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OCCURRENCE AND X-RAY-INDUCED REDUCTION OF AFLATOXIN M1 IN MONGOLIAN DRIED CURD (AARUUL) AND MILK

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Abstract. Aflatoxin M1 (AFM1), a carcinogenic metabolite of aflatoxin B1 excreted into milk, resists conventional dairy processing and may become concentrated in dried products. Traditional Mongolian dried curd (*aaruul*) is widely consumed, yet data on its AFM1 contamination and on non-thermal decontamination methods are lacking. This study aimed to determine AFM1 in homemade and commercial dried curd and in milk, and to evaluate X-ray irradiation for reducing AFM1 and microbial load. AFM1 was quantified by competitive ELISA. Samples were irradiated at 1, 3 and 5 kGy with an RS 1800 X-ray irradiator, with the dose verified by ionization-chamber dosimetry, and total bacterial count, protein, total sugar and antioxidant activity were determined. Mean AFM1 was 70.9 ± 24.3 ng/kg in homemade ($n = 20$) and 37.6 ± 17.1 ng/kg in commercial ($n = 9$) dried curd ($p < 0.05$). Of these samples, 80% and 22.2%, respectively, exceeded the Mongolian maximum level of 50 ng/kg, whereas milk levels were low (5.7 ng/L). Irradiation reduced AFM1 by 3.8–21.0% in curd and 7.0–23.3% in milk, and reduced bacterial counts by up to 97%, but lowered measured protein and total sugar by 34–51% at 3–5 kGy. A dose of 3 kGy offered the best compromise, although no dose reduced AFM1 below 50 ng/kg. The study provides the first data on AFM1 in Mongolian dried curd and shows that X-ray irradiation can complement, but not replace, the prevention of feed contamination and the regular monitoring of dairy products.

Keywords: aflatoxin M1; dried curd (aaruul); ELISA; X-ray irradiation; food safety

INTRODUCTION

Milk and dairy products are a central part of the traditional Mongolian diet, especially in rural communities where homemade products predominate. Among these, dried curd (*aaruul*), prepared by heating soured milk, pressing the resulting curd and drying it in the open air, is one of the most widely consumed dairy foods, particularly during the Lunar New Year festival (*Tsagaan Sar*). Dried curd is made from the milk of cows, yaks, goats, sheep and camels; it contains approximately 90% dry matter and up to 30% fat, and is valued for its long shelf life and ease of transport [1]. In recent years, the industrial production of dairy products has increased, but food safety regulation and quality control have not fully kept pace, and a large share of the dried curd on the market is still produced at the household level.

One of the major chemical hazards in dairy products is aflatoxin M1 (AFM1). When lactating animals ingest feed contaminated with aflatoxin B1 (AFB1), produced mainly by *Aspergillus flavus* and *A. parasiticus*, AFB1 is hydroxylated by hepatic cytochrome P450 enzymes, and approximately 0.3–6.2% of the ingested dose is excreted into milk as AFM1 [2]. AFB1 is the most potent naturally occurring hepatocarcinogen and has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) [3]; aflatoxin exposure has been estimated to contribute to 4.6–28.2% of all hepatocellular carcinoma cases worldwide [4]. AFM1 is relatively stable during processing; conventional thermal treatments such as pasteurization and ultra-high-temperature (UHT) treatment do not reliably eliminate it, and it therefore persists in cheese, yogurt and other dairy products [5, 6]. IARC has classified AFM1

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as possibly carcinogenic to humans (Group 2B) [3]. Accordingly, maximum levels have been established, for example 50 ng/kg for raw and heat-treated milk in the European Union [7] and 50 ng/kg for dairy products in Mongolian standard MNS 6361:2012 [8].

Various strategies have been investigated to reduce AFM1 in milk, including ozonation, ultraviolet light and pulsed electric fields [9], as well as adsorbents and lactic acid bacteria [5, 6]. Ionizing radiation is of particular interest because it is a non-thermal process that can simultaneously degrade mycotoxins and inactivate spoilage and pathogenic microorganisms [10]. Most previous studies have used gamma irradiation, whereas X-ray irradiation, which has comparable applications in food processing but does not require a radioactive source, has received considerably less attention [10]. Recent reviews have highlighted electron-beam, gamma and X-ray irradiation as promising tools for improving the microbial safety and shelf life of cheese and other dairy products [11]. Food irradiation at overall average doses of up to 10 kGy is recognized by the Codex Alimentarius Commission [12], and the World Health Organization has concluded that food irradiated to doses appropriate to achieve the intended technological objective is both safe and nutritionally adequate [13].

