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The Influence of Lactic Acid Bacteria Fermentation on the Bioactivity of Crayfish (*Faxonius limosus*) Meat

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Abstract: In recent years, new raw materials have been sought for use in processing. This category certainly includes invasive crayfish *Faxonius limosus*. One of the problems associated with their use is their short microbiological shelf life. Therefore, in the research presented here, an attempt was made to ferment crayfish meat with strains of *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactobacillus casei*, and yogurt culture. The analyses included an evaluation of changes in the microbial quality of the material, the content of free amino acids, reducing sugars, ascorbic acid, and the antioxidant properties of the fermented meat. Changes in the canthaxanthin content and the number of sulfhydryl groups and disulfide bridges were also evaluated. The study showed that carrying out lactic fermentation resulted in a decrease in meat pH (8.00 to 7.35–6.94, depending on the starter culture). Moreover, the meat was characterized by an increase in FRAP (2.99 to 3.60–4.06 mg AAE/g), ABTS (2.15 to 2.85–3.50 µmol Trolox/g), and reducing power (5.53 to 6.28–14.25 µmol Trolox/g). In addition, the study showed a favorable effect of fermentation on the content of sulfhydryl groups in the meat as well as for ascorbic acid content. The results obtained can serve as a starting point for the further development of fermented products based on crayfish meat.

Keywords: crayfish; *Faxonius limosus*; lactic acid bacteria; bioactive compounds; fermentation; biotransformation; antioxidants

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1. Introduction

In recent years, an increase in the food industry's interest in unconventional raw materials that can be used to develop new bioactive and functional foods has been observed. This category certainly includes freshwater crayfish. At the beginning of the 20th century, the noble crayfish (*Astacus astacus*) and the swamp crayfish (*Astacus leptodactylous*) were the most common in European waters. The meat of crayfish is characterized by a delicate taste and pleasant texture, which is why they are enjoyed and have gained popularity at the table [1]. The interest in them was so great that it was decided to enrich the local species of crayfish with others from America: spiny-cheek crayfish (*Faxonius limosus*) and signal crayfish (*Pacifastacus leniusculus*). No one at the time could have guessed what effects this action would have. The imported foreign species turned out to be strongly invasive against the native species [1–3]. This is due to the life strategy of these species and resistance to the disease known as the crayfish plague. According to Śmietana [2], the number of spiny-cheek crayfish habitats, which was four sites in 1902 in Pomerania (Poland), had increased to 865 sites in 2014. Ways to protect native species are still being researched. One of them may be the use of crayfish as a raw material in the food industry. An earlier study by Śmietana et al. [4] showed that the meat of *F. limosus* is characterized by a high protein content (18.23%) and low-fat content (0.23%). An analysis of the fatty acid profile of meat from the abdomen of *F. limosus*, which is the main source of edible

raw material, showed that, on average, 44% of the fatty acids contained in the abdomen are polyunsaturated fatty acids, 28.8% saturated and 26.8% monounsaturated fatty acids. By analyzing the parameters of culinary quality, it was shown that meat derived from the abdomen of *F. limosus* is soft, consistent, juicy, and resilient, and no foreign tastes or odors are observed during its consumption. The conducted studies also confirmed that the consumption of crayfish meat is toxicologically safe and nutritionally complete [5].

Despite the undoubtedly favorable characteristics of the raw material of crayfish meat, one of the problems that could arise in their processing is the short shelf life of the raw material. Crayfish, due to the high pH of their meat, are susceptible to the action of microorganisms. The current state of knowledge suggests that a process that extends the shelf life of *F. limosus* meat, as well as positively influences sensory and textural parameters, could be fermentation. Fermentation is one of the oldest methods used to extend the shelf life of food while affecting its color, texture, and taste [6–8]. The strains that are most used for meat fermentation are lactic acid bacteria (LAB) [6]. The effect of their application is to inhibit the formation of pathogenic microflora and prolong the shelf life of meat [9]. Until now, for aquatic animal sources, this process has been successfully used in the production technology of fermented sausages and pastes in which the main material is the meat of fish and shellfish animals [6–14].

Thus far, there is no information about crayfish fermentation; therefore, in the research described here, we attempted to use three probiotic strains and a yogurt culture for the fermentation of *F. limosus* meat. In addition, changes in the bioactive properties of the fermented meat were studied.

2. Materials and Methods

2.1. Materials and Reagents

F. limosus was caught in Lake Sominko (54°4′47″ N 17°52′48″ E) in September 2022. A yogurt starter culture (YO 122, consisting of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was procured from Biochem Srl (Via Salaria, Italy). Strains of *Lactiplantibacillus plantarum* ATCC®8014, *Lacticaseibacillus rhamnosus* ATCC®53103, and *Lacticaseibacillus casei* ATCC®393 were obtained from Microbiologics (St. Cloud, MN, USA). MRS agar and broth were procured from Merck (Darmstadt, Germany). Ethanol, chloroform, and methanol were delivered by Chempur (Chempur, Piekary Śląskie, Poland. 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium dihydrogen phosphate, sodium phosphate dibasic heptahydrate, potassium hexacyanoferrate (III), trichloroacetic acid (TCA), iron (II) chloride, glacial acetic acid, cadmium chloride, 2,2-dihydroxyindane-1,3-dione (ninhydrin), glycine, sodium acetate, acetic acid, 3,5-dinitrosalicylic acid (DNS reagent), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS reagent), diammonium salt sodium hydroxide, potassium sodium tartrate tetrahydrate, tris, EDTA buffer pH 8.0, Trolox, ascorbic acid, urea, and 5,5′-dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent), 2,6-dichlorophenolindophenol, and oxalic acid were purchased from Merck (Darmstadt, Germany).

