ՀՀ ԿՐԹՈՒԹՅԱՆ, ԳԻՏՈՒԹՅԱՆ, ՄՇԱԿՈՒՅԹԻ ԵՎ ՍՊՈՐՏԻ

ՆԱԽԱՐԱՐՈՒԹՅՈՒՆ

ԵՐԵՎԱՆԻ ՊԵՏԱԿԱՆ ՀԱՄԱԼՍԱՐԱՆ

ԳՐԻԳՈՐՅԱՆ ՀԱՍՄԻԿ ԳՐԻԳՈՐԻ

ՄԱԿԱՐԴՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԽՈՋԱՄՍԻ ԿԵՆՍԱՔԻՄԻԱԿԱՆ ՑՈՒՑԱՆԻՇՆԵՐԻ ԵՎ ԱՂԱԴՐՄԱՆ ԳՈՐԾԸՆԹԱՑԻ ՎՐԱ

Գ.00.04 - «Կենսաքիմիա» մասնագիտությամբ կենսաբանական գիտությունների թեկնածուի գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ – 2026

MINISTRY OF EDUCATION, SCIENCE, CULTURE AND SPORTS OF RA

YEREVAN STATE UNIVERSITY

GRIGORYAN HASMIK GRIGOR

EFFECT OF STARTER CULTURES ON THE BIOCHEMICAL PARAMETERS OF PORK AND THE SALTING PROCESS

SYNOPSIS

of dissertation for conferring a scientific degree of Candidate of Biological Sciences (PhD) specialty 03.00.04 – «Biochemistry»

YEREVAN – 2026

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝ կ.գ.դ., դոցենտ Գայանե Յուրիի Մարմարյան

Պաշտոնական ընդդիմախոսներ՝ կ.գ.դ., դոցենտ Սեդա Վիկտորի Մարության
ան.գ., թ., դոցենտ Հայկ Աշոտի Հարությունյան

Առաջատար կազմակերպություն՝ ՀՀ ԳԱԱ Հ. Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2026թ. դեկտեմբերի 20-ին ժամը 13^{00}-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲԿԳԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:
Ատենախոսության սեղմագիրն առաքված է 2026թ. նոյեմբերի 20-ին:

051 մասնագիտական խորհրդի
գիտական քարտուղար, կ.գ.դ., դոցենտ՝ Մ. Ա.Փարսադանյան

The theme of dissertation has been approved at Yerevan State University

Scientific supervisor: D. Sc., Assoc. Prof. Gayane Marmaryan

Official opponents: D. Sc., Assoc. Prof. Seda Marutyan

Ph.D., Assoc. Prof. Hayk Harutyunyan

Leading organization: H. Buniatyan Institute of Biochemistry of NAS RA

The defense of the dissertation will be held on 22th of December, 2026, at 13^{00} at the session of 051 Scientific Specialized Council on Biophysics of HESC of RA at Yerevan State University. Address: 1 Alex Manoogian, 0025 Yerevan, RA, YSU, Faculty of Biology

The dissertation is available at the library of Yerevan State University.

The synopsis has been sent on 20th of November, 2026.

Scientific Secretary of 051 Specialized Council
D.Sc., Assoc. Prof. M. A. Parsadanyan

GENERAL OVERVIEW OF THE DISSERTATION

Relevance of the Research Topic. Pork is one of the most widely consumed types of meat in the Republic of Armenia. According to the Statistical Committee of the Republic of Armenia, pork consumption totalled 37.3 thousand tonnes in 2024, equivalent to an annual per capita consumption of 13.1 kg (ARMSTAT, 2026). FAO data indicate that the global average annual per capita food supply of pork is 15.4 kg, ranking second only to poultry meat (FAO, 2025). The widespread consumption of pork underscores the importance of continuously improving the nutritional value and quality of pork products through the application of scientific research findings in production processes (Pandey et al., 2024).

Salting and maturation are key processes in the production of meat products, including ham. The biochemical and microbiological transformations occurring during these processes determine the nutritional value, safety, sensory quality, and shelf life of the finished product (Zhou & Zhao, 2007; Blesa et al., 2008). Although the BactoFlavor® starter culture is used in meat processing in the Republic of Armenia, its effects on the nutritional value of the semi-finished product and the biochemical parameters underlying maturation and flavour development have not been investigated. Therefore, a comprehensive investigation of this culture, together with the testing and comparative evaluation of alternative starter cultures, is timely and relevant.

In the present study, the lactic acid bacterial strains *Lacticaseibacillus rhamnosus* 20-12 (MDC 9631) and *Lactobacillus plantarum* 66 (MDC 9619) were tested alongside BactoFlavor® during pork salting. Their effects on pork maturation time, biochemical parameters associated with nutritional value and flavour development, safety, and shelf life were investigated. These comparative studies may provide a basis for selecting the most effective starter culture and scientifically substantiating its application in ham production, thereby contributing to reductions in processing time, production expenses, and the unit cost of the finished product.

The aim of this study is to investigate the effects of different starter cultures on pork maturation time and on biochemical parameters associated with nutritional value and flavour development, including changes in these parameters during maturation. Additional objectives are to elucidate the molecular mechanisms underlying the proteolytic, lipolytic, and antioxidant activities of the most effective starter culture through genomic analysis, and to assess microbiological safety and the growth of pathogenic microorganisms. From an applied perspective, the study aims to identify the most effective starter culture and provide a scientifically substantiated recommendation for its use in meat production.

To achieve this aim, the following objectives were established:

1. To select starter cultures for pork salting based on their salt tolerance and ability to grow at low temperatures.
2. To investigate the effects of the commercial starter culture BactoFlavor® and the lactic acid bacterial strains *Lacticaseibacillus rhamnosus* 20-12 and *Lactobacillus plantarum* 66 on pork maturation time.
3. To examine the effects of the starter cultures on biochemical parameters indicative of the intensity of pork maturation.
4. To assess the effects of the starter cultures on the amino acid composition of pork at different stages of maturation.
5. To evaluate the fatty acid composition of pork at different stages of maturation under the influence of the starter cultures.
6. To assess the effects of the starter cultures on the total antioxidant activity of pork.

7. To elucidate the molecular mechanisms underlying proteolytic, lipolytic, and antioxidant activities through genomic analysis of the most effective starter culture.
8. To analyse the genome of the most effective starter culture with regard to microbiological safety and the growth of pathogenic microorganisms.

Scientific Novelty of the Study

For the first time, the effects of the *L. rhamnosus* 20-12 strain on pork salting and maturation were comprehensively investigated in comparison with the commercial starter culture BactoFlavor® and the *L. plantarum* 66 strain. Maturation time, biochemical parameters associated with nutritional value and flavour development, and microbiological safety were assessed. The use of *L. rhamnosus* 20-12 reduced maturation time by two days compared with BactoFlavor®, from seven to five days, and by one day compared with *L. plantarum* 66. Samples treated with the lactic acid bacterial strains exhibited extensive glycogen breakdown and lactic acid accumulation, whereas pyruvic acid predominated in the sample treated with BactoFlavor®.

Analysis of changes in amino acids, creatinine, and fatty acids revealed that *L. rhamnosus* 20-12 promoted an increase in amino acid content within a shorter period than BactoFlavor®, with glutamic acid, aspartic acid, serine, and alanine predominating. This was accompanied by marked decreases in creatinine and methionine levels. Palmitic, stearic, linoleic, and oleic acids were the predominant fatty acids in the semi-finished pork product. The sample containing *L. rhamnosus* 20-12 exhibited the highest oleic acid content and antioxidant activity among the starter cultures tested. The experimental results also demonstrated suppression of E. coli growth in the semi-finished pork product treated with *L. rhamnosus* 20-12.

For the first time, whole-genome sequencing and bioinformatic characterisation of *L. rhamnosus* 20-12 were performed. Genome annotation identified 41 genes associated with lipogenic and lipolytic systems involved in lipid metabolism, as well as sequences encoding more than 21 distinct proteases. Genes encoding peroxidases and NADH oxidases involved in antioxidant defence, gene clusters associated with terpene biosynthesis, and a type III polyketide synthase (T3PKS) system were identified. The detection of gene clusters associated with bacteriocins and ribosomally synthesised and post-translationally modified peptides (RiPPs) revealed a potential genetic basis for the strain antimicrobial activity.