In Mongolia, the effects of ionizing radiation have been studied in several biological systems. Ionizing radiation damages DNA through single- and double-strand breaks and base damage, and the in vitro response of human cancer cell lines to ^{60}Co gamma rays and to high-energy electron and X-ray beams from a clinical linear accelerator has been shown to depend on the dose, the cell type and the beam characteristics [14, 15]. For the RS 1800 X-ray irradiator at the Institute of Veterinary Medicine, the spatial distribution of the absorbed dose rate was determined with an ionization chamber at its three sample positions, giving mean dose rates of 9.92, 14.82 and 31.98 Gy/min at distances of 22.2, 13.8 and 6.2 cm from the X-ray tube, respectively [16]. Using this irradiator, low-dose X-ray treatment (100–200 Gy) inhibited the sprouting of ‘Gala’ potato tubers stored at room temperature for 60 days, and 150 Gy was identified as the most suitable dose [17]. More recently, X-ray irradiation of *Khorkhoi aaruul* at 1, 3 and 5 kGy reduced the total bacterial count by up to 95.5% (at 5 kGy) without significant changes in its mineral composition, vitamin C content or sensory properties; moreover, the irradiated samples showed no mold growth during three months of storage at room temperature, whereas the non-irradiated controls began to mold within the first month [18].

Nevertheless, little information is available on AFM1 contamination of traditional Mongolian dried curd, and, to our knowledge, no study has evaluated X-ray irradiation for reducing AFM1 in this solid, low-moisture dairy matrix. Therefore, this study aimed (i) to determine AFM1 levels in homemade and commercial dried curd and in milk; (ii) to characterize the microbial (total bacteria and molds) and compositional (protein, total sugar and antioxidant activity) quality of dried curd; and (iii) to evaluate the effects of X-ray irradiation at 1, 3 and 5 kGy on AFM1, microbial load and compositional parameters, in order to identify a dose that balances decontamination efficacy with product quality. We hypothesized that X-ray irradiation would reduce AFM1 in a dose-dependent manner, but less effectively in the low-moisture curd matrix than in milk or in matrix-free standard solutions, and that a dose could be identified that reduces the microbial load without substantially altering product composition.

Materials and Methods

Sample collection

Homemade dried curd samples (n = 20) were collected in February 2025, immediately before *Tsagaan Sar*, which corresponds to the peak sales period. Eighteen samples were purchased from independent vendors at four markets in Ulaanbaatar (Narantuul, Dunjingarav, Asa Circus (Tsagaan Sar fair) and Bars market), and the remaining two were obtained from a private household. According to the vendors, the homemade samples had been produced in seven

provinces (Arkhangai, Bayankhongor, Bulgan, Khentii, Khovd, Khuvsgul and Uvs); of these, 11 samples were made from yak milk, six from cow milk and three from goat milk (Table 1). For comparison, nine commercial dried curd samples produced by six companies (Table 1) were collected. Approximately 200 g of each sample was placed in a sealed sterile zip-lock polyethylene bag and stored at 4 °C until analysis.

Milk samples were collected in April 2025, during the calving season. Three household (raw) milk samples were obtained from vendors at the Amgalan and Bayanzurkh markets and from a herder in Nalaikh district, Ulaanbaatar. In addition, four commercial milk samples from three companies were purchased. Details of the commercial milk products are given in Table 1.

Samples 21, 25 and 27 were used for the X-ray irradiation experiments.

X-ray irradiation

Milk and dried curd samples were stored at 4 °C until irradiation. X-ray treatment was performed with an RS 1800 Q4 biological irradiator (Rad Source Technologies, USA) at the Laboratory of Infectious Diseases and Immunology, Institute of Veterinary Medicine (Ulaanbaatar, Mongolia), operated at 160 kV and 25 mA, with a source-to-sample distance of 222 mm. Samples were exposed to absorbed doses of 1, 3 and 5 kGy; at the measured dose rate, the corresponding exposure times were approximately 101, 302 and 504 min. Non-irradiated samples served as controls (0 kGy). For dried curd, three samples with similar AFM1 concentrations (Nos. 21, 25 and 27; Table 1) were selected, and a portion of each sample was treated at each dose (n = 3 per dose). Milk and the AFM1 standard solutions supplied with the ELISA kit (40 and 80 ng/L) were irradiated at the same doses in order to compare AFM1 degradation in a liquid matrix and in the absence of a food matrix.