2.2. Fermentation and Preparation of the Samples

Immediately after being caught, the crayfish were placed in a container filled with lake water at a temperature of 6 °C and transported to the laboratory. While awaiting the analysis, the specimens were stored at 4 °C. The analysis began by cleaning the crayfish and subjecting them to a 12-min heat treatment in 100 °C water with 2% NaCl. The *F. limosus* were then cooled and the meat contained in the claws and abdomen was collected. The collected research material was then frozen for further research. The purchased bacterial strains: *L. plantarum* ATCC 8014, *L. rhamnosus* ATCC 53103, and *L. casei* ATCC 393 were cultured overnight in MRS broth under anaerobic conditions at 37 °C for 24 h. After the incubation stage, the broth with the grown microorganisms was transferred to sterile Falcon tubes. The whole thing was then centrifuged (6000 rpm for 5 min). The resulting bacterial biomass was then suspended in 4 mL of 0.9% saline to a density of 2 McFarland. The inoculation with yogurt culture was performed by adding the culture to the sample at

a density of 2 McFarland. Before inoculation, the meat samples were homogenized and mixed using a domestic blender. The prepared bacterial suspensions were inoculated separately into the meat of the crayfish (60 g), then the samples were mixed thoroughly. All samples were fermented for 24 h at 37 °C.

2.3. Microbiological Analyses, Determination of pH, Titratable Acidity (TA) and Total Solids Content (TSC)

With the aim of determining the TA and microbial counts, 1 g of samples were taken, then diluted with 9 mL of sterile saline (0.9% NaCl) followed by serial dilutions. The content of LAB was examined by inoculation with MRS agar. The prepared samples were cultured at 37 °C under anaerobic conditions for 72 h. The resulting number of viable cells was expressed as a CFU/g sample. After sampling for microbiological analysis, the meat in saline was centrifuged (6000 rpm), after which 5 mL was taken, then titrated with a 0.001 M sodium hydroxide solution using phenolphthalein as an indicator, and expressed as mg of lactic acid per 1 g of sample. A pH meter (CP-411, Elmetron, Zabrze, Poland) was used to determine the pH of the samples by immersing the device's probe directly into the homogenized meat (control) as well as the fermented samples. The Association of Official Agricultural Chemists (AOAC) standard method (No. 925.23) was used to evaluate the total solids content (TSC) of the samples [15]. All analyses were carried out with a triplicate determination.

2.4. Preparation of Extracts

The methodology for preparing the extracts has been described in detail in previous studies [16]. The whole experimental *F. limosus* meat samples were subjected to a freeze-drying process for 24 h in a Beta 2-8 LSC plus freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) under the following conditions: chamber pressure of 0.190 mbar, shelf temperature T_{min} = −35 °C, T_{max} = 20 °C, condenser temperature −85 °C. To prepare the water–ethanol extracts, 50 mL of the water–ethanol mixture (50/50) was added to 0.5 g of the sample. The whole mixture was sonicated for 30 min (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) and then centrifuged (6000 rpm for 5 min). The mixture was then filtered through nylon membrane filters (0.22 µm; Sigma-Aldrich, Darmstadt, Germany) into sterile Falcon tubes, and used for further analyses.

2.5. Determination of Reducing Sugars Content (RSC) and Total Free Amino Acids Level (TFAAL)

The content of RSC in the samples was assayed according to the methodology previously described by Łopusiewicz et al. [17]. In total, 1 mL of 0.05 M acetate buffer (pH 4.8) and 3 mL of DNS reagent were mixed with 1 mL of extract. The whole mixture was shaken vigorously, then incubated in boiling water for 5 min, and subsequently cooled to room temperature. The absorbance was measured using a microplate reader (Synergy LX, BioTek, Winooski, VT, USA) at a wavelength of 540 nm. Glucose (0.01–10 mg/mL) in an acetate buffer was used as the standard.

The total free amino acids (TFAAL) were analyzed using a Cd-ninhydrin reagent. In total, 1 mL of extract was combined with 2 mL of ninhydrin. The mixture was vortexed and placed in a water bath for 5 min at 84 °C followed by cooling in ice. The absorbance was measured at 507 nm. The results are presented in mg of glycine (Gly) per gram of sample.

2.6. Determination of Reducing Rower, DPPH, FRAP, ABTS, and Ascorbic Acid Content

To determine the reducing power (RP), 100 µL of supernatant was added to 250 µL of phosphate buffer solution (0.2 M, pH 6.6) and 250 µL of 1% potassium ferricyanide. The samples were incubated for 20 min at 50 °C and then 250 µL of 10% trichloroacetic acid was added to the total. After centrifugation (3000 rpm for 10 min; centrifuge 5418 Eppendorf, Warsaw, Poland), 500 µL of the supernatant was diluted with the same volume of deionized water. Finally, 25 µL of 0.1% ferric chloride solution was added. The absorbance was measured at 700 nm. The standard curve was prepared using Trolox [17].

