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INVESTIGATION OF ANTIOXIDANT ACTIVITY DURING THE RIPENING OF PORK MEAT AS INFLUENCED BY STARTER CULTURES

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Abstract

This study evaluates the antioxidant activity of pork meat during maturation as influenced by different starter cultures. The performance of *Lacticaseibacillus rhamnosus* 2012 MDC 9631, *Lactobacillus plantarum* 66 MDC 9619, and a commercial BactoFlavor® culture was compared with traditional salting. Antioxidant activity was measured using the DPPH radical scavenging assay.

Results showed that all samples exhibited concentration-dependent antioxidant activity. Traditionally salted pork showed the highest activity, but *L. rhamnosus* 2012 MDC 9631 also demonstrated strong antioxidant potential, surpassing the other two cultures. Its proteolytic and antimicrobial properties suggest practical applicability in dry-cured ham production.

The findings support the use of *L. rhamnosus* MDC 9631 as a natural biopreservative that can enhance product quality, improve oxidative stability, and reduce maturation time in pork processing.

Keywords: Pork meat, Lactic acid bacteria cultures, salting, antioxidant.

Introduction

Starter cultures used in meat production can be described as viable microorganisms capable of multiplying within meat products, thereby extending their shelf life, ensuring sanitary and hygienic safety, improving food quality, and being harmless to the consumer [1-2]. Lactic acid bacteria (LAB) have been used by humans for millennia to preserve perishable foods such as milk, fermented sausages, and others.This is mainly due to their ability to synthesize lactic acid and antimicrobial compounds, which inhibit the growth of spoilage-causing microorganisms[3].The effectiveness of lactic acid bacteria (LAB) has been scientifically proven from a health and safety perspective, and they are known to have great potential for use as biopreservatives to improve the quality and extend the shelf life of various food products. In meat production, starter cultures are used for the enzymatic conversion of various components present in the raw material, which in turn contributes to the development of the flavor, stable color, and quality characteristics of the meat products.The biochemical composition of raw materials and the caloric content of finished meat products depend on the ratio of different tissue types and the technological processes used [4]. Starter cultures can reduce the amount of biogenic amines in fermented meat products.The accurate selection of cultures during the maturation process of raw materials makes it possible to shorten the

maturation time and obtain a semi-finished product with high biological value.Oxidative processes in meat lead to quality deterioration. Meat has endogenous antioxidants and prooxidants and living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px).Catalase and glutathione peroxidase (GSH-Px) are considered major peroxide-removing enzymes located in the cytosol[5].The biological value and shelf life of meat are determined by its maturation process.

Conflict Setting

Currently, starter cultures have become widely used and have established their unique role in the field of meat production.Total antioxidant activity is also considered an indicator of meat preservation and quality characteristics, as it provides insight into pork defects, including fatty acid oxidation.LAB as a bio-preservative alternative to chemical and physical preservatives, are used in various food products to eliminate pathogenic and spoilage microorganisms.The impact of lactic acid bacteria (LAB) on the microbiological safety of food is due to their production of various compounds, such as organic acids (lactic acid, pyruvic acid, oxo-acids), bacteriocins, diacetyl, acetyl-methyl-carbinol, hydrogen peroxide, carbon dioxide, and other yet-undescribed substances that inhibit foodborne pathogens and spoilage microorganisms[6].Currently, there is a growing emphasis on reducing the duration of the maturation process and optimizing the conditions for the use of starter cultures in technological processes.

This study evaluated the optimal exposure time, salting process, and antioxidant activity of different starter cultures during pork maturation. Salting is one of the oldest preservation methods, during which meat acquires a number of sensory characteristics beneficial for production. It is noteworthy that salting of meat is carried out to achieve the desired consumer and technological properties of the finished product (flavor, aroma, color), as well as to prevent microbiological defects [7-8].

