B. Munkhbat[1,] , A. Tursukh[1] , N. Otgonpurev[1] ,
Ts. Javzandolgor[2] , R. Chinzorig[3*] , G. Manlaijav[3]

[1]Nuclear Research Center, National University of Mongolia, Ulaanbaatar, Mongolia
[2]Institute of Veterinary Medicine, Ulaanbaatar, Mongolia
[3]School of Engineering and Technology, National University of Mongolia, Ulaanbaatar, Mongolia
*e-mail: chinzorig@num.edu.mn

Elemental analysis and X-ray irradiation effect
on the Mongolian dairy product – "*Khorkhoi Aaruul*"

Abstract. *Aaruul* is a traditional dairy product from Mongolia, recognized for its nutritional qualities and potential health benefits. Traditionally produced through manual milk processing at home, *aaruul* is increasingly mass-produced for commercial markets. This study aimed to investigate the effects of X-ray irradiation on *Khorkhoi aaruul*, a widely consumed small-curd variety. Samples were exposed to irradiation doses of 1 kGy, 3 kGy, and 5 kGy to evaluate reductions in bacterial contamination, extension of shelf life, and compliance with international food safety standards. The changes in vitamin C content and organoleptic properties (taste, texture, and appearance) were systematically assessed. Trace element composition was quantified using Energy-Dispersive X-ray Fluorescence (ED-XRF) spectrometry, focusing on phosphorus (P), calcium (Ca), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), and bromine (Br). Results indicated a substantial reduction in bacterial counts, achieving a 95.5% decrease at the 5 kGy dose (from 4.2×10^6 to 2×10^5 CFU). Critically, irradiation did not induce significant alterations in chemical composition, sensory quality, or vitamin C levels. These findings demonstrate the effectiveness of X-ray irradiation as a viable approach to enhancing microbial safety and prolonging the shelf life of traditional Mongolian dairy products without compromising their nutritional or sensory characteristics.

Keywords: Aaruul, irradiation, trace elements, ED-XRF, colony formation unit.

Introduction

Aaruul is one of the most cherished traditional dairy products in Mongolia. This non-fermented, hard-dried cheese-like food varies widely in size, shape, and ingredients, reflecting its diverse regional origins. Although typically made from cow's milk, aaruul can also be produced from sheep, goat, yak, or camel milk. The traditional preparation involves curdling the milk, filtering it through cotton cloth, manually pressing out the whey, and sun-drying the curds.

In recent years, the consumption of dairy products has increased substantially, leading many producers to commercialize traditional *aaruul*. Among these, the small, white curd known as *Khorkhoi Aaruul* has gained immense popularity, often enjoyed as a snack and frequently flavored with sugar and fruits. While milk products are recognized for their high nutritional value, the specific health benefits of *aaruul* remain under-researched. When stored properly, *aaruul* can be preserved for years; however, it is prone to mold in dark and humid conditions. This susceptibility suggests that *aaruul* may harbor pathogens, particularly since it is often produced in rural settings and under household conditions.

Food sanitation is a crucial concern in Mongolia. Between 2017 and 2019, the prevalence of severe food insecurity within the population was 5.9%, while moderate to severe food insecurity affected 27.5% of the total population. Consequently, enhancing food hygiene and sanitation has become an urgent issue in the country [1]. In many instances, particularly in rural areas, *aaruul* is stored for extended periods without refrigeration, which often results in mold growth. Walter et al. investigated the levels of aflatoxin in *aaruul* products, posing the possibility that food-derived aflatoxins could exacerbate viral hepatitis in Mongolia. However, their analysis revealed that aflatoxin levels were undetectable in 17 samples of *aaruul* products [2].

Irradiation technology presents a viable, multipurpose solution for enhancing food safety, food security, and trade biosecurity when applied at the appropriate doses. Food irradiation is effective against a wide range of living microorganisms, including protozoa, insects, and bacteria. Among the various irradiation methods, X-ray irradiation is a relatively novel technique that exposes food to high-energy photons, potentially allowing for deeper penetration than electron beams and gamma rays [3]. This study aimed to reduce the overall microbial load in *aaruul* through the application of X-ray radiation, thereby extending its shelf life and ensuring compliance with international hygiene standards. Following irradiation, we analyzed the changes in the chemical and physical properties of the *aaruul* samples. Additionally, we quantified trace elements such as P, Ca, Zn, Fe, Mn, Cu, and Br, taking into account their potential role in fostering microbial growth, particularly the proliferation of molds. It is widely recognized that mold requires several trace elements, including iron, zinc, and copper, for its growth. Therefore, analyzing the mineral composition of food and feed products is crucial for assessing the risk of mold growth. To address this objective, we utilized Energy-Dispersive XRF Analysis [4]. XRF spectrometry is a well-established, cost-effective, and efficient analytical technique applied across various research fields and industries. In Mongolia, this method is predominantly used within geology, while measurements of biological samples remain infrequent, and the concentration of trace elements in *aaruul* has not been previously assessed [4-6].