The radical scavenging activity of DPPH was determined as described elsewhere [16]. The DPPH was determined by mixing 1 mL of supernatants with 1 mL of 0.01 mM methanolic DPPH solution and the absorbance was measured with a UV–VIS Thermo Scientific Evolution 220 spectrophotometer (Waltham, MA, USA) at 517 nm.

The FRAP was analyzed by combining 20 µL methanol extracts with 280 µL FRAP reagent. The whole mixture was stirred on a plate for 10 s. A Thermo Scientific Evolution 220 UV-VIS spectrophotometer (Waltham, MA, USA) was used to measure the absorbance at 507 nm. The standard curve was prepared using ascorbic acid [17].

The ABTS was determined according to previous studies [18]. In total, 25 µL supernatant was mixed with 1.5 mL of ABTS reagent. The mixture was incubated in the dark for 6 min and then the absorbance was measured at 734 nm. The standard curve was prepared using Trolox.

The total ascorbic acid content was determined according to the methodology described in the previous work [19]. The method is based on the titration of samples with 2,6-dichlorophenolindophenol. To 2 mL of the supernatant, 2 mL of 2% oxalic acid was added and vigorously mixed. The mixture was titrated with 2,6-dichlorophenolindophenol until it turned pink for 30 s. The result was expressed in milligrams of ascorbic acid per gram of sample.

2.7. Determination of Canthaxanthin

Chloroform extracts were used to study changes in the canthaxanthin (carotenoid) content. Extracts were prepared by combining 0.25 g of the lyophilized sample with 10 mL of chloroform. The whole mixture was sonified for 30 min (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany). The extracts were centrifuged (6000 rpm for 5 min). The detection of spectra was carried out using a UV-Vis spectrophotometer at 400–800 nm.

2.8. FTIR Analyses

The FTIR spectra of the samples were obtained at room temperature by attenuated total reflection with an FTIR spectrometer (Perkin Elmer Spectrophotometer 100, Waltham, MA, USA). In total, 40 scans of samples in the range of 650 cm^{-1} to 4000 cm^{-1} were carried out. The obtained spectra were analyzed using SPECTRUM software (v10, Perkin Elmer, Waltham, MA, USA).

2.9. Determination of Sulfhydryl Groups (–SH) and Disulfide Bonds (–S–S–) Contents

Studies on the content of sulfhydryl groups (–SH) and disulfide bonds (–S–S–) were carried out according to the methodology previously described by Gong et al. [20]. To 180 mg of lyophilized crayfish meat samples, 30 mL of Tris-Glycine buffer and 14.414 g of urea were added. The whole mixture was stirred for 30 min, and subsequently, centrifuged at 10,000 g for 10 min. The supernatant was collected to use in further tests. To measure the content of sulfhydryl –SH groups, 160 mL of Ellman's reagent was added to 4 mL of supernatant. The absorbance was measured at 412 nm. To measure –S–S– content, 8 mL of β-mercaptoethanol was added to 4 mL of supernatant. Then, the mixture was incubated at 25 °C for 2 hr. Subsequently, 10 mL of 12% trichloroacetic acid (TCA) was added and incubated again at 25 °C for another 1 h. The whole mixture was centrifuged (6000 rpm for 10 min). The resulting precipitate was washed three times with TCA buffer and dissolved with 6 mL of Tris-Glycine buffer. Then, 4 mL of the resulting supernatant was combined with 160 mL of Ellman's reagent (4 mg/mL). The absorbance was determined at 412 nm. The—SH and –S–S– contents were calculated using the following equations:

$$\text{–SH } (\mu\text{mol/g}) = \frac{7.53 \times A_{412}}{C}$$

$$\text{–S–S–}(\mu\text{mol/g}) = \frac{Q_1 - Q_2}{2}$$

where A_{412} is the absorbance at 412 nm; C is the sample concentration, and Q_1 and Q_2 stand for the –SH contents before and after β-mercaptoethanol addition in the supernatants, respectively.

2.10. Color Measurements

The color of the test samples was measured using a Konica Minolta CR-5 colorimeter with the Hunter LAB color system (Konica Minolta, Osaka, Japan). The color was expressed in accordance with the CIELab system and the parameters determined were: lightness (L* = 0 (black) and L* = 100 (white), and chromatic components: a* (−a* = greenness and +a* = redness) and b* (−b* = blueness and +b* = yellowness). All analyses were conducted in three independent trials and the results are presented as means with ± standard deviation.

2.11. Statistical Analysis

All obtained results were statistically analyzed using Statistica version 13 software (StatSoft Poland, Krakow, Poland) and presented with ± standard deviation (SD). Two-way ANOVA and Fisher's NIR test were used to determine statistical significance. Results were considered statistically significant when the *p*-value was less than <0.05.