Practical Significance of the Study. The reduction in maturation time achieved using *L. rhamnosus* 20-12, the changes in biochemical parameters associated with nutritional value and flavour development, and the strain antioxidant and antimicrobial properties provide a basis for its application in ham production. Based on the research findings, an invention entitled “Method for Pork Maturation” was developed and granted short-term patent No. 1077 Y of the Republic of Armenia, based on application AM20260083Y. A prospective technological model for the application of the strain was proposed.

Main Provisions Submitted for Defence

- Lactic acid bacterial strains for pork salting and maturation were selected based on their salt tolerance and ability to grow at low temperatures, in accordance with the technological requirements of meat production.
- Among the starter cultures investigated for pork maturation, *L. rhamnosus* 20-12 was the most effective. Its application reduced maturation time by two days, from seven to five days, compared with the commercial starter culture BactoFlavor®.
- Lactic acid predominated in the semi-finished pork samples containing *L. rhamnosus* 20-12 and *L. plantarum* 66, whereas pyruvate predominated in the sample containing the commercial starter culture BactoFlavor®. The superior meat-fermenting capacity of the lactic acid

bacterial strains was further supported by analysis of the complete genome sequence of *L. rhamnosus* 20-12.

- Compared with BactoFlavor®, *L. rhamnosus* 20-12 increased amino acid content and decreased creatinine levels in the semi-finished pork product within a shorter period, demonstrating pronounced proteolytic potential. The L-glutamic acid content increased from 2.9% to 4.0%, while the contents of L-lysine, L-leucine, and L-serine reached 1.23%, 1.43%, and 1.57%, respectively. The increase in glutamic acid was accompanied by a decrease in methionine.
- Analysis of the complete genome and individual genes of L. rhamnosus 20-12 identified genes encoding more than 21 distinct proteases, including proteinases, aminopeptidases, dipeptidases, and endopeptidases. Their presence indicates a genetic basis for the strain proteolytic and peptidolytic potential and its capacity to generate free amino acids.
- The semi-finished pork product treated with L. rhamnosus 20-12 exhibited the highest oleic acid content (44.27%) among the starter cultures tested, together with high antioxidant activity. The strain genome contained 41 genes encoding enzymes involved in lipid metabolism (KEGG modules M00082 and M00083), genes encoding peroxidases and NADH oxidases, gene clusters involved in terpene precursor biosynthesis, and a T3PKS pathway.
- Whole-genome sequencing and annotation of L. rhamnosus 20-12 revealed the genetic basis of its proteolytic, lipolytic, antioxidant, and antimicrobial potential. Its potential application as a biopreservative was supported by experimentally confirmed antagonistic activity against E. coli, the presence of genetic elements associated with anti-listerial activity, and the assessment of microbiological parameters during pork maturation.

Publications. The results of the research conducted within the framework of this dissertation have been published in four scientific articles, including one single-authored article and three articles in Scopus-indexed journals ranked in quartiles Q2–Q4.

The main results of the dissertation were presented at the 35th FFC International Conference–23rd ASFFBC International Conference, held in Yerevan, Armenia, with the presentation "Genetic and Biochemical Characterization of Lacticaseibacillus rhamnosus 20-12 (MDC 9631) and Its Potential Application in Raw Pork Processing.", and Interuniversity Conference of Young Scientists entitled "Youth and the Horizons of Natural Sciences," held at Khachatur Abovian Armenian State Pedagogical University, with the presentation "Effects of Starter Cultures on the Biochemical Parameters of Pork and the Salting Process" (April 2025).

Structure and Length of the Dissertation. The dissertation comprises 121 pages and includes 8 figures, 16 tables, 5 charts, and 5 appendices. It consists of the following sections: List of Abbreviations, Introduction, Literature Review, Materials and Methods, Results and Discussion, Summary, Conclusions, and References.

CHAPTER 1. LITERATURE REVIEW

Chapter 1 reviews the starter cultures used in meat production and their technological and biopreservative significance. It describes the biochemical transformations of carbohydrates, proteins, and lipids during pork salting and maturation, as well as their role in determining the nutritional value and flavour and aroma characteristics of raw meat and finished products. The proteolytic, lipolytic, antimicrobial, and antioxidant properties of starter cultures are discussed, together with their effects on the quality, safety, and shelf life of raw meat. The chapter also highlights the importance of genomic studies in assessing the technological potential of strains and guiding their selection for specific applications.

CHAPTER 2. MATERIALS AND METHODS

Study Materials. The study used porcine *biceps femoris* muscle tissue, the commercial starter culture BactoFlavor® BFL-T03, comprising strains of *Pediococcus pentosaceus* and *Staphylococcus carnosus*, and the strains *Lactobacillus plantarum* 66 (MDC 9619) and *Lacticaseibacillus rhamnosus* 20-12 (MDC 9631). The latter two strains were obtained from the Microbial Depository Center of the Scientific and Production Center "Armbiotechnology" SNCO. Starter cultures were selected based on their tolerance to 3.5% NaCl and the ability to grow at 4–6 °C. The strains were grown in MRS medium under anaerobic conditions at 37 °C for 24 h. The cultures were lyophilised at −40 ± 2 °C for 48 h, and their viability was assessed by plating the reconstituted cultures and counting the resulting colonies.

Sample Preparation and Salting. Pork thigh muscle was trimmed of visible fat and divided into 150 g samples. Samples treated with the commercial starter culture BactoFlavor® BFL-T03, *L. plantarum* 66, or *L. rhamnosus* 20-12 were compared, with a traditionally salted sample serving as the negative control. A brine containing 11.13% NaCl was used for salting. The bacterial cultures were applied at a titre of 10^9 CFU/mL, and fructose was added to all treatment groups at 0.1% of the raw meat mass. Samples were analysed on days 1 and 4 of maturation and at the end of maturation. To monitor the process, pH was measured every 24 h using a Jenway 3540 pH meter.

Determination of Amino Acid and Fatty Acid Composition. Amino acid composition was determined by gas chromatography–mass spectrometry (GC–MS) using a Bruker Scion 450-GC gas chromatograph coupled to a Scion SQ mass spectrometer. Amino acid contents were expressed as percentages. For fatty acid analysis, lipids were extracted using a chloroform–methanol solvent system according to Folch et al. (1957), followed by conversion of the fatty acids into their methyl esters using a modified version of the method described by Ichihara and Fukubayashi (2010). The analysis was performed at the "Standard Dialog" laboratory using a Shimadzu GCMS-QP2010 system. The results were expressed as percentages of the total identified fatty acids.

Determination of Glycogen, Lactic Acid, Pyruvate, and Creatinine. Glycogen content was determined using the glucose oxidase–peroxidase method. Lactic acid and pyruvate were determined using a lactate dehydrogenase-based spectrophotometric method by monitoring changes in NADH absorbance at 340 nm. Creatinine content was assessed using the Jaffé reaction by measuring the absorbance of the coloured complex formed with picric acid under alkaline conditions at 520 nm.

Assessment of Antioxidant Activity. The antioxidant activity of aqueous extracts of pork samples was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Hambardzumyan et al., 2020). Following incubation of the reaction mixtures in the dark for 30 min, absorbance was measured at 517 nm using a Thermo Scientific Genesys 50 UV–Vis spectrophotometer. The percentage of radical scavenging activity and the IC_{50} value were calculated.

Evaluation of the Technological and Probiotic Properties of Starter Cultures. The salt tolerance of the strains was assessed in MRS broth containing 2–12% NaCl, while viability under different acidity conditions was evaluated over a pH range of 2–9. Adhesion capacity, proteolytic activity, and antibiotic resistance were also investigated. Antimicrobial activity was determined by measuring growth inhibition zones against the test strains *Salmonella typhimurium* G-38, *Bacillus subtilis* 17-89, and *Escherichia coli* K12, according to Parente et al. (1995). Viable cell counts were determined by serial tenfold dilution and plating on MRS agar.

