





## DEVELOPMENT OF A FUNCTIONAL HORSE-MEAT ROULADE USING PLANT-DERIVED COMPONENTS

<sup>1</sup>SH.Y. KENENBAY\* , <sup>1</sup>B.A. RSKELDIYEV ,  
<sup>1</sup>A.S. KRASNIKOV , <sup>2</sup>Z.N. NIYAZBEKOVA 

(<sup>1</sup>JSC «Almaty Technological University», Kazakhstan, 050012, Almaty, Tole bi str., 100,

<sup>2</sup>NPJSC «Kazakh National Agrarian Research University», Kazakhstan, Almaty)

Corresponding author e-mail: sh.kenenbai@atu.edu.kz

*The aim of the study was to provide a scientific justification for the formulation and processing technology of a horse-meat roulade using plant-based functional ingredients – calamus rhizome, a humic–fulvic acid complex and pumpkin seed cake – and to evaluate their effects on the nutritional value, technological properties and sensory characteristics of the product. Within the framework of the study, control and experimental samples of horse-meat roulade were developed, for which the chemical composition, energy value, pH, TBA index, cooking loss, total viable count (TVC), amino acid profile and sensory attributes were determined. In the experimental roulade, the protein content decreased from 28,2% to 19,12%, and the fat content from 14,8% to 8,7%, while carbohydrates (5,6%) were detected, which reduced the energy value of the meat product from 246,0 to 177,2 kcal/100 g. A decrease in pH from 6,1 to 5,8 and in the TBA value from 0,45 to 0,31 mg MDA/kg was observed, and cooking loss was reduced from 32,8% to 17,01%. The TVC in the experimental roulade sample amounted to  $7,2 \times 10^2$  CFU/g versus  $8,1 \times 10^2$  CFU/g in the control sample, which was below the limit specified by the regulatory document ( $1 \times 10^3$  CFU/g). The amino acid score for key essential amino acids exceeded 130%, and the biological value of the meat product reached 139,86%. At the same time, the sensory scores for appearance, colour and taste of the experimental roulade were higher than those of the control sample.*

**Keywords:** horse meat, meat roulade, calamus rhizome, humic–fulvic acids, pumpkin seed cake, functional meat product.

## РАЗРАБОТКА ФУНКЦИОНАЛЬНОГО МЯСНОГО РУЛЕТА ИЗ КОНИНЫ С ИСПОЛЬЗОВАНИЕМ РАСТИТЕЛЬНЫХ КОМПОНЕНТОВ

<sup>1</sup>Ш.Ы. КЕНЕНБАЙ\*, <sup>1</sup>Б.А. РСКЕЛДИЕВ, <sup>1</sup>А.С. КРАСНИКОВ, <sup>2</sup>Ж.Н. НИЯЗБЕКОВА

(<sup>1</sup>АО «Алматинский технологический университет», Республика Казахстан,  
050012, г. Алматы, ул. Толе би, 100,

<sup>2</sup>НАО «Казахский национальный аграрный исследовательский университет,  
Республика Казахстан, г.Алматы)

Электронная почта автора-корреспондента: sh.kenenbai@atu.edu.kz

*Цель исследования заключалась в научном обосновании рецептуры и технологии приготовления мясного рулета из конины с использованием растительных функциональных ингредиентов – корневища айра, комплекса гуминно-фульвовых кислот и жмыха тыквенных семечек, а также оценке их влияния на пищевую ценность, технологические и органолептические показатели продукта. В рамках исследования были разработаны контрольные и опытные образцы мясного рулета, для которых определяли химический состав, энергетическую ценность, pH, ТБЧ, потери массы при тепловой обработке, КМАФАНМ, аминокислотный профиль и органолептические показатели. В опытном рулете содержание белка снизилось с 28,2% до 19,12%, и жира с 14,8% до 8,7%, а также были обнаружены углеводы (5,6%), что уменьшило энергетическую ценность мясного продукта с 246,0 до 177,2 ккал/100 г. Отмечено снижение pH с 6,1 до 5,8 и ТБЧ с 0,45 до 0,31 мг МДА/кг, а потери массы сократились с 32,8% до 17,01%. КМАФАНМ в опытном образце мясного рулета составило  $7,2 \times 10^2$  КОЕ/г и  $8,1 \times 10^2$  КОЕ/г в контрольном образце, что было ниже нормы по НД ( $1 \times 10^3$  КОЕ/г). Аминокислотный скор по ключевым незаменимым аминокислотам превышал 130%, и биологическая ценность мясного продукта достигла 139,86%. при этом органолептические оценки внешнего вида, цвета и вкуса опытного рулета были выше контрольного образца.*

**Ключевые слова:** конина, мясной рулет, корневище айра, гуминно-фульвовые кислоты, жмых тыквенных семечек, функциональный мясной продукт.

