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MICROBIOLOGICAL SAFETY OF HORSEMEAT INTESTINAL BY-PRODUCTS: EFFECT OF ULTRASOUND AND CITRIC ACID TREATMENTS

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This study evaluated the microbiological safety of horse intestinal by-products “Qarta” before and after processing, with particular attention to changes during refrigerated storage at 4 °C. Intestinal segments were subjected to different treatments: washing only (control), immersion in citric acid solutions (0.5% and 1.0%), ultrasound treatment (35 kHz, 200 W, 5 min) and a combined treatment (1.0% citric acid followed by ultrasound). Microbiological parameters included total plate count (TPC), pH and the presence of pathogenic microorganisms (Salmonella spp., Listeria monocytogenes, Escherichia coli). The raw intestines exhibited a high initial microbial load (2.6×10^5 CFU/g), confirming the inherent hygienic risks of this by-product. The combined citric acid and ultrasound treatment was the most effective, reducing TPC to 6.0×10^2 CFU/g and maintaining the lowest microbial counts during refrigerated storage for up to 9 days, while no target pathogens were detected in any treated samples. The pH values remained within the acceptable technological range (5.26-6.12), supporting product stability. Additional evaluation of chemical and radiological safety (toxic elements, pesticide residues, Cs-137, Sr-90) confirmed full compliance with TR CU 021/2011 «On Food Safety» and relevant GOST standards. Overall, the results indicate that properly processed horse intestinal by-products can meet microbiological and food safety requirements and can be considered suitable raw materials for the production of delicatessen meat products.

Keywords: microbiological safety, ultrasound treatment, citric acid, horsemeat, intestinal by-products, “Qarta”.

МИКРОБИОЛОГИЧЕСКАЯ БЕЗОПАСНОСТЬ КИШЕЧНОГО СЫРЬЯ КОНИНЫ: ВЛИЯНИЕ УЛЬТРАЗВУКОВОЙ И ЛИМОННОКИСЛОТНОЙ ОБРАБОТКИ

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В работе исследована микробиологическая безопасность кишечного сырья лошадей «Қарта» до и после обработки, с особым акцентом на изменения в процессе холодильного хранения (4 °C). Кишечные отрезки подвергали различным видам обработки: только промывке (контроль), выдержке в растворах лимонной кислоты (0,5% и 1,0%), ультразвуковой обработке (35 кГц, 200 Вт, 5 мин) и комбинированной обработке (1,0% лимонной кислоты с последующим воздействием ультразвука). Определяли общую бактериальную обсеменённость (КМАФАнМ), значения pH и наличие патогенных микроорганизмов (Salmonella spp., Listeria monocytogenes, Escherichia coli). В исходном сырье отмечался высокий уровень микробной контаминации ($2,6 \times 10^5$ КОЕ/г), что подтверждает характерные риски для данного побочного продукта. Наиболее эффективной оказалась комбинированная обработка лимонной кислотой и ультразвуком, при которой КМАФАнМ снижалась до $6,0 \times 10^2$ КОЕ/г и сохранялась на минимальном уровне в течение холодильного хранения до 9 суток, при этом целевые патогенные микроорганизмы во всех обработанных образцах не выявлялись. Значения pH находились в технологически допустимых пределах (5,26-6,12), что обеспечивало стабильность продукта. Дополнительная оценка химической и радиационной безопасности (токсичные

элементы, остатки пестицидов, цезий-137, стронций-90) показала полное соответствие требованиям ТР ТС 021/2011 «О безопасности пищевой продукции» и соответствующих ГОСТ. Полученные результаты подтверждают, что правильно обработанное кишечное сырьё лошадей соответствует требованиям микробиологической и пищевой безопасности и может рассматриваться как перспективное сырьё для производства деликатесных мясных изделий.

Ключевые слова: микробиологическая безопасность, ультразвуковая обработка, лимонная кислота, конина, кишечное сырьё, «Қарта».