Genome Sequencing and Bioinformatic Analysis of *L. rhamnosus* 20-12. Genomic DNA was extracted using the phenol–chloroform method. DNA quality was assessed by spectrophotometry and agarose gel electrophoresis. Whole-genome sequencing was performed on the Oxford Nanopore Technologies MinION platform using a Flongle R10.4.1 flow cell and the SQK-LSK114 kit. De novo genome assembly was performed using Flye within the EPI2ME Desktop environment, followed by annotation with Prokka. Genes associated with lipid metabolism were analysed using KEGG GhostKOALA, while genes involved in antioxidant defence were identified through targeted searches of the annotated genome. Gene clusters involved in bacteriocin and secondary metabolite biosynthesis were identified using BAGEL4 and antiSMASH, respectively.

Statistical Analysis. Biochemical and microbiological parameters were measured in at least triplicate for each sample. Results were expressed as the mean ± standard deviation (M ± SD). Data processing and graph generation were performed using GraphPad Prism 8.0.3 and Microsoft Excel.

CHAPTER 3. RESULTS AND DISCUSSION

Starter Culture Selection and Maturation Time. The effects of *L. plantarum* 66 and *L. rhamnosus* 20-12 on the rate and progression of pork maturation were evaluated against samples treated with the commercial starter culture BactoFlavor® (positive control) and traditionally salted samples without starter culture addition (negative control). Maturation was assessed by monitoring changes in pH and biochemical and microbiological parameters. In meat processing, pH is a key indicator of maturation, with its profile depending on both the product being manufactured and the type of raw meat used. In this study, a pH range of 4.9–5.1 was used as the criterion for pork maturation. Changes in pork pH during maturation are presented in Table 1.

Table 1. Changes in Pork pH During Maturation

Maturation day	Traditional salting	BactoFlavor®	*L. plantarum* 66	*L. rhamnosus* 20-12
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*	—	—	—

* Sample considered mature upon first reaching the pH range of 4.9–5.1 used as the maturation criterion in this study.

On day 1 of maturation, all samples had a pH of 6.2. Differences in acidification became more pronounced on day 4, when the pH values were 5.3 and 5.4 in samples treated with *L. rhamnosus* 20-12 and *L. plantarum* 66, respectively, compared with 5.6 for BactoFlavor® and 5.9 for traditional salting.

The sample treated with *L. rhamnosus* 20-12 reached the specified maturation pH range on day 5 at pH 5.1, whereas those treated with *L. plantarum* 66 and BactoFlavor® reached this range on days 6 and 7, respectively, at pH 5.0. The traditionally salted control sample reached the same range on day 8. Thus, *L. rhamnosus* 20-12 shortened maturation time by three days (37.5%) compared with traditional salting and by two days (approximately 28.6%) compared with BactoFlavor®. These findings support its selection for further investigation as a starter culture capable of accelerating pork maturation under the conditions used in this study.

Table 2. Viability of Lactic Acid Bacterial Strains Under Different Conditions

Strain	NaCl 2–12%	pH 3–8, %	OD_{590} 30 °C	OD_{590} 37 °C	OD_{590} 42 °C
L. rhamnosus 20-12	+	90	2.6	2.6	2.9
L. plantarum 66	+	80	3.3	2.7	2.7

MRS: de Man–Rogosa–Sharpe medium; OD: optical density; "+": growth observed

Table 3. Functional Properties of Lactic Acid Bacteria Strains

Strain	Proteolytic activity	IC_{50}, µL	Antibiotic resistance, % (n = 20)	Antimicrobial activity		
				S. Typhimurium G-38	*B. subtilis* 17-89	*E. coli* K-12
L. rhamnosus 20-12	+	59.6	34.0	2000	4000	2500
L. plantarum 66	+	21.0	55.6	1200	3000	1200

The last three columns show antimicrobial activity at pH 4.5–5.5, expressed in $AU \cdot mL^{-1}$. IC_{50}: the volume of culture supernatant required to scavenge 50% of DPPH radicals in a 0.1 mM DPPH solution.

Strain Viability and Antimicrobial Activity. Both strains exhibited salt tolerance and proteolytic activity. Across different pH values, *L. rhamnosus* 20-12 showed higher viability than *L. plantarum* 66, with viability levels of 90% and 80%, respectively. The antimicrobial activity of *L. rhamnosus* 20-12 against all three test strains exceeded that of *L. plantarum* 66 (Tables 1 and 2).



Figure 1. Assessment of viability at different salt concentrations: (a) *L. rhamnosus* 20-12; (b) *L. plantarum* 66; (c) BactoFlavor®; (d) traditional salting.

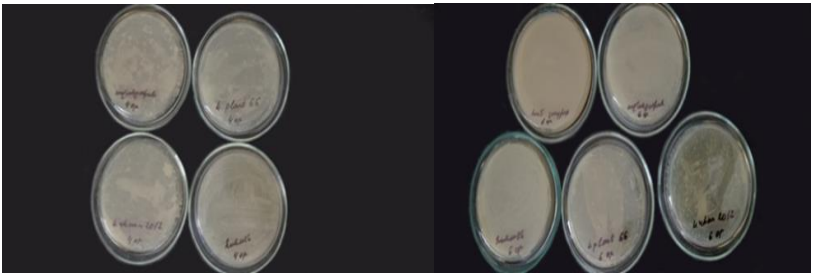


Figure 2. Viability of starter cultures in salted pork at different stages of maturation.

Table 4. Antimicrobial efficacy of lactic acid bacteria strains and BactoFlavor® BFL-T03 at different stages of pork maturation.

Type of salting	Total microbial count, CFU·mL⁻¹			*E. coli*, CFU·mL⁻¹		
	1st day	4rd day	Maturated	1-ին օր	4-րդ օր	Maturated
L. rhamnosus 20-12	$3{,}5 \times 10^2$	$1{,}2 \times 10^1$	0	$1{,}6 \times 10^2$	0	0
L. plantarum 66	$3{,}6 \times 10^2$	0	0	$2{,}0 \times 10^2$	0	0
BactoFlavor®	$4{,}5 \times 10^2$	$2{,}5 \times 10^1$	$2{,}2 \times 10^1$	$1{,}4 \times 10^2$	$2{,}0 \times 10^1$	0
Traditional salting (NaCl)	$1{,}4 \times 10^3$	$2{,}0 \times 10^1$	$1{,}5 \times 10^1$	$3{,}0 \times 10^2$	$3{,}5 \times 10^2$	$1{,}2 \times 10^3$

Mature samples: *L. rhamnosus* 20-12, day 5; *L. plantarum* 66, day 6; BactoFlavor®, day 7; control, day 8. "0" indicates non-detection as reported in the source. CFU: colony-forming unit.

E. coli was undetectable in samples treated with the lactic acid bacteria strains by day 4, whereas it was undetectable in the BactoFlavor®-treated sample on day 7 of maturation. In the control, its count increased from 3.0×10^2 to 1.2×10^3 CFU·mL⁻¹. These findings indicate that refrigerated storage or traditional salting alone is insufficient to ensure effective control of *E. coli*. The antagonistic activity of the starter cultures against *E. coli* is shown in Figure 3.

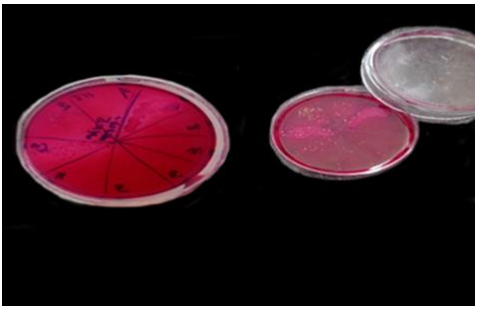
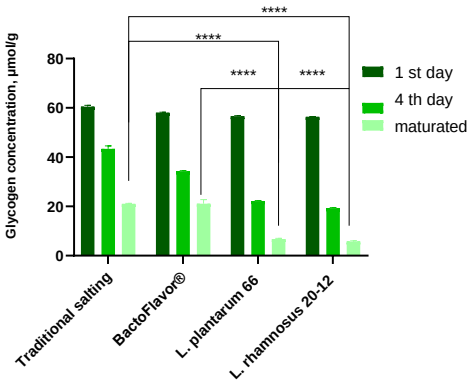
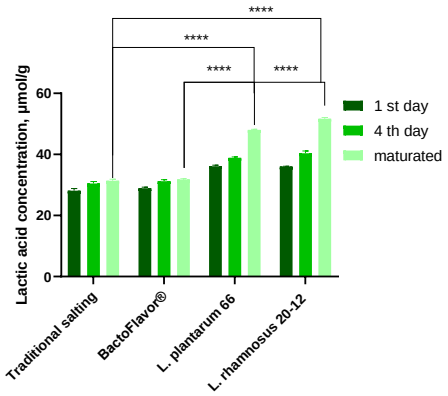


Figure 3. Antagonistic activity of starter cultures against *E. coli*.





