Сандугаш Сайлауовна Толеубекова – техника ғылымдарының кандидаты; қауымдастырылған профессор; «Биотехнология» кафедрасы, Шәкәрім университеті, Семей қаласы, Қазақстан Республикасы; e-mail: saltosha-sandu@mail.ru. ORCID: https://orcid.org/0000-0003-2658-9514.

Айнур Толеухановна Қабденова – биотехнология магистрі; «Биотехнология» кафедрасы, Шәкәрім университеті, Семей қаласы, Қазақстан Республикасы; e-mail: ain_arik@mail.ru. ORCID: https://orcid.org/0009-0001-6558-1868.

Диана Сергеевна Свидерская – техника ғылымдарының кандидаты; доцент; «Сәулет және дизайн» кафедрасы, Торайғыров университеті, Павлодар қаласы, Қазақстан Республикасы; e-mail: sofilsev@rambler.ru. ORCID: https://orcid.org/0000-0003-3329-1126.

Information about the authors

Aigerim Bepeyeva* – PhD; Department of Biotechnology; Shakarim University, Semey city, Republic of Kazakhstan; e-mail: bepeyeva1987@gmail.com. ORCID: https://orcid.org/0000-0003-0097-8466.

Yerlan Zharykbasov – PhD; Associate Professor; Department of Biotechnology, Shakarim University, Semey city, Republic of Kazakhstan; e-mail: erlan-0975@mail.ru. ORCID: https://orcid.org/0000-0001-9707-0539.

Sandugash Toleubekova – Candidate of Technical Sciences; Associate Professor; Department of Biotechnology, Shakarim University, Semey city, Republic of Kazakhstan; e-mail: saltosha-sandu@mail.ru. ORCID: https://orcid.org/0000-0003-2658-9514.

Ainur Kabdenova – Master of Biotechnology; Department of Biotechnology, Shakarim University, Semey city, Republic of Kazakhstan; e-mail: ain_arik@mail.ru. ORCID: https://orcid.org/0009-0001-6558-1868.

Diana Sviderskaya – Candidate of Technical Sciences; Associate Professor; department of «Architecture and design», Toraighyrov University, Pavlodar city, Republic of Kazakhstan; e-mail: sofilsev@rambler.ru. ORCID: https://orcid.org/0000-0003-3329-1126.

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S.E. Ibraimova[1*], A.N. Serikbaeva[1], G.Sh. Jumabekova[2], R.A. Izteliyeva[1], Sh.S. Amanova[1]
[1]Almaty Technological University,
[2]International Engineering and Technology University
050012, Republic of Kazakhstan, Almaty, Tole bi street, 100
*e-mail: canek21@mail.ru

ENSURING MICROBIOLOGICAL SAFETY OF BREAD WITH THE ADDITION OF CRUSHED JUNIPER FRUIT

Abstract: *This study investigates the impact of incorporating powdered juniper berries into the formulation of wheat-based bakery products on their microbiological stability during storage. Juniper berries are rich in natural antimicrobial and antioxidant compounds, which makes them promising as a functional ingredient in food production. The aim of the research was to evaluate the effectiveness of adding 3% powdered juniper berries to wheat bread. A comparative analysis was conducted between a control sample (without additives) and an experimental sample containing the juniper berry powder. Microbiological indicators were assessed under room temperature conditions and at elevated temperature (37 °C) over a defined storage period. No signs of "potato disease" caused by* Bacillus subtilis *and* Bacillus mesentericus *were observed in the experimental samples. Furthermore, compared to the control, the juniper-enriched bread showed a significant reduction in the total count of mesophilic aerobic and facultative anaerobic microorganisms (TAMC), mold fungi, and coliform bacteria. The results confirm that the use of powdered juniper berries contributes to enhanced microbiological safety and prolonged shelf life of bread products without the need for synthetic preservatives. The obtained results open up prospects for the development of functional bakery products with enhanced hygienic, technological, and consumer characteristics, aligned with the principles of healthy nutrition in the baking industry.*

Key words: *juniper berries, bakery products, microbiological stability, antimicrobial activity, shelf life.*

Introduction

One of the key strategic directions of the state policy in the Republic of Kazakhstan is the promotion of principles of rational and balanced nutrition aimed at protecting public health and ensuring food security. In this context, the expansion of the range of high-quality and microbiologically safe bakery products becomes particularly important. According to expert estimates, approximately 25% of food products become unsuitable for consumption annually due to the activity of microorganisms. This is especially true for bakery products, which serve as a favorable nutrient medium for microbial growth [1, 2, 3].

