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Evaluation of the Technological and Functional Properties of a Fermented Hemp Seed Product and Its Application in a Fermented Dairy Drink

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Abstract

The development of innovative fermented beverages relies on microbial fermentation to enhance the nutritional and functional potential of plant-derived ingredients. This study aimed to evaluate the effects of lactic acid bacteria (LAB) fermentation on the composition, technological and functional properties of a fermented hemp seed product (FHSP), and to assess its suitability for fermented dairy drink enrichment. The hemp seed product was fermented with *Lactocaseibacillus paracasei*, *Lactiplantibacillus plantarum*, or *Limosilactobacillus fermentum*. LAB growth, acidification, technological properties, bile- and cholesterol-binding capacities, total phenolic content, and antioxidant activity were determined. The selected FHSP was incorporated into fermented dairy drink, which was evaluated for physicochemical and sensory properties. LAB counts increased during the first 48 h and stabilised after 72 h. *L. paracasei* showed the highest viability during fermentation (1.38×10^9 CFU/g) and after freeze-drying (9.02×10^8 CFU/g). Fermentation increased water swelling capacity and, depending on the strain, water retention capacity. The *L. fermentum* FHSP exhibited the highest bile-binding capacity, whereas the products fermented with *L. paracasei* and *L. plantarum* showed the highest total phenolic content and ABTS radical-scavenging activity. Considering its highest LAB viability together with its high total phenolic content and DPPH radical-scavenging activity, the *L. paracasei* FHSP was selected for fermented dairy drink enrichment. Increasing its level significantly enhanced total phenolic content and antioxidant activity but also increased syneresis and darkened the product. The drink containing 4 g/100 g of FHSP received the highest sensory scores. Overall, LAB fermentation improved the technological and functional potential of hemp seed product and supported its use in fermented dairy beverages.

Keywords: *Cannabis sativa* L.; lactic acid bacteria; fermentation; techno-functional properties; antioxidant activity; volatile profile; fermented dairy product



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1. Introduction

Industrial hemp (*Cannabis sativa* L.) has attracted considerable interest as a versatile raw material not only in biotechnology and sustainable materials science but also in food production. Although hemp has traditionally been associated with fibre and oil production, recent research has increasingly focused on its higher-value applications in the development of food ingredients, biomaterials and biologically active compounds [1–3]. Hemp seeds and

the fractions obtained after oil extraction are particularly promising for food applications because of their nutritional composition and potential technological functionality.

Hemp seeds contain substantial amounts of protein, polyunsaturated fatty acids, dietary fibre, minerals and various bioactive compounds. Hemp-derived ingredients are therefore increasingly used in the development of nutritionally enriched foods, with particular attention being paid to hemp seed proteins as both nutrients and technologically relevant components of food systems [3]. Hemp seed proteins consist mainly of edestin and albumin and are characterised by a favourable amino acid composition. Together with the relatively sustainable cultivation of hemp, these properties support the potential use of hemp seed proteins in plant-based beverages, meat alternatives and other protein-enriched foods [2,3]. Commercial hemp seed flours and protein-rich hemp seed products may also provide water- and oil-retention and emulsifying properties, although their composition and technological behaviour vary according to the degree of dehulling, oil removal and milling [4].

Despite their valuable composition, hemp seed ingredients have certain limitations, including a characteristic colour and earthy, bitter or astringent sensory attributes. Their application may also be restricted by relatively low protein solubility under certain pH conditions, variable emulsifying properties and a high proportion of insoluble dietary fibre, which can affect product dispersibility, texture and physical stability [4]. Consequently, processing methods that improve the nutritional, technological, and sensory properties of hemp seed ingredients are needed.

Fermentation is a promising method for modifying plant-derived raw materials. During fermentation, microorganisms utilise fermentable carbohydrates, produce organic acids, and promote various biochemical transformations in the substrate. These processes may improve protein digestibility, increase the availability of phenolic compounds, and enhance antioxidant activity [1]. Fermentation of hemp seeds and their processing fractions has also been associated with changes in antinutritional compounds, phenolic profiles, and selected technological properties [5,6].

Lactic acid bacteria (LAB) are widely used for the controlled fermentation of plant-derived materials because of their acidification capacity and diverse enzymatic activities. Microbial and endogenous enzymes may promote protein hydrolysis, modify interactions between dietary fibre and other constituents, and facilitate the release of phenolic compounds from bound forms. As a result, fermentation may influence protein solubility, water and oil retention, swelling, emulsion stability and biological activity. However, the magnitude and direction of these changes depend on the bacterial strain, fermentation conditions and raw-material composition [5,6]. Plant-derived matrices have been shown to support the growth, viability and stability of LAB with probiotic potential, while their fermentation may also reduce antinutritional factors and improve the physicochemical, textural and volatile properties of plant-based beverages and yoghurt-like products [7].

Hemp seed substrates have been shown to support LAB growth, although the resulting acidification, antioxidant activity and other biochemical properties are strongly strain-dependent [6]. A screening of ten LAB strains on hemp bran demonstrated marked strain-dependent differences in fermentation performance, with strains of *L. plantarum* and *L. fermentum* showing high growth capacity, while *L. rhamnosus* exhibited the strongest acidification performance [8]. Similarly, fermentation of hemp seed milk with *L. plantarum*, *L. paracasei* and *L. rhamnosus* resulted in distinct metabolite and volatile profiles, together with differences in the biological activity of the fermented products [9].

Among these fermentation-induced changes, modification of the volatile profile is particularly relevant to the sensory quality of hemp seed ingredients. The formation or degradation of alcohols, aldehydes, ketones, organic acids, esters and terpenes may alter the

aroma of the fermented product. Previous research on fermented hemp seed bran demonstrated that appropriately selected LAB strains may reduce compounds associated with undesirable odours while promoting the formation or retention of potentially favourable volatile compounds [10]. Therefore, volatile-profile analysis is important when evaluating fermented hemp seed ingredients intended for incorporation into foods.

Fermented hemp seed products may be suitable for enriching fermented dairy products with protein, dietary fibre and phenolic compounds. Fermented dairy drink and drinking yoghurt represent a convenient food system for incorporating such ingredients; nevertheless, the addition of plant-derived materials may affect colour, whey separation, texture and sensory acceptability. Research on hemp-containing yoghurt-like products has shown that hemp flour can increase protein and fibre contents, while LAB fermentation may improve antioxidant properties and reduce the perceived intensity of the characteristic earthy flavour [11]. However, available studies have mainly focused on entirely plant-based yoghurt-like alternatives rather than on the incorporation of a separately fermented and freeze-dried hemp seed ingredient into fermented dairy drink.

Despite growing interest in fermented hemp seed ingredients, information remains limited regarding the simultaneous strain-dependent effects of LAB fermentation on the chemical composition, technological properties, in vitro biological activity, and volatile compound profile of partially defatted hemp seed flour. Moreover, the suitability of such a fermented product for enriching fermented dairy drinks and its effects on product quality during refrigerated storage have not been sufficiently investigated.

Therefore, this study aimed to evaluate the suitability of LAB strains with probiotic potential—*Lacticaseibacillus paracasei* subsp. *paracasei* DSM 4905, *Lactiplantibacillus plantarum* DSM 24624 and *Limosilactobacillus fermentum* DSM 20055—for the fermentation of partially defatted hemp seed flour and to determine the resulting changes in its chemical composition, technological properties, selected in vitro functional characteristics and volatile compound profile. LAB growth during fermentation and survival after freeze-drying were also assessed. A selected fermented hemp seed product (FHSP) was subsequently incorporated into fermented dairy drink at different concentrations, and its effects on the physicochemical, textural, and sensory properties of the drink during refrigerated storage were evaluated.

2. Materials and Methods

2.1. Hemp Seed Flour

A commercially available partially defatted hemp seed flour, marketed as hemp protein (Žolynėlis, UAB Acorus Calamus, Kaunas, Lithuania), was purchased from a local retail store. According to the manufacturer's information, the product was obtained by milling hemp seeds after partial oil removal.

2.2. Fermentation of Hemp Seed Flour with Lactic Acid Bacteria

Partially defatted hemp seed flour was sterilized at 121 °C for 15 min and mixed with sterile water at a flour-to-water ratio of 1:2 (*w/w*) in a sterile bottle under aseptic conditions. The resulting substrate was separately inoculated with 1% (*v/w*) overnight-cultivated suspensions of *Lacticaseibacillus paracasei* subsp. *paracasei* DSM 4905 (*L. paracasei*), *Lactiplantibacillus plantarum* DSM 24624 (*L. plantarum*), or *Limosilactobacillus fermentum* DSM 20055 (*L. fermentum*). Each strain was used in a separate fermentation treatment. Three independent fermentation batches were prepared for each treatment.

Fermentation was carried out for 72 h under each strain's optimal growth conditions. The substrate inoculated with *L. paracasei* was incubated at 37 °C, whereas the substrates

inoculated with *L. plantarum* and *L. fermentum* were incubated at 30 °C. Samples were collected at 0, 24, 48, and 72 h to determine viable LAB counts and measure the pH.

After fermentation, the fermented hemp seed products (FHSP) were freeze-dried using a laboratory freeze-dryer (ZIRBUS Technology GmbH, Bad Grund (Harz), Germany), transferred into sealed containers, and stored until further analysis. A non-inoculated control was prepared in parallel and subjected to the same sterilization, mixing, incubation, sampling, and freeze-drying procedures as the fermented samples, but without adding LAB.

2.3. Determination of Proximate Composition

Moisture content was determined according to AOAC Method 925.10 [12]. Approximately 1 g of sample was dried at 105 °C to a constant mass, and the results were expressed as g/100 g. Crude protein content was determined by the Kjeldahl method, following AOAC Method 978 [12]. Approximately 1 g of sample was digested with concentrated sulfuric acid, and the nitrogen content was converted to protein using a conversion factor of 6.25. Total lipid content was determined according to AOAC Method 948.22 [12] by Soxhlet extraction. Approximately 2 g of sample was extracted for 3 h using an automated extraction system (Behr Labor-Technik GmbH, Düsseldorf, Germany), and the lipid content was calculated gravimetrically. Ash content was determined based on AOAC Method 930.05 by incinerating approximately 1 g of sample in a muffle furnace at 550 °C.

Soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined using a commercial total dietary fibre assay kit (Megazyme Ltd., Wicklow, Ireland) according to AACC Method 32-07.01 and AOAC Method 991.43 [13]. Briefly, 0.25 g of sample was dispersed in 10 mL of MES–Tris buffer (pH 8.2) and sequentially digested with thermostable α -amylase at 95 °C for 30 min, protease at 60 °C for 30 min, and amyloglucosidase at 60 °C for 30 min after adjustment of the pH to 4.1–4.8. IDF was collected by vacuum filtration, washed with hot water, and dried at 105 °C. SDF was precipitated from the filtrate by adding four volumes of ethanol, collected by filtration, and dried to a constant mass. Protein and ash corrections were applied to both fractions, and total dietary fibre was calculated as the sum of SDF and IDF.

