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Next-generation safe, tasty, and healthy fermented Baltic herring products (*Clupea harengus membras*)

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ABSTRACT

As food, Baltic herring is an underutilized fish species. In this study, fermentation was applied to produce a new type of tasty and safe Baltic herring product with high nutritional value. Baltic herring fillets were fermented using a sourdough starter B-7, and a yoghurt starter Y482F. A fermentation temperature of 32 °C and 50 °C was used for B-7 and Y482F, respectively. Following periods of 2- and 5-days' fermentation time the fillets were subjected to 14-days cold storage at 2 °C. The fermented samples were tested for microbiological and chemical safety, lipid oxidation, protein degradation levels, and sensory quality. A moderate level of lipid oxidation and low level of protein degradation was observed. Depending on the starters applied, a clear difference in taste and texture between the fermented fish fillets was observed in the sensory evaluation: the products fermented with the B-7 were scored as saltier, fresher, fishier, and sourer than those fermented with Y482F. Although the products' salt concentration was low (0.4 %), a sufficiently salty taste was still detected. According to the results, the fermentation process developed enables the production of next-generation microbiologically and chemically safe, tasty, low-salt fish food products from an underutilized, vast fish resource.

1. Introduction

Fish is part of a sustainable and healthy diet, with a lower environmental footprint than land-based animal protein. Moreover, fish is rich in proteins, polyunsaturated fatty acids (PUFAs), minerals and essential vitamins, offering potential health benefits. However, the sustainable supply of fish has become critical issue, as the global consumption of fish is increasing. Consequently, to sustainably meet the growing demand for fish, the European Commission recommends the consumption of local, seasonal, and sustainably harvested fish products (European Commission, 2021). To tackle this challenge, new solutions are needed to turn the fish species that are classified underutilized into palatable food products.

In the Baltic Sea, Baltic herring (*Clupea harengus membras*) is one of the major fish species, accounting for 94 % of the volume of the commercial marine fishery catch in Finland. However, only 3 % of the Baltic herring catch was used for food in Finland in 2023 (Saarni et al., 2024), even though its nutritional value is excellent, containing protein, PUFAs (EPA, DHA, α-linoleic acid, linoleic acid), minerals (Ca, P, Mg, iodine, selenium), and vitamins (A, E, D, B9, B12) (Fineli, 2024), for example. One of the challenges regarding the food use of Baltic herring is that especially younger consumers perceive the smell and taste of herring as unappealing (Hopia & Lundén, 2021). New food processing solutions for improving the sensory quality of Baltic herring, would unlock the potential of this vast fish source as a sustainable, nutritional food source.

Salt (NaCl) is usually added to fish products to improve the taste and texture, as well as to ensure the microbiological safety (Hafez et al., 2019). Traditional fermented fish products contain typically high contents of salt, while dietary guidelines recommend reducing salt intake in the diets (Hunter et al., 2022). Thus, new solutions for reducing salt content in fish products without compromising taste and safety are needed, and recently, this issue has gained an increasing interest in research (e.g. Zhang et al., 2022; Li et al., 2023; Ghayoomi et al., 2023)

Fermentation is an ancient and widely used method to prepare and preserve food, fish (Belleggia & Osimani, 2023) among others. Globally, various fermented fish products such as solid (whole fish), sauces and

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pastes are consumed especially in Asian, African and Northern European countries. In Asia, traditional fermented commercial fish products include fish sauce, such as Thailand's nampla, (Chan et al., 2023 Elsewhere, in Europe, *surströmming* in Sweden (Skåra et al., 2015) represents a traditional fermented fish product, while garum is a traditional fish sauce in southern Europe (Grainger, 2020). Fish fermentation is a complex process which may result in histamine production (Barbieri et al., 2019), but histamine synthesis is affected by the fermentation parameters (temperature, time, pH, salt content) and can be lowered by applying controlled fermentation with selected lactic acid bacteria (LAB) strains (Barbieri et al., 2019).

Fish fermentation can be carried out by either spontaneous fermentation or controlled fermentation (Chan et al., 2023). Most traditional fermented fish products are produced through spontaneous fermentation, in which fermentation occurs through naturally present microbes in fish and other raw materials (Belleggia & Osimani, 2023). Spontaneous fermentation processes vary, depending on fish species, salt content, temperature, storage technique, and raw material handling (Belleggia et al., 2020). In contrast, controlled fermentation is carried out by inoculating specific known starter cultures with a single strain or a mixture of microorganisms (Belleggia & Osimani, 2023). Controlled fermentation processes are widely used in the food industry – most commonly, in the dairy sector.

To the best of our knowledge, fermentation has not been studied in order to produce ready-to-eat, low-salt convenience foods. Thus, this study investigates controlled fermentation for turning Baltic herring into low-salt, ready-to-eat fish food. For this aim, the current study (i) investigates two commercial LAB starters for fermenting Baltic herring fillets, (ii) determines optimal fermentation parameters for improving the sensory quality of Baltic herring fillets and (iii) measures the effects of fermentation on the nutritional quality, histamine levels and lipid oxidation in Baltic herring fillets.

2. Material and methods

2.1. Pre-processing of baltic herring fillets

Fresh Baltic herring fillets were purchased from a local supermarket (Forssa, Finland), transported on an ice bath to Luke Jokioinen (Jokioinen, Finland), and frozen (individually in a freezer bag) at −20 °C for two weeks before use. According to the pretests fish fillets turned brown and were soft in structure when autoclaved. Thus, to retain light colour in the fish fillets and firm structure, they were autoclaved (Systec VX-75, Systec GMbH&Co, Germany) as frozen. The frozen fillets were weighed into glass jars (1.5 l, purchased from Clas Ohlson, Finland) (approximately 250 g/a jar, 24 jars divided in 8 batches consisting of triplicates) and covered with 350 ml of 2 % NaCl-solution (w/v) at room temperature (22 °C). Then, the jars were covered with aluminium foil and autoclaved (Systec VX-75, Systec GMbH&Co, Germany) subsequently at 121 °C/20 min. After cooling on ice to 32 °C (12 jars) or 50 °C (12 jars), the NaCl solution was poured out of the glass jars.

2.2. Fermentation and cold storage

The fermentation of the fillets was started by aseptically adding the starter cultures to the pre-processed fillets. Two different commercial LAB starters, the sourdough culture Lyoflora B-7 (B-7) and the yoghurt culture Lyofast Y 482 F (Y482F) (Sacco, Italy), containing both homo- and heterofermentative strains and purely homofermentative LAB strains, respectively (Table 1) were applied in the fermentation. The suitable concentrations of the starters were determined by pre-tests the aim of which was to achieve pH ≤ 4.4 at the end of fermentations periods, as this it is considered a safe pH to limit the growth of *Listeria monocytogens* strains in ready-to-eat products (EU 2073/2005) and also inhibit the growth of the other severe pathogen *Clostridium botulinum* (Majou, 2024). The starter cultures were prepared by dissolving 0.1 g of B-7 or 1.1 g of Y482F in sterilised tap water, followed by a 2 % inoculation in a 4 % glucose (w/v) solution, resulting in microbial counts of approximately 6.89 log (B-7) and 8.06 log (Y482F) cfu/mL in the final volume of the fish fillet brine. To accelerate the growth of the starters in the fermentation, glucose was used because the carbohydrate content in Baltic herring fillets is negligible (Fineli, 2024).