Charts 1 and 2. Glycogen breakdown (left) and lactic acid accumulation (right) under the influence of starter cultures.

<u>Biochemical Indicators of Maturation Intensity</u>. Biochemical Indicators of Maturation Intensity. Following slaughter, endogenous autolytic enzymatic processes contribute to meat maturation, the duration of which depends on animal species, temperature, and the intensity of postmortem metabolic processes (Han et al., 2024). Glycogen, lactic acid, and pyruvate contents were determined on days 1 and 4 and in the semi-finished product at the end of maturation. Since the meat samples treated with different starter cultures reached maturity on different days (Table 1), comparison of these parameters at the final stage of maturation is particularly important.

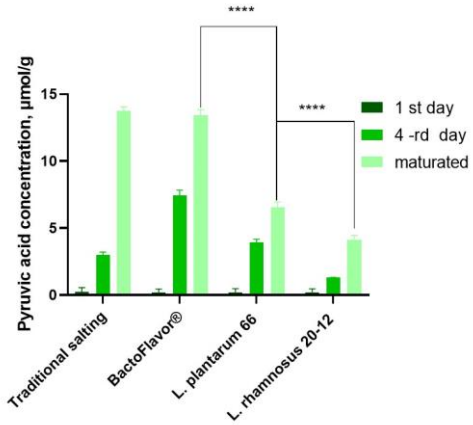


Chart 3. Changes in pyruvic acid content during maturation.

Glycogen content decreased during maturation in all samples, with the most pronounced reductions observed in those treated with the lactic acid bacteria strains. In the mature samples treated with *L. rhamnosus* 20-12 and *L. plantarum* 66, glycogen contents were 5.7 and 6.8 µmol/g, respectively. Lactic acid content increased from 36.0 to 51.7 µmol/g and from 36.1 to 48.0 µmol/g, respectively ($p < 0.0001$). In the mature samples treated with *L. rhamnosus* 20-12, *L. plantarum* 66, and BactoFlavor®, and in the control, pyruvate contents were 4.1, 6.5, 13.4, and 13.7 µmol/g, respectively. The high lactic acid and relatively low pyruvate contents in samples containing lactic acid bacteria are consistent with enhanced acid production and a rapid decrease in pH.

Amino Acid Composition and Changes in Creatinine Content. Free amino acids and their degradation products are key contributors to the nutritional value of meat and also enhance the sensory properties of meat and meat products, particularly flavour and aroma. Changes in free amino acid content were assessed at three stages of maturation.

Table 5. Changes in free essential amino acid content during pork maturation under the influence of starter cultures.

Amino acid / day	Traditional salting	BactoFlavor®	*L. plantarum* 66	*L. rhamnosus* 20-12
Isoleucine 1st day	0.70±0.03	0.71±0.03	0.69±0.03	0.70±0.04
4th day	0.78±0.04	0.65±0.03	0.81±0.04	0.78±0.04
Maturated*	1.01±0.05	0.56±0.03	0.97±0.05	0.81±0.04
Leucine 1st day	0.32±0.02[a]	0.36±0.02[a]	0.34±0.02[a]	0.36±0.02[a]
4th day	0.39±0.03	0.64±0.04	1.15±0.07	0.50±0.02
Maturated	1.30±0.08[a]	1.37±0.06[a]	1.77±0.11[a]	1.43±0.06[a]
Threonine 1st day	0.68±0.04	0.65±0.04	0.68±0.03	0.65±0.04
4th day	0.81±0.04	0.68±0.04	0.78±0.05	0.68±0.04
Maturated*	0.93±0.05	0.68±0.04	0.82±0.05	0.72±0.04
Histidine 1st day	0.52±0.03	0.52±0.03	0.50±0.03	0.52±0.03
4th day	0.58±0.02	0.50±0.03	0.58±0.03	0.58±0.03
Maturated	0.65±0.03	0.50±0.03	0.60±0.03	0.64±0.03
Lysine 1st day	0.44±0.03	0.48±0.03[a]	0.50±0.03[a]	0.48±0.03[a]
4th day	0.47±0.03	0.67±0.05	0.71±0.05	0.52±0.03
Maturated*	0.48±0.03	1.93±0.14[a]	1.33±0.09[a]	1.23±0.04[a]
Methionine 1st day	0.60±0.04	0.60±0.04	0.61±0.04[a]	0.60±0.04[a]
4th day	0.55±0.04	0.68±0.05	0.55±0.04	0.42±0.03
Maturated*	0.44±0.03	0.72±0.05	0.35±0.02[a]	0.29±0.02[a]

* Mature samples: traditional salting, day 8; BactoFlavor®, day 7; *L. plantarum* 66, day 6; *L. rhamnosus* 20-12, day 5.

[a] Indicates a statistically significant difference ($p < 0.05$) in horizontal comparison of each amino acid concentration across maturation days within the same salting treatment.

Among the essential amino acids, leucine, lysine, and arginine predominated, with contents ranging from 1.30–1.77%, 0.48–1.93%, and 1.13–1.54%, respectively, in mature samples subjected to traditional salting or treatment with starter cultures. Leucine and arginine predominated in the semi-finished product treated with *L. plantarum* 66, accounting for 1.77% and 1.47%, respectively, whereas the highest lysine content (1.93%) was recorded in the sample containing the commercial starter culture BactoFlavor®. As maturation progressed, statistically significant increases in the predominant amino acids were generally observed under both traditional salting and starter culture treatments ($p < 0.05$).

Table 6. Changes in free non-essential amino acid content during pork maturation under the influence of starter cultures.

Amino acid / day	Traditional salting	BactoFlavor®	*L. plantarum* 66	*L. rhamnosus* 20-12
Proline 1st day	0.57±0.03	0.60±0.03	0.59±0.03	0.59±0.03
4th day	0.61±0.03	0.63±0.03	1.03±0.05	0.61±0.03
Maturated*	1.20±0.06	0.98±0.05	1.17±0.06	0.92±0.04
Serine 1st day	0.47±0.03	0.50±0.03[a]	0.55±0.03[a]	0.50±0.03[a]
4th day	0.49±0.05	1.55±0.09	1.71±0.10	1.54±0.06
Maturated	0.83±0.05	1.58±0.08[a]	1.76±0.11[a]	1.57±0.05[a]
Aspartic acid 1st day	0.65±0.04[a]	0.69±0.04[a]	0.73±0.04[a]	0.71±0.04
4th day	0.70±0.14	0.88±0.05	0.84±0.05	0.79±0.05
Maturated	1.94±0.11[a]	1.50±0.08[a]	1.58±0.20[a]	1.20±0.06
Glutamic acid 1st day	1.94±0.13[a]	2.03±0.14[a]	2.10±0.15[a]	2.9±0.05[a]
4th day	2.08±0.03	2.46±0.17	2.20±0.15	2.41±0.06
Maturated	2.93±0.20[a]	5.79±0.04[a]	3.82±0.27[a]	4.00±0.06[a]
Tyrosine 1st day	0.32±0.02	0.35±0.02[a]	0.35±0.02	0.35±0.02
4thday	0.35±0.02	0.66±0.03	0.68±0.05	0.68±0.04
Maturated	0.37±0.02	0.80±0.06[a]	0.75±0.05	0.70±0.04
Arginine 1st day	0.90±0.06[a]	0.91±0.06	0.99±0.07	0.91±0.05
4th day	1.01±0.07	1.14±0.08	1.25±0.09	1.05±0.05
Maturated	1.54±0.10[a]	1.25±0.09	1.47±0.10	1.13±0.05
Alanine 1st day	0.85±0.06[a]	0.85±0.06	0.85±0.06[a]	0.89±0.06
4th day	0.88±0.06	1.10±0.08	0.85±0.06	0.93±0.05
Maturated	1.54±0.10[a]	1.48±0.10	1.75±0.12[a]	1.42±0.12

