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THE INFLUENCE OF *LACTOBACILLUS PARACASEI* LPC-37 ON SELECTED PROPERTIES OF FERMENTED SAUSAGES

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ABSTRACT

Fermented sausages rank among non-heat-treated meat products. Their nutritional properties are similar to the raw material, simultaneously their microbial safety and stability is ensured using additives and specific microbial cultures. The use of probiotic cultures can positively affect the processing of fermented sausages, resulting in the new technological properties and beneficial effect on human health. However, commercial application of probiotic microorganisms in fermented sausages is not common yet. *Lactobacillus paracasei* LPC-37 is a gram-positive, non-spore forming, homofermentative rod, which according to studies may modulate immune responses in human organism and survives the passage through the gastrointestinal tract. The main object of this work was to evaluate technological properties of *L. paracasei* LPC-37, which have not been fully examined. Two groups of fermented sausages were analysed in this work. The first group of fermented sausages was prepared using lyophilized starter culture (Lyocarni RHM-33). The second group of fermented sausages was prepared by the combination of lyophilized starter culture and potential probiotic culture *Lactobacillus paracasei* LPC-37. The processing and ripening of sausages were carried out in meat processing plant to simulate real conditions of production. The changes of the products (water activity, pH, concentration of organic acids and microbial growth) were evaluated during ripening (3 weeks), while sensory analysis was carried out in the final stage of the process and during storage (3 weeks). It was found that the environment of raw-fermented sausages is suitable for the growth and survival of *Lactobacillus paracasei* LPC-37 and the microbiological quality of the final product was very good (absence of *Salmonella* and *Listeria monocytogenes*). The counts of lactobacilli reached 10^7 CFU/g of the product, which meet the requirements for functional foods. The results of the sensory evaluation showed that the overall quality of raw-fermented sausages with *Lactobacillus paracasei* LPC-37 was satisfactory and differed mainly in two taste descriptors (meaty and sour).

Keywords: fermented sausage; ripening; probiotic culture; starter culture

INTRODUCTION

The quality and microbial stability of the fermented meat products depends on the raw meat quality, additives, conditions of production, and at least, on microbial cultures used in their processing. The starter cultures that are used in production of fermented sausages basically consist of staphylococci bacteria mainly in combination with lactobacilli or pediococci, *which* control the safety of processing and positively influence the sensory characteristics of the product. The sausage matrix, non-heat-treatement processing, and possible storage up to 24 °C represent optimal conditions for growth and another reprodution of beneficial bacteria. This fact allows to use probiotic cultures for achieving the new technological and nutritional properties of fermented sausages.

Lactobacillus paracasei LPC-37 is a gram-positive, non-spore forming, homofermentative rod **(Trautvetter et al., 2012).**

L. paracasei LPC-37 has been the subject of several research works focused mainly on its probiotic effects. In **2007, Roessler et al.**, conducted a double-blind, placebo-controlled, randomized crossover study in healthy adults and patients with atopic dermatitis to examine the impact of a probiotic drink containing a combination of the probiotics *Lactobacillus paracasei* LPC-37 (3.9×10^8 CFU/g), *Lactobacillus acidophilus* 74-2 and *Bifidobacterium lactis* 420, on clinical and immunological parameters and their ability to survive the passage through the human GIT. *L. paracasei* LPC-37 was able to colonize transiently the intestine and was found in high numbers in faeces. No significant improvement of the skin conditions in patients with atopic dermatitis results from the study but the symptoms were described as "allayed" **(Roessler et al., 2007).** The study of **Paineau et al. (2008)**, indicates that *L. paracasei* LPC-37 (1×10^{10} CFU) and several other probiotic strains may modulate immune responses of human organism (tested with oral vaccination).

The placebo controlled study of **Engelbrektson et al. (2009)**, showed that *Lactobacillus paracasei* LPC-37 in combination with *Bifidobacterium lactis* Bl-04, *Bifidobacterium lactis* Bi-07, *Lactobacillus acidophilus* NCFM and *Bifidobacterium bifidum* Bb-02 (4×10^{10} CFU/g) minimized the disruption of faecal microbiota of healthy subjects undergoing antibiotic therapy. A similar work, which ranks among the largest,

placebo controlled studies with probiotics and antibiotic associated diarrhoea, was made by **Ouwehand et al. (2014)**. The same type of probiotic mixture as the above-mentioned was used. High doses of mixture consumed: 1.70×10^{10} CFU/g resulted in reduce of the incidence of antibiotic associated diarrhoea from 24.6 to 12.5%, significant reduce of abdominal pain and bloating.