3. Results and Discussion

3.1. The Changes in pH, TA and TSC

One of the most important factors contributing to the shortened shelf life of *F. limosus* meat is the high pH proven in previous studies [4]. Changes in pH and titratable acidity (TA) are among the main indicators for proof of fermentation. In the tests conducted, the initial pH of the meat was 8.00 ± 0.01 (Table 1). It was noted that a decrease in pH occurred in all of the fermented variants that were used for fermentation ($p < 0.05$). The highest decrease was noticed in the yogurt culture used (6.94 ± 0.02). Similar results were noted by Hu et al. [21], who fermented silver carp meat sausage in their study, as well as by Nursyam [22], who fermented tuna fish sausage. As the pH of the samples decreased, a significant increase in titratable acidity was observed. The highest increase in TA was observed for meat fermented with the strain *L. casei* ATCC 393 (0.93 ± 0.25 mg/g). The observed changes in acidity could be due to the production of lactic acid by LAB. It has been proven in the literature that LAB can produce organic acids, thus, contributing to lowering the pH of the samples. The observed phenomenon is a natural result of microorganisms' metabolism [17]. The different levels of acidity changes in the samples depending on the cultures used may have been due to the individual characteristics of the strains. Nie et al. [23] focused on fermented carp sausage in their study. The researchers showed a significant decrease in the pH of the meat as a result of fermentation with *L. plantarum* and the *Saccharomyces cerevisiae* yeast strains. This is a result that is consistent with studies conducted on crayfish meat. The highest ability of the yogurt culture to lower the pH may be due to the composition of the applied culture, which includes two synergistic strains: *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. In addition, the differences in pH reduction between the strains used may have been due to the characteristics of the heterofermentative strains. Yin et al. [24] investigated the fermentability of minced mackerel meat by different types of lactic acid bacteria strains. They showed significant differences depending on the strains used. The authors recorded the highest decrease in pH when fermented with *L. plantarum* and *L. lactis* strains. According to Szparaga et al., heterofermentative bacteria are capable of producing CO_2, acetic acid, acetaldehyde, and/or ethanol. In addition, they are characterized by different pathways of carbohydrate fermentation mechanisms which may explain the differences observed in the study [25]. Analyzing changes in dry matter as a result of fermentation, a decrease was noted. The lowest decrease was noticed when the yogurt culture was used (19.26 ± 0.02%) with an initial value of 19.59 ± 0.58%. In the case of the samples fermented with the other strains, the dry matter content was similar, ranging from 16.66 ± 0.15% for *L. casei* ATCC 393 to 16.81 ± 0.25% for *L. plantarum* ATCC 8014.

Table 1. Changes in total solid content (TSC), pH, and titratable acidity (TA).

	0	1
TSC (%)		
Control	19.59 ± 0.58 [a]	
Lactiplantibacillus plantarum ATCC 8014		16.81 ± 0.25 [b]
Lactobacillus casei ATCC 393		16.66 ± 0.15 [b]
Lacticaseibacillus rhamnosus ATCC 53103		16.74 ± 0.21 [b]
Yogurt culture		19.26 ± 0.16 [a]
pH		
Control	8.00 ± 0.01 [a]	
Lactiplantibacillus plantarum ATCC 8014		7.35 ± 0.02 [d]
Lactobacillus casei ATCC 393		7.01 ± 0.03 [b]
Lacticaseibacillus rhamnosus ATCC 53103		7.11 ± 0.02 [c]
Yogurt culture		6.94 ± 0.01 [b]
TA (mg/g)		
Control	0.18 ± 0.23 [a]	
Lactiplantibacillus plantarum ATCC 8014		0.40 ± 0.02 [ab]
Lactobacillus casei ATCC 393		0.93 ± 0.25 [c]
Lacticaseibacillus rhamnosus ATCC 53103		0.52 ± 0.06 [b]
Yogurt culture		0.62 ± 0.04 [b]

Values are means ± standard deviation of triplicate determinations. Means with different lowercase letters are significantly different at $p < 0.05$.

3.2. Changes in LAB Content

Changes in the number of LAB are shown in Figure 1. The viability of microorganisms has a significant impact on the quality of meat and could also extend its shelf life. The content of LAB is congruent with the observed changes in sample acidity. The highest increase relative to the unfermented sample was observed in the sample with the yogurt culture ($p < 0.05$). This may be due to the symbiotic composition of the strains forming it. The increase in LAB content is consistent with the results previously obtained by Sakhare et al. [26]. These researchers showed that in mutton meat fermented with a mixture of *L. plantarum*, *L. casei*, and *L. lactis* strains, there was a significant increase in lactic acid bacteria after 24 h of fermentation. Similar results were obtained by Riebroy et al. [27], when studying the changes that occured during the fermentation of Som-fug paste produced from various species of marine fish.

3.3. The Changes in Reducing Sugars Content and Total Free Amino Acids Level

Table 2 shows the results of changes in the reducing sugar content (RSC). RSC statistically significantly decreased ($p < 0.05$) as a result of the fermentation. This behavior has been reported in previous studies by Li et al. [28] in trimming pork hydrolysate after fermentation with LAB. These researchers demonstrated a significant decrease in reducing sugars over four days of fermentation. They also noted differences in the RSC content between the strains used. The observed decrease in the amount of reducing sugars in crayfish meat may be because microorganisms use the sugars as a substrate necessary for the fermentation process. The greatest reduction was observed in the case of fermentation with the strain *L. rhamnosus* ATTC 53103 (from 12.25 ± 0.06 mg/g to 11.10 ± 0.51 mg/g). According to available reports [16–18], the complete breakdown of reducing sugars occurs around 14 days. Choi et al. [29] showed that the highest decrease in reducing sugars in

kimchi fermented with anchovy sauce of different nitrogen contents occurred on day 14. On the first day of fermentation, the breakdown of complex sugars into disaccharides begins. LAB cannot completely utilize them, which would explain the relationship between the amount of LAB to the degree of reducing sugars.

