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Original Paper

Effects of Lactic Acid Bacteria on Pork Meat Maturation

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Process the purpose of reduction of the maturation process of pork meat, the bio preservation of meat products and the prevention of microbial contamination, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* strains were used. The application of Lactic Acid Bacteria strains proved to impact on the duration of the maturation process of salt-cured pork by shortening the process as opposed to samples of pork salt-cured in a traditionally way and containing a commercial BactoFlavor® culture used in production. The maturation process of pork was accompanied by decreased glycogen levels, increased lactic acid dominant in samples containing *L. plantarum* and *L. rhamnosus* strains. The maturation also triggered a shift in pH making it more acidic. The tendency towards glycogenolysis in samples containing LAB strains, prior to the formation of lactic acid, is determined by LAB strains producing lactic acid too, which comes to explain the low level of pyruvate. The application of LAB strains as protective starter cultures with bactericidal activity during the *in situ* fermentation of meat demonstrates that the use of strains leads to the decrease of total contamination by 2 Log CFU·ml^{-1}, while using commercial starter brings to the decrease of total contamination by 1 Log CFU·ml^{-1}. After 4 days of meat maturation, *L. rhamnosus* and *L. plantarum* inhibited the growth of *E. coli* by 2 Log CFU·ml^{-1}, after 8 days the use of LAB and the BactoFlavor® led to the absence of *E. coli*. The broad antimicrobial and bacteriocins spectrum of LAB stimulated their application in the food industry as natural preservatives.

Keywords: pork meat, maturation, lactic acid, glycogen, pyruvate, lactic acid bacteria

1 Introduction

The use of meat and various meat products has always been an important part of the diet for humans, as they contain various essential nutrients that promote human health. In 2024, decreasing maturation period of meat products and producing nutritious, safe and functional food remains among the production challenges. In this context, the accurate selection of starter cultures can contribute to the production of safe semi-processed pork with a high biological value (HBV) a much shorter timeframe. Given that probiotics offer multiple functions, Lactic Acid Bacteria (LAB) improves the colour, favour, and pH of meat products and inhibits the growth of harmful microorganisms during the fermentation

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process (Besser et al., 2019). They also decompose large molecules of protein, fat, and carbohydrates in the meat to generate numerous of aromatic substances such as alcohols, aldehydes, acids, and esters, as well as amino acid and small molecule peptides, which can be easily absorbed by the body and produce lactic acid, lactic acid enzymes, associated to the food maturation, development of the sensory characteristics. LAB have an ability to degrade nitrites, provide an antioxidant activity, and inhibit the growth of pathogenic bacteria. They also reduce the moisture content in the meat, which effectively improves product safety and extends shelf life (Wang et al., 2022).

The health concern with food safety issues and demand for functional, less processed, or preservative-free food products from consumers is increasing. At the same time, the use of chemicals in preservation of food products brings to the development of different human allergic diseases (Bouarab et al., 2019). In this context, the development of new preservation technologies as alternatives to chemical additives is urgent and topical. In recent years, using microorganisms or their metabolites to control unwanted organisms and extend food shelf-life and increase food safety has gained increasing interest (Yépez et al., 2017; Quattrini et al., 2019).

Starter cultures have a number of advantages: they are of known quantity and quality; reduce the ripening time; they increase safety by outcompeting undesirable microorganisms; they enable the manufacture of a product of constant quality all year round in any climatic zone, as long as proper natural conditions or fermenting/drying chambers are available. Starter cultures play an essential role in the manufacture of fermented food products, improving the organoleptic qualities, nutritional characteristics and textural profile of these food products. Additionally, microbial substances, namely, bacteriocins, produced by Gram-positive species of the LAB group, such as, for example, nisin and other antibiotics or pediocin – like bacteriocins, have an antimicrobial role with an effect on preservation and safety (Laranjo et al., 2017). In addition, in most cases, meat and meat products are susceptible to contamination by microorganisms, resulting in enhanced health risks for the customers and economic loss for the industry. Among processed food, meat and meat products represents a serious challenge for the food industry, as the possible microbial contamination of fresh meat and meat products by various harmful bacteria, such as *Listeria monocytogenes*, cannot be handled by physical ways only, such as lowering the pH, freezing, and salting. Fermented meat products are obtained by fermenting raw meat with specific microorganisms or enzymes under natural or artificial intervention conditions to induce a series of biochemical reactions and physical changes. Most of the current fermented meat products are fermented in a natural environment, and the problems with their use mainly include an unclear source of fermentation strains, uncontrollable microbial flora, easy contamination by harmful pathogenic bacteria, and other food safety problems. Hence, such problems are produced by common food degrading microorganisms. One of the most used forms to address such problems is the use of LAB and intelligent use of their properties, including production of bacteriocins and production of primary metabolite lactic acid, which in turn can decrease the pH, inhibiting the growth of a wide variety of food spoilage organisms mainly *L. monocytogenes*, which leads to extension of the expiry date of fermented foods (Da Costa et al., 2019; Lahiri et al., 2022).

