

Does the application of a protective microbial culture influence the quality of cooked fish sausages?

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Abstract

In this study, meat from freshly slaughtered African sharp-tooth catfish (*Clarias gariepinus*) was used to assess whether the technological application of a protective microbial culture (PMC) could influence the quality and hygienic evaluation of a model fish meat product. The fillets were processed into cooked fish sausages prepared either from freshly killed fish (F – fresh meat) or from meat stored for 3 days at 2 °C (S – stored meat). Each of these two variants was further divided into subgroups according to the application of a commercially available protective culture (*Pediococcus acidilactici*, P) or without it (control, C), resulting in a 2 × 2 factorial design. Significant differences ($P < 0.05$) were observed only in texture indicators of sausages prepared from stored fish meat. However, the application of PMC had no significant effect ($P > 0.05$) on the microbiological or overall quality indicators of fish meat and cooked sausages.

Food safety, microorganism, sensory analysis, Clarias gariepinus, African sharp-tooth catfish

Fish and fish-derived meat are generally considered highly perishable raw materials for meat product processing. This requires rapid handling, optimization of processing speed, and strict control of storage conditions within a properly established and implemented Hazard Analysis and Critical Control Points (HACCP) system. In recent years, increasing attention has been directed toward the application of protective microbial cultures (PMCs) in food production. These cultures can serve as a natural safeguard against *Listeria monocytogenes* and other microorganisms through their direct and indirect antimicrobial activity. However, some concerns remain that their use could complicate the interpretation of product hygiene or raw material freshness, as protective cultures may suppress specific indicator microorganisms or delay early spoilage manifestations, even though they are not expected to outcompete an already established spoilage microbiota.

According to the Food Safety and Nutrition Strategy 2030 of the Czech Republic, the key objective is to ensure that consumers have access to safe and high-quality food products. The document highlights that the most frequent foodborne diseases are caused by bacteria such as *Campylobacter* spp., *Salmonella* spp., Shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes* (EAGRI 2021). Protective cultures are microorganisms capable of inhibiting undesirable species through the production of antagonistic substances such as organic acids, bacteriocins, or hydrogen peroxide. Their mechanism of action includes competition for nutrients and ecological niches (Chaillou et al. 2014; Di Gioia et al. 2016; Xu et al. 2021), which can contribute to improved microbiological safety and extended shelf life of perishable foods (Holzapfel et al. 1995).

The quality indicators of perishable raw materials can fluctuate within hours, not only days. When additional factors – such as temperature management, sanitation effectiveness,

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and proper cleaning of processing and storage facilities – are not adequately maintained, the control of product quality becomes challenging, even under a certified HACCP system (Dušková et al. 2020). Furthermore, the modern meat industry is under continuous pressure from consumers to replace synthetic additives with natural and healthier alternatives (Bobko et al. 2025).

Instead of evaluating the antimicrobial effectiveness of a protective microbial culture (PMC), the objective of this study was therefore to assess whether its technological application to fish meat prior to processing could influence commonly used quality and hygienic indicators. Particular attention was paid to the total viable count (TVC), physicochemical properties, and sensory attributes, in order to determine whether the use of a protective culture could complicate the interpretation of raw material quality or mask potential hygiene-related differences in a thermally processed fish product.

Materials and Methods

Table 1. Recipe for fish sausages.

Ingredient	kg/100 kg
<i>Clarias gariepinus</i> meat	90.54
Water in form of ice	5.20
Nitrite curing salt*	2.00
Spice blend	0.96
FORRO PE*	0.30
Functional fibre	1.00

*Nitrite curing salt ingredients: vacuum salt (min. 99.6% NaCl) 99.3%, anticaking agent sodium ferrocyanide (E535) 4.0–12.0 mg/kg, potassium iodate (KIO₃) 36.0–56.0 mg/kg, equivalent iodine (I) 21.0–33.0 mg/kg, sodium nitrite E 251 (NaNO₂) 0.5–0.6%; FORRO PE (Gewürzmüller FORRO-PE BG BA, Germany): Polyphosphates E 452, dextrose, glucono-delta-lactone E 575, ascorbic acid E 300, citric acid E 330, spice extracts.