2.4. Determination of Reducing Sugar

Reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method described by Miller [14] with minor modifications. Briefly, 0.25 g of sample was mixed with 12 mL of distilled water and stirred for 10 min at room temperature. The suspension was centrifuged at 5000 rpm for 15 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). Subsequently, 1 mL of the supernatant was mixed with 1 mL of DNS reagent, heated at 95 °C for 5 min, rapidly cooled, and diluted with 6 mL of distilled water. Absorbance was measured at 540 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A glucose calibration curve was prepared over the concentration range of 0–0.5 mg/mL using a 1 mg/mL glucose stock solution. The results were expressed as g/100 g dry matter.

2.5. Determination of Soluble Protein Content

Soluble protein content was determined using the Bradford assay [15]. Briefly, 0.16 g of sample was mixed with 8 mL of distilled water and homogenised for 1 min at 10,000 rpm using an IKA® T25 digital Ultra-Turrax homogeniser (IKA-Werke GmbH & Co., Staufen, Germany). The suspension was centrifuged at 6000 rpm for 15 min (BOECO U320, Boeckel + Co. (GmbH + Co.), Hamburg, Germany), and the supernatant was filtered.

An aliquot of 0.1 mL of the filtrate was mixed with 3 mL of Bradford reagent and incubated for 10 min at room temperature. Absorbance was measured at 595 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A calibration curve was prepared using bovine serum albumin solutions in the 0–0.8 mg/mL concentration range. Soluble protein content was calculated from the calibration curve and expressed as mg/g of sample.

2.6. Determination of Viable Lactic Acid Bacteria Counts

Viable lactic acid bacteria (LAB) counts were determined using the surface plating method in accordance with ISO 15214:1998 [16], with modifications. Serial decimal dilutions of the samples were prepared up to 10^{-7} . Aliquots of 100 μ L from the 10^{-5} , 10^{-6} , and 10^{-7} dilutions were spread onto MRS agar plates. Plates inoculated with *Lactocaseibacillus paracasei* subsp. *paracasei* DSM 4905 were incubated at 37 °C, whereas those inoculated with *Lactiplantibacillus plantarum* DSM 24624 and *Limosilactobacillus fermentum* DSM 20055 were incubated at 30 °C for 72 h. After incubation, the colonies were counted, and the results were expressed as colony-forming units per gram of sample (CFU/g).

2.7. Determination of Hydration Properties

Water swelling capacity and water retention capacity were determined as described by Yu et al. [17] with modifications. Briefly, 0.2 g of sample was mixed with 6 mL of distilled water in a graduated centrifuge tube and hydrated for 18 h at room temperature. The volume occupied by the dry sample was recorded before hydration, and the volume of the hydrated sample was measured after 18 h.

The suspension was centrifuged at 5000 rpm for 20 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). The hydrated sediment was weighed together with the centrifuge tube and subsequently dried at 105 °C to a constant mass.

Water swelling capacity was expressed as mL/g dry matter, and water retention capacity as g of retained water per g of dry matter.

2.8. Determination of Oil Retention Capacity

Oil retention capacity was determined as described by Yu et al. [17] with modifications. Briefly, 0.2 g of sample was mixed with sunflower oil and maintained at room temperature for 1 h. The mixture was centrifuged at 1000 rpm for 10 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). The supernatant was carefully decanted, and the remaining sediment was weighed. Oil retention capacity was calculated as the mass of retained oil per gram of sample dry matter and expressed as g/g dry matter.

2.9. Determination of Sodium Cholate-Binding Capacity

Sodium cholate-binding capacity was determined as described by Xu et al. [18] with modifications. Briefly, 0.1 g of sample and 0.2 g of sodium cholate were mixed with 50 mL of 125 mmol/L NaCl solution adjusted to pH 7.0. A control was prepared under the same conditions without adding the hemp seed product. The mixtures were incubated at 37 °C for 2 h in a shaking water bath at 120 rpm, then centrifuged at 4000 rpm for 20 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany).

The concentration of unbound sodium cholate in the supernatant was determined colorimetrically. An aliquot of 0.5 mL of the supernatant was mixed with 4.5 mL of 42% (*w/w*) sulfuric acid and incubated at 70 °C for 20 min. Absorbance was measured at 387 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A calibration curve was prepared using sodium cholate solutions in the concentration range of 0.1–1.0 mg/mL. Sodium cholate-binding capacity was calculated as follows:

$$\text{Sodium cholate-binding capacity (mg/g)} = \frac{(C_c - C_s)}{m} \times V \quad (1)$$

where C_c is the sodium cholate concentration in the control (mg/mL), C_s is the sodium cholate concentration in the sample (mg/mL), V is the total sample volume (mL), and m is the sample mass (g).

2.10. Determination of Cholesterol-Binding Capacity

Cholesterol-binding capacity was determined as described by Zhang et al. [19] with modifications. Egg yolk was diluted ninefold (w/w) with distilled water. A 10 mL portion of the diluted egg yolk suspension was mixed with 0.2 g of sample. Control preparations were prepared under the same conditions without adding the hemp seed product. The pH of the mixtures was adjusted to either 2.0 or 7.0, after which the samples were incubated at 37 °C for 2 h in a shaking water bath at 120 rpm.

Following incubation, 0.25 mL of each mixture was combined with 1 mL of 96% ethanol and centrifuged at 5000 rpm for 20 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). The remaining unbound cholesterol in the supernatant was determined using a colorimetric method. An aliquot of 1 mL of the supernatant was mixed with 4 mL of o-phthalaldehyde reagent prepared by dissolving 0.05 g of o-phthalaldehyde in 100 mL of glacial acetic acid. After 10 min, 2 mL of concentrated sulfuric acid was added. The mixture was maintained for a further 10 min, and absorbance was measured at 550 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). A reagent blank was prepared by replacing the supernatant with 1 mL of ethanol.

A calibration curve was prepared using cholesterol solutions in ethanol over the concentration range of 0.010–0.180 mg/mL. Cholesterol-binding capacity was calculated as follows:

$$\text{Cholesterol-binding capacity (mg/g)} = \frac{(C_c - C_s)}{m} \times V \quad (2)$$

where C_c is the cholesterol concentration in the control (mg/mL), C_s is the cholesterol concentration in the sample (mg/mL), V is the total sample volume (mL), and m is the sample mass (g).

2.11. Determination of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with minor modifications [20]. Briefly, 0.5 g of sample was mixed with 4.5 mL of distilled water, homogenised for 1 min at 10,000 rpm, and centrifuged at 6000 rpm for 15 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). Total phenolic content was determined in the obtained extract.

An aliquot of 0.1 mL of the supernatant was mixed with 3 mL of 3.3% aqueous sodium carbonate solution and 0.1 mL of Folin–Ciocalteu reagent. The mixture was incubated for 30 min at room temperature, and absorbance was measured at 760 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A calibration curve was prepared using gallic acid solutions in the concentration range of 0.02–1.0 mg/mL. Total phenolic content was calculated from the calibration curve and expressed as mg gallic acid equivalents per gram of sample (mg GAE/g).

2.12. Determination of Antioxidant Activity

Aqueous extracts were prepared by mixing FHSP with distilled water at a 1:10 (w/v) ratio, then centrifuged at 6000 rpm for 15 min using a BOECO U320 centrifuge (Boeckel + Co. (GmbH + Co.), Hamburg, Germany). The resulting supernatants were used for the antioxidant activity assays.

ABTS radical-scavenging activity was determined as described by Re et al. [21] with modifications. The ABTS radical solution was prepared by mixing equal volumes of 7 mM

ABTS and 2.45 mM potassium persulfate solutions and incubating the mixture in the dark at room temperature for 16 h. Before analysis, the solution was diluted with methanol to an absorbance of 0.700 at 734 nm.

An aliquot of 50 μL of the sample extract was mixed with 3950 μL of the diluted ABTS solution and incubated in the dark for 30 min. Absorbance was measured at 734 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). A Trolox calibration curve was prepared over the concentration range of 0.05–1.00 mg/mL. The results were expressed as mg Trolox equivalents per gram of sample (mg TE/g).

DPPH radical-scavenging activity was determined as described by Brand-Williams et al. [22] with modifications. A 0.004 mM DPPH solution was prepared in methanol. An aliquot of 0.5 mL of the sample extract was mixed with 1.5 mL of the DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was subsequently measured at 515 nm using a GENESYS 10 UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A Trolox calibration curve was prepared over the concentration range of 0–50 $\mu\text{mol/L}$. The standards were analysed under the same conditions as the samples. The results were expressed as μmol Trolox equivalents per gram of sample ($\mu\text{mol TE/g}$).

2.13. Determination of Volatile Compounds

The composition of volatile compounds of fermented and non-fermented hemp seed products was analysed by gas chromatography—time-of-flight mass spectrometry (GC–TOFMS). Analyses were performed on a comprehensive gas chromatography time-of-flight mass spectrometry (GC $\times$ GC–TOFMS) LECO Pegasus 4D system (LECO Corp., St. Joseph, MI, USA) equipped with an Agilent 7890A gas chromatograph, a GERSTEL Multipurpose Sampler MPS autosampler (GERSTEL GmbH & Co., Mülheim an der Ruhr, Germany), and a high-speed TOFMS detector (LECO Corp., St. Joseph, MI, USA).

Volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME). For the analysis 0.1 $\pm$ 0.001 g of fermented and non-fermented hemp seed product was placed in a 20 mL SPME glass vial, sealed with a magnetic cap fitted with a sili-cone/polytetrafluoroethylene septum and subjected to HS-SPME with a divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS; 50/30 μm , 1 cm length, needle size 23 Ga; Supelco, Inc., Bellefonte, PA, USA). The vials were equilibrated at 40 $^{\circ}\text{C}$ for 10 min, followed by fiber exposure to the sample headspace at the same temperature for 30 min. Afterwards, the fiber was desorbed into the GC injector port for 120 s.

Chromatographic separation was performed using a BPX-5 primary column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness) and a BPX-50 secondary column (1.8 m $\times$ 0.10 mm $\times$ 0.10 μm ; SGE Analytical Science Pty Ltd., Ringwood, Australia). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The primary oven temperature was initially maintained at 40 $^{\circ}\text{C}$, increased to 220 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$, held for 1 min, and then increased to 280 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$, with a final holding time of 5 min. The secondary oven followed the same temperature program with a +5 $^{\circ}\text{C}$ offset, while the modulator temperature offset was +15 $^{\circ}\text{C}$.

The GC injector port temperature was initially set at 180 $^{\circ}\text{C}$ then at rate a 12 $^{\circ}\text{C}/\text{s}$ ramped to 250 $^{\circ}\text{C}$ with a desorption time of 5 min. The transfer line and ion source temperatures were maintained at 250 $^{\circ}\text{C}$. The detector voltage was 1550 V. Mass spectra were acquired over an m/z range of 30–500 at an acquisition rate of 10 spectra/s. The injection was performed in splitless mode for the first 30 s, followed by purging at 20 mL/min. Data acquisition and processing were conducted using ChromaTOF v.4.22 software (LECO Corp., St. Joseph, MI, USA) after a solvent peak delay of 300 s.