The health-promoting properties of probiotic LAB strains and their beneficial effects on consumer's health make them attractive for industrial application in the composition of functional foods and beverages. Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts (Arihara, 2006). Probiotics have been used in food products, food supplements and pharmaceutical products. By using probiotics as starter microorganisms, potential health benefits can be introduced to meat products, and it is already possible to produce probiotic meat products (Rouhi et al., 2013). Most meat starter cultures commercially available are combined cultures of LAB (mainly *Lactobacillus* spp. and *Pediococcus* spp.) and Gram-positive catalase-positive cocci (GCC+, primarily *Staphylococcus* spp. and *Kocuria* spp.). These bacteria are responsible for the microbial reactions that occur during meat fermentation, such as acidification, catalase activity, and bacteriocin production (Fontana et al., 2015).

Lactobacillus plantarum plays a crucial role in the meat industry by offering biocontrol and preservation benefits. Studies have shown that *L. plantarum* can reduce pH values and inhibit potential pathogenic microorganisms in fermented sausages, enhancing their safety and shelf life. Additionally, *L. plantarum* forms biofilms that prevent the growth of harmful microorganisms on meat products, thus improving their quality and extending shelf life. Overall, *L. plantarum* ability to withstand adverse conditions, its probiotic properties, and its antimicrobial effects make it a versatile and beneficial component in the meat industry for preservation and quality enhancement (Lahiri et al., 2022).

It was shown that abundant values of various flavour substances increased with protein hydrolysis when the fermentation time reached 9 days, in addition to which different proportions of salt also affected

the flavour, pH, and moisture content of the fermented sausages (Chen et al., 2021). It was reported that *L. plantarum*'s enzyme system can break down myogenic fibronectin and sarcoplasmic proteins into small active peptides and free the amino acids through the action of proteases (Fadda et al., 1999). In general, proteins undergo oxidative deamination and decarboxylation reactions under the action of a series of enzymes to eventually produce flavour precursors, such as aldehydes, alcohols, and aromatic substances. *L. plantarum* could reduce the sausage pH, accelerate the acidification and gelatinization process, and add good colour to the meat products and reduce the content of nitrite residues in the sausage, as well as prevent fat oxidation, protein decomposition, and myoglobin oxidation, and increase the content of free amino acids (Wang et al., 2022).

The probiotic strain *L. rhamnosus* LOCK900 used for fermenting the loin and pork sausage produces significant amounts of organic acids modifying the pH of the meat product. The increase in the acidity of the products during refrigerated storage suggests that the addition of a probiotic had a beneficial effect on the fermentation process. Lower pH values in the probiotic loin compared to the control loin, which underwent spontaneous fermentation, showed that the probiotic strain used probably improved the fermentation process by producing more organic acids, especially lactic acid (Neffe-Skocińska et al., 2020).

In recent decades, different advanced formats for application of LAB bacteriocins in food bio preservation have been developed:

a) by inoculation of food with LAB – starter or protective cultures for *in situ* production of bacteriocins;
b) use of previously fermented food by fermentative of a bacteriocin-producing strains as an ingredient in food processing;
c) by addition of semi- or purified LAB bacteriocins.

In situ production is readily cost-effective provided that the bacteriocin producers are technologically suitable (García et al., 2010).

As demonstrated in many studies, glycogen level plays a key role in post-mortem glycogenolysis that determined much meat quality traits. Glycogen content influences ultimate pH which determined much meat quality traits as colour, drip loss, water holding capacity, cooking loss and sensory attributes (Rosenvold & Andersen, 2003). Lactic acid is produced during the fermentation of LAB, giving the product a special flavour, and its enzyme system produces esterase, protease, and peroxidase; these enzymes and endogenous enzymes in the meat products synergize, inducing biochemical changes in the protein and fat of raw meat, thereby producing small molecules of amino acids, esters, peptides, and short-chain fatty acids and other small molecules (Wang et al., 2022).

The research study aim is to monitor and explore the impact of probiotic LAB strains and commercial starter cultures on the maturation process of pork meat under the same conditions as opposed to traditional salting method from the perspective of reducing the duration of the maturation process and producing safe semi-processed food.

2 Material and Methods

2.1 Examined Material and Starter Cultures

The experimental material of the research study is the leg of 7 months old sows. Lactic Acid Bacteria strains *Lactobacillus rhamnosus* 2012 (MDC 9631) and *Lactobacillus plantarum* 66 (MDC 9619) used in the current study were taken from the culture collection of Microbial Depository Center (MDC) of SPC "Armbiotechnology" NAS RA, included in WDCM (World Data Centre for Microorganisms) database (https://armbiotech.am; www.wfcc.info). For comparison purposes, the BactoFlavor® BFL-T03 Hansen commercial starter was used. For cultivation of LAB strains and BactoFlavor® BFL-T03 Hansen the Man Rogosa Sharpe (MRS) agar and broth (Merck, Germany), ISO (Italy), HiMedia (India) media was used. LAB strains maintained as frozen stock at -20 °C in the MRS broth containing 40% Glycerol. Before use, were transferred twice into the appropriate medium and incubated during 24 hours at temperature 37 °C.

