lipid content of oats makes them unique from other cereals and one of the goals of this study was to evaluate whether fermentation with *Lactobacillus delbrueckii* could enhance the lipid content and fatty acid profile of oat flour (Pokhrel et al., 2022). The fatty acid profile of oats comprises saturated, monounsaturated and polyunsaturated fatty acids, which contribute to the unique nutritional characteristics (Kouřimská et al., 2018; Pokhrel et al., 2022). Fermentation of cereal grains is often associated with an improved flavour profile due to the favourable compounds formed from the release of free fatty acids (FFA) by the lipolytic enzymes in LAB. (Tuccillo et al., 2026).

The content of the saturated fatty acid, palmitic acid (C16:0), after fermentation had a significant ($p < 0.05$) increase to 4.28 ± 0.06% d.w.b. across all fermentation conditions from 2.89 ± 0.03% d.w.b. in oat flour. This change (Fig. 4a) was also accompanied by a similar and significant ($p < 0.05$) increase in the monounsaturated fatty acid, oleic acid, to 6.36 ± 0.03% from 4.48 ± 0.04%, and both polyunsaturated fatty acids, linoleic and linolenic acid, to 8.69 ± 0.10% from 5.98 ± 0.08% d.w.b. and 0.33 ± 0.00% d.w.b. from 0.20 ± 0.04% d.w.b., respectively. Polyunsaturated and monounsaturated fatty acids are both well known for being beneficial to the cardiovascular health of humans, hence, their presence in higher fractions than saturated fatty acids in oats, is a key factor that makes oats more beneficial from a nutritional perspective (Sterna et al., 2016; Gebauer et al., 2019). Although changes in content were observed in stearic acid, from 0.20 ± 0.04% d.w.b. to 0.27 ± 0.01% d.w.b., they were not significant ($p > 0.05$).