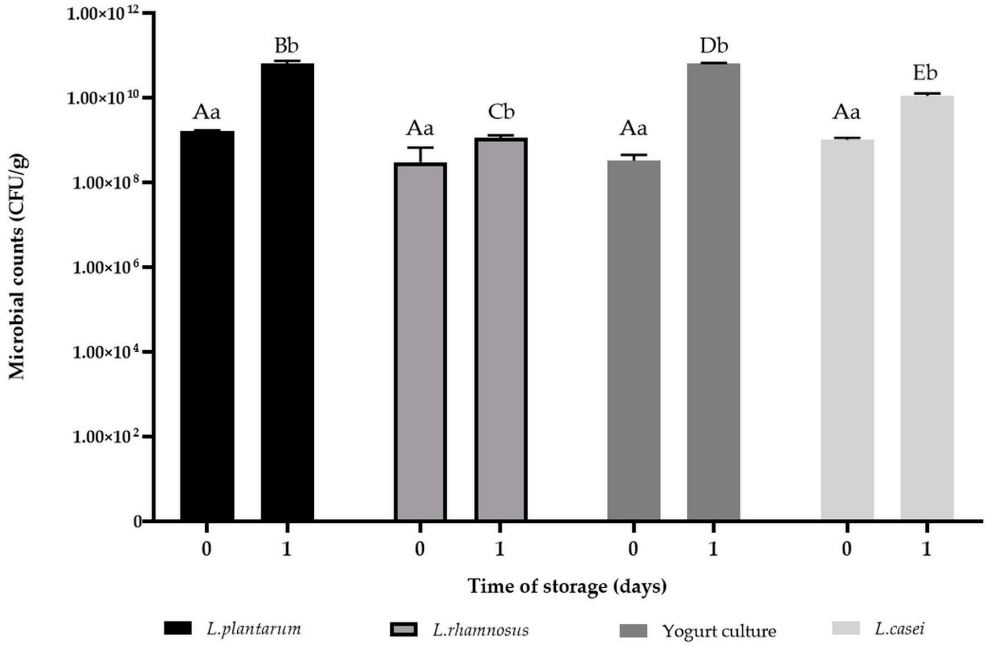

Figure 1. Lactic acid bacteria (LAB) counts. Values are means ± standard deviation of triplicate determinations. Means with different lowercase letters are significantly different between the strains at $p < 0.05$. Means with different uppercase letters are significantly different between days.

Table 2. Changes in Reducing Sugars Content (RSC) and Total Free Amino Acids Level (TFAAL).

	RSC (mg/g)
Control	12.25 ± 0.06 ^{a}
Lactiplantibacillus plantarum ATCC 8014	11.74 ± 0.22 ^{c}
Lactobacillus casei ATCC 393	11.39 ± 0.22 ^{bc}
Lacticaseibacillus rhamnosus ATCC 53103	11.10 ± 0.51 ^{b}
Yogurt culture	11.49 ± 0.06 ^{bc}
	TFAAL (mg/g)
Control	7.89 ± 0.12 ^{a}
Lactiplantibacillus plantarum ATCC 8014	4.68 ± 0.03 ^{b}
Lactobacillus casei ATCC 393	5.15 ± 0.37 ^{b}
Lacticaseibacillus rhamnosus ATCC 53103	9.15 ± 0.54 ^{a}
Yogurt culture	6.01 ± 0.32 ^{b}

Values are means ± standard deviation of triplicate determinations. Means with different lowercase letters are significantly different at $p < 0.05$.

The changes in TFAAL are shown in Table 2. A statistically significant decrease in TFFAL was observed among most of the fermented meat samples ($p < 0.05$). The only slight increase was observed in the *L. rhamnosus* ATTC 53103 fermented sample. However, this change was not statistically significant ($p > 0.05$). The decrease in free amino acid content may have been due to the short fermentation period of the samples. Similar results were obtained by Limsuwan et al. [30] who, in their work, studied the effect of the starter cultures used on the free amino acid content of fermented beef sausages. These researchers observed a significant decrease in TFAAL after 5 days of fermentation.

3.4. The Changes in Sulfhydryl Groups (–SH), Disulfide Bonds (–S–S–), Cantahxanthin and Functional Groups

Changes in the content of sulfhydryl groups are a characteristic feature of protein transformations. The –SH group is one of the more reactive groups in proteins. Amino acids containing sulfur in their composition are susceptible to oxidation by hydroxyl radicals. This process results in the formation of intra- or intermolecular disulfide bonds [31–33]. Sulfhydryl groups are part of the tertiary structure of proteins and participate in weak secondary bonds. The study showed an increase in –SH groups in all fermented meat samples (Table 3). The highest increase was obtained in samples subjected to fermentation with *L. casei* ATCC393 (97.69 ± 3.06 µmol/g). The obtained results may be due to differences in the proteolytic activity of the strains used for fermentation [34]. As demonstrated by Vaithiyanathan et al. [35], sulfhydryl groups have the ability to scavenge free radicals. The higher content of –SH groups in fermented meat may indicate that the strains used for fermentation compete with sulfhydryl groups for free radical capture, thereby protecting sulfhydryl groups from oxidation. The obtained results are consistent with those reported previously by Ge et al. [36] and Dai et al. [37], who showed an increase in the content of sulfhydryl groups in sausage with increasing fermentation time. Piao et al. [38] observed an increase in the content of –SH groups during 28 days of fermentation in their study on *Raja kenojei*. In addition, they proved a beneficial effect on meat texture.