2.2 LAB Species Identification

Species identification of LAB was confirmed by 16S rDNA gene sequencing method (Kalendar et al., 2021). The nucleotide sequence of the obtained amplified 16S rDNA was determined by "MACROGEN" (Korea). Strain identification was performed using the online BLAST software (www.ncbi.nlm.nih.gov/BLAST).

2.3 Freeze-drying of LAB Strains (Lyophilization) and Survival Test

Before lyophilization lactic acid bacteria were grown in MRS broth, incubated anaerobically at 37 °C for 24 (stationary phase). LAB were frozen to -20 °C. The frozen LAB strains were dried-frozen (temperature -40 ±2 °C; vacuum pressure 10^{-1} torr for 48 h) using a bench-top lyophilizer (Modulyo bench top freeze dryer, Edwards, Burgess Hill, UK). Viable cell counts were checked after freeze drying of LAB by the standard plate count method.

To this end, the dried powder was rehydrated in sterile saline solution (NaCl, 0.85%) at room temperature for 10 min. One ml aliquots were serially diluted and plated; following this, MRS agar medium was poured. After incubation for 2–3 days at 37 °C under anaerobic conditions, the colonies were counted and results were expressed as log CFU·ml^{-1} (Jalali et al., 2012; Yeo et al., 2018).

2.4 Determination of Probiotic Properties and Antimicrobial Activity of LAB Strains

Determination of probiotic and antimicrobial activity was carried out according to the generally accepted methods (Shenderov, 2013; Linares et al., 2017). Antimicrobial activity of samples (aliquots of 100 µl) assessed by spot-on-lawn method, measuring the size of the inhibition zone (diameter) of test culture growth (Ø, mm) after 24 h incubation in the thermostat at 30 °C. The antimicrobial activity was calculated according to Parente (Parente et al., 1995) and expressed in arbitrary units (AU·ml^{-1}).

2.5 Sample Preparation for in situ Testing

The pork leg was obtained from a supermarket and N5 approval document is available. One of the primary and key stages of the production process is the safe handling and transportation of raw meat produce, therefore all procedures are regulated by Regulation 034/2013 "On Safety of Meat and Meat Products" Technical Regulations of the Customs Union (TR CU) https://mineconomy.am/page/444. In accordance with the technical regulations, the raw meat produce sent to the production line must be of slaughterhouse origin and have accompanying documents/certificates https://www.arlis.am/DocumentView.aspx?docid=118821. The availability of such documents confirms the safety of manufacturing and transportation of the raw meat produce.

For purpose of the sample preparation four types of salting were performed: traditional salting, salting proposed by the manufacturer with the application of BactoFlavor® culture and salting recommended by the current research with the application of the following strains of lactic acid bacteria (LAB) *Lactobacilus plantarum* 66 and *Lactobacilus rhamnosus* 2012. The pork leg was split into four segments, each weighing 150 grams. For salting, brine (a saline solution) was prepared (50 ml) with the salt concentration being 11.3%, the density being 1.077, at room temperature +15 ±2 °C (in line with GOST state standards for meat products). The biochemical study was implemented on day 1, 4 and 8 of maturation. The saline solution was injected by applying 10% of the solution on every 1 cm surface. The *Lactobacilus plantarum* 66 and *Lactobacilus rhamnosus* 2012 lactic acid bacteria culture amounted to 1 litre, the titer of the culture – the initial cell count of LAB strains was 1.0×10^8–1.0×10^9 CFU·ml^{-1}. Sucrose in the amount of 0.1% was added to the strains of *L. rhamnosus* 2012 and *L. plantarum* 66 based on the weight of the raw meat produce. The final cell count of LAB strains in the tested samples was 1.0×10^6–1.0×10^7 CFU.ml^{-1}. In the case of BactoFlavor® BFL-T03 Hansen commercial starter culture, fructose was added based on the weight of the raw meat produce of 0.1%.

2.6 Analytical Methods

Glycogen, lactic acid, pyruvic acid study of pork meat samples were carried out immediately when the meat maturation period is started – on the 1st, 4th, and 8th days of refrigerated storage. The pH measurement was carried out once every 24 hours. The levels of the glycogen, lactic acid and pyruvic acid were determined by means of enzymatic method, spectrophotometrically, in accordance with glucose oxidase and lactate dehydrogenase activity respectively (Pesce, 1984; Moreno et al., 2020). Determination of the biochemical markers were conducted in triplicate. The measurements were carried out using a Jenway™ 7315 UV/Visible Single Beam spectrophotometer (Jenway™ 7315, Stone, Staffordshire, United Kingdom). The pH was measured by the digital Jenway 3540 pH-meter/conductivity meter (Jenway 3540, Keison Products, Essex, England, UK). The pH readings were recorded at exactly 4 min after the insertion of the electrode into the sample. The pH meter was standardized with buffer solutions at pH 2.0, 4.0, 7.0, and 10.0.