Table 1
Bacterial strains, fermentation conditions and quality evaluation timepoints used in the study.

Starter	Bacterial LAB strains included	Fermentation	Quality evaluation
B-7	Homofermentative strains: *Lactobacillus acidophilus, Lacticaseibacillus paracasei, Lactiplantibacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus*	32 °C/2 days 32 °C/2 days 32 °C/5 days 32 °C/5 days	AFP 14-DS AFP 14-DS
	Heterofermentative strains: *Levilactobacillus brevis, Limosilactobacillus fermentum, Leuconostoc mesenteroides* ssp. *cremoris.*		
Y482F	Homofermentative strains: *Streptococcus thermophilus* *Lactobacillus delbrueckii* spp. *bulgaricus*	50 °C/2 days 50 °C/2 days 50 °C/5 days 50 °C/5 days	AFP AFP, 14-DS AFP AFP, 14-DS

AFP: After fermentation period, 14-DS: after 14 days of cold storage at 2 °C.

To obtain the anaerobic conditions the jars were closed with lids followed by the fermentation in the growth chambers (Heratherm Refrigerated Incubator, Thermo Scientific) for 2 and 5 days at 32 °C fermenting the fillets with B-7, and at 50 °C with the Y482F. Additionally, the fermented fillets were also subjected to two weeks' cold storage at 2 °C to determine the quality and stability of the product during storage (Table 1). All the fermentation were done in triplicate.

These fermentation temperatures were selected based on the literature that certain sourdough processes are usually carried out at a fermentation temperature of >30 °C (Arora et al., 2021), and the growth of *C. botulinum* is inhibited at 48 °C in the laboratory conditions (Majou, 2024). Because certain sourdough processes last for 2–5 days, the fermentation periods of two and five days were selected (De Vuyst & Neysens, 2005). Following the fermentation and storage periods, microbiological, chemical, and sensory quality (Sections 2.3–2.7) of the fillets were analysed (Table 1).

2.3. Microbiological quality and safety

Ten grams of the Baltic herring fillet in the 90 ml of Ringer solution (Ringer Solution Tablets, Neogen) were homogenized (260 rpm, 2 × 1min) using a Stomacher 400 Circulator (Seward, West Sussex, UK), and ten-fold serial dilutions were prepared in Ringer solution and used for the microbiological analyses.

The enterobacteria counts were determined by a pour plate technique on Violet Red Bile Glucose Agar (VRBGA, Neogen, UK). After a 24-h incubation period at 37 °C, the colonies were counted. LAB were enumerated on de Man, Rogosa, and Sharpe agar (MRSA, BD Difco, France) plates, and the colonies were counted after incubation in anaerobic conditions at 30 °C for 72 h. The number of yeasts was measured on Dichloran Rose Bengal Chloramphenicol agar (DRBCA, Becton, Dickinson and Company, France) plates supplemented with 50 μg/mL of oxytetracycline hydrochloride (AppliChem BioChemica, Germany) incubated at 25 °C for 3–5 days. Each sample was determined in duplicate, and the cell count was measured as cfu/g.

L. monocytogenes, sulphite-reducing bacteria, hydrogen sulphide-producing bacteria and coagulase-positive staphylococci were tested in the KVVY Tutkimus Oy (Tampere, Finland) accredited laboratory. The number of *L. monocytogenes* was determined using the RAPID'L.mono method (Bio-Rad), which is validated by AFNOR (https://nf-validation.afnor.org//en/wp-content/uploads/sites/2/2014/03/Synt-BRD-07-0

4-09-98_en.pdf). The number of sulphite-reducing clostridia was determined according to the method of the Nordic Committee on Food Analysis NMKL 56:2015 5. Ed., while the number of hydrogen-sulphide-producing bacteria was determined according to the method of the NMKL 184:2006. The number of coagulase-positive staphylococci were determined by the 3M Petrifilm Staph Express System (https://multimedia.3m.com/mws/media/2412800/petrifilm-staph-express-interpretation-guide.pdf).

The samples' pH values were measured using a pH meter (Mettler Toledo, SevenCompact^{Tm} S210, Switzerland), and the measurements were made for fillets in duplicate.

2.4. Chemical quality and safety

2.4.1. Histamine

The histamine concentrations in a 5 g of sample were determined using an enzymatic method with a Histamine automated analyser BioSystems Y15 (BioSystems S.A., Spain) in the Net-Foodlab Oy accredited laboratory (Turku, Finland). This test is approved by AOAC (AOAC performance tested method 072001, (Tobeña et al., 2021). In all the matrixes, the limit of quantification (LOQ) is 10 mg/kg.

2.4.2. Nutritional composition

After fermentation, the softened fish samples were mixed into a smooth mass using a spoon and frozen for later analysis. For nutritional content analysis, the frozen samples were thawed and heated to 37 °C in a water bath to soften the fat contained in the sample. Before analysis, the thawed sample was mixed using a spoon into a homogeneous mass. The fish samples were analysed as three parallel samples/fermentation-storage treatments.

For moisture and ash, approximately 1 g of the thawed sample was weighed. Moisture and ash were determined using a TGA701 Thermogravimetric Analyser (Leco Corporation, St. Joseph, MI, USA) at 105 °C and 650 °C, respectively. For total protein determination about 1 g of fish sample was used. The total protein content was determined by measuring the nitrogen content of the fish sample using a Kjeltec TM8400 analyser (Foss Analytical Ab, Höganäs, Sweden) based on an in-house Kjeldahl method (ISO 5983-2, 2009, ISO 20483, 2013), wand a 6.25 as a conversion factor was used to calculate the total protein content. For lipid content determination 1–2 g of fish sample was used. Lipid content was measured using the SoxCap TM 2047 in combination with the Soxtec TM 2050 extraction system (Foss A/B, Hillerød, Denmark), with diethyl ether as a solvent (ISO 6492, 1999).

Energy densities (kJ/g) on a wet mass basis for fermented fish samples were calculated from their composition using energy equivalents of 37 kJ/g and 17 kJ/g for lipid and protein respectively (EU 1169, 2011). Carbohydrates were omitted, as they are an insignificant component of fish.