Food safety concerns arising from microbial contamination and the resulting spoilage of products are common and present a major challenge. Most control measures are focused on extending shelf life and ensuring the microbiological safety of food products. Prioritizing food safety, many microbiologists working in the food industry dedicate significant time to studying spoilage microorganisms [4, 5, 6].

Although the exact economic losses due to food spoilage are difficult to quantify, they are undoubtedly substantial. According to some estimates, up to one-quarter of the global food supply is lost due to microbial activity [7]. For cereals and legumes, global losses are estimated to account for at least 10% of agricultural production, while losses for grains, vegetables, and fruits can reach up to 50%. The range of microbial contamination is extensive. Bacteria are the main cause of spoilage in protein-rich foods such as meat, poultry, fish, milk, and dairy products [8, 9, 10].

The primary spoilage organisms include Gram-negative rods (e.g., *Pseudomonas*, *Shewanella*), Gram-positive spore-forming bacteria (*Bacillus*, *Clostridium*), lactic acid bacteria, and other Gram-positive bacteria (*Brochothrix*, *Micrococcus*). Additionally, *Enterobacteriaceae* represent a large group of bacteria that include both spoilage and pathogenic strains. These bacteria are often used as indicators of quality and food safety [11].

Although the growth of yeasts and molds is generally slower than that of bacteria, they can survive under harsher conditions and are thus considered potential hazards in food spoilage.

The causes of microbiological spoilage of food can be categorized as «intrinsic», «extrinsic», «technological», and «latent». Intrinsic factors include the physicochemical and structural properties of the food (e.g., water activity, pH, redox potential, availability of nutrients for microorganisms, and natural antimicrobial compounds). Extrinsic factors refer to storage conditions such as temperature, humidity, and the composition of the surrounding atmosphere. Technological factors involve physicochemical processing methods that influence the properties of the food and its microbiota. Latent factors are related to the interactions between microorganisms under the influence of the above-mentioned conditions, including symbiotic, synergistic, and antagonistic relationships [12, 13].

Technologies that ensure food safety and prevent foodborne illness play a vital role in improving the nutritional value of food products. On a global scale, the control and regulation of microbiological contamination remain a pressing issue [14].

The quality of bakery products depends on the properties of the ingredients used, the processes of dough preparation and baking, and storage conditions. During storage, adverse microbiological processes may occur, leading to a significant decline in product quality. Microbial spoilage of bread is commonly referred to as «bread disease».

One of the most well-known forms of bread spoilage is «potato disease», caused by spore-forming bacteria such as *Bacillus mesentericus* and *Bacillus subtilis*. These microorganisms are widely distributed in nature (in air, soil, plants) and are commonly found in grain and flour. The spores remain viable during baking (surviving heating up to 130 °C or up to 6 hours at 100 °C). Conditions favorable for their germination include high moisture content, neutral pH, and temperatures of 37-40 °C. These bacteria possess strong amylolytic (e.g., α-amylase) and proteolytic (e.g., protease, polypeptidase, dipeptidase) enzyme complexes, which cause noticeable structural changes in bread crumb during spoilage [15].

Initially, affected bread loses its characteristic flavor and aroma. As spoilage progresses, the odor becomes more intense and unpleasant. The crumb turns sticky and viscous, producing slimy threads when torn apart. For this reason, the disease is also known as «ropy bread».

This condition is also referred to as «mucous bread disease» (E. Boyko, 1936) or «bread chain disease» (S. A. Balakshin, K. A. Popov, 1933). The disruption of bread structure is the result of the bacteria’s ability to hydrolyze starch into soluble carbohydrates, sugars, and dextrins. According to V. A. Nikolaev (1932), in heavily spoiled bread, starch content can drop from 63% to as low as 16%. Other components of bread also undergo changes [16].