dextrose, glucono- δ -lactone, ascorbic acid, citric acid), nitrite curing salt, and functional fibre (MASOPROFIT a.s., Praha, Czech Republic). The mixture was then finely minced (8 mm plate) and stuffed (HTS 95, HTS Fleischereimaschinen, Thalgau, Austria) into natural pork casings (30/32 mm; MASOPROFIT a.s.).

The sausages were hung on racks and subjected to smoking and heat treatment in a programmable chamber (Bastramat B 850 FR, BASTRA GmbH, Arnsberg, Germany) until the internal temperature reached 70 °C for 10 min. After thermal processing, the products were cooled to 5 °C, stored at 2 °C for 24 h, and subsequently vacuum-packed (Boxer 35, Henkelman, Netherlands). All analyses were performed at the end of the declared shelf life (21 days under vacuum storage at 4 °C).

The PMC used in this study was a commercially available strain of *P. acidilactici* (B-LC-20; Novonesis, Lyngby, Denmark), applied at a dosage of 20 g/100 kg of fish meat in accordance with the manufacturer's recommendation. The viable cell concentration (CFU/g) of the added culture was not determined, as the study was not designed to monitor the growth, survival, or antimicrobial activity of the protective culture itself.

The culture was applied under technological conditions in which its metabolic activity was not expected. *Pediococcus acidilactici* exhibits a minimum growth temperature of approximately 15 °C and an optimal growth temperature around 45 °C. Therefore, no proliferation or antimicrobial effect of the culture could be assumed during chilled storage of the raw material at 2 °C or after thermal processing of the sausages (70 °C for 10 min). The purpose of including the protective culture was to evaluate whether its technological application could influence standard quality and hygiene indicators or complicate their interpretation in a cooked fish product.

Sausage production

Boneless fillets of African sharptooth catfish (*Clarias gariepinus*) were obtained from the aquaculture facilities of the Department of Zoology, Fisheries, Hydrobiology, and Apiculture at Mendel University in Brno and transported under chilled conditions (2 ± 1 °C) to the pilot plant of the Department of Food Technology (CZ 22067). Four sausage variants were prepared in two independent batches (10 kg each) according to the formulation presented in Table 1.

Fresh fish meat slaughtered on the day of production and chilled to 2 °C within 30 min was used for the first variant (C0-F; control, fresh meat). The second variant (C1-S; control, stored meat) contained *C. gariepinus* meat stored for 72 h at 2 °C prior to processing. The third variant (P0-F) was prepared from freshly killed fish meat treated with PMC (*Pediococcus acidilactici*; Novonesis, Lyngby, Denmark). The PMC was applied according to the manufacturer's instructions (20 g/100 kg of meat). The viable cell concentration expressed as CFU/g of meat was not quantified in this study. The fourth variant (P1-S) was produced identically to P0-F, except that the inoculated meat was stored for 72 h at 2 °C before processing. During storage, all samples were covered with polyethylene film to prevent surface drying.

Before processing, fish meat was trimmed of skin and visible connective tissue, coarsely minced (12 mm; TMP 23-98, Braher, Andoain, Spain), and mixed (RC-10, Manca, Barcelona, Spain) with the spice blend, FORRO PE functional additive (polyphosphates,

Chemical analysis of fish fillets and cooked sausages

The chemical composition of the fish meat and cooked sausage samples was determined to evaluate differences resulting from storage and the application of PMC. For each experimental group, approximately 250 g of homogenized sample material was analysed in triplicate to ensure accuracy and reproducibility.

