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COMPARATIVE ANALYSIS OF ANTIBIOTICS IN BROILER MEAT USING DIFFERENT METHODS

¹ZH.T. AKHMET* , ¹G.N. ZHAKSYLYKOVA , ²A.D. SERIKBAYEVA 

(¹Almaty Technological University, Kazakhstan, 050000, Almaty, 44 Baitursynov Str.)
(² Kazakh National Agrarian Research University, Kazakhstan, 28 Abay Avenue, Almaty, 050011)
Corresponding author e-mail: zhan.ahmetova@mail.ru*

The poultry industry is one of the fastest-growing industries worldwide, and broiler chicken meat is one of the main sources of meat. To speed up the growth of chickens in a short period of time, poultry farmers use antibiotics that prevent disease and stimulate growth by increasing the rate of feed intake and reducing mortality from pathogen attacks. This study may be useful in analyzing antibiotic residues and aid in scientific research. Samples of broiler chicken meat from different producer firms were purchased from the Almaty market. The analysis was done by chromatographic method and enzyme immunoassay. The Ridascreen test kit was used for the immunoassay. This article presents the results of the study of meat of broiler chickens of domestic and foreign producers for the presence of antibiotic residues used as growth stimulants. The researches have shown that imported meat of broiler chickens from the USA has 10-20% higher antibiotic content than the permitted level by the amount of antibiotic residues, and meat from Ukraine is 10-13% higher in antibiotics than the permitted level. The content of chloramphenicol is within the norm in all samples. Kazakhstan and Russian samples of broiler chicken meat meet the requirements of regulatory documents in force on the territory of the EAEU and the results showed that they contain only traces of antibiotics chloramphenicol and tetracycline. Analysis of imported broiler chicken meat (USA, Ukraine) showed the presence of antibiotic residues such as tetracycline and chloramphenicol, exceeding the maximum allowable level. To find out the effect of heat treatment on reducing the amount of antibiotics in broiler chicken meat, pates were made using different modes of heat treatment. As a result of heat treatment, the antibiotic content of poultry muscle tissue is significantly reduced.

Keywords: antibiotic, poultry meat, tetracycline, chloramphenicol, streptomycin, enzyme immunoassay, chromatography.

БРОЙЛЕР ЕТІНДЕГІ АНТИБИОТИКТЕРДІ ӘРТҮРЛІ ӘДІСТЕРМЕН АНЫҚТАУҒА САЛЫСТЫРМАЛЫ ТАЛДАУ

¹Ж.Т. АХМЕТ, ¹Г.Н. ЖАҚСЫЛЫҚОВА, ²А.Д. СЕРІКБАЕВА

(¹ Алматы технологиялық университеті, Қазақстан, 050000, Алматы, Байтұрсынов көшесі, 44)
(² Қазақ ұлттық аграрлық зерттеу университеті, Қазақстан, 050011, Алматы, Абай даңғылы 28)
Автор-корреспонденттің электрондық поштасы: zhan.ahmetova@mail.ru*

Күс шаруашылығы – әлемдегі ең жылдам дамып келе жатқан салалардың бірі, ал бройлер тауықтарының еті – еттің негізгі көздерінің бірі. Қысқа уақыт ішінде тауықтардың өсуін жылдамдату мақсатында шаруалар антибиотиктерді аурудың алдын-алу және азық-түлік мөлшерін көбейту, патогендік микроорганизмдерден болатын өлімді азайту арқылы өсуді ынталандыру үшін қолданады. Бұл зерттеу антибиотиктердің қалдықтарын анықтау үшін пайдалы болуы мүмкін және ғылыми зерттеулерге көмектеседі. Тауық етінің үлгілері Алматының базарларынан алынды. Талдау иммуноферменттік талдау әдісімен және хроматографиялық әдіспен жүргізілді. Иммуноферменттік талдауды Ridascreen тест-жүйісін пайдалана отырып жасадық. Бұл мақалада отандық және шетелдік өндірушілердің бройлер тауықтарының етін өсу стимуляторлары ретінде қолданылатын антибиотиктердің қалдықтарын зерттеу нәтижелері келтірілген. Осы көрсеткіш бойынша АҚШ-тан импортталатын ет рұқсат етілген нормадан 10-20% - ға, ал Украинадан импортталатын күс еті рұқсат етілген деңгейден 10-13% - ға асып түсетіні белгілі болды. Антибиотик хлорамфеникол құрамы барлық үлгілерде нормадан

