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БАЯНДАМАЛАРЫ

ДОКЛАДЫ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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IDENTIFICATION AND RESEARCH OF THE PROPERTIES OF LACTIC ACID BACTERIA STRAINS USED AS STARTER CULTURES FOR MAKING RAW-SMOKED HORSEMEAT SAUSAGES

Abstract. This article is about identification of flora of raw smoked sausages produced in the course of study, which will enable a further study of its properties and use in the production of raw smoked sausage from horse meat. The article describes the current state of the world market for horse meat and meat products; and production of sausages, including raw smoked sausages. There is a technology that has been developed in order to produce horse meat products using starter cultures: ready cooked smoked *Kazy* and raw smoked sausage. The hip portion, which remains after *jaya* is cut off, is used as the main stock for the production of raw smoked sausage. We compared the quality characteristics of the finished products, and studied the amino acid and fatty acid composition. We provided a comparative description for the organoleptic parameters of raw smoked sausages with starter cultures and a reference sample added. The samples were compared for: pH value, content of mass fraction of protein and microbiological characteristics. We tested elective nutrient medium for lactic acid bacteria in order to see if there was any significant microbial flora in raw smoked sausages.

Key words: Flora in raw smoked sausages, fermented meat products, starter cultures, lactic acid bacteria strains, amino acids, fatty acids, identification of microorganisms.

The world market for meat and meat products is one of the major sectors of the food market. One of the main and traditional sources of raw meat in our country is horse meat. According to the data, the Republic of Kazakhstan has sufficient resources of horse meat, which makes it prospective and interesting to be used in the production of national meat products for the population of Kazakhstan, as well as for export. The horse headcount in March 2018 in virtually all of the regions of Kazakhstan reached a maximum over the last 25 years in relative and absolute terms. According to the Statistics Committee of the Republic of Kazakhstan, as of March 1, 2018, there were 2,356.9 thousand horses in Kazakhstan, and as of this April 1, the number of these animals reached 2,463,500 heads. Despite the significant share of horse meat, the associated meat production is largely hampered by the lack of appropriate process solutions that would enable an effective use of this type of resource based on promising types of processing [1].

An analysis of the world horse meat market shows that horse meat production is less than 0.25% of total world meat production, with 700,000 tons of horse meat produced in total per year. If you look across continents, Asia, America and Europe produced 46%, 30% and 18% of the world's horse meat, respectively, with China, Kazakhstan and Mexico as the largest producers. Asia, as the largest producer, has almost no exports or imports, while North and South America exports most of the horse meat to Europe and Japan [2.11].

Despite having so many resources of live weight, Kazakhstan is the main importer of Russian meat products. Russia holds the main share in the structure of imports of meat products (up to 95%).

Today, if we look at European countries, horse meat is widely used in France, Belgium, Italy and Sweden, where horse meat exceeds lamb in sales volumes. In France, horse meat consumption accounts for 0.4 percent of all meat consumed. In Italy, traditional fermented sausage is made without the use of microbial starters, with the end of the product maturation characterized by a slight sourness and an elastic, semi-solid consistency. *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* are most commonly found lactobacilli in meat and meat products, including fermented sausages processed by various technologies. These include the most often isolated species - *Lactobacillus sakei* [3,4,10].

The main product derived from processing various types of raw meat is sausages. The different types of sausages include raw smoked sausage as the most natural sausage, which belongs to a unique meat products class consisting of only spice added meat. Raw smoked sausages represent a class of traditional meat products that are not heat-treated, that is, produced without pasteurization [3]. High-quality raw smoked products are based on the use of modern biotechnological techniques. Currently, most raw smoked sausages are produced via an accelerated technology using starter cultures, which may include microorganisms of various genera, including *Lactobacillus spp., Pediococcus spp., Staphylococcus spp., Micrococcus spp.* [2, 13].

Meat products are perishable products, which makes finding harmless and effective drugs to increase their shelf life an urgent matter.

One of the most important effects of starter bacterial cultures is an extended shelf life of meat products. The strains used for the meat industry can have a significant impact on the shelf life and quality of fermented foods by suppressing unwanted flora. Undesirable flora is suppressed and beneficial flora is produced due to the release of antibacterial substances, such as organic acids, carbon dioxide, hydrogen peroxide, diacetyl, and bacteriocins [13,14].

Purpose: to expand the range of raw smoked horse meat sausages with the possibility of using the portion of the hip, which usually remains after *jaya* is cut off; to improve organoleptic parameters and ensure the safety of the finished product; to shorten the maturation period for the product.