Volatile compounds were identified by comparing their mass spectra with data from the NIST, RepLib, MainLib, and Adams mass spectral libraries and by comparing experimentally determined retention indices (RI) with literature values [23]. Retention indices were calculated using a C₇–C₃₀ *n*-alkanes standard analysed on a non-polar BPX-5 column. Compound identification was considered positive when a good match of the mass spectrum and retention index was obtained. The signal-to-noise (S/N) threshold was set at 50, and the minimum acceptable spectral similarity was 700.

Analyses were performed in triplicate. Results were expressed semi-quantitatively as percentages of the total chromatographic (GC) peak area for comparative purposes across samples. Odour descriptors were obtained from The Good Scents Company Information System.

2.14. Preparation of Fermented Dairy Drink

Commercial cow's milk (ROKIŠKIO NAMINIS, UAB „Rokiškio pieno gamyba“, Utena, Lithuania) was purchased from a local retail store. Fermented dairy drink was prepared using a commercial direct-vat-set starter culture (YoFlex® YC-180, Chr. Hansen A/S, Hørsholm, Denmark) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Approximately 5 L of fermented dairy drink was produced using a laboratory-scale fermented products processing unit (OMVE Netherlands B.V., De Meern, The Netherlands).

The milk was heated to 42–44 °C and inoculated with 260 mg of the starter culture. Fermentation was continued until a pH of 4.79 and the fluid consistency characteristic of a drink were achieved. The drink was subsequently divided into five portions and mixed with the hemp seed product fermented with *Lactocaseibacillus paracasei* subsp. *paracasei* DSM 4905 (*L. paracasei* FHSP) at concentrations of 0, 2, 4, 6, and 8 g/100 g of fermented dairy drink. The prepared formulations were stored at 5 °C until further analysis. All analytical determinations were performed in triplicate.

2.15. Quality Assessment of Fermented Dairy Drink During Storage

The pH of the drink samples enriched with *L. paracasei* FHSP was measured immediately after preparation and after 4 and 9 days of refrigerated storage using a PB-11 pH meter (Sartorius AG, Göttingen, Germany).

The colour parameters of the drink samples were measured using a CR-410 portable colorimeter (Konica Minolta, Inc., Sakai, Japan) and expressed according to the CIE *Lab** colour system. The *L** value indicates lightness, ranging from 0 (black) to 100 (white), whereas positive and negative *a** values indicate red and green tones, respectively, and positive and negative *b** values indicate yellow and blue tones, respectively.

Syneresis was determined by transferring 20 mL of each drink sample into a graduated tube. The volume of whey separated on the drink surface was recorded after 24 and 48 h. Syneresis was calculated as follows:

$$\text{Syneresis(\%)} = \frac{V_s}{V_t} \times 100 \quad (3)$$

where *V_s* is the volume of separated whey (mL), and *V_t* is the initial fermented dairy drink volume (mL).

2.16. Sensory Evaluation of Fermented Dairy Drink

The acceptability of the selected sensory attributes of the drink samples enriched with *L. paracasei* FHSP were evaluated by 30 assessors [24]. Before the evaluation, the assessors participated in a familiarisation session during which the sensory attributes, scoring criteria, and rating procedure were explained. Only individuals who voluntarily

agreed to participate and reported no temporary conditions that could impair their sensory perception at the time of testing were included.

The acceptability of the selected sensory attributes was evaluated using a seven-point scale, with the endpoints representing the lowest and highest degree of acceptability. Each sample (50 mL) was identified by a randomly assigned three-digit code and presented individually in monadic order at room temperature. Water was provided for palate cleansing between successive samples.

2.17. Statistical Analysis

All analyses were performed in triplicate, and the results are presented as mean values $\pm$ standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) in STATGRAPHICS Centurion 19. Differences among mean values were evaluated using Fisher's least significant difference (LSD) post hoc test. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Chemical Composition of Hemp Seed Flour

The nutritional composition of hemp seed flour is presented in Table 1. Dietary fibre was the predominant component of hemp seed flour and consisted mainly of the insoluble fraction, with an approximate insoluble-to-soluble dietary fibre ratio of 14:1. Protein was the second most abundant component, followed by fat and ash, whereas sugars represented only a minor proportion of the flour composition.

Table 1. Chemical composition of hemp seed flour.

Nutrient	Hemp Seed Flour
Moisture, g/100 g	6.63 $\pm$ 0.19
Protein, g/100 g d.m.	28.05 $\pm$ 0.61
Fat, g/100 g d.m.	8.90 $\pm$ 0.20
Insoluble dietary fibre, g/100 g d.m.	45.15 $\pm$ 0.06
Soluble dietary fibre, g/100 g d.m.	3.19 $\pm$ 0.06
Ash, g/100 g d.m.	6.38 $\pm$ 0.08
Total carbohydrates *, g/100 g d.m.	8.3
Reducing sugars, g/100 g d.m.	2.46 $\pm$ 0.01

* Carbohydrates = 100 – (crude protein + lipids + ash + total dietary fibre). Values are presented as mean $\pm$ standard deviation ($n = 3$).

A similarly pronounced predominance of insoluble dietary fibre was reported by Mikulec et al. [25], who determined 45.18 g/100 g total dietary fibre in partially defatted hemp seed flour, including 44.00 g/100 g insoluble and 1.37 g/100 g soluble dietary fibre. The high proportion of insoluble dietary fibre in the present sample indicates that dietary fibre-rich structural seed fractions remained after oil extraction and milling. This interpretation is consistent with findings showing that whole hemp seeds contain considerably more fibre than dehulled seeds, whereas dehulling increases the relative proportions of protein and fat [26].

The protein content determined in the present study was higher than the 25.90 g/100 g reported by Cabral et al. [27] for hemp seed meal obtained from an oil-processing stream, although it was lower than the 36.25 g/100 g dry matter reported by Mikulec et al. [25] for another partially defatted hemp seed flour. Absi et al. [4]. reported protein contents ranging from 31.34 to 34.41 g/100 g in organic and conventional hemp seed flours, which were also higher than the value determined in the present study.

The fat content was lower than the 9.10 g/100 g reported for partially defatted hemp seed flour by Mikulec et al. [25] and to the range of 9.93–12.31 g/100 g observed in commercial hemp seed flours and protein-rich products by Absi et al. [4]. The ash content of 6.38 g/100 g was consistent with the composition of commercially available hemp seed flour. Absi et al. [4] reported an average ash content of 6.68 g/100 g for commercial hemp seed flours, whereas Cabral et al. [27] determined 7.61 g/100 g in hemp seed meal.

Such differences are expected because the composition of commercial hemp seed ingredients is affected by the cultivar, inclusion or removal of the seed hull, oil-extraction efficiency, milling conditions, and any subsequent protein-enrichment treatment. Considerable compositional differences have also been reported among commercial products marketed either as hemp seed flour or hemp seed protein concentrate [4,27].

3.2. Effect of Fermentation on Hemp Seed Flour Acidification and LAB Viability

Hemp seed flour was fermented for 72 h using three lactic acid bacteria (LAB) strains. LAB counts and changes in pH were evaluated at 24 h intervals (Table 2, Figure 1).

Table 2. LAB counts during 72 h of hemp seed flour fermentation, CFU/g.

Bacterial Strain	0 h	24 h	48 h	72 h
<i>L. paracasei</i>	$2.8 \pm 0.20 \times 10^7$ a,A	$1.25 \pm 0.98 \times 10^9$ a,B	$1.38 \pm 0.94 \times 10^9$ a,B	$1.20 \pm 0.70 \times 10^9$ a,B
<i>L. plantarum</i>	$3.1 \pm 0.25 \times 10^7$ b,A	$3.81 \pm 0.20 \times 10^8$ b,B	$4.10 \pm 0.60 \times 10^8$ b,B	$3.80 \pm 0.55 \times 10^8$ b,B
<i>L. fermentum</i>	$4.5 \pm 0.10 \times 10^7$ c,A	$4.30 \pm 0.80 \times 10^8$ c,B	$4.60 \pm 0.70 \times 10^8$ c,B	$4.72 \pm 0.60 \times 10^8$ c,B

Within the same column, mean values marked with different lowercase letters differ significantly among LAB strains ($p < 0.05$). Within the same row, mean values marked with different uppercase letters differ significantly among fermentation times ($p < 0.05$).

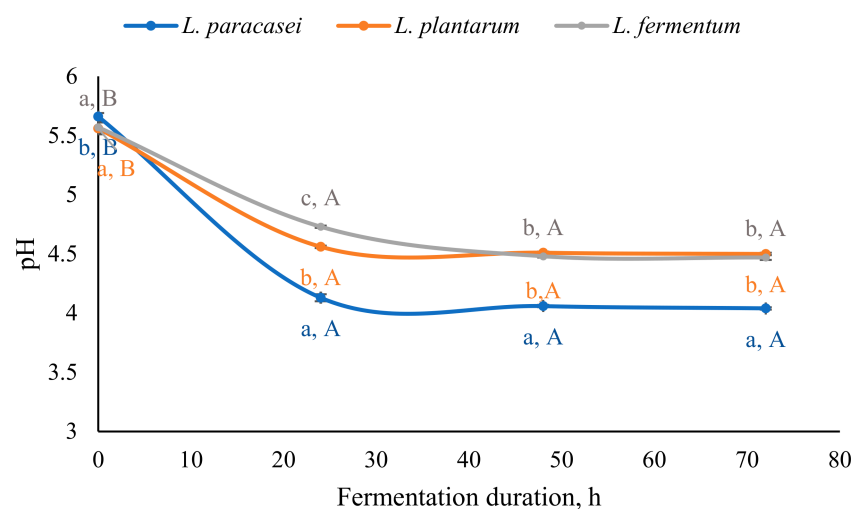


Figure 1. Changes in the pH of hemp seed flour substrates during 72 h of fermentation with different LAB strains. Values are presented as mean $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate significant differences between LAB strains at the same time point, and different uppercase letters indicate significant differences between fermentation times within the same LAB strain ($p < 0.05$).

A pronounced increase in viable LAB counts was observed during the first 24 h of fermentation. The greatest increase occurred during fermentation with *L. paracasei*, in which viable counts reaching 10^9 CFU/g after 24 h. In comparison, the counts of *L. plantarum* and *L. fermentum* reached the order of 10^8 CFU/g. Only minor changes were observed during the subsequent fermentation period. The viable counts of *L. paracasei* and *L. plantarum* increased slightly up to 48 h and subsequently decreased, whereas the count of *L. fermentum* continued to increase slightly throughout the 72 h period.

Alongside the increase in viable LAB counts, the pH of the hemp seed flour decreased during fermentation (Figure 1).

The initial pH values ranged from 5.56 to 5.66. The most pronounced acidification occurred during the first 24 h of fermentation, corresponding to the period of the greatest increase in viable LAB counts. However, the extent of acidification was dependent on the bacterial strain.

Fermentation with *L. paracasei* resulted in the greatest decrease in pH, reaching 4.13 after 24 h, and remained the most acidic throughout the fermentation period. Fermentation with *L. plantarum* and *L. fermentum* resulted in less pronounced pH reductions during the first 24 h. During fermentation with *L. fermentum*, a further noticeable decrease in pH occurred between 24 and 48 h, whereas the changes observed with the other strains during this period were smaller.