## ӨСІМДІК КОМПОНЕНТТЕРІН ҚОЛДАНА ОТЫРЫП ЖЫЛҚЫ ЕТІНЕН ФУНКЦИОНАЛДЫ ЕТ РУЛЕТІН ӘЗІРЛЕУ

<sup>1</sup>Ш.Ы. КЕНЕНБАЙ\*, <sup>1</sup>Б.А. РСКЕЛДИЕВ, <sup>1</sup>А.С. КРАСНИКОВ, <sup>2</sup>Ж.Н. НИЯЗБЕКОВА

(«Алматы технологиялық университеті» АҚ, Қазақстан Республикасы,  
050012, Алматы қ., Төле би көш., 100,  
«Қазақ ұлттық аграрлық зерттеу университеті» АҚ, Қазақстан Республикасы, Алматы қ.)  
Автор-корреспонденттің электрондық поштасы: sh.kenenbai@atu.edu.kz

*Зерттеудің мақсаты – өсімдік текті функционалдық ингредиенттер – аир тамырсабағы, гумин-фульвилік қышқылдарының кешені және асқабақ дәні күнжарасын пайдала отырып әзірленген жылқы етінен дайындалатын ет рулетінің рецептурасын және технологиясын ғылыми тұрғыдан негіздеу, сондай-ақ өсімдік текті функционалдық ингредиенттердің өнімнің тағамдық құндылығына, технологиялық және органолептикалық көрсеткіштеріне әсерін бағалау. Зерттеу аясында ет рулетінің бақылау және тәжірибелік үлгілері әзірленіп, олар үшін химиялық құрамы, энергетикалық құндылығы, рН, ТБК, жылулық өңдеу кезіндегі масса жоғалуы, КМАФАНМ, аминқышқылдық профилі және органолептикалық көрсеткіштері анықталды. Тәжірибелік рулетте ақуыз мөлшері 28,2%-дан 19,12%-ға, ал май мөлшері 14,8%-дан 8,7%-ға дейін төмендеді, сонымен қатар көмірсулар (5,6%) құрамы анықталды, бұл ет өнімінің энергетикалық құндылығын 246,0-ден 177,2 ккал/100 г дейін азайтты. рН мәні 6,1-ден 5,8-ге және ТБК көрсеткіші 0,45-тен 0,31 мг МДА/кг-ға дейін төмендегені байқалды, ал масса жоғалуы 32,8%-дан 17,01%-ға дейін қысқарды. Тәжірибелік ет рулетінің КМАФАНМ мәні -  $8,1 \times 10^2$  КОЕ/г, ал бақылау үлгісінде -  $7,2 \times 10^2$  КОЕ/г құрады, бұл НҚ бойынша қойылатын нормадан ( $1 \times 10^3$  КОЕ/г) төмен. Негізгі алмастырылмайтын аминқышқылдар бойынша аминқышқылдық скор 130%-дан асты, ал ет өнімінің биологиялық құндылығы 139,86%-ға жетті. Сонымен бірге тәжірибелік рулеттің сыртқы түрі, түсі және дәмі бойынша органолептикалық бағалары бақылау үлгісіне қарағанда жоғары болды.*

**Негізгі сөздер:** жылқы еті, ет рулеті, аир тамыр сабағы, гумин-фульвилік қышқылдары, асқабақ дәні күнжарасы, функционалды ет өнімі.

### **Introduction**

Horse meat occupies a distinctive place in the dietary culture and meat market of Kazakhstan, where it is used for traditional products such as kazy and other boiled and smoked delicacies and ranks among the leading meat types in per capita consumption. From a nutritional standpoint, horse meat is characterised by a high protein content of approximately 21–24%, relatively low intramuscular fat (4–9%) and an advantageous profile of polyunsaturated fatty acids, including linoleic and  $\alpha$ -linolenic acids, together with elevated iron levels compared with beef and pork. These attributes position horse meat as a promising raw material for the development of functional meat products [1, 2].

Among potential phytochemical modifiers of meat quality, *Acorus calamus L.* rhizome represents an interesting bioactive source. The rhizome contains a complex mixture of essential oils (including  $\beta$ -asarone), phenylpropanoids and polyphenols with documented antioxidant, anti-inflammatory and antimicrobial effects in vitro and in vivo. Extracts of *acorus calamus* have demonstrated significant radical-scavenging activity (DPPH, ABTS) and inhibition of lipid peroxidation in model food systems and bakery matrices, while also suppressing the growth of spoilage and pathogenic microorganisms. In aquatic

species, dietary inclusion of sweet flag extract improved growth performance, blood biochemistry and meat quality, suggesting a capacity to modulate oxidative and metabolic status of animal tissues. These data indicate that *calamus* rhizome infusion may act as a natural antioxidant and antimicrobial co-factor in brines for meat curing [3-5].

