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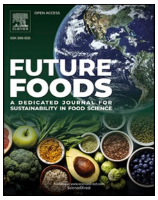

Inhibition of *Bacillus cereus* growth by fermentation of sunflower seed oil press cake for cheese alternatives

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ABSTRACT

The reformulation of dairy products with plant ingredients poses new food safety challenges, particularly microbiological risks. Due to their proximity to the soil, plant ingredients are susceptible to contamination with spore-forming bacteria such as *Bacillus spp.*, with toxin-producing *Bacillus cereus* posing a health risk. Here, we examined the contamination of a plant-based raw material used for a cheese alternative with *B. cereus* at various stages of the production process and how it can be inhibited by fermentation. The raw material consisted of a paste containing 40% shelled sunflower seed oil press cake. The concentration of *B. cereus* in the untreated paste was below the quantification limit of 50 CFU/g vegetative cells or spores. However, after heat treatment and incubation at room temperature, the vegetative cell counts increased to potentially toxin-forming concentrations of $>10^5$ CFU/g within 22 h if no simultaneous fermentation with lactic acid bacteria took place. Simultaneous mesophilic fermentation to a final pH of 4.9 reproducibly inhibited the growth of *B. cereus* but not at a final pH of 5.7. During the subsequent one- and two-month storage at 4 °C, the *B. cereus* numbers in all fermented masses dropped sharply to <200 CFU/g, despite previous potentially toxigenic *B. cereus* levels of $>10^5$ CFU/g. The decrease in vegetative cells was not associated with a corresponding increase in spores. Based on these findings, we recommend monitoring *B. cereus* in new dairy alternatives both after heat treatment and immediately following the end of fermentation to support the development of a safe production process.

1. Introduction

The increase in plant-based diets, motivated by potential health benefits, environmental concerns, and ethical reasons, has significantly expanded the market for plant-based products and innovative alternatives to dairy products. The reformulation of traditional animal-based products with plant ingredients introduces new food safety challenges, particularly microbiological risks related to changes in the microbial profile, notably spore-forming bacteria. Spore-forming *Bacillus* species are common contaminants in tofu and other plant-based products. In particular, those in the *Bacillus cereus* group can pose health risks due to their ability to produce toxins (Ananchaipattana et al., 2012; Rouzeau-Szynalski et al., 2020; Kyrylenko et al., 2023). In a recent study of vegan alternative products in England, *B. cereus* was detected at borderline levels in 2 out of 246 vegan cheese alternatives (Willis et al., 2024). In 2022, the Swedish food company Oatly recalled a drink in Finland and Estonia due to *B. cereus* contaminations (Whitworth, 2022).

The *B. cereus* group comprises several closely related species, including *B. cereus sensu stricto* (commonly known as *B. cereus*), *Bacillus anthracis, B. thuringiensis, B. weihenstephanensis, B. mycoides, B. pseudomycoides, B. cytotoxicus*, and the recently described *B. toyonensis* (EFSA_BIOHAZ_Panel, 2016). In routine microbiological analyses, isolates from this group are collectively referred to as presumptive *B. cereus*; thus, we use the term *B. cereus* for the whole group in this study. These species share up to 99% similarity in their 16S rRNA sequences and are mainly differentiated by phenotypic and genotypic characteristics, including their toxin gene profiles (EFSA_BIOHAZ_Panel, 2005; Ehling-Schulz et al., 2019). Nearly all *B. cereus* strains carry the genes to produce diarrheal enterotoxins in the small intestine, while only a minority could potentially produce emetic heat-resistant toxins (e.g., cereulide) directly in the food matrix (Rouzeau-Szynalski et al., 2020; EFSA_BIOHAZ_Panel, 2016).

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According to scientific reports and regulatory guidelines, *B. cereus* contamination at levels $\geq 10^5$ CFU/g is strongly linked to toxin production and a higher risk of foodborne illness outbreaks (BfR, Aktualisierte Stellungnahme Nr. 048, 2020; EFSA_BIOHAZ_Panel, 2016). Some outbreak cases have also been reported at lower levels, between 10^3 and 10^4 CFU/g, although these are less common. However, legislation regarding *B. cereus* contamination is only available for dried infant formula and dried dietary food for special medical purposes intended for infants before six months of age (EC No 1441/2007: <500 CFU/g *B. cereus*) (European Commission, 2007). By contrast, contamination levels $\leq 10^3$ CFU/g are generally considered safe from a hygiene and food safety perspective, particularly in ready-to-eat foods (BfR, Aktualisierte Stellungnahme Nr. 048, 2020; EFSA_BIOHAZ_Panel, 2016). The risk depends on multiple factors, including the strain's toxin gene profile, processing conditions, and post-processing storage that may result in spore germination, bacterial growth, and toxin formation, particularly cereulide. Given strain variability in toxin production and the high heat resistance of cereulide, the health risk must be assessed on a case-by-case basis, especially for foods stored at temperatures of 4–48 °C and a pH range of 4.9–9.3 permissive to *B. cereus* growth (BfR, Aktualisierte Stellungnahme Nr. 048, 2020; Kharel et al., 2024b).

Soil is the main source of *Bacillus* species and can contain up to 10^5 spores of *B. cereus* per gram, making plant ingredients naturally prone to contamination (Christiansson et al., 1999; Dufrenne et al., 1995). A 2023 study examined microbial contamination in 88 plant-based ingredients commonly used in plant-based dairy and meat alternatives. The ingredients included legumes (pea, faba bean, chickpea, mung bean), cereals and pseudocereals (oat, rice, amaranth, quinoa), and drupes (coconut, almond, cashew). *Bacillus spp.* made up 60% of all microbial isolates in the samples. After heat treatment, 87% of aerobic mesophilic spore counts and 40% of thermophilic spore counts belonged to *Bacillus* representatives (Kyrylenko et al., 2023).

Sunflower press cake, a by-product of sunflower oil pressing used in this study, may also serve as a reservoir for heat-resistant bacterial spores (Mangieri et al., 2023; Rohm et al., 2025) as has been reported for various plant-based ingredients (Karamcheti et al., 2025; Kyrylenko et al., 2023). Although it has mainly been used as animal feed, its potential as a valuable ingredient in human nutrition is currently being investigated (Hadidi et al., 2023). The valorisation of sunflower press cake for the manufacture of cheese-like plant-based alternatives aligns with sustainability goals and circular food system approaches, as it promotes the upcycling of agro-industrial side streams into value-added foods. However, the use of such plant-based side streams may also introduce new food safety challenges, particularly due to the possible presence of heat-resistant spores. In this study, we used oil press cake made from hulled sunflower seeds, as the absence of hulls makes it more suitable for human consumption by reducing contamination risk and enhancing nutritional value (Lužaić et al., 2025; Robertson and Thomas, 1976). Sunflower press cake contains up to 50% protein and has a complete amino acid profile, although it is low in lysine. It is rich in glutamic acid and methionine, a sulfur-containing amino acid that may contribute to the development of ripening aromas in cheese alternatives (Petraru et al., 2021). However, its application is limited by the presence of antioxidant phenolic compounds, particularly chlorogenic acid, which can reach concentrations of approximately 2–4 g per 100 g of seeds (Jia et al., 2022). When oxidised, these compounds can cause undesirable brown or green discoloration (Hadidi et al., 2023; Wildermuth et al., 2016). Fermentation with specific lactic acid bacteria has shown the potential to degrade or convert phenolic compounds, thereby reducing their negative effects (Fritsch et al., 2016; Gaur and Ganzle, 2023; Michalska-Ciechanowska et al., 2024).