After 48 h, only minor changes in pH were observed in all samples. The final pH values ranged from 4.04 following fermentation with *L. paracasei* to 4.50 and 4.47 following fermentation with *L. plantarum* and *L. fermentum*, respectively. Similarly, viable LAB counts did not change significantly after 24 h of fermentation, despite slight fluctuations during the subsequent period. The limited changes in pH after 48 h and in viable LAB counts after 24 h indicate that the main changes in these parameters occurred during the earlier stages of fermentation.

Overall, the hemp seed flour supported the growth and metabolic activity of all three LAB strains. The most pronounced increase in viable LAB counts and the greatest reduction in pH occurred during the first 24 h. Further acidification was observed up to 48 h, particularly during fermentation with *L. fermentum*, whereas extending fermentation from 48 to 72 h resulted in only minor changes in both viable LAB counts and pH. Therefore, a fermentation period of 48 h appears to be sufficient to achieve stable LAB counts and pH values, while extending fermentation to 72 h provides limited additional benefit.

Bartkiene et al. (2016) [28] investigated the fermentation of hulled and unhulled hemp seeds with *Pediococcus acidilactici* and *Pediococcus pentosaceus* and reported that, after 48 h, the pH decreased to approximately 4.75, while LAB counts reached about 7.72 log CFU/g, corresponding to 5.25×10^7 CFU/g [28]. In the present study, acidification was more pronounced, particularly in the substrate fermented with *L. paracasei*, along with a higher maximum viable count of 1.38×10^9 CFU/g. The suitability of hemp seed flour for LAB fermentation was also demonstrated by Čižeikienė et al. [6]. After 48 h of fermentation, LAB counts reached 7.9×10^9 CFU/g with *L. plantarum*, 3.3×10^9 CFU/g with *L. brevis* and 2.9×10^9 CFU/g with *L. acidophilus*. However, the corresponding pH values differed markedly, reaching 4.72, 5.52 and 4.92, respectively, from an initial pH of 6.65. The lower final pH obtained with *L. paracasei* in the present study may also be associated with differences in flour composition, water content, inoculum size and fermentation duration.

The viable counts of *L. paracasei* and *L. plantarum* reached their highest values after 48 h of fermentation and subsequently decreased slightly or remained relatively stable until 72 h. In contrast, the population of *L. fermentum* continued to increase gradually throughout fermentation, suggesting slower adaptation to the substrate or more gradual utilisation of available nutrients. A similar fermentation pattern was observed in hemp seed bran, where the pH decreased rapidly during the first 24–48 h and subsequently remained relatively stable. Depending on the LAB strain, the pH of fermented hemp seed bran ranged from 4.13 to 4.96 after 24 h and from 4.01 to 4.49 after 48 h, indicating that most acidification occurred during the early stages of fermentation [10]. The similar stabilisation of pH after 48 h in both studies indicates that the major acidification occurred during the earlier stages of fermentation, whereas the extent of the pH decrease remained dependent on the LAB strain and the fermentation system.

Although the extent of acidification and final LAB populations differed between the studies, both demonstrate that hemp seed-based substrates can support LAB growth. These differences may be attributed to the processing and composition of the hemp raw material, the initial inoculum level, the bacterial strains used, and the applied fermentation conditions. Previous studies have shown that LAB produce lactic acid and, depending on their metabolic pathways, other organic acids, including acetic acid, during the fermentation of plant-based substrates. The accumulation of these metabolites results in substrate acidification and may reduce the pH of fermented plant products to approximately 3.5–4.0. Under favourable fermentation conditions, viable LAB counts may reach approximately 10^9 CFU/g [29,30]. These findings are consistent with the intensive acidification and LAB growth observed in the present study, particularly in the hemp seed flour substrate fermented with *L. paracasei*.

The viability of LAB in the FHSP after freeze-drying is presented in Table 3.

Table 3. Effect of fermentation and freeze-drying on LAB counts and soluble compound content in the fermented hemp seed product (FHSP).

Sample	LAB, CFU/g	Reducing Sugars, g/100 g d.m.	Soluble Protein, g/100 g d.m.	Soluble Dietary Fibre, g/100 g d.m.
<i>L. paracasei</i>	$9.02 \pm 0.21^c \times 10^8$	1.94 ± 0.02^c	0.37 ± 0.04^a	3.79 ± 0.12^b
<i>L. fermentum</i>	$1.85 \pm 0.21^a \times 10^8$	1.36 ± 0.02^a	0.55 ± 0.01^b	2.17 ± 0.11^a
<i>L. plantarum</i>	$2.45 \pm 0.71^b \times 10^8$	1.61 ± 0.01^b	0.38 ± 0.01^a	3.91 ± 0.15^b
Non-fermented	-	2.33 ± 0.02^d	0.73 ± 0.03^c	3.81 ± 0.04^b

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$).

Freeze-drying reduced viable LAB counts compared with those determined at the end of fermentation. However, the counts remained above 10^8 CFU/g in all FHSPs. The highest viability after freeze-drying was observed in the product fermented with *L. paracasei*, whereas the lowest viable count was recorded in the product fermented with *L. fermentum*. These results indicate that LAB survival during freeze-drying was strain-dependent.

High LAB viability after drying has also been reported in other fermented plant-based materials. Ayub et al. [31] found that freeze-dried fermented quinoa flour retained approximately 9–10 log CFU/g of *Lactiplantibacillus plantarum*. Similarly, Santos et al. [32] reported LAB counts of approximately 8.5–8.6 log CFU/g after freeze-drying cereal sourdoughs. In the present study, post-freeze-drying counts ranged from approximately 8.27 to 8.96 log CFU/g, which were within or close to the values reported for these fermented plant-based materials. Differences between studies may relate to the LAB strain, the composition of the fermented material, and the applied freeze-drying conditions.

The reduction in LAB viable counts may be related to membrane damage caused by freezing, osmotic stress and dehydration. Proteins, residual carbohydrates, lipids and dietary fibre in the hemp seed product may have provided partial protection to the bacterial cells [33]. Nevertheless, the relatively high LAB viable counts retained after freeze-drying demonstrate that the FHSP provided suitable conditions for maintaining LAB viability during processing.

3.3. Effect of LAB Fermentation on the Chemical Composition of the Hemp Seed Product

The reducing sugar, soluble protein, and soluble dietary fibre contents of the FHSP and non-fermented hemp seed flour are presented in Table 3.

All fermented samples had significantly lower reducing sugar contents than the non-fermented control. The extent of the reduction was strain-dependent, with the lowest value

observed in the product fermented with *L. fermentum*, followed by those fermented with *L. plantarum* and *L. paracasei*.

The decrease in reducing sugars may be attributed to their utilisation by LAB as substrates for growth and energy production [34]. However, the extent of reducing sugar reduction did not correspond directly to the degree of acidification. Although the product fermented with *L. paracasei* retained the highest reducing sugar content among the fermented samples, this treatment resulted in the greatest decrease in pH. This difference may reflect strain-specific carbohydrate metabolism and differences in the relative formation of lactic acid and other fermentation products [34–37].

All fermented products contained significantly less soluble protein than the non-fermented sample. Among the fermented samples, the highest soluble protein content was determined in the product fermented with *L. fermentum*, whereas no significant difference was observed between the products fermented with *L. paracasei* and *L. plantarum*.

The reduction in soluble protein content may have resulted from the combined effects of fermentation and technological processing. Acidification during fermentation may have shifted hemp seed proteins towards their isoelectric region, promoting protein aggregation and precipitation and consequently reducing their solubility under the applied analytical conditions [38].

Hemp seed fibre consists mainly of cellulose, hemicellulose, and lignin, which form the major part of the insoluble dietary fibre fraction and are relatively resistant to LAB fermentation [39]. Therefore, insoluble dietary fibre remained the predominant fraction after fermentation. The small differences among the samples should not necessarily be interpreted as the formation or degradation of dietary fibre. They may instead reflect changes in the relative proportions of product components following the utilisation of reducing sugars and other fermentable compounds, as well as analytical variation.

3.4. Effect of Fermentation on the Technological Properties of the Hemp Seed Product

The technological properties evaluated in the hemp seed products included water swelling capacity (WSC), water retention capacity (WRC), and oil retention capacity (ORC). The effects of fermentation with different LAB strains on WSC, WRC, and ORC are presented in Table 4.

Table 4. Effect of LAB fermentation on the technological properties of the fermented hemp seed product (FHSP).

Sample	WSC, mL/g d.m.	WRC, g/g d.m.	ORC, g/g d.m.
<i>L. paracasei</i>	0.26 ± 0.02 ^b	2.94 ± 0.10 ^b	1.28 ± 0.04 ^a
<i>L. fermentum</i>	0.26 ± 0.02 ^b	2.58 ± 0.02 ^a	1.65 ± 0.04 ^b
<i>L. plantarum</i>	0.26 ± 0.02 ^b	2.95 ± 0.09 ^b	1.30 ± 0.07 ^a
Non-fermented	0.15 ± 0.01 ^a	2.55 ± 0.09 ^a	1.23 ± 0.04 ^a

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$). d.m., dry matter.

All FHSP exhibited significantly greater WSC capacity than the non-fermented hemp seed flour. No significant differences were observed among the products fermented with the three LAB strains.

The effect of fermentation on WRC was strain-dependent. Products fermented with *L. paracasei* and *L. plantarum* had significantly greater WRC than the non-fermented sample, and the product fermented with *L. fermentum*. In contrast, the WRC of the product fermented with *L. fermentum* did not differ significantly from that of the non-fermented sample.

ORC was significantly increased only in the product fermented with *L. fermentum*. The other fermented products did not differ significantly from the non-fermented hemp seed flour. Therefore, the effects of fermentation on WRC and ORC were strain-specific: *L. paracasei* and *L. plantarum* increased WRC, whereas *L. fermentum* increased ORC.

The WRC of the FHSPs ranged from 2.58 to 2.95 g/g. Lower values of approximately 0.83–1.05 g/g have been reported for hemp seed protein isolates [40,41]. ORC values reported for hemp seed protein isolates ranged from approximately 1.28 to 1.81 g/g and were therefore comparable with those obtained in the present study [40,41]. Nevertheless, direct comparison is limited because the present study evaluated a multicomponent hemp seed product containing a considerable amount of dietary fibre rather than an isolated protein fraction. In addition to proteins, dietary fibre may have contributed substantially to the WRC and ORC.

Differences in the technological properties of hemp seed protein preparations have previously been associated with protein composition, including the relative proportions of edestin, albumin, and vicilin, as well as protein solubility [40,41]. Structural changes during fermentation may alter the exposure of hydrophilic and hydrophobic groups and consequently affect interactions with water and lipids. In addition to proteins, the predominantly insoluble dietary fibre fraction may contribute substantially to these properties. Although fermentation did not increase soluble dietary fibre content, the higher WSC and, in some samples, WRC may reflect structural modifications of the insoluble fibre fraction rather than changes in its quantitative distribution. Changes in fibre morphology, porosity, crystallinity and the accessibility of hydrophilic groups may enhance interactions with water without necessarily resulting in a measurable increase in soluble dietary fibre content [42].

3.5. Effect of Fermentation on the Functional Properties of the Hemp Seed Product

The functional properties evaluated in the hemp seed products included sodium cholate-binding capacity, cholesterol-binding capacity at pH 2 and pH 7, total phenolic content, and antioxidant activity determined using the ABTS and DPPH assays. The results are presented in Table 5.