Humic and fulvic acids constitute another class of biologically active substances with potential relevance for meat systems. Humic substances are heterogeneous macromolecules derived from the humification of plant and microbial biomass and are rich in aromatic, phenolic and carboxylic groups that confer strong metal-chelating and electron-donating properties. Numerous animal-nutrition studies have shown that dietary humic acid improves growth performance, immune status and antioxidant capacity, and can lower TBARS values and microbial counts in poultry meat during refrigerated storage. A recent review emphasised that humic substances enhance nutrient digestibility and mineral utilisation and may improve overall meat quality through modulation of oxidative processes and gut microbiota. Given their redox and chelating characteristics, humic-fulvic complexes added via brine may interact with muscle proteins and transition

metals, thereby influencing pH, lipid oxidation and possibly tenderness of cooked meat products [6,7].

Pumpkin seed cake, obtained as a press-cake by-product of pumpkin seed oil extraction, is increasingly recognised as a high-value functional ingredient. Pumpkin seeds and their oil cakes contain 14,05–39,75 % protein, substantial amounts of dietary fibre, minerals (Zn, Mg) and bioactive compounds such as tocopherols, carotenoids and phenolic acids. Functional studies have shown that pumpkin seed kernel flour exhibits high water- and oil-holding capacities and can act as a fat replacer and structuring agent in meatballs and beef patties, decreasing total fat and energy value while maintaining or improving texture and sensory acceptance. Moreover, incorporation of pumpkin seed oil or seed-based emulsions into cooked meat products has been reported to reduce lipid oxidation during storage and to enhance nutritional and technological quality. These findings suggest that pumpkin seed cake can serve both as a carrier of antioxidants and as a physical barrier limiting moisture and fat migration during thermal processing [8-12].

Therefore, the aim of the present study was to develop and scientifically substantiate the formulation and processing conditions of a horse-meat roulade enriched with calamus rhizome, a humic–fulvic acid complex and pumpkin seed cake, and to evaluate their combined effects on the proximate composition, pH and lipid oxidation indices, cooking loss, microbiological safety, amino-acid profile and sensory characteristics of the finished product.

#### **Materials and methods**

For the investigation of the impact of plant components on the quality attributes of meat products, horse meat-based roll (roulade) samples were prepared at the Educational and Scientific Center of Meat Processing of the Almaty Technological University.

The following components of plant origin were purchased for the experiment:

- Calamus Rhizome - dried crushed raw material with a specific aroma and bitter-spicy flavor.
- Complex of humic and fulvic acids ‘T8 Tayga Stone’ - a natural concentrated product containing active humic and fulvic acids with antioxidant properties.
- Pumpkin seed cake is a secondary plant product after oil pressing, characterized by a high content of dietary fibre, minerals and antioxidants.

For the study, horse meat was purchased from the local market. After acquisition, the meat was subjected to technological operations: cutting, deboning. Next, the meat was cut into 200 g pieces

with a weight of about 1 cm thick and prepared for the wet salting process.

A brine base including 1.5 litres of water, 75 g table salt and 0.3 g sodium nitrite was made to prepare the brine. Portions of meat were placed in a specially purchased plastic dish (GN1/6 polypropylene/Schneider/Germany) and poured 100 ml of the prepared brine base. Then, humic-fulvic acid complex and, previously brewed in hot water and cooled to room temperature, calamus rhizome (100 ml) were introduced into the brine. The amount of plant-based components was calculated according to the experimental matrix as a percentage of the meat weight (200 g). The total volume of brine for each sample was 200 ml.

The brining process was carried out at  $2\pm 2^\circ\text{C}$  and lasted 24 hours.

After completion of the salting process, the meat samples were removed from the brine and further prepared for heat treatment. The pumpkin seed cake was ground to powder using a nano-crusher (Grindomix GM 200, Retsch, Germany) to obtain a homogeneous fine structure. Then, water was gradually added to the obtained powder, mixing thoroughly until a homogeneous paste-like consistency was achieved, which was convenient for application to the surface of meat.

The obtained paste from pumpkin seeds evenly covered the surface of each meat sample in the amount of 5% of the weight of meat raw material, after which the products were formed in the form of rolls. The prepared rolls were hermetically wrapped in thin aluminium foil and placed in a heat chamber (UK-3\1M100, Techtron+, Russia). Heat treatment was carried out at  $110^\circ\text{C}$  until the temperature in centre of the roll reached  $78^\circ\text{C}$ , which was monitored using a thermometer with a probe.

After completion of thermal treatment, the rolls were cooled to room temperature ( $20\pm 2^\circ\text{C}$ ) in air. Then the finished samples were placed in storage at refrigerator temperature ( $2\pm 2^\circ\text{C}$ ) until the organoleptic evaluation and laboratory tests aimed at studying the effect of plant components on the quality parameters of the meat product.

Laboratory analyses of the final products were conducted at the accredited testing laboratory “Food Safety” of Almaty Technological University (Certificate accreditation No.KZ. T.02.E1158).