Materials and Methods

In our studiey the selection of strains was based on salt tolerance (3.5%) and psychrophilicity (4–6°C).Based on the above criteria, the following starter cultures were selected: the widely used BactoFlavor® BFL-T0 culture from ***Hansen (Denmark)***, which includes the starter cultures *Pediococcus pentosaceus* and *Staphylococcus carnosus*, as well as the strains *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* 2012 MDC 9631.these were compared with traditional salting. The latter two strains, *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* 2012 MDC 9631 (previous Lactobacillus rhamnosus MDC 9631), were obtained from the Microbial Depository Center (MDC) of the ArmBiotechnology Scientific and Production Center, National Academy of Sciences of the Republic of Armenia. All lactic acid bacteria are stored in sterilized milk with a 40% glycerine content at 20 ˚C. The viability of LAB is up to 6 months.

The strains of LAB are maintained on de Man, Rogosa and Sharpe (MRS) agar medium under refrigerated conditions (+4–6 °C). Subculturing is performed once every 1–2 months.

The sensitivity of LAB strains to NaCl was tested in MRS broth containing various salt concentrations (2–12%) at 37°C for 24 hours of incubation [9-10].

The strains *Lacticaseibacillus rhamnosus* 2012 MDC 9631 and *Lactobacillus plantarum* 66 MDC 9619 are resistant to NaCl concentrations up to 8%, as they were isolated from salted cheese samples. The bacterial activity is evident in almost all technological stages of raw smoked meat processing.

Table 1

H.G. Grigoryan

INVESTIGATION OF ANTIOXIDANT ACTIVITY DURING THE RIPENING OF PORK MEAT AS INFLUENCED BY STARTER CULTURES

Characterization of LAB strains: *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* 2012 MDC 9631

N	Species Affiliation	Characterization of LAB strains								
		Proteolytic activity	Antioxidant activity (%)	Antibiotic Resistance, (%)	NaCl Tolerance (6%)	Antimicrobial activity pH=4,5-5,5 U/ml			Adhesion ability	Viability in pH range 2–9, %
						S.typhimurium G-38.	*B.subtilis* 17-89	*E.coli* K12		
1	*L. rhamnosus* 2012 *MDC* 9631	+	59.6	34.0	+	2000	4000	2500	+	90
2	*L. plantarum 66 MDC 9619*	+	21.0	55.6	+	1200	3000	1200	+	80

Notably, strains of *Pediococcus pentosaceus* and *Staphylococcus carnosus* have a positive effect on meat color development, as they contribute to the conversion of nitrate to nitrite through nitrate reductase activity. Moreover, staphylococci are capable of producing the catalase enzyme, which helps prevent oxidative defects during storage by breaking down the resulting hydrogen peroxide.Hydrogen peroxide and other peroxides are strong oxidants that react with myoglobin, causing it to lose color [11-12] Notably, the above-mentioned cultures can survive at temperatures below the optimal level, but they do not multiply under such conditions.

The research sample was collected from the thigh of a 7-month-old female pig. Four types of salting were performed: traditional salting, salting using the BactoFlavor level recommended by the manufacturer, and salting with lactic acid bacteria *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* 2012 MDC 9631 strains. The pork thigh was divided into four parts, each weighing 150 g. For all variants, a brine solution (50 ml) was prepared, with a salt concentration of 11.13% and a density of 1.077, under conditions of 15 °C. Injection was performed using 10% of the prepared 50 ml brine solution, applied to each 1 cm² of the surface. The quantity of lactic acid bacteria *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* 2012 MDC 9631 was 1 ml, with a culture titer of 10^9 CFU/ml. In addition, sucrose was added at 0.1% of the raw meat weight. The titer of the BactoFlavor culture was 10^7 CFU/ml, and in the case of this culture, fructose was also added at 0.1% of the raw meat weight. The objective is to establish identical conditions including temperature, brine density, and the same anatomical part of the meat while applying different starter cultures simultaneously. The aim is to compare the resulting changes with traditional salting, focusing on aspects such as reduced maturation time, increased biological value, enhanced antioxidant activity, and improved safety indicators.