The analyses included determination of moisture (g/100 g, expressed as loss on drying), crude protein (g/100 g), fat (g/100 g), and sodium chloride (g/100 g) contents. All determinations were performed according to standardized and validated procedures established by AOAC (2016).

Moisture content was measured gravimetrically by drying the samples to constant weight at 105 °C. Protein content was determined using the Kjeldahl method (conversion factor 6.25), and fat was quantified by Soxhlet extraction with petroleum ether. Salt content was measured potentiometrically using a chloride-selective electrode after sample homogenization in distilled water.

The data obtained were used to evaluate compositional differences between the four experimental variants (C0-F, C1-S, P0-F, P1-S) and to assess potential interactions between storage time and the application of PMC on proximate chemical composition.

pH

The pH of fish meat and sausage samples was determined using a calibrated pH meter (HI981036, Hanna Instruments, Rhode Island, USA) equipped with a penetration electrode suitable for semisolid samples. Measurements were performed at three stages of processing: (i) in the raw fish meat prior to mixing, (ii) immediately after heat treatment, and (iii) after 21 days of vacuum storage at 4 °C. Each measurement was conducted in five replicates per variant, and the electrode was rinsed and recalibrated between samples. Unless stated otherwise, pH values used for subsequent evaluation correspond to measurements performed after 21 days of vacuum storage at 4 °C. The results are presented as mean \pm SD.

Texture properties

The texture characteristics of cooked fish sausages were evaluated using a TIRATEST 27 025 texture analyser (TIRA Maschinenbau GmbH, Schalkau, Germany) equipped with the Meullenet-Owens Razor Shear (MORS) blade set. Cylindrical sausage samples were cut into pieces of 20 mm length and tempered to room temperature (20 ± 1 °C) prior to measurement.

The analysis was performed at a crosshead speed of 50 mm/min with a penetration depth of 10 mm. The maximum shear force (N) was recorded for each measurement. Ten replicates were analysed per treatment group to ensure statistical reliability.

The MORS method was selected as it provides a sensitive measure of surface firmness and internal structural integrity, allowing comparison of textural differences potentially induced by meat storage or application of PMC.

Colour measurement

The colour of the fish sausages was assessed using a CM-3500d spectrophotometer (Konica Minolta, Tokyo, Japan) operating within the CIELAB colour space. The instrument was calibrated against a white reference standard prior to each measurement. Colour parameters were expressed as L* (lightness), a* (red–green coordinate), and b* (yellow–blue coordinate).

Measurements were conducted under standard illumination D65 (6500 K) in the Specular Component Excluded mode using an 8 mm aperture. Each sample was evaluated both on the surface and on the freshly cut cross-section after equilibration to room temperature (20 ± 1 °C).

The total colour difference (ΔE^*_{ab} , 1976) was calculated using the C0-F variant as the reference sample, according to the formula Neves et al. (2025). Interpretation of ΔE^*_{ab} followed the visual scale proposed by Neves et al. (2025), where ΔE^*_{ab} values < 1 are considered imperceptible, 1–2 slight, 2–3 noticeable, and > 3 clearly perceivable. All measurements were performed in triplicate, and results are expressed as mean \pm SD.

Microbiological analysis

Microbiological evaluation was performed to determine the TVC, *Escherichia coli*, and other coliform bacteria in both fish meat and cooked sausage samples. Analyses followed the standardized protocol described by Kalhotka et al. (2012) for sample preparation and enumeration.

For the raw material, samples were collected within 1 h after slaughter (C0-F and P0-F) and after 72 h of chilled storage at 2 °C (C1-S and P1-S). The corresponding sausage variants were analysed after 21 days of vacuum storage at 4 °C.

Serial dilutions were prepared in sterile saline, and 1 ml aliquots were plated on Plate Count Agar (PCA; Biokar Diagnosti cs, Paris, France) for determination of total aerobic counts. Plates were incubated at 30 °C for 72 h. *Escherichia coli* and other coliform bacteria were enumerated using ECC agar (Biokar Diagnostics, Paris, France) after incubation at 37 °C for 48 h. The results are expressed as log CFU/g (mean \pm SD). The detection limit of the method was 10^2 CFU/g.