2.4.3. Salt concentration

For Na content determination 1–2 g of thawed fish sample (2.4.2) was used. The samples' Na content was measured using plasma emission spectrometry with an ICP-OES (PerkinElmer Optima 8300, Shelton, CT, USA). The amount of salt (NaCl) was calculated by multiplying the Na content by 2.5 (Kumpulainen & Paakki, 1987).

2.5. Lipid oxidation

In the fermented Baltic herring fillets, the level of lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS) as described in Logrén et al. (2022). Briefly, the Baltic herring fillets samples (á 100 mg) were subjected to alkaline hydrolysis, with a subsequent protein precipitation, after which the supernatants were reacted with thiobarbituric acid (TBA) to form a pink adduct and analysed with UHPLC at 532 nm. Chromatographic analyses were performed in duplicate from each of the subsamples ($n \geq 6$).

2.6. Protein size distribution

The degradation of proteins into peptides was monitored using size-exclusion chromatography (SEC). SEC of the fermented fish samples was performed on an Äkta pure 25 M chromatographic system (Cytiva Sweden AB, Uppsala, Sweden) equipped with an ALIAS Bio autosampler (DURATEC Analysentechnik GmbH, Hockenheim, Germany) using a Superdex Peptide HR 10/30 column (GE Healthcare Life Sciences, Uppsala, Sweden) as described by Mäkinen et al. (2022). In this study, the SEC sample was prepared as follows: 0.5 g of the sample and 7.5 mL of the running buffer (100 mM sodium phosphate buffer, pH 7.0) were weighed into a 20 mL decanter and mixed for 3 h using a magnetic stirrer. Then, the sample was then centrifuged at 10,000×*g* for 15 min. The supernatant was diluted 1/10 with running buffer and filtered through a 0.45 μm filter (Acrodisc, Pall Life Sciences) before injection (100 μl). The total surface area of the chromatograms was integrated and separated into four molecular weight (MW) ranges (>10,000, 1000–10,000, 200–1,000, <200 Da), and the results were expressed as a percentage of the total area. Here, the mutual effects of different treatments on MW distribution were compared. The analyses were performed in triplicate.

2.7. Sensory profile

After microbiological and chemical safety was ensured, the sensory properties of the fermented Baltic herring fillet samples were evaluated at Luke's sensory laboratory in Jokioinen, Finland, using a quantitative descriptive method.

Luke's sensory panellists have years of experience of sensory analysis of various foods, and before the first session the panel was calibrated in a round-table session discussing the attributes using a reference sample (Baltic herring).

The first evaluations (two sessions) consisted of the following samples: B-7 2 days' fermentation, B-7 5 days' fermentation, Y482F 2 days' fermentation, and Y482F 5 days' fermentation. The second evaluations (two sessions) consisted of samples after 14 days of cold storage at 2 °C: B-7 2 days' fermentation, stored; B-7 5 days' fermentation, stored; Y482F 2 days' fermentation, stored; and Y482F 5 days' fermentation, stored. The samples were all duplicates.

The samples were coded with random three-digit numbers and served at 21 °C in randomised order. A maximum of six samples was evaluated in each of the four evaluation sessions, and all tasters tasted all the samples. In each session, the number of panellists ranged between 10 and 12, from a pool with a total of 16 judges. Using a scale of 1–5, the panel evaluated the intensity of colour (lightness), odour (acidic, metallic, fresh, and fish), taste (sweetness, sourness, saltiness, bitterness, and umami), flavour (freshness, fish, and metallic), texture (juiciness and firmness), and overall acceptance (a hedonic attribute) An open text field was provided for additional notes, and Webropol software (Version 3.0; Webropol, Finland) was used for data collection.

2.8. Statistical analysis

A three-way ANOVA were used to compare the measured variables means on groups (two different starters, two different fermentation times and two storage times), and Tukey's HSD test was used in pairwise comparisons. T-test was used to test initial values against measured values on the LAB counts. The exploratory data analysis on sensory panel data (averaged over judges for each sample) was conducted using unsupervised classification procedure principal component analysis (PCA). All the statistical analyses were performed using *R* Statistical Software (v4.3.0; R Core Team (2023)).

3. Results and discussion

3.1. Fermentation

The ability of the selected starters to ferment the fish material was assessed by the decrease in pH and the growth of LAB (Fig. 1).

When using the B-7 sourdough starter for the fermentation, the pH values were 4.36 and 3.81 after two days and five days of fermentation, respectively, and did not change significantly ($p = 0.958$) during the two-week storage at 2 °C. The LAB counts with starter B-7 were 6.89 log cfu/mL at the beginning and increased to 8.98 log cfu/g and 8.64 log cfu/g after two days and five days of fermentation ($p < 0.002$), respectively. After 14 days of cold storage, the LAB counts were 7.28 log cfu/g after 14 days of cold storage (Fig. 1).

According to the pH and LAB count results, the B-7 sourdough culture consisting of both homofermentative and heterofermentative LAB strains (Table 1) performed well when used to ferment Baltic herring fillets.

The pH values in the present study are lower in comparison to the values reported for spontaneously fermented fish sauce and paste (pH 5.10–7.53) (Julmohammad et al., 2025), but they are comparable to typical pH levels in sourdough fermentation (pH 3.7–4.0) (Van Kerrebroeck et al., 2016). Lower pH values in comparison to the spontaneously fermented fish results putatively from the controlled fermentation process, which enables the LAB strains to produce organic acids efficiently during fermentation, while in the spontaneous fermentation yeasts and mould are typically present and may consequently reduce the formation of organic acids by LAB and enhance the formation of ammonia (Julmohammad et al., 2025).

Compared with the initial LAB count of 8.06 log cfu/mL ($p < 0.002$), these LAB numbers are low, and they are also, lower in comparison to the LAB counts of the Baltic herring fillets fermented with the B-7 strains (Fig. 1). The results indicate a weaker performance of the LAB strains of Y482F (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) for fermenting Baltic herring fillets, in comparison to B-7 starter with various homofermentative and heterofermentative strains (Table 1). According to the previously published results, *S. thermophilus* has been identified from fermented fish. However, reports concerning *L. delbrueckii* subsp. *bulgaricus* in fermented fish are lacking (Belleggia & Osimani, 2023). Putative factors affecting the growth potential of Y482F strains in the current study are the fermentation temperature of 50 °C, as the maximum temperature 47–50 °C has been reported for *S. thermophilus* (https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/streptococcus-thermophilus).

Although spontaneous fermentation of fish materials is commonly used and widely studied (Belleggia & Osimani, 2023), information on controlled fermentation of fish is scarce including only a few studies concerning the production of fish paste (Semjonovs et al., 2015) and fish pieces (Hua et al., 2020).