Several causes of potato disease in bread can be identified: high spore count of *Bacillus mesentericus* and *Bacillus subtilis* (1-5 thousand spores per gram of wheat flour); use of contaminated flour and inadequate sanitation practices at baking facilities; reuse of contaminated bread; violation of technological parameters during production (e.g., acidity, moisture, crumb structure); Improper storage conditions (e.g., storing warm, uncooled bread in high-humidity environments).

The potential impact of spore-forming bacteria on human health is a serious concern. Potato disease alters the physicochemical, biological, and organoleptic properties of bread, rendering it nutritionally deficient and unfit for consumption. Spoiled bread has been shown to be toxic, causing death in up to 70% of experimental animals (mice) [17].

Therefore, technologies aimed at improving the nutritional quality of food must also ensure microbiological safety and prevent foodborne illnesses. To prevent bread spoilage, it is essential to monitor raw materials and finished products for microbial contamination. A variety of technological, bacteriological, physical, biochemical, and other methods are used to ensure microbiological safety.

Various methodological approaches are used to assess resistance to microbiological spoilage, as shown in Figure 1.

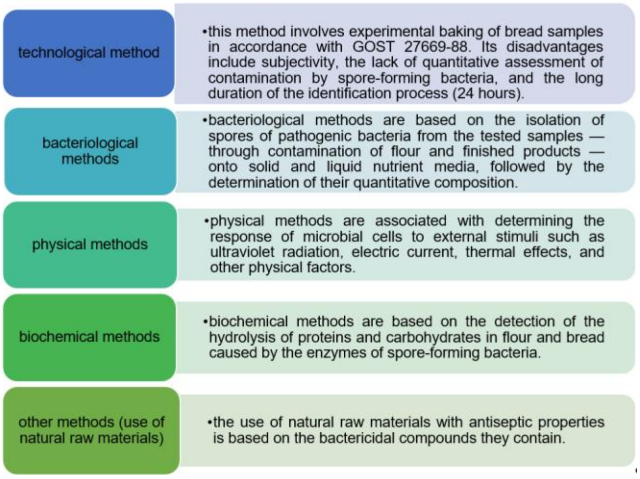


Figure 1 – Main methods for evaluating the microbiological stability of bread

Materials and methods

In the study of the microflora of long shelf-life products, classical microbiological methods were employed, including sampling and preparation techniques for microbiological analysis, as well as methods for cultivating microorganisms. Investigations were conducted to detect coliform bacteria (Coliforms), pathogenic microorganisms including Salmonella, and molds [18].

The total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) was determined in accordance with GOST 10444.15-94. The count of coliform bacteria (Coliforms) was determined following GOST 10444.2-94. Detection of pathogenic microorganisms, including Salmonella, was carried out in accordance with GOST 31747-2012.

Results

To confirm the feasibility of incorporating juniper (Juniperus) into the formulation of wheat bread in order to enhance its microbiological stability, experimental laboratory trials were conducted. The trials involved inducing Bacillus subtilis and Bacillus mesentericus spore-forming bacteria, based on a total spore count of 10^2 spores/g of wheat flour. After baking, the bread samples were wrapped in moist paper and incubated at a temperature of 37 °C and a relative humidity of 85–90% for 36 hours. Following incubation, the development of potato disease in the bread was assessed organoleptically. The results are presented in Table 1.

According to the organoleptic evaluation, no signs of potato spoilage were observed in the bread sample made with contaminated flour supplemented with juniper. In addition to improving microbiological stability, the incorporation of 3% juniper berry powder positively influenced the sensory characteristics of the product. The bread with juniper demonstrated a more pronounced pleasant aroma with subtle resinous and spicy notes characteristic of juniper berries. The crumb had a light brownish hue, uniform porosity, and a slightly firmer texture compared to the control, while maintaining elasticity and freshness throughout storage. The taste was harmonious, mildly tart, and refreshing, without bitterness or off-flavors.

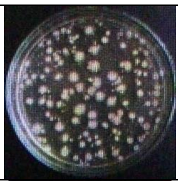
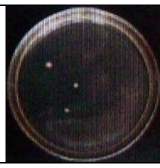
Overall, the inclusion of juniper berry powder not only suppressed the development of *Bacillus*-related «potato disease» but also enhanced the sensory appeal of the bread, contributing to a distinctive flavor profile and improved freshness perception.