Sensory analysis

Sensory evaluation of the cooked fish sausages was conducted in the accredited sensory laboratory of the Department of Food Technology, designed in accordance with ISO 8589 requirements. The assessments were performed in individual booths under standardized illumination and controlled temperature (22 ± 1 °C) to eliminate external influences.

A trained panel of ten assessors, qualified in accordance with ISO 8586-1, carried out the evaluation. Prior to testing, panellists received a briefing and reference training to ensure consistency in scoring. Each sample was evaluated for seven hedonic descriptors: overall appearance, colour, texture, aroma, taste, acidity, and overall acceptability.

Unstructured 100 mm line scales were used for scoring, where 0 corresponded to ‘unsatisfactory’ and 100 to ‘excellent’. Sausage samples were cut into 2 mm slices, equilibrated to room temperature, and served immediately. Water and unsalted bakery products were provided as palate cleansers between samples.

Each variant was assessed in duplicate sessions, and the data were processed statistically as mean \pm SD.

Statistical analysis

All data were analysed using STATISTICA software, version 14 (TIBCO Software Inc., Palo Alto, USA). The normality of data distribution was verified using Shapiro-Wilk test. One-way analysis of variance (ANOVA) was applied to determine differences among the experimental groups, followed by Tukey’s HSD *post hoc* test for multiple comparisons.

Results were considered significant at $P < 0.05$. All measurements are presented as mean \pm standard deviation (SD). Graphical outputs were prepared using the same software to visualize differences in sensory descriptors and instrumental parameters.

Results

The proximate composition of fresh fish meat and cooked sausage samples is summarized in Table 2. The results show a distinct reduction in moisture content after storage of fish meat for 72 h at 2 °C, which can be attributed to partial surface drying during chilled storage. Correspondingly, fat and protein concentrations slightly increased as a consequence of this water loss.

Table 2. Chemical analysis of fish meat and sausages (mean \pm SD in g/100 g).

	FFM ($\bar{x} \pm$ SD)	C0-F ($\bar{x} \pm$ SD)	C1-S ($\bar{x} \pm$ SD)	P0-F ($\bar{x} \pm$ SD)	P1-S ($\bar{x} \pm$ SD)	<i>P</i>
Water	78.56 \pm 1.88 ^a	71.82 \pm 1.16 ^b	73.02 \pm 1.13 ^b	71.22 \pm 1.12 ^{bc}	70.44 \pm 1.17 ^c	0.05
Protein	18.24 \pm 1.30	19.07 \pm 1.32	18.03 \pm 1.20	18.57 \pm 1.17	19.28 \pm 1.35	0.68
Fat	2.92 \pm 0.15 ^a	4.54 \pm 0.19 ^b	4.81 \pm 0.17 ^b	4.55 \pm 0.15 ^b	5.16 \pm 0.25 ^b	0.05
NaCl	ND	2.49 \pm 0.08	2.36 \pm 0.06	2.41 \pm 0.07	2.58 \pm 0.06	

$\bar{x} \pm$ SD = mean \pm standard deviation; ND – not detected; FFM = fresh fish meat *Clarias gariepinus* fillets after slaughtering, C0-F – control group (fresh meat without protective microbial culture, PMC), C1-S (stored meat without PMC), P0-F (fresh meat with PMC), P1-S (stored meat with PMC); ^{a-c} different superscripts within the same row indicate significant differences ($P < 0.05$)

The application of PMC (*P. acidilactici*) did not significantly affect the basic chemical composition of either the raw meat or the cooked sausages ($P > 0.05$). The salt content in all sausage variants remained consistent, confirming uniform formulation and processing conditions.

The observed differences in moisture among variants (particularly between C0-F and P1-S) may influence the interpretation of texture indicators and microbial load, as surface drying can limit microbial proliferation during storage.