To ferment sterilised minced Baltic herring supplemented with glucose or sucrose and NaCl (1–4 %), Semjonovs et al. (2015) applied a single probiotic strain *Bifidobacterium animalis* subsp. *lactis* Bb12 (Chris. Hansen A/S, Denmark). Fermentation for 48h resulted in a *B. lactis* Bb12 count of up to 8 $\log_{10}$ cfu/g, and a pH of 3.9–5.0. In their study, Hua et al. (2020), have fermented pieces of raw silver carp with chopped fresh red chili, salt and other ingredients at a temperature of 25 °C for a total of 8 weeks using the starter cultures (Sacco, Italy) SBM-52 containing *Staphylococcus xylosus, Staphylococcus carnosus, P. pentosaceus, Pediococcus lactis* and WBX-43 containing *S. xylosus*, *S. carnosus*. After one week of fermentation the LAB counts were 7.88 $\log_{10}$ and 7.6 $\log_{10}$, respectively, and after 8 weeks fermentations 6.37 $\log_{10}$ and 6.31 $\log_{10}$, respectively. A pH of approximately 4 was reported after one week of fermentation, and similarly, after 8 weeks of fermentation. The results of the current study are in line with the previous studies, showing that slightly lower pH values and comparable LAB counts can be obtained by controlled fermentation of Baltic herring fillets with commercial lactic acid bacteria starter B-7.

3.2. Microbiological quality and safety

To evaluate microbiological food safety, the presence of foodborne pathogens and food spoilage microbes was measured.

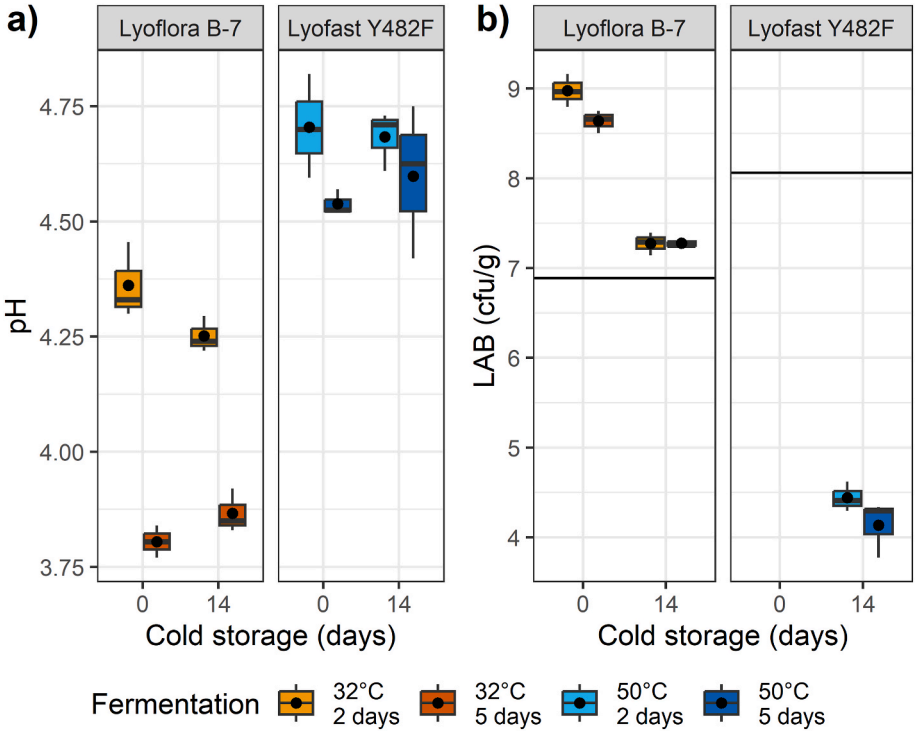

Fig. 1. Boxplot of the pH and number of LAB in the Baltic herring fillets after fermentation and two weeks of cold storage at 2 °C. Averages are indicated with black dots. Horizontal lines in b) indicate initial LAB counts of the starter cultures.

The microbiological quality of the fermented products is presented in Table 2. In all samples *L. monocytogenes* was absent/25 g, and sulphite-reducing clostridia, hydrogen-sulphide-producing bacteria, coagulase-positive staphylococci, and enterobacteria remained below the detection limit of 1 $\log_{10}$ cfu/g, with the number of yeasts below the detection limit of 2 $\log_{10}$ cfu/g).

In the present study, the presence of a severe foodborne pathogens *L. monocytogenes* was studied based on EU regulation (EC) 1441/2007, according to which in ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes *L. monocytogenes* must be absent in 25 g. As regulation (EC) 1441/2007 lacks the guideline values for other microbes than *L. monocytogenes,* sulphite-reducing clostridia, coagulase-positive staphylococci, enterobacteria and yeasts, counts were studied according to the Finnish Food and Drink Industries' Federation's recommendation for the microbiological quality of cooked fish products and hot-smoked fish foods on the last day of use (ETL, 2022). Hydrogen sulphide-producing bacteria were included, since these spoilage bacteria are naturally present in fish (Fogarty et al., 2019).

According to the results, the fermented Baltic herring products can be considered safe, since in all the samples, *L. monocytogenes* was absent/25 g and the counts of sulphite-reducing clostridia, coagulase-positive staphylococci and enterobacteria and also yeasts were within the recommended limits (m < 1.0 $\log_{10}$ cfu/g), (m < 2.0 $\log_{10}$ cfu/g), and (m < 3.0 $\log_{10}$ cfu/g), respectively, and hydrogen sulphide-producing bacteria counts were below 1.0 $\log_{10}$ cfu/g.

The main factors affecting microbiological quality were presumably autoclaving applied as a pre-treatment and the controlled fermentation process. Autoclaving is known to inactivate vegetative microbes effectively, and therefore, in this study this was applied to ensure the rapid growth for the LAB strains of the starters applied, because the low salt concentration (2 % in brine) is inadequate to inhibit the growth of food pathogens and spoilage bacteria such as *L. monocytogenes* (Aalto-Araneda et al., 2020) and *C. botulinum* (Lindström et al., 2006) and *Shewanella* and *Pseudomonas* strains (Serio et al., 2014) respectively. In the traditional fermented fish product, e.g. *surströmming*, the high salt concentration (17 % salt in brine) and the long fermentation time (15–18 °C for 3–4 weeks) effectively inhibits the growth of harmful microbes (Skåra et al., 2015). However, options for producing new fish products with low salt content are needed to provide healthier options. During fermentation, a drop in pH (see Section 3.3) indicates that the LAB strains in the starters produced organic acids, including lactic acid, which is known to inhibit microbial growth.

3.3. Chemical safety: histamine

The results of histamine concentration (mg/kg) in the fermented products are presented in Table 3.