The following objectives were set in order to address this purpose:

- ✓ production of raw smoked horse meat sausages using starter cultures;
- ✓ production of raw smoked horse meat sausages without using starter cultures;
- ✓ comparison of quality characteristics and chemical composition of readymade product;
- ✓ identification of technologically significant microflora of finished raw smoked sausages for further study of their properties and use in production.

The following served as the research targets:

1) **starter cultures: a)** BLC -78 - *Staphylococcus carnosus, Pediococcus acidilacti;* б) Flora italia LC – *Lactobacillus sakei, Pediococcus acidilacti, Staphylococcus carnosus;*

2) **food products made with and without a starter culture.**

Research methods: pH value was determined by a potentiometric method; protein content in the product was determined by the content of protein nitrogen, which is found by the difference between the amount of total and non-protein nitrogen, based on nitrogen conversion to protein; a capillary gas chromatography with a flame ionization detector (FID) and a quartz column was used for gas chromatographic studies; microbiological research was based on testing elective nutrient media for lactic acid bacteria (medium for singling out lactic acid bacteria according to A. Netrusov's method) in order to identify technologically significant flora of ready-made raw smoked sausages (MRS nutrient medium).

Results. The research made it possible to:

- design a technology for the production of raw smoked horse meat sausages using starter cultures;
- design a technology for the production of cooked smoked product - *Kazy* branded as "Almaty" with the addition of starter cultures.

Starter cultures were added together with salt and seasonings at the rate of 0.5 g per 1 kg of minced meat, and they were also added to the brine. Microorganisms, introduced with starter cultures, acted through enzymes to change the structure of meat products, building new substances that contribute better product quality indicators.

Figure 1 – Cooked-smoked product – Kazy "Almaty" with the addition of starter cultures

The results of the study of the quality characteristics of cooked smoked *kazy* with the addition of starter cultures versus a reference sample are presented below (table 1). The production of cooked smoked *kazy* was based on horse meat from the rib section cooled for 48 hours at a temperature of (2-4) °C. What makes this process essential different is the use of a bacterial preparation when treated with brine, which makes it possible to intensify biochemical and microbiological processes during meat ripening. This reduces the duration of treatment with brine, reduces the amount of residual nitrite and improves the organoleptic characteristics of the finished product.

Table 1 – Organoleptic assessment of cooked smoked *Kazy* "Almaty"

Sample	Outer appearance	Appearance and color in cross section	Smell	Taste	Consistency	Juiciness	General score
Reference sample	7.1±0.3	6.7±0.2	6.8±0.3	6.4±0.2	6.4±0.3	6.1±0.3	6.6±0.3
Kazy with starter cultures added	7.5±0.3	7.4±0.3	7.0±0.4	7.4±0.2	7.0±0.4	6.6±0.4	2±0.3

We can see from Table 1 that *Kazy* samples with the addition of starter cultures received high scores compared to reference. *Kazy* made using starter cultures are distinguished by a denser consistency and a pronounced specific taste and aroma.

One of the components impacting taste and aroma are amino acids and fatty acids. The amino acid composition of cooked smoked *kazy* is presented in Table 2. These substances are formed and accumulated as a result of the breakdown of proteins, as well as peptides belonging to extractive substances of muscle tissue.

Table 2 - Amino Acid composition of the product: boiled and smoked Kazy "Almaty"

№	amino acid	Height	Area	Mass fraction in %	Concentration mg/l
1	Arginine	1,712	68,87	0,90±0,36	78,0
2	Lysine	0,243	2,415	0,014±0,004	1,20
3	Tyrosine	3,336	154,2	1,84±0,55	160,0
4	Phenylalanine	1,251	45,92	0,51±0,15	44,0
5	Leucine+isoleucine	3,124	233,3	0,98±0,25	85,0
6	Methionine	1,275	64,73	0,62±0,21	54,0
7	Valine	2,355	122,7	0,93±0,37	81,0
8	Proline	2,659	117,3	0,83±0,22	72,0
9	Threonine	1,547	63,64	0,47±0,19	41,0
10	Serine	1,215	59,0	0,36±0,009	31,0
11	Alanine	2,978	145,6	0,71±0,19	62,0
12	Glycine	3,794	225,7	0,89±0,30	77,0

Analysis temperature: 30°C;
Wavelength: 254
Calculation method: absolute calibration of the instrument.

The rate of accumulation of potential predecessor of taste and aroma which are built altogether in the process of cooking is associated with the rate of degradation of high molecular substances of muscle tissue, especially proteins. This is related to the depth of proteolysis [1].