Microbiological results for both fish meat and cooked sausage samples are presented in Table 3. Total viable counts in fresh fish meat ranged from 3.1 to 5.91 log CFU/g, indicating a satisfactory hygienic quality of the raw material immediately after slaughter. After 72 h of storage at 2 °C, a moderate increase in microbial load was observed in the control group; however, this difference was not significant ($P > 0.05$).

Table 3. Microbiological evaluation of fish meat (mean \pm SD in log CFU/g).

Bacterial group and sample		C0-F ($\bar{x} \pm$ SD)	C1-S ($\bar{x} \pm$ SD)	P0-F ($\bar{x} \pm$ SD)	P1-S ($\bar{x} \pm$ SD)	<i>P</i>
Total plate count	fresh fish meat	3.09 \pm 0.22 ^b	3.91 \pm 0.11 ^b	5.87 \pm 0.24 ^a	5.91 \pm 0.23 ^a	0.012
	fish sausage	3.92 \pm 0.11	3.64 \pm 0.03	3.89 \pm 0.09	4.26 \pm 0.70	0.051
<i>Escherichia coli</i>	fresh fish meat	ND	ND	ND	ND	
	fish sausage	ND	ND	ND	ND	
Other coliforms	fresh fish meat	ND	ND	ND	ND	
	fish sausage	0.85 \pm 0.21	1.00 \pm 0.10	1.47 \pm 0.66	1.74 \pm 0.27	0.628

$\bar{x} \pm$ SD = mean \pm standard deviation; ND – no detected; C0-F – control group (fresh meat without protective microbial culture, PMC), C1-S (stored meat without PMC), P0-F (fresh meat with PMC), P1-S (stored meat with PMC); ^{a-b} different letters within the same row indicate significant differences ($P < 0.05$)

Following heat treatment and 21 days of vacuum storage at 4 °C, all sausage samples exhibited comparable microbial counts, typically below 4.5 log CFU/g, confirming effective pasteurization and stable storage conditions. *Escherichia coli* and other coliform bacteria were not detected in any variant, demonstrating adequate hygiene during processing and no recontamination during storage.

The application of PMC did not result in measurable differences in total aerobic counts or coliform presence. This finding indicates that PMC had no significant antimicrobial effect in cooked fish sausages under the tested conditions. It should be noted that TVC represents a non-selective microbiological indicator reflecting the overall aerobic microbial population and does not allow differentiation between intentionally added protective cultures and background microflora. Therefore, comparable TVC values observed between control and PMC-treated samples do not indicate an absence of the protective culture but rather confirm that its application did not result in measurable changes in the total aerobic microbial load under the applied storage and processing conditions.

The physico-chemical characteristics of the cooked fish sausages are presented in Table 4. After 21 days of vacuum storage at 4 °C, the pH values of all variants were comparable, ranging between 6.0 and 6.1, with no significant differences observed ($P > 0.05$). This stability indicates that the PMC did not induce acidification in the heat-treated products, which is consistent with its inactivation during cooking.

Table 4. Physicochemical properties (pH, texture, colour parameters) of cooked fish sausages after 21 days of vacuum storage at 4 °C (mean \pm SD).