The mass fraction of protein in the pâté samples was determined in accordance with GOST 25011-2017 “Meat and meat products. Protein determination methods”.

The mass fraction of fat was determined according to GOST 23042-2015 “Meat and meat products. Methods of fat determination”.

The mass fraction of carbohydrates was determined by the permanganometric method, the principle of which is based on the oxidation of organic reducing substances in the sample by a potassium permanganate solution in an acidic medium, with subsequent calculation of carbohydrate content from the volume of oxidant consumed relative to a standard solution.

The amino acid composition was determined by ion-exchange (or capillary) chromatography using a procedure based on GOST R 55569-2013 “Feedstuffs, compound feeds, feed raw materials. Determination of proteinogenic amino acids using capillary electrophoresis”, adapted for meat systems.

Thiobarbituric acid number (TBA) was determined according to GOST R 55810-2013. The method is based on spectrophotometric measurement of the intensity of colouration arising from the interaction of fat oxidation products with thiobarbituric acid, which allows to assess the degree of oxidative damage of the fat component of meat.

The pH value was determined by potentiometric method. The essence of the method is the direct measurement of hydrogen ion activity using a glass electrode potentiometer, which allows to quickly and accurately determine the acidity of the meat sample under study.

The mass loss of meat samples was calculated from the sample weight values before heat treatment, and the sample weight values after heat treatment. The mass loss reflected the weight of the sample after heat treatment (B) as a

percentage of the weight of the sample before heat treatment (A), as shown by the equation below:

$$\text{Mass loss (\%)} = \left[ \frac{A-B}{A} \right] \times 100 \quad (1),$$

The organoleptic evaluation of the meat product was carried out in accordance with GOST 9959-2015 “Meat and meat products. General requirements for organoleptic evaluation.” This method involves the systematic assessment of sensory characteristics such as appearance, color, smell, texture, and taste. The samples were subjected to organoleptic evaluation using a 5-point scale.

The total viable count of mesophilic aerobic and facultative anaerobic microorganisms (TVC, KMAFAnM) was determined in accordance with GOST 21237-75 “Meat. Methods of bacteriological analysis”.

### Results and discussion

The introduction of plant-derived components markedly modified the basic chemical composition of the horse-meat roulade (Table 1). In the experimental sample, protein content decreased from 28,2% to 19,12%, which corresponds to a reduction of approximately 32,2%. Fat content was reduced even more substantially – from 14,8% in the control to 8,7% in the experimental sample (–41,2%). At the same time, the incorporation of plant ingredients contributed 5,6% carbohydrates, which were absent in the control formulation. As a consequence, the calculated energy value of the control sample amounted to about 246,0 kcal/100 g, whereas the experimental roulade provided approximately 177,2 kcal/100 g, the caloric density was lowered by nearly 28,0%.

Table 1. Physicochemical and microbiological characteristics of horse meat roulade samples

Indicator	Control	Experimental
Physico-chemical parameters:		
Protein content, %	28.2±0.42	19.12±0.29
Fat content, %	14.8±0.21	8.7±0.13
Carbohydrates content, %	-	5.6
pH, units	6.1	5.8
TBA, mg MDA/kg	0,45	0,31
Mass loss, %	32,8	17,01
Microbiological parameter:		
TVC (CFU/g)	8.1×10 <sup>2</sup>	7.2×10 <sup>2</sup>

The use of plant components also influenced technological and oxidative stability parameters. The pH value decreased from 6,1 in the control to 5,8 in the experimental sample, indicating a slight acidification of the meat matrix, which is generally favourable for microbiological stability and colour retention. The thiobarbituric acid (TBA) index, reflecting the level of secondary lipid oxidation products, decreased from 0,45 to 0,31 mg MDA/kg, which corresponds to a reduction of about 31,1% and confirms the antioxidant potential of the added plant ingredients. Thermal losses during cooking were also markedly reduced: cook loss decreased from 32,8% in the control to 17,01% in the experimental roulade (-48,1%), indicating

improved water- and fat-binding capacity and a more stable structure during heat treatment.

Microbiological evaluation showed that the total viable count (TVC) remained within acceptable limits for ready-to-eat meat products. TVC values were  $7,2 \times 10^2$  CFU/g for the control and  $8,1 \times 10^2$  CFU/g for the experimental sample, both remaining below the regulatory threshold of  $1 \times 10^3$  CFU/g established by the normative documentation. A slight increase in TVC in the experimental product does not exceed the permissible level and, in combination with the lower pH and reduced TBA values, indicates that the inclusion of plant components does not compromise microbiological safety under the tested storage conditions.