The potential contamination of raw material with heat-resistant bacterial spores is a challenge to produce plant-based alternatives and has an influence on the design of the entire manufacturing process. Similar to milk-based products, the production of plant-based dairy alternatives often involves heat treatments, such as pasteurisation, to eliminate foodborne pathogens and spoilage microorganisms. Common heat treatments, such as boiling or pasteurisation, effectively kill vegetative cells of the *B. cereus* group but do not fully eliminate their spores, which can survive and pose a risk during further processing and storage. The heat resistance of *B. cereus* spores is strongly dependent on the food matrix in which the spores are located. A sterilisation treatment at 121 °C for 3 min is sufficient to inactivate all spores (BfR, Aktualisierte Stellungnahme Nr. 048, 2020; EFSA_BIOHAZ_Panel, 2016). By contrast, heat treatments below sterilisation may not only fail to eliminate spores but can also trigger their germination and support the growth of vegetative cells, particularly when competing microbial flora have been reduced (Luu et al., 2015; Wen et al., 2022). Typically, *B. cereus* poisoning occurs when foods are inadequately cooled for several hours after cooking, as elevated temperatures promote growth of vegetative cells after germination (Kameník et al., 2025; Tallent et al., 1998).

This risk is particularly relevant in the production of fermented plant-based dairy alternatives. Even with a low initial spore load, the combination of heat treatment and warm conditions during fermentation can promote the growth of *B. cereus* (Daelman et al., 2013; Kameník et al., 2025). Although fermentation temperatures may support the growth of germinated spores, the resulting acidification and the presence of competing lactic acid bacteria can inhibit the growth of *B. cereus* (Nout et al., 1987). The effect of acidification and fermentation on the suppression of *B. cereus* growth has been investigated for tempeh made from different beans (Ashenafi and Busse, 1991; Nout et al., 1987), rice noodle products (Yang et al., 2008), and cow's milk (Røssland et al., 2003). In tempeh production, however, mixed fermentation of soaked sterile soybeans with *Lactobacillaceae* and *Rhizopus oligosporus* did not inhibit the growth of *B. cereus* strains, which were inoculated at a low concentration of 30 CFU/g of beans (Nout et al., 1987). Interestingly, the growth of the same *B. cereus* strains was inhibited during soaking of raw soybeans when spontaneous lactic acid fermentation lowered the pH to 4.9 after 24 h at 25 °C. By contrast, when the final pH remained between 5.7 and 6.0, *B. cereus* growth was not suppressed, despite similar final levels of lactic acid bacteria at 10^9 CFU/g (Nout et al., 1987). Røssland et al. (2003) found that the suppression of *B. cereus* in skim milk is closely linked to the speed of pH reduction during early fermentation. Lactococcus strains that reduced the pH to 5.3 or lower within 7 h were more effective at inhibiting *B. cereus* than some *Lactobacillaceae* strains, which required up to 24 h to reach a pH below 5.0 (Røssland et al., 2003). Together, these findings indicate that early and rapid acidification is critical for inhibiting the growth of *B. cereus* during lactic acid fermentation.

To support the valorisation of sunflower press cake as a sustainable, upcycled ingredient for plant-based cheese alternatives while addressing relevant food safety concerns, we investigated the inhibition of *B. cereus* growth in dehulled sunflower seed oil press cake. The primary objective was to assess the potential of traditional lactic acid fermentation, using a *Lactococcus lactis spp.*-based culture, to effectively inhibit the growth of *B. cereus*. Our study monitored *B. cereus* contamination throughout all stages of processing, from the initial raw material to the storage of the processed press cake.

2. Materials and methods

2.1. Ingredients and microorganisms

Sunflower oil press cake was obtained from Ölmühle Florin AG (Muttenz, Switzerland) from trials conducted in July 2024 using a single-stage mechanical cold-pressing process. The raw material consisted of hulled organic sunflower seeds. The resulting press cake was a dry, coarse-grained powder with 6% (w/w) residual moisture. It contained approximately 35% (w/w) fat, 35% (w/w) protein, about 10–15% (w/w) fibre, 6% (w/w) water-soluble carbohydrates, and, according to the literature, about 2–4% antioxidant phenolic compounds, particularly chlorogenic acid (Jia et al., 2022). The 6% (w/w) soluble carbohydrates comprised predominantly sucrose (4% w/w), with the remaining fractions presumably being oligosaccharides, such as raffinose and stachyose (Kuo et al., 1988).

For fermentation, a vegan-based version of the commercial RSW 901 starter culture by Liebefeld Kulturen AG, Switzerland, named RSW 901 vegan, was used with approximately 7.7 × 10^8 CFU/ml. The RSW 901 starter includes *Lactococcus lactis* subsp. *lactis, L. cremoris*, and *L. lactis* subsp. *lactis* biovar *diacetylactis*.

2.2. *Sunflower oil press cake processing protocol*

2.2.1. *Preparation*

Sunflower press cake was processed using a heated food processor equipped with a stirrer (Artisan Cook Processor, KitchenAid Europa Inc., Strombeek-Bever, Belgien). Prior to the experiment, the mixing bowl was thoroughly cleaned with dishwashing detergent, disinfected using Halades Alco containing 631 mg/g 2-propanol (Halag, Aadorf, Switzerland), and subsequently rinsed with boiling tap water. This procedure established standard hygienic conditions as used in typical food processing environments, although it did not achieve sterility.

2.2.2. *Processing*

Oil press cake from hulled organic sunflower seeds (Florin AG) was used as the main ingredient (40% w/w). Depending on the experimental condition (Fig. 1, formulation A–D), either 1.1% (w/w) glucose monohydrate (Sigma-Aldrich, Merck, Darmstadt, Germany) and/or 1% (w/w) NaCl (JuraSel #1473, Schweizer Salinen, Pratteln, Switzerland) were added. These ingredients were added to tap water, which had been preheated to 80 °C in the above-described food processor. The mixture was stirred, and the temperature was held at 77–83 °C for 3 min and thereof at least 80 °C for 1 min before transferring the mixture to a disinfected stainless-steel container for cooling. The mass was subsequently cooled to 40 °C within 10 min using a cold-water bath while stirring continuously.

2.2.3. *Inoculation of culture*

The mass was divided into two disinfected containers. One batch was inoculated with 1% (v/w) RSW 901 vegan (Liebefeld Kulturen AG, Bern, Switzerland), resulting in an initial microbial concentration of approximately 7.7 × 10^6 CFU/g. The second batch remained uninoculated for incubation only.

2.2.4. *Incubation*

Aliquots of the prepared mixtures (30 g each) were transferred into sterile 50 mL skirted conical centrifuge tubes, sealed, and incubated in an incubator at 25 °C for varying time periods, as shown in Fig. 1. After incubation, the samples were cooled in a refrigerator to 4 °C for microbiological analysis, which was conducted within 3 days of storage at this temperature.