Table 1 – Organoleptic evaluation of potato spoilage manifestation in bread

Sample name	Potato disease symptoms
Bread baked from wheat flour	Characteristic aroma of wheat bread; no signs of disease in the crumb.
Bread baked from wheat flour (10^3 spores/g flour)	Sticky crumb; presence of specific off-odor.
Bread with the addition of 3% juniper powder (10^3 spores/g flour + ground juniper berries)	No signs of disease in the crumb.

To determine the inhibitory effect of plant ingredients, the quantitative composition of *Bacillus subtilis* and *Bacillus mesentericus* colonies was assessed using a bacteriological method. This method is based on determining the number of visible colonies growing in the sample by inoculating it onto a solid nutrient medium. The results are presented in Table 2.

Table 2 – Number of visible colonies of *Bacillus subtilis* and *Bacillus mesentericus* in the samples

Dilution	Wheat flour bread (10^3 spores/g flour)		Wheat flour bread (10^3 spores/g flour + ground juniper berries)	
10^{-1}		confluent growth		confluent growth
10^{-2}		99		11
10^{-3}		27		3

Based on the evaluation of the data presented in Table 2, the following conclusion can be drawn: the addition of ground juniper berries to the formulation of wheat flour bread contributes to the reduction of potato disease pathogens. This effect may be associated with the increased acidity of the dough containing ground juniper berries, as the organic acids present in juniper act as natural preservatives.

The baked bread samples were stored under ambient conditions (t = 20 ÷ 25 °C) and under induced spoilage conditions (t = 37 ÷ 38 °C, high humidity).

The results obtained are presented in Table 3.

The results of the conducted study (Table 3) showed that in the bread sample containing 3% ground juniper berries, no mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) were detected after 72 hours of storage. In contrast, in the control sample, the growth of MAFAM was observed after just 24 hours of storage. Mold development was first noted on the control sample after 2 days, whereas in the sample containing 3% ground juniper berries, mold appeared on the 4th day, yet did not exceed the permissible limits established by TR CU 021/2011. After 72 hours of storage, the total viable bacterial count (TVBC) in the control sample remained within acceptable limits, whereas in the sample with 3% ground juniper berries, the TVBC remained below the regulatory threshold even after 96 hours. These findings demonstrate the positive effect of juniper on the microbiological safety of bread products.

Table 3 – Changes in microbiological indicators of the test samples during storage (R≥0.95, n=3)

Microbiological indicator		Bread samples		TR CU 021/2011 requirements
		Control	Bread with 3% ground juniper berries	
the number of mesophilic aerobic and facultative anaerobic microorganisms, expressed in colony-forming units per gram (CFU/g)	24 h	not found	not found	$5{,}0\times10^3$
	48 h	$(5{,}0\pm0{,}5)\times10^2$	not found	
	72 h	$(2{,}5\pm1{,}5)\times10^4$	not found	
	96 h	$(8{,}5\pm1{,}5)\times10^4$	$(5{,}3\pm0{,}4)\times10^2$	
Mold, CFU/g	24 h	not found	not found	50
	48 h	< 10	not found	
	72 h	(80±7)	not found	
	96 h	(135±15)	< 30	
Coliform bacteria (Coliforms), g	24 h	not found	not found	1,0
	48 h	0,01	not found	
	72 h	0,1	not found	
	96 h	1,0	0,01	

The results obtained indicate that the inclusion of ground juniper berries in bread not only enhances the nutritional value of the final product but also extends its shelf life. Thus, bread products containing ground juniper berries can be stored safely for up to 72 hours.

Conclusion

The conducted research demonstrated that the addition of 3% juniper berry powder to the wheat flour bread formulation exhibits a pronounced antimicrobial effect. Microbiological analysis revealed that in the experimental bread sample, no growth of mesophilic aerobic and facultative anaerobic microorganisms (Total Viable Count, TVC) was observed during 72 hours of storage, whereas active microbial growth in the control sample began as early as 24 hours. Moreover, mold development in the control bread occurred after 48 hours, while in the juniper-enriched sample it appeared only after 96 hours, and the levels did not exceed the limits established by TR CU 021/2011. It was also found that the addition of juniper powder inhibited the manifestation of «potato disease» symptoms: the treated sample showed no crumb stickiness or off-odors.