Among the non-essential amino acids, serine, aspartic acid, alanine, and glutamic acid were present at the highest levels, with glutamic acid predominating. Among the starter culture treatments, the highest glutamic acid contents were recorded in samples treated with the commercial culture BactoFlavor® and *L. rhamnosus* 20-12, reaching 5.79 ± 0.04% and 4.0 ± 0.06%, respectively. These values were attained on day 7 with BactoFlavor® and on day 5 with *L. rhamnosus* 20-12. Thus, *L. rhamnosus* 20-12 demonstrated greater efficiency in promoting glutamic acid accumulation within a shorter maturation period.

In addition to amino acids, changes in creatinine content were investigated at different stages of pork maturation under the influence of starter cultures. The results are presented in Table 7. A statistically significant decrease in creatinine content was observed in all samples as maturation progressed ($p < 0.005$).

Table 7. Changes in creatinine content at different stages of pork maturation under the influence of starter cultures (µmol/g).

Maturation day	Traditional salting	BactoFlavor®	*L. plantarum* 66	*L. rhamnosus* 20-12
1st day	4.47 ± 0.10a	6.13 ± 0.37a	4.15 ± 0.10a	6.80 ± 0.10a
4 th day	3.48 ± 0.08	4.80 ± 0.06	3.51 ± 0.14	3.80 ± 0.05
Maturated	2.43 ± 0.04a (8th day)	2.70 ± 0.05a (7th day)	1.94 ± 0.08a (6 th day)	2.15 ± 0.10a (5 th day)

* Results are expressed as the mean ± standard deviation (M ± SD).

[a] Indicates a statistically significant difference ($p < 0.005$) across maturation stages within each salting treatment.

The results showed that mature samples treated with *L. rhamnosus* 20-12 and *L. plantarum* 66 had lower creatinine contents—2.15 and 1.94 µmol/g, respectively—within shorter maturation periods than samples treated with BactoFlavor® or subjected to traditional salting. Compared with day 1, creatinine content decreased by 68.4% on day 5 in the *L. rhamnosus* 20-12-treated sample, by 53.3% on day 6 in the *L. plantarum* 66-treated sample, by 56.0% on day 7 in the BactoFlavor®-treated sample, and by 45.6% on day 8 in the traditionally salted sample.

An association was observed between changes in creatinine content and those in arginine and methionine, possibly reflecting their metabolic interrelationship, as these amino acids participate in creatine synthesis. In the sample containing *L. rhamnosus* 20-12, the decrease in creatinine was accompanied by a 24.2% increase in arginine and a 51.7% decrease in methionine relative to their initial values. In the sample treated with *L. plantarum* 66, arginine content increased by 48.5%, while methionine content decreased by 42.6%. In samples treated with BactoFlavor®, the decrease in creatinine was accompanied by increases in both arginine and methionine, although these increases were not statistically significant ($p > 0.05$).

Assessment of Pork Fatty Acid Composition at Different Stages of Maturation Under the Influence of Starter Cultures. To assess the effects of starter cultures on pork fatty acid composition, the relative contents of saturated, monounsaturated, and polyunsaturated fatty acids were investigated in samples treated with BactoFlavor®, *L. plantarum* 66, and *L. rhamnosus* 20-12, as well as in traditionally salted samples without starter culture addition. Analysis of the fatty acid profiles of the maturated samples revealed that palmitic (C16:0) and stearic (C18:0) acids predominated among saturated fatty acids, whereas oleic (C18:1 cis-9) and linoleic (C18:2 cis-9,12) acids were the predominant monounsaturated and polyunsaturated fatty acids, respectively. Further analysis therefore focused on changes in the relative contents of these four major fatty acids at different stages of maturation (Table 8, 9).The relative contents of all four fatty acids increased during maturation in every treatment, including traditional salting. In the maturated samples, palmitic acid content ranged from 22.44% to 24.56%. The values were similar in samples treated with BactoFlavor® and *L. rhamnosus* 20-12 (24.49% and 24.56%, respectively), whereas the lowest value was recorded in the *L. plantarum* 66 sample (22.44%).

Table 8. Fatty Acid Composition of Maturated Pork as Influenced by Starter Cultures (%)

Fatty acid, %	Traditional salting (8th day)	BactoFlavor® (7th day)	*L. plantarum* 66 (6th day)	*L. rhamnosus* 20-12 (5th day)
Saturated fatty acids (SFA)				
C8:0 Caprylic acid	0.18 ± 0.012	0.19 ± 0.009	0.16 ± 0.012	0.33 ± 0.023
C10:0 Capric acid	0.09 ± 0.006	0.11 ± 0.005	0.07 ± 0.008	0.11 ± 0.008
C11:0 Undecanoic acid	0.24 ± 0.016	0.22 ± 0.015	0.18 ± 0.012	0.36 ± 0.025
C12:0 Lauric acid	0.07 ± 0.005	0.07 ± 0.005	—	—
C13:0 Tridecanoic acid	0.21 ± 0.014	0.18 ± 0.012	0.15 ± 0.010	0.32 ± 0.022
C14:0 Myristic acid	1.03 ± 0.072	1.14 ± 0.08	0.98 ± 0.068	1.08 ± 0.075
C15:0 Pentadecanoic acid	0.13 ± 0.009	0.10 ± 0.007	0.10 ± 0.007	0.18 ± 0.012
C16:0 Palmitic acid	23.40 ± 0.28	24.48 ± 0.30	22.40 ± 0.30	24.5 ± 0.30
C17:0 Margaric acid	0.29 ± 0.014	0.25 ± 0.017	0.23 ± 0.016	0.20 ± 0.014
C18:0 Stearic acid	12.13 ± 0.20	13.02 ± 0.23	12.03 ± 0.22	12.75 ± 0.23
C20:0 Arachidic acid	0.15 ± 0.007	0.17 ± 0.011	0.18 ± 0.012	0.17 ± 0.011
C22:0 Behenic acid	0.25 ± 0.017	0.18 ± 0.014	0.22 ± 0.015	0.25 ± 0.017
C24:0 Lignoceric acid	0.09 ± 0.006	0.08 ± 0.005	0.19 ± 0.009	—
Monounsaturated fatty acids (MUFA)				
C16:1 n-7 Palmitoleic acid	2.08 ± 0.14	2.83 ± 0.19	2.18 ± 0.15	2.84 ± 0.19
C18:1 trans-9 Elaidic acid	0.08 ± 0.005	0.13 ± 0.009	0.16 ± 0.011	0.11 ± 0.007
C18:1 cis-9 Oleic acid	43.31 ± 0.20	42.82 ± 0.52	43.89 ± 0.50	44.27 ± 0.58
Polyunsaturated fatty acids (PUFA)				
C18:2 trans-9,12 Linolelaidic acid	0.15 ± 0.007	0.11 ± 0.006	0.16 ± 0.008	0.16 ± 0.008
C18:2 cis-9,12 Linoleic acid	9.61 ± 0.18	7.42 ± 0.17	10.12 ± 0.20	5.28 ± 0.15
C18:3 n-3 α-Linolenic acid	0.81 ± 0.048	0.08 ± 0.048	0.50 ± 0.031	0.56 ± 0.033

Stearic acid content ranged from 12.03% to 13.02%, with the lowest value observed in the *L. plantarum* 66 sample and the highest in the BactoFlavor® sample. Oleic acid was the predominant fatty acid among those examined in all maturated samples, with relative contents ranging from 42.82% to 44.27%. In the sample treated with *L. rhamnosus* 20-12, its content increased from 27.15% on the first day to 38.55% on the fourth day and 44.27% upon maturation on the fifth day. The corresponding final values were 43.89% for *L. plantarum* 66, 43.31% for traditional salting, and 42.82% for BactoFlavor®. Linoleic acid content increased from 3.00% to 5.28% in the *L. rhamnosus* 20-12 sample and from 5.80% to 10.12% in the *L. plantarum* 66 sample. In the traditionally salted and BactoFlavor® samples, it increased from 5.28% to 9.61% and from 4.10% to 7.42%, respectively. Thus, although linoleic acid content increased during maturation in all treatments, its final value was lowest in the L. rhamnosus 20-12 sample and highest in the L. plantarum 66 sample. Overall, *L. rhamnosus* 20-12 yielded the highest final relative content of oleic acid, whereas L. plantarum 66 yielded the highest final relative content of linoleic acid. These endpoint comparisons refer to samples reaching maturation on different days: day 5 for L. rhamnosus 20-12, day 6 for *L. plantarum* 66, day 7 for BactoFlavor®, and day 8 for traditional salting.