However, there is still a need to acomplish the other relevant, randomized, double blind clinical studies to investigate the beneficial effect of *Lactobacillus paracasei* LPC-37 on human organism.

MATERIAL AND METHODOLOGY

1. Sample preparation

The first group of samples was prepared by using a standard lyophilized starter culture (Lyocarni RHM-33 produced by the Sacco company, Italy) consisting of *Staphylococcus xylosus* and *Pediococcus pentosaceus.* The second group of samples was prepared by using the combination of a standard lyophilized starter culture (Lyocarni RHM-33) and probiotic *Lactobacillus paracasei* LPC-37 (produced by Danisco). The samples were made from beef (28.7%), pork meat (33.6%), bacon (33.6%), antioxidant, sodium nitrate and flavouring substances. The processes of grinding, chopping, mixing and 3 weeks of ripening (16 – 23 °C, relative humidity: 95 – 80%) were carried out in a meat processing plant. The samples were collected over a period of one week (7 days) during the ripening process.

2. Extraction of the samples for isotachophoresis (ITP)

Sample preparation: 5 g of sample was homogenized in a grinder (3 minutes at 6000 rpm) to obtain a homogeneous mixture. Afterwards, homogeneous sample was extracted with 50 ml of hot (80 °C) deionized water, stirred for 2 minutes and tempered 1 hour at 80 °C. The supernatant was cooled to room temperature and filtered by Filtrak 388 ø filter paper. The filtrate was amended to volume of 50 ml by deionized water. 1 ml of the solution was transferred into 10 ml of volumetric flask and amended by deionized water. The final supernatant was transferred into reagent tubes, which have been frozen afterwards **(Pereira et al., 2012)**.

2.1 The conditions of ITP analyses

The samples were slowly defrosted and filtered by 0.45 μm microfilter prior to injection of the samples.

Leading electrolyte (LE): 10 mM HCL + ε-aminocaproic acid + 0.1% methylhydroxyethylcellulose

Terminating electrolyte (TE): 5×10^{-3} M caproic acid

Standards for lactic acid: solutions of lithium lactate at levels 2, 6 and 10×10^{-4} mol.dm^{-3}.

Standards for acetic acid: solutions of sodium acetate at levels 2, 6 and 10×10^{-4} mol.dm^{-3}.

Selectivity: The standard solutions of acids were measured and it was found that the relative step height value (RSH) for analysed acids did not change.

3. Microbial analyses

The samples with *Lactobacillus paracasei* LPC-37 were collected for microbial analyses during the fermentation process (0, 1st, 2nd and 3rd week).

3.1 Detection of *Lactobacillus*

Microbial analysis was made according to ISO Standard 15214 - horizontal method for the enumeration of mesophilic lactic acid bacteria -colony-count technique at 30 °C.

3.2 Detection of *Listeria*

Microbial analysis was made according to ISO Standard 11290-2 - horizontal method for the detection and enumeration of *Listeria monocytogenes*.

3.3 Detection of *Salmonella*

Microbial analysis was made according to ISO Standard 6579 - horizontal method for the detection of *Salmonella*, including *Salmonella typhi* and *Salmonella paratyphi*.

4. pH measurement

pH measurement was carried out according to ISO Standard 2917-using digital pH-meter OP-211/1 (Radelkis, Hungary) with a direct electrode injection into a sample.

5. a_w measurement

a_w measurement was carried out using Rotronic Hygroskop DT a_w meter after the samples were allowed to reach room temperature.

6. Sensory analysis

The sensory evaluation was performed by 9 panelists made up of males and females, selected from the Department of Food Science and Technology. The consistency, colour and appearance on cut, aroma, and taste were evaluated by hedonic test. The intensity of following taste descriptors was also described by hedonic test: meaty, salty, spicy, sour and smoked taste.

RESULTS AND DISCUSSION

ITP analysis

The results of ITP analyses are presented in Figure 1 and Figure 2. The lactic acid was found as the predominant acid created during the process of fermentation. The highest values of lactic acid in sausages was detected in the 3rd week of ripening. These values represented 16.94 g.kg^{-1} of dry matter in sausages with starter culture Lyocarni RHM-33 (Figure 1) and 18.64 g.kg^{-1} of dry matter (Figure 2) in sausages with addition of *Lactobacillus paracasei* LPC-37, which represented difference at 9%. Differences between samples became more apparent in the 2nd week of ripening which confirmed higher fermentative activity of *Lactobacillus paracasei* LPC-37 in fermented sausages.