Four salting samples were studied at different stages of maturation: on the 1st day, the 4th day, and in the fully matured state, which varies depending on the starter culture used.The pH value was considered an indicator of maturation, and the experiments were conducted in triplicate[13].

In the technological process of raw smoked products, when the salt content increases and the moisture level in the meat decreases, the species composition of the microflora changes

depending on various factors: raw material, technological process, and time. During salting, an increase in the number of lactic acid bacteria (LAB) is observed, which contributes to meat maturation. The strains of *Lactobacillus plantarum* and *Streptococcus lactis* show resistance to high salt concentrations [14].

Table 2

Physiological data of *Pediococcus pentosaceus* and *Staphylococcus carnosus Subs. Utilis*

Culture composition	***Pediococcus pentosaceus***	***Staphylococcus carnosus Subs. utilis***
Growth temperature Opt/max/min	35ºC/48 ºC/15 ºC (95 ºF/118 ºF/59 ºF)	30ºC/45ºC/10ºC (86 ºF/113 ºF/50 ºF)
Salt limit	7% salt-in-water	16% salt-in-water
Characteristics	Facultative anaerobic DL(+/-)- lactic acid producing	Facultative anaerobic Catalase positive Nitrate reductase positive Lipolitic Proteolytic
Fermentable sugars Glucose (dextrose) Fructose Maltose Lactose Saccharose (sucrose) Starch	 + + + (+) + -	 + + - + - -

The antioxidant capacity was evaluated during the different stages of pork maturation. Several methods exist for assessing antioxidant capacity, including ABTS, FRAP, CUPRAC, DPPH, and others[15]. For evaluating antioxidant activity in pork, the spectrophotometric method using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent is considered the most suitable[16].

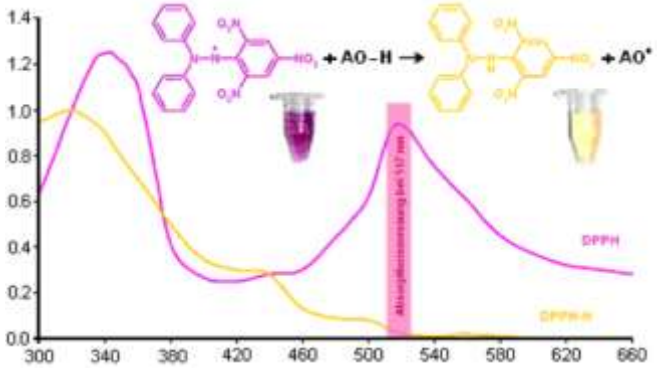


Fig. 1 Changes in the spectrophotometric absorption intensities of the DPPH• radical

Photometric measurements were carried out using a Thermo Scientific Genesys 50 UV-Vis spectrophotometer, at a wavelength of 517 nm.

During data analysis, the violet chromogenic radical is reduced by the antioxidant compound to 2,2-diphenyl-1-picrylhydrazine (DPPHH), which exhibits a yellow coloration. The degree of decolorization is directly proportional to the antioxidant activity the greater the decolorization, the stronger the antioxidant properties (Figure 1). Measurements were

performed using a spectrophotometer in the visible range at a wavelength of 517 nm, corresponding to the absorption intensity of the DPPH color.

To determine the IC50 values for DPPH radical scavenging, stock solutions with a concentration of 0.1 g/mL, prepared from the homogenized aqueous extracts of the final stage of pork maturation of each variant, were used.Subsequently, 250, 300, and 600 µL aliquots from each variant were diluted to a final volume of 2000 µL and mixed with 2000 µL of 0.2 mM DPPH solution in methanol-water. The mixtures were vortexed and incubated in the dark for 30 minutes. The absorbance (A1) was then measured at 517 nm.In addition, the DPPH solution was replaced with an equal volume of absolute methanol, and its absorbance (A2) was measured. Besides the test samples, a control measurement was performed using an equal volume of distilled water to determine the initial absorbance of the DPPH solution (A0). The DPPH radical scavenging activity was calculated using the following equation.