Table 9. Changes in major fatty acid contents at different stages of pork maturation under the influence of starter cultures (%).

Type of salting	Maturation day	(Palmitic acid) C16:0	(Stearic acid) C18:0	(Oleic acid) C18:1	(Linoleic acid) C18:2
Traditional salting	1st day	17.21 ± 0.25	10.26 ± 0.18	22.44 ± 0.18	5.28 ± 0.12
	4th day	21.58 ± 0.30	11.05 ± 0.22	31.38 ± 0.22	7.50 ± 0.15
	Maturated (8th day)	23.44 ± 0.28	12.13 ± 0.20	43.31 ± 0.20	9.61 ± 0.18
BactoFlavor®	1st day	17.88 ± 0.24	10.98 ± 0.19	20.75 ± 0.32	4.10 ± 0.11
	4th day	23.51 ± 0.31	11.80 ± 0.21	30.25 ± 0.38	5.90 ± 0.14
	Maturated (7 th day)	24.49 ± 0.33	13.02 ± 0.23	42.82 ± 0.52	7.42 ± 0.17
***L. plantarum* 66**	1 st day	18.0 ± 0.26	10.56 ± 0.18	23.89 ± 0.36	5.80 ± 0.13
	4 th day	20.52 ± 0.29	10.98 ± 0.20	33.14 ± 0.42	8.10 ± 0.16
	Maturated (6th day)	22.44 ± 0.30	12.03 ± 0.22	43.89 ± 0.56	10.12 ± 0.20
***L. rhamnosus* 20-12**	1st day	17.85 ± 0.25	10.44 ± 0.18	27.15 ± 0,38	3.00 ± 0.10
	4 th day	22.19 ± 0.30	12.00 ± 0.21	38.55 ± 0.45	4.20 ± 0.12
	Maturated (5th day)	24.56 ± 0.34	12.75 ± 0.23	44.27 ± 0.58	5.28 ± 0.15

* Results are expressed as the mean ± standard deviation (M ± SD).

Thus, the use of *L. rhamnosus* 20-12 resulted in the highest relative oleic acid content in the semi-finished product within the shortest maturation period of five days.

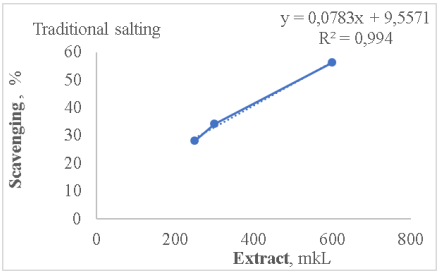
Assessment of Total Antioxidant Activity at Different Stages of Pork Maturation Under the Influence of Starter Cultures. Antioxidant activity was assessed using the DPPH assay with extract volumes of 250, 300, and 600 µL. Absorbance, radical scavenging activity, and IC_{50} values were compared between the initial and mature samples.

Table 9. Absorbance measurements of pork samples on day 1 and at the end of maturation.

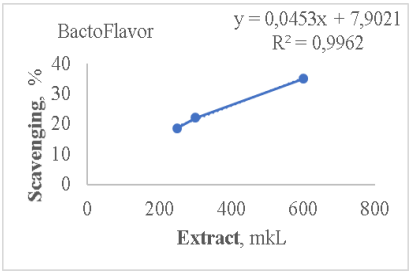
Type of salting	Maturation day	250 µL	300 µL	600 µL
Traditional salting	1 st day	1.05	0.94	0.68
	8th day	0.82	0.75	0.44
BactoFlavor®	1st day	0.96	0.94	0.72
	7th day	0.95	0.89	0.66
L. plantarum 66	1st day	1.07	1.03	0.72
	6th day	1.00	0.98	0.80
L. rhamnosus 20-12	1st day	1.01	0.88	0.76
	5th day	0.95	0.94	0.60

* Note: The table presents the arithmetic means of three absorbance measurements at 517 nm (M, n = 3), which were used to calculate IC_{50} values.

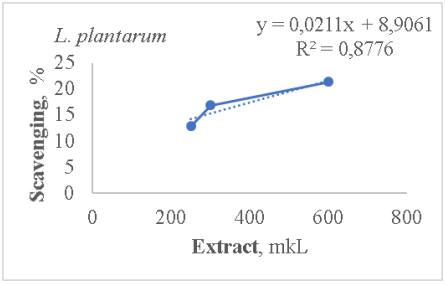
a)



b)



c)



d)

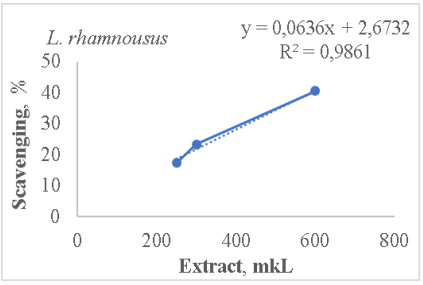


Chart 4. DPPH radical scavenging activity as a function of extract volume: (a) traditional salting; (b) BactoFlavor®; (c) *L. plantarum* 66; (d) *L. rhamnosus* 20-12.

Table 10. Antioxidant activity of pork samples on day 1 of maturation and in the semi-finished product.

Type of salting	Maturation day	250 µL	300 µL	600 µL	IC_{50}, mg
Traditional salting	1st day	8.0±0.6	16.3±1.1	32.7±2.1	-
	8th day	28.0±2.4	34.2±2.5	56.3±3.6	51.8
BactoFlavor®	1st day	17.0±1.0	18.0±1.2	28.3±2.0	-
	7th day	18.7±1.2	22.0±1.4	35.0±2.6	92.9
L. plantarum 66	1st day	6.7±0.5	11.9±0.8	21.6±1.5	-
	6th day	12.8±0.8	16.8±1.0	21.3±1.4	94.7
L. rhamnosus 20-12	1st day	10.2±0.7	13.8±0.9	24.7±1.6	-
	5th day	17.2±1.3	23.2±1.6	40.6±3.0	74.4

Among the starter cultures, *L. rhamnosus* 20-12 exhibited the highest antioxidant activity, with an IC_{50} of 74.4 mg, compared with 92.9 mg for BactoFlavor® and 94.7 mg for *L. plantarum* 66. The control without a starter culture had an IC_{50} of 51.8 mg. Thus, the advantage of *L. rhamnosus* 20-12 is limited to the comparison among starter cultures.

Characterisation of the Genomic Potential of *L. rhamnosus* 20-12. Genome sequencing of *L. rhamnosus* 20-12 yielded an assembly with a total length of 3,007,568 nucleotides, comprising 40 contigs, two of which were characterised as plasmid-like structures. Genome annotation identified 21 entries associated with proteolytic systems, as well as sequences encoding aminopeptidases, dipeptidases, and endopeptidases (Figure 4). These findings indicate a potential genetic basis for the degradation of protein and peptide substrates, complementing the experimental data on free amino acid formation.