According to the results, the concentration of histamine was below the LOQ <10 mg/kg in all samples. Our results are quite similar to those of Köse et al. (2012), who found that in *surströmming* products, histamine concentrations ranged between 9 and 80 ppm. Histamine can cause food poisoning reactions with allergic-like symptoms burning sensation, headache and dizziness, nausea, a drop in blood pressure or breathing difficulties (Wójcik et al., 2021). Moreover, histamine is thermally stable and cannot be removed once it has been formed (Feng et al., 2016).

Table 3
The histamine concentration (mg/kg) in the fermented samples.

B-7 fermentation	Histamine, mg/kg	Y482F fermentation	Histamine, mg/kg
32 °C/2 days, AFP	<10	50 °C/2 days, AFP	<10
32 °C/2 days, 14-DS	<10	50 °C/2 days, 14-DS	<10
32 °C/5 days, AFP	<10	50 °C/5 days, AFP	<10
32 °C/5 days, 14-DS	<10	50 °C/5 days, 14-DS	<10

AFP: After fermentation period, 14-DS: after 14 days of cold storage at 2 °C.

According to the Finnish food safety authorities the risk for histamine poisoning is low, since fresh Baltic herring contains no, or a very low level of histamines (<1 mg/kg) (Ruokavirasto, 2023). In this study, the level of histamine was analysed, since it may be formed during LAB fermentation (Barbieri et al., 2019). The results revealed that the risk for histamine poisoning is low, and thus our fermented products can be considered safe. In the EU legislation guideline, there is a lack of values regarding the accepted histamine levels for fishery products from fish species associated with a low amount of histidine, but the maximum histamine level accepted for fishery products from fish species associated with a high amount of histidine is 200 mg/kg (EC) No. 2073/2005.

3.4. Nutritional composition

The effect of LAB starters, fermentation time, and storage on the proximate composition (moisture, protein, fat, and ash) and energy of the fermented fish Baltic herring are shown in Fig. 2.

The average moisture content ranged from 74.2 % to 79.6 % and from 76.0 % to 77.1 % with B-7 and Y482F respectively. When fermentation was carried out with the B-7 starter and the fermentation time was extended from 2 to 5 days, the fillets' moisture content increased when fermentation was carried out with the B-7 starter and the fermentation time was extended from 2 to 5 days, and the moisture content increased further during the two-week cold storage period ($p = 0.002$). Fermentation with Y482F did not show a similar effect on the fillets' moisture content ($p = 0.86$). This may be attributed to the differences of the bacterial composition of the starters and their characteristics. As the fermentation time was extended from 2 to 5 days, the moisture content of the fish products increased with the B-7 starter, while with the Y482F starter, it remained at about the same level. During cold storage, the moisture content of the fermented fillets increased ($p = 0.02$), although only very slightly in fillets fermented for five days with Y482F. The higher moisture content of the B-7 fermented

Table 2
Microbiological quality of the fermented products presented as not detected/25 g or Log_{10} colony forming units per gram or.

Samples	*L.mono-cytogenes*	Sulphite-reducing Clostridia	Coagulase-positive staphylococci	Hydrogen sulphide-producing bacteria	Yeast	Entero-bacteria
B-7 fermentation						
32 °C/2 days, AFP	not detected/25 g	<1	<1	<1	<2	<1
32 °C/2 days, 14-DS	not detected/25 g	<1	<1	<1	<2	<1
32 °C/5 days, AFP	not detected/25 g	<1	<1	<1	<2	<1
32 °C/5 days, 14-DS	not detected/25 g	<1	<1	<1	<2	<1
Y482F fermentation						
50 °C/2 days, AFP	not detected/25 g	<1	<1	<1	<2	<1
50 °C/2 days, 14-DS	not detected/25 g	<1	<1	<1	<2	<1
50 °C/5 days, AFP	not detected/25 g	<1	<1	<1	<2	<1
50 °C/5 days, 14-DS	not detected/25 g	<1	<1	<1	<2	<1

AFP: After fermentation period, 14-DS: after 14 days of cold storage at 2 °C.