Scavenging activity (%)=[1-((A1-A2))/A0]*100

Research Results

During the determination of antioxidant activity by the DPPH method, an important indicator is the neutralization (scavenging) of 50% of the DPPH reagent by the antioxidant substance (IC50). Tab. 3 presents the results of the interaction between extracts obtained from the application of various starter cultures in pork and the DPPH reagent.

Table 3

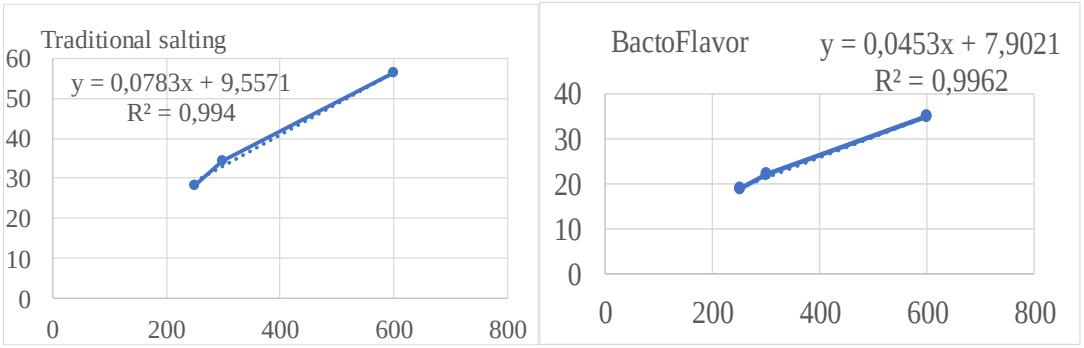
Results of optical density measurements of pork samples at the start and at the matured stages

Type of salting	Maturation phase	D_{517}		
		V, mkl 250	V, mkl 300	V, mkl 600
Traditional salting	Start	1.0	0.9	0.6
	8th day	0.8	0.7	0.4
BactoFlavor®	Start	0.9	0.9	0.7
	7th day	0.9	0.8	0.6
L. plantarum 66 MDC 9619	Start	1.0	1.0	0.7
	6th day	1.0	0.9	0.8
L. rhamnosus 2012 MDC 9631	Start/	1.0	0.8	0.7
	5th day	0.9	0.9	0.6

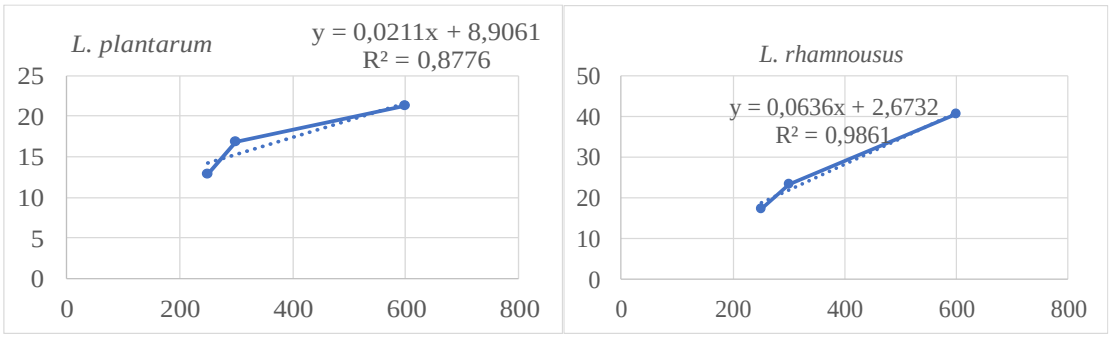
The obtained results show that the antioxidant activity (the ability to neutralize the DPPH radical) in the samples with traditional salting and applied starter cultures is directly proportional to the concentration of the tested meat extract. Specifically, at 600 µL of extract, all samples exhibited higher activity compared to the 250 µL or 300 µL concentrations.