Materials and Methods

A total of 500 grams of *Khorkhoi aaruul* was acquired from a local supermarket in Ulaanbaatar. The sample was stored in a freezer at +4°C until it was ready for use. For the experiment, the *aaruul* was divided into four portions of 120 grams each. According to the product label, this type of *aaruul* has a relatively short shelf life of 14 days at room temperature due to its high moisture and nutrient content, making it vulnerable to microbial spoilage, including mold growth.

Irradiation

The samples were irradiated using an RS1800 Q4 biological irradiator, with the irradiation area, diameter, and dosimetry having been previously established. In this study, the distance from the X-ray focal point to the samples was set at 222 mm, while the X-ray tube operated at a voltage of 160 kV and a current of 25 mA. Each of the 120 gram *aaruul* samples received doses of 1 kGy, 3 kGy, and 5 kGy. The actual doses were measured using a PTW Unidos 10002 electrometer and a PTW30004 camera [7]. The average irradiation dose rate of the irradiator was determined to be 9.92 ± 0.5 Gy/min. Following irradiation, all samples were stored at room temperature for a duration of three months.

ED-XRF Elemental analysis

All control and X-ray irradiated samples were dried at 105°C for 24 hrs in a drying oven until the weight was stabilized and milled using an automatic mortar grinder for 15 minutes to get a homogenized powder. Ten grams of the homogenized powder from each sample were packed into a small polymer container with a diameter of 32 mm, then all the packed samples were loaded into the ED-XRF spectrometer, and measurements were carried out. Blank samples and certified reference standards were included in the analysis sequence. The fluorescence intensity was measured on the ED-XRF spectrometer SPECTRO XEPOS SPECTRO Analytical Instruments GmbH, equipped the setup with a palladium (Pd) anode. Measurements were performed for 5 minutes for each sample. The x-ray tube voltage and current of the spectrometer when using secondary targets were 40 kV, 0.88 mA for Mo, 35 kV, 0.90 mA for Co, 49.5 kV, 0.7 mA for Al2O3, and in the case of highly oriented pyrolytic graphite (HOPG), 17.5 kV, 2 mA, respectively. Biological standard reference materials of the milk powder and the rye flour were chosen for similarity to that of the *aaruul* matrix and used as calibration samples. For all elements, the K α lines were used as analytical lines to determine the element concentration in the sample. We used the method of external standards. The standard reference materials, such as milk powder A-11 and rye flour V-8, produced by the IAEA, were used as calibration samples and calculated by the following equation [8-10] :

$$C_x = C_{st} \times \frac{I_x}{I_{st}}$$

Where: C_x, C_{st} are the concentrations of the element of interest in the sample and standard reference material; I_x, I_{st} are the analytical lines intensities of the element from the sample and standard reference material.

Organoleptic analysis and Vitamin C content

To study the physicochemical changes in curd after irradiating with X-ray radiation, the content of vitamin C, minerals, and changes in color, smell, and taste were determined. Vitamin C is measured 2,6-dichloroindophenol titrimetric method [11]. Sensory assessment of the *aaruul* samples was done after irradiation for 24 hours. Five persons, including students and researchers, were panelists who assessed the sensory properties of *aaruul* samples. Each panelist evaluated samples using scorecards, assigning points for flavor (25 points), appearance (15 points), and texture (10 points).

Total microbial count

Measurements were carried out on the total colony formation count of bacteria, mold, and coliform count in the irradiated and control samples according to the American Public Health Association, standard methods for the examination of dairy products [12].

Statistical analysis

All data are expressed as the means ±S.D. The statistical analyses were performed using Microsoft Excel 2016 and SPSS 22 software. One-way ANOVA with Duncan's multiple-range test was used to examine differences among groups. *P* values ≤ 0.05 were considered significant, if not otherwise stated.