Despite the homofermentative character of *Lactobacillus paracasei* LPC-37, small amounts of acetic acid were identified in sausages mainly in the 1st week of ripening which was probably due to the activity of heterofermentative, naturally presented microflora in a meat at the initial stage of ripening.

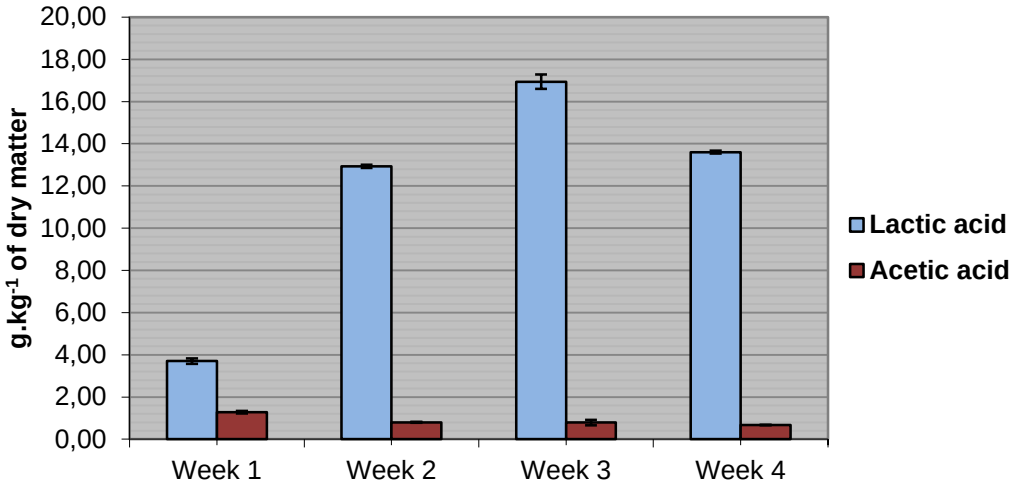

Figure 1 The concentration of the organic acids in sausages with starter culture Lyocarni RHM-33.

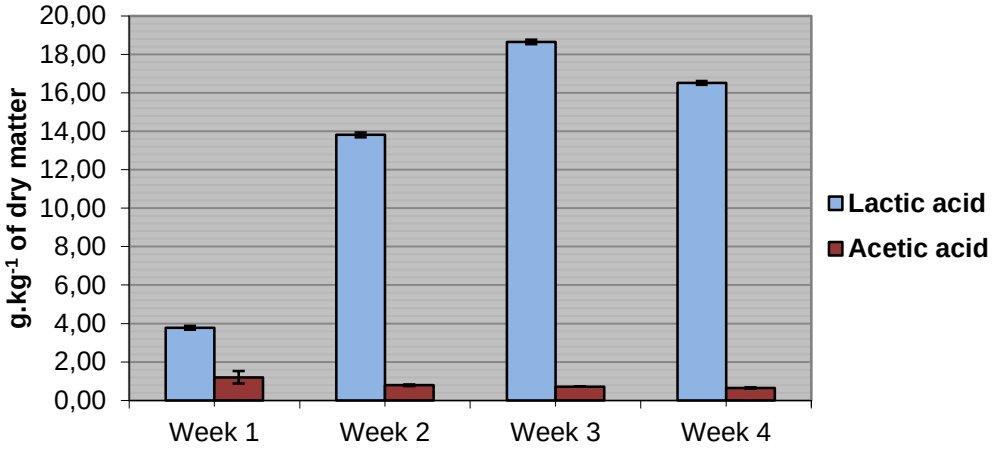

Figure 2 The concentration of the organic acids in sausages with starter culture Lyocarni RHM-33 and *Lactobacillus paracasei* LPC-37.