Table 2. Amino acid composition of a horse meat roulade experimental sample

Component	Height	Start	End	Area	Concentration, mg/L	Amino acid mass fraction, %
Arginine	1.267	3.375	4.300	15.38	22.0	1.407±0.563
Lysine	3.608	4.430	4.548	30.12	35.0	2.401±0.630
Tyrosine	0.808	4.545	4.661	13.75	13.0	0.893±0.372
Phenylalanine	0.583	4.648	4.768	15.13	18.0	0.911±0.455
Histidine	0.663	4.876	4.950	16.01	21.0	0.916±0.475
Leucine + Isoleucine	1.560	4.948	5.078	31.42	26.0	1.138±0.458
Methionine	0.770	4.995	5.103	11.42	9.5	0.786±0.267
Valine	1.157	5.078	5.162	21.06	17.0	1.703±0.396
Proline	1.630	5.105	5.182	27.49	22.0	1.736±0.569
Threonine	1.511	5.242	5.323	26.12	14.0	1.159±0.384
Serine	2.450	5.323	5.392	49.45	21.0	1.738±0.452
Alanine	2.678	5.558	5.632	50.18	17.0	1.407±0.478

Chromatographic analysis of the experimental horse-meat roulade showed a nutritionally valuable and well-balanced amino acid profile (Table 2). Among the essential amino acids, lysine had the highest mass fraction (2,401±0,630%), confirming the product as a rich source of lysine, which is critical for growth and muscle protein turnover. Noticeable contributions were also observed for valine (1,703±0,396%), threonine (1,159±0,384%) and the leucine+isoleucine fraction (1,138±0,458%), providing branched-chain amino acids involved in energy metabolism and regulation of protein synthesis. Methionine showed the lowest level among indispensable amino acids (0,786±0,267%), but

remains sufficient when the product is considered as part of a mixed diet.

The non-essential and conditionally essential amino acids further characterise the protein matrix of the roulade. Elevated levels of proline (1,736±0,569%) and serine (1,738±0,452%) suggest a significant proportion of collagen-derived and structurally organised proteins typical of horse meat. Alanine and arginine, together with histidine, complement the profile with amino acids participating in buffering, nitric oxide metabolism and maintenance of metabolic homeostasis, indicating that the incorporation of plant components did not deteriorate the overall amino acid quality of the product.

Table 3. Amino acid score of a horse meat roulade experimental sample

Amino acid	A <sub>j</sub> , g/100 g protein	A <sub>ref</sub> , g/100 g protein	AAS, %	ΔAAS, %	CAAS, %	BV, %	a <sub>j</sub>
Lysine	12,56	5,5	228,32	174,21	98,19	139,86	1
Tyrosine + Phenylalanine	9,44	6	157,25	103,14			0,69
Leucine + Isoleucine	5,95	11	54,11	0			0,24
Methionine	4,11	3	137,03	82,92			0,6
Valine	8,91	4,8	185,56	131,45			0,81
Threonine	6,06	4	151,54	97,43			0,66

The amino acid score analysis of the experimental horse-meat roulade confirms the high biological value of its protein and highlights certain features of its limiting amino acid pattern (Table 1). Lysine shows the highest amino acid score (AAS - 228,32%), indicating a pronounced excess relative to the reference pattern and confirming the product as an excellent source of this essential amino acid. Valine, tyrosine+phenylalanine, threonine and methionine also exceed 100%, which means that their supply in 100 g protein of the roulade fully covers and even surpasses physiological requirements. In contrast, the

leucine+isoleucine pair demonstrates the lowest score (54,11%) and therefore acts as the first limiting amino acid combination in the protein system. Its normalized coefficient (a<sub>j</sub>- 0,24) reflects the fact that the effective utilisation of other indispensable amino acids will be constrained primarily by this fraction. The coefficient of amino acid score differences (CAAS) reaches 98,19%, while the biological value calculated by the amino acid index method (BV) equals 139,86 %, which allows the protein of the experimental roulade to be classified as highly valuable.

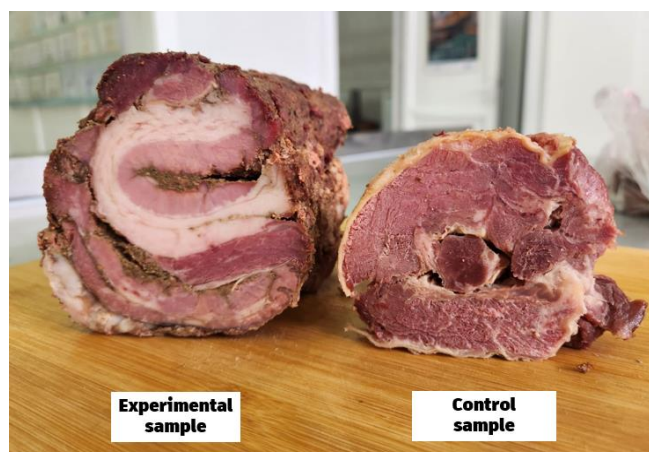


Figure 1. Cross-sectional surface of horse meat roulade samples

Evaluation of the roulade cross-sections showed clear visual differences between the control and experimental samples (Fig.1). The control sample has a more compact, irregular structure with loosely arranged muscle bundles and less clearly separated layers of lean and fatty tissue. In contrast, the experimental roulade demonstrates

a well-defined rolled architecture with distinct alternating layers of darker muscle and lighter intermuscular fat, as well as a clearly visible band of plant coating between the layers. This layered structure gives the experimental sample a more uniform shape, a cleaner cut surface and a visually “crafted” appearance.