Table 5. Effect of LAB fermentation on the functional properties of the fermented hemp seed product (FHSP).

Sample	Sodium Cholate-Binding Capacity, mg/g	Cholesterol-Binding Capacity at pH 2, mg/g	Cholesterol-Binding Capacity at pH 7, mg/g	Total Phenolic Content, mg GAE/g	ABTS Antioxidant Activity, mg TE/g	DPPH Antioxidant Activity, μ mol TE/g
<i>L. paracasei</i>	13.00 $\pm$ 0.50 ^b	2.77 $\pm$ 0.06 ^a	1.87 $\pm$ 0.06 ^a	5.22 $\pm$ 0.02 ^c	11.61 $\pm$ 0.002 ^b	1.50 $\pm$ 0.12 ^c
<i>L. fermentum</i>	15.33 $\pm$ 0.76 ^d	4.60 $\pm$ 0.10 ^c	1.77 $\pm$ 0.06 ^a	3.87 $\pm$ 0.01 ^a	10.82 $\pm$ 0.004 ^a	1.35 $\pm$ 0.06 ^b
<i>L. plantarum</i>	12.33 $\pm$ 0.29 ^b	4.43 $\pm$ 0.15 ^c	5.13 $\pm$ 0.06 ^b	4.77 $\pm$ 0.01 ^b	11.79 $\pm$ 0.003 ^b	1.22 $\pm$ 0.21 ^a
Non-fermented	10.33 $\pm$ 0.29 ^a	4.13 $\pm$ 0.06 ^b	5.07 $\pm$ 0.06 ^b	3.78 $\pm$ 0.01 ^a	10.89 $\pm$ 0.005 ^a	1.25 $\pm$ 0.15 ^a

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$). GAE, gallic acid equivalents; TE, Trolox equivalents.

Sodium cholate-binding capacity differed significantly among samples. The highest value was determined in the product fermented with *L. fermentum*, whereas the lowest value was observed in the non-fermented hemp seed flour. Fermentation with *L. paracasei* and *L. plantarum* resulted in intermediate values. The high sodium cholate-binding capacity of the *L. fermentum*-FHSP coincided with its significantly increased ORC. Since bile acid adsorption by dietary fibre-rich materials is strongly influenced by hydrophobic interactions, fermentation-induced exposure of hydrophobic binding sites may have contributed to both the increased oil retention and sodium cholate binding observed in this sample [43].

At pH 2, the products fermented with *L. fermentum* and *L. plantarum* showed the highest cholesterol-binding capacities, with no significant difference between them. The non-fermented sample exhibited a lower value. The *L. paracasei* FHSP showed the lowest cholesterol-binding capacity under acidic conditions. Thus, fermentation with *L. fermentum* and *L. plantarum* improved cholesterol binding at pH 2, whereas fermentation with *L. paracasei* reduced it.

A different pattern was observed at pH 7. The *L. plantarum* FHSP and the non-fermented hemp seed flour showed the highest cholesterol-binding capacities and did not differ significantly. Products fermented with *L. paracasei* and *L. fermentum* showed considerably lower values. These results show that cholesterol-binding capacity depended on both the LAB strain and the system pH.

The binding of bile salts and cholesterol may be associated with the physicochemical properties of dietary fibre, proteins and phenolic compounds. Dietary fibre can entrap lipid-associated compounds or interact with bile acids and cholesterol through hydrophobic and other non-covalent interactions, potentially reducing their availability in the gastrointestinal tract [44,45]. Fermentation-induced proteolysis may also release low-molecular-weight peptides and expose hydrophobic amino acid residues capable of interacting with bile salts and cholesterol-containing micelles [46].

The cholesterol- and sodium cholate-binding capacities determined in the present study were lower than the values previously reported for selected berry pomace powders. Cholesterol-binding capacities of approximately 21–23 mg/g at pH 7 and 14–21 mg/g at pH 2, as well as bile salt-binding capacities ranging from 24 to 75 mg/g, have been reported for these materials [45]. Differences between the studies may be related to raw material composition and structure, while in the present study the observed variation among samples may additionally reflect LAB fermentation-induced changes in dietary fibre, proteins, phenolic compounds and the accessibility of binding sites.

The highest value of total phenolic content was found in *L. paracasei* FHSP. The lowest total phenolic contents (TPC) were recorded in *L. fermentum* FHSP and in the non-fermented sample.

The TPC determined in the present study were higher than the values of approximately 1.13–1.27 mg GAE/g previously reported for fermented cereal- and hemp-based beverages [1]. Direct comparison is nevertheless limited because of differences in raw materials, product concentration, extraction procedures, analytical conditions and fermentation parameters. LAB fermentation may release phenolic compounds from bound forms through enzymatic activity and disruption of plant structures, thereby increasing the amount detected by the Folin–Ciocalteu assay [1]. However, this method also responds to other reducing compounds; therefore, the results represent total reducing capacity expressed as GAE rather than the concentration of specifically identified phenolic compounds.

The ABTS antioxidant activity was significantly higher in the products fermented with *L. paracasei* and *L. plantarum* than in the other samples, with no significant difference between these two fermented products. Fermentation with *L. fermentum* did not significantly alter ABTS antioxidant activity compared with the non-fermented sample.

According to the DPPH assay, the highest antioxidant activity was determined in *L. paracasei* FHSP, followed by *L. fermentum* FHSP. The lowest activity was found in *L. plantarum* FHSP and the non-fermented sample. Thus, fermentation with *L. paracasei* produced the most pronounced increase in DPPH radical-scavenging activity.

Previous studies have shown that LAB fermentation may increase the antioxidant activity of hemp- seed based products, in some cases by up to approximately 140% [47]. Such changes may result from the release of bound phenolic compounds, transformation of phenolic structures, formation of antioxidant peptides or production of other reducing

metabolites. Nevertheless, the magnitude and direction of these changes depend on the bacterial strain, substrate composition and fermentation conditions [47]. In the present study, the highest total phenolic content in *L. paracasei*-FHSP coincided with the highest DPPH antioxidant activity, suggesting that the greater availability of phenolic compounds may have contributed to its radical-scavenging capacity. However, differences between the ABTS and DPPH assays indicate that the antioxidant response was also influenced by the types of antioxidant compounds present and their reactivity in the respective assay systems.

Overall, the effect of fermentation on the functional properties of the hemp seed product was strongly strain-dependent. Fermentation with *L. fermentum* resulted in the highest sodium cholate-binding capacity and, together with *L. plantarum*, the highest cholesterol-binding capacity at pH 2. The product fermented with *L. plantarum* exhibited the highest cholesterol-binding capacity at pH 7, although it did not differ significantly from the non-fermented sample. Fermentation with *L. paracasei* resulted in the highest total phenolic content and DPPH antioxidant activity, while the products fermented with *L. paracasei* and *L. plantarum* showed the highest ABTS antioxidant activity. These differences indicate that fermentation affected the individual functional properties through different strain-dependent biochemical changes. Therefore, none of the investigated LAB strains produced the highest values for all functional properties.

3.6. Effect of Fermentation on the Volatile Compound Profile of the Hemp Seed Product

The volatile compound profiles of the FHSPs and the non-fermented hemp seed flour were analysed by gas chromatography—time-of-flight mass spectrometry (GC–TOFMS). As a result, volatile data in this study are reported semi-quantitatively as percentages (%) of the total chromatographic (GC) peak area for comparative purposes across samples. No internal or external standards were employed. The headspace volatile composition of the hemp seed products is presented in Table 6.

Table 6. Headspace volatile compound composition (% of the total GC peak area) of the fermented hemp seed product (FHSP).

No #	Compound ^A	RI Exp. ^B	RI Lit. ^C	% of the Total GC Peak Area ^D				Odour Type: Description ¹
				<i>L. fermentum</i>	<i>L. plantarum</i>	<i>L. paracasei</i>	Non-Fermented	
1	Ethyl acetate	633	628	14.70 ± 0.50 ^c	4.74 ± 0.20 ^a	12.75 ± 0.55 ^b	19.28 ± 1.05 ^d	Ethereal: ethereal, fruity, sweet, pineapple, weedy, green, musty
2	Acetic acid	649	645	38.25 ± 1.77 ^b	44.38 ± 1.73 ^c	50.50 ± 2.15 ^d	9.61 ± 0.51 ^a	Acidic: sharp, pungent, vinegar, sour
3	(<i>E</i>)-2-Butenal	669	665	tr ^E	0.05 ± 0.01 ^a	– ^F	0.07 ± 0.01 ^a	Pungent, sharp
4	3-Methylbutanal	670	669	–	–	–	0.45 ± 0.04	Aldehydic: ethereal, aldehydic, chocolate, peach, fatty
5	1-Penten-3-ol	691	684	0.37 ± 0.09 ^a	0.16 ± 0.07 ^a	0.29 ± 0.01 ^a	0.54 ± 0.23 ^a	Green: ethereal, horseradish, green, radish, chrysanthemum, vegetable, tropical, fruity
6	Pentanal	700	704	1.34 ± 0.09 ^b	0.64 ± 0.07 ^a	0.64 ± 0.01 ^a	1.40 ± 0.23 ^b	Fermented: fermented, breadly, nutty, berry
7	Acetoin	711	711	–	6.37 ± 0.21 ^b	0.11 ± 0.02 ^a	–	Buttery: sweet, buttery, creamy, dairy, milky, fat
8	<i>n</i> -Propyl acetate	714	716	–	2.76 ± 0.13 ^c	0.05 ± 0.00 ^a	0.23 ± 0.02 ^b	Fruity: solvent, celery, fruity, fusel, raspberry, pear, sweet
9	Isopentyl alcohol	736	731	1.49 ± 0.22 ^b	1.96 ± 0.18 ^b	0.69 ± 0.06 ^a	–	Fermented: fusel, alcoholic, pungent, ethereal, cognac, whiskey, fruity, banana, molasses
10	2-Methyl-1-butanol	740	739	–	–	–	tr	Ethereal: ethereal, fusel, alcoholic, fatty, greasy, winey, whiskey, leathery, cocoa
11	Dimethyl disulfide	747	746	0.26 ± 0.02 ^b	0.45 ± 0.07 ^c	0.34 ± 0.06 ^{bc}	0.09 ± 0.01 ^a	Sulfurous: sulfurous, vegetable, cabbage, onion
12	2-Methylpropanoic acid	752	753	–	–	0.31 ± 0.06	–	Acidic: sour, cheesy, dairy
13	Pentan-1-ol	768	771	0.26 ± 0.04 ^a	0.66 ± 0.04 ^b	0.25 ± 0.03 ^a	0.67 ± 0.05 ^b	Fermented: pungent, fermented, breadly, yeasty, fusel, winey and solvent-like

Table 6. Cont.