Table 3. Determination of Sulfhydryl Groups (–SH) and Disulfide Bonds (–S–S–).

	–SH (µmol/g)	**–S–S– (µmol/g)**
Control	37.56 ± 0.91 ^{a}	13.64 ± 0.49 ^{a}
Lactiplantibacillus plantarum ATCC 8014	53.33 ± 0.57 ^{b}	17.83 ± 0.32 ^{b}
Lactobacillus casei ATCC 393	97.69 ± 3.06 ^{c}	28.20 ± 0.93 ^{c}
Lactobacillus rhamnosus ATCC 53103	91.52 ± 2.35 ^{cd}	9.46 ± 1.29 ^{d}
Yogurt culture	94.29 ± 3.21 ^{d}	25.47 ± 1.75 ^{e}

Means with different lowercase letters in the same column are significantly different at $p < 0.05$.

The FTIR spectroscopy technique is supported by measuring the spectrum and the intensity of infrared absorption by the sample under study. The full FTIR spectra of *F. limosus* are shown in Figure 2. The results obtained show two main areas of absorption increase at 1625 cm^{-1} and 1535 cm^{-1}. The fermentation resulted in an increase in absorbance in these areas. Differences in the amide 1 area (1625 cm^{-1}) and amide 2 areas (1535 cm^{-1}) indicate changes in the secondary structure of the proteins [39]. The amide I band (1600–1700 cm^{-1}) is comprised of overlapped band components of the protein's secondary structure, which is often utilized to estimate protein secondary structural information containing α-helix, β-sheet, β-turn, and random coil [40–43]. The increase in absorbance observed in the amide I and amide II areas are indicative of changes in the β-sheet [44]. According to the literature, 80% C=O stretching, 10% N-H bending, 10% C-N stretching can account for these changes [44]. The same authors see 60% N-H bending, and 40% C-N stretching as the cause of changes in the amide II area.

Most carotenoids show the highest absorbance in the spectral region, between 400 and 500 nm [45]. As demonstrated by Czeczuga et al. [46], the predominant carotenoid in the muscle and carapace in *F. limosus* is canthaxanthin. This carotenoid is characterized by its reddish-orange color. Canthaxanthin exhibits antioxidant properties and is capable of trapping reactive oxygen species and quenching singlet oxygen [47,48]. The study showed that *F. limosus* samples had the highest absorbance at 483 nm (Figure 3). The highest decrease was recorded for the sample fermented by *L.rhamnosus* ATCC53103 (1.074). The results obtained are in line with an earlier study by Rezzesy-Kun et al. [49], who found a decrease in carotenoid content after 24 h of carrot juice fermentation. They attributed the reason for this phenomenon to differences in the metabolism of the bacteria included

in the starter cultures. A second potential reason for the decrease in carotenoid content as a result of fermentation can be considered the conditions under which it takes place (temperature, pH of environment) [50]. The observed changes in canthaxanthin content as a result of fermentation may indicate a change in the conformation of this carotenoid. In the natural environment, canthaxanthin occurs in a stable trans form. The change of acidity in the meat observed during fermentation may contribute to the transformation of the trans form of canthaxanthin into the less stable cis form, which, due to its conformation, is more susceptible to degradation [48,51].

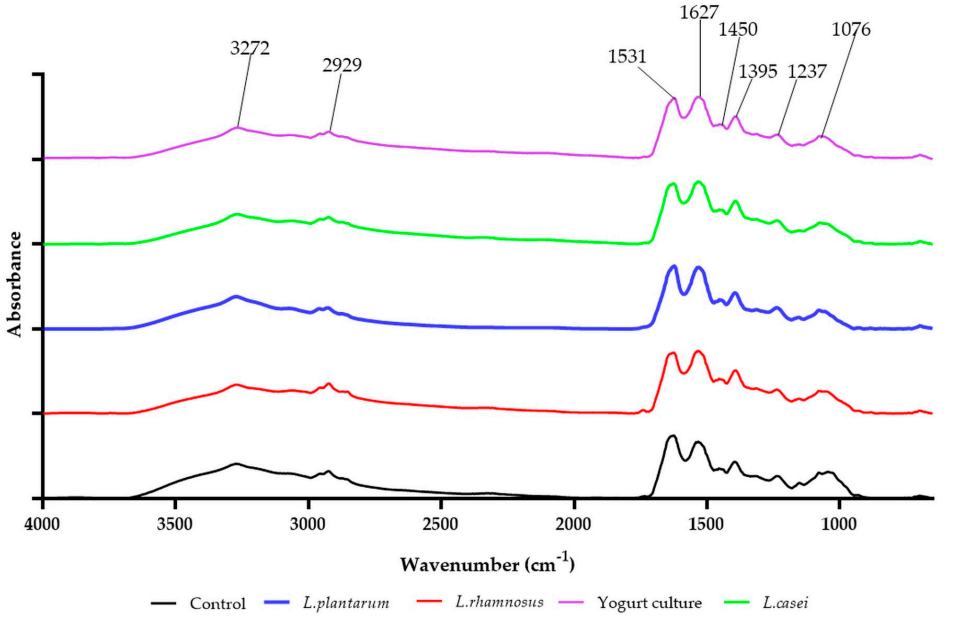

Figure 2. FTIR spectra of fermented *F. limosus* meat.