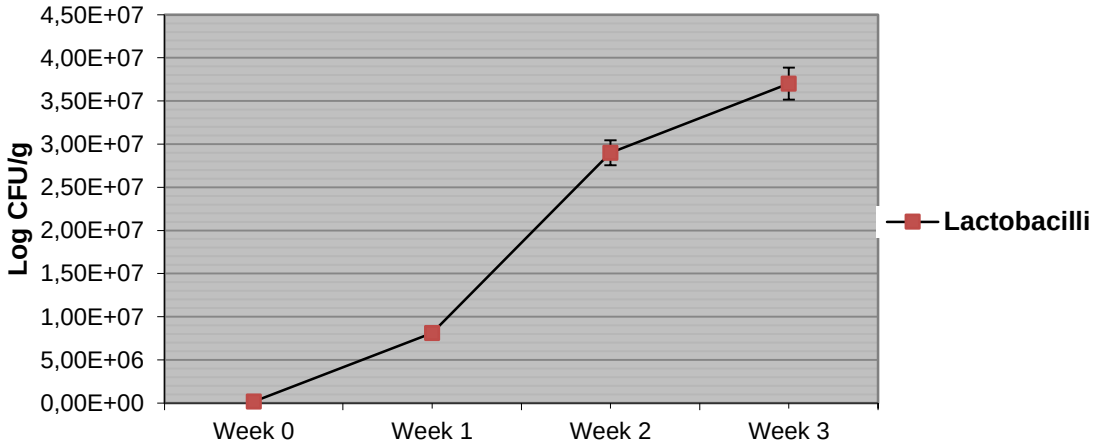

Figure 3 Evolution of the *lactobacilli* counts in sausages with Lyocarni RHM-33 and *Lactobacillus paracasei* LPC.

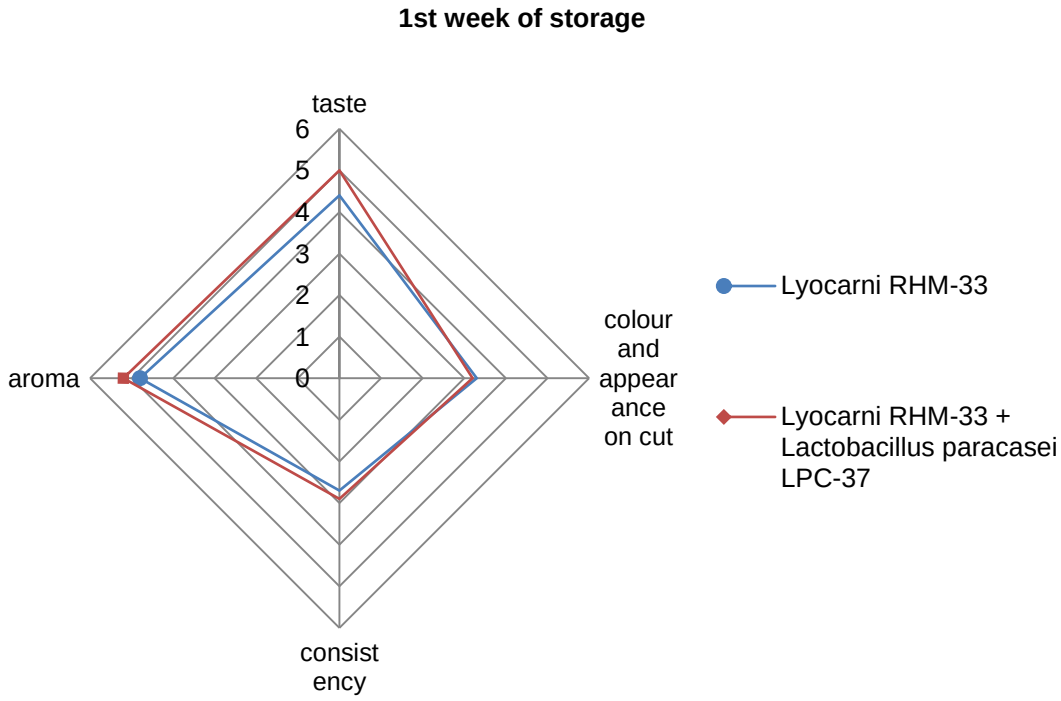

Figure 4 Sensory evaluation of sausages in the 1st week of storage.