2.2.5. *Storage*

Samples collected after 22 h of fermentation or incubation were

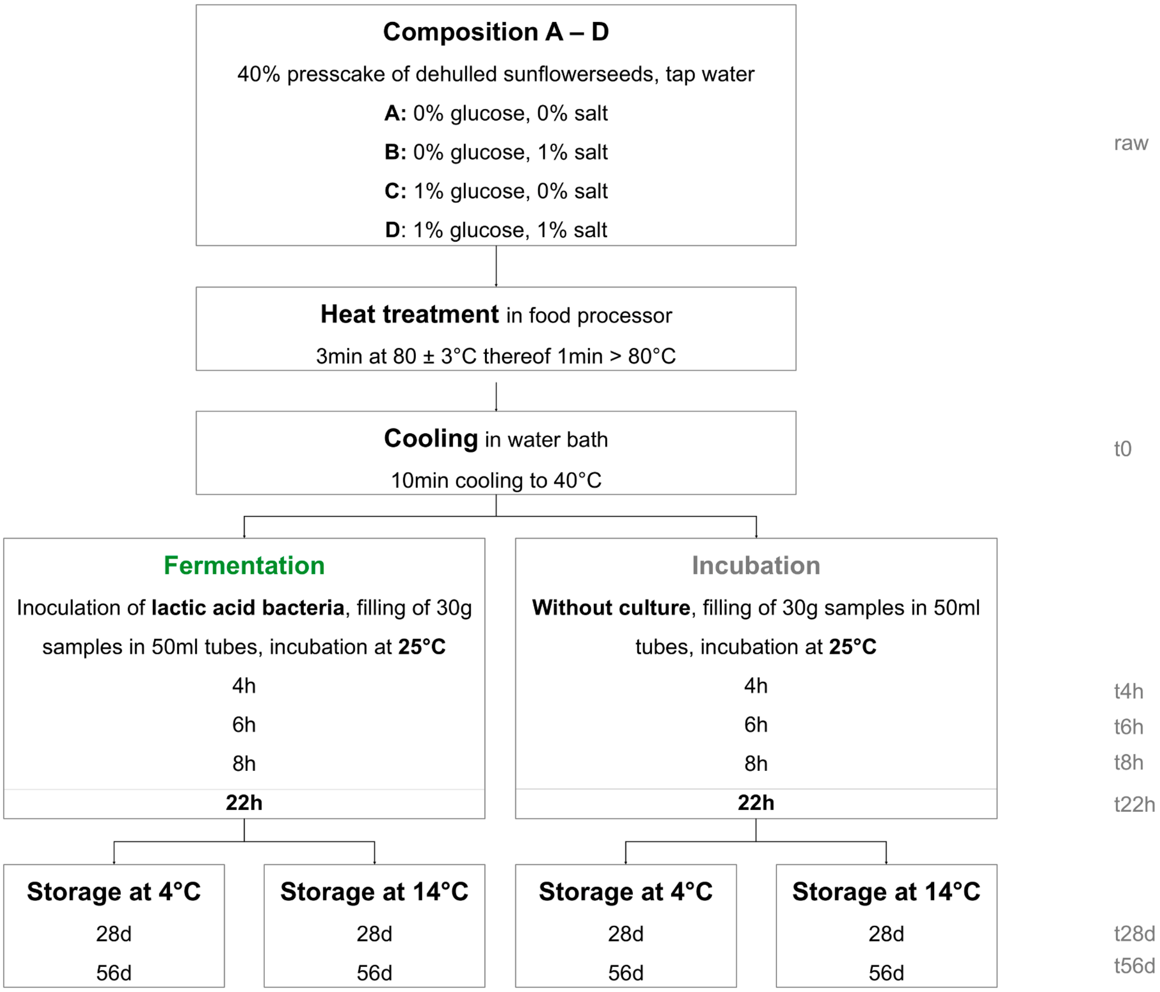

Fig. 1. Flow chart of all processing steps of sunflower press cake from raw material to storage. Contamination with *B. cereus* was analysed at all levels marked with the sample name.

stored at either 4 °C or 14 °C for extended periods (28 or 56 days). These long-term storage samples were analysed for product stability and quality at the end of their respective storage durations.

The experiments were performed in two biological replicates in consecutive weeks.

2.3. Microbiological analysis

2.3.1. Enumeration of B. cereus

The cells of the *B. cereus* group were counted using standard procedures. Briefly, 10 g of each sample was diluted with 90 mL of peptone saline diluent and homogenised in TEMPO bags for 1 min using a stomacher (AESAP1064, BioMerieux, France). *B. cereus* were counted using the TEMPO BC (*B. cereus*) kit (BioMerieux, France), which is a miniaturized and automated MPN system. It has been validated after ISO 16,140–2:2016 against the standard procedure ISO 7932:2004 to count presumptive *Bacillus cereus* (third party validation report available from the manufacturer).

Analysis was done after the manufacturer's instructions. In short, the primary dilution of the sample was used to prepare a 1:200 dilution with the TEMPO BC medium to gain a *B. cereus* cell count between 50 and 2.45×10^5 CFU/g. TEMPO cards were incubated for 22–27 h at 30 ± 1 °C.

In parallel, ready-to-use mannitol egg yolk polymyxin (MYP) agar plates (Oxoid, Switzerland) were used after the standard protocol of ISO 7932 (International Organization for Standardization, 2004), as a disadvantage of TEMPO is the lacking possibility of gaining isolates. Using the same primary dilutions of samples as for TEMPO, MYP plates were inoculated with 0.1 mL or dilutions here of in NaCl-Peptonwater), and incubated for 18–24 h at 30 ± 1 °C. Presumptive *Bacillus cereus* colonies were identified by their characteristic pink colouration and surrounding lecithinase-induced precipitate according to ISO 7932. Individual colonies were confirmed by MALDI-TOF MS analysis (Doellinger et al., 2020). This confirmation step is not part of the standard procedures after ISO 7932.

The limit of detection was 100 CFU/g and the limit of quantification was 1000 CFU/g using colony counts on MYP agar.

Enumeration of spores was done with the same procedures as described above after heating the homogenised samples to 80 °C for 10 min.

2.3.2. Inhibitory activity of RSW 901 culture supernatant against Bacillus subtilis and Bacillus cereus

The inhibitory activity of substances such as bacteriocins in the RSW 901 vegan culture supernatant was tested against *B. subtilis* and *B. cereus.* The bacterial culture RSW 901 vegan was cultivated in 50 mL of liquid medium M17-X (M17 broth, Millipore, with 5 g/L glucose, Merck) at 30 °C for 24 h. Following incubation, the culture was transferred into 50 mL tubes and centrifuged at 3010 g for 25 min. The supernatant was carefully transferred to a new sterile 50 mL tube, and the pellet was discarded. The supernatant was then filtered sequentially—first through a 0.45 μm cellulose-acetate filter (Sartorius Minsart, Goettingen, Germany), followed by a second filtration using a 0.22 μm cellulose-acetate filter (Sartorius Minsart, Goettingen, Germany)—to ensure the complete removal of bacterial cells. The filtered supernatant was stored at −40 °C until further use. For the agar well diffusion assay, *B. subtilis* and *B. cereus* were grown under shaking conditions at 30 °C overnight and subsequently diluted with physiological peptone solution (0.8% NaCl (Merck, Darmstadt, Germany) and 1 g/L pancreatic digested casein peptone (Merck, Darmstadt, Germany)) to an OD_{600} of 1.0. A sterile cotton swab was used to inoculate a uniform bacterial lawn onto SC agar plates. Using a sterile metal ring (5 mm diameter), up to six wells were punched into each agar plate. Each well was filled with 50 μL of the prepared supernatant. The plates were incubated at 30 °C overnight, and zones of inhibition were assessed the following day.

2.3.3. Lactic acid bacteria

For the re-isolation of lactic acid bacteria (RSW 901 vegan), M17-X plates (M17 Agar, Millipore with 5 g/L Glucose, Merck) were used. The samples were diluted serially in physiological peptone solution and spread onto the plates. The highest dilution (10^7) was plated three times. Plates were incubated at 30 °C for up to 48 h in anaerobic jars (bioMérieux, Switzerland) using GENbox anaer (bioMéieux Switzerland) for anaerobic conditions.