аспады. Қазақстандық және ресейлік құс етінің үлгілері ЕАЭО аумағында қолданылатын нормативтік құжаттардың талаптарына сәйкес келеді және нәтижелер олардың құрамында стрептомицин және тетрациклин антибиотиктері ғана бар екенін көрсетті. Шикі тауық етін талдау нәтижесінде антибиотиктердің қалдықтары (тетрациклин, стрептомицин, хлорамфеникол) анықталды. Біз термиялық өңдеудің әртүрлі режимдерін қолдана отырып, құс етінен жасалған пащеттерін дайындадық. Термиялық өңдеу нәтижесінде құстың бұлшықет тініндегі антибиотиктердің мөлшері айтарлықтай төмендейді.

Негізгі сөздер: антибиотик, бройлер еті, тетрациклин, хлорамфеникол, стрептомицин, иммуноферментті талдау, хроматография.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ОПРЕДЕЛЕНИЯ АНТИБИОТИКОВ В МЯСЕ БРОЙЛЕРОВ РАЗЛИЧНЫМИ МЕТОДАМИ

¹Ж.Т. АХМЕТ*, ¹Г.Н. ЖАКСЫЛЫКОВА, ²А.Д. СЕРИКБАЕВА

¹Алматинский Технологический Университет, Казахстан, 050000, Алматы, ул. Байтурсынова 44

²Казахский Национальный Аграрный Исследовательский Университет,
Казахстан, 050011, Алматы, проспект Абая 28)

Электронная почта автора корреспондента: zhan.ahmetova@mail.ru*

Птицеводство является одной из наиболее быстро развивающихся отраслей во всем мире, а бройлеры - одни из основных источников мяса. Чтобы ускорить рост цыплят в короткие сроки, птицеводы используют антибиотики, которые предотвращают заболевания и стимулируют рост за счет увеличения скорости поглощения корма и снижения смертности от атак патогенов. Данное исследование может быть полезно для анализа остатков антибиотиков и поможет в научных исследованиях. Образцы мяса цыплят-бройлеров от разных фирм-производителей были закуплены на рынке города Алматы. В данной статье приведен сравнительный анализ результатов двух методов исследования мяса цыплят-бройлеров отечественных и зарубежных производителей на наличие остатков антибиотиков, используемых в качестве стимуляторов роста. Анализ проводился с помощью хроматографического метода и иммуноферментного анализа. Для проведения иммуноферментного анализа использовали тест-набор Ridascreen. Исследования показали, что импортное мясо цыплят-бройлеров из США по количеству остатков антибиотиков на 10-20% выше допустимой нормы, а мясо птицы из Украины превышает допустимый уровень на 10-13%. Содержание стрептомицина находится в пределах нормы во всех пробах. Казахские и российские образцы мяса цыплят-бройлеров соответствуют требованиям нормативных документов, действующих на территории ЕАЭС. Результаты показали, что они содержат только следы антибиотиков хлорамфеникола и тетрациклина. Анализ импортного мяса цыплят-бройлеров (США, Украины) показал наличие остатков антибиотиков, таких как тетрациклин и хлорамфеникол, превышающих предельно допустимый уровень. Для выяснения влияния термообработки на снижение концентрации антибиотиков мяса цыплят-бройлеров были изготовлены пащеты с применением разных режимов термической обработки. В результате тепловой обработки содержание антибиотиков в мышечной ткани птицы значительно снижается.

Ключевые слова: антибиотик, мясо бройлеров, тетрациклин, хлорамфеникол, стрептомицин, иммуноферментный анализ, хроматография.

Introduction.

In recent decades, the poultry industry has undergone substantial expansion, necessitating an increased reliance on specific pharmaceuticals and nutritional supplements for disease prevention, treatment, and growth promotion. An inherent drawback associated with the excessive utilization of antimicrobial agents lies in their accumulation within the tissues and organs of animals, manifesting as residues that ultimately integrate into the food chain. Consequently, the unwarranted use of these agents has been deemed illicit and has been

proscribed by regulatory bodies overseeing food products and public health in numerous countries. This research article scrutinizes the utilization of diverse antimicrobial agents in poultry farming. It delves into their dispersion and accumulation within various anatomical regions and tissues, subsequently addressing their potential ramifications on human health [1, 2].