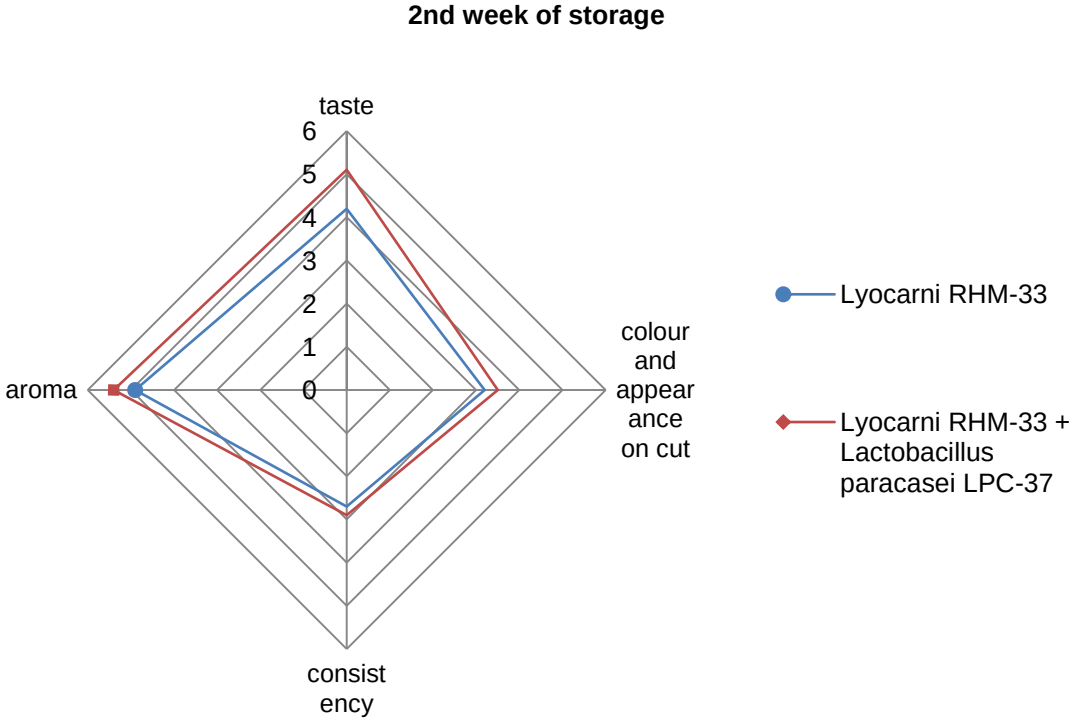

Figure 5 Sensory evaluation of sausages in the 2nd week of storage.