2.4. Biochemical characterisation

2.4.1. Glucose, sucrose, lactic acid, acetic acid, and OPA

Sugars, acetic acid, and primary amines of free amino acids and small peptides (o-phthaldialdehyde method, OPA) were analysed on a Gallery UV/VIS automate (Thermo, Switzerland) by applying enzymatic kits (glucose, sucrose, acetic acid: R-Biopharm, Pfungstadt, Germany; lactic acid: Thermo, Switzerland) or an Agroscope in-house method (OPA). Briefly, after grinding the samples using a knife mill (Grindomix, Retsch, Switzerland), 5 g of the sample was weighed in 50 mL tubes. For the analysis of sucrose, glucose, lactate, and acetate, 10 mL of pure H_2O were added to the samples, and they were homogenised using an OMNI PREP homogeniser (Labforce, Switzerland) at 15,000 rpm for 60 s. After adding another 10 mL of H_2O and repeated homogenisation, the samples were clarified by Carrez solutions: 1.25 mL Carrez I (potassium hexacyanoferrate-(II) $[K_4Fe(CN)_6 \cdot 3H_2O]$, 170 mmol/L), 1.25 mL Carrez II (zinc sulfate $[ZnSO_4 \cdot 7H_2O]$, 500 mmol/L), 1.25 mL of sodium hydroxide (NaOH, 0.4 mol/L). The samples were vigorously mixed between each addition. The sample volume was completed with pure H_2O to a final volume of 50 mL. Thereafter, samples were centrifuged (3000×g, 10 min), the supernatant was filtered through a paper filter and used for analysis, using the above-mentioned enzyme kits.

The OPA value (mmol/kg) quantifies the concentration of free primary amino groups (mainly from free amino acids and small peptides) in a sample, expressed as mmol per kg, where low values indicate little protein hydrolysis and higher values reflect increasing degrees of proteolysis. To determine the OPA value, 8 mL of pure H_2O was added to the samples, and they were homogenised at 13,000 rpm for 40 s. After repeating the H_2O addition and homogenisation step, the samples were precipitated using perchloric acid (2.5 mL, 5 mol/L). After mixing and adding another 5 mL of H_2O, samples were passed through a paper filter and used for analysis as previously described (Sousa et al., 2023). Briefly, the clear supernatant was diluted (if needed) with perchloric acid (0.5 mol/L) and 8 μL of sample were mixed with a reagent solution (0.05 mol/L borate, 10 g/L laurylsulfate, 0.8 g/L OPA, 5 g/L Na-MES, and 5 g/L Triton X-100) at a ratio of 1:30, incubated at room temperature in the dark for 40 min, and measured at 340 nm. The results were calculated based on a glutamic acid standard curve.

2.4.2. pH measurement

The pH profiles during fermentation were measured in two parallel samples under identical conditions in a water bath and recorded as technical replicates using a data logger (Almemo 710; AHLBORN, Holzkirchen, Germany). To account for probe-specific baseline deviations, the recorded pH values were corrected by applying a fixed offset to match the respective offline-measured initial pH values (6.05 for samples with NaCl and 6.35 for samples without NaCl).

2.4.3. Water activity and dry matter

Water activity was analysed according to ISO 18,787:2017 using a system from Novasina (Lachen, Switzerland) (International Organization for Standardization, 2017). For the assessment of dry matter content, the samples were dried for 3 h at 105 °C in a prepASH 340 Analysator (Precisa Gravimetrics AG, Dietikon, Switzerland) and analysed according to ISO 5984:2002 (International Organization for Standardization, 2022).

2.5. Data and statistics

If not stated otherwise, experiments were performed with two independent biological replicates conducted in separate weeks. Data are presented as mean ± standard deviation. Graphs were created using GraphPad Prism version 10.5.0 (GraphPad Software, USA). To visualise *B. cereus* results below the quantification limit of 50 CFU/g, a value of 25 CFU/g was used. Raw data are stored in the Zenodo open research data repository (https://doi.org/10.5281/zenodo.17404495).

3. Results

To support the safe production of a plant-based cheese alternative made from sunflower seed oil press cake, we systematically investigated *B. cereus* contamination across all stages of processing, from raw press cake to shelf life of processed press cake (Fig. 1). The main objective was to determine whether traditional lactic acid fermentation could effectively suppress *B. cereus* growth. For this purpose, four formulations (A–D) were tested (Table 1).

All paste formulations (A–D) contained 40% (w/w) shelled sunflower seed oil press cake in tap water, but formulations C and D also included 1% (w/w) glucose. Glucose can be utilised by the *L. lactis spp.*-based acidification culture RSW 901 vegan. Sunflower seeds naturally contain mainly sucrose, which was not metabolized by the acidification culture (Supplementary Figure 1). Furthermore, formulations B and D included 1% (w/w) NaCl. The a_w-value was slightly lower for the NaCl-containing batches (0.982–0.984) than for those without NaCl (0.993–0.998). The dry matter after the heating step in the food processor and potential water evaporation was still close to the initial range of 40–42 g/100 g.

3.1. Bacillus cereus contamination of sunflower press cake

The oil press cake with added cold or hot water showed very low contamination of the raw material with vegetative *B. cereus* cells or spores, close to or below the quantification limit of 50 CFU/g (Table 2). The vegetative cells or spore counts of *Bacillus spp.* other than *B. cereus* determined by the plate count method were below the limit of quantification of 1000 CFU/g. Given the strong overall agreement between the counts of the *B. cereus* group (vegetative cells and spores) obtained using TEMPO and MYP agar (data not shown), only the TEMPO counts are presented here. On very few rare occasions, *Bacilli* strains other than those of the *B. cereus* group were observed on MYP plates (e.g., a morphologically different colonies of *B. subtilis*), which we do not consider relevant for our study. TEMPO detected elevated counts of up to 4000 CFU/g of vegetative *B. cereus* after heating the formulations (A–D) to 80 ± 3 °C for 3 min in a food processor. However, when the oil press cake paste was incubated at 25 °C, the number of vegetative *B. cereus* increased to $>10^5$ /g within 22 h independent of the treatment (Table 2).

Table 1
Four different formulations (A–D) of paste made from dehulled sunflower seed oil cake.

			A	B	C	D
Additions	Press cake	g/100 g	40.0	40.0	40.0	40.0
	Glucose	g/100 g	0	0	1.0	1.0
	NaCl	g/100 g	0	1.0	0	1.0
Measured parameters	Dry matter	g/100 g	39.4 ± 0.1	40.7 ± 0.1	40.5 ± 0.1	41.7 ± 0.1
	a_w-value	-	0.996 ± 0.003	0.984 ± 0.000	0.993 ± 0.000	0.982 ± 0.000
	Glucose	g/100 g	0.0	0.0	1.1 ± 0.02	1.1 ± 0.01
	Sucrose	g/100 g	1.6 ± 0.04	1.5 ± 0.01	1.7 ± 0.01	1.6 ± 0.03

Additions: Initial composition of the raw formulations A–D (Fig. 1, timepoint raw),.
Measured parameters: After the heating step (Fig. 1, timepoint t0).

Table 2
Bacillus cereus contamination in paste formulations (A–D) containing 40% (w/w) oil press cake from dehulled sunflower seeds in water. *B. cereus* counts of paste was analysed after three different treatments: addition of cold tap water to press cake (“Raw”), addition of boiling tap water to press cake (“Heated”), or heating and stirring of paste in the food processor (“Heated in food processor”).

		After treatment (formulations A–D)		After incubation (22 h/25 °C)
	Treatment	Vegetative (CFU/g)	Spores (CFU/g)	Vegetative (CFU/g)
Raw	Cold water added	<50 (n = 5)	<50 (n = 5)	5×10^5–1×10^7 (n = 3)*
Heated	Boiling water added: initial 70 °C	<50 (n = 4) 52 (n = 1)	<50 (n = 5)	3×10^5–4×10^6 (n = 3)*
Heated in food processor	3 min 77–83 °C (1 min >80 °C)	<50 (n = 2) 52–4000 (n = 6)	<50 (n = 8)	2×10^7–3×10^8 (n = 8)

n: number of analysed biological replicates.
* 40% (w/w) oil press cake in water only (Formulation A).