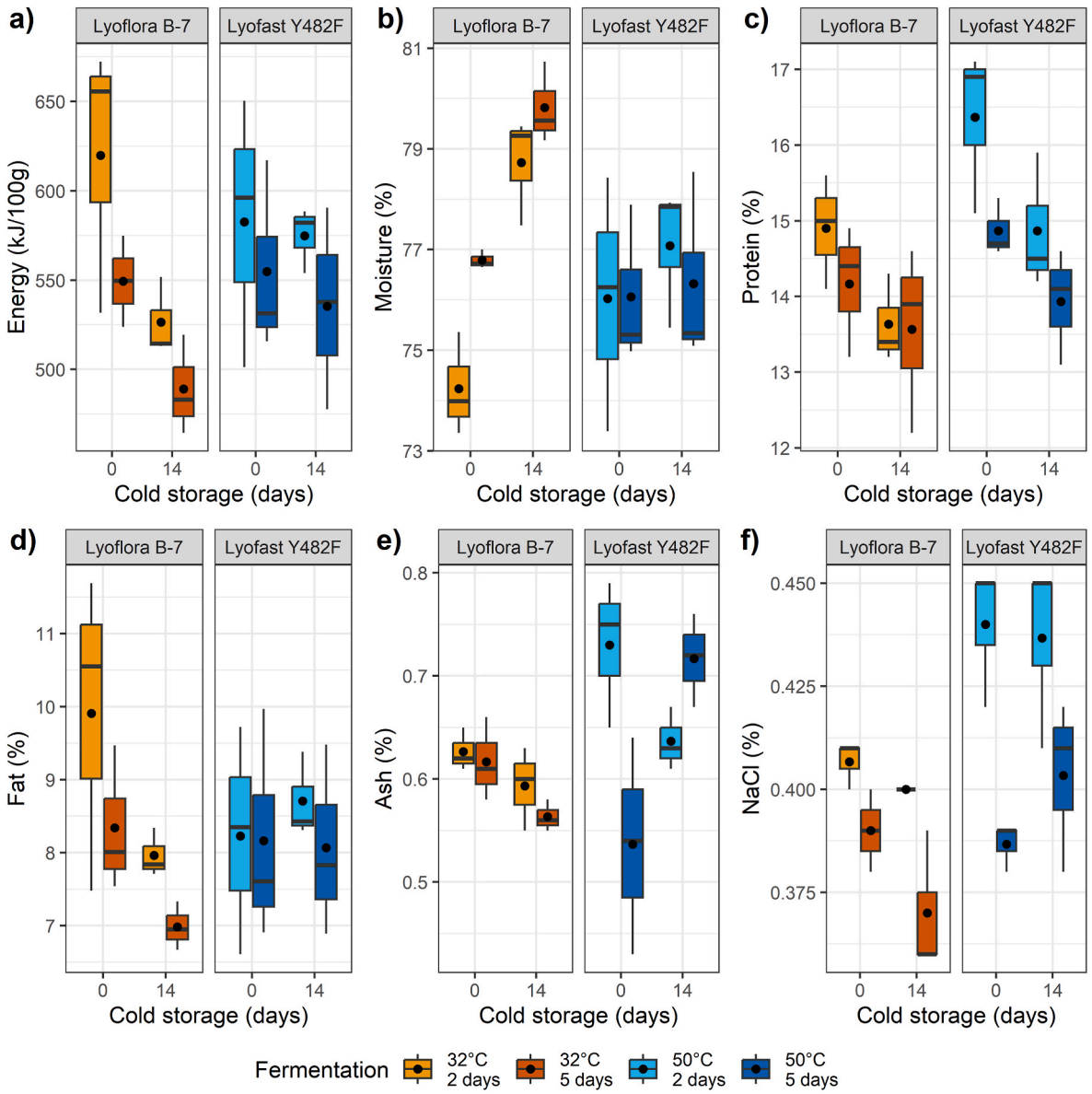

Fig. 2. Boxplot of the energy, proximate composition (moisture, protein, fat, ash), and NaCl content in Baltic herring fillets after fermentation and cold storage at 2 °C. The results are expressed as fresh weight basis. Averages are indicated with black dots.

fillets was also reflected in the sensory quality of the fish, as in the sensory panel the juiciness of the fish fillets was rated as higher with B-7 than with Y482F fermented fillets (see Section 3.8).

The average protein content of the B-7 fermented Baltic herring ranged between 13.6 % and 14.9 %, while with Y482F, it ranged between 13.9 % and 16.4 % (Fig. 2c). The average fat content of the fermented Baltic herring fillets ranged from 7.0 % to 9.9 % and 8.1 %–8.7 % for the B-7 and Y482F fermentations respectively (Fig. 2d). With fermentation time and cold storage, the ash content of the fish fillets decreased with fermentation time and cold storage, except for five-day fermented and 14-day stored fillet fermented with Y482F (Fig. 2e). The overall average ash content was practically the same with B-7 and Y482F, at 0.66 % and 0.60 % respectively.

Overall, in the fillets fermented with B-7 starter, the energy, protein, fat, NaCl, and ash content was reduced as the fermentation time was extended from 2 to 5 days and further during cold storage. This is at least partly attributed to the increase in moisture content (Fig. 2). However, reductions in the protein and NaCl content were also observed in fillets fermented with the Y482F starter, although no increase in moisture content was observed (Fig. 2b). Indeed, when the protein content of the fermented fish fillets was evaluated on a dry matter basis (data not shown), the protein content was observed to increase when the fermentation time was extended, and it increased further during storage. The only exception was with those fillets fermented with Y482F and stored for two weeks: in this case, the protein content per dry matter did not increase during storage; the reason for this remained unclear. As the protein content in the fillets increased (on a dry matter basis), and the protein molecular weight distribution was stable during fermentation and storage (see Section 3.7), the results taken together indicate the potential of the LAB strains in B-7 and Y482F starters to retain high nutritional quality protein in fish fillets.

The nutritional composition of the fermented herring fillets produced in this study differed from that of *surströmming*. According to Skåra et al. (2015), *surströmming* typically contains 11.8 % protein and 3.8 % fat, whereas the fermented fillets in this study had protein and fat contents of 13.4–16.9 % and 7.0–9.9 %, respectively. Furthermore, according to the national Food Composition Database in Finland, Fineli (2024), fresh Baltic herring fillets contain 16.0 % protein and 8.9 % fat.

Compared with these average values in Fineli (2024), the protein and fat content of the fermented fillets in this study was lower except after the 2-day fermentation with Y482F and 2-day fermentation with B-7 respectively. Generally, however, the composition of the fermented fillets produced in this study was closer to that of fresh herring fillets than *surströmming*.

3.5. Salt concentration

Fig. 2f shows that the Baltic herring fillets fermented with B-7 and Y482F starters have a low salt content (0.36–0.45 %) compared with *surströmming*, for example, which has been reported to have a salt content of 6.49–8.88 % (Belleggia et al., 2020). It is noteworthy that the salt content in the fermented fillets was only about 2–3 times higher compared to the salt content of about 0.16 % in fresh Baltic herring fillets (Fineli, 2024). Despite the low salt content, the fillets showed a sufficiently salty taste in a sensory evaluation (see 3.8)., Based on these results the objective of this study to develop a low-salt, tasty fish product was achieved.

3.6. Lipid oxidation

Fish fat contains a high amount of PUFAs. Malondialdehyde (MDA) has been broadly used to represent the degree of lipid oxidation in various meat products. MDA is formed in the second phase of lipid oxidation, during which peroxide is oxidised to aldehydes and ketones (Parisi, 2016). In the present study, we analysed how different LAB starters, fermentation conditions, and storage affected the content of MDA in Baltic herring fillets. The fermented Baltic herring fillets showed MDA content indicating a moderate level of lipid oxidation (Fig. 3). Fillets fermented with B-7 showed MDA content between 6 and 18 nmol/g, while the corresponding values for the fillets fermented with Y482F ranged between 3 and 18 nmol/g. Overall, five days of fermentation resulted in higher levels of MDA than two days of fermentation ($p = 0.0007$). Moreover, a two-week cold storage of the fillets increased the MDA content ($p = 0.0002$). Both microbial starters showed a similar effect on lipid oxidation, as no statistically significant difference was observed between the starters applied ($p = 0.72$) (Fig. 3). Minor differences in the MDA content did not affect sensory quality (see Section 3.8).

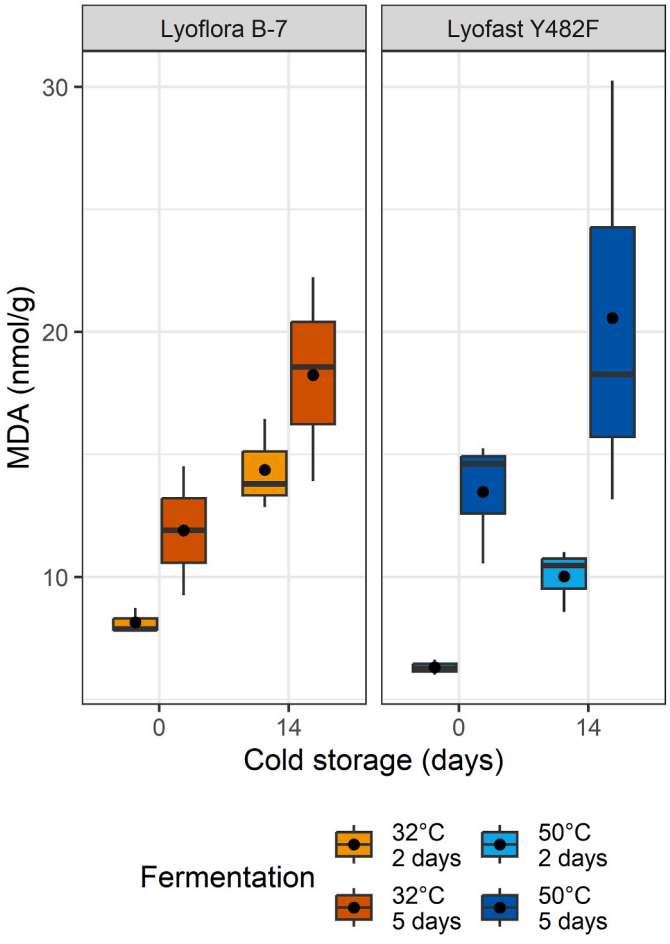

Fig. 3. Boxplot of the MDA content of Baltic herring fillets after fermentation and two weeks of cold storage at 2 °C. Averages are indicated with black dots.