The microbiological analyses of pork meat samples were carried out immediately after the meat ripening period on the 1st, 4th, and 8th days of refrigerated storage. The analyses were carried out using the traditional plate method with dedicated culture media for a tested group of microorganisms: for lactic acid bacteria: MRS agar, incubation parameters 37 °C per 48 h), ENT (microorganism from Enterobacteriaceae family): Nutrient agar (Himedia, India), selective medium Endo agar (Himedia, India; Merck, Germany), incubation parameters 30 °C per 24–48 h. All microbiological analysis was carried out in accordance with ISO (International Standards Organization) guidelines for the selected test, respectively. The results obtained are given in colony forming units per gram or millilitre of study product (CFU·g^{-1} or CFU·mL^{-1}). Count of survived LAB cells as well as total contamination was determined by the serial dilution method (Ben-David & Davidson, 2014). For this purpose, 1 gr of meat samples was rehydrated in sterile saline solution (NaCl, 0.85%) at room temperature for 10 min. One ml aliquots were serially diluted and plated; then agar medium was poured. After incubation for 2–3 days at 30 or 37 °C under

anaerobic conditions, the colonies were counted. Sample preparation, biochemical experiments were carried out in the Food Quality Control Laboratory of the Armenian National Agrarian University. Microbiological analyses were conducted in the Probiotics Production Sector of the Institute of Biotechnology – Research Unit of the Scientific and Production Center "Armbiotechnology" NAS RA.

2.7 Statistical Analysis

Statistical analysis was performed using MS Office Word 10 and MS Office Excel 2010 software applications. The experiments' results were pointed as mean value. The obtained differences were considered statistically significant at $p < 0.05$ and calculated using one-way ANOVA.

3 Results and Discussion

3.1 pH as an Indicator of Meat Maturation

It is important to note that the salt-cured pork is considered mature when the pH is within the range of 5.0 (Feiner, 2010). Table 1 illustrates the maturation dynamics of the salt-cured pork depending on the applied starter cultures and the pH range ($p < 0.05$). The obtained data show that on day 1, the pH of all samples was within the range of 6.2. On day 2 of the maturation, the pH decreased to 5.9 in samples with the added LAB strains, while in the case of BactoFlavor® culture used in production the pH of the sample is 6.0 and 6.1 respectively, compared to traditional salting method. The monitoring of the maturation dynamics shows that on day 4, the pH of the sample containing *L. rhamnosus* 2012 strain decreased to 5.3, than that of *L. plantarum* 66 to 5.4, and the sample containing BactoFlavor® decreased to 5.6; a slower maturation was detected in the sample which was salt-cured in traditionally, whose pH within that period was 5.9. The research findings show that the maturation of pork samples containing *L. plantarum* 66 and *L. rhamnosus* 2012 strains were recorded on day 6, while the maturation impacted by the strain used in production was detected on day 7, and in the case of traditional salting the maturation was recorded on day 8. It has been shown that the application of two LAB strains has shortened the maturation period of the salt-cured pork by one day as compared to the BactoFlavor® method and by two days as compared to traditional salting method. The data in the literature demonstrate that parallel to the meat maturation process, the pH marker decreases, which at the same time increases the resistance to the microorganisms that cause putrefaction (Neffe-Skocinska et al., 2020).

3.2 Glycogen, Lactic and Pyruvic Acids Level Depending on Meat Maturation Period According to the Starter Cultures

During the research the glycogen, lactic and pyruvic acids levels were examined at different maturation stages (1st, 4th and 8th day) of the samples. The objective was to determine, apart from the pH, the intensity of maturation processes from the perspective of biochemistry, as well as to monitor the level of the lactic acid which impacts the meat flavour quality wise, considering the application of LAB strains that realistically impact the formation of lactic acid.

Figure 1 displays the glycogen level based on cultures and maturation stages. The data obtained shows that the glycogen breakdown with *L. plantarum* 66 and *L. rhamnosus* 2012 cultures occurs more intensively as opposed to the sample salt-cured in a traditional way and containing BactoFlavor® widely applied in production. The difference in the intensity of glycogen breakdown and, therefore, the maturation process of the pork becomes more apparent on the 4th and 8th day of maturation when *L. rhamnosus* 2012 strain becomes dominant. Thus, in the sample containing *L. rhamnosus* 2012 strain, the process of glycogenolysis is 1.7 times higher in intensity on the 4th day of maturation than

Table 1 Dynamics of meat maturation determined by pH

Maturation time (day)	Maturation methods (pH)			
	traditional salting	hansen/bacto flavor	*L. plantarum* 66	*L. rhamnosus* 2012
1	6.2	6.2	6.2	6.2
2	6.1	6.0	5.9	5.9
3	6.1	6.0	5.9	5.9
4	5.9	5.6	5.4	5.3
5	5.6	5.4	5.2	5.1
6	5.4	5.2	5.0	4.9
7	5.2	5.0		
8	5.0			