ЖЫЛҚЫ ІШЕГІ ШИКІЗАТЫНЫҢ МИКРОБИОЛОГИЯЛЫҚ ҚАУІПСІЗДІГІ: УЛЬТРАДЫБЫСТЫҚ ЖӘНЕ ЛИМОН ҚЫШҚЫЛЫМЕН ӨҢДЕУ ӘСЕРІ

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Бұл жұмыста жылқы ішек шикізатының «қарта» микробиологиялық қауіпсіздігі өңдеуге дейін және өңдеуден кейін зерттелді, әсіресе тоңазытқышта (4 °С) сақтау кезінде болатын өзгерістерге назар аударылды. Ішек сегменттері әртүрлі өңдеу нұсқаларынан өткізілді: тек жуу (бақылау), лимон қышқылының ерітінділерінде (0,5% және 1,0%) өңдеу, ультрадыбыстық өңдеу (35 кГц, 200 Вт, 5 мин) және біріктірілген өңдеу (1,0% лимон қышқылы, содан кейін ультрадыбыс). Зерттеу барысында жалпы микробтық ластану деңгейі (КМАФАнМ), pH мәндері және патогенді микроорганизмдердің (Salmonella spp., Listeria monocytogenes, Escherichia coli) болуы анықталды. Бастапқы шикізатта микробтық контаминация деңгейі жоғары болды (2,6 × 10^5 КОЕ/г), бұл осы қосалқы өнімге тән тәуекелдердің бар екенін растады. Лимон қышқылы мен ультрадыбысты біріктіріп қолдану ең тиімді өңдеу нұсқасы болып анықталды: КМАФАнМ 6,0 × 10^2 КОЕ/г деңгейіне дейін төмендеп, тоңазытқышта 9 тәулікке дейін сақтау барысында ең төмен деңгейде сақталды, ал барлық өңделген үлгілерде мақсатты патогенді микроорганизмдер анықталған жоқ. pH мәндері технологиялық тұрғыдан рұқсат етілген шектерде (5,26–6,12) болды, бұл өнімнің тұрақтылығын қамтамасыз етті. Қосымша түрде химиялық және радиациялық қауіпсіздік көрсеткіштері (токсинді элементтер, пестицид қалдықтары, цезий-137, стронций-90) бағаланып, олардың КО ТС 021/2011 «Тамақ өнімдерінің қауіпсіздігі туралы» талаптарына және тиісті мемлекеттік стандарттарға (МЕМСТ) толық сәйкес келетіні анықталды. Зерттеу нәтижелері дұрыс өңделген жылқы ішек шикізаты микробиологиялық және азық-түлік қауіпсіздігі талаптарына сай келетінін және деликатес ет өнімдерін өндіруге пайдалануға болатынын көрсетті.

Түйін сөздер: микробиологиялық қауіпсіздік, ультрадыбыстық өңдеу, лимон қышқылы, жылқы еті, ішек шикізаты, «Қарта».

Introduction. Horsemeat and its offal play an important role in the traditional diet of Central Asia, where intestinal segments are widely used to produce delicatessen products such as “Qarta”. However, intestinal raw materials are very sensitive to microbial contamination due to their anatomical origin and nutrient-rich composition. The rapid increase in the total amount of food on plates and the potential presence of pathogenic microorganisms such as Salmonella spp., Listeria monocytogenes and Escherichia coli pose serious problems for food safety and limit their shelf life.

Various canning strategies have been studied to improve the microbiological stability of meat and casings. Organic acids, including lactic and citric, are widely known as natural antimicrobial agents capable of reducing surface contamination and lowering pH. Ultrasound (US) has recently attracted the attention of meat scientists as a non-thermal processing method that enhances microbiological inactivation due to cavitation and improves mass

transfer, while preserving the taste and structural properties of products. Studies have shown that combining ultrasound with moderate chemical treatment can lead to synergistic antimicrobial effects and improved product quality [1].

Despite these achievements, there is limited information about the microbiological characteristics of raw materials from horse intestines and the potential for treating organic acids with ultrasound to stabilize them. In particular, little attention is paid to the processing of “Qarta” in the scientific literature, despite its importance in regional cuisine and commercial potential as a delicacy product [2].

Therefore, the purpose of this study was to evaluate the microbiological quality of horse intestines when stored in a refrigerator and evaluate the effectiveness of ultrasound and citric acid treatment, alone or in combination, to improve microbiological safety, pH stability and shelf life [3].

Ultrasound is considered as being one of the few processing technologies with the potential to differentiate into a range of industry segments; as such, it has been widely explored over the last decade. Extensive review articles have shown the multiple applications of ultrasound within the food industry, most being focused on improving product quality and extending the shelf life of fresh and processed products [3].

Well-known applications of ultrasound incorporate the murdering or repressing of bacterial development. It has been detailed that high-intensity ultrasound of recurrence 20–100 kHz and vitality escalated 10–100 W cm^{-1} creates gradients of intense pressure that can modify the structure of microorganisms in food.

Microbial inactivation has been observed using ultrasound [4]. Ultrasound treatment at low energy intensity caused a quick reduction within the number of viable microbes. When used in combination with moderate heat, ultrasound can accelerate the decontamination rate of food and decrease both the duration and intensity of heat treatments and their resulting damage [5].

Ultrasound is an innovative technology and it is based on the application of mechanical waves at a frequency ranging from 20 kHz to 10 MHz which can be classified into two frequency ranges: low-intensity ultrasound (LIUS) which uses high frequencies between 100 kHz and 10 MHz at intensities lower than 1 Wcm^{-2}, whereas high-intensity ultrasound (HIUS), uses intensities higher than 1 Wcm^{-2} at low frequencies between 20 kHz and 100 kHz [6].