Quality indicator and sample		C0-F ($\bar{x} \pm$ SD)	C1-S ($\bar{x} \pm$ SD)	P0-F ($\bar{x} \pm$ SD)	P1-S ($\bar{x} \pm$ SD)	<i>P</i>
pH		6.05 \pm 0.05	6.08 \pm 0.04	6.01 \pm 0.05	6.09 \pm 0.06	0.665
Shear force (N)		6.89 \pm 1.19 ^a	7.58 \pm 1.01 ^a	5.54 \pm 1.02 ^b	6.23 \pm 0.92 ^{ab}	0.001
<i>L</i> *	surface	36.24 \pm 3.10 ^a	37.08 \pm 2.17 ^a	38.18 \pm 2.46 ^a	41.30 \pm 1.30 ^b	0.007
	cut	51.92 \pm 0.82	54.07 \pm 3.61	55.15 \pm 1.10	54.77 \pm 1.78	0.069
<i>a</i> *	surface	17.71 \pm 1.23	17.84 \pm 0.70	17.43 \pm 1.07	17.19 \pm 1.26	0.738
	cut	8.49 \pm 0.97	8.05 \pm 1.21	8.27 \pm 0.82	8.50 \pm 0.56	0.812
<i>b</i> *	surface	21.78 \pm 2.55	22.32 \pm 1.39	22.50 \pm 2.82	24.96 \pm 0.69	0.064
	cut	9.48 \pm 0.86	9.93 \pm 1.08	10.27 \pm 0.79	12.23 \pm 1.18	0.001
ΔE_{*ab}	surface	0.00	1.01	2.09*	6.00**	
	cut	0.00	2.24*	3.33*	3.96*	

$\bar{x} \pm$ SD = average \pm standard deviation; C0-F – control group (fresh meat without protective microbial culture, PMC), C1-S (stored meat without PMC), P0-F (fresh meat with PMC), P1-S (stored meat with PMC); ^{a-b} different superscripts within the same row indicate significant differences ($P < 0.05$); ΔE_{*ab} = variant C0-F is the chosen template for calculation with other groups as a control; *noticeable difference, **medium difference

In terms of texture measured by MORS, sausages produced from fresh fish meat (C0-F and P0-F) exhibited slightly lower values compared to those prepared from stored meat. The highest shear force was recorded in the C1-S variant, whereas a significant softening effect ($P < 0.05$) was noted in the P0-F samples. These results suggest that limited proteolytic or structural changes may occur during short-term storage, potentially influenced by moisture redistribution rather than microbial activity.

Colour measurements revealed a clear increase in lightness (L^*) on the surface of sausages produced from stored meat with the protective culture (P1-S). The total colour difference (ΔE^*_{ab}) reached values above 3, corresponding to visually perceptible changes. However, no significant differences were detected in redness (a) or yellowness (b^*) parameters across treatments. The results indicate that storage time prior to processing, rather than the application of PMC, primarily affected surface brightness of the final products.

The results of the sensory evaluation are summarized in Fig. 1. Overall, panellists rated all fish sausage variants positively, with mean scores exceeding 70 mm on the 100 mm hedonic scale, confirming good overall acceptability of the products.

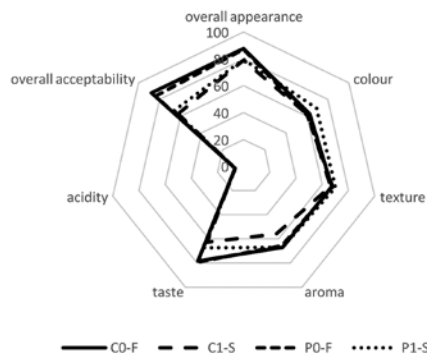


Fig. 1. Sensory analysis of fish sausages depending on treatment before production

C0-F – control group (fresh meat without protective microbial culture, PMC), C1-S (stored meat without PMC), P0-F (fresh meat with PMC), P1-S (stored meat with PMC)

Sausages prepared from fresh meat (C0-F and P0-F) received slightly higher scores for appearance, aroma, and overall acceptability compared with those made from stored meat (C1-S and P1-S). The effect of storage time was significant for overall appearance and aroma ($P < 0.05$), suggesting that even short-term chilled storage may influence the sensory freshness of fish meat used for processing.

Conversely, the application of PMC did not significantly affect any of the assessed sensory descriptors ($P > 0.05$). The trained assessors did not perceive differences in flavour intensity, acidity, or texture that could be attributed to the presence of *P. acidilactici*.