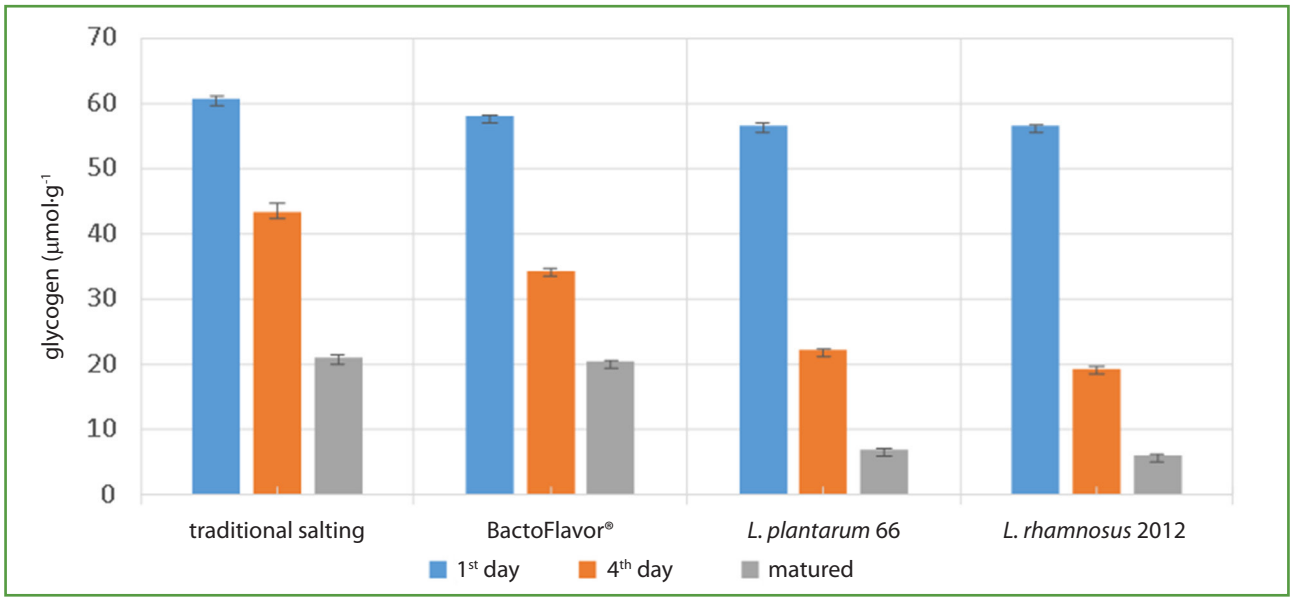

Figure 1 Glycogen level in pork meat depending on cultures and maturation stages

of the BactoFlavor® containing sample; and on the 8th day it is higher by 3.6 times. Compared to the traditionally salt-cured sample, the intensity of glycogen breakdown exceeds 2.3 and 3.6 times, respectively. This fact comes to prove that the glycogen breakdown is directly correlated to pH range dynamics during the maturation process of the pork.

The results in Figure 2 reveal that the tendency of the lactic acid formation in pork samples with various cultures based on maturation stages. The findings show that the formation of the lactic acid is in direct correlation with the breakdown of the glycogen. The predominance of the lactic acid in samples containing *L. plantarum* 66 and *L. rhamnosus* 2012 strains is obvious in contrast to samples containing either BactoFlavor® or samples salted in traditionally, with LAB strains triggering it. It is worth mentioning that the lactic acid is not only an indicator of the maturation process of the pork meat, but also an indicator of quality and taste characteristics. Grajales-Lagunes et al. (2012) demonstrated the impact of the lactic acid on the qualitative aspect and the taste characteristics of pork meat. Murata (2022) shows a direct correlation between the amount of the lactic acid and the taste of the meat.

Figure 3 shows the dynamics of the pyruvate level in research samples, determined by the maturation stages of the pork. It is to be noted that a very low level of pyruvate, almost next to none, was recorded