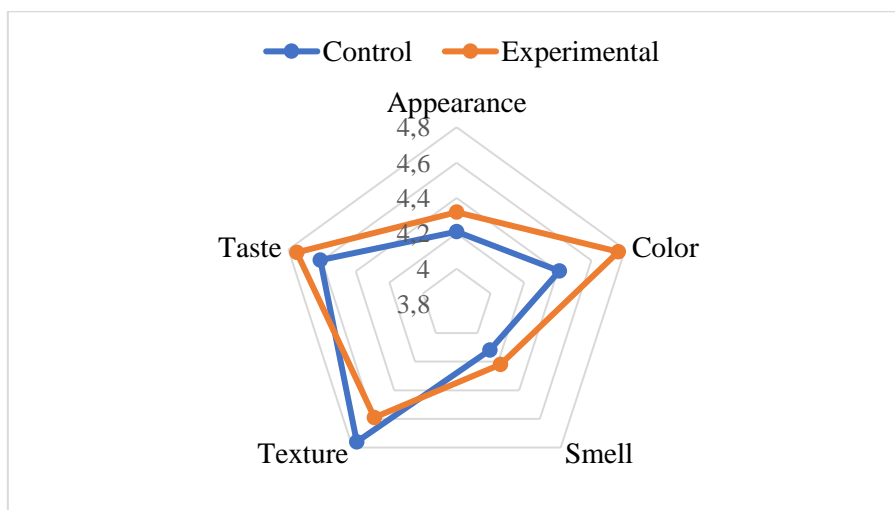


Figure 2. Profilogram of sensory profile of horse meat roulade samples

The sensory profile, assessed on a 5-point scale (Fig.2), confirms the visual impression. The experimental sample received slightly higher scores for appearance, colour and smell, indicating that the use of plant components improved surface colour intensity and aromatic perception without introducing off-flavours. Texture scores were high for control sample (control – 4,76; experimental – 4,59) but differed only marginally, suggesting that the partial replacement of animal raw material did not negatively affect perceived juiciness or tenderness. The integrated sensory score increased from 4,42 in the control to 4,53 in the experimental sample, which indicates that the experimental sample of roulade is at least not inferior, and in several attributes slightly superior, to the traditional product in overall consumer acceptability.

Partial replacement of meat and fat by plant ingredients and additional bound moisture reduced the protein content and the fat content of the experimental sample. At the same time, the appearance of carbohydrates (5,6%) is explained by the presence of non-starch polysaccharides and residual soluble carbohydrates from pumpkin seed cake and calamus infusion. As a result, the calculated energy value decreased, which indicates a successful reduction of caloric density while preserving a high amino-acid score. Similar changes in proximate composition were reported when pumpkin seed kernel flour or paste was used as a fat replacer or functional ingredient in meatballs and cooked meat batters, where Öztürk et al. and Ferrer-González et al. observed decreased fat content and energy value alongside an improved fatty-acid profile and enhanced nutritional functionality of the products[13, 14].

The slight but consistent decrease in pH in the experimental roulade reflects the cumulative

acidifying effect of the humic–fulvic complex and phenolic acids from the calamus rhizome. Humic and fulvic acids are weak polyelectrolytes rich in carboxyl and phenolic groups, which can donate protons and shift the equilibrium toward lower pH, while plant polyphenols additionally contribute to acidification and buffering of the muscle matrix. A moderate decrease of ultimate pH in this range is generally associated with improved water-holding capacity and reduced exudative losses due to more favourable protein hydration, as shown for pork and mutton by Jankowiak et al. and Moustafa et al., which is in line with the lower cooking loss observed in our experimental variant[15, 16].

Lipid oxidation, assessed by the TBA index, was substantially reduced in the experimental sample compared with the control, representing a decrease of about 31%. This effect is consistent with the high radical-scavenging capacity of calamus rhizome extracts, which are rich in phenolic compounds and asarone derivatives, and have been shown by Shukla et al. and Dinev et al. to exhibit pronounced antioxidant activity in model food systems and against mycotoxigenic fungi[17, 18].

The most pronounced technological effect of the plant complex was the almost two-fold reduction in cooking loss, from 32,8% in the control to 17,01% in the experimental sample. This can be explained by several complementary mechanisms. First, the external paste from pumpkin seed cake forms a semi-continuous plant “coating” around the roulade, which acts as a barrier to moisture and fat migration during heating, analogous to the protective effect of pumpkin seed paste and oleogel systems on yield and juiciness in cooked meat batters reported by Ferrer-González et al. Second, the slightly lower pH, together with the presence of humic–fulvic

acids capable of binding metal ions and interacting with muscle proteins, likely improves the water-holding capacity of myofibrillar structures and stabilises the gel network formed during heating, in accordance with classical relationships between pH, protein hydration and drip loss described for different types of meat. Finally, dietary fibre and protein from the pumpkin seed cake can absorb exuded juices and retain them within the plant–meat matrix, further limiting mass loss [19, 20].