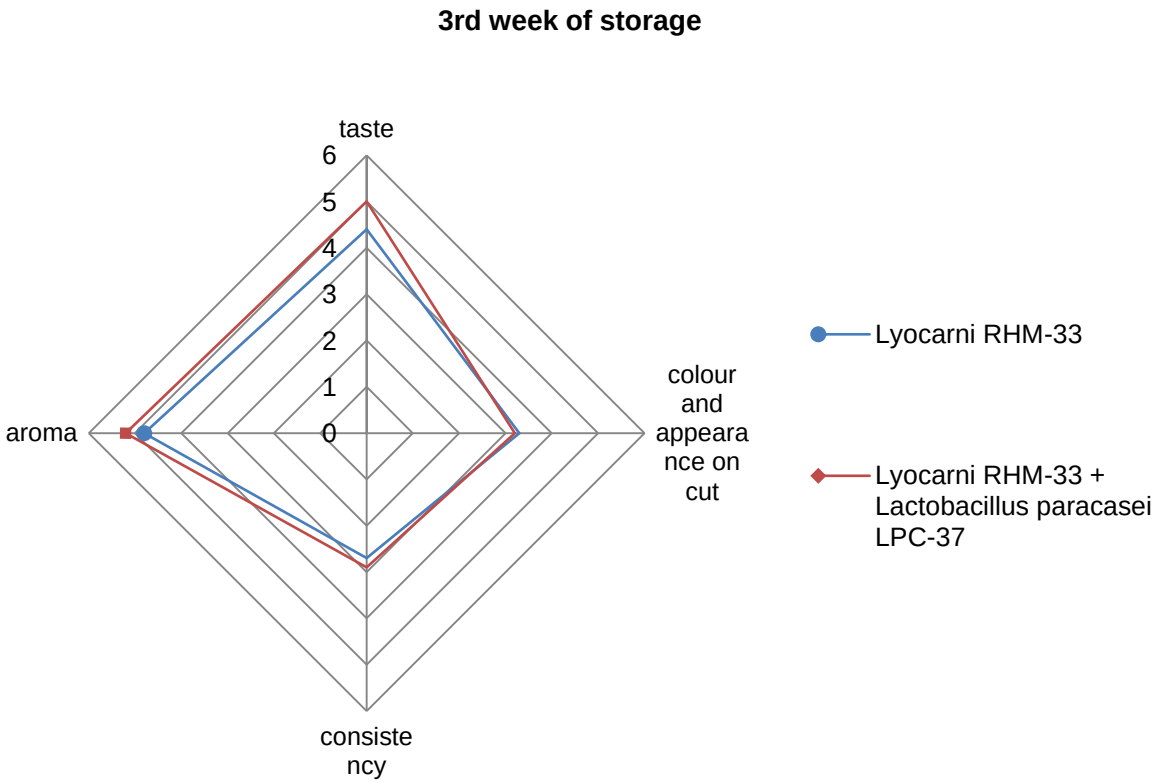

Figure 6 Sensory evaluation of sausages in the 3rd week of storage.

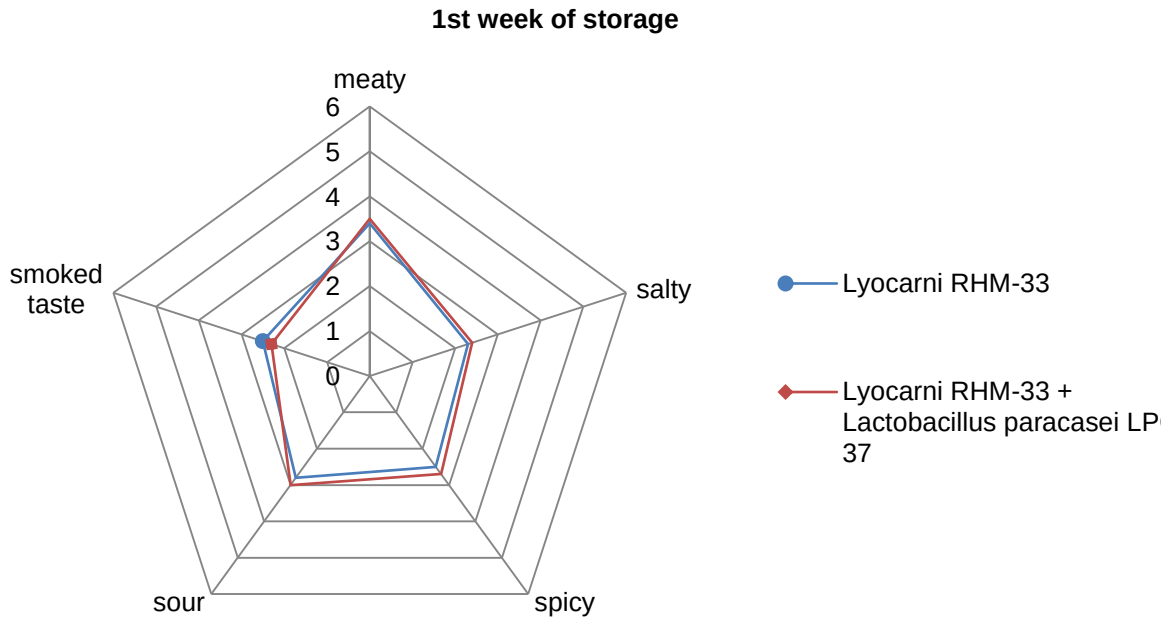

Figure 7 Sensory evaluation of sausages in the 1st week of storage - intensity of taste descriptors.