In poultry farming, antibiotics represent the most extensively employed class of pharmaceuticals, administered via various routes including parenteral, intravenous, local, and oral administra-

tion. Antibiotic agents are typically employed for three primary purposes in poultry husbandry:

1) Therapeutic use: This involves the administration of high doses of antibiotics to individual animals or small groups over a relatively short period;

2) Prophylactic use: This pertains to the application of moderate doses of antimicrobial agents to animals over an extended period as a preventive measure;

3) Growth promotion: Antibiotics are administered in subtherapeutic doses, for example, at 10 or 100 times less than therapeutic doses, over an extended period, sometimes throughout the entire lifespan of the animals, to stimulate growth.

Antibiotics are known to exert their effects through various mechanisms. They can suppress DNA replication, inhibit the synthesis of ribonucleic acid (RNA) and proteins, impede cell division, interfere with cell differentiation and development, affect the directed metabolism of folic acid, or disrupt the cellular membrane and synthesis of the cell wall in microorganisms responsible for infection propagation [3, 4].

In developing countries, the use of antibiotics is a fairly common practice. The most frequently employed antibiotics include tetracycline, gentamicin, neomycin, tylosin, erythromycin, virginiamycin, ceftiofur, and bacitracin, which are typically effective in reducing and preventing respiratory diseases and necrotic enteritis infections. Fluoroquinolones or quinolone compounds are utilized to treat gastroenteritis, skin infections, or soft tissue infections. Sulfonamide compounds are applied as preventive and chemotherapeutic agents against coccidiosis, typhoid, coryza, and pullorum. Piperazine, oxytetracycline, amoxicillin, amprolium, ciprofloxacin, and sulfonamide preparations are used for the treatment of coccidiosis. However, the consumption of meat without knowledge of the withdrawal periods of antibiotics can lead to the accumulation of their residues in edible tissues and animal products (such as eggs) in various concentrations, thereby adversely affecting human health [5, 6].

Even traces of chemical compounds can accumulate and exert adverse effects on the human body, such as the development of resistance and impact on beneficial gut microflora. Elevated levels of residues pose a serious threat to public health and contribute to the emergence of antibiotic resistance. The consumption of meat containing antibiotic residues disrupts the functionality of the human gut microflora, promoting the proliferation of

pathogenic bacteria, thereby leading to heightened organism sensitivity and disturbances in the normal gut microflora [7, 8].

Therefore, the development and implementation of relevant epidemiological studies, integrated with the monitoring of broiler meat product samples, will be a crucial step in assessing the risk of chemical residue in broiler meat products. Additionally, such studies will play a vital role in their control and mitigation [9, 10].

The detection frequency of antibiotic substances in products depends on various factors, including the time elapsed since the completion of animal treatment, climatic and territorial conditions, seasonal variations, and so forth. In most countries worldwide, there are stringent regulations governing the use of antibiotic agents, along with comprehensive controls at all stages of food production and processing, extending up to the point of sale [11, 12].

Materials and research methods.

Various instrumental research methods, such as radioimmunoassay, chromatography, spectrometry, and others, are employed to determine the antibiotic content. These methods are labor-intensive, require expensive equipment, are complex to set up, and are typically applicable only in laboratory settings. From a practical standpoint, the most suitable methods are enzyme-linked immunosorbent assay (ELISA) and chromatographic techniques due to their high sensitivity [7, 8].

This study aims to assess the residues of antibiotics (streptomycin, tetracycline, chloramphenicol (levomycetin)) in broiler chicken meat available in the Kazakhstan market. The research was conducted at the "Antigen" laboratory in Almaty. The analysis was performed using enzyme-linked immunosorbent assay (ELISA) with the Ridascreen test kit and high-performance liquid chromatography (HPLC) method.

A total of 105 samples of broiler chicken meat were analyzed for the presence of tetracycline, streptomycin, and levomycetin.

The preparation of chicken pâté under laboratory conditions was conducted to determine temperature regimes that contribute to reducing the quantity of residues in the meat.