For milk irradiation, aliquots were dispensed into a standard 96-well cell culture microplate at 200 μL per well. The plate was sealed with a single layer of Parafilm to minimize evaporation and positioned at the center of the ionization chamber platform. For dried curd irradiation, 10 g of sample was placed in a standard glass Petri dish (9 cm diameter, 18 mm internal depth) and manually spread to form a visually uniform layer.

The delivered dose was verified using a PTW UNIDOS 10002 electrometer coupled with a PTW 30004 ionization chamber (PTW, Freiburg, Germany); at this sample position, the average dose rate was 9.92 ± 0.50 Gy/min [16]. No significant temperature change was observed during irradiation. After treatment, samples were kept at room temperature and analyzed within 24 h.

Sample preparation for ELISA

Milk samples (20 mL) were cooled and centrifuged at 6000 rpm for 10 min; the upper fat layer was removed, and 100 μL of the defatted milk was used for ELISA. For dried curd, 5 g of finely grated sample was mixed with 20 mL of 70% methanol in a capped tube, shaken at 50 °C for 30 min and centrifuged at 6000 rpm for 10 min. Subsequently, 2 mL of the supernatant was transferred to a new tube, mixed with 2 mL of hexane for 10 s and centrifuged again at 6000 rpm for 10 min, and 100 μL of the lower aqueous phase was used for ELISA [19]. The dilution factor of the extraction procedure was taken into account in calculating AFM1 concentrations.

ELISA

AFM1 was quantified with a competitive enzyme immunoassay (RIDASCREEN® Aflatoxin M1, Art. No. R1121; R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions. Absorbance was measured at 450 nm, and AFM1 concentrations were calculated from a calibration curve constructed with the kit standards. Briefly, 100 μL of anti-AFM1 antibody solution was added to each well and incubated for 15 min at 25 °C; the wells were then emptied, tapped dry and washed three times with 250 μL of washing buffer. Standards and samples (100 μL) were added and incubated for 30 min at 25 °C. After washing, 100 μL of enzyme conjugate was added (15 min, 25 °C), the wells were washed

Table 1 – Dried curd and milk samples analyzed in this study

No.	Sample type	Collection site / producer	Stated origin (province, soum)	Milk species	Shape
Homemade dried curd ($n = 20$)					
1	Homemade curd	Narantuul market	Arkhangai (Bulgan soum)	Cow	Small, curly
2	Homemade curd	Narantuul market	Arkhangai	Yak	Small, square
3	Homemade curd	Narantuul market	Khovd	Cow	Khuruud (large pieces)
4	Homemade curd	Narantuul market	Khentii	Cow	Small, flower-shaped
5	Homemade curd	Narantuul market	Bayankhongor	Goat	Square
6	Homemade curd	Narantuul market	Khuvsgul	Yak	Large, flat
7	Homemade curd	Narantuul market	Arkhangai	Yak	Khuruud
8	Homemade curd	Dunjingarav	Arkhangai (Tariat soum)	Goat	Round, khorkhoi (worm-shaped)
9	Homemade curd	Dunjingarav	Arkhangai	Yak	Long, with central hole
10	Homemade curd	Dunjingarav	Arkhangai	Yak	Short, with central hole
11	Homemade curd	Dunjingarav	Arkhangai (Tariat soum)	Goat	Khorkhoi
12	Homemade curd	Asa Circus (Tsagaan Sar fair)	Arkhangai (Khangai soum)	Yak	Large, flower-shaped
13	Homemade curd	Asa Circus (Tsagaan Sar fair)	Arkhangai (Khangai soum)	Yak	Small, cone-shaped
14	Homemade curd	Bars market	Uvs	Cow	Square
15	Homemade curd	Bars market	Arkhangai	Yak	Flat khuruud
16	Homemade curd	Bars market	Arkhangai	Yak	Small, square
17	Homemade curd	Bars market	Khuvsgul	Yak	Large, flat
18	Homemade curd	Bars market	Arkhangai	Yak	Khuruud
19	Homemade curd	Household	Uvs	Cow	Khuruud
20	Homemade curd	Household	Bulgan	Cow	Thin, flat
Commercial dried curd ($n = 9$)					
21	Commercial curd	Producer A	–	Cow	Khorkhoi
22	Commercial curd	Producer A	–	Cow	Round khorkhoi
23	Commercial curd	Producer B	–	Cow	Khorkhoi, with fruit
24	Commercial curd	Producer B	–	Cow	Khorkhoi
25	Commercial curd	Producer C	–	Cow	Khorkhoi
26	Commercial curd	Producer D	–	Cow	Khorkhoi
27	Commercial curd	Producer E	–	Cow	Small
28	Commercial curd	Producer F	–	Cow	Khorkhoi
29	Commercial curd	Producer B	–	Cow	Khorkhoi
Milk ($n = 7$)					
M1	Commercial milk	Producer C	–	Cow	–
M2	Commercial milk	Producer B (brand 1)	–	Cow	–
M3	Commercial milk	Producer B (brand 2)	–	Cow	–
M4	Commercial milk	Producer G	–	Cow	–
M5	Household (raw) milk	Herder, Nalaikh district	–	Cow	–
M6	Household (raw) milk	Bayanzurkh market	–	Cow	–
M7	Household (raw) milk	Amgalan market	–	Cow	–