These findings are consistent with the instrumental results, confirming that the protective culture had no detectable technological or sensory impact under the applied processing and storage conditions.

Discussion

Prevention of contamination and the control of *L. monocytogenes* growth remain major challenges in food safety due to the pathogen's high mortality rate and ability to persist in processing environments (EFSA and ECDC 2024). The prevalence of *L. monocytogenes* in raw fish is estimated to be around 2.2% in Europe, with values exceeding 15% in ready-to-eat fishery products (Zakrzewski et al. 2024). This highlights the importance

of developing preservation strategies that minimize microbial risks while maintaining the sensory and nutritional quality of products.

Protective microbial cultures such as *P. acidilactici* are increasingly studied for their ability to inhibit pathogenic and spoilage microorganisms through the production of lactic acid and bacteriocins (pediocins). These effects are most pronounced in raw, fermented, or mildly processed foods, where the microorganisms remain viable and metabolically active (Komora et al. 2021). In the present study, however, the product was subjected to thermal processing at 70 °C for 10 min, which likely inactivated *P. acidilactici* cells. As a result, no antimicrobial or acidifying effect could be expressed, and the protective culture did not influence pH, microbial counts, or sensory characteristics.

The TVC is a non-selective microbiological indicator reflecting the overall aerobic microbial population and cannot be used to confirm the presence, survival, or metabolic activity of a protective culture. In the present study, PMC was applied under technological conditions in which its growth or antimicrobial activity could not be expected, due to chilled storage of the raw material and subsequent thermal processing.

Consequently, comparable TVC values observed between control and PMC-treated samples should not be interpreted as evidence of culture absence or inefficacy, but rather as confirmation that the application of PMC did not interfere with standard hygienic indicators in a thermally processed fish product. A similar interpretation of conventional quality and microbiological indicators was reported by Jůzl et al. (2025), who demonstrated that modifications in product formulation, such as salt reduction, do not necessarily lead to proportional changes in TVCs or sensory acceptability in thermally processed meat products. From a technological and regulatory perspective, this finding indicates that the use of protective cultures cannot mask insufficient raw material quality or inadequate hygiene when conventional microbiological criteria are applied.

The minor differences observed in texture (MORS) and surface lightness (L^*) can be attributed mainly to moisture loss during the 72-h storage of raw fish meat prior to processing. Storage time rather than the application of protective culture was therefore the key factor affecting these quality attributes. Similar findings were reported by Jůzl et al. (2026) and Dušková et al. (2024), who demonstrated that the effectiveness of protective cultures depends strongly on product type, temperature, and processing intensity.

From a technological perspective, the results demonstrate that the application of *P. acidilactici* PMC did not influence the quality or microbiological indicators of the product under the conditions applied in this study. The absence of any detectable effect, including in the raw material prior to heat treatment, indicates that the culture did not establish or express metabolic activity in the fish meat matrix. This finding suggests that factors such as product composition, storage temperature, and background microflora may critically limit the functionality of protective cultures in fish-based products. Therefore, no conclusions can be drawn from the present data regarding their effectiveness in other product categories, and future studies should specifically address the conditions required for the growth and activity of protective cultures in minimally processed fish products.

In conclusion, the application of *P. acidilactici* PMC in the production of cooked African sharp-tooth catfish sausages did not significantly affect the physico-chemical, microbiological, or sensory quality indicators of the products. The culture's potential antimicrobial activity could not be expressed due to the applied heat treatment.

Minor variations in texture and surface colour were associated primarily with the storage time of fish meat prior to processing rather than with the use of the culture itself. These findings confirm that PMCs cannot mask the low quality of raw materials or inadequate hygiene conditions when applied in thermally processed products.

Future research should focus on evaluating the effectiveness of such cultures in minimally processed or fermented fish products, where microbial viability and metabolic activity can contribute to both safety and sensory improvement.