HIUS has the ability to change the chemical and physical properties of food in filtration, drying, sterilization, extraction, food preservation, emulsification, treating, bleaching, etc. The benefits of HIUS applications in the food industry are increased efficiency, made strides mass and heat transfer, reduced processing time, expanded physical blending, lower processing temperature, selective extraction, expanded surrender, etc. [7].

Due to the non-invasive and non-destructive nature of LIUS, this innovation is suitable for following the food processes, giving an evaluation of physicochemical changes of food during processing, as well as for food analysis and detection. Different from LIUS, HIUS has a strong physicochemical effect on foods.

Many studies have shown that HIUS has a positive impact on the quality of meat [8]. Be that as it may, there are reports of antagonistic impacts of this innovation on the organoleptic and physicochemical properties of meat. As a case, lipid oxidation in meat preparing for the most part comes about within the generation of off-flavors and off-odors, driving to adverse sensory properties. It has been demonstrated that HIUS energizes oxidation of both, lipid and protein [9,10]. An issue that must be tended to is that the retained vitality of HIUS may lead to warm era rising to hoisted temperatures and hence coming about in warm harm to meat [11,12].

The impacts of HIUS on water holding capacity and trickle misfortune of meat can be assessed emphatically or contrarily, the changes depend on fiber sort and orientation, biochemical properties as well as control and time of ultrasonication.

It has also been reported that Gram-positive

microorganisms are more resistant to ultrasound treatments than Gram-negative microorganisms. Research studies have primarily focused on the deactivation of Listeria monocytogenes, Escherichia coli, a series of Salmonella spp., Staphylococcus aureus and some other microorganisms [13].

In recent times, food industries of developing countries within the wake of successfully preserving food are implementing hurdle innovation. To increase food, save it from microorganisms and prolong shelf-life of energy-sensitive, nutritional, and organoleptic characteristics, the use of ultrasound is of extraordinary significance.

In this study, characteristics of the physicochemical and microbiological quality of meat treated with high-intensity ultrasound were evaluated in order to decide the impact of different sonication treatment times on the meat during storage.

This paper discusses the application of ultrasound to the processing of Qarta horsemeat secondary intestinal raw materials in the meat industry. It will consider the issues of de-oiling and processing of intestinal raw materials, physicochemical, organoleptic and microbiological parameters, as well as evaluating their influence on product quality and justifying the choice of one of the types of intestinal raw materials.

In the present study, particular attention was given to evaluating how ultrasound treatment (35 kHz, 200 W, 5 min) and mild citric acid immersion (0.5-1.0%) influence the microbiological safety, pH stability, and structural characteristics of horse intestinal by-products («Qarta»). These processing techniques were selected due to their potetntial to enhance product quality and reduce microbial contamination while maintaining the natural properties of the raw material.

Materials and methods. Sample preparation. According to information of Stanisławczyk et al. horse age divided to group I-foals and young horses up to 2 years of age, group II-adult horses from 2 to 10 years of age, and group III-older horses from 10 to 20 years of age.

Since the Kazakhs were good cattle breeders, they had a classification of horses - by suit, by purpose, by age for slaughtering Table 1.

Table 1 - Classification of horses by age and the corresponding Kazakh terminology

Age of horses	Description	The name in the Kazakh language for each type
Up to 6 months old	Foal	Qulyn
6-7 months	Plump foal	Jabagy
Over a 1 year old	Foal	Tay
2-2,5 years old	Young male, accustomed to the saddle	Qunan
2-2,5 years old	Young mare	Qunazhun
3-4 years old	Young virgin male horse	Donen
3-4 years old	Young virgin female mare	Baytal
5 years old	Adult horse	Aygyr
5 years old	Adult mare	Bie

A total of 3 different age categories of the Kazakh horse meat breed took part in the study. In this study, the secondary intestinal raw materials of horse meat of the Kazakh breed «Qarta» of 3 age categories of slaughtering (over a 1-year-old – Tay; 2-2,5 years old – Qunan, 3-4 years old - Baytal)

were considered, as this is the best suitable intestinal raw material for further processing and use in the production of a new food product.

The intestine of a slaughter animal is a part of the digestive tube of an animal, following the stomach and ending in the anus. Fig.1 «Qarta» is intestinal secondary raw material of horse meat with the presence of cream or yellow colored fatty tissue. The Qarta is the raw rectum consisting of an intestine diameter of 80 to 200 mm and a length of 0.3 to 1.0 m. Characteristic features of this type of colon clean and dry surface, without lesions or stains. Since the beginning of the development of industrial sausage production both in the Republic of Kazakhstan and worldwide, intestinal casings have been used in the production of cooked, smoked sausages, sausages, wieners and other products. They meet most of the requirements for sausage casings: they have sufficient strength, elasticity, smoke and moisture permeability, as well as resistance to high temperatures; give products the necessary commodity properties that allow to determine the type of finished product; protect the product from external factors that can lead to its spoilage. To preserve the quality of intestinal raw materials under production and storage conditions, they are preserved, mainly by salting or drying.