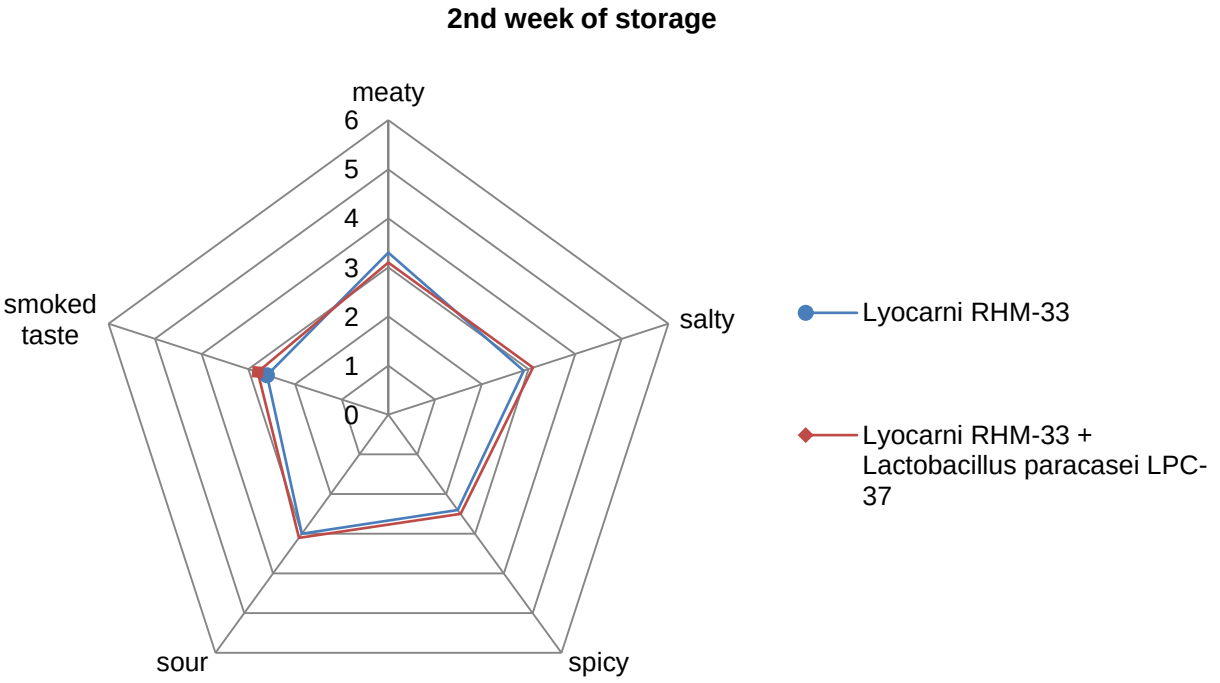

Figure 8 Sensory evaluation of sausages in the 2nd week of storage - intensity of taste descriptors.

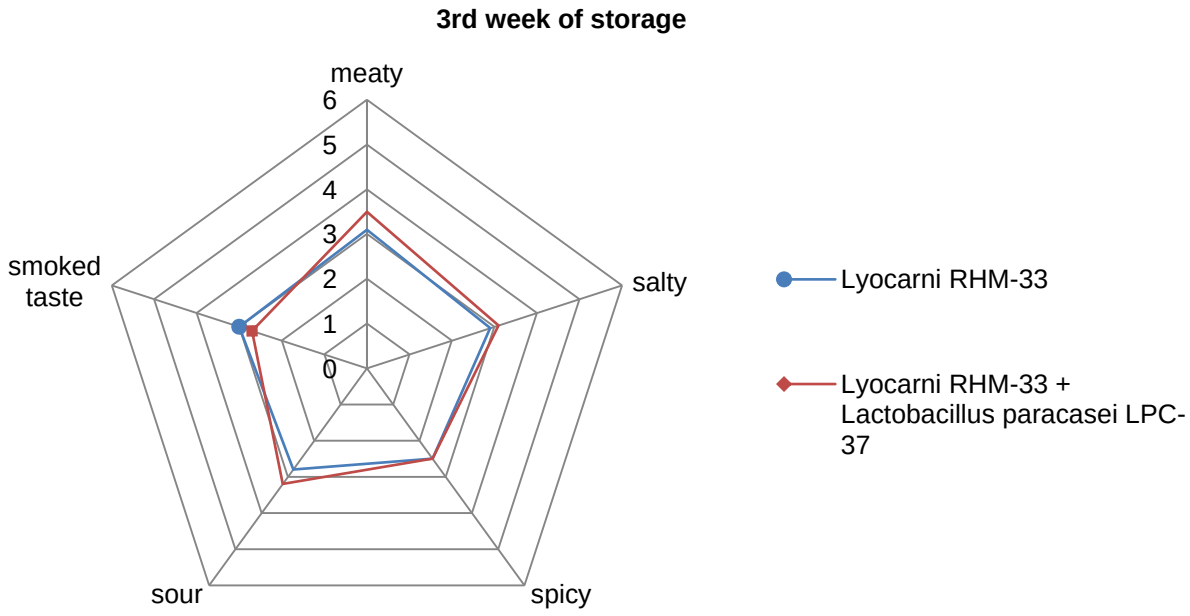

Figure 9 Sensory evaluation of sausages in the 3rd week of storage - intensity of taste descriptors.