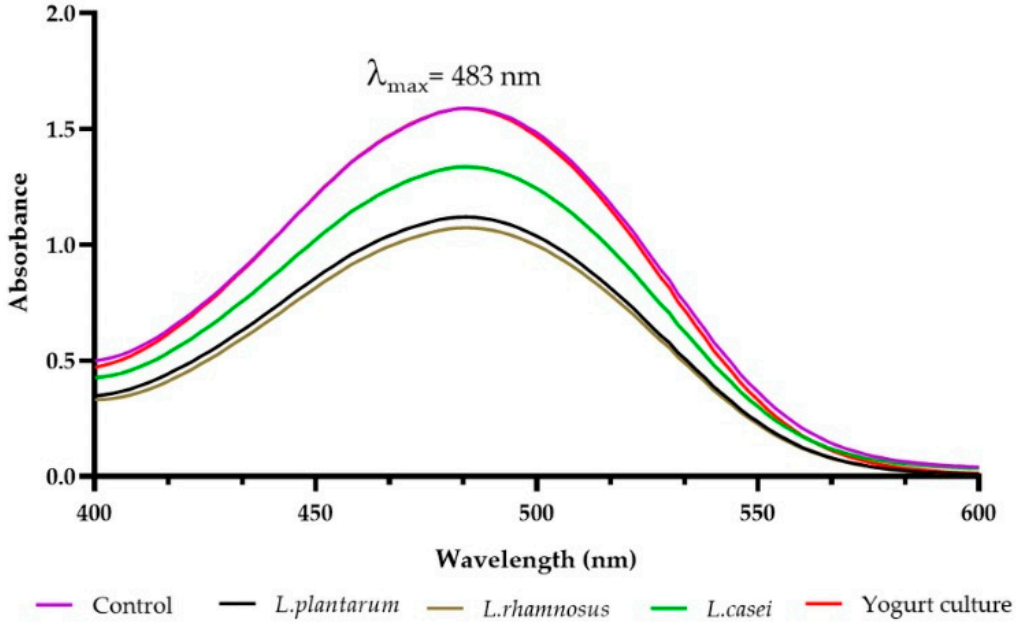

Figure 3. Changes in canthaxanthin absorbance.

3.5. Color Changes

Changes in the color of the samples are shown in Table 4. The *F. limosus* samples after fermentation were significantly lighter in color (higher L* parameter). In addition, the fermentation process significantly affected the decrease in the yellowing of the samples (b*)

and the increase in their redness (a*). The increase in redness of the samples may be due to changes in the conformational transformation of canthaxanthin occurring during fermentation. In addition, changes in the color of the meat might be a result of changes occurring in proteins. This process can result in the breakdown of natural pigments released from proteins by protease enzymes [52–56]. The increase in the L* parameter may have been influenced by the progressive denaturation of the heme proteins [27].

Table 4. Color changes of the samples.

	0			
	L*	**a***	**b***	**ΔE**
Control	58.90 ± 0.01 [a]	18.48 ± 0.01 [a]	21.81 ± 0.01 [a]	Used as a standard
	1			
Lactiplantibacillus plantarum ATCC 8014	61.26 ± 0.01 [b]	19.94 ± 0.00 [b]	20.82 ± 0.01 [b]	2.95 ± 0.01 [a]
Lactobacillus casei ATCC 393	59.92 ± 0.01 [c]	19.99 ± 0.01 [c]	21.28 ± 0.01 [c]	1.90 ± 0.01 [b]
Lacticaseibacillus rhamnosus ATCC 53103	60.44 ± 0.01 [d]	20.60 ± 0.01 [d]	21.53 ± 0.01 [d]	2.64 ± 0.00 [c]
Yogurt culture	59.26 ± 0.01 [e]	20.28 ± 0.01 [e]	21.31 ± 0.01 [e]	2.49 ± 0.01 [d]

Means with different lowercase letters in the same column are significantly different at $p < 0.05$.

3.6. The Changes in Antioxidant Activity

The changes in antioxidant activity are presented in Table 5. FRAP, ABTS, DPPH, and reducing power determinations were used to assay the antioxidant activity of the samples. The compounds with antioxidant potential may promote the protection, prevention, or reduction of the effects of oxidative stress. Fermentation with all strains increased the FRAP values ($p < 0.05$). The highest increase was observed for the sample fermented with yogurt culture (4.06 ± 0.17 mg AAE/g). This result may be due to the composition of the yogurt culture, which is composed of two bacterial strains that can contribute to the reduction of Fe^{3+} ions. The obtained values may indicate an increase in the antioxidant activity of fermented crayfish meat. The resulting increase in FRAP was consistent with the results of an earlier study by Faithong et al. [57]. These researchers showed an increase in FRAP throughout the 12-month storage period of the fermented shrimp paste. They showed that hydrolysis combined with LAB fermentation can lead to the production of active peptides that can interact with free radicals and terminate the autooxidation chain reaction. It has been reported in the literature that peptides from fermented fish products have antioxidant properties [58]. The study showed an increase in the ability of the samples to scavenge the ABTS cation radical as a result of fermentation. The highest increase was observed in the sample fermented with the *L. plantarum* strain. The results obtained are consistent with those obtained previously by Geeta et al. [59] on fermented chicken sausage. They showed an increase in the ABTS radical as a result of 24 h fermentation with *L. plantarum*.