Results and Discussion

Elemental analysis

Milk contains 0.7-0.8 % inorganic elements, mainly associated with casein micelles (Ca, Mg, P, Zn), citrate and phosphate complexes (Ca, Mg, Na, K), chlorides (Na, K), milk fat membrane (Fe, Cu), and enzymes (Fe, Mn, Zn) [13]. We have determined a total of 8 elements of the *aaruul*.

Figure 1 shows the X-ray spectrum of *Aaruul* and reference samples measured with a Spectro XEPOS spectrometer using molybdenum (Mo) as a secondary target. From this spectrum, trace elements such as P, K, and Ca have a clear content in *Aaruul*. Additionally, other trace elements such as Fe, Zn, Cu, Mn, and Br were evaluated by XRF.

The XRF spectrum clearly showed the presence of trace elements such as P, K, and Ca in the *aaruul* samples, indicating their substantial contribution to the product's mineral composition.

Table 1 displays the certified concentrations of elements in the reference materials A-11 milk powder and V-8 rye flour, which served as calibration standards for the ED-XRF analysis of the *aaruul* samples. These reference values provide a basis for comparing the elemental concentrations detected in *Aaruul*. According to the measurement results, there was no difference in the amount of minerals in the irradiated and non-irradiated samples.
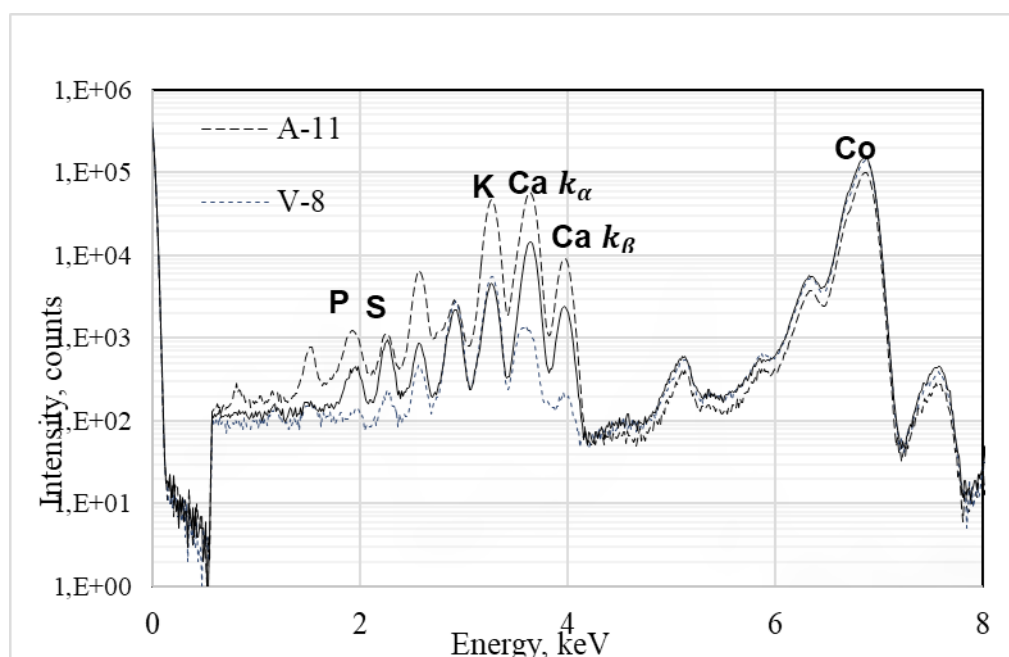

Figure 1 – XRF spectrum of *aaruul* and reference samples (t_{meas}=300s)

Table 1 – Certified concentration amounts of elements in the reference materials A-11 milk powder and V-8 rye flour used as standard samples, (mg/kg)

Element	Certified content values of the RMs	
	A-11	V-8
P	9100±6	592±1
K	17170±2,5	1925±3
Ca	12900±2,0	149±2
Mn	0.257±0.0	2.06±0.2
Zn	38.9±7.3	2.53±0.7
Fe	3.65±1.7	4.1±1.3
Cu	0.378±0.0	0.95±0.4
Br	n.d.	0.38±0.0

n.d. non-identified

Table 2 – The elemental concentrations of the *aaruul* (mean and 95 % confidence interval), and their detection limits; and others' results (mg/kg)