H.G. Grigoryan

INVESTIGATION OF ANTIOXIDANT ACTIVITY DURING THE RIPENING OF PORK MEAT AS INFLUENCED BY STARTER CULTURES



a – Traditional salting b- BactoFlavor



c- *L. plantarum* d- *L. rhamnousus*

Fig. 2 Dependence of antioxidant activity on concentration: a – traditional salting, b – BactoFlavor, c – *L. plantarum*, d – *L. rhamnosus*

Table 4

Antioxidant activity of pork samples during the initial and maturation stages

Type of salting	Maturation phase	Sqavenging %		
		V, mkl 250	V, mkl 300	V, mkl 600
Traditional salting	Start	8.0	16.3	32.7
	8th day	28.0	34.2	56.3
BactoFlavor®	Start	17.0	18.0	28.3
	7th day	18.7	22.0	35.0
L. plantarum 66 MDC 9619	Start	6.7	11.9	21.6
	6th day	12.8	16.8	21.3
L. rhamnosus 2012 MDC 9631	Start	10.2	13.8	24.7
	5th day	17.2	23.2	40.6

Our findings demonstrate that among the four tested samples, the traditionally salted meat exhibited the highest antioxidant activity. Interestingly, while the sample inoculated with *Lacticaseibacillus rhamnosus* 2012 MDC 9631 showed slightly lower activity compared to the traditionally salted variant, it nevertheless surpassed those prepared with BactoFlavor® and *Lactobacillus plantarum* 66 MDC 9619 starter cultures. Considering the recorded antioxidant

activity of the *Lacticaseibacillus rhamnosus* 2012 MDC 9631 strain in the sample during pork maturation, along with the strain's proteolytic and lipolytic activities, as well as its demonstrated antagonistic properties, strong prerequisites are established for the practical application of the *L. rhamnosus* strain. Consequently, a technological prospective model for the production of dry-cured ham incorporating the use of *L. rhamnosus* strain will be proposed.

Technological recommendation

Based on the proposed technological scheme, the integration of *Lacticaseibacillus rhamnosus* 2012 MDC 9631 into ham production can enhance both antioxidant stability and overall product quality. The recommended technological process is as follows (Figure 3):

1. Raw Meat Preparation (Step 1): Select high-quality pork cuts, ensuring uniformity in size and anatomical location to maintain consistency in maturation.
2. Salting / Brine Injection (Step 2): Prepare a brine solution with controlled salt concentration and add the *L. rhamnosus* culture (10^9 CFU/mL). Inject the brine uniformly into the meat to achieve optimal distribution of starter cultures, while optionally including sugars to support bacterial activity.
3. Fermenter Cultivation of L. *rhamnosus* (Step 3): The *L. rhamnosus* 2012 MDC 9631 strain is cultivated in a bioreactor under optimized conditions—pH 5.8–6.0, temperature 37 °C, and gentle aeration—to achieve maximum viable cell density. The fermentation process continues until the culture reaches the target cell concentration suitable for inoculation ($\geq 10^9$ CFU/mL).
4. Cell Harvesting by Flow Centrifugation (Step 4): After cultivation, the bacterial biomass is separated from the culture medium using a continuous flow centrifuge. The concentrated cell suspension is then either directly incorporated into the brine solution or stored short-term at 4 °C before use to ensure cell viability and metabolic activity.
5. Additive/Dosing System (Step 5): Additional natural antioxidants or flavor-enhancing agents can be introduced during brine preparation, promoting both microbial growth and oxidative stability.
6. Maturation (Drying/Oven and Smoking) (Steps 6–7): Maintain controlled temperature and humidity conditions to allow for optimal bacterial activity and enzymatic reactions. L. rhamnosus contributes to the development of flavor, color stabilization, and antioxidant activity, reducing lipid oxidation and enhancing shelf life. Additional natural antioxidants or flavor-enhancing agents can be introduced during brine preparation, promoting both microbial growth and oxidative stability.
7. Packaging (Step 8): Once the desired maturation stage is reached, vacuum-pack or otherwise appropriately package the product to prevent contamination and preserve the functional properties conferred by the starter culture.