The technological process for producing chicken pâté consists of the following stages under laboratory conditions: thawing broiler chicken, washing, blanching, coating the meat, grinding, slicing, packaging, sealing, washing, checking the jars for airtightness, and sterilization. Chicken meat containing antibiotics above the

permissible norm was used for pâté preparation. Other ingredients (salt, black pepper, and vegetable oil) were purchased from a store. To compare the impact of temperature regimes, four different meat samples with the same amount of antibiotic residues were blanched. After the preliminary treatment, the poultry meat was ground to a paste-like consistency. Subsequently, the pastes were mixed with varying concentrations of water, salt, onions, phosphate mix, vegetable oil, vinegar, wheat bran, oil emulsion, soy isolate, pea flour, semolina, spice mix, and black pepper. The mixtures were thoroughly blended in a cutter to obtain the pâté [13, 14].

Samples of poultry meat were examined for the presence of antibiotic residues both before and after heat treatment. The ELISA method was employed for the detection of residues.

The broiler chicken meat underwent blanching in boiling water until the temperature in the muscle thickness reached 80°C, followed by exposure for 11, 13, and 19 minutes [15].

Results and discussion.

The results of the study on the content of antibiotics – streptomycin, tetracycline, and chloramphenicol (levomycetin) in samples from foreign (USA, Russia, Ukraine, Belarus) and domestic (firms "Alel," "Vkus kus," "Alatau kus") production are presented in Table 1.

It was determined that in Sample №1, the average concentration of chloramphenicol (levomycetin) was 0.022 mg/kg, and tetracycline was 0.018 mg/kg, exceeding the standard set by the Technical Regulation (Table 1). In Sample №3, the concentration of chloramphenicol (levomycetin) was 0.018 mg/kg, and tetracycline was 0.011 mg/kg.

Table 1 – Results of antibiotic determination using the ELISA method.

Sample	Producer Country	Tetracycline, mg/kg		Levomycetin (chloramphenicol), mg/kg		Streptomycin, mg/kg	
		permissible levels	obtained concentrations	permissible levels	obtained concentrations	permissible levels	obtained concentrations
1	USA	0,01	0,018	0,01	0,022	0,5	0,000
2	Russia	0,01	0,001	0,01	0,007	0,5	0,000
3	Ukraine	0,01	0,011	0,01	0,018	0,5	0,000
4	Belarus	0,01	0,001	0,01	0,008	0,5	0,000
5	Kazakhstan («Alel»)	0,01	0,005	0,01	0,009	0,5	0,000
6	Kazakhstan («Vkus kus»)	0,01	0,007	0,01	0,009	0,5	0,000
7	Kazakhstan («Alatau kus»)	0,01	0,003	0,01	0,007	0,5	0,000

The average concentration of tetracycline in samples of broiler chicken meat from the USA and Ukraine exceeded the permissible concentration by 1-1.8 times, and for levomycetin (chloramphenicol) - by 1.8-2 times. The level of streptomycin in all samples does not exceed the

permissible norm.

The residual amounts of antibiotics in broiler chicken meat were insignificant and below the permissible concentration limits, as indicated by the results obtained through HPLC (Table 2).

Table 2 – Results of antibiotic determination using HPLC.

Sample	Producer Country	Tetracycline, mg/kg		Levomycetin (chloramphenicol), mg/kg		Streptomycin, mg/kg	
		permissible levels	obtained concentrations	permissible levels	obtained concentrations	permissible levels	obtained concentrations
1	USA	0,01	0,0001	0,01	0,00002	0,5	0,00001
2	Russia	0,01	0,0002	0,01	0,00001	0,5	0,00001
3	Ukraine	0,01	0,0002	0,01	0,00002	0,5	0,00001
4	Belarus	0,01	0,0001	0,01	0,00001	0,5	0,00001
5	Kazakhstan («Alel»)	0,01	0,0005	0,01	0,00001	0,5	0,00001
6	Kazakhstan («Vkus kus»)	0,01	0,0003	0,01	0,00001	0,5	0,00001
7	Kazakhstan («Alatau kus»)	0,01	0,0002	0,01	0,00001	0,5	0,00001

The widespread use of antibiotics in poultry farming without knowledge of their withdrawal periods leads to the accumulation of small amounts of antibiotics in broiler meat products.