Thus, the results confirm that incorporating juniper berry powder into bread formulations not only improves microbial resistance and safety but also contributes to extending shelf life. The practical significance of the study lies in the potential industrial application of juniper berry powder as a natural preservative and functional additive in bakery production. This approach can support the development of clean-label bakery products with enhanced quality and extended freshness. Further research should focus on optimizing dosage levels, studying the impact on sensory characteristics, and assessing scalability in industrial baking conditions.

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С.Е. Ибраимова[1]*, А.Н. Серикбаева[1], Г.Ш. Джумабекова[2], Р.А. Изтелиева[1], Ш.С. Аманова[1]
[1]Алматы технологиялық университеті,
[2]Халықаралық инженерлік-технологиялық университеті
050012, Қазақстан Республикасы, Алматы қаласы, Төле би көшесі, 100
*e-mail: canek21@mail.ru

ҰНТАҚТАЛҒАН АРША ЖЕМІСІ ҚОСЫЛҒАН НАННЫҢ МИКРОБИОЛОГИЯЛЫҚ ҚАУІПСІЗДІГІНІҢ КӨРСЕТКІШТЕРІ

Бұл зерттеуде бидай ұнынан жасалған нан өнімдерінің сақтау мерзімінің микробиологиялық тұрақтылығына ұнтақталған арша жемісін қосу әсері қарастырылды. Арша жемістері табиғи антимикробтық және антиоксиданттық қосылыстарға бай болғандықтан, оларды тағам өндірісінде функционалдық ингредиент ретінде қолдану перспективалы бағыт болып табылады. Зерттеудің мақсаты – бидай нанының құрамына 3% ұнтақталған арша жемісін қосудың тиімділігін бағалау. Қоспасыз дайындалған бақылау үлгісімен және ұнтақталған арша жемісі қосылған тәжірибелік үлгімен салыстырмалы талдау жүргізілді. Микробиологиялық көрсеткіштер бөлме температурасында және 37 °С жоғары температурада белгілі бір сақтау мерзімінде бағаланды. Тәжірибелік үлгілерде Bacillus subtilis және Bacillus mesentericus бактериялары туындататын «картоп ауруының» белгілері байқалмады. Сонымен қатар, бақылау үлгісімен салыстырғанда, арша қосылған нан мезофильді аэробты және факультативті анаэробты микроорганизмдердің жалпы санының (МАжФАМС), зең саңырауқұлақтарының және ішек таяқшасы тобы бактерияларының айтарлықтай азаюын көрсетті. Алынған нәтижелер ұнтақталған арша жемісін қолдану синтетикалық консерванттарды пайдаланбастан, нан өнімдерінің микробиологиялық қауіпсіздігін арттыруға және сақтау мерзімін ұзартуға ықпал ететінін дәлелдейді. Алынған нәтижелер нан өнімдерін өндіру саласында жоғары гигиеналық, технологиялық және тұтынушылық сипаттамаларға ие, салауатты тамақтану қағидаттарына сай келетін функционалдық нан-тоқаш өнімдерін әзірлеудің перспективаларын айқындайды.

Түйін сөздер: *арша жемістері, нан өнімдері, микробиологиялық тұрақтылық, антимикробтық белсенділік, сақтау мерзімі.*

С.Е. Ибраимова[1]*, А.Н. Серикбаева[1], Г.Ш. Джумабекова[2], Р.А. Изтелиева[1], Ш.С. Аманова[1]
[1]Алматинский технологический университет,
[2]Международный инженерно-технологический университет
050012, Республика Казахстан, город Алматы, улица Толе би, 100
*e-mail: canek21@mail.ru