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References

- AOAC 2016: Official methods of analysis of AOAC International, 20th edition. Gaithersburg, MD, USA: Association of Official Analytical Chemists
- Bobko M, Mesárošová A, Kročko M, Hanuska A, Bobková A, Poláková K, Vörösová D, Švecová T, Jurčaga L 2025: Cascara, the unusual antioxidant option for meat industry. *J Microbiol Biotechnol Food Sci* **14**: e11662
- Chaillou S, Christeans S, Rivollier M, Lucquin I, Champomier-Vergès MS, Zagorec M 2014: Quantification and efficiency of *Lactobacillus sakei* strain mixtures used as protective cultures in ground beef. *Meat Sci* **97**: 332-338
- Di Gioia D, Mazzola G, Nikodinoska I, Aloisio I, Langerholc T, Rossi M, Raimondi S, Melero, Rovira J 2016: Lactic acid bacteria as protective cultures in fermented pork meat to prevent *Clostridium* spp. growth. *Int J Food Microbiol* **235**: 53-59
- Dušková M, Morávková M, Mrázek J, Florianová M, Vorlová L, Karpíšková R 2020: Assessment of antibiotic resistance in starter and non-starter lactobacilli of food origin. *Acta Vet Brno* **89**: 401-411
- Dušková M, Dorotíková K, Macharáčková B, Ježek F, Kameník J, Šedo O 2024: The shelf life of cooked sausages with reduced salt content. *Acta Vet Brno* **93**: 115-121
- EAGRI 2021: Food Safety and Nutrition Strategy 2030, Ministry of Agriculture of the Czech Republic Website, Available at: https://www.bezpecnostpotravin.cz/UserFiles/publikace1/Strategie_EN_web.pdf. Last modified March 29, 2021. Accessed September 11, 2025
- EFSA and ECDC 2024: The European Union One Health 2023 zoonoses report. *EFSA Journal* **22**: e9106
- Jůzl M, Kameník J, Saláková A, Macharáčková B, Ježek F, Janík Piechowiczová M, Slováček J 2025: The effect of salt reduction on sensory, physicochemical, and microbial quality in selected meat products. *Foods* **14**: 3150
- Jůzl M, Kalhotka L, Kameník J, Dušková M, Ondruchová S, Slováček J 2026: Protective cultures applied in meat products: Technological functions, safety aspects and current advances: A review. *Processes* **14**: 425
- Kalhotka L, Cwíková O, Čírtková V, Matoušová Z, Přichystalová J 2012: Changes in counts of microorganisms and biogenic amines production during the manufacture of fermented sausages Poličan. *J Microbiol Biotechnol Food Sci* **2**: 667-683
- Komora N, Maciel C, Amaral RA, Fernandes R, Castro SM, Saraiva JA, Teixeira P 2021: Innovative hurdle system towards *Listeria monocytogenes* inactivation in a fermented meat sausage model - high pressure processing assisted by bacteriophage P100 and bacteriocinogenic *Pediococcus acidilactici*. *Food Res Int* **148**: 110628
- Holzappel WH, Geisen R, Schillinger U 1995: Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int J Food Microbiol* **24**: 343-362
- Neves BV, Fernandes AS, Nass P, Jacob-Lopes E, Zepka LQ, Mazzo TM, Longo E, Braga ARC, de Rosso VV 2025: Exploring different food-grade bigel systems for delivering bioactive carotenoids: Part 1 - Evaluation of color parameters. *Food Hydrocoll* **169**: 111625
- Xu MM, Kaur M, Pillidge CJ, Torle PJ 2021: Evaluation of the potential of protective cultures to extend the microbial shelf-life of chilled lamb meat. *Meat Sci* **181**: 108613
- Zakrzewski AJ, Gajewska J, Chajęcka-Wierzchowska W, Załuski D, Zadernowska A 2024: Prevalence of *Listeria monocytogenes* and other *Listeria* species in fish, fish products and fish processing environment: A systematic review and meta-analysis. *Sci Total Environ* **907**: 167912