No #	Compound ^A	RI Exp. ^B	RI Lit. ^C	% of the Total GC Peak Area ^D				Odour Type: Description ¹
				<i>L. fermentum</i>	<i>L. plantarum</i>	<i>L. paracasei</i>	Non-Fermented	
14	Isobutyl acetate	772	772	0.35 ± 0.05 ^b	0.24 ± 0.02 ^{ab}	0.19 ± 0.01 ^a	0.60 ± 0.00 ^c	Fruity: sweet, fruity, ethereal, banana, tropical
15	(2Z)-Pentenol	772	773	–	–	–	0.67 ± 0.05	Green: green, phenolic, nasturtium, ethereal, medicinal, aldehydic, cherry, narcissus, metallic, fruity
16	2-Hexanone	791	792	tr	–	0.06 ± 0.00	tr	Fruity: fruity, fungal, meaty, buttery
17	Hexanal	801	801	27.51 ± 1.63 ^b	20.67 ± 1.49 ^a	21.41 ± 2.13 ^a	44.75 ± 1.34 ^c	Green: fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty
18	Methylpyrazine	830	826	–	tr	tr	–	Nutty: nutty, cocoa, roasted, chocolate, peanut, green, musty, earthy
19	2-Methylbutanoic acid	848	832	–	–	0.07 ± 0.01	–	Acidic: pungent, acid, sour, cheesy, fruity, fermented
20	(2E)-Hexenal	855	855	0.12 ± 0.01 ^a	0.14 ± 0.01 ^b	0.17 ± 0.01 ^{bc}	0.21 ± 0.02 ^c	Green: fresh, green, banana, aldehydic, fatty, cheesy, fruity, herbal, spicy
21	n-Hexanol	868	870	1.03 ± 0.10 ^b	1.57 ± 0.19 ^c	0.17 ± 0.01 ^a	1.16 ± 0.15 ^b	Herbal: ethereal, fusel, oily, fruity, alcoholic, sweet, green
22	Isopentyl acetate	876	876	0.21 ± 0.01 ^a	0.16 ± 0.00 ^a	0.31 ± 0.03 ^b	0.44 ± 0.33 ^{ab}	Fruity: sweet, fruity, banana, solvent, ripe, estery
23	2-Heptanone	891	892	0.22 ± 0.04 ^a	0.54 ± 0.04 ^b	0.31 ± 0.06 ^a	0.87 ± 0.11 ^c	Cheesy: fruity, spicy, sweet, herbal, coconut, woody, ketonic, green banana
24	Styrene	896	896	–	0.07 ± 0.00	–	–	Balsamic: sweet, balsam, floral, plastic
25	Heptanal	902	901	0.69 ± 0.04 ^b	0.54 ± 0.04 ^a	0.83 ± 0.11 ^c	1.26 ± 0.15 ^d	Green: fresh, aldehydic, fatty, green, herbal, wine-lee, ozone
26	γ-Butyrolactone	918	917	0.05 ± 0.01 ^a	0.10 ± 0.01 ^{ab}	0.12 ± 0.01 ^b	0.08 ± 0.01 ^a	Creamy: creamy, oily, fatty, caramel
27	α-Pinene	939	939	0.31 ± 0.03 ^a	0.47 ± 0.04 ^b	0.34 ± 0.02 ^a	0.68 ± 0.05 ^c	Herbal: fresh, camphor, sweet, pine, earthy, woody
28	(2E)-Heptenal	958	954	0.32 ± 0.02 ^a	0.73 ± 0.08 ^c	0.51 ± 0.05 ^b	0.26 ± 0.02 ^a	Green: pungent, green, vegetable, fresh, fatty, fruity
29	Benzaldehyde	967	966	tr	0.14 ± 0.01 ^a	0.60 ± 0.06 ^b	0.45 ± 0.05 ^b	Fruity: almond, powdery, nutty, fruity, cherry
30	1-Octen-3-one	978	977	0.26 ± 0.03 ^a	0.34 ± 0.05 ^a	0.26 ± 0.01 ^a	0.25 ± 0.30 ^a	Earthy: herbal, mushroom, earthy, musty, dirty
31	Hexanoic acid	980	979	2.43 ± 0.24 ^{ab}	2.67 ± 0.29 ^b	2.07 ± 0.06 ^a	2.24 ± 0.30 ^{ab}	Fatty: sour, fatty, sweaty, cheesy
32	β-Pinene	983	979	0.39 ± 0.03 ^a	0.49 ± 0.08 ^{ab}	0.46 ± 0.08 ^{ab}	0.64 ± 0.08 ^b	Herbal: cooling, woody, pine, terpenic, fresh, minty, eucalyptus, camphoreous, spicy, peppery, nutmeg
33	3-Octanone	987	983	–	–	0.43 ± 0.04	–	Herbal: fresh, herbal, lavender, sweet, mushroom, fermented, musty, ketonic, cheesy, green, vegetable
34	6-Methyl-5-hepten-2-one	988	985	0.24 ± 0.03 ^a	0.24 ± 0.04 ^a	0.34 ± 0.04 ^a	0.61 ± 0.06 ^b	Citrus: fruity, apple, musty, ketonic, creamy, cheesy, banana, citrus, lemongrass
35	2-Octanone	992	991	0.08 ± 0.01 ^a	0.23 ± 0.01 ^b	0.043 ± 0.04 ^a	0.39 ± 0.05 ^b	Earthy: earthy, weedy, natural, woody, herbal, cheesy, dairy
36	Myrcene	992	991	0.52 ± 0.03 ^{ab}	0.53 ± 0.05 ^{ab}	0.46 ± 0.05 ^a	0.61 ± 0.04 ^b	Spicy: terpenic, herbal, woody, rose, celery, carrot
37	2-Pentylfuran	993	993	–	–	–	0.86 ± 0.09	Fruity: fruity, green, earthy, beany, vegetable, metallic
38	Decane	1000	1000	0.17 ± 0.01 ^a	0.22 ± 0.02 ^a	0.18 ± 0.03 ^a	0.49 ± 0.03 ^b	Alkane
39	Octanal	1004	1003	0.08 ± 0.02 ^a	0.14 ± 0.01 ^{ab}	0.14 ± 0.02 ^{ab}	0.23 ± 0.00 ^b	Aldehydic: aldehydic, waxy, citrus, orange peel, green, herbal, fresh, fatty
40	(2E,4E)-Heptadienal	1014	1007	0.08 ± 0.01 ^a	0.08 ± 0.00 ^a	0.08 ± 0.01 ^a	–	Fatty: fatty, green, oily, aldehydic, vegetable
41	δ-3-Carene	1015	1011	0.18 ± 0.03 ^a	0.22 ± 0.02 ^a	0.16 ± 0.02 ^a	0.46 ± 0.05 ^b	Citrus: citrus, terpenic, herbal, pine, solvent, resinous, phenolic, cypress, medicinal, woody
42	p-Cymene	1029	1024	0.06 ± 0.00 ^a	0.09 ± 0.01 ^{ab}	0.07 ± 0.01 ^a	0.16 ± 0.02 ^b	Terpenic: chemical, woody, terpenic, fresh, citrus, lemon, spicy, cumin, origanum, cilantro
43	Limonene	1033	1029	0.80 ± 0.06 ^a	0.81 ± 0.05 ^a	0.64 ± 0.07 ^a	1.32 ± 0.07 ^b	Citrus: citrus, orange, fresh, sweet, terpenic
44	1,8-Cineole	1037	1031	0.12 ± 0.01 ^a	0.14 ± 0.00 ^a	0.12 ± 0.02 ^a	0.28 ± 0.02 ^b	Herbal: eucalyptus, herbal, camphor, minty, medicinal
45	(3E)-Octen-2-one	1040	1035	0.47 ± 0.02 ^b	0.56 ± 0.04 ^{bc}	0.19 ± 0.02 ^a	0.62 ± 0.06 ^c	Earthy: earthy, spicy, herbal, sweet, mushroom, hay, blueberry

Table 6. Cont.

No [#]	Compound ^A	RI Exp. ^B	RI Lit. ^C	% of the Total GC Peak Area ^D				Odour Type: Description ¹
				<i>L. fermentum</i>	<i>L. plantarum</i>	<i>L. paracasei</i>	Non-Fermented	
46	Isopentyl butanoate	1056	1052	tr	–	tr	–	Fruity: fruity, green, apricot, pear, sweet, banana
47	(<i>E</i>)-2-Octenal	1061	1054	–	0.09 ± 0.01 ^a	0.07 ± 0.01 ^a	–	Fatty: fresh, cucumber, green, leafy, herbal, banana, fatty, waxy
48	(<i>E,E</i>)-3,5-Octadien-2-one	1073	1072	0.35 ± 0.02 ^b	0.13 ± 0.01 ^a	0.21 ± 0.02 ^a	1.14 ± 0.11 ^c	Fruity: grassy, green, fruity
49	<i>p</i> -Cymene	1095	1091	–	–	–	0.24 ± 0.01	Phenolic: phenolic, spicy, styrene, clove, guaiacol
50	Linalool	1096	1095	0.42 ± 0.03 ^a	0.54 ± 0.04 ^a	0.42 ± 0.03 ^a	0.96 ± 0.11 ^b	Floral: citrusy, orange, floral, sweet, waxy, rose
51	Undecane	1100	1100	0.06 ± 0.01 ^a	0.19 ± 0.01 ^b	0.12 ± 0.01 ^b	0.18 ± 0.03 ^b	Alkane
52	Nonanal	1105	1104	0.10 ± 0.01 ^a	0.17 ± 0.02 ^b	0.08 ± 0.01 ^a	0.22 ± 0.03 ^b	Aldehydic: waxy, aldehydic, rose, fresh, orris, orange peel, fatty, citrus, cucumber, green
53	Camphor	1155	1146	–	0.06 ± 0.01	–	–	Camphoraceous: camphoraceous
54	Menthone	1161	1152	0.22 ± 0.03 ^a	0.30 ± 0.03 ^b	0.21 ± 0.03 ^a	0.42 ± 0.05 ^b	Minty: minty
55	Isomenthone	1172	1162	0.07 ± 0.01 ^a	0.09 ± 0.02 ^a	0.05 ± 0.01 ^a	0.15 ± 0.03 ^a	Mentholic: minty, cool, peppermint, sweet, refreshing
56	Estragole	1204	1195	–	tr	–	–	Anisic: sweet, phenolic, anise, spicy, green, herbal, minty
57	Carvone	1252	1243	0.21 ± 0.01 ^a	0.38 ± 0.01 ^b	0.26 ± 0.03 ^a	0.55 ± 0.06 ^c	Herbal: spicy, green, sweet, spearmint, minty, bread-like, caraway, dill
58	Tridecane	1300	1300	0.06 ± 0.01 ^a	0.07 ± 0.01 ^a	tr	0.07 ± 0.01 ^a	Alkane
59	Triacetin	1345	1347	–	–	–	0.05 ± 0.00	Fruity: mild, clean, tropical, fruity
60	2,4-Di- <i>tert</i> -butylphenol	1514	1519	tr	tr	tr	0.10 ± 0.00	Phenolic: leathery, woody, medicinal, dry, spicy, smoky
	Total identified, %			94.70	96.19	98.67	96.98	
	Total GC peak area, AU × 10 ⁶			2821.87 ± 163.71	2594.31 ± 173.19	2916.43 ± 137.89	1527.43 ± 143.42	

[#] Compounds are listed in order of their elution from a nonpolar BPX-5 capillary column.