Note: Origin and milk species of homemade curd are as stated by the vendors. Commercial producers are coded (A–G); the same letter denotes the same company for curd and milk. n.s., not stated on the label.

again, and 100 μL of substrate/chromogen was added (15 min, 25 °C). The reaction was terminated with 100 μL of stop solution, and absorbance was read within 15 min. All standards and samples were analyzed in duplicate wells. The kit standards contained 0, 5, 10, 20, 40 and 80 ng/L AFM1, and the detection limit stated by the manufacturer for milk is 5 ng/kg [20].

Total bacterial count and mold identification

The total bacterial count of dried curd was determined by ten-fold serial dilution and plate counting [21] on nutrient agar: 1 g of grated sample was homogenized in 9 mL of sterile 0.89% NaCl solution and serially diluted, and 200 μL of each dilution was spread-plated and incubated at 37 °C for 24 h (triplicate plates); results were expressed as colony-forming units per gram (CFU/g). For mold enumeration and identification, samples were plated (200 μL of the 10^{-1} dilution) on Sabouraud dextrose agar (SDA) with chloramphenicol and incubated at 30 °C for 5 days. Pure cultures were obtained by point inoculation of individual colonies onto fresh SDA plates and incubation at 30 °C for 5 days. Mold genera were identified on the basis of colony and microscopic morphology using the slide culture technique [22]. Briefly, a block of SDA (approximately 0.5 cm thick) was placed on a sterile glass slide supported on two sticks in a Petri dish, inoculated on its four sides, covered with a sterile coverslip and incubated at 30 °C. After sufficient growth, the coverslip was lifted, the agar block was removed, a drop of methylene blue was applied, and the coverslip was replaced; the preparation was examined under a light microscope with 40× and 100× objectives.

Total sugar

Total sugar (total carbohydrate) content was determined by the phenol–sulfuric acid method [23]. One gram of dried curd was homogenized in 9 mL of deionized water. One milliliter of the homogenate was mixed with 0.5 mL of 5% phenol solution and 2.5 mL of concentrated sulfuric acid, shaken thoroughly and left to stand for 30 min, after which absorbance was measured at 490 nm. Results were calculated from a glucose standard curve and expressed as mg glucose equivalents per gram (mg/g). The glucose standard curve was prepared from a 1 mg/mL stock solution diluted to 0, 0.125, 0.25, 0.5, 0.75 and 1 mg/mL. Each sample was analyzed in triplicate.