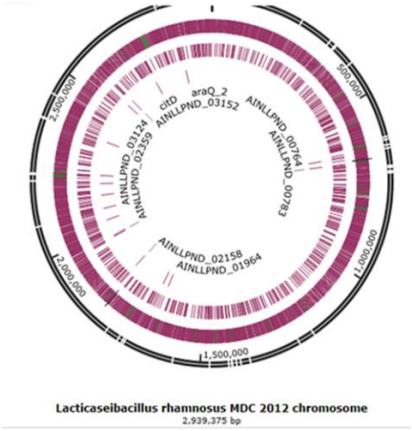


Figure 4. Circular map of the *Lacticaseibacillus rhamnosus* strain 20-12 chromosome, composed of 38 contigs. The chromosome was assembled using the chromosome of *L. rhamnosus* strain GR-1 as a reference.

The identification of 41 genes associated with lipid metabolism and the fatty acid biosynthesis modules KEGG M00082 and M00083 further characterises the strain metabolic potential. Genes encoding NADH oxidases, peroxidases, and reductases associated with protection against oxidative stress were also identified, together with clusters related to terpene precursor biosynthesis and the T3PKS system.

Annotations of sequences encoding LcnD proteins involved in bacteriocin secretion indicate the strain potential antimicrobial capacity. Together with the experimentally observed antagonistic activity against *E. coli*, these findings support further evaluation of the strain for biopreservative applications. Genomic analysis further suggests that the strain may exhibit antimicrobial activity against *Listeria monocytogenes* and thereby contribute to the microbiological safety of meat products.

CONCLUSIONS

1. Salting pork with *L. rhamnosus* 20-12 reduced maturation time by two days, from seven to five days, compared with the commercial starter culture BactoFlavor®, without reducing the nutritional value of the semi-finished product.
2. In samples treated with *L. rhamnosus* 20-12 and *L. plantarum* 66, maturation was accompanied by enhanced glycogenolysis and substantial lactic acid accumulation. Pyruvate accumulation was observed in the BactoFlavor®-treated sample. The genetic basis of the fermentative potential of *L. rhamnosus* 20-12 was characterised through genomic analysis.
3. Compared with BactoFlavor®, the use of *L. rhamnosus* 20-12 resulted in high amino acid contents, including the flavour-associated amino acids glutamic acid, aspartic acid, serine, and alanine, within a shorter period, together with a rapid decrease in creatinine content. These findings indicate the strain pronounced proteolytic potential.
4. Genomic analysis of the strain identified more than 21 annotations associated with the proteolytic system, including proteinases, aminopeptidases, dipeptidases, and endopeptidases. These findings indicate a potential genetic basis for the degradation of protein and peptide substrates and the formation of free amino acids.
5. The relative contents of the predominant fatty acids—palmitic, stearic, oleic, and linoleic acids—increased during maturation. Compared with samples treated with the other starter cultures, the sample treated with *L. rhamnosus* 20-12 exhibited the highest oleic acid (ω-9) content and antioxidant activity.
6. Genome annotation identified 41 genes associated with lipid metabolism, the fatty acid biosynthesis modules KEGG M00082 and M00083, genes encoding peroxidases and NADH oxidases involved in protection against oxidative stress, and clusters associated with terpene precursor biosynthesis and the T3PKS system. These findings characterise the potential genetic basis of the strain lipid metabolism and antioxidant defence.
7. Genomic data indicate potential mechanisms of lipid remodelling and oleic acid accumulation (DOI: 10.17632/smpvh236g3.2). Clusters associated with bacteriocins and RiPP systems suggest antimicrobial potential, including possible anti-listerial activity, which, together with the ability to suppress *E. coli* growth, may contribute to the safety of pork products.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. **Grigoryan H.,** Marmaryan G., Dashtoyan A., Karapetyan K., Kasarda R., Paloyan A., Fik M., Marmaryan Y. Effects of Lactic Acid Bacteria on Pork Meat Maturation. Acta Fytotechnica et Zootechnica. 2025. Vol. 28(2). p. 122–132. DOI: 10.15414/afz.2025.28.02.122-132.
2. **Grigoryan H. G.** Investigation of Antioxidant Activity During the Ripening of Pork Meat as Influenced by Starter Cultures. Bulletin of High Technology. 2025. No. 3 (35). p. 54–64. DOI: 10.56243/18294898-2025.3-54.
3. **Grigoryan H**., Marmaryan G., Soghomonyan T., Khoyetsyan L., Paloyan A., Tkhruni F., Karapetyan K., Hambardzumyan A. Genetic and biochemical characterization of Lacticaseibacillus rhamnosus MDC 2012 and its potential in processing of raw pork meat. Bioactive Compounds in Health and Disease. 2025. Vol. 8 (11), p. 462–473. DOI: 10.31989/bchd.8i11.1757.
4. **Grigoryan H.,** Marmaryan G., Marmaryan Y., Karapetyan K., Mkhitaryan A., Navasardyan D., Kovacik A., Hambardzumyan A. Impact of Starter Cultures on Lipid Oxidation and Fatty Acid Profile of Pork Meat Maturation. Journal of Microbiology, Biotechnology and Food Sciences. 2026. Vol. 16 (2) e14611. – DOI: 10.55251/jmbfs.14611.

ԳՐԻԳՈՐՅԱՆ ՀԱՍՄԻԿ ԳՐԻԳՈՐԻ

ՄԱԿԱՐԴՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԽՈԶԱՄՍԻ ԿԵՆՍԱՔԻՄԻԱԿԱՆ ՑՈՒՑԱՆԻՇՆԵՐԻ ԵՎ ԱՂԱԴՐՄԱՆ ԳՈՐԾԸՆԹԱՑԻ ՎՐԱ

ԱՄՓՈՓԱԳԻՐ

Բանալի բառեր՝ խոզամիս, աղադրում, *L. rhamnosus* 20-12, *L. plantarum* 66, BactoFlavor®, ամբողջական գենոմի սեքվենավորում

Հետազոտության նպատակն է. համեմատել արտադրական BactoFlavor® կուլտուրայի և կաթնաթթվային բակտերիաների՝ *Lacticaseibacillus rhamnosus* 20-12 և *Lactobacillus plantarum* 66 շտամների ազդեցությունը խոզամսի հասունացման տևողության, սննդային արժեքն ու համային որակը պայմանավորող կենսաքիմիական ցուցանիշների վրա՝ ընտրելով առավել արդյունավետ մեկնարկային կուլտուրան: Համադրվել են կուլտուրաների տեխնոլոգիական, կենսաքիմիական ու հակամանրէային հատկությունները և ընտրված շտամի գենոմային ներուժը: Ավանդական աղադրմամբ նմուշը ծառայել է որպես առանց մեկնարկային կուլտուրայի՝ բացասական ստուգիչ: *L. rhamnosus* 20-12-ի կիրառմամբ խոզամսի հասունացման տևողությունը BactoFlavor®-ի համեմատ կրճատվել է երկու օրով՝ յոթից հասնելով հինգ օրվա: *L. plantarum* 66-ով մշակված և ստուգիչ նմուշները հասունացել են համապատասխանաբար վեցերորդ և ութերորդ օրերին: *L. rhamnosus* 20-12-ով նմուշում pH-ի նվազումը մինչև 5.1 ուղեկցվել է գլիկոգենի ինտենսիվ տրոհմամբ, կաթնաթթվի կուտակմամբ: Հասունացած նմուշում գլիկոգենի քանակը կազմել է 5.7 մկմոլ/գ, իսկ կաթնաթթվինը սկզբնական 36.0-ից աճել է մինչև 51.7 մկմոլ/գ: BactoFlavor®-ով մշակված նմուշը հակված է պիրուվատի բարձր կուտակմանը: *L.*