Tests on *F. limosus* meat showed that the fermentation process induced an increase in inhibition of the DPPH radical. However, these changes were not statistically significant. The reason for this could be the short fermentation period of the samples. According to the literature, differences in radical scavenging capacity may be related to the length of peptides and differences in amino acid composition [60]. Li et al. [61] reported that high DPPH radical scavenging activity for protein hydrolysates or peptides is usually associated with a high content of hydrophobic amino acids. A study by Zhang [62] showed an increase in DPPH values in beef sausage stuffing during a 28-day fermentation process. Similar results were obtained by Akhter et al. [63], who dealt with the fermentation of low-salted squid jeotgal. They showed that the greatest increase in DPPH was observed when a strain of *L. plantarum* was used for fermentation.

Table 5. Changes in FRAP, ABTS, DPPH, Reducing Power (RP), and Ascorbic Acid Content (AAC).

	FRAP (mg AAE/g)
Control	2.99 ± 0.04 [a]
Lactiplantibacillus plantarum ATCC 8014	3.60 ± 0.06 [b]
Lactobacillus casei ATCC 393	3.69 ± 0.02 [b]
Lactobacillus rhamnosus ATCC 53103	3.78 ± 0.01 [bc]
Yogurt culture	4.06 ± 0.17 [c]
	ABTS (µmol Trolox/g)
Control	2.15 ± 0.04 [a]
Lactiplantibacillus plantarum ATCC 8014	3.50 ± 0.20 [b]
Lactobacillus casei ATCC 393	3.05 ± 0.38 [b]
Lactobacillus rhamnosus ATCC 53103	2.85 ± 0.47 [ab]
Yogurt culture	3.02 ± 0.04 [b]
	DPPH (µmol Trolox/g)
Control	2.81 ± 0.64 [a]
Lactiplantibacillus plantarum ATCC 8014	4.01 ± 0.27 [a]
Lactobacillus casei ATCC 393	3.26 ± 0.55 [a]
Lactobacillus rhamnosus ATCC 53103	4.03 ± 0.39 [a]
Yogurt culture	3.79 ± 1.05 [a]
	RP(µmol Trolox/g)
Control	5.53 ± 0.05 [a]
Lactiplantibacillus plantarum ATCC 8014	9.91 ± 0.56 [b]
Lactobacillus casei ATCC 393	6.28 ± 0.41 [a]
Lactobacillus rhamnosus ATCC 53103	12.66 ± 0.48 [c]
Yogurt culture	14.55 ± 0.25 [d]
	AAC (mg/g)
Control	5.56 ± 0.02 [a]
Lactiplantibacillus plantarum ATCC 8014	8.24 ± 0.04 [c]
Lactobacillus casei ATCC 393	7.92 ± 0.06 [b]
Lactobacillus rhamnosus ATCC 53103	7.91 ± 0.13 [b]
Yogurt culture	11.60 ± 0.17 [d]

Means with different lowercase letters in the same column are significantly different at $p < 0.05$.

In addition to the increase in DPPH and ABTS, the increase in the antioxidant potential of meat is also evidenced by the observed differences in reducing power. A significant increase in RP was observed in all fermented meat samples. The greatest increase was observed in samples fermented with the yogurt culture (14.55 ± 0.25 µmol Trolox/g). Similar observations were provided by Giri et al. [64]. They showed that miso paste prepared from squid flesh fermented with an *Aspergillus oryzae* strain was characterized by an intense increase in the iron-reducing capacity of the samples during the first 15 days of fermentation. For the next 365 days of the experiment, the reducing power factor remained stable.

The study showed that the fermentation process significantly increased ascorbic acid in the meat samples (Table 5). Ascorbic acid in food shows strong antioxidant activity [65]. It effectively quenches singlet oxygen and other radicals. In the literature, there are reports about the increase in ascorbic acid content due to fermentation in plant products [66–69]. To date, no one has attempted to study the content of ascorbic acid in crayfish and shellfish meat. The increase in ascorbic acid content as a result of fermentation confirms the increase in antioxidant activity of meat as evidenced by the increase in FRAP, ABTS, and reducing power.

4. Conclusions

Spiny-cheek crayfish are the most abundant species of crayfish in European waters. Until now, their use in the food industry has been limited. One of the main reasons for this was the short shelf life of the meat. The research described in this paper is the first attempt,

to date, to conduct the fermentation of *F.limosus* crayfish meat. As a result of the applied fermentation process, there was a significant increase in acidity as well as in the number of lactic acid bacteria. In addition, it was shown that the use of various microorganisms in *F. limosus* meat can beneficially increase the antioxidant activity of the meat. The results obtained in the present work may contribute to the development of fermented products based on crayfish meat in the future.

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