Microbiological analysis showed low total viable counts in both samples, with a slight advantage of the experimental roulade and both values remain below the regulatory limit of  $1 \times 10^3$  CFU/g for this category of cooked meat products, but the 11% reduction in TVC suggests a mild preservative effect of the plant complex. Calamus rhizome extracts and essential oil demonstrate broad-spectrum antifungal and antibacterial activity against food-spoiling microorganisms, as shown by Shukla et al. and other authors, due to membrane-active phenylpropanoids and phenolic compounds. Humic substances also display antibacterial properties related to their polyphenolic fragments and ability to chelate essential ions, as reported by Verrillo et al., while pumpkin seed components have been proposed as natural preservatives that improve colour and shelf-life of meat products. Thus, the slightly lower microbial load of the experimental roulade can be attributed to the synergistic antimicrobial action of calamus phenolics, humic–fulvic acids and pumpkin seed bioactives superimposed on the thermal lethality of the cooking process [21, 22].

### Conclusion

The incorporation of calamus rhizome, a humic–fulvic acid complex and pumpkin seed cake into the horse-meat roulade led to a substantial reformulation of its nutritional and technological profile. Partial replacement of meat and fat by plant ingredients reduced protein from 28,2% to 19,12% and fat from 14,8% to 8,7%, while introducing 5,6% carbohydrates and lowering the calculated energy value from approximately 246,0 to 177,2 kcal/100 g. These compositional shifts were accompanied by a decrease in pH from 6,1 to 5,8 and a pronounced reduction in lipid oxidation, with TBA values declining from 0,45 to 0,31 mgMDA/kg. At the same time, cooking loss was almost halved, from 32,8 % in the control to 17,01 % in the experimental variant, indicating improved water- and fat-binding and a more stable structure during heat treatment. TVC values were  $7,2 \times 10^2$  CFU/g for the control and  $8,1 \times 10^2$  CFU/g for the experimental sample, both remaining below the regulatory threshold of  $1 \times 10^3$

CFU/g established by the normative documentation, confirming that the plant complex did not compromise, and may modestly enhance microbiological safety under the tested conditions.

Sensory evaluation demonstrated that these technological and biochemical modifications were not achieved at the expense of consumer-relevant quality. The experimental roulade showed equal or superior scores for appearance, colour, smell and taste compared with the control, with only a minor and non-critical decrease in texture score. Taken together, the data indicate that the combined use of calamus rhizome, humic–fulvic acids and pumpkin seed cake enables the development of a horse-meat roulade with reduced caloric density, attenuated lipid oxidation, lower cooking losses and stable microbiological quality, while maintaining high sensory acceptability.

### Acknowledgments, conflict of interest (funding)

The research was carried out within the framework of the project IRN BR24993234 “Innovative technologies for the production of national products: intensification and digitalization of meat and dairy products.”

### REFERENCES

1. Abilmazhinov Y. et al. Physical-Chemical and Amino Acid Composition of Horsemeat //International Journal of Pharmaceutical Research (09752366). – 2020. – T. 12. – №. 3. <https://doi.org/10.31838/ijpr/2020.12.03.256>
2. Stanisławczyk R., Rudy M., Rudy S. The quality of horsemeat and selected methods of improving the properties of this raw material //Processes. – 2021. – T. 9. – №. 9. – C. 1672. <https://doi.org/10.3390/pr9091672>
3. Zhao Y. et al. Ethnic, botanic, phytochemistry and pharmacology of the Acorus L. genus: A review //Molecules. – 2023. – T. 28. – №. 20. – C. 7117. <https://doi.org/10.3390/molecules28207117>
4. Kongkham B., Duraivadivel P., Hariprasad P. Acorus calamus L. rhizome extract and its bioactive fraction exhibits antibacterial effect by modulating membrane permeability and fatty acid composition //Journal of Ethnopharmacology. – 2024. – T. 331. – C. 118323. <https://doi.org/10.1016/j.jep.2024.118323>
5. Velichkova K., Sirakov I., Stoyanova S. Growth efficiency, biochemical blood parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.), fed with supplement of sweet flag extract (*Acorus calamus* L.) //Bulgarian Journal of Agricultural Science. – 2020. – T. 26. URL: [agrojournal.org/26/01s-22.pdf](http://agrojournal.org/26/01s-22.pdf)
6. Arif M. et al. Humic acid as a feed additive in poultry diets: A review //Iranian journal of veterinary research. – 2019. – T. 20. – №. 3. – C. 167. URL: <https://pubmed.ncbi.nlm.nih.gov/31656520/>
7. Marcinčák S. et al. Humic substances as a feed supplement and the benefits of produced chicken meat