The growth of *B. cereus* during incubation at 25 °C was investigated in more detail for the oil press cake pastes with formulations A–D heated in the food processor (Fig. 2). For all formulations without the addition of an acidification culture, the numbers of vegetative *B. cereus* increased to $>10^7$/g within 22 h (grey circles, Fig. 2). The addition of glucose or NaCl in formulations A–D had no impact on this result. Thus, the *B. cereus* counts after 22 h at room temperature were well above the potentially toxigenic *B. cereus* concentrations of $>10^5$/g (red dashed line, Fig. 2). Even a low initial contamination of the raw material led to potentially toxin inducing numbers $>10^5$/g of *B. cereus* after storage at room temperature, despite heat treatment. These counts were reached within 6 h for glucose-containing formulation C, and within 8 h for glucose and NaCl containing formulation D and oil press cake only formulation A. The time to achieve a potentially toxin-inducing level of *B. cereus* was longest for formulation B, which contained only NaCl.

3.2. Inhibition of Bacillus cereus growth during fermentation

During the first 4 h of incubation at 25 °C, the increase in the concentration of *B. cereus* was comparable for both fermentation with acidification culture and incubation without any culture (Fig. 2). After 4 h, fermentation with RSW 901 vegan effectively inhibited the growth of *B. cereus* in formulations C and D, which contained an additional 1% (w/w) glucose. For these formulations, the final concentration of *B. cereus* after 22 h of fermentation with culture was comparable to the initial concentration after heat treatment. The initial concentration was approximately within the range of the considered safe level of 1000 CFU/g (see Fig. 2, orange line). By the end of fermentation, the growth of *B. cereus* was inhibited by at least 4.5 log compared to the control incubation without culture.

By contrast, fermentation without added glucose could not sufficiently inhibit *B. cereus* growth. During the 22-h fermentation period, *B. cereus* counts in these samples (formulations A and B) increased to levels above 10^5 CFU/g, which could potentially lead to toxin formation. The pH decrease during fermentation was less pronounced in samples

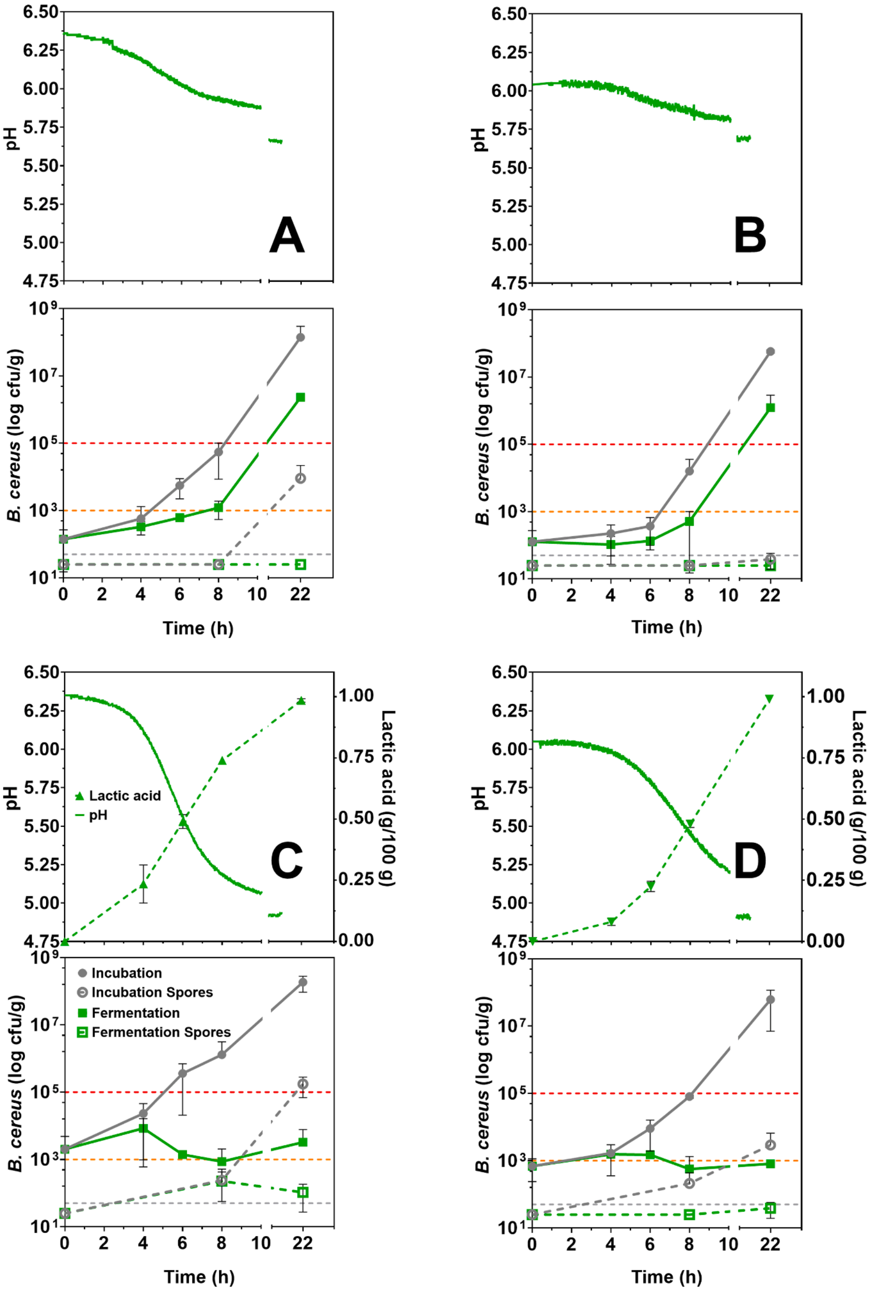

Fig. 2. Growth of *B. cereus* either during fermentation with lactic acid bacteria (green squares) or during incubation without culture (grey circles) at 25 °C for formulations A–D. A: 0% glucose, 0% salt; B: 0% glucose, 1% salt; C: 1% glucose, 0% salt; D: 1% glucose, 1% salt. The measured time course of pH is given for fermentation only and lactic acid (triangles) only conditions for fermentations C and D. Data are shown as mean ± SD from two independent biological replicates; error bars represent standard deviations. Horizontal dotted lines indicate the quantification limit of 50 CFU/g *B. cereus* (black; values below set to 25 CFU/g), the considered safe level (<1000 CFU/g, orange); and the concentration at which *B. cereus* may produce toxins (>10^5 CFU/g, red) (EFSA_BIOHAZ_Panel, 2016).

without glucose (Fig. 2A, B) compared to those with added glucose (Fig. 2C, D). Without glucose, the pH dropped slightly to 5.7 (formulation A) and 5.8 (formulation B). By contrast, with 1% glucose addition, the pH decreased to 4.9 (formulation C) and 4.8 (formulation D), with the latter also containing an additional 1% NaCl.

The addition of 1% NaCl influenced both the initial pH levels and acidification rates; however, formulation B, which contained 1% NaCl but not glucose, could not inhibit *B. cereus* growth. The initial pH was lower in NaCl-containing samples (6.05) than in samples without NaCl (6.35). However, the final pH after fermentation was in a comparable range in both cases. NaCl addition slowed down the acidification process: in formulation D with 1% NaCl, a pH below 5 was achieved only after 13 h, whereas in formulation C without added NaCl, this level was reached after 10 h. The slower pH decreases also corresponded to a slower increase in lactic acid concentration (Fig. 2C, D, upper graph). However, at the end of fermentation, the lactic acid concentration was similar with and without 1% NaCl. Acetic acid concentration increased slightly during fermentation by 12–17 mg/100 g and continued to rise during subsequent storage at 4 °C and ripening at 14 °C (Supplementary Figure 1). Free amino acids measured using the OPA (o-phthaldialdehyde) assay remained constant (formulation D) or initially decreased (formulation C) during fermentation (Supplementary Figure 1). During subsequent storage at 4 °C and ripening at 14 °C, free amino acids increased slightly, which indicates that protein has been cleaved.

In addition to the inhibition of *B. cereus* growth fermentation also had a positive effect on the colour of the sunflower seed oil press cake.