The MDA content in the Baltic herring fillets fermented for two days were slightly lower than in commercial food grade protein concentrates, while the Baltic herring fillets fermented for five days showed MDA content at the same level as commercial food grade protein hydrolysates (Partanen et al., 2023). For example, a significantly higher level of lipid oxidation and MDA content has been reported for Atlantic mackerel (*Scomber scombrus*) muscle after nine days of cold storage at 4 °C (500 μmol/g) (Standal et al., 2018) and sardine (*Sardinella gibbosa*) muscle after 15 days storage at 4 °C (600 μg/g ≈ 8 μmol/g) (Chaijan et al., 2006). Higher MDA levels have also been reported for Baltic herring fillets stored in weak acids (1–2 μmol/g) (Logrén et al., 2022). The current study's results therefore indicate that LAB strains in B-7 and Y482F starters can inhibit lipid oxidation in Baltic herring fillets. Previous studies have also indicated that LAB may contribute to lipid stability and inhibit the development of rancidity (Skåra et al., 2015).

3.7. Protein size distribution

During the fermentation of meat products, one of the main biochemical reactions that occur is protein degradation. In fermented meat products, protein degradation is influenced both by endogenous enzymes in muscle tissue and by proteases produced by microorganisms involved in the fermentation process (Wang et al., 2022). Protein degradation can be monitored by determining the change in protein molecular size distribution, for example, by using SEC.

The protein size distribution of the Baltic herring fillets after fermentation and cold storage are expressed in Fig. 4. According to the results, the molecular weight distribution in the fillets was quite stable, as no significant changes during fermentation and storage were observed for MW size >10 kDa. The fillets fermented with B-7 for two days showed a slightly different molecular weight profile than the other samples: a minor fraction (about 3 % of total proteins) with MW sizes ranging from 1000 to 10,000 Da was observed. The results show that the fermentation process and storage treatment applied in the current study had no significant proteolytic effects on the Baltic herring fillets.

3.8. Sensory profile

In the current study, a trained panel evaluated the sensory properties (colour, odour, taste, flavour, texture, and overall acceptance) of the fermented Baltic herring fillets at two timepoints: at day 0 (=1 day after the fermentation periods); and after two weeks of cold storage. The samples' sensory profiles can be seen in Fig. 5. To describe the intensity of specific characteristics of fish products, we used the attributes freshness and fishiness. For fish odour, the reference is trimethylamine, while fresh refers to shelf-life properties.

PCA (Fig. 6) showed clear separation in the samples' sensory profiles originating from the B-7 and Y482F starters, cold storage, and different fermentation periods. The first two principal components dimensions explained 60 % of the total variance, with the first component appearing to be characterised mainly by texture and taste properties. The PCA components are presented in Supplemental Table S1.

Between the samples, the results of the sensory analysis showed differences in sourness, saltiness, and freshness, as well as in firmness

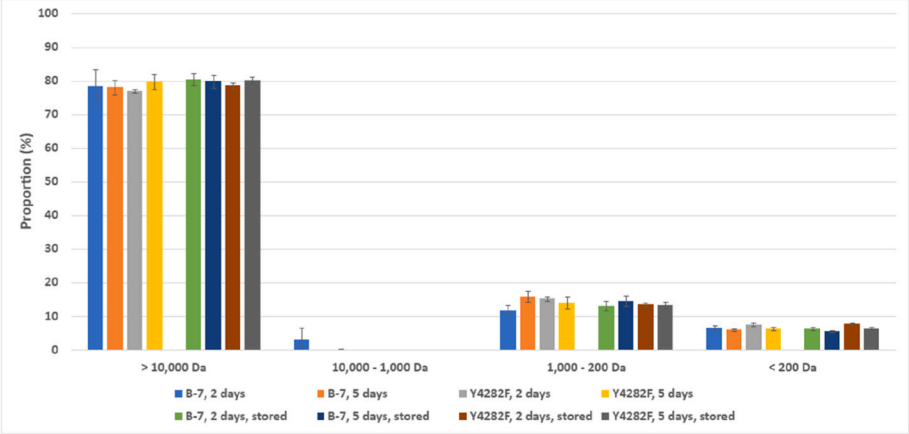

Fig. 4. Protein size distribution of the Baltic herring fillets after fermentation and cold storage for 2 weeks at 2 °C with average and standard deviation.

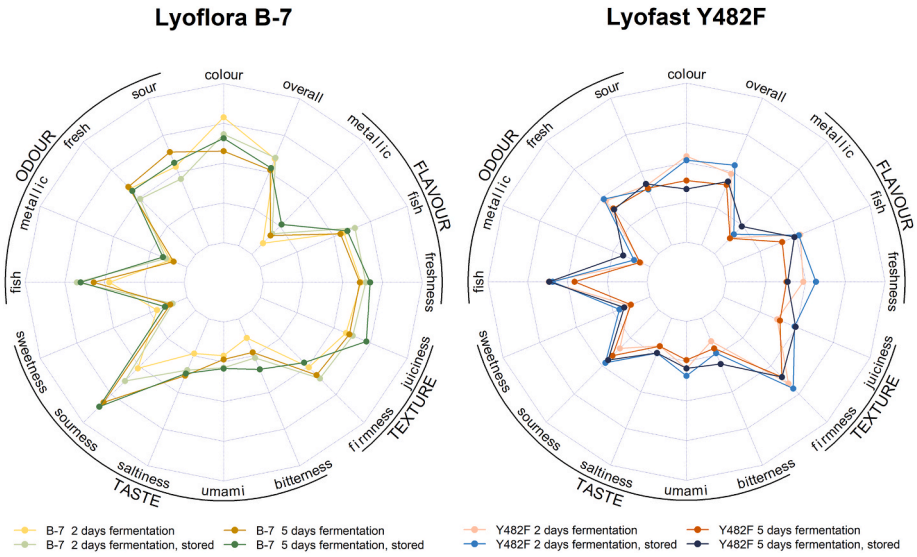

Fig. 5. The sensory profiles of the samples fermented by B-7 (a) and Y482F starters (b) after 2- and 5-day fermentation and after 14-day cold storage.

and juiciness. However, no significant differences in the sweetness, bitterness, or umami taste of the samples were observed.