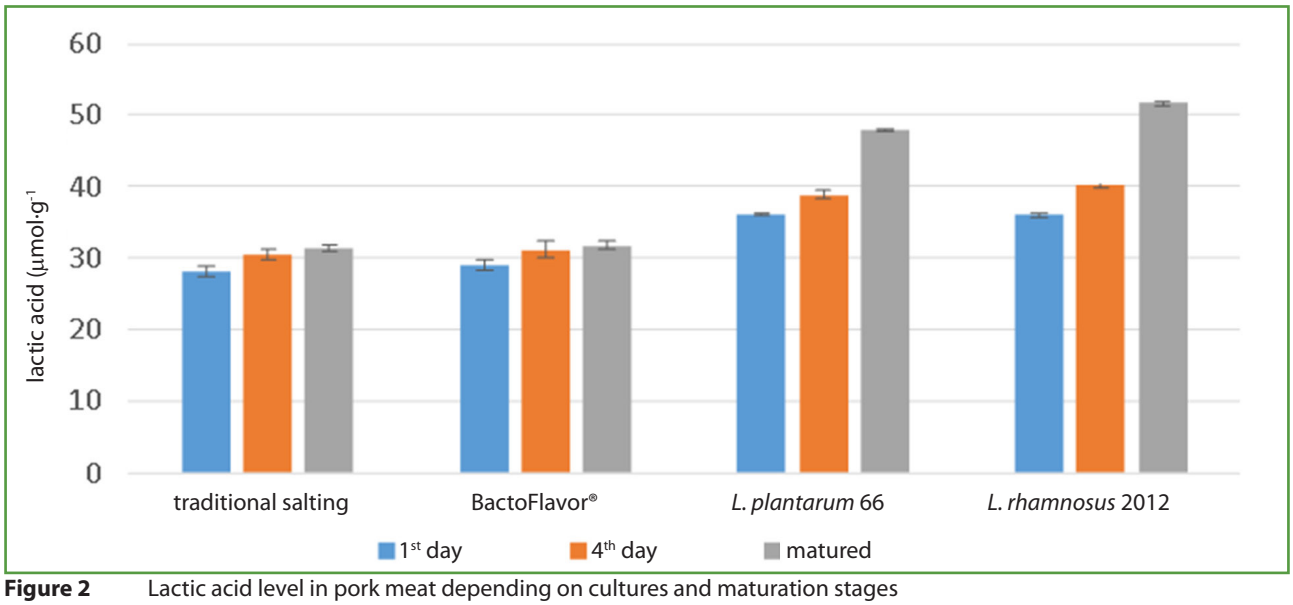

Figure 2 Lactic acid level in pork meat depending on cultures and maturation stages

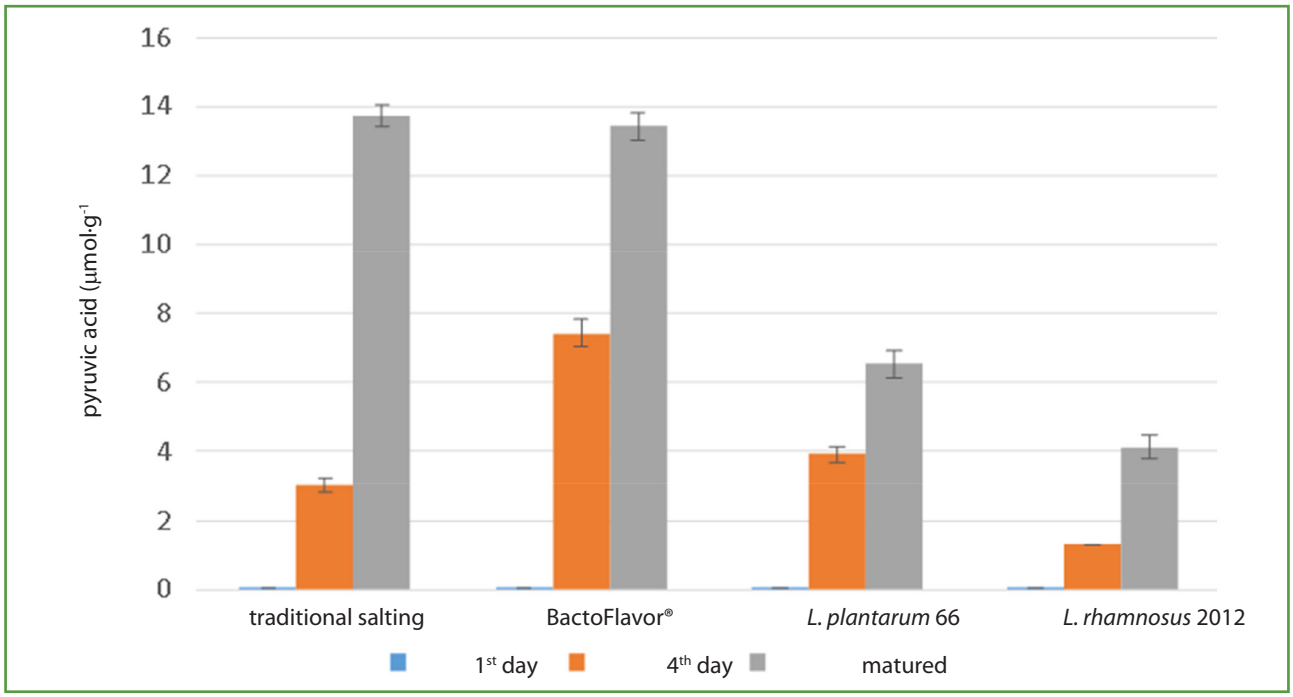

Figure 3 Pyruvic acid level in pork meat depending on cultures and maturation stages

on the 1st day of maturation. Despite the fact that the pyruvate concentration increases alongside the maturation, determined by glycogen breakdown, the process of glycogenolysis is more prone to the formation of lactic acid, therefore, in samples containing *L. plantarum* and *L. rhamnosus*, pyruvate is significantly inferior to lactic acid in its quantity. It is noteworthy that the aforementioned strains too trigger the production of lactic acid. The research results provide grounds to conclude that both, the level of lactic acid and pyruvate in samples with BactoFlavor® culture and samples cured traditionally is almost the same. However, if the pyruvate concentration in those samples exceeds the samples containing *L. plantarum* 66 and *L. rhamnosus* 2012 strains, in the case of lactic acid it is the contrary.

The autolytic maturation of the meat is determined by the breakdown of muscle glycogen and the formation of lactic acid. As is known, after the slaughter of the animal, muscle cells no longer receive oxygen; as a result, the glycogen synthesis stops, and the remaining glycogen begins to break down and after some changes transforms into lactic acid which accumulates in muscle tissue. As a result of the accumulation of a significant amount of lactic acid, the process of glycogenolysis stops (Ramanathan et al., 2011). The initial screening and selection of probiotics includes testing of the following important criteria: phenotype and genotype stability; carbohydrate and protein utilization patterns; tolerance both to the highly acidic conditions present in the stomach and to concentrations of bile salts found in the small intestine; survival and growth; intestinal epithelial adhesion properties for colonization and any direct interactions between the probiotic and host cells leading to the competitive exclusion of pathogens and/or modulation of host cell responses; production of antimicrobial substances; antibiotic resistance patterns and immunogenicity (Shenderov, 2013; Linares et al., 2017).