^A An identification based on GC-TOF/MS spectra comparison with NIST, RepLib, MainLib and Adams mass spectra libraries and calculated RI with those reported in NIST, Adams, PubChem, and ChemSpider databases. ^B Retention indices calculated against C₇–C₃₀ *n*-alkanes on nonpolar BPX-5 column. ^C Retention indices on nonpolar column reported in the literature [23] or NIST (<https://webbook.nist.gov> (accessed on 15 July 2026)), PubChem (<https://pubchem.ncbi.nlm.nih.gov> (accessed on 15 July 2026)), and ChemSpider (<https://www.chemspider.com> (accessed on 15 July 2026)) databases. ^D Results are expressed as mean ± SD (n = 3). ^E tr—trace compounds (≤0.04%). ^F “–” indicates that the compound was not detected. ¹ Odour description from <https://www.thegoodscentscompany.com> (accessed on 17 September 2026). Different lowercase letters in the same row indicate significantly different values (*p* < 0.05).

A total of 60 volatile compounds were identified in the headspace of FHSPs and the non-fermented sample. The total GC peak area, representing the cumulative abundance of volatile compounds detected in the sample headspace, was significantly higher for the FHSPs (from 2594.31 ± 173.19 × 10⁶ AU to 2916.43 ± 137.89 × 10⁶ AU) compared to the non-fermented sample (1527.43 ± 143.42 × 10⁶ AU). The identified compounds accounted for 94.70–98.67% of the total GC peak area, indicating that the major part of the volatile profile was characterised (Table 6).

The volatile profiles were dominated by ethyl acetate, acetic acid and hexanal, although their relative proportions differed considerably among the samples. Together, these three compounds accounted for approximately 70–85% of the identified volatile compounds, depending on the LAB strain and treatment.

The non-fermented hemp seed flour was characterised primarily by hexanal, which represented 44.75 ± 1.34% of the total relative peak area (718.65 ± 71.08 × 10⁶ AU). After fermentation, the relative proportion of hexanal decreased to 20.67–27.51%, that was equivalent to 560.16 ± 55.28 × 10⁶–824.03 ± 85.85 × 10⁶ AU. The lowest value was determined in the *L. plantarum* FHSP, whereas the highest value among the fermented products was observed for *L. fermentum* FHSP (Table 6).

Hexanal is commonly associated with lipid oxidation and contributes green, grassy leafy, fatty, aldehydic, fruity and fresh plant-like aroma notes. Its lower relative proportion in the fermented samples suggests that fermentation substantially modified the aldehyde-dominated volatile profile of the hemp seed flour.

The most pronounced change following fermentation was observed in the relative proportion of acetic acid. Acetic acid accounted for only $9.61 \pm 0.51\%$ of the total volatile profile in the non-fermented sample ($159.98 \pm 13.78 \times 10^6$ AU), whereas its peak area increased from $1140.31 \pm 14.94 \times 10^6$ to $1506.40 \pm 52.37 \times 10^6$ AU reaching 38.25–50.50% in the fermented products. The highest proportion was observed in the *L. paracasei* FHSP, followed by the products fermented with *L. plantarum* and *L. fermentum*.

The accumulation of acetic acid is consistent with the acidification observed during fermentation and indicates active microbial metabolism. However, the extent of its formation was strain-dependent, demonstrating that the selected LAB strains followed different metabolic pathways or differed in their capacity to utilise compounds present in the hemp seed substrate.

In contrast to acetic acid, the relative proportion of ethyl acetate decreased after fermentation. Its percentage of the total GC peak area ($309.26 \pm 28.89 \times 10^6$ AU) was $19.28 \pm 1.05\%$ in the non-fermented sample and ranged from 4.74 to 14.70% in the fermented products. The highest abundance among the fermented products was observed for *L. fermentum* FHSP ($439.43 \pm 25.96 \times 10^6$ AU), while the lowest was found for *L. plantarum* FHSP ($128.58 \pm 11.22 \times 10^6$ AU).

Ethyl acetate is generally associated with fruity, ethereal and sweet aroma notes. Therefore, its reduction, together with the increase in acetic acid, suggests that fermentation shifted the aroma profile from a predominantly aldehydic and ester-like character towards a more acidic profile.

Some volatile compounds showed a particularly strain-dependent distribution. Acetoin accounted for $6.37 \pm 0.21\%$ ($172.15 \pm 6.12 \times 10^6$ AU) of the volatile profile in the *L. plantarum* FHSP, whereas only a small proportion was found in the *L. paracasei* FHSP and it was not detected in either the *L. fermentum* FHSP or the non-fermented sample. Acetoin is associated with buttery, sweet, milky, fat, creamy and dairy-like aroma notes and may therefore have contributed to the distinct aroma profile of the *L. plantarum* FHSP.

Other alcohols, aldehydes, ketones, esters, acids and terpenes were detected in smaller proportions. Several terpenes characteristic of hemp, including α -pinene, β -pinene, myrcene, limonene, linalool, menthone and carvone, remained detectable after processing and fermentation, although their relative proportions were low compared with those of acetic acid, hexanal and ethyl acetate.

Previous studies have indicated that aldehydes, particularly hexanal, are characteristic of non-fermented hemp materials and are associated predominantly with lipid oxidation. Nissen et al. (2021) similarly reported that aldehydes represented a major volatile compound group in non-fermented hemp bran and decreased during LAB fermentation [10].

LAB fermentation of hemp bran has also been associated with increased concentrations of volatile organic acids, including acetic, propionic and butyric acids. These changes have been attributed to the utilisation of fermentable substrates and the metabolic activity of LAB during fermentation [48].

Overall, the non-fermented control was characterised by fresh green-aldehydic and fruity-ethereal odour types dominated by hexanal and ethyl acetate. Fermentation substantially changed this volatile profile to acidic odour type by increasing the relative proportion of acetic acid and decreasing the relative proportions of hexanal and ethyl acetate. The magnitude of these changes depended on the bacterial strain, with *L. paracasei* producing

the most acid-dominated profile, while *L. plantarum* was distinguished by the formation of acetoin, which has a distinctive buttery odour type.

3.7. Evaluation of Fermented Dairy Drink Enriched with Fermented Hemp Seed Product

In the next stage of the study, fermented dairy drink was prepared and enriched with different concentrations of *L. paracasei* FHSP. This FHSP was selected because it retained the highest LAB count after freeze-drying and exhibited the highest total phenolic content and DPPH antioxidant activity, as well as high ABTS antioxidant activity. Five drink samples were prepared containing 0, 2, 4, 6, and 8 g of *L. paracasei* FHSP per 100 g of drink.

Fermented dairy drink samples were analysed for total phenolic content and antioxidant activity using the ABTS and DPPH assays. The results are presented in Table 7.

Table 7. Total phenolic content and antioxidant activity of fermented dairy drink enriched with the fermented hemp seed product (FHSP).

Content of FHSP g/100 Drink	Total Phenolic Content, mg GAE/g	ABTS Antioxidant Activity, mg TE/g	DPPH Antioxidant Activity, μ mol TE/g
0	0.88 $\pm$ 0.01 ^a	0.31 $\pm$ 0.01 ^a	0.075 $\pm$ 0.004 ^a
2	1.11 $\pm$ 0.03 ^b	0.32 $\pm$ 0.01 ^a	0.103 $\pm$ 0.002 ^b
4	1.29 $\pm$ 0.03 ^c	0.54 $\pm$ 0.02 ^b	0.129 $\pm$ 0.002 ^c
6	1.44 $\pm$ 0.02 ^d	0.66 $\pm$ 0.01 ^c	0.150 $\pm$ 0.002 ^d
8	1.72 $\pm$ 0.01 ^e	0.96 $\pm$ 0.02 ^d	0.168 $\pm$ 0.002 ^e

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$). GAE, gallic acid equivalents; TE, Trolox equivalents.

As expected, increasing the concentration of the FHSP in the drink resulted in a progressive increase in total phenolic content and antioxidant activity. The control sample without FHSP showed the lowest total phenolic content, whereas the highest value was observed in the drink containing 8 g/100 g FHSP. A similar concentration-dependent trend was obtained for both ABTS and DPPH radical-scavenging activity. In the ABTS assay, the sample without FHSP and the sample containing 2 g/100 g FHSP did not differ significantly, indicating that this concentration was insufficient to produce a measurable increase, whereas higher FHSP concentrations significantly enhanced antioxidant activity. In contrast, the DPPH assay revealed significant differences among all formulations, with radical-scavenging activity increasing gradually with increasing FHSP concentration. Overall, the highest total phenolic content and antioxidant activity were consistently observed in the drink containing 8 g/100 g FHSP.

Similar trends have been reported by Beşir et al. (2022) [49] who studied an ayran-type beverage enriched with a hemp seed -derived ingredient and observed that increasing the level of hemp seed additive increased both total phenolic content and antioxidant activity. The increase observed in the present study was primarily associated with the greater amount of phenolic and antioxidant compounds introduced with the FHSP.

This concentration-dependent response is also consistent with the properties of the *L. paracasei*-FHSP before its incorporation into the drink, as this FHSP showed the highest total phenolic content and DPPH antioxidant activity among the hemp seed products. Thus, the increase in these parameters in the enriched drinks reflects the growing contribution of the selected FHSP to the final product composition. The different response observed at the lowest addition level in the ABTS and DPPH assays further shows that the magnitude of the measured antioxidant effect depended on the analytical method used [49].

Overall, enrichment with the FHSP improved the functional properties of the fermented dairy drink in a concentration-dependent manner. The most pronounced effect was

observed at the addition level of 8 g/100 g, resulting in the highest total phenolic content and antioxidant activity in both ABTS and DPPH assays.

Changes in the pH of fermented dairy drink supplemented with the FHSP during 9 days of refrigerated storage are presented in Table 8.

Table 8. Changes in the pH of fermented dairy drink supplemented with the fermented hemp seed product (FHSP) during storage.

Content of FHSP g/100 Drink	Day 0	Day 4	Day 9
0	4.84 ± 0.02 ^c	4.03 ± 0.02 ^a	3.88 ± 0.01 ^c
2	4.84 ± 0.01 ^c	4.11 ± 0.02 ^c	3.86 ± 0.01 ^{bc}
4	4.82 ± 0.02 ^{bc}	4.08 ± 0.01 ^b	3.88 ± 0.01 ^c
6	4.81 ± 0.01 ^{ab}	4.05 ± 0.01 ^a	3.85 ± 0.01 ^b
8	4.79 ± 0.01 ^a	4.09 ± 0.01 ^{bc}	3.83 ± 0.01 ^a

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$).

A clear decrease in pH was observed in all fermented dairy drink samples during storage, indicating continued acidification. The most pronounced change occurred during the first 4 days of storage, while a further decrease was observed up to day 9. At the end of storage, the lowest pH value was recorded in the fermented dairy drink containing 8 g/100 g FHSP (3.83 ± 0.01), whereas the sample without FHSP and the sample containing 4 g/100 g showed the highest final pH values (3.88 ± 0.01). Although significant differences were observed among some formulations at individual storage times, no consistent concentration-dependent relationship between the amount of FHSP and pH was evident. Therefore, the level of FHSP appeared to have a relatively limited effect on the overall acidification pattern compared with the effect of storage time.