Tay **Qunan** **Baytal**

Fig.1 - The secondary intestinal raw materials of horse meat of the Kazakh breed «Qarta» of 3 age categories of slaughtering (over a 1 year old - Tay; 2-2,5 years old - Qunan, 3-4 years old - Baytal)

Taking into account the identified progressive technical solutions in improving the technology of natural casing processing, as which is used intestinal raw material of horsemeat «Qarta» for the development of a complex meat product, as well as modern scientific trends concerning the preservation of quality during processing and ensuring the microbiological safety of products, studies have been conducted to create a new technological method of processing, which has a significant antimicrobial effect.

Microbiological safety indicators, including total plate count and the presence of Salmonella, Listeria monocytogenes and Escherichia coli, were evaluated using standard culture-based methods. Microbiological evaluation was performed in accordance with TR CU 021/2011 «On Food Safety» and the requirements of GOST 10444.15-94 and GOST 10444.2-94 [14,15,16].

Bacterial cultures and microbiological analysis of raw materials before treatment (or samples not treated with ultrasound). Samples of intestinal raw materials were prepared according to the following protocol of Serial Dilution technique [17]. Fig.2. As stock solution was used NaCl salt solution 0.85 %.3 samples of intestine raw materials collected from Kazakh breed horse.

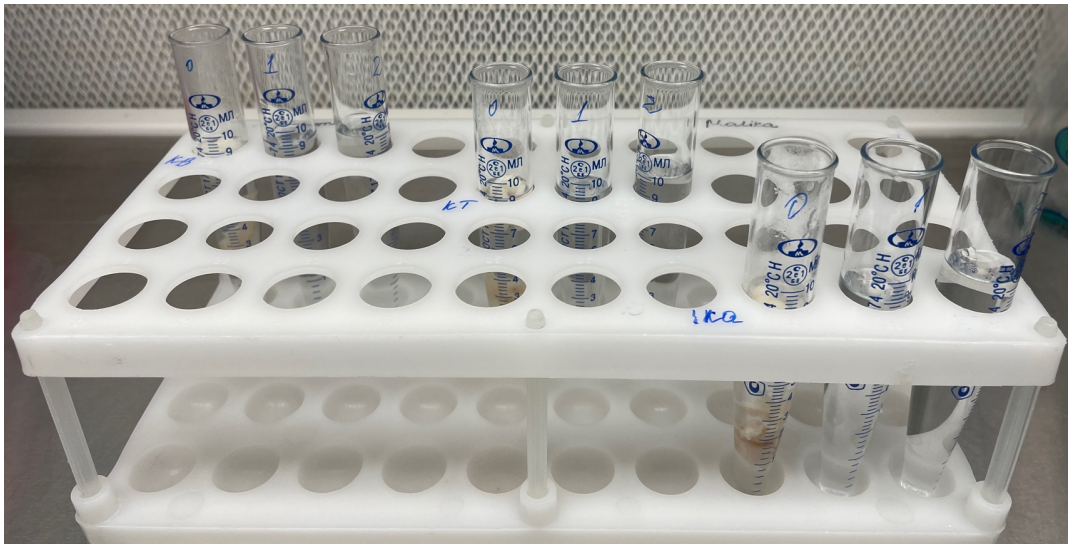
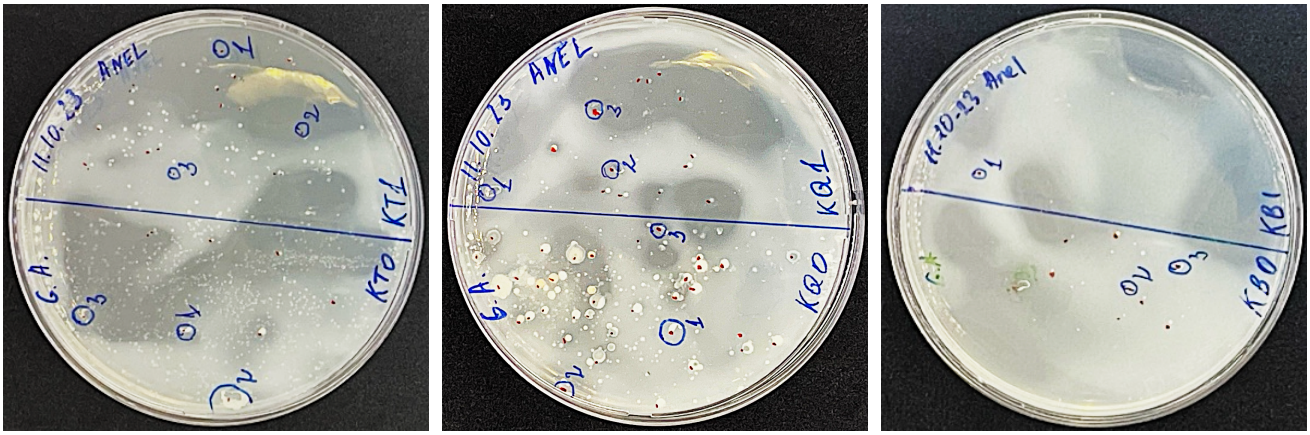