Incorporating *L. rhamnosus* 2012 MDC 9631 into the process not only ensures improved antioxidant activity and oxidative stability but also supports safer microbiological quality by inhibiting spoilage microorganisms. This approach allows for a shorter maturation period without compromising the sensory and nutritional quality of the final ham product [17].

H.G. Grigoryan

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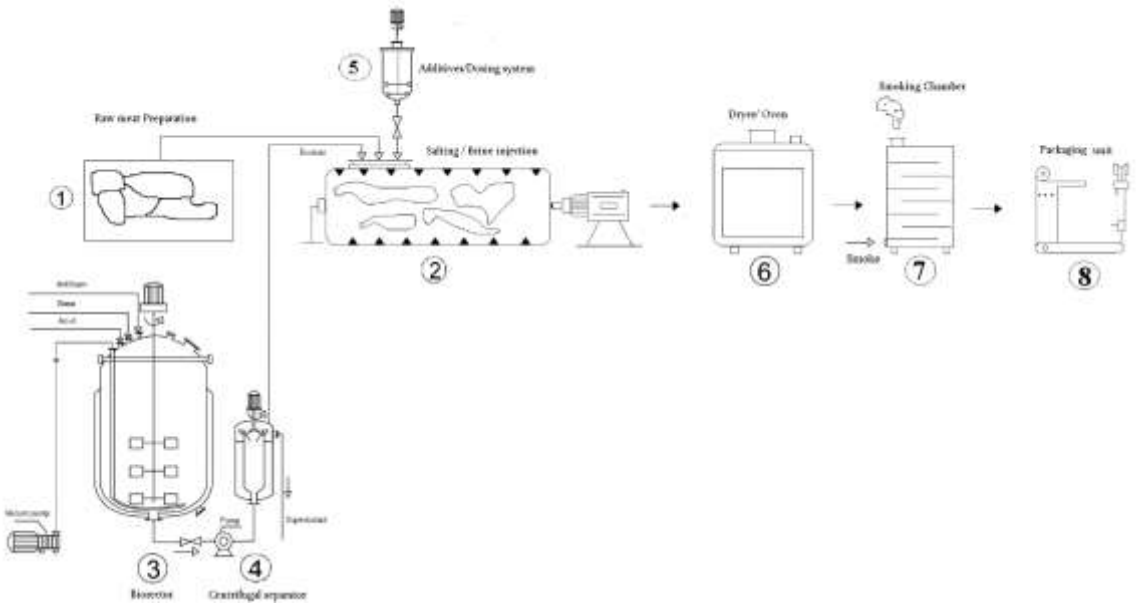

Fig 3. Technological Scheme of Ham Production with Integration of *Lacticaseibacillus rhamnosus* 2012 MDC 9631.

Conclusions

The study demonstrated that the antioxidant activity of pork meat during maturation is significantly influenced by the type of starter culture used. Among the tested variants, traditionally salted meat exhibited the highest antioxidant activity, while the sample inoculated with *Lacticaseibacillus rhamnosus* 2012 MDC 9631 showed slightly lower but still superior activity compared to those treated with BactoFlavor® and *Lactobacillus plantarum* 66 MDC 9619. The results indicate that the proteolytic and lipolytic activities, along with the antagonistic properties of *L. rhamnosus* 2012 MDC 9631, contribute to the maintenance of meat quality and oxidative stability during ripening. The study also confirmed that antioxidant activity is directly proportional to extract concentration, highlighting the importance of starter culture selection for optimizing meat maturation processes.

From a practical perspective, *L. rhamnosus* 2012 MDC 9631 demonstrates strong potential for application in the production of dry-cured ham and other pork products, providing a natural biopreservative effect while enhancing biological value and safety. The use of this starter culture could contribute to reduced maturation times, improved oxidative stability, and extended shelf life of meat products, representing an effective alternative to conventional chemical preservatives. These findings provide a technological basis for the incorporation of selected lactic acid bacteria in meat processing, supporting both product quality and consumer safety.