Baltic herring fillets fermented using the B-7 starter were rated sourer than those fermented with Y482F ($p < 0.001$). With both starters, sourness increased when the fermentation time was extended from two to five days. The sensory results are therefore in line with the pH measured in the products. In yoghurt production, post-acidification caused by the LAB strains similar to those in Y482F can lead to a bitter taste (Leroy & De Vuyst, 2004), but this was not observed in the current experiment.

Sodium chloride (NaCl) enhances the flavour and texture of fermented fish (Petit et al., 2019). Indeed, saltiness and flavour generally attract people to consuming fermented fish (Chan et al., 2023). Though, the salt concentration of the fermented Baltic herring fillets was low (0.4 %), a sufficiently salty taste was still detected in the sensory analysis. The salty taste was found to be correlated with an acidic taste (0.82, data not shown). In the current study, the panel scored the fillets fermented with the B-7 starter as saltier and at the same time sourer than those fermented with Y482F ($p < 0.001$).

Products fermented with the B-7 starter were also scored as fresher and fishier than products fermented with Y482F ($p < 0.01$), and their overall score was slightly higher. The fish fermented with Y482F were scored as firmer ($p < 0.001$) and less juicy ($p < 0.001$) than the fish fermented with B-7, indicating that LAB selection also affected the texture of the product. Although lipid oxidation was found to increase slightly during storage (see Section 3.6, Fig. 3), but these changes were relatively minor and did not affect the sensory scores. The sensory results indicate a high potential for the fermentation process developed in the current study to produce tasty and healthy new fish products. Before commercialization, consumer tests will be needed to assess the market potential and preferences of different consumer groups.

4. Conclusion

In the present study, controlled fermentation with two commercial LAB starters was investigated to develop a new type of low-salt, fresh tasting solid fish product from Baltic herring fillets.

Although the results indicated, that both starters are applicable to ferment the Baltic herring fillets, the sensory analysis showed B-7 to result in fresher and juicier fillets in comparison to the fermentation with Y482F starter. With both starters applied, the salt content of the fermented Baltic herring fillets was low (<0.4 %), yet showing a sufficient salty taste. Food safety assessment indicated that the fermentation process developed results in microbiologically and chemically safe products. Overall, this study shows for the first time that the commercial LAB starter B-7 can positively affect the flavour of the Baltic herring and can be applied to produce new types of low-salt, solid, fish products from Baltic herring fillets. However, further studies are needed to elucidate

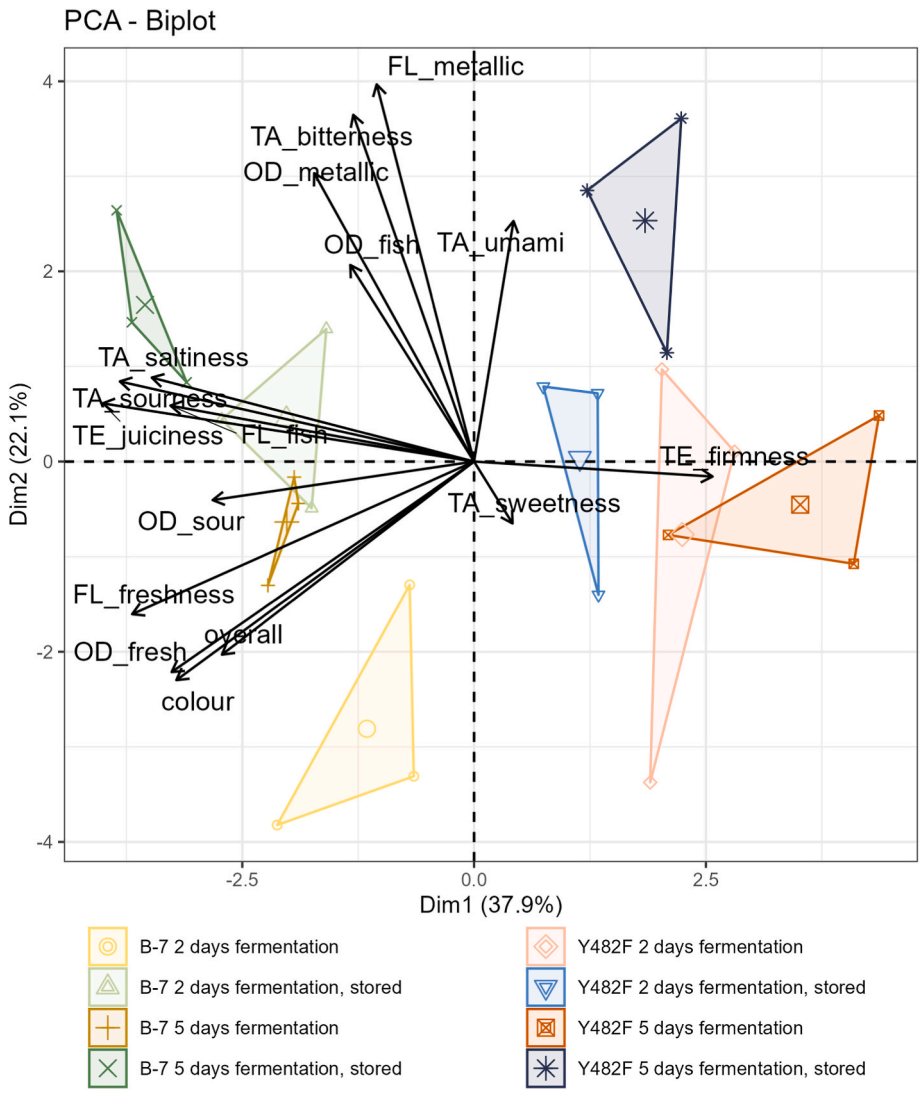

Fig. 6. Principal component analysis (PCA) biplot based on evaluated sensory properties showing loading vectors, scores, and convex hull of sample groups (clusters).

the biochemical phenomena associated with the flavour formation, for comprehensive texture investigation and to scale up the fermentation process for other underutilized fish species. Moreover, this will enable the wide utilization of the developed fermentation process in the production of new low-salt fish products.

CRediT authorship contribution statement

Anna-Liisa Välimaa: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Minna Kahala:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Jaakko Hiidenhovi:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Susanna Rokka:** Writing – review & editing, Methodology, Investigation. **Jouni Karhu:** Writing – review & editing, Software, Methodology, Formal analysis. **Sari Mäkinen:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118760.

Data availability

Data will be made available on request.

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