rhamnosus 20-12-ով մշակված խոզի մսային հումքում գլյուտամինաթթվի քանակը 2.9%-ից աճել է և կիսապատրաստուկում կազմել 4.0%, իսկ լիզինի, լեյցինի և սերինի քանակները հինգերորդ օրը հասել են համապատասխանաբար 1.23%, 1.43% և 1.57%-ի: Կրեատինինի մակարդակը նվազել է 68.4%-ով: BactoFlavor® պարունակող նմուշում գլյուտամինաթթվի առավել բարձր՝ 5.79% քանակն արձանագրվել է յոթերորդ օրը. ուստի, *L. rhamnosus* 20-12-ի առավելությունն այս համատեքստում՝ հասունացման առավել կարճ տևողությունն է: Հասունացման ընթացքում աճել են առանցքային ճարպաթթուների՝ պալմիտինաթթվի, ստեարինաթթվի, օլեինաթթվի և լինոլաթթվի հարաբերական քանակները: *L. rhamnosus* 20-12-ով նմուշում օլեինաթթվի քանակը 1-ին օրվա 27.15%-ից կիսապատրաստուկում հասել է 44.27%-ի՝ հինգերորդ օրը գրանցելով ուսումնասիրված նմուշների առավել բարձր վերջնական արժեքը: ԴՖՊՀ մեթոդով գնահատված հակաօքսիդանտային ակտիվությամբ *L. rhamnosus* 20-12-ով նմուշը գերազանցել է մյուս մեկնարկային կուլտուրաներով մշակված նմուշներին, սակայն զիջել ավանդական աղադրմամբ ստուգիչին: Մանրէաբանական հետազոտությամբ *E. coli* չի հայտնաբերվել *L. rhamnosus* 20-12-ով նմուշում չորրորդ և հինգերորդ օրերին, իսկ BactoFlavor®-ով նմուշում՝ յոթերորդ օրը: Ավանդական աղադրման տարբերակում (ստուգիչ) դրա բջջաքանակն աճել է:

L. rhamnosus 20-12-ի գենոմի սեկվենավորումը կատարվել է Oxford Nanopore MinION հարթակով: Ստացվել է 3 007 568 նուկլեոտիդ ընդհանուր երկարությամբ հավաքում՝ 40 կոնթիգով, որոնցից երկուսը մեկնաբանվել են որպես պլազմիդանման կառուցվածքներ: Անոտավորմամբ հայտնաբերվել են պրոտեոլիտիկ համակարգերին առնչվող 21 CDS-գրառում, ամինո-, դի- և էնդոպեպտիդազներ կոդավորող հաջորդականություններ, լիպիդային նյութափոխանակությանն առնչվող 41 գեն և ճարպաթթուների կենսասինթեզի KEGG M00082 ու M00083 մոդուլները: Նույնականացվել են նաև օքսիդատիվ սթրեսից պաշտպանությանը մասնակցող NADH-օքսիդազների, պերօքսիդազների ու ռեդուկտազների գեներ, T3PKS և տերպենային նախորդների կենսասինթեզին առնչվող հնարավոր կլաստերներ: Բակտերիոցինի արտազատման համակարգին առնչվող lcnD_1 և lcnD_2 անոտացիաները, բակտերիոցինային ու RiPP հնարավոր կլաստերները լրացնում են շտամի հակամանրէային ներուժի գենետիկական բնութագիրը: Գենոմային տվյալների հիման վրա ենթադրվում է նաև *Listeria monocytogenes*-ի նկատմամբ հնարավոր ակտիվություն: Արդյունքների համադրումը հիմնավորում է *L. rhamnosus* 20-12-ի ընտրությունը որպես խոզամսի աղադրման և հասունացման հեռանկարային մեկնարկային կուլտուրա: Հասունացման տևողության կրճատումը, կենսաքիմիական ցուցանիշների փոփոխությունները, հակաօքսիդանտային և անտագոնիստական հատկությունները հիմք են ստեղծում խոզապուխտի արտադրությունում շտամի կիրառման տեխնոլոգիական մոդելի հետագա փորձարկման համար:

ГРИГОРЯН АСМИК ГРИГОРЬЕВНА

ВЛИЯНИЕ ЗАКВАСОЧНЫХ КУЛЬТУР НА БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ СВИНИНЫ И ПРОЦЕСС ПОСОЛА

РЕЗЮМЕ

Ключевые слова: свинина, посол, ***L. rhamnosus* 20-12, *L. plantarum* 66, BactoFlavor®**, **полногеномное секвенирование**.

Целью исследования являлось сравнительное изучение влияния промышленной культуры BactoFlavor® и штаммов молочнокислых бактерий *Lacticaseibacillus rhamnosus* 20-12 и *Lactobacillus plantarum* 66 на продолжительность созревания свинины и биохимические показатели, определяющие её пищевую ценность, вкусовые свойства, с целью выбора наиболее эффективной стартовой культуры. Сопоставлены технологические, биохимические и антимикробные свойства культур, а также геномный потенциал выбранного штамма. Образец традиционного посола без внесения стартовой культуры использован в качестве контроля. Применение *L. rhamnosus* 20-12 сократило продолжительность созревания свинины на двое суток по сравнению с BactoFlavor® — с семи до пяти суток. Созревание образцов, обработанных *L. plantarum* 66, и контрольных образцов завершилось соответственно на шестые и восьмые сутки. В образце с *L. rhamnosus* 20-12 снижение pH до 5.1 сопровождалось интенсивным распадом гликогена и накоплением молочной кислоты. В полуфабрикате содержание гликогена составило 5.7 мкмоль/г, а содержание молочной кислоты увеличилось с исходных 36.0 до 51.7 мкмоль/г. В образце с BactoFlavor® отмечено сравнительно более выраженное накопление пирувата. В свинине, обработанной *L. rhamnosus* 20-12, содержание глутаминовой кислоты увеличилось с 2.9 до 4.0%, а содержание лизина, лейцина и серина на пятые сутки достигло соответственно 1.23, 1.43 и 1.57%. В полуфабрикате содержание креатинина снизилось на 68.4%. Более высокое содержание глутаминовой кислоты в образце с BactoFlavor® — 5.79% — зарегистрировано на седьмые сутки. Следовательно, преимущество *L. rhamnosus* 20-12 в данном сравнении заключается в меньшей продолжительности созревания. В ходе созревания увеличивалось относительное содержание основных жирных кислот: пальмитиновой, стеариновой, олеиновой и линолевой. В образце с *L. rhamnosus* 20-12 доля олеиновой кислоты возросла с исходных 27.15 до 44.27% на пятые сутки, достигнув наибольшего конечного значения среди исследованных образцов. Наиболее высокое содержание линолевой кислоты отмечено в образце с *L. plantarum* 66 — 10.12%. По антиоксидантной активности, оценённой методом DPPH, образец с *L. rhamnosus* 20-12 превосходил образцы, обработанные другими стартовыми культурами, однако уступал контрольному образцу традиционного посола. При микробиологическом исследовании *E. coli* не обнаружена в образце с *L. rhamnosus* 20-12 на четвёртые и пятые сутки, а в образце с BactoFlavor® — на седьмые сутки. В контрольном образце численность *E. coli* увеличивалась.

Секвенирование генома *L. rhamnosus* 20-12 выполнено на платформе Oxford Nanopore MinION. Получена сборка общей длиной 3 007 568 нуклеотидов, включающая 40 контигов, два из которых интерпретированы как плазмидоподобные структуры. При аннотировании выявлена 21 CDS-запись, связанная с протеолитическими системами. Также обнаружены последовательности, кодирующие амино-, ди- и эндопептидазы, и 41 ген, связанный с липидным метаболизмом, а также модули биосинтеза жирных кислот KEGG M00082 и M00083. Идентифицированы гены NADH-оксидаз, пероксидаз и редуктаз,

участвующих в защите от окислительного стресса, и предполагаемые кластеры, связанные с T3PKS и биосинтезом предшественников терпенов. Аннотации lcnD_1 и lcnD_2, связанные с системой секреции бактериоцина, а также предполагаемые бактериоциновые и RiPP-кластеры дополняют характеристику генетических основ антимикробного потенциала штамма. На основании геномных данных предполагается также антимикробная активность в отношении *Listeria monocytogenes*. Совокупность полученных результатов: сокращение продолжительности созревания, изменения биохимических показателей, антиоксидантные и антагонистические свойства обосновывает выбор *L. rhamnosus* 20-12 в качестве перспективной стартовой культуры для дальнейшей апробации технологической модели применения штамма в производстве ветчины.