Fig.2 - Serial Dilution Method of Estimating Viable Count of Bacteria

The basic medium that was utilized for the cultivation of intestine of a slaughter horse microorganisms was composed of the following constituents (g/L): peptone (4); yeast extract (1); gelatin (15); agar powder (15), pH 7.0. A gelatin agar medium was used for screening the microorganisms on plates.

Fig.3 series of dilutions from 1:10 to 1:100 were prepared and seeding on the plate with gelatin agar and were incubated overnight at 37 ◦C to obtain single colonies.



Tay **Qunan** **Baytal**

Fig.3 - Serial Dilution Method of Estimating Viable Count of Bacteria

The plates were flooded with Trcichloracetic Acid (TCA), and clear zones around the bacterial growth were recorded as positive for collagenolytic activity. The new medium was tested with a variety of anaerobic bacteria, and the results were compared with data obtained with the conventional technique for detecting collagenolytic activity. Overall, there was satisfactory agreement in the detection of collagenolytic activity. The following equation was used to calculate the number of colony-forming units (CFU) mL^{-1} in the exudate of each samples:

$$CFUmL^{-1} = \text{number of bacteria/mL} \quad (1)$$

$$CFUmL^{-1} = A * DF * 10 \quad (2)$$

where A is the average number of colonies in a dilution and DF is dilution factor, which depends in the dilution used, as follows: 1 for 1:0, 10 for 1:10, 100 for 1:100, etc.

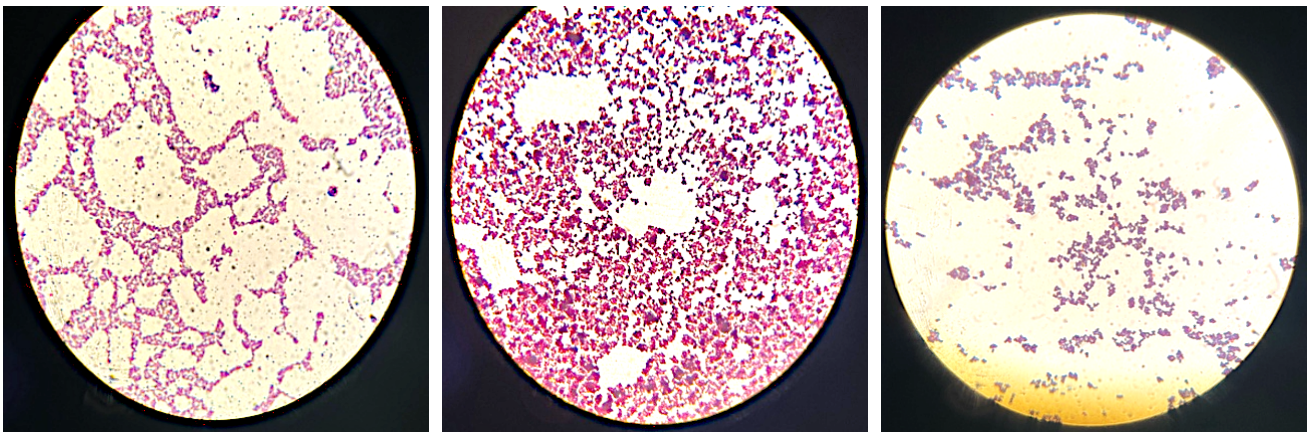
$$\text{Plate with Tay 1:100 (KT1)} = 50 \cdot 1 \cdot 10 = 5 \cdot 10^2 \text{ CFU mL}^{-1}$$

$$\text{Plate with Qunan 1:100 (KQ1)} = 20 \cdot 1 \cdot 10 = 2 \cdot 10^2 \text{ mL}^{-1}$$

$$\text{Plate with Baytal 1:100 (KB1)} = 1 \cdot 1 \cdot 10 = 10^1 \text{ mL}^{-1}$$

Due to the fact that the age category of 3-4 years old grew few colonies is explained by the fact that in developed and older species of slaughter animals gastrointestinal tract is better developed, as well as in this species of horse meat intestinal raw material is wider in diameter, which facilitates further use as a natural casing for the development of delicacy meat product, unlike young animals, so it was decided to continue work with the species Baytal.