The initial brown colour became increasingly yellow as fermentation progressed (Fig. 3). This effect was observed for all four formulations (A–D). However, it persisted only as long as the fermented samples were not opened and exposed to oxygen. Upon exposure to oxygen, the yellow colour faded to a greyish colour within minutes, although the colour remained lighter than for the non-fermented samples. Samples incubated for 22 h without culture retained their original brown colour (Fig. 3, Inc.).

3.3. Inhibited sporulation during fermentation

Spore formation of *B. cereus* differed between the control incubation and fermentation conditions across all formulations. In the non-fermented control incubation, spore counts increased, with the highest levels observed in formulation C (1% glucose, 0% NaCl), whose spore levels reached nearly 10^5 CFU/g after 22 h (Fig. 2). This suggests that the presence of glucose promotes sporulation in the absence of salt. By contrast, formulation D (1% glucose, 1% NaCl) showed considerably lower spore levels under incubation, indicating that NaCl may suppress sporulation despite glucose availability. In formulation A (no glucose, no salt), spores increased only moderately during incubation, while formulation B (no glucose, 1% NaCl) exhibited low sporulation, supporting the idea that salt reduces sporulation efficiency and that glucose enhances it.

Under fermentation conditions, spore counts were consistently lower than in control incubation across all formulations (Fig. 2). In formulations C and D, spore levels remained at or near the quantification limit throughout the 22 h period, coinciding with rapid acidification and high lactic acid production. This suggests that fermentation, particularly the resulting drop in pH, effectively inhibited sporulation, even in the presence of glucose. In formulations A and B, which exhibited slower or less pronounced acidification, some sporulation still occurred during fermentation, but the levels remained well below those observed under non-fermented control conditions.

3.4. Decrease in B. cereus counts during storage and ripening

In this study the 4 °C storage simulated a product shelf life study, while the 14 °C storage investigated changes during potential ripening (Fig. 4). After one and two months of storage at 4 °C, all fermented formulations showed *B. cereus* counts <200 CFU/g, even in cases where potentially toxin-forming *B. cereus* numbers >10^5 CFU/g were detected at the end of incubation (A, B). The reduction in vegetative *B. cereus* was not reflected in a corresponding increase in the detected spores (Fig. 4). Therefore, the decrease in vegetative *B. cereus* in the fermented formulations cannot be explained by sporulation. During storage of the fermented paste at 14 °C (ripening), the counts of vegetative *B. cereus* decreased more slowly, and the counts of spores increased slightly, although not to the extent of the decrease in vegetative cells. Storage of non-fermented pastes showed a decrease but not below the potential toxin formation threshold of *B. cereus* of 10^5 CFU/g (Fig. 4, lower graph). Further, the number of spores did not increase in the non-fermented pastes.

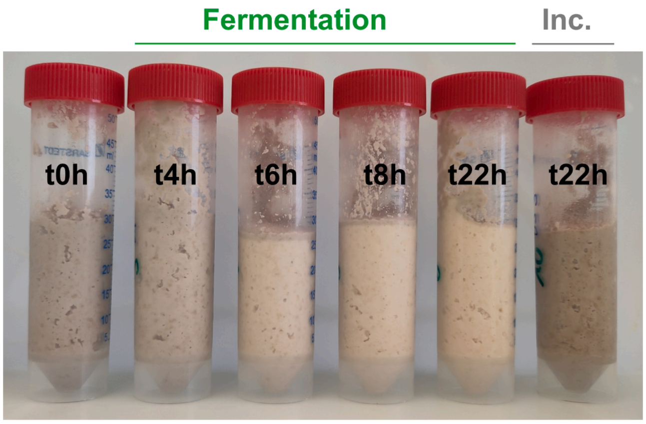

Fig. 3. Example of colour changes during fermentation with *Lactococcus lactis spp.* Example of formulation A, repetition 2 with culture (“Fermentation”) and without (“Inc.”). Samples incubated for 22 h without culture were considered negative controls.

4. Discussion

The rapid expansion of plant-based alternatives to meat and dairy products has outpaced the development of standardised food safety regulations and analytical methods. Although some safety specifications—such as EC 2073/2005—cover pathogens such as *Salmonella spp.*, *Listeria* spp., and *B. cereus*, many regulations remain country-specific and are only tailored for plant-based protein isolates (EUR-Lex, 2020). Notably, spore-forming bacteria, such as *Bacillus spp.* and *Clostridium* spp., have been identified as the most frequent contaminants across 88 processed plant-based ingredients, even after processing (Kyrylenko et al., 2023). Furthermore, spore loads in plant-based materials have been reported at levels up to 1000 spores/g, far exceeding the 1–10 spores/mL typically found in raw milk (NIZO Food Research, 2023). These factors raise an important question: What microbial specifications are appropriate for plant-based alternatives, and is there a need to revalidate existing food safety thresholds reflecting new raw materials and processing conditions?

In our study, *B. cereus* was initially not quantifiable (<50 CFU/g) in either vegetative or spore form in sunflower oil press cake used as raw material for cheese alternatives. However, with mild heat treatment (80 ± 3 °C, 3 min), vegetative cell counts increased up to 4 × 10^3 CFU/g immediately after heat treatment. The observed increase in vegetative cell counts after heat treatment may be attributed to multiple factors. The oily matrix likely hindered accurate microbial quantification at lower temperatures, while 80 °C treatment in the food processor might have facilitated spore dissolution. Mild heat treatment (80 °C, 3 min) could have led to spore germination, and the presence of germinants (e.g., amino acids or inosine) could have further contributed to the observed spike, as supported by Freire et al. (2024) and Løvdal et al. (2011). Further, additional contamination with spores from the food processor cannot be ruled out; however, the press cake that did not undergo food processor treatment also showed *B. cereus* growth after incubation (Table 2). The presence of spores resistant to standard cleaning protocols may also present a challenge later in production facilities. These findings emphasise the need for a better understanding of *B. cereus* contamination and careful consideration of heat treatments in regard to activation/inactivation of spores, which might vary depending on matrix composition and initial contamination levels (Løvdal et al., 2011; Wen et al., 2022).

The safety of plant-based food products is a challenge due to their sensitivity to heat and elevated exposure to environmental spores. Many plant proteins denature at elevated temperatures, affecting the taste, texture, and nutritional quality of the final product. Moreover, spore-forming bacteria present in these ingredients can survive even the most intense heat treatments (Christiansson et al., 1999; Dufrenne et al., 1995). This dual limitation reduces the effectiveness of conventional food safety interventions in plant-based systems. The hurdle technology concept has been proposed as a solution for the control of spore-forming bacteria (Aaliya et al., 2021; Reineke and Mathys, 2020; Shymialevich et al., 2024). This approach allows food safety to be improved step-by-step without compromising the structural challenges of heat-sensitive plant proteins. According to Aaliya et al. (2021), combining thermal and non-thermal methods can produce synergistic effects against food spoilage microorganisms, overcoming the limitations of individual treatments (Aaliya et al., 2021). Combining thermal and non-thermal processes can enhance spore activation, as each