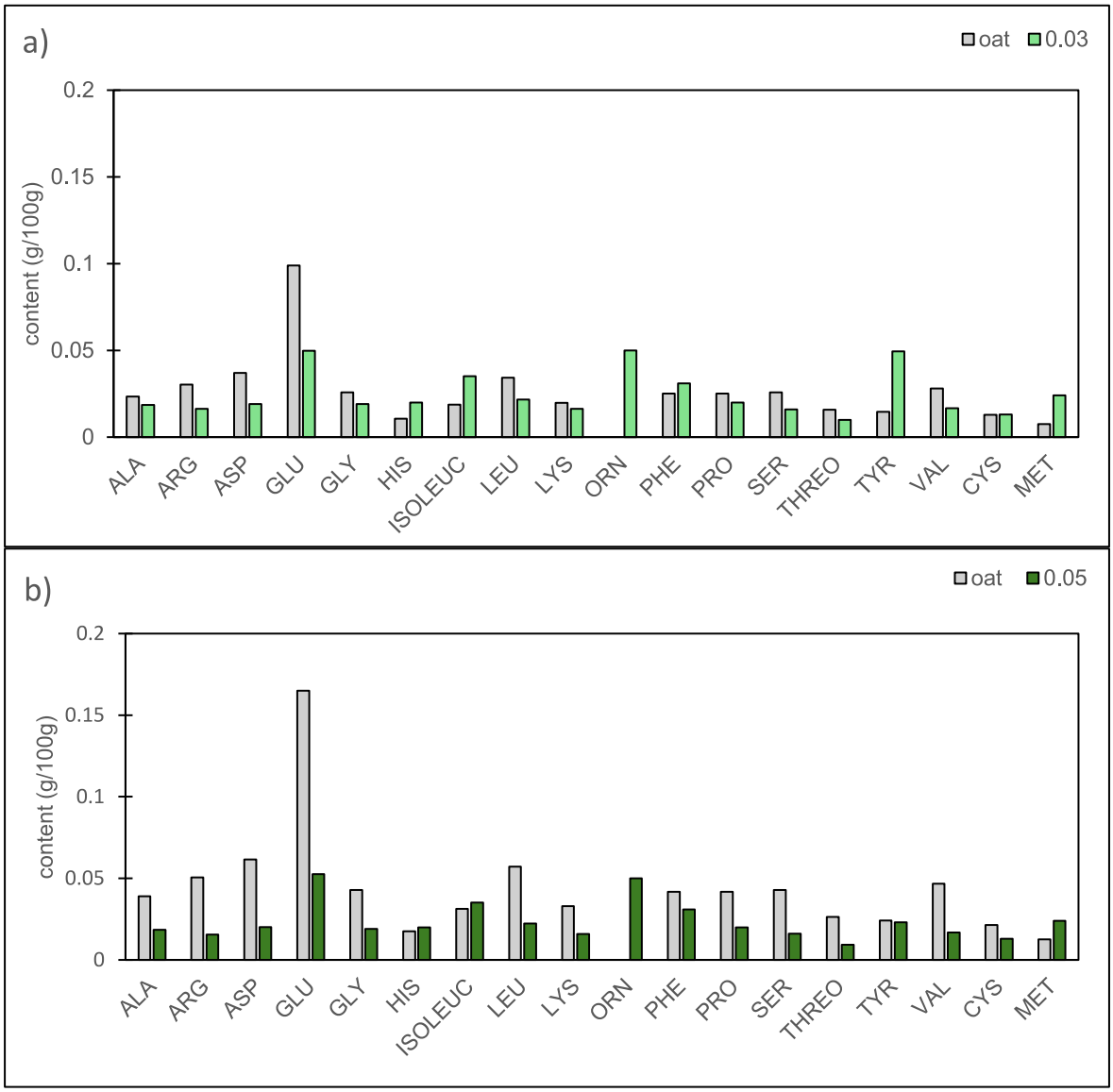

Fig. 6. The amino acid content in untreated oat flour and fermented oat fractions 37°C. **a.** for solid loading of 3% oats. **b.** for solid loading of 5% oats. Amino acid data are based on a single analytical replicate (n = 1) and should therefore be interpreted with caution.

The oat flour used in this study had an already favourable lipid content of 13.85 ± 0.12% d.w.b., which significantly ($p < 0.05$) increased to 19.55 ± 0.82% d.w.b. and 19.91 ± 0.46% d.w.b. at 3% solid loading, and to 19.98 ± 0.43% d.w.b. and 20.23 ± 0.45% d.w.b. at 5% solid loading after fermentation at 37°C and 30°C, respectively (Fig. 4b). This marked increase in the total fatty acid content in the fermented fractions compared to the oat flour is presumed to be a result of possible loss of carbohydrates during the pre-treatment of the oat substrate before fermentation leading to relative enrichment instead of absolute increase of total fat content from the contribution of microbial biomass after fermentation. Thus, despite significant increase in the post-fermentation total fatty acid content, only minor difference was observed between the lipid contents after fermentation at optimum growth temperature (37°C) and EPS producing temperature (30°C) or the solid loadings at 3% or 5%. (tables in supplementary).

3.5. Protein content and amino acid profile

Oat flour is a highly processed final product that goes through harsh processes of removal of husks and kernels followed by kilning and milling. These pretreatments result in a maximum of 17% crude protein content in the oat flour, which again is dependent on the cultivar and other factors that could influence the production of oat flour (Ekelund et al., 2024). The crude protein content increased to 48.71 ± 0.17% d.w.b. and 47.86 ± 1.87% d.w.b. at 30°C and 37°C in 3% solid loading after hydrolysis and fermentation, from 12.50 ± 0.10% d.w.b. in untreated oat flour. Similarly, the protein content increased to 49.86 ± 1.82% d.w.b. and 50.48 ± 1.42% d.w.b. at 30°C and 37°C in 5% oat base (Fig. 5). Similar to the total fat content, the significant ($p < 0.05$) increase in protein content in the fermented fractions can be attributed to the pre-treatment of the oat substrate prior to fermentation, which may have led to loss of carbohydrates and moisture in both solid loading conditions, as compared to untreated oat flour. Besides, oat proteins at low pH are less soluble, thus, are not susceptible to the proteolytic degradation during the growth of LAB (Li and Xiong, 2021, Lille et al., 2026).

Hence, despite the increase in crude protein content compared to untreated oat flour, negligible difference ($p > 0.05$) in the protein content among the different fermentation conditions was seen. This suggests that the effect of pre-treatment on the solid loadings

contributed primarily to the tremendous crude protein increase instead of microbial biomass, thus, leading to relative enrichment. The conversion factor of 5.54 for cereal proteins was used, instead of the commonly used factor of 6.25, to estimate the crude protein from the nitrogen content measured in order to avoid an over-estimation of the crude protein (Ekelund et al., 2024).