Light Microscopy. The microstructure of the intestine samples before and after ultrasound-assisted brining was observed with an optical microscope. The meat samples were searched by Gram Staining [18]. Gram staining is a differential staining method since more than one dye is used as primary and secondary dyes. In gram staining, gram-negative bacteria lose their crystal violet iodine (CV-I) complex and become colorless after washing, but gram-positive bacteria retain the CV-I complex and remain purple. The color of the purple gram-positive bacteria isn't affected from safranin, the discolored gram-negative bacteria are dyed pink with safranin. This test differentiates the bacteria into gram-positive and gram-negative bacteria, which helps in the classification and differentiation of microorganisms. In this practice, gram staining procedure are applied on the bacteria after preparation of smear of seeding plates with intestinal raw materials of horsemeat "Qarta". Samples were observed using a Zeiss Primo Star light microscope (Carl Zeiss Ltd., Jena, Germany) after Gram staining (Fig.4).



Tay **Qunan** **Baytal**

Fig.4 - Microstructural characteristics of the «Qarta» samples before treatmnets. Images were obtained using a Zeiss Primo Star (Carl Zeiss Ltd., Jena, Germany) light microscope after Gram staining

Ultrasound treatment was carried out in a 10-L ultrasonic bath (Sapphire UZV-28 TTC, Moscow, Russia) at a frequency of 35 kHz an power of 200 W. Samples were placed at a distance of 3-5 cm from the emitter and fully submerged in distilled water. The temperature of the medium did not exceed 25°C, which was controlled by periodic mixing of the bath. Total sonication time was 5 minutes.

Citric acid solutions (0.5% and 1.0%) were prepared in distilled water at 20-22°C. Samples were immersed in the solution for 5 minutes at a raw-material-to-solution ratio of 1:5 to ensure uniform diffusion. After treatment, samples were rinsed under running water for 1 minute.

Results and Discussions. Horse intestines were prepared by washing, segmenting, and packaging according to standard microbiological protocols. During the main experiment, the samples were analyzed for the total number of plates (TPC), pH and the presence of pathogenic microorganisms (Salmonella spp., Listeria monocytogenes and Escherichia coli) when stored in the refrigerator (4 °C) for 13 days.

In addition to the control group, additional procedures were performed to evaluate modern processing methods. The intestines were immersed in citric acid solutions (0.5% and 1.0%, IV) for 5 minutes, and treated with ultrasound (35 kHz, 200 W, 5 min) or subjected to a combined treatment consisting of citric acid (1.0%, 5 min) followed by ultrasound (5 min). After processing, all samples were washed in running water, drained, and stored under identical conditions.

Ultrasound treatment was carried out in a Sapphire UZV-28TTC bath (Moscow, Russia) at a frequency of 35 kHz and power of 200 W, which corresponds to the standard high-intensity sonication mode used for protein-containing raw materials. These parameters were selected because they ensure effective cavitation without excessive heating of the tissue; the temperature of the medium did not exceed 25–28 °C during treatment. The samples were placed at a fixed distance of 3–4 cm from the ultrasonic emitter to guarantee uniform energy distribution. The temperature of the ultrasonic bath was monitored throughout processing to avoid thermal denaturation of the sample.

Microbiological analyses included determination of the total number of bacteria on the tablet (TPC), pH measurement and detection of pathogenic microorganisms (Salmonella spp., Listeria monocytogenes, Escherichia coli). The studies were conducted on the 1^{st}, 5^{th}, 9^{th} and 13^{th} days of storage in the refrigerator.

To provide a clear comparison of the microbiological safety of the samples after various treatments, the key parameters were summarized in Table 2. Since the main objective of the study was to evaluate the effectiveness of citric acid and ultrasound in reducing contamination, the table highlights the presence or absence of target pathogens and the corresponding pH values. Quantitative TPC enumeration was available only for the untreated control and the combined treatment, which demonstrated the most significant reduction in microbial load. For other treatments, qualitative microbiological assessment confirmed the absence of pathogens and a decrease in contamination levels.

As shown in Table, the greatest reduction of microbial load and lipid oxidation was observed in the combined treatment group. This treatment also omproved texture parameters and extended the shelf life to ≥9 days. Citric acid alone and ultrasound alone provided moderate improvements, while potassium sorbate and other acids showed limited effectiveness.

The observed reduction in microbial load after ultrasound treatment is associated with acoustic cavitation, which generates localized pressure changes capable of damaging bacterial cell membranes. Citric acid, in turn, decreases pH and disrupts bacterial membrane permeability. When combined (citric acid and ultrasound), a synergetic antimicrobial effect was observed, resulting in the lowest TPC values throughout refrigerated storage.

In addition to the control group (washed intestines without treatment), additional studies were conducted to evaluate modern processing technologies:

Citric acid (CA): soaking in 0.5% or 1.0% solution (IV) for 5 minutes.

Ultrasound: immersion in distilled water and exposure to ultrasound with a frequency of 35 kHz and a power of 200 Watts for 5 minutes using a 10-liter ultrasonic bath (Sapphire UZV-28 TTC, Moscow, Russia).