No	Element	Our result				Y.W.Park *et al.*		The corresponding detection limits
		Mean value	Confidence interval	Highest value	Lowest value	Highest value	Lowest value	
1	P	5498	4341–6656	6473	4711	8934	2419	3
2	Ca	3804	3715–3892	3871	3755	5183	1236	10
3	K	1673	1628–1718	1715	1653	6989	1350	10
4	Zn	36.6	35.1–38.1	37.5	35.4	34.1	15.6	0.5
5	Fe	4.32	4.12–4.52	4.5	4.2	17.3	5.2	1
6	Mn	1.93	1.81–2.05	1.99	1.82	0.787	0.704	1
7	Cu	0.48	0.43–0.53	0.51	0.45	8.4	7.53	0.5
8	Br	0.56	0.51–0.61	0.59	0.52	n.d.	n.d.	0.5

n.d. non-identified

Accordingly, Table 2 summarizes our findings, including the mean, confidence interval, and the highest and lowest concentrations, in comparison to the results reported by Y.W. Park et al. [7]. Since Cd and Pb were not detected, we suggest that their concentrations fell below the detectable range of the spectrometer. Y.W.Park *et al* measured 20 major and trace minerals by an Inductively Coupled Plasma Optical Emissions Spectrometer using argon in *aaruul* samples marketed at retail stores in Mongolia, and the highest amounts of minerals were Ca 5,183 mg/kg, P 8,934 mg/kg, K 6,989 mg/kg, Na 1,523 mg/kg, Mg 440 mg/kg. Our results confirmed the results of Y.W.Park *et al.*, so our data demonstrate that *aaruul* is particularly rich in P, Ca, and K. In Table 4, Significant variability was observed in phosphorus (P) concentrations. This variability arises primarily from the low-energy X-ray emissions of elements like phosphorus, making them more susceptible to absorption by the sample matrix or detector window, thereby increasing measurement uncertainty and reducing reproducibility. Additionally, inconsistencies in sample preparation, such as variations in particle size, homogeneity, and sample positioning within the spectrometer, likely contributed further to measurement variability. The complex organic and inorganic matrix of *aaruul* samples exacerbates these measurement challenges. Natural differences in phosphorus content due to variations in milk origin, processing conditions, and moisture content may also account for observed fluctuations. Therefore, the considerable variations

in phosphorus concentrations observed in our study are attributable to both technical limitations of the ED-XRF method and inherent heterogeneity of the *aaruul* samples.

Total microbial counts

Takeda *et al.* investigated the probiotic potential of lactic acid bacteria isolated from Mongolian dairy products. Lactic acid bacteria are considered to be probiotics with properties beneficial to health. This study suggests that the heat treatment the tarag (yogurt) goes through (boiling and drying) when making *aaruul* kills off the lactic acid bacteria from the tarag (yogurt), and therefore the lactic acid bacteria found in *aaruul* are of environmental origin [14]. This indicates hygienically, *aaruul* may expose pathogens, especially when *aaruul* is prepared in rural areas without proper sanitation conditions.

The effect of X-ray irradiation on the total microbial colony formation unit count is presented in Table 3. Before and after irradiation, we cultured total bacteria, mold, and coliforms, but mold and coliforms were not detected in any of the samples. The reduction of the bacterial total count was significantly different ($p \leq 0.05$) in control and 5 kGy treated samples, and microbial count was reduced from 4.4×10^6 to 2×10^5 or a 95.5% reduction. Ionizing radiation produces well-documented biological effects, including DNA strand breaks [15], which contribute to microbial inactivation.

Table 3 – The total colony formation unit (CFU/g) count of *aaruul* samples

Aaruul samples	Mean log CFU/g	Reduction %
Control	4.4±1.6[a]	0
1 kGy	3.2±1.8[ab]	27.3
3 kGy	1.2±0.8[ab]	72.7
5 kGy	0.2±0.1[b]	95.5

Different superscripts [a] and [b] are considered significantly different at $p \leq 0.05$

Organoleptic analysis

Table 4 – Organoleptic examination of *aaruul* samples

Aaruul samples	Organoleptic scores			
	Flavor (25)	Appearance (15)	Texture (10)	Total scores (50)
Control	24.2±1.17*	13.6±1.02*	9.6±0.49*	47.4
1 kGy	24.2±0.75*	13.8±0.75*	9.8±0.40*	47.8
3 kGy	24±0.63*	14.2±0.75*	9.4±0.49*	47.6
5 kGy	23.2±0.75*	13.8±1.17*	8.8±0.75*	45.8

*There was no significant difference between the irradiated groups and the control ($p \leq 0.05$).

During irradiation, there were no significant differences in temperature, and physical state changes were observed in the *aaruul* samples. In organoleptic analysis, panelists' scores indicated there are no differences in flavor, texture, and appearance between irradiated and non-irradiated samples in Table 4.