Protein

Protein content was determined by the Lowry method [24, 25]. The Lowry reagent was prepared by mixing solution A (2.86 g NaOH and 14.31 g Na_2CO_3 in 500 mL of distilled water), solution B (1.42 g $CuSO_4{\cdot}5H_2O$ in 100 mL) and solution C (2.85 g sodium tartrate dihydrate, $Na_2C_4H_4O_6{\cdot}2H_2O$, in 100 mL) at a ratio of 100:1:1, and the Folin–Ciocalteu reagent (2 N) was diluted by mixing 5 mL with 6 mL of distilled water. To 0.5 mL of diluted sample (the sample was equilibrated to room temperature and diluted 10-fold with distilled water), 0.7 mL of Lowry reagent was added, mixed thoroughly and incubated at room temperature for 20 min. Then, 0.1 mL of Folin–Ciocalteu reagent was added, and the mixture was shaken and incubated for a further 30 min at room temperature in the dark. Absorbance of 300 μL aliquots in a 96-well plate was measured at 750 nm, and protein content was calculated from a bovine serum albumin (BSA) standard curve (0, 50, 100, 200, 500, 1000 and 2000 μg/mL). Each sample was analyzed in triplicate.

Antioxidant activity

Antioxidant activity was assessed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [26]. Diluted sample (40 μL) was mixed with 260 μL of DPPH solution (0.1 mM in ethanol, prepared by dissolving 4 mg of DPPH in 100 mL of ethanol), and absorbance was read at 517 nm. Results were calculated from an ascorbic acid standard curve (0, 0.25, 0.5, 0.75 and 1 μg/mL) and expressed as μg ascorbic acid equivalents (AAE) per gram. Each sample was analyzed in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Unless otherwise stated, all analyses were performed in triplicate. Differences between homemade and commercial samples were assessed using Student's t-test. Differences among irradiation treatment groups, including the non-irradiated control, were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. All statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

AFM1 in milk and dried curd

Mean AFM1 concentrations were 5.7 ± 0.9 ng/L in household milk and 3.4 ± 2.8 ng/L in commercial milk (Figure 1a). In dried curd, the mean AFM1 concentration was 70.9 ± 24.3 ng/kg in homemade samples and 37.6 ± 17.1 ng/kg in commercial samples, and this difference was statistically significant ($p < 0.05$; Figure 1b). MNS 6361:2012 sets a maximum level of 50 ng/kg for AFM1 in dairy products [8], which equals the EU maximum level for milk [7]; no specific EU maximum level exists for dried curd. The mean AFM1 content of homemade dried curd exceeded this level, with 16 of 20 homemade samples (80%) and 2 of 9 commercial samples (22.2%) containing more than 50 ng/kg. Mean AFM1 levels in both milk groups were far below 50 ng/L. However, these milk concentrations are close to or below the detection limit stated by the manufacturer for milk (5 ng/kg) [20] and should therefore be regarded as semi-quantitative estimates.

AFM1 contamination was more pronounced in homemade dried curd than in commercial samples, whereas AFM1 levels in milk were comparatively low. This pattern may reflect differences in raw milk quality, feed contamination and the degree of quality control during processing. Commercial dairy products are generally manufactured from milk collected under more standardized conditions, whereas homemade products may show greater variation in milk sourcing, hygiene and processing practices. These factors may contribute to the higher mean AFM1 levels and the greater proportion of samples exceeding the maximum level in homemade dried curd. A similar pattern has been reported for yogurt in Iran, where traditional products contained more AFM1 than industrial ones (mean 33.6 vs 24.6 ng/kg) [27]. In addition, most homemade samples in this study were made from yak milk (11 of 20), which is richer in protein and dry matter than cow milk [28]; because AFM1 associates with casein, the milk species may also have contributed to the higher AFM1 levels in homemade curd, although the number of samples per species was too small to test this statistically.

AFM1 concentrations in dried curd were considerably higher than those in milk. AFM1 binds preferentially to casein and therefore remains associated with the protein-rich curd fraction during coagulation and whey separation [29, 30]; subsequent dehydration further concentrates it. Consequently, even low AFM1 levels in raw milk may lead to markedly higher concentrations in dried dairy products. Because the milk and dried curd samples in this study were not obtained from the same sources, a quantitative concentration factor could not be determined; nevertheless, these findings highlight the importance of monitoring AFM1 not only in liquid milk but also in processed and dehydrated dairy matrices. For comparison, AFM1 in Iranian kashk, a dried fermented dairy product, averaged 21 ng/kg, and only 1 of 20 samples exceeded 50 ng/kg [31], whereas the mean level in homemade Mongolian dried curd in the present study was more than three times higher.