ПОКАЗАТЕЛИ МИКРОБИОЛОГИЧЕСКОЙ БЕЗОПАСНОСТИ ХЛЕБА С ДОБАВЛЕНИЕМ ПОРОШКА ПЛОДОВ МОЖЖЕВЕЛЬНИКА

В данном исследовании рассматривается влияние добавления порошка ягод можжевельника в рецептуру хлебобулочных изделий из пшеничной муки на их микробиологическую устойчивость в процессе хранения. Ягоды можжевельника богаты природными антимикробными и антиоксидантными соединениями, что делает их перспективным функциональным ингредиентом в пищевом производстве. Целью работы было оценить эффективность внесения 3% порошка ягод можжевельника в состав пшеничного хлеба. Проведен сравнительный анализ между контрольным образцом (без добавок) и опытным образцом с добавлением порошка можжевельника. Микробиологические показатели оценивались при комнатной температуре и при повышенной температуре (37 °С) в течение установленного срока хранения. В опытных образцах не выявлено признаков развития «картофельной болезни», вызываемой Bacillus subtilis и Bacillus mesentericus. Кроме того, по сравнению с контролем, хлеб с можжевельником показал значительное снижение общего количества мезофильных аэробных и факультативно-анаэробных микроорганизмов (КМАФАнМ), плесеней и бактерий группы кишечной палочки. Полученные результаты подтверждают, что использование порошка ягод можжевельника способствует повышению микробиологической безопасности и увеличению срока годности хлебобулочной продукции без применения синтетических консервантов. Полученные результаты открывают перспективы для разработки функциональных хлебобулочных изделий, отличающихся высокими гигиеническими, технологическими и потребительскими характеристиками, а также ориентированных на принципы здорового питания в хлебопекарной отрасли.

Ключевые слова: *ягоды можжевельника, хлебобулочные изделия, микробиологическая устойчивость, антимикробная активность, срок годности.*

Авторлар туралы мәліметтер

Сания Ерболатовна Ибраимова* – PhD, «Тағам өнімдерінің сапасы және қауіпсіздігі» кафедрасының ассисент-профессоры, Алматы технологиялық университеті, Алматы, Қазақстан Республикасы; e-mail: canek21@mail.ru. ORCID: https://orcid.org/0000-0002-9439-7461.

Аяна Нургалиевна Серикбаева – PhD, «Тағам өнімдерінің сапасы және қауіпсіздігі» кафедрасының ассисент-профессоры; Алматы технологиялық университеті, Қазақстан Республикасы; e-mail: ayana-sn@mail.ru. ORCID: https://orcid.org/0000-0001-8252-1034.

Гульзира Шабаевна Джумабекова – Техника ғылымдарының магистрі, «Тағам өндірістерінің техникасы және технологиясы» кафедрасының лекторы, Халықаралық инженерлік-технологиялық университеті, Алматы, Қазақстан Республикасы; e-mail: guzika_j@mail.ru. ORCID: https://orcid.org/0000-0002-6375-3128.

Раушан Акмуратовна Изтелиева – PhD, «Тағам өнімдерінің сапасы және қауіпсіздігі» кафедрасының қауымдастырылған профессоры; Алматы технологиялық университеті, Алматы, Қазақстан Республикасы; e-mail: iztelieva80@mail.ru. ORCID: https://orcid.org/0000-0001-9129-2798.

Шолпан Сапаровна Аманова – PhD, «Тағам өнімдерінің сапасы және қауіпсіздігі» кафедрасының ассисент-профессоры, Алматы технологиялық университеті, Алматы, Қазақстан Республикасы; e-mail: amanova_sh@mail.ru. ORCID: https://orcid.org/0000-0001-7964-7736.

Сведения об авторах

Сания Ерболатовна Ибраимова* – PhD, ассистент-профессор кафедры «Безопасность и качество пищевых продуктов», Алматинский технологический университет, Алматы, Республика Казахстан; e-mail: canek21@mail.ru. ORCID: https://orcid.org/0000-0002-9439-7461.

Аяна Нургалиевна Серикбаева – PhD, ассистент-профессор кафедры «Безопасность и качество пищевых продуктов», Алматинский технологический университет, Республика Казахстан; e-mail: ayana-sn@mail.ru. ORCID: https://orcid.org/0000-0001-8252-1034.

Гульзира Шабаевна Джумабекова – Магистр технических наук, лектор кафедры «Техника и технология пищевых производств», Международный инженерно-технологический университет, Алматы, Республика Казахстан; e-mail: guzika_j@mail.ru. ORCID: https://orcid.org/0000-0002-6375-3128.