Vitamin C content

Table 5 – The mean moisture and vitamin C content of *aaruul* samples

Aaruul samples	Moisture (%)	Vitamin C (mg in 100g)
Control	7.78±1.11*	2.28±0.37*
1 kGy	6.07±0.38*	2.11±0.04*
3 kGy	6.62±0.32*	2.06±0.14*
5 kGy	6.21±0.43*	2.14±0.10*

*There was no significant difference between the irradiated groups and the control ($p \leq 0.05$).

Dairy nutrition is the key factor since it is an essential part of the food chain. After irradiation, the moisture content is reduced in all irradiated samples in Table 5. Similar results were reported by Salwa. A *et al*. and Faith Nyamakwere *et al*., after irradiation of Egyptian Karish cheese and Artisanal hard cheese. After X-ray irradiation, the total moisture content decreased gradually but not significantly [16]. This may be the effect of ionization energy. Pryke *et al*. stated that in a high dose range, the temperature of the irradiated product increases by a few degrees centigrade [17]. Vitamin C content was not decreased after 5 kGy irradiation; thus, we expected that above 1 kGy irradiation may result decrease of vitamins by approximately 10% depending on the previously reported study [18]. It is well known that irradiation dose-dependently shows a destructive effect on milk enzymes and lipids of natural products, depending on the absorbed radiation dose or exposure time [3].

Conclusion

This work represents the first comprehensive analysis of *aaruul* samples in Mongolia, focusing on their elemental composition, microbial safety, and the impact of X-ray irradiation on both nutritional and sensory attributes. While studies on the elemental composition of dairy products worldwide have utilized XRF spectrometry [18-19], the concentrations of minerals and trace elements in aaruul have not been previously measured. Our study reveals that *aaruul* is rich in trace elements, particularly containing 4.32 mg/kg of iron, 36.6 mg/kg of zinc, and 0.48 mg/kg of copper. These trace minerals are not only essential nutrients that enhance the nutritional value of food products but also play a crucial role in microbial metabolism. Specifically, elements such as iron, zinc, and copper are known to be necessary cofactors for enzymes involved in fungal growth and proliferation. Therefore, the relatively high concentrations of these minerals in *aaruul* provide favorable conditions for

potential mold contamination, particularly when stored improperly under conditions of high humidity and limited ventilation. Thus, the presence of iron, zinc, and copper in *aaruul* represents a dual-effect scenario: while they contribute positively to the product's nutritional value as essential micronutrients, they may also support undesirable mold growth under poor storage conditions. This underscores the importance of proper handling and storage practices to maintain product safety and quality. Steele et al. researched *Aspergillus ochraceus* and found that the optimal conditions for the production of ochratoxin A in a synthetic medium were between 1.2-24 mg/kg of iron, 0.055-262 mg/kg of zinc, and 0.004-0.04 mg/kg of copper[19]. Suhr et al. also demonstrated that *Penicillium caseifulvum* can grow on cheese with a minimum requirement of 0.5 mg/kg of iron, 0.3 mg/kg of copper, and 150 mg/kg of manganese [20].

After irradiation with a dose of 3 kGy, the total number of bacteria in *aaruul* is decreased by 3-4 times. At 5 kGy irradiation, the total number of bacteria was greatly reduced. Any food irradiated up to an overall average dose of 10 kGy is safe and wholesome; the dose should not exceed 10 kGy except for a legitimate technological purpose according to the Codex Alimentarius issued a General Standard for Irradiated Food (1983, revised 2003) [21]. After irradiation, we kept our samples in petri dishes at room temperature for 3 months for further observation, and from the first month, control samples started molding, but none other groups. Therefore, we suggest ionization radiation is the best way to improve hygiene and extend the shelf life of the *aaruul* without losing its nutritional quality.

Acknowledgments

References

1.   Organization, W.H., *The state of food security and nutrition in the world 2020: transforming food systems for affordable healthy diets*. Vol. 2020. 2020: Food & Agriculture Org.

2.   Popp, W., et al., *Aflatoxin exposure may not play a role in liver cancer development in Mongolia*. Digestion, 2014. 89(4): p. 268-271.

3.   Odueke, O.B., et al., *Irradiation applications in dairy products: a review*. Food and Bioprocess Technology, 2016. **9**: p. 751-767.