Effect of X-ray irradiation on AFM1

In milk, X-ray irradiation decreased AFM1 content by 7.0%, 10.7% and 23.3% at 1, 3 and 5 kGy, respectively (Figure 2a), from 6.4 ng/L in the control to 6.0, 5.7 and 4.9 ng/L. In dried curd, AFM1 decreased from 80.2 ± 2.6 ng/kg in the control to 77.2 ± 3.7, 68.7 ± 4.2 and 63.3 ± 3.1 ng/kg, i.e.,

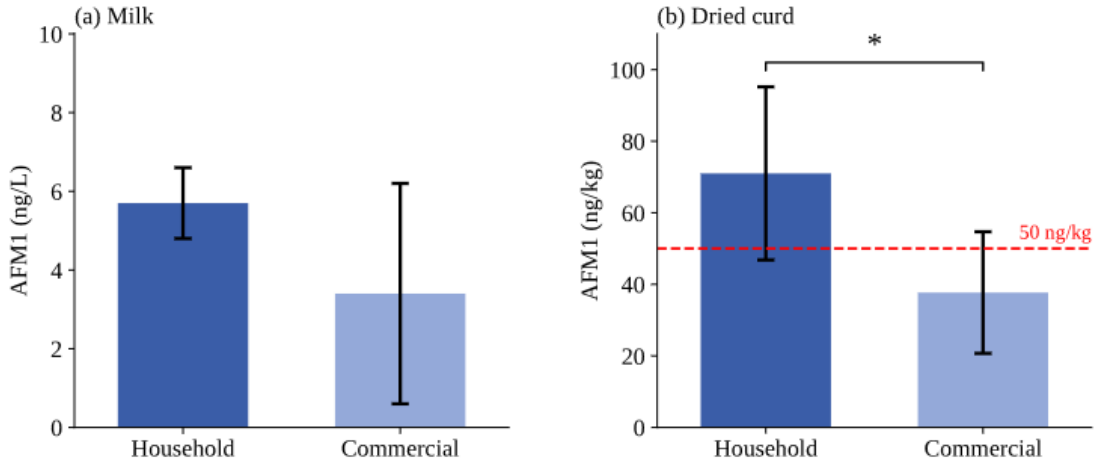


Figure 1 - AFM1 concentrations in (a) household (n = 3) and commercial (n = 4) milk and (b) homemade (n = 20) and commercial (n = 9) dried curd. Values are mean ± SD. In (b), the dashed line indicates the maximum level of 50 ng/kg (MNS 6361:2012). The asterisk indicates a significant difference ($p < 0.05$, Student's t-test).

by 3.8%, 14.4% and 21.0% at 1, 3 and 5 kGy, respectively, and the reductions at 3 and 5 kGy were statistically significant compared with the control ($p < 0.05$; Figure 2b). Nevertheless, AFM1 in the irradiated dried curd remained above 50 ng/kg at all doses tested (approximately 69 and 63 ng/kg at 3 and 5 kGy, respectively).

To assess the effect of irradiation in the absence of a food matrix, the AFM1 standards supplied with the kit were also irradiated. AFM1 decreased by 17%, 23% and 32% in the 80 ng/L standard (to 66.6, 61.8 and 54.4 ng/L) and by 27%, 34% and 53% in the 40 ng/L standard (to 29.2, 26.3 and 18.7 ng/L) at 1, 3 and 5 kGy, respectively (Figure 2c), suggesting that the relative reduction was greater at the lower AFM1 concentration.

X-ray irradiation reduced AFM1 in both milk and dried curd, and the reduction increased with dose. Ionizing radiation degrades mycotoxins mainly through reactive species generated by the radiolysis of water, such as hydroxyl radicals, hydrated electrons and hydrogen atoms, which can attack the terminal furan ring and the lactone moiety of aflatoxin molecules [10]. The reduction efficiency in dried curd and milk was lower than that in the AFM1 standard solutions. This difference is likely due to matrix effects: the low water content of dried curd limits the formation of radiolytic radicals, and proteins, fats and other solids compete for the reactive species and may partially shield the toxin. The larger relative reduction observed in the 40 ng/L than in the 80 ng/L standard is consistent with a limited yield of reactive species per unit dose acting on a smaller number of target molecules. It should be noted that ELISA measures the immunoreactivity of AFM1; the identity and toxicity of radiolytic degradation products were not determined in this study.