Table 1 pH of the sausages during ripening.

	Lyocarni RHM-33 Mean ± SD	Lyocarni RHM-33 + L.paracasei LPC-37 Mean ± SD
Week 0	6.23 ±0.03	6,21 ±0.02
Week 1	5.82 ±0.04	5.77 ±0.01
Week 2	5.65 ±0.02	5.45 ±0.09
Week 3	4.39 ±0.01	4.30 ±0.02

Table 2 Water activity of the sausages during ripening.

	Lyocarni RHM-33 Mean ±SD	Lyocarni RHM-33 + L.paracasei LPC-37 Mean ±SD
Week 0	0.97 ±0.01	0.97 ±0.01
Week 1	0.96 ±0.01	0.94 ±0.01
Week 2	0.93 ±0.00	0.93 ±0.00
Week 3	0.93 ±0.01	0.93 ±0.01

Microbial analyses

The results of the microbial analyses are presented in Figure 3. The population of lactobacilli was monitored during fermentation up to the 3rd week. Week 0 represented a day in which the fermented sausages were stuffed into casings and the 3rd week represented the final week of ripening. Overall, the population of lactobacilli increased throughout fermentation from 10^5 CFU/g to more than 10^7 log CFU/g. This suggests that *Lactobacillus paracasei* LPC-37 grew rapidly and was probably dominant culture in sausages. According to **Grajek et al. (2005)**, at least 10^6 log CFU/g viable cells must reach the intestine for potential health benefits or therapeutic effect which seems to be possible in this type of culture used.

The presence of *Salmonella* and *Listeria monocytogenes* was found negative during the time of ripening which confirmed controlled and safety proceesing.

pH measurement

The initial pH of the sausages with starter culture was 6.23 and in samples with combination of Lyocarni RHM-33 and *Lactobacillus paracasei* LPC-37 represented 6.21 (Table 1). The lowest pH was detected in the 3rd week of ripening in sample with combination of Lyocarni RHM-33 and *Lactobacillus paracasei* LPC-37. The pH progressively increased until the end of ripening due to the proteolytic activity generated by the microorganisms. Bacterial proteases induce proteolytic degradation, generating amino acids, peptides and amines which have a buffering effect on the organic acids. The final pH of the sample with *Lactobacillus paracasei* LPC-37 was 4.35, which comply with specific requirements for this type of ready-to-eat meat products.

a_w measurement

Water activity a_w (presented in Table. 2) decrease was observed during ripening in all samples. The initial water activity in fermented sausages was 0.97. Higher water activity level (0.96) in the 1st week of ripening was determined in the samples with Lyocarni RHM-33. The samples with combination of Lyocarni RHM-33 and *Lactobacillus paracasei* LPC-37 had slightly lower water activity (0.94), which is in the correlation with fact that lowering of the pH reduce the water binding properties of the meat and reduce the drying time of fermented meat products **(Holck et al., 2011)**.

The values of water activity became equivalent (0.93) in the 2nd week of ripening in all samples and reached the legislative claims.

Sensory analysis

The sensory evaluation revealed no significant differences between the consistency, colour and appearance on cut, aroma, and taste during the storage of sausages. The evaluation of the intensity of taste descriptors showed some differences in meaty and sour taste during the 3rd week of storage. The fermented sausages with *Lactobacillus paracasei* LPC-37 had higher mean score (differ by 0.40 point) in both of the taste descriptors.

CONCLUSION

The objective of this study was to evaluate the water activity, pH, concentration of organic acids, microbiological quality and sensory acceptability of raw-fermented sausages with *Lactobacillus paracasei* LPC-37. The obtained data (pH, a_w and microbial quality) confirm the beneficial effects of fermentation with probiotic bacteria application in terms of the health safety of the product. Based on determined counts of lactobacilli (more than 10^7 CFU/g) represented mainly by *L. paracasei* LPC-37, product can be considered as functional food. An analysis of the sensory evaluation results showed that the overall post-fermentation quality was positively correlated with the aroma and taste of fermented sausages and the intensity of two taste descriptors (meaty and sour) was higher than in sausages with starter culture only. The results of this study showed that raw-fermented sausages with *L. paracasei* LPC-37 potential probiotic strain are of good microbiological quality. The environment of raw-fermented sausages is suitable for the growth and survival of *L. paracasei* LPC-37 potential probiotic strain.

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