Oat flour is abundant in glutamic acid, a non-essential amino acid, as it primarily consists of the starchy endosperm, as the higher protein containing parts such as the bran containing the aleurone layer could be removed during the production of oat flour. Thus, the resulting oat flour is lower in essential amino acids such as lysine (Pomeranz et al., 1976). Only methionine and isoleucine out of the essential amino acids (lysine, leucine, isoleucine, methionine, threonine, and phenylalanine), went through some minor improvements. This trend agrees with studies that found cereals fermented with a lactic acid bacteria resulted in an improvement in the amino acid profile, especially, with regards to methionine (Shenkin, 2006; Das and Maria, 2017). This especially would aid in the enhancement of methionine (Fig. 6), given that it is a primary limiting amino acid in oat protein (World Health and United Nations, 2007, Rafique et al., 2022).

However, the decrease in many amino acids across the profile post fermentation in both solid loadings, is expected as a result of the LAB utilising certain amino acids for growth and metabolism (Nygren et al., 2026). As oat is rich in glutamic acid, its marked decrease is observed in the fermented fraction, which, according to the study of Gänzle (2015) was utilised by the LAB for growth at low pH. A similar trend is also observed in arginine. However, the amino acid profiling was done as an exploratory analysis and not for definitive conclusions due to the lack of replicates.

4. Conclusion

In this study, it has been observed that fermentation using *Lactobacillus delbrueckii* after enzymatic pre-treatment of oat flour has had a significant ($p < 0.05$) impact on the fat and protein content of oat flour thus resulting in enrichment of protein and lipid fractions post-fermentation. Significant ($p < 0.05$) changes in both the total fatty acid content and fatty acid profile were observed where fermentation maintained the trend of a higher PUFA (linoleic and linolenic acid) and MUFA (oleic acid) as compared to SFA (palmitic and stearic acid). Similarly, protein content improved significantly ($p < 0.05$) in the fermented oats with modest improvements in the essential amino acid content. EPS concentrations extracted from the different fermentation conditions also were in alignment with studies where suboptimal growth temperature (30°C in this case) tended to have higher EPS concentrations. However, the improvements in the nutritional profile in this case are result of both pre-treatment of the oat flour leading to loss of carbohydrate and moisture content, and microbial biomass rather than solely the latter. Overall, the results demonstrate that controlled fermentation after enzyme hydrolysis can be an effective strategy for improving the nutritional attributes of oat-based substrates, even when the primary objective is not probiotic viability.

CRediT authorship contribution statement

Sharmeen Mustafa: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Swarnima Agnihotri:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis. **Patrik R. Lennartsson:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

The authors report that there is no funding associated with the work featured in this article.

Declaration of competing interests

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.

Acknowledgements

The authors would like to express their gratitude to the University of Borås for their technical and financial support of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2026.104571.

Data availability

Data will be made available on request.

References

Babini, E., Taneyo-Saa, D.L., Tassoni, A., Ferri, M., Kraft, A., Grän-Heedfeld, J., Bretz, K., Roda, A., Michelini, E., Calabretta, M.M., Guillon, F., Tagliazucchi, D., Martini, S., Nissen, L., Gianotti, A., 2020. Microbial fermentation of industrial rice-starch byproduct as valuable source of peptide fractions with health-related activity. Microorganisms 8. https://doi.org/10.3390/microorganisms8070986.

Cavonius, L.R., Carlsson, N.-G., Undeland, I., 2014. Quantification of total fatty acids in microalgae: comparison of extraction and transesterification methods. Anal. Bioanal. Chem. 406, 7313–7322.

Cerning, J., Bouillanne, C., Landon, M., Desmazeaud, M., 1992. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. J. Dairy Sci. 75 (3), 692–699.

Chervaux, C., Ehrlich, S.D., Maguin, E., 2000. Physiological study of Lactobacillus delbrueckii subsp. bulgaricus strains in a novel chemically defined medium. Appl. Environ. Microbiol. 66 (12), 5306–5311.

Daba, G.M., Elnahas, M.O., Elkhateeb, W.A., 2021. Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical, and medical applications. Int. J. Biol. Macromol. 173, 79–89.

Das, G., Maria, J.M., 2017. Nutrient profile of fermented oats. Int. J. Food Sci. Nutr. 2, 69–71.