3.3 *Comparative evaluation of probiotic characteristics and antimicrobial properties of L. rhamnosus 2012 and L. plantarum 66 LAB strains*

It was reported that *L. plantarum*'s enzyme system can break down myogenic fibronectin and sarcoplasmic proteins into small active peptides and free the amino acids through the action of proteases (Fadda et al., 1999). The probiotic strain *L. rhamnosus* LOCK 900 used for fermenting the loin and pork sausage produces significant amounts of organic acids modifying the pH of the meat product. The increase in the acidity of the products during refrigerated storage suggests that the addition of a probiotic had a beneficial effect on the meat fermentation process (Neffe-Skocinska et al., 2020).

The main probiotic characteristics of *L. rhamnosus* 2012 and *L. plantarum* 66 LAB strains, were investigated. Briefly, bacterial suspensions were incubated sequentially in solutions simulating the gastric and intestinal

compartments (24 hrs, MRS broth, OD at μ = 590). Results are summarized in Table 2.

As it seen from the given results, LAB strains differ by their resistance to proteolytic enzymes, viability after influence of pH and bile (had resistance towards various concentrations of bile (0.3–0.8%). *L. plantarum* 66 strain shown better viability during the growth at different pH.

Thus, high antimicrobial activity, tolerance to NaCl and growth in various temperature and pH conditions allows using of selected strains in meat fermentation to prevent contamination of meat products by food spoilage microorganisms.

At present, the BactoFlavor® BFL-T0, a commercial starter consist of mixture of some LAB strains offered by Hansen, is one of the most widely used starters in food production in Armenia. LAB cultures include in starter are: *Pediococcus pentosaceus*, *Staphylococcus carnosus* subsp. *utilis* which accelerate the ripening process, ensure low acidity, colour stabilization, while imparting a certain flavour to meat products. The main characteristics of LAB cultures include in starter are presented in Table 3. The above-mentioned cultures can be maintained below the specified temperature, but do not multiply. Combination of properties of both strains brings to better efficiency of meat treatment process. Microbial activity is essential in almost all technological stages of meat

Table 2 Comparative evaluation of some probiotic properties of LAB strains

LAB strains	NaCl tolerance 0.4–12%	Growth in MRS broth (°C) (OD, 590)			Resistance to enzymes (typsin, pepsin, proteinase K)	Stability to bile (%)	Survival at different pH values (pH 3-8)	Proteolytic activity	Antimicrobial activity (AU·ml^{-1})		
									Salmonella typhimurium G-38	*Bacillus subtilis* 17-89	*E. coli* K 12
		30	37	42							
L. rhamnosus 2012	100	2.6	2.6	2.9	+	80	70	+	3,000	5,000	1,500
L. plantarum 66	80	3.3	2.7	2.7	+	70	90	+	2,000	4,500	1,000

MRS – Man Rogosa Sharpe broth; OD – optical density; AU·ml^{-1} – arbitrary units per ml

Table 3 Physiological characteristics of LAB strains of Hansen BactoFlavor® BFL-T0

LAB strains	Characteristics				
	growth temperature (°C)			NaCL tolerance (%)	main enzymatic activity (U·mL^{-1})
	minimum	optimum	maximum		
Pediococcus pentosaceus	15	35	48	7	DL (+/-) – lactic acid producer
Staphylococcus carnosus subsp. *utilis*	10	30	45	16	catalase nitrate-reductase positive lipolytic proteolytic

Table 4 Evaluation of antimicrobial activity of selected LAB strains and BactoFlavor® BFL-T0 during meat maturation

Starter cultures/ maturation methods	Maturation time (day)					
	1	4	8	1	4	8
	total contamination (CFU·ml^{-1})			*E. coli* cell count (CFU·ml^{-1})		
L. rhamnosus 2012	centre	1.2×10^1	0	3.5×10^2	0	0
L. plantarum 66	3.6×10^2	0	0	2.0×10^2	0	0
BactoFlavor/Hansen	4.5×10^2	2.5×10^1	2.2×10^1	1.4×10^2	2.0×10^1	0
Traditional ripening (NaCl)	1.4×10^3	2.0×10^1	1.5×10^1	3.0×10^2	3.5×10^2	1.2×10^3
Control (untreated meat)	2.1×10^3	4.7×10^3	5.2×10^3	4.5×10^2	2.4×10^3	1.2×10^4