Total bacterial count and mold genera

The total bacterial count of non-irradiated dried curd was 1100 ± 597 CFU/g and decreased to 600 ± 352, 86.7 ± 20.8 and 33.3 ± 23.1 CFU/g after irradiation at 1, 3 and 5 kGy, respectively (Figure 3). The reductions at 3 and 5 kGy (92.1% and 97.0%, respectively) were statistically significant ($p < 0.05$), whereas the 45.5% reduction at 1 kGy was not.

Molds were detected in 12 of the 29 dried curd samples (41.4%; 11 homemade and 1 commercial), with a mean count of 2207 ± 4739 CFU/g. On the basis of morphology, the isolates were identified as *Penicillium* (8 samples, 67%), *Paecilomyces* (2 samples, 17%), *Mucor* (1 sample, 8%) and *Bispora* (1 sample, 8%) (Figure 4). *Aspergillus* was not detected.

Irradiation was also highly effective against bacteria, reducing the total bacterial count by

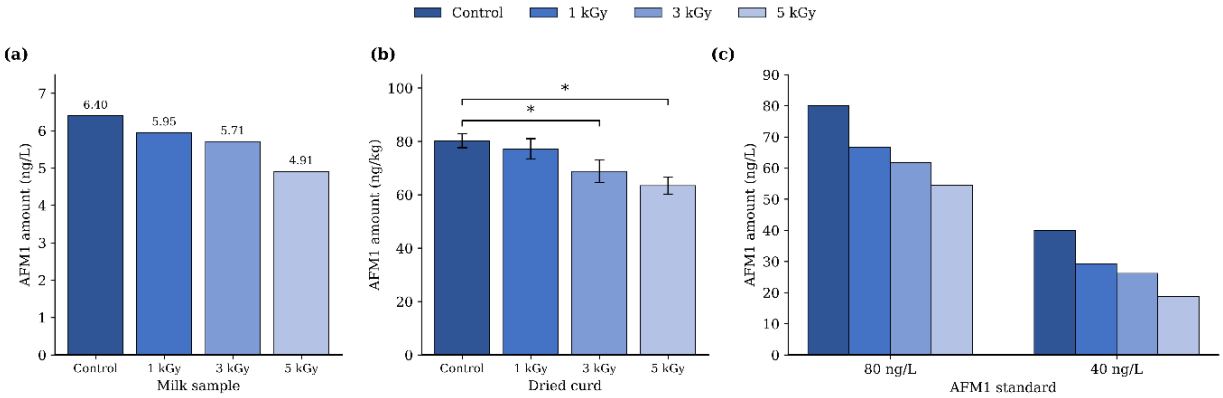


Figure 2 - Effect of X-ray irradiation (1, 3 and 5 kGy) on AFM1 content in (a) milk, (b) dried curd and (c) AFM1 standard solutions (80 and 40 ng/L). In (b), values are mean ± SD (n = 3), and asterisks indicate significant differences from the control ($p < 0.05$).

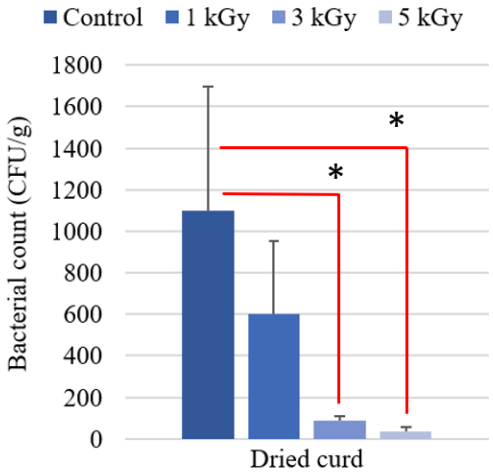


Figure 3 - Total bacterial count in non-irradiated (control) and X-ray-irradiated (1, 3 and 5 kGy) dried curd. Values are mean ± SD; asterisks indicate significant differences from the control ($p < 0.05$).