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ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒՄԸ ԽՈԶԱՄՍԻ ՀԱՍՈՒՆԱՑՄԱՆ ԸՆԹԱՑՔՈՒՄ ԿԱԽՎԱԾ ՄԵԿՆԱՐԿԱՅԻՆ ԿՈՒԼՏՈՒՐԱՆԵՐԻՑ

Հ.Գ. Գրիգորյան

Հայաստանի Ազգային Ագրարային Համալսարան

Հետազոտության նպատակն էր գնահատել խոզի մսի հակաօքսիդանտային ակտիվության օրինաչափությունը հասունացման ընթացքում՝ տարբեր մեկնարկային կուլտուրաների ազդեցության պայմաններում: Համեմատվել է *Lacticaseibacillus rhamnosus* 2012 MDC 9631, *Lactobacillus plantarum* 66 MDC 9619 և արտադրությունում կիրառվող BactoFlavor® կուլտուրաների արդյունավետությունը՝ ավանդական աղադրման մեթոդի հետ: Հակաօքսիդանտ ակտիվության գնահատման համար կիրառվել է DPPH ռադիկալների կապման թեստը:

Արդյունքները ցույց են տվել, որ բոլոր փորձանմուշները դրսևորել են կոնցենտրացիայից կախված հակաօքսիդանտային ակտիվություն: Ամենաբարձր արժեքները արձանագրվել են ավանդական եղանակով աղադրված մսի դեպքում, սակայն *L. rhamnosus* 2012 MDC 9631- ը նույնպես ցուցաբերել է էական հակաօքսիդանտային ակտիվություն՝ գերազանցելով մյուս մեկնարկային կուլտուրաներին: Վերջինիս արտահայտված պրոտեոլիտիկ և հակամանրէային հատկությունները վկայում են դրա գործնական կիրառելիության մասին խոզապուխտի արտադրության մեջ: Ընդհանուր առմամբ, ստացված տվյալները հաստատում են, որ *L. rhamnosus* 2012 MDC 9631–ը կարող է դիտարկվել որպես բնական կենսապահպանիչ, որն ունակ է բարձրացնել արտադրանքի որակը, ամրապնդել օքսիդատիվ կայունությունը և կրճատել մսամթերքի հասունացման տևողությունը:

Բանալի բառեր. խոզամիս, կաթնաթթվային կուլտուրաներ, աղադրում, հակաօքսիդանտ:

H.G. Grigoryan

INVESTIGATION OF ANTIOXIDANT ACTIVITY DURING THE RIPENING OF PORK MEAT AS INFLUENCED BY STARTER CULTURES

ИССЛЕДОВАНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ В ПРОЦЕССЕ СОЗРЕВАНИЯ СВИНИНЫ ПОД ВЛИЯНИЕМ СТАРТОВЫХ КУЛЬТУР

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Целью исследования являлась оценка закономерностей антиоксидантной активности свинины в процессе созревания под воздействием различных стартовых культур. Сравнивалась эффективность *Lacticaseibacillus rhamnosus* 2012 MDC 9631, *Lactobacillus plantarum* 66 MDC 9619 и промышленной культуры BactoFlavor® с традиционным методом посола. Для определения антиоксидантной активности был применён тест связывания радикалов DPPH. Результаты показали, что все образцы проявили концентрационно-зависимую антиоксидантную активность. Наибольшие значения зафиксированы у традиционно посоленного мяса, однако *L. rhamnosus* 2012 MDC 9631 также продемонстрировал значительный антиоксидантный потенциал, превысив остальные стартовые культуры. Его выраженные протеолитические и антимикробные свойства свидетельствуют о практической применимости в производстве сыровяленой свинины.

В целом, полученные данные подтверждают, что *L. rhamnosus* 2012 MDC 9631 может рассматриваться как природный биоконсерват, способный повышать качество продукции, усиливать окислительную стабильность и сокращать сроки созревания мясопродуктов.

Ключевые слова: свинина, молочнокислые культуры, посол, антиоксидант.

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