Combined treatment (citric acid and ultrasound): soaking in 1.0% citric acid solution for 5 min, followed by ultrasound treatment at the same parameters (35 kHz, 200 W, 5 min).

Table 2 - Microbiological safety and pH of horse intestinal raw materials («Qarta») after different processing treatments

Treatment	Pathogens (Salmonella, L.monocytogenes, E.coli)	pH	Comment
Control (untreated)	Present in raw material	6.12	High initial contamination
Citric acid 0.5%	Not detected	5.83	Reduced microbial risk
Citric acid 1.0%	Not detected	5.76	Stronger acid effect
Ultrasound	Not detected	5.61	Non-thermal treatment
Citric acid+Ultrasound	Not detected	5.32	Most effective combined approach

Note: Quantitative TPC enumeration was performed only for the control and combined treatment groups. All other treatments were assessed qualitatively based on the absence of pathogenic microorganisms and pH changes.

After processing, the samples were washed in running water for 1 min, transferred to sterile absorbent paper, packaged and stored at a temperature of 4°C [19].

The aim of this work was to apply ultrasound on samples in order to see the changes in raw materials properties at experimental level in an attempt to view the effect of sonication over the whole of the intestinal secondary raw material of horsemeat “Qarta”. It is well known that pH is one of the parameters with great influence on meat quality and is directly related to characteristics such as WHC, color and texture of meat.

It is known that WHC in any muscle is minimal at low hH, but it tends to increase with maturation owng to protein degradation and changes in electric charges by intramolecular rearrangement. In the present study the performance of WHC can be explained by the pH decrease observed throughout the storage period. WHC is related to many physicochemical characteristics of myofibrillar and protein components. The results of the present study agree partially with the results of others, who also found an increase in WHC of sonicated meat [20].

One of the most important ”barriers” in the production of meat products is a low pH value. It is difficult to overestimate the value of pH in the technology of meat products, largely due to the fact that it is fast and informative. The high informativeness of this indicator is explained by the fact that the change in its values significantly affects the properties of meat raw materials, in particular: moisture-binding capacity, colour, consistency (friability, rigidity, etc.) odour, taste, the rate of penetration of salting substances and stability during storage. Considering that the pH value for cooked meat products should not be lower than 5,8 and not higher than 6,4 (Table 3).

Hygienic standards for microbiological indicators include control of four groups of microorganisms: sanitary indicator microorganisms, which include the number of mesophilic aerobic and facultative anaerobic microorganisms (NMAFAnM) and E. coli bacteria; opportunistic

microorganisms (E. coli, S. Aureus, Proteus, B. Cereus and sulfuric acid bacteria). E.coli, S. Aureus, Proteus, B. Cereus and sulphite-reducing clostridia) pathogenic microorganisms, including Salmonella, Listeria monocytogenes; spoilage microorganisms.

Microbiological examination of the meat product allows us to assess its condition and suitability for use in the production of meat products.

As a result of microbiological analysis the compliance of microbiological indicators with the established indicators according to the normative documentation was established. Coliforms, L.monocytogenes, pathogenic microorganisms including salmonella were not detected. Microbiological parameters of intestinal raw horse meat after ultrasonic treatment are shown in Table 4. It can be seen that at 30 degrees all microorganisms cease their activity.

Table 3 - Organoleptic and physicochemical characteristics of horse intestinal raw materials

Name of indicators	Characteristics and standards	Results
1	2	3
Appearance	Moderately moist, not contaminated with foreign matter	Moderately moist, not contaminated with foreign matter
Colour	Ccream, light beige, beige	Beige
Odour	Characteristic of good quality products, with no offensive odour	Characteristic of good quality products, with no offensive odour
pH meat extract, not less	No more than 6,2	6,12
Mass fraction of fat	-	9,86
Ammonia nitrogen determination	negative reaction	negative reaction
Protein determination	-	9,035

Microbiological analysis after ultrasound treatment. In order to ensure product safety, a system of hazard analysis and critical control points (HACCP) is in place at the enterprises.

Table 4 - Microbiological parameters of intestinal raw horse meat after ultrasonic treatment

Name of indicators	Characteristics and standards	Results
1	2	3
NMAFAnM, CFU/mL, not more	$5x10^6$	$3,2x10^4$
Presence of coliforms	not allowed	absent
Presence of sulphite-reducing clostridia in 0.1 g of product	not allowed	absent
Pathogenic, including salmonellae in 25g	not allowed	absent

As a result of microbiological analysis, compliance of microbiological indicators with the established indicators according to the regulatory documentation was established.

Microbiological analysis of intestinal raw

materials showed that in the process of heat treatment contamination of raw materials is significantly reduced (from 260 000 to 600 microbes in 1 g of the product), such microorganisms as salmonellae, proteins, sulfite reducing, clostridia, coaguladepositive staphylococci and other pathogenic microflora after heat treatment are not detected.