Djorgbenoo, R., Hu, J., Hu, C., Sang, S., 2023. Fermented oats as a novel functional food. Nutrients 15 (16), 3521.

Ekelund, S., Gladkauskas, E., Krause, S., Sirijovski, N., Adlercreutz, P., Rayner, M., Grey, C., Tullberg, C., 2024. Oat kilning and its effects on liquid oat-base production. Foods 13 (24), 4083.

Galle, S., Schwab, C., Arendt, E., Gänzle, M., 2010. Exopolysaccharide-forming weissella strains as starter cultures for sorghum and wheat sourdoughs. J. Agric. Food Chem. 58 (9), 5834–5841.

Gancel, F., Novel, G., 1994. Exopolysaccharide production by Streptococcus salivarius ssp. Thermophilus cultures. 2. Distinct modes of polymer production and degradation among clonal variants. J. Dairy Sci. 77 (3), 689–695.

Gänzle, M.G., 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr. Opin. Food Sci. 2, 106–117.

Gebauer, S., Harris, W.S., Kris-Etherton, M., Etherton, T.D., 2019. Dietary n-6: n-3 fatty acid ratio and health. Healthful Lipids. AOCS Publishing, pp. 221–248.

Gotcheva, V., Hristozova, E., Hristozova, T., Guo, M., Roshkova, Z., Angelov, A., 2002. Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. Food Biotechnol. 16 (3), 211–225.

Juodeikiene, G., Klupsaite, D., Zadeike, D., Cizeikiene, D., Vidziunaite, I., Bartkiene, E., Cernauskas, D., 2016. Bioconversion of agro-industrial by-products to lactic acid using Lactobacillus sakei and two pediococcus spp. strains. Int. J. Food Sci. Technol. 51 (12), 2682–2691.

Karetkin, B.A., Panfilov, V.I., Khromova, N.Y., Baurin, D.V., Shakir, I.V., 2017. OPTIMIZATION OF WHEAT FLOUR ENZYMATIC HYDROLYSIS FOR