//Life. – 2023. – Т. 13. – №. 4. – С. 927. <https://doi.org/10.3390/life13040927> (ENG)

8. Hadidi M. et al. Pumpkin seed as a sustainable source of plant-based protein for novel food applications //Critical Reviews in Food Science and Nutrition. – 2025. – С. 1-26. <https://doi.org/10.1080/10408398.2025.2505235>

9. Xie Y. et al. Pumpkin Seed Proteins: The Potentially Alternative Protein Supplements for Food Applications //Foods. – 2025. – Т. 14. – №. 22. – С. 3969. <https://doi.org/10.3390/foods14223969>

10. Serdaroğlu M. et al. Evaluation of the quality of beef patties formulated with dried pumpkin pulp and seed //Korean journal for food science of animal resources. – 2018. – Т. 38. – №. 1. – С. 1. <https://doi.org/10.5851/kosfa.2018.38.1.001>

11. Kaisarova, A., Shingisov, A., Mukhametov, A., & Kenenbay, S. (2024). Development of Technology for Producing Combined Meat Chips With Reduced Sodium Content. Journal of Food Process Engineering, (Volume 47, Issue 12, December 2024) (2024), J Food Process Eng, 47: e70004. <https://doi.org/10.1111/jfpe.70004>

12. Kenenbay Sh.Y., Korzeniowska M., Tortai A.N., Krasnikov A.S. Study of the influence of plant – based components on the physicochemical and organoleptic properties of meat product, Vestnik KazUTB, vol. 4, issue. 29, December 2025, doi:10.58805/kazutb.v.4.29-1119. p.485-495. <https://doi.org/10.58805/kazutb.v.4.29-1119>

13. Öztürk T., Turhan S. Physicochemical properties of pumpkin (Cucurbita pepo L.) seed kernel flour and its utilization in beef meatballs as a fat replacer and functional ingredient //Journal of food processing and preservation. – 2020. – Т. 44. – №. 9. – С. e14695. <https://doi.org/10.1111/jfpp.14695>

14. Ferrer-González B. M., García-Martínez I., Totosaus A. Textural properties, sensory acceptance and fatty acid profile of cooked meat batters employing pumpkin seed paste or soybean oil oleogel as fat replacers //Grasas y Aceites. – 2019. – Т. 70. – №. 3. – С. e320-e320. <https://doi.org/10.3989/gya.1055182>

15. Jankowiak H., Cebulska A., Bocian M. The relationship between acidification (pH) and meat

quality traits of polish white breed pigs //European Food Research and Technology. – 2021. – Т. 247. – №. 11. – С. 2813-2820. <https://doi.org/10.1007/s00217-021-03837-4>

16. Moustafa A. K. et al. Effect of Plant Polyphenols and Ascorbic Acid on Physio-Chemical Characteristics of Sausage //Alexandria Journal of Veterinary Sciences. – 2021. – Т. 69. – №. 1. <https://doi.org/10.5455/ajvs.38728>

17. Shukla R. et al. Efficacy of Acorus calamus L. essential oil as a safe plant-based antioxidant, Aflatoxin B1 suppressor and broad spectrum antimicrobial against food-infesting fungi //International Journal of Food Science and Technology. – 2013. – Т. 48. – №. 1. – С. 128-135. <https://doi.org/10.1111/j.1365-2621.2012.03168.x>

18. Dinev T. et al. Antifungal and antioxidant potential of methanolic extracts from Acorus calamus L., Chlorella vulgaris Beijerinck, Lemna minuta Kunth and Scenedesmus dimorphus (Turpin) Kützing //Applied Sciences. – 2021. – Т. 11. – №. 11. – С. 4745. <https://doi.org/10.3390/app11114745>

19. Hussain A. et al. A Comprehensive review of functional ingredients, especially bioactive compounds present in pumpkin peel, flesh and seeds, and their health benefits //Food Chemistry Advances. – 2022. – Т. 1. – С. 100067.

20. Hricikova S. et al. Humic substances as a versatile intermediary //Life. – 2023. – Т. 13. – №. 4. – С. 858. <https://doi.org/10.3390/life13040858>

21. Verrillo M. et al. Antibacterial and antioxidant properties of humic substances from composted agricultural biomasses //Chemical and Biological Technologies in Agriculture. – 2022. – Т. 9. – №. 1. – С. 28. <https://doi.org/10.1186/s40538-022-00291-6>

22. Bayat H. et al. Comparative effects of humic and fulvic acids as biostimulants on growth, antioxidant activity and nutrient content of yarrow (Achillea millefolium L.) //Scientia Horticulturae. – 2021. – Т. 279. – С. 109912. <https://doi.org/10.1016/j.scienta.2021.109912>