The table 5 presents the results of the safety assessment of horsemeat intestinal by-products, including the levels of toxic elements (Pb, Cd, Hg, As), organochlorine pesticides (HCH isomers, DDT and its metabolites) and radionuclides (Cs-137, Sr-90). All indicators were evaluated in accordance with the requirements of TR CU 021/2011 «On Food Safety». Analytical measurements were carried out using standardized methods: atomic absorption spectrometry for toxic elements (GOST 30178-96), gas-liquid chromatography for pesticide residues (GOST 23452-2015), and gamma-spectrometric analysis for radionuclides (GOST 32161-2013). The obtained results demonstrate that all contaminants were absent or significantly below the permissible limits, confirming full compliance of the products with food safety standards.

Table 5 - Chemical, toxicological and radiological safety indicators of horsemeat intestinal by-products in accordance with TR CU 021/2011 and relevant GOST standards

Indicator	Regulatory limit (TR CU 021/2011 / GOST)	Result	Compliance
Pesticides, mg/kg, not more:			
HCH (α, β, γ isomers)	≤ 0.1 (GOST 23452-2015)	absent	Meets requirements
DDT and metabolites	≤ 0.1 (GOST 23452-2015)	absent	Meets requirements
Toxic elements, mg/kg, not more:			
Lead (Pb)	≤ 0.5 (GOST 30178-96)	absent	Meets requirements
Arsenic (As)	≤ 0.1 (GOST 30178-96)	absent	Meets requirements
Cadmium (Cd)	≤ 0.03 (GOST 30178-96)	0.03	Meets requirements
Mercury (Hg)	≤ 0.1 (GOST 30178-96)	absent	Meets requirements
Radionuclides, Bq/kg, not more:			
Cesium-137	≤ 200 (GOST 32161-2013)	0.0 ± 12.2	Below limit
Strontium-90	no limit in TR CU	0.0 ± 1.0	Safe

According to the results of the analysis of food safety indicators the meat product complies with the food safety norms, the content of radionuclides - caesium-137 and strontium – 90 does not exceed the maximum permissible norm (200 bq/kg) and corresponds to 0,0±12,2; 0,0±1,0 bq/kg. All the results of the food safety analysis indicate that the intestinal raw material after ultrasonic bath treatment has no effect on the food safety indicators and is ready for further sale.

Thus, the optimum ratio of processing of intestinal raw materials, which have a positive effect on microbiological, physico-chemical and food safety parameters, was identified. The research methods were carried out according to the normative documents in the RSE ”Centre of Sanitary-Epidemiological Expertise” of the Medical Centre of the Medical Centre of the Presidential Administration Medical Centre of the Presidential Administration of the Republic of Kazakhstan” on the right of economic management, Astana, Kazakhstan.

Comparative analysis of the treatment groups revealed distinct differences in microbial dynamics and physicochemical stability. The control samples demonstrated the fastest increase in TPC values, exceeding 6.0 log CFU/g by day 9. Samples treated with citric acid (0.5-1.0%) showed a slower growth rate and maintained TPC below 5.0 log CFU/g at the same storage point. Ultrasound treatment alone resulted in an additional reduction of 0.3-0.5 log CFU/log compared with citric acid treatment. The combined citric acid and ultrasound treatment demonstrated the strongest effect, maintaining the lowest TPC values (below 4.0 log CFU/log on day 9) and ensuring the most stable pH profile throughout storage. These results confirm a synergistic antimicrobial interaction between ultrasound and citric acid. The synergistic reduction in microbial load is attributed to cavitation-induced structural damage of bacterial cells and the acidification effect of organic acids, which together enhance membrane permeability and accelerate bacterial inactivation.

Conclusion. The study showed that intestinal offal from horse meat (“Qarta”) is very sensitive to microbiological contamination, which limits their safe use as raw materials for the production of delicatessen products. Microbiological assessment confirmed that untreated intestines carry a high microbial load, while treatment with ultrasound and citric acid significantly increases safety. Combined treatment with 1.0% citric acid (5 min) followed by ultrasound (35 kHz, 200 W, 5 min) was recognized as the most effective method to reduce the total number of viable microorganisms from 2.6×10^5 to 6.0 × 102 CFU/g and maintain a low level of microbial growth during 13 days of storage. in the fridge. After treatment, pathogenic microorganisms such as Salmonella spp., Listeria monocytogenes and Escherichia coli were not detected. In addition, the pH values remained stable within the acceptable technological range (5.26–5.52), which confirms the stability of the product. These results indicate that treatment with citric acid using ultrasound can serve as a promising approach to ensure microbiological safety and prolong the shelf life of horse intestines, which will allow them to be used in the production of high-quality delicatessen meat products. The combined use of ultrasound and mild organic acid treatment can therefore be considered a promising and technologically feasible approach for improving the microbiological safety «Qarta» and similar intestinal by-products.

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