LACTOBACILLUS RHAMNOSUS SUBMERGED FERMENTATION, Sofia. Surveying Geology & Mining Ecology Management (SGEM).
Kaur, N., Dey, P., 2023. Bacterial exopolysaccharides as emerging bioactive macromolecules: from fundamentals to applications. Res. Microbiol. 174 (4), 104024.
Khalil, M.A., Sonbol, F.I., Al-Madboly, L.A., Aboshady, T.A., Alqurashi, A.S., Ali, S.S., 2022. Exploring the therapeutic potentials of exopolysaccharides derived from lactic acid bacteria and bifidobacteria: Antioxidant, antitumor, and periodontal regeneration. Front. Microbiol. 13, 803688.
Kouřimská, L., Sabolová, M., Horčička, P., Rys, S., Božik, M., 2018. Lipid content, fatty acid profile, and nutritional value of new oat cultivars. J. Cereal. Sci. 84, 44–48.
Kumar, H., Salminen, S., 2016. Probiotics. In: Caballero, B., PM Finglas, Toldrá, F. (Eds.), Encyclopedia of Food and Health. Academic Press, Oxford.
Li, R., Xiong, Y.L., 2021. Sensitivity of oat protein solubility to changing ionic strength and pH. J. Food Sci. 86 (1), 78–85.
Lille, M., Janssen, F., Juvonen, R., Wouters, A.G.B., 2026. Determination of the extractable protein content of (fermented) plant-based raw materials and foods. In: Wouters, A.G.B., De Bondt, Y., Katina, K., Courtin, C.M. (Eds.), Characterisation of Fermented Grain-based Raw Materials and Foods. Leuven University Press, pp. 55–68.
Loponen, J.R., Katariina, 2020. Method of Preparing Liquid Oat Base. W. o. W. I. P. Organization. WO 2020/025856.
Mårtensson, O., 2002. Lactic Acid Bacteria Fermentations in Oat-based Suspensions.
Mårtensson, O., Öste, R., Holst, O., 2002. Texture promoting capacity and EPS formation by lactic acid bacteria in three different oat-based non-dairy media. Eur. Food Res. Technol. 214 (3), 232–236.
Minari, G.D., Piazza, R.D., Sass, D.C., Contiero, J., 2024. EPS production by Lacticaseibacillus casei using glycerol, glucose, and molasses as carbon sources. Microorganisms 12. https://doi.org/10.3390/microorganisms12061159.
Murugu, J., Narayanan, R., 2024. Production, purification, and characterization of a novel exopolysaccharide from probiotic lactobacillus amylovorus: mtcc 8129. Indian J. Microbiol. 64 (3), 1355–1365.
Nygren, H., Maiorova, N., Juvonen, R., Sözer, N., 2026. Determination of free amino acids and total amino acid composition in plant-based raw materials. In: Wouters, A. G.B., De Bondt, Y., Katina, K., Courtin, C.M. (Eds.), Characterisation of Fermented Grain-based Raw Materials and Foods. Leuven University Press, pp. 37–54.
Pokhrel, K., Kouřimská, L., Pazderů, K., Capouchová, I., Božik, M., 2022. Lipid content and fatty acid profile of various European and Canadian hulled and naked oat genotypes. J. Cereal. Sci. 108, 103580.
Pomeranz, Y., Shands, H.L., Robbins, G.S., Gilbertson, J.T., 1976. Protein content and amino acid composition in groats and hulls of developing oats (Avena sativa). J. Food Sci. 41 (1), 54–56.
Rafique, H., Dong, R., Wang, X., Alim, A., Aadil, R.M., Li, L., Zou, L., Hu, X., 2022. Dietary-nutraceutical properties of oat protein and peptides. Front. Nutr. 9, 950400.
Schober, I., Koblitz, J., Sardà Carbasse, J., Ebeling, C., Schmidt, M.L., Podstawka, A., Gupta, R., Ilangovan, V., Chamanara, J., Overmann, J., Reimer, L.C., 2025. BacDive in 2025: the core database for prokaryotic strain data. Nucleic Acids Res. 53 (D1), D748–d756.
Shenkin, A., 2006. Micronutrients in health and disease. Postgrad. Med. J. 82 (971), 559–567.
Sterna, V., Zute, S., Brunava, L., 2016. Oat grain composition and its nutrition benefice. Agric. Agric. Sci. Procedia 8, 252–256.
Tarique, M., Ali, A.H., Kizhakkayil, J., Gan, R.-Y., Liu, S.-Q., Kamal-Eldin, A., Ayyash, M., 2023. Investigating the biological activities and prebiotic potential of exopolysaccharides produced by Lactobacillus delbrueckii and lacticaseibacillus rhamnosus: implications for gut microbiota modulation and rheological properties in fermented milk. Food Hydrocolloids for Health 4, 100162.
Torino, M.I., Font de Valdez, G., Mozzi, F., 2015. Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. Front. Microbiol. 6.
Tuccillo, F., Cera, S., Maina, N.H., Lampi, A.M., Katina, K., 2026. Free fatty acid analysis in fermented cereal- and pulse-based matrices. In: Wouters, A.G.B., De Bondt, Y., Katina, K., Courtin, C.M. (Eds.), Characterisation of Fermented Grain-based Raw Materials and Foods. Leuven University Press, pp. 251–258.
Wang, Y., Ames, N.P., Tun, H.M., Tosh, S.M., Jones, P.J., Khafipour, E., 2016. High molecular weight barley β-glucan alters gut microbiota toward reduced cardiovascular disease risk. Front. Microbiol. 7, 129.
Weiss, N., Schillinger, U., Kandler, O., 1983. Lactobacillus lactis, lactobacillus leichmannii and Lactobacillus bulgaricus, subjective synonyms of Lactobacillus delbrueckii, and description of Lactobacillus delbrueckii subsp. lactis comb. Nov. and Lactobacillus delbrueckii subsp. bulgaricus comb. nov. Syst. Appl. Microbiol. 4 (4), 552–557.
World Health, O., United Nations, U., 2007. Protein and Amino Acid Requirements in Human Nutrition. World Health Organization.
Wu, T., Guo, S., Kwok, L.-Y., Zhang, H., Wang, J., 2025. Temperature-dependent metabolic interactions between Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus in milk fermentation: insights from gas chromatography–ion mobility spectrometry metabolomics. J. Dairy Sci. 108 (1), 242–256.
Xie, J., Gänzle, M., 2023. Microbiology of fermented soy foods in Asia: can we learn lessons for production of plant cheese analogues? Int. J. Food Microbiol., 110399