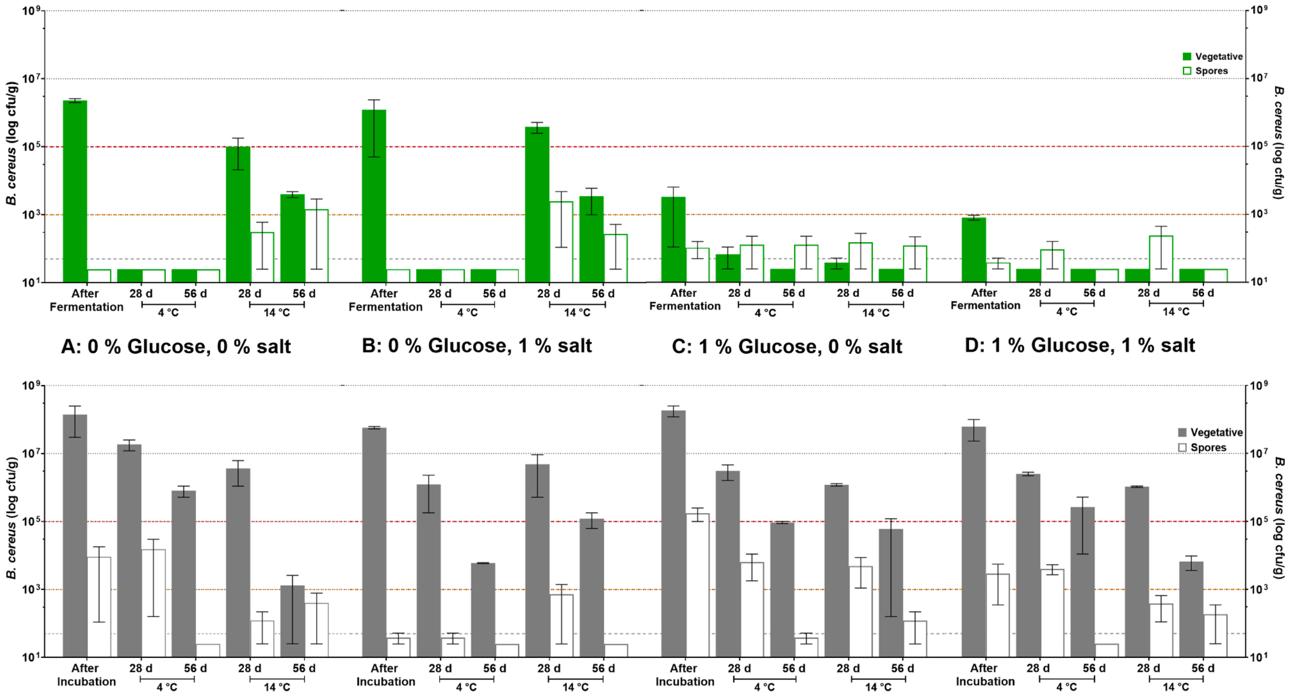

Fig. 4. Changes in vegetative cells and spore counts of *B. cereus* during storage at 4 °C and 14 °C. The upper graph shows the storage of previously fermented samples for formulations A–D, while the lower part of the graph displays samples without culture. The filled columns represent the average values of vegetative *B. cereus*, and the empty columns show the average values of spores. Data are shown as mean ± SD from two independent biological replicates; error bars represent standard deviations. Horizontal dotted lines indicate the quantification limit of 50 CFU/g *B. cereus* (black; values below set to 25 CFU/g), the considered safe level (<1000 CFU/g, orange), and the concentration at which *B. cereus* may produce toxins (>10^5 CFU/g, red) (EFSA_BIOHAZ_Panel, 2016).

method targets different structural or molecular features critical for spore germination and stability in the dormant state (Reineke and Mathys, 2020).

Fermentation is a well-established, non-thermal method for improving microbial stability and shelf life by lowering pH and increasing acidity (Bourdichon et al., 2021). In our study, we inoculated sunflower oil press cake blends with a lactic acid bacteria culture (RSW 901 vegan, *Lactococcus* spp.) with and without glucose and/or NaCl to investigate the effect of culture under different conditions on *B. cereus* growth. The optimal pH for the growth of *B. cereus* is between 4.9 and 5.3 (Kharel et al., 2024a). In the absence of glucose (formulations A and B), the starter culture achieved limited growth due to its inability to metabolise sucrose, which is the main carbon source naturally present in the sunflower oil press cake. Consequently, the formation of lactic acid was low, with the pH remaining above the threshold required to fully suppress *B. cereus* (Kharel et al., 2024a). Nevertheless, in both formulations, a 2-log decrease in B. *cereus* counts was observed after 22 h in comparison with the unfermented sample. However, this inhibition was not sufficient to reach food safety standards. Adding 1% NaCl alone (formulation B) did not significantly affect *B. cereus* growth. This aligns with prior reports showing that *B. cereus* tolerates mild salt stress and can grow up to 5% NaCl (Den Besten Heidy et al., 2009). Additionally, salt slightly reduced the activity of the starter culture (Fig. 2), possibly affecting its competitive advantage. Although NaCl can lower water activity and affect sporulation (Jakobsen and Murrell, 1977), the concentration used here (1%) was likely too low to create a relevant hurdle effect on its own.

By contrast, fermentation under the conditions in formulations C and D—both which contained 1% glucose—resulted in robust growth of RSW 901 vegan, a pronounced drop in pH (to ~4.9), lactic acid production (~1 g/100 g after 22 h), and a slight increase of acetic acid (0.12–17 mg/100 g). This acidification corresponded with inhibition of *B. cereus* growth, in line with previous studies showing that vegetative cell growth is suppressed at a pH <5.6 in the presence of 0.9 g/100 g lactic acid (Ceuppens et al., 2012; Wong and Chen, 1988) or if a pH of <5.3 was reached within 7 h of fermentation with *Lactococcus* spp. culture (Røssland et al., 2003). However, acetic acid has been found to inhibit even at a higher pH than lactic acid (Nout et al., 1987; Wong and Chen, 1988). After 22 h of fermentation, the added glucose (1 g/100 g) was completely utilised, whereas the native sucrose present in the sunflower oil press cake remained available (Supplementary Figure 1). The chosen *Lactococcus* spp. Culture RSW 901 is not able to metabolise the native sucrose present in the sunflower press cake whereas *B. cereus* can. Consequently, *B. cereus* grew to high counts in samples containing native sucrose only (formulation A and B). This suggests that by the end of fermentation of formulation C and D, growth of *B. cereus* was effectively inhibited even though *Lactococcus* spp. had no glucose left to grow, while *B. cereus* could still use the remaining sucrose. However, the growth of *B. cereus* was effectively inhibited in formulation C (final pH 4.9) and D (final pH 4.8). Reduction of pH corresponds with *B. cereus* inhibition but is not seen as the only factor.

Depletion of oxygen by the vegan RSW 901 *Lactococcus spp.* Culture (Tachon et al., 2010) may be an additional factor contributing to the inhibition of *B. cereus* growth. Although *B. cereus* is a facultatively anaerobic organism capable of growing under aerobic and low-oxygen conditions, growth is favoured in the presence of oxygen (De Sarrau et al., 2012). However, under comparable conditions without the *Lactococcus* spp. culture, sufficient oxygen was available, allowing *Bacillus cereus* to reach counts exceeding 10^7 CFU/g within 22 h at 25 °C (Fig. 2).

In addition to the inhibition of *B. cereus*, improvements in product quality attributes were associated with fermentation, including the development of flavours characteristic of lactic acid fermentation and colour modification. The colour change observed during fermentation represents a beneficial side effect that may increase consumer acceptance (Fig. 3). In plant-based matrices, such changes are commonly driven by pH shifts, organic acid production, and microbial metabolism (Fernández-Varela et al., 2024; Fritsch et al., 2016). Fermentation of sunflower press cake is often linked to colour lightening when

Lactobacillaceae strains degrade or reduce chlorogenic acid derivatives via esterase activity (Fritsch et al., 2016; Santos et al., 2018; Verde et al., 2022). By contrast, *Lactococcus* spp. have not yet been reported to directly reduce oxidised phenolics in this context. However, oxygen consumption by *Lactococcus* strains and changes in redox potential have been documented during milk fermentation (Cachon et al., 2002; Larsen et al., 2015), which could potentially contribute to the lightening effect during the fermentation of sunflower press cake.