Aromatic compounds obtained as a result of the activity of microorganisms are known to have a significant effect on the formation of the aggregated aroma of fermented meat products. In the development process, starter cultures release enzymes that catalyze the breakdown of carbohydrates, with organic acids, acetone, diacetyl now accumulated, which participate in the formation of taste and aroma of meat products [6.13].

One of the informing and accessible indicators for taste and aroma of meat products is the mass fraction of volatile fatty acids (table 3), which was identified by removing it with direct steam from acidified water extract followed by refining a distillate with alkali [5,8.12].

Table 3 - Fatty Acid composition of the boiled and smoked product: Kazy "Almaty"

№	Time, min.	Fatty acids	Height	Area, %
1	9,43	Oil	0,697	0,6387
2	14,41	Myristic	1,653	0,3807
3	18,78		0,692	0,1012
4	22,23	Stearic	0,724	0,0860
5	22,83		0,624	0,0483
6	26,21		0,689	0,0347
7	27,30		2,903	0,5578
8	29,06		2,393	0,3740
9	30,12		1,891	0,2537
10	30,74	Oleic	6,482	1,2961
11	33,36		5,038	0,8760
12	42,68		5,591	0,7667
13	47,34		24,661	5,1761
14	53,64	Linoleic	1,894	0,3788
15	56,96	Linolenic	7,580	1,8901
16	74,05	Pentadecane	3,170	1,1702
			66,682	13,4763

Chromatograph type – Crystallux – 4000M

Figure 2 – Smoked sausage made from horse meat

Formation of meat products' aroma is predominantly determined by the fermentation of fats, which results in di- and monoglycerides, volatile fatty acids and associated decomposition products [5,15].

Jaya, *kazy* and *karta* are the most valuable things in horse meat. We used portion of a horse hip, which remains after we cut out jaya, as the main stock for production of raw smoked sausage. The two following types of starter cultures were used in the manufacture of raw smoked sausages: BLC -78 — *Staphylococcus carnosus*, *Pediococcus acidilacti*; Flora italia LC - *Lactobacillus sakei*, *Pediococcus acidilacti*, *Staphylococcus carnosus*. The strains for microorganisms present in the composition of these

starter cultures are intended for the production of various types of raw smoked sausages, and are significantly different in quality from those starter cultures that are available in the market of our country (were purchased in Bulgaria during research training).

The work produced: a reference sample without a fermentation starter added; a test sample with bacterial fermentation starter BLC -78 added; a test sample with bacterial fermentation starter Flora italia LC. The results of the study into the organoleptic characteristics of raw smoked sausage with an addition of starter cultures versus reference sample are presented below (table 4).

Table 4 - Organoleptic assessment of raw smoked sausages

Assessment based on five score scale						
Sausage samples	cross section appearance	color	smell	taste	consistency	general
Test sample with BLC -78 added;	4.9	4.8	4.7	4.8	4.8	4.8
Test sample with Flora italia LC added	4.8	4.8	4.8	4.7	4.9	4.8
Reference (no ferment added)	4.5	4.5	4.6	4.5	4.5	4.52

From table 4 we can see that sausage samples made with starter cultures are rated higher than reference samples in terms of organoleptic indicators. Test samples had a more tender consistency and sourish taste versus reference. Samples of finished sausages also differed in pH:

test sample No. 1 – 4.74;

test sample No. 2 – 4.86;

reference – 4.91.

One of the most important values is the proteolytic activity of microorganisms used as starter cultures. It is determined by the degree of protein breakdown in meat. This principle contributes to higher quality characteristics of raw meat stock. Proteolytic activity of enzymes is about a change in the amount of protein in the final product [8,9.15]. The following data was obtained once the mass fraction of protein in the finished raw smoked sausages was determined (table 5):

Table 5 – Content of mass fraction of protein in finished raw smoked sausages

No.	Protein mass fraction, %	Actual results	Trial regulations
1	Test sample No. 1	28.51	GOST 25011-81
2	Test sample No. 1	28.15	GOST 25011-81
3	Reference	31.0	GOST 25011-81

At the next stage of the research, we tested elective nutrient media for lactic acid bacteria in order to identify technologically significant flora of ready-made raw smoked sausages.

The research revealed that no lactic acid microorganisms were detected in test sample No. 1, since only denitrifying cocci were found in the starter culture used: *Staphylococcus carnosus, Pediococcus acidilacti*. Test sample No. 2 revealed the number of lactic acid microorganisms, CFU/g - $12*10^7$. The reference sample revealed CFU/g – $6*10^7$ of lactic acid microorganisms. At the next stage of research we will single out pure cultures of denitrifying cocci, study the physiological and biochemical properties of the singled out strains and create new starter cultures.