4.   Zuzaan, P. and D. Bolortuya, *X-Ray Fluorescence Studies of Biological Objects in Mongolia*. X-Ray Fluorescence in Biological Sciences: Principles, Instrumentation, and Applications, 2022: p. 591-607.

5.   Damdinsuren, Z., P. Zuzaan, and B. Damdinsuren, *Brief overview of x-ray fluorescence applications in Mongolian brown coal*. X-Ray Spectrometry, 2024. 53(2): p. 153-158.

6.   Bolortuya, D. and P. Zuzaan, *X-Ray Fluorescence Analysis of Human Hair.* X-Ray Fluorescence in Biological Sciences: Principles, Instrumentation, and Applications, 2022: p. 405-418.

7.   Манлайжав, Г., et al., *RS-1800 Рентген шарах төхөөрөмжийн цацрагийн тунг иончлолын камераар тодорхойлох.* Физик сэтгүүл, 2023. 34(577): p. 31-34.

8.   Byrne, A., et al., *Results of a co-ordinated research programme to improve the certification of IAEA milk powder A-11 and animal muscle H-4 for eleven "difficult" trace elements.* Fresenius' Zeitschrift für analytische Chemie, 1987. 326: p. 723-729.

9.   Dybczynski, R., O. Suschny, and A. Veglia, *Report on the intercomparison run A-11 for the determination of inorganic constituents of milk powder.* 1980: IAEA.

10.   Pszonicki, L., et al., *Report on intercomparison V-8 of the determination of trace elements in rye flour.* 1982, International Atomic Energy Agency.

11.   Nielsen, S.S. and S.S. Nielsen, *Vitamin C determination by indophenol method.* Food analysis laboratory manual, 2017: p. 143-146.

12.   Marshall, R.T., *Standard methods for the examination of dairy products.* 1992.

13.   Pashkova, G.V., *X-ray fluorescence determination of element contents in milk and dairy products.* Food Analytical Methods, 2009. 2: p. 303-310.

14.   Takeda, S., et al., *Efficacy of oral administration of heat-killed probiotics from Mongolian dairy products against influenza infection in mice: alleviation of influenza infection by its immunomodulatory activity through intestinal immunity.* International immunopharmacology, 2011. 11(12): p. 1976-1983.

15.   Myagmarjav, O., et al., *Effects of gamma radiation on lung and colon cancer cell viability.* International Journal of Mathematics and Physics, 2024. 15(2): p. 42-48.

16.   Aly, S.A., D. Farag, and E. Galal, *Effect of gamma irradiation on the quality and safety of Egyptian Karish cheese.* J Am Sci, 2012. **8**(10): p. 761-766.

17.   Pryke, D. and R. Taylor, *The use of irradiated food for immunosuppressed hospital patients in the United Kingdom.* Journal of human nutrition and dietetics, 1995. 8(6): p. 411-416.

18.   Roberts, P.B., *Food irradiation is safe: Half a century of studies.* Radiation Physics and Chemistry, 2014. 105: p. 78-82.

19.   Steele, J., N. Davis, and U. Diener, *Effect of zinc, copper, and iron on ochratoxin A production.* Applied microbiology, 1973. 25(5): p. 847-849.

20.   Suhr, K.I., et al., *Factors affecting growth and pigmentation of Penicillium caseifulvum.* Journal of dairy science, 2002. 85(11): p. 2786-2794.

21.   CODEX, S., *STAN 106-1983.* Codex Alimentarius Commission: Rome, Italy, 1983.

Information about authors:

Byambajav Munkhbat – Doctor of Engineering, Associate Professor, National University of Mongolia (Ulaanbaatar, Mongolia, e-mail: munkhbat.b@num.edu.mn).

Amgalan Tursukh – PhD student, National University of Mongolia (Ulaanbaatar, Mongolia, e-mail: tursukh.amgalan@gmail.com).

Nergui Otgonpurev – Researcher, Nuclear Research Center, National University of Mongolia (Ulaanbaatar, Mongolia, e-mail: otgonpurev@num.edu.mn).

Tserendorj Javzandolgor – PhD, Researcher, Institute of Veterinary Medicine (Ulaanbaatar, Mongolia, e-mail: tsjavzandolgor@gmail.com).

Radnaabazar Chinzorig (corresponding author) – Doctor of Biology, Associate Professor, National University of Mongolia (Ulaanbaatar, Mongolia, e-mail: chinzorig@num.edu.mn).

Gunaajav Manlaijav – PhD student, National University of Mongolia (Ulaanbaatar, Mongolia, e-mail: manlaijav@gmail.com).