Storage at 4 °C sharply reduced the number of vegetative cells of *B. cereus* in all fermented formulations to <200 CFU/g, although counts in formulations A and B exceeded 10^5 CFU/g at the end of fermentation due to insufficient inhibition in the absence of glucose (Fig. 4, upper graph). The decrease was not accompanied by increased spore counts, suggesting loss of culturability or lysis of vegetative cells rather than sporulation. In contrast, under non-fermented control conditions (Fig. 4, lower graph), the reduction was considerably less pronounced, with *B. cereus* counts remaining above 10^5 CFU/g after 28 days at 4 °C. These findings indicate that low temperature alone cannot explain the strong reduction observed in fermented samples, although the effect was less pronounced at 14 °C. Instead, the data suggest a synergistic effect of exposure time, fermentation conditions, and refrigerated storage. A plausible explanation is that sublethal stress induced by fermentation (pH ~5.7 and antimicrobial metabolites) cannot be effectively repaired during refrigerated storage, resulting in the accumulation of cellular damage and a progressive decline in vegetative cells. This interpretation is supported by previous studies. Røssland et al. (2005) demonstrated that fermentation at moderately acidic conditions (final pH ~5.5) initially allowed growth of *B. cereus*, but extended fermentation (48–72 h) resulted in a substantial reduction from $>10^5$ to ~70 CFU/mL. Similarly, Shen et al. (2020) reported a 3–3.5 log reduction of *B. cereus* during 10 days of storage at 5 °C in fermented milk adjusted to pH 6.4, attributing this decrease to antimicrobial factors other than pH. Pokorski and Trzaskowska (2023) further observed a 2–4 log reduction of *B. cereus* during storage of fermented brewers' spent grain and demonstrated inhibitory activity of the cell-free supernatant, indicating antimicrobial bacteriocin production. In our study, culture supernatant from RSW 901 vegan grown in M17-X medium showed no inhibitory activity against *B. cereus* or *B. subtilis* (data not shown); however, differences in the medium and native strains present in sunflower press cake mean that the potential role of bacteriocins cannot be excluded. Importantly, lactic acid bacteria persisted at high levels during storage in fermented samples (formulation D with initial glucose). After 28 days at 4 °C and 14 °C, lactic acid bacteria counts reached 5×10^9 and 3×10^9 CFU/g, respectively (data available on Zenodo open research data repository https://doi.org/10.5281/zenodo.17404495). These levels are comparable to those observed in traditional Raclette cheese after 30 days, which contains $\sim 1 \times 10^9$ CFU/g LAB (Schär et al., 1992). The sustained lactic acid bacteria population may have contributed to *B. cereus* suppression via ongoing metabolic activity (e.g., antimicrobial compound production), even if no further acidification or strong nutrient competition occurs.

Although *B. cereus* counts in fermented samples decreased during storage at 4 °C to below 200 CFU/g, the product may still not be safe. During fermentation with formulations A and B, *B. cereus* levels exceeded 10^5 CFU/g, which could have allowed the formation of emetic heat-resistant toxins that may persist in the product independently of viable cell counts. Therefore, post-storage *B. cereus* analysis alone may underestimate the associated risk. To support the development of a safe production process, it is therefore essential to monitor *B. cereus* contamination immediately after fermentation, when maximum cell counts are expected, rather than relying solely on end-of-shelf-life assessments.

In this study, sporulation occurred primarily during incubation at 25 °C rather than during storage at 4 °C or 14 °C. The reduction in vegetative *B. cereus* during storage was not accompanied by a corresponding increase in spores, although a slight rise in spore counts was observed for formulations A and B at 14 °C. Spore counts mainly increased during incubation at 25 °C without acidification culture and the presence of additional glucose (formulations C and D) or in the absence of salt (formulation A, Fig. 2). Under fermentation conditions, spore counts were consistently lower than in the incubation without acidification culture across all formulations (Fig. 2). These findings indicate that fermentation not only suppressed vegetative growth but also limited the sporulation capacity of *B. cereus.* Sporulation is generally most efficient under optimal growth conditions, including temperature, pH, and water activity, with yields decreasing as these parameters deviate from the optimum, as previously reported for various *Bacillus spp.* (Bressuire-Isoard et al., 2018). However, the sporulation efficiency can vary depending on the strain, sporulation medium, and incubation parameters. In the *B. cereus* group, sporulation can be reduced by low oxygen availability, depletion of essential divalent cations such as Ca^{2+} and Mg^{2+}—which are critical for proper spore coat formation—and elevated salinity, while the presence of glucose has been shown to promote sporulation (Bressuire-Isoard et al., 2018; Jakobsen and Murrell, 1977; Mazas et al., 2009).

Together, these findings underscore the pivotal role of sugar availability—particularly added glucose—in supporting starter culture activity and ensuring microbial safety. Only fermented samples with glucose supplementation fully suppressed *B. cereus* growth and remained stable during extended storage.

5. Conclusion

In this study, we confirmed that *Bacillus cereus* is present in sunflower oil press cake. Before heat treatment, the number of vegetative cells and spores was below the quantification limit of the method. They became detectable only after the sample was heated to 80 ± 3 °C for 3 min. This underscores the need for a better understanding of *B. cereus* contamination in plant-based matrices and to integrate further post-heating analyses into production processes to accurately assess initial contamination during production.

The growth of *B. cereus* during processing was effectively suppressed by fermentation with a starter culture containing *Lactococcus spp.*, which was added immediately after heat treatment. This led to acidification from pH 6.35 to 5.0 within the first 10 h, resulting in a final pH of 4.9 after 22 h. While the addition of 1% salt helped slow down *B. cereus* growth, salt addition at 1% on its own was too low to prevent the proliferation of *B. cereus*. Using *Lactococcus* spp. in sunflower oil press cake requires glucose supplementation, since these bacteria cannot metabolise the native available sucrose in the raw material. The addition of external glucose enables control of final pH, but fermentation parameters must be carefully optimised to suppress the outgrowth of germinated *B. cereus* after heat treatment.

During storage at 4 °C for up to two months, vegetative *B. cereus* counts in fermented samples declined sharply to below 200 CFU/g, even if counts above 10^5 CFU/g were observed at the end of fermentation. By reaching levels above 10^5 CFU/g during processing, there is a potential risk that *B. cereus* will produce stable toxins that remain in the product until the end of its shelf life, even if *B. cereus* is no longer detectable. To support the development and establishment of a safe production process, it is therefore essential to monitor *B. cereus* contamination immediately after fermentation, not just at the end of the product's shelf life.

The current study demonstrates that a combined strategy of thermal treatment and fermentation can enhance the microbial safety of plant-based foods, in line with the core principles of hurdle technology. In many practical applications, raw materials are subjected to heat, either for pasteurisation or as part of the processing steps. This thermal exposure may induce the sporulation of *Bacillus cereus*, while subsequent fermentation effectively inhibits the outgrowth of vegetative cells, thereby reducing potential safety risks. However, further studies are needed to evaluate the current safety thresholds not only by quantifying bacterial loads but also by assessing toxin production to provide a more comprehensive understanding of the contamination risks and toxin formation potential in plant-based alternative matrices.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Perplexity and ChatGPT to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Ethical statement

The research presented does not involve any animal or human study (including sensory evaluation and customer surveys).

CRediT authorship contribution statement

Verena Looser: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Gabriela Purtschert-Montenegro:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Ueli von Ah:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Dieter Weik:** Investigation. **Elvira Wagner:** Investigation. **Jörg Hummerjohann:** Writing – review & editing, Methodology, Conceptualization. **Lotti Egger:** Writing – review & editing, Methodology. **Helena Stoffers:** Writing – review & editing, Resources, Conceptualization. **Emmanuelle Marie Arias:** Writing – review & editing, Conceptualization. **Jan-Erik Ingenhoff:** Writing – review & editing, Supervision. **Remo S. Schmidt:** Writing – review & editing, Supervision, Conceptualization. **Hans-Peter Bachmann:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ghazal Nemati:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2026.101017.

Data availability

https://doi.org/10.5281/zenodo.17404495 (Data will be made available on).

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