Based on the provided data, a comparative analysis of two methods (ELISA, HPLC) for determining residues of tetracycline, streptomycin, and chloramphenicol (levomycetin) was conducted in various samples (Table 3).

Table 3 – Comparative analysis of the residual concentrations of antibiotic substances in broiler chicken meat.

Antibiotic	Method	Sample						
		1	2	3	4	5	6	7
Tetracycline	HPLC	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	ELISA	0,018	0,001	0,011	0,001	0,005	0,007	0,003
Levomycetin (chloramphenicol)	HPLC	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	ELISA	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Streptomycin	HPLC	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	ELISA	0,022	0,007	0,018	0,008	0,009	0,009	0,007

The analysis of the residual concentrations of antibiotic substances in broiler chicken meat using HPLC revealed trace amounts of antibiotics. The difference between the results of the two methods can be explained by the fact that the HPLC method allows for the determination of antibiotic content in the range of 0.01-100 mg/kg, while ELISA covers the range of 0.0001-100 mg/kg.

Traces of antibiotics and their metabolites in broiler meat products can accumulate, thereby

causing adverse effects on the health of consumers [16].

The results obtained by us through the ELISA method differ from the analysis results conducted using HPLC. We have determined that the method of analyzing the concentration of residual antibiotic substances through ELISA is more accurate compared to the HPLC method.

Table 4 – Reduction of antibiotic residues after thermal processing, %

Duration, minutes	Antibiotic Concentration Reduction Level, %	
	Tetracycline	Levomycetin (chloramphenicol)
11	4	8
13	15	12
19	35	32

Applying different temperature regimes, we found that the antibiotic content decreases with prolonged blanching of broiler chicken carcasses for one hour. Additionally, we observed a reduction in the initial levels of chloramphenicol and tetracycline proportionally to the duration of temperature exposure. For levomycetin (chloramphenicol), this reduction averaged 8%, 12%, and 32% for durations of 11, 13, and 19 minutes, respectively, while for tetracycline, it was 4%, 15%, and 35%.

Based on our observations, the greatest reduction is associated with prolonged exposure of antibiotics to high temperatures, leading to coagulation and precipitation of proteins along with antibiotics on the walls of containers. However, brief and instantaneous blanching of poultry meat results in the destruction of antibiotics by approximately 4-8%. Blanching chicken carcasses for one hour reduces the initial

amount of antibiotics by 32-35%. Blanching is a necessary stage in the preparation of canned meat products, including pâtés. Therefore, if broiler meat exceeds the permissible limits of tetracycline and chloramphenicol, industrial processing, such as blanching chunks of muscle tissue weighing no more than 2 kg for 19 minutes, reaching an internal temperature of at least 80°C, can be recommended for further broiler implementation. The antibiotic, along with muscle juice, transfers from muscle fibers to the broth; hence, the broth after blanching should be disposed of.

Conclusion

The issue of contamination of poultry products with antibiotics is a subject of discussion both at the national and international levels. The widespread, often unauthorized use of these medicinal preparations in veterinary practice leads to their accumulation in food products and the surrounding environment. Approximately 80% of antibiotics

produced worldwide are used in poultry and livestock farming. Some poultry farmers currently employ subtherapeutic concentrations of antibiotics for various purposes, such as growth stimulation, accelerated weight gain, improved digestion, increased feed conversion ratio, as well as prevention or reduction of disease outbreaks. Since antibiotics cannot be completely eliminated from the organism, residues of veterinary drugs can be present in food products, even if their use is regulated.

We investigated the content of three antibiotics in broiler chicken meat. Based on the studied data, the following conclusion can be drawn: the concentration of tetracycline predominated over other investigated groups of antibiotics in all meat samples. It was found that the minimum residue levels of tetracycline were observed in domestically produced samples, while the maximum levels were detected in samples from the USA. The established results of exceeding the permissible concentration and the regimes of heat treatment can be utilized to address the issue of more rational use of chicken meat that received tetracycline group preparations before slaughter.