CFU·ml^{-1} – colony forming units per ml

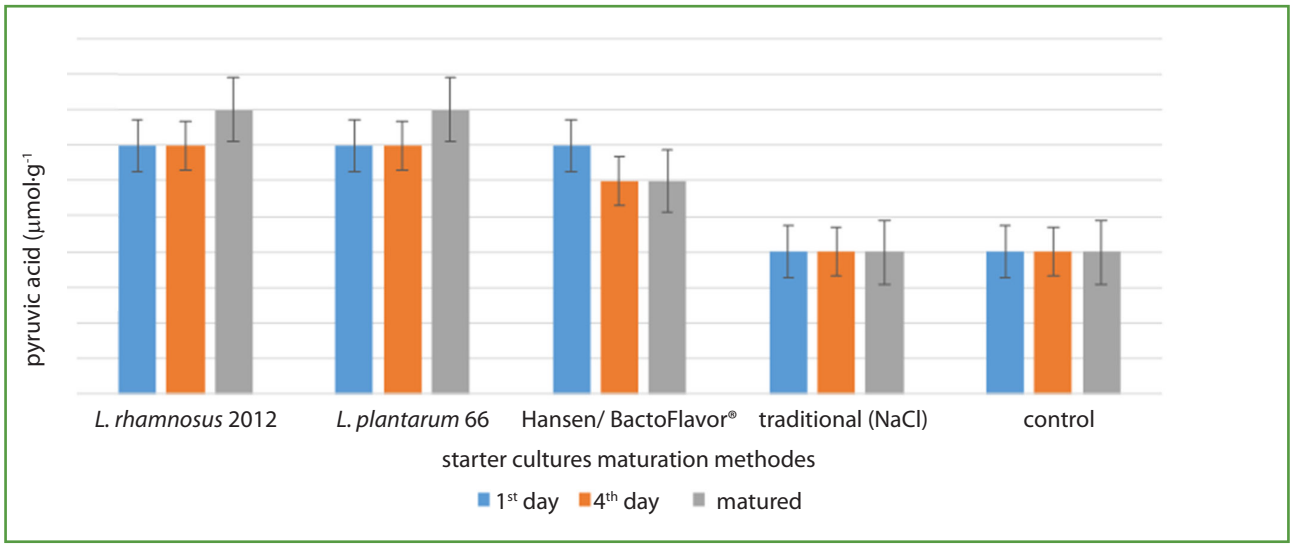

Figure 4 Evaluation of survival of LAB strains and BactoFlavor® BFL-T0 during meat maturation
CFU·ml^{-1} – colony forming units per ml

products processing. The strains of *Staphylococcus* species have a positive effect on the colour formation process, as they contribute to the conversion of nitrate to nitrite through nitrate reductase, moreover, staphylococci can produce the enzyme catalase, which helps prevent meat products from oxidative spoilage during storage, as it destroys the resulting hydrogen peroxide.

The probiotic strains *L. rhamnosus* 2012 (MDC 9631) and *L. plantarum* 66 (MDC 9619) were used during the pork meat maturation. During the meat ripening process, the coli titter and common contamination of meat was determined. The efficiency of selected LAB strains as starter cultures in meat production were compared with commercial starter BactoFlavor®/Hansen. The results are presented in Table 4.

As is seen from the given results, the growth of *E. coli* was more effectively suppressed by using of *L. plantarum* 66 strains as a starter culture. The survival of LAB strains during meat ripening was investigated. The results are presented in Figure 4. As it seen from the given data investigated *L. rhamnosus* 2012 and *L. plantarum* 66 LAB strains, as well as strains of BactoFlavor®/Hansen shown high survival at meat maturation conditions and after 8 days of maturation the increased cell count are observed.

4 Conclusion

The maturation of salt-cured pork meat impacted by *L. plantarum* 66 and *L. rhamnosus* 2012 strains shortened of the ripening process, it was recorded on the 6th day, compared to the traditional salting with the maturation on the 8th day and the BactoFlavor® culture with the maturation recorded on the 7th day. This comes to demonstrate one of the expected and desired changes, which is the shorted of the maturation process. The maturation of the pork, alongside a decrease in pH range, was accompanied by a decrease in the level of glycogen and an increase in lactic acid, dominating in samples with *L. plantarum* 66 and *L. rhamnosus* 2012 probiotic LAB strains. This fact is substantiated by an increase in the intensity of the glycogenolysis process impacted by LAB strains. The glycogenolysis is most likely to occur prior to the formation of lactic acid, therefore, in samples containing *L. plantarum* 66 and *L. rhamnosus* 2012 probiotic LAB strains the pyruvate is significantly lower than the lactic acid. It was established that the samples of pork meat with *L. plantarium* 66 and *L. rhamnosus* 2012 strains displayed antagonism against food spoilage *E. coli* and contaminating microflora, which will also contribute to extending the shelf life of the food item.

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Authors Contribution

Hasmik Grigoryan was engaged in performing of the biochemical analysis, investigation, data analysis, interpretation of the results. G. Marmaryan contributed supervision of the research, processing of interpretation of the resutls, drafting the article. Anna Dashtoyan contributed to the sample preparation stage, according to the proper technological processes, requirements. Kristina Karapetyan was engaged in performing of the microbiological experiments and the data interpretation. Ani Paloyan contributed microbiological data interpretation and soft analysis. Radovan Kasardra suggested research methodology. Martin Fik was involved in correction of the article before publication. Yuri Marmaryan was involved in the conceptualization and implementation, reviewing the manuscript. All authors contributed to the final review and approval of the manuscript to be published.

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