As a conclusion to the work done to study the effect of lactic acid bacteria strains used as starter cultures on the quality characteristics of fermented meat products, we can now state that microorganisms in a starter culture perform many indispensable functions, such as: accelerating biochemical processes occurring in raw meat when treated with brine, and proteolysis of muscle proteins driving the rate of maturation of meat products; improvement of organoleptic characteristics of the finished products; optimal pH reduction; improved sanitary conditions; all of these also play an important role in the formation of taste and aroma.

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ЖЫЛҚЫ ЕТІНЕН ҚАҚТАЛҒАН ШҰЖЫҚ ДАЙЫНДАУДА ҚОЛДАНЫЛАТЫН ҰЙЫТҚЫ РЕТІНДЕ ПАЙДАЛАНЫЛАТЫН СҮТ ҚЫШҚЫЛДЫ БАКТЕРИЯЛАР ШТАММДАРЫН БӨЛІП АЛУ ЖӘНЕ ҚАСИЕТТЕРІН ЗЕРТТЕУ

Аннотация. Бұл мақала зерттеу барысында дайындалған, қақталған шұжық өнімдерінен технологиялық тұрғыдан маңызды микрофлораны бөліп алуға, қасиеттерін зерттеуге және алдағы уақытта жылқы етінен қақталған шұжық өнімдерін өндіруде пайдалануға арналған. Мақалада жылқы еті және ет өнімдерінің дүниежүзілік нарықтағы бүгінгі күнгі жағдайы; шұжық өнімдерінің, соның ішінде қақталған шұжық өнімдерінің өндірісі баяндалған. Жылқы етінен ұйытқы қосылған ет өнімдерін дайындау технологиясы құрастырылған: пісіріліп-ысталған Қазы және қақталған шұжық. Қақталған шұжық дайындау үшін, жаяны кесіп алғаннан кейін қалатын, жылқының сан еті пайдаланылды. Дайын өнімдердің сапалық көрсеткіштерін өзара салыстыру, аминқышқылдық және майқышқылдық құрамын зерттеу жүргізілді. Ұйытқы қосылып дайындалған және ұйытқысыз дайындалған, қақталған шұжық өнімдерінің органолептикалық көрсеткіштері салыстырмалы түрде сипатталған. Үлгілерде салыстырмалы түрде: pH мөлшері, ақуыздың салмақтық үлесі және микробиологиялық қасиеттері сипатталған. Дайын қақталған шұжық өнімдерінің технологиялық тұрғыдан маңызды микрофлорасын бөліп алу мақсатында, сүт қышқылды микроорганизмдерді элективті қоректік орталарға себу жүргізілген

Түйін сөздер: қақталған шұжық өнімдерінің микрофлорасы, ферменттелген ет өнімдері, ұйытқы, сүт қышқылды бактериялар штаммдары, аминқышқылдары, май қышқылдары, микроорганизмдерді бөліп алу.

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ИДЕНТИФИКАЦИЯ И ИЗУЧЕНИЕ СВОЙСТВ ШТАММОВ МОЛОЧНОКИСЛЫХ БАКТЕРИЙ, ПРИМЕНЯЕМЫХ В КАЧЕСТВЕ СТАРТОВЫХ КУЛЬТУР ДЛЯ ИЗГОТОВЛЕНИЯ СЫРОКОПЧЕНЫХ КОЛБАС ИЗ КОНИНЫ

Аннотация. Данная статья посвящается идентификации технологически значимой микрофлоры изготовленных в ходе исследования сырокопченых колбас, для дальнейшего изучения их своиств и применения в производстве сырокопченых колбас из конского мяса. В статье описаны: нынешнее состояние мирового рынка конского мяса и мясных продуктов; производство колбасных изделии, в том числе сырокопченых колбас. Разработана технология производства мясных изделии из конины с использованием стартовых культур: варено-копченая Казы и сырокопченая колбаса. В качестве осовного сырья для производства сырокопченой колбасы использован задний окорок (сан ет) конины, который остается после срезания жая. Проведено сравнение качественных характеристик готовых продуктов, исследование аминокислотного и жирнокислотного состава. Сравнительно описаны органолептические показатели изготовленных сырокопченых колбас с добавлением стартовых культур и контрольного образца. Сравнительно описаны в образцах: величина pH, содержание массовой доли белка и микробиологические характеристики. Произведен посев на элективные питательные среды для молочнокислых бактерий, с целью выявления и идентификации технологически значимой микрофлоры готовых сырокопченых колбас.

Ключевые слова: Микрофлора сырокопченых колбас, ферментированные мясные продукты, стартовые культуры, штаммы молочнокислых бактерии, аминокислоты, жирные кислоты, идентификация микроорганизмов.

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