This study has shown that the results of antibiotic residue determination in broiler chickens differ when using two methods. While enzyme-linked immunosorbent assay (ELISA) indicates an average antibiotic concentration ranging from 0.001 to 0.022 mg/kg, high-performance liquid chromatography (HPLC) only detects traces of antibiotics. The levels of antibacterial substances, such as tetracycline and streptomycin, in broiler chicken meat identified by the HPLC method were only 0.00001-0.00028 mg/kg, consistent with data from most literature sources.

The insights gained from this study can be practically utilized by research institutions and production laboratories for assessing quantitative indicators of tetracycline content in broiler chicken meat. This information is valuable for making informed decisions regarding the further distribution of such poultry products.

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ФЕРМЕНТТЕЛГЕН ЖАРТЫЛАЙ ЫСТАЛҒАН ШҰЖЫҚТЫҢ ТАҒАМДЫҚ ЖӘНЕ БИОЛОГИЯЛЫҚ ҚҰНДЫЛЫҒЫ

У.А. РЫСПАЕВА* , Ш.Б. БАЙТУКЕНОВА , С.Б. БАЙТУКЕНОВА 

(С.Сейфуллин атындағы Қазақ агротехникалық университеті,
Қазақстан, Z11F9K, Астана қ., Жеңіс даңғылы 62)

Автор-корреспонденттің электрондық поштасы: ulzhan.ryspaeva@bk.ru*

Бұл мақалада ферменттелген жартылай ысталған шұжықтың тағамдық құндылығы мен биологиялық құндылығының зерттеу нәтижелерін талдау қарастырылады. Бірінші және екінші сортты сиыр етін пропион қышқылды микроағзамен өңдей отырып ет шикізатының сапасының өзгеруі зерттеледі. Ет шикізатын жетілу сатысы кезінде пропион қышқыл микроағзаның 0,1%-мен өңдеді. Стартерлік микроағзалар ет биополимерлерінің жылдам гидролизденуін тудырады, сол арқылы технологиялық процесті интенсификациялауға мүмкіндік береді. Отандық және шетелдік ғалымдар қазіргі заманауи биотехнологияны, нақты айтқанда ферментті дайын шұжық өнімінің шығымын жоғарылату, термиялық өңдеу ұзақтығын қысқарту, биологиялық құндылығы мен тағамдық құндылықтарын арттыру мақсатында қолданудың дұрыс бағыт екендігін дәлелдеген. Пропион қышқылды микроағзалары бос аминқышқылды мен ұшпа май қышқылдының айтарлықтай түзілуіне әкелетіндігі, сол арқылы дайын шұжық өнімінің дәмі мен органолептикалық көрсеткіштерінің жақсаруы мен өнімнің консистенциясының қалыптасуын жеделдететіндігі анықталады. Пропион қышқылдының төмен температурада өсетіндігі, патогенді микрофлораға жоғары белсенді екендігі және де бос май қышқылдары мен, аминқышқылдарының, витаминдер мен минералды заттарды жетілдіру қабілеттілігі ескеріліп микроағзалар ет шикізатын өңдеуде стартерлі бактерия ретінде қолдану тиімді болып табылады.

Негізгі сөздер: ферменттелген жартылай ысталған шұжық, пропион қышқылды, стартерлік бактерия, аминқышқыл құрамы, өнімнің тағамдық және биологиялық құндылығы.

БИОЛОГИЧЕСКАЯ И ПИЩЕВАЯ ЦЕННОСТЬ ФЕРМЕНТИРОВАННОЙ ПОЛУКОПЧЕНОЙ КОЛБАСЫ

У.А. РЫСПАЕВА*, Ш.Б. БАЙТУКЕНОВА, С.Б. БАЙТУКЕНОВА

(Казахский агротехнический университет имени С.Сейфуллина»,
Казахстан, Z11F9K, г.Астана, пр. Жеңіс 62)

Электронная почта автора корреспондента: ulzhan.ryspaeva@bk.ru*

В данной статье анализируются результаты исследований пищевой и биологической ценности ферментированных полукопченых колбас. Исследовано изменение качества мясного сырья при обработке говядины первого и второго сорта с пропионовокислыми бактериями. Мясное сырье обрабатывали в количестве 0,1% на стадии созревания. Бактериальные культуры вызывают быстрый гидролиз биополимеров мяса, что позволяет интенсифицировать технологический процесс. Отечественными и зарубежными