Similar trends were reported by Kanabus et al. (2024) [50], who found that the pH of fermented milk beverages containing hemp seed—derived ingredients was approximately 4.55–4.65 after fermentation and decreased to approximately 4.40–4.55 after 24 h, followed by a further decline during storage. The authors also reported that the extent of acidification depended on the amount and type of hemp seed ingredient used, with some formulations showing slower acidification and higher pH values. Compared with the values reported by Kanabus et al. [50], the fermented dairy drinks containing FHSP in the present study reached lower pH values during storage, which may be related to differences in product formulation, starter culture and storage conditions. The progressive decrease in pH during refrigerated storage may be attributed to post-acidification caused by continued metabolic activity of the fermented dairy drink starter cultures and the associated accumulation of organic acids [50].

Syneresis in drinks containing different concentrations of FHSP was evaluated after 24 and 48 h. The results are presented in Table 9.

Table 9. Syneresis of fermented dairy drink enriched with fermented hemp seed product (FHSP).

Content of FHSP g/100 Drink	After 24 h, %	After 48 h, %
0	0.00 ^a	0.67 ± 0.29 ^a
2	1.17 ± 0.29 ^b	1.33 ± 0.29 ^b
4	2.17 ± 0.29 ^c	2.83 ± 0.29 ^c
6	3.83 ± 0.29 ^d	5.50 ± 0.50 ^d
8	5.33 ± 0.29 ^e	10.33 ± 0.58 ^e

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$).

Syneresis increased consistently as FHSP concentration increased. No whey separation was observed in the drink without FHSP after 24 h, whereas the sample containing 8 g/100 g FHSP exhibited the highest syneresis. The same concentration-dependent pattern was observed after 48 h, with syneresis ranging from 0.67% in the drink without FHSP to 10.33% in the drink containing 8 g/100 g FHSP.

Syneresis also increased between 24 and 48 h in all FHSP-enriched drink samples. The increase was particularly pronounced in the formulation containing 8 g/100 g FHSP, in which the proportion of separated whey almost doubled. These results indicate that both the concentration of the FHSP and the duration of storage contributed to the physical destabilisation of the fermented dairy drink.

Beşir et al. (2022) [49] reported a similar trend in an ayran-type beverage, in which increasing the concentration of a hemp seed-derived ingredient increased syneresis during storage. The addition of hemp seed product increased the total solids content; therefore, the greater whey separation cannot be attributed to a reduction in total solids. Similar increases in syneresis have also been reported in fermented dairy products enriched with plant-derived ingredients, where the effect was related to disruption or contraction of the casein network and to the limited water-holding capacity of the added material; however, the opposite effect has also been observed for fibre-rich ingredients with greater water-retention capacity, indicating that syneresis is strongly influenced by the structural and hydration properties of the incorporated plant material [51,52].

The colour characteristics of fermented dairy drink supplemented with FHSP are presented in Table 10.

Table 10. Colour parameters of fermented dairy drink supplemented with fermented hemp seed product (FHSP).

Content of FHSP g/100 Drink	L^*	a^*	b^*
0 g	90.41 ± 0.10 ^e	−3.47 ± 0.10 ^a	8.50 ± 0.01 ^a
2 g	77.70 ± 0.47 ^d	−0.48 ± 0.01 ^b	10.91 ± 0.05 ^b
4 g	73.36 ± 0.35 ^c	0.06 ± 0.01 ^d	12.31 ± 0.06 ^c
6 g	69.59 ± 0.22 ^b	0.19 ± 0.01 ^d	12.93 ± 0.08 ^d
8 g	66.91 ± 0.63 ^a	0.24 ± 0.01 ^d	13.30 ± 0.01 ^e

Within the same column, mean values marked with different lowercase letters differ significantly ($p < 0.05$). L^* represents lightness, ranging from 0 (black) to 100 (white); positive and negative a^* values indicate red and green tones, respectively, whereas positive and negative b^* values indicate yellow and blue tones, respectively.

The results showed that increasing the amount of FHSP in fermented dairy drink significantly affected all CIE Lab^* colour parameters. The L^* value decreased from 90.41 in the control to 66.91 in the sample containing 8 g/100 g FHSP, indicating progressive darkening of the fermented dairy drink. The a^* values increased from −3.47 to 0.24, showing a shift from a greenish tone towards a more neutral and slightly reddish colour. At the same time, the b^* value increased from 8.50 to 13.30, indicating a greater contribution of yellow tones. Overall, increasing the concentration of the FHSP resulted in a darker fermented dairy drink with a more brownish-yellow appearance. A comparable shift in colour characteristics has been reported for commercial hemp seed-based yoghurt, which showed lower L^* and less negative a^* values than dairy yoghurt, although its b^* value was lower, indicating that the colour response may depend on the composition and form of the hemp seed ingredient used [53].

3.8. Effect of Fermented Hemp Seed Product on the Sensory Properties of Fermented Dairy Drink

Sensory evaluation was performed to assess the acceptability of selected attributes in fermented dairy drink enriched with FHSP. The acceptability scores are presented in Figure 2.

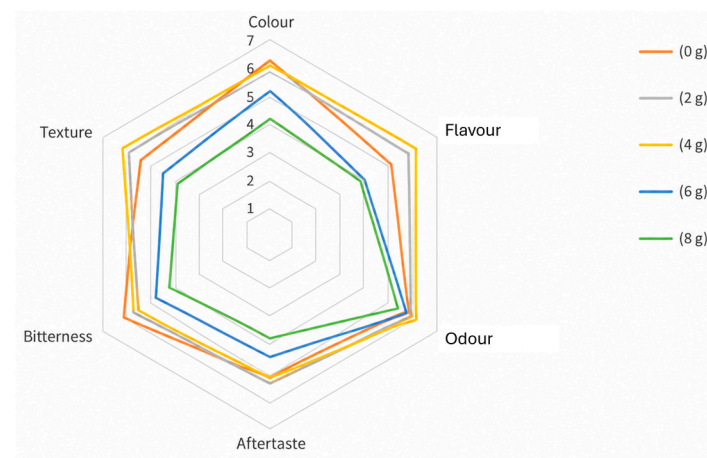


Figure 2. Sensory acceptability profile of fermented dairy drink enriched with different concentrations of fermented hemp seed product (FHSP).

The formulation containing 4 g/100 g of FHSP showed the most balanced sensory acceptability. Colour acceptability was highest for the drink containing 2 g/100 g FHSP, which received a score of 6.5, followed closely by the sample containing 4 g/100 g FHSP, with a score of 6.3.

The drink containing 4 g/100 g FHSP received the highest flavour acceptability score of 6.0. Odour acceptability was similar among the samples containing 2, 4 and 6 g/100 FHSP, with scores ranging from 5.4 to 5.6.

The sample containing 2 g/100 g FHSP had the most acceptable aftertaste, with a score of 5.1. The control drink without FHSP received the highest acceptability score for bitterness (5.4), indicating that bitterness associated with higher FHSP concentrations reduced acceptance of this attribute. The highest texture acceptability was recorded in the drink containing 4 g/100 g FHSP, with a score of 6.3.

The assessors generally evaluated the enriched fermented dairy drink positively. Their comments indicated that the characteristic earthy flavour was perceived as distinctive and was sometimes associated with products such as matcha. Most comments concerning flavour were favourable, particularly for the formulation containing 4 g/100 g FHSP.

The addition of 2 g/100 g FHSP was considered insufficient by some assessors because the characteristic hemp flavour was weak. In contrast, the sample containing 8 g/100 g FHSP was perceived as excessively intense by some assessors, although others preferred its more pronounced flavour. Differences in aroma acceptability among the samples containing 2–8 g/100 g FHSP were considered relatively small.

The fermented dairy drink became progressively darker as the FHSP concentration increased, in agreement with the instrumental colour measurements. The texture of the formulations was generally considered appropriate for a dairy drink. However, aftertaste acceptability tended to decrease at higher FHSP concentrations, most likely because of the increasingly intense bitterness and persistent hemp flavour. A similar sensory response has been reported for other hemp seed-enriched fermented products: Kanabus et al. [50] found that fermented dairy drinks containing dried hemp seeds were characterised by more pronounced grassy and bitter notes, while the highest hemp seed addition received the lowest overall sensory quality score.

Overall, the formulation containing 4 g/100 g FHSP provided the most favourable balance between sensory acceptability of hemp flavour, colour, and texture. Lower FHSP addition levels produced a less distinctive product, whereas the highest concentration resulted in an excessively intense flavour and aftertaste for some assessors.

4. Conclusions

Hemp seed flour, characterised by a high content of dietary fibre and protein, was a suitable substrate for lactic acid fermentation. All three tested lactic acid bacteria (LAB) strains grew successfully in the hemp seed flour substrate, with the most pronounced increase in viable counts and acidification occurring during the first 24–48 h. Fermentation with *Lactocaseibacillus paracasei* resulted in the highest viable LAB count, the lowest final pH, and the highest bacterial survival after freeze-drying. Fermentation reduced the reducing sugar content and altered protein solubility.

Fermentation affected the technological and functional properties of the hemp seed product in a strain-dependent manner. Fermentation with *L. paracasei* and *Lactiplantibacillus plantarum* resulted in the highest water-retention capacity, whereas fermentation with *Limosilactobacillus fermentum* produced the highest oil-retention capacity. Fermentation with *L. fermentum* resulted in the highest sodium cholate-binding capacity and, together with *L. plantarum*, the highest cholesterol-binding capacity at pH 2. At pH 7, the highest cholesterol-binding capacity was observed in the product fermented with *L. plantarum*. Fermentation with *L. paracasei* resulted in the highest free total phenolic content and DPPH antioxidant activity, whereas products fermented with *L. paracasei* and *L. plantarum* showed the highest ABTS antioxidant activity. Fermentation also modified the volatile profile by increasing the relative proportion of acetic acid and reducing that of hexanal compared with the non-fermented hemp seed flour.

Based on high LAB viability, phenolic content, and antioxidant activity, the hemp seed product fermented with *L. paracasei* was selected for incorporation into a fermented dairy drink. Increasing its concentration enhanced the total phenolic content and antioxidant activity of the fermented dairy drink, but also caused a darker colour and greater syneresis. The addition level had no consistent effect on pH or instrumental texture, and all formulations remained drinkable after 9 days of storage.

Sensory evaluation showed that the hemp seed-derived sensory attributes became more pronounced as the addition level increased. Although the drink containing 8 g/100 g of fermented hemp seed product had the highest phenolic content and antioxidant activity, it also exhibited the most intense hemp-related sensory characteristics and the greatest syneresis. The formulation containing 4 g/100 g of fermented hemp seed product achieved the highest acceptability of sensory attributes and provided the most favourable balance between improved functional properties, physical stability, and sensory quality. Overall, the results demonstrate the potential of *L. paracasei*-fermented hemp seed product for application in fermented dairy drinks, allowing enhancement of phenolic content and antioxidant activity while maintaining acceptable sensory and physical properties at an appropriate addition level.

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Abbreviations

The following abbreviations are used in this manuscript:

FHSP	Fermented hemp seed product
LAB	Lactic acid bacteria
ORC	oil retention capacity
WSI	water solubility index
WRC	water retention capacity
WSC	water swelling capacity

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