Раушан Акмуратовна Изтелиева – PhD, ассоциированный профессор «Безопасность и качество пищевых продуктов», Алматинский технологический университет, Республика Казахстан; e-mail: iztelieva80@mail.ru. ORCID: https://orcid.org/0000-0001-9129-2798.

Шолпан Сапаровна Аманова – PhD, ассистент-профессор кафедры «Безопасность и качество пищевых продуктов», Алматинский технологический университет, Республика Казахстан; e-mail: amanova_sh@mail.ru. ORCID: https://orcid.org/0000-0001-7964-7736.

Information about the authors

Saniya Ibraimova* – Assistant Professor, Department of Food Safety and Quality, Almaty Technological University, Republic of Kazakhstan; e-mail: canek21@mail.ru. ORCID: https://orcid.org/0000-0002-9439-7461.

Ayana Serikbaeva – Assistant Professor, Department of Food Safety and Quality, Almaty Technological University, Republic of Kazakhstan; e-mail: ayana-sn@mail.ru. ORCID: https://orcid.org/0000-0001-8252-1034.

Gulzira Jumabekova – Master of Technical Sciences, Lecturer at the Department of «Technique and technology of the Food Production», International Engineering and Technology University, Almaty, Republic of Kazakhstan; e-mail: guzika_j@mail.ru. ORCID: https://orcid.org/0000-0002-6375-3128.

Raushan Izteliyeva –PhD, associate professor, Department of Food Safety and Quality, Almaty Technological University, Almaty, Republic of Kazakhstan; e-mail: iztelieva80@mail.ru. ORCID: https://orcid.org/0000-0001-9129-2798.

Sholpan Amanova – Assistant Professor, Department of Food Safety and Quality, Almaty Technological University, Republic of Kazakhstan; e-mail: amanova_sh@mail.ru. ORCID: https://orcid.org/0000-0001-7964-7736.

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А.К. Базанова*, А.К. Какимов, Н.К. Ибрагимов, М.М. Ташыбаева
Шәкәрім университет,
071412, Республика Казахстан, г. Семей, ул. Глинки, 20 А
*e-mail: arayka.bazanovak@mail.ru

ПРИМЕНЕНИЕ ПРИРОДНЫХ СОРБЕНТОВ В УСОВЕРШЕНСТВОВАННОЙ УСТАНОВКЕ ДЛЯ ОЧИСТКИ МОЛОКА ОТ ТОКСИЧНЫХ ЭЛЕМЕНТОВ

Аннотация: *Молоко является одним из наиболее значимых и питательных продуктов питания, однако его качество во многом зависит от экологической обстановки. Загрязнение окружающей среды приводит к накоплению тяжёлых металлов в пищевой продукции, что представляет угрозу для здоровья населения. Среди них наибольшую опасность представляют свинец (Pb), кадмий (Cd), мышьяк (As) и другие элементы. Целью работы являлось определение содержания свинца, кадмия и мышьяка в образцах сырого коровьего молока и исследование эффективности адсорбционной очистки с использованием природных сорбентов. Анализ проб из трёх населённых пунктов проводился методом инверсионно-вольтамперометрии. Установлено, что содержание кадмия в молоке из села Саржал превышает предельно допустимую концентрацию (0,067 мг/кг при норме 0,03 мг/кг), тогда как уровни свинца и мышьяка находились в пределах нормы. Для очистки молока применялась усовершенствованная экспериментальная установка с тремя сорбционными колоннами, загруженными шунгитом, цеолитом и кокосовым активированным углём. Наибольшая эффективность достигнута при комбинации 1+3 колонна (шунгит + кокосовый активированный уголь), обеспечившей снижение концентраций свинца на 91 %, кадмия на 75 % и мышьяка на 84 %. Полученные результаты подтверждают целесообразность применения природных сорбентов для очистки молока и демонстрируют перспективность разработанной установки для использования в лабораторных и малых производственных условиях.*

Ключевые слова: *сырое коровье молоко, тяжёлые металлы, инверсионно-вольтамперометрический метод, шунгит, цеолит, кокосовый активированный уголь.*

Введение

Современная молочная промышленность сталкивается с проблемами производства экологически безопасной продукции, что во многом связано с возможным загрязнением молочного сырья токсичными элементами [1]. Коровье молоко рассматривается как