92% at 3 kGy and 97% at 5 kGy, in agreement with the established use of ionizing radiation for microbial decontamination of foods [10] and with the 95.5% reduction previously reported for X-ray-irradiated *Khorkhoi aaruul* at 5 kGy [18]. Molds were detected mainly in homemade samples, which may reflect less controlled drying and storage conditions. *Aspergillus* was not isolated, and the molds present in the final product are not the source of AFM1, which originates from AFB1 in animal feed. However, *Penicillium*, the predominant genus, includes species capable of producing other mycotoxins, so mold contamination of dried curd remains a quality and safety concern [10].

Total sugar, protein and antioxidant activity

The total sugar content was 61.1 ± 62.1 mg/g in homemade and 123.7 ± 27.4 mg/g in commercial dried curd ($p < 0.05$; Figure 5a). The protein content was 96.5 ± 42.6 mg/g in homemade and 133.2 ± 38.1 mg/g in commercial samples (corresponding to 9.7% and 13.3%; $p < 0.05$; Figure 5b). Antioxidant activity did not differ significantly between homemade (0.0042 ± 0.0022 μg AAE/g) and commercial (0.0045 ± 0.0031 μg AAE/g) samples (Figure 5c).

According to MNS 4230:2005, the protein content of dried curd should be 14–25% [32]. Only 12 of the 29 samples met this requirement, whereas 17 samples (58.6%) had protein contents below 14% (Figure 6).

Irradiation decreased the measured total sugar content from 94.3 ± 17.4 mg/g in the control to 82.7 ± 13.4, 61.6 ± 13.6 and 46.7 ± 15.5 mg/g, i.e., by 12.3%, 34.7% and 50.5% at 1, 3 and 5 kGy, respectively (Figure 7a), and the measured protein content from 15.1 ± 1.6% to 14.4 ± 2.1%, 9.9 ± 1.1% and 8.1 ± 1.3%, i.e., by 4.4%, 34.4% and 46.4%, respectively (Figure 7b). For both parameters, the differences between the control and the 3 and 5 kGy groups were statistically significant ($p < 0.05$), whereas those at 1 kGy were not.

The higher total sugar content of commercial

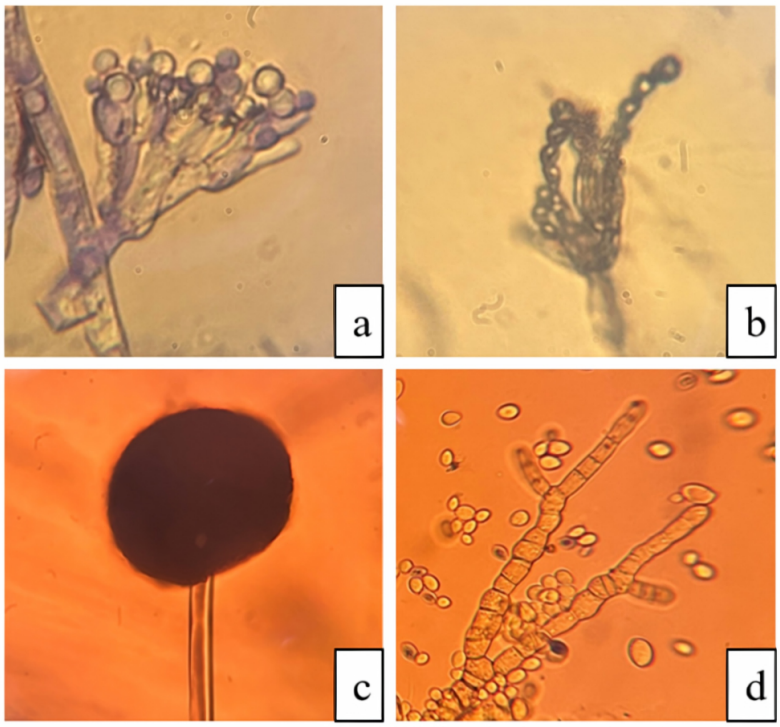


Figure 4 - Molds isolated from dried curd samples (slide culture, light microscopy, methylene blue staining; 40× and 100× objectives): (a) Penicillium sp., (b) Paecilomyces sp., (c) Mucor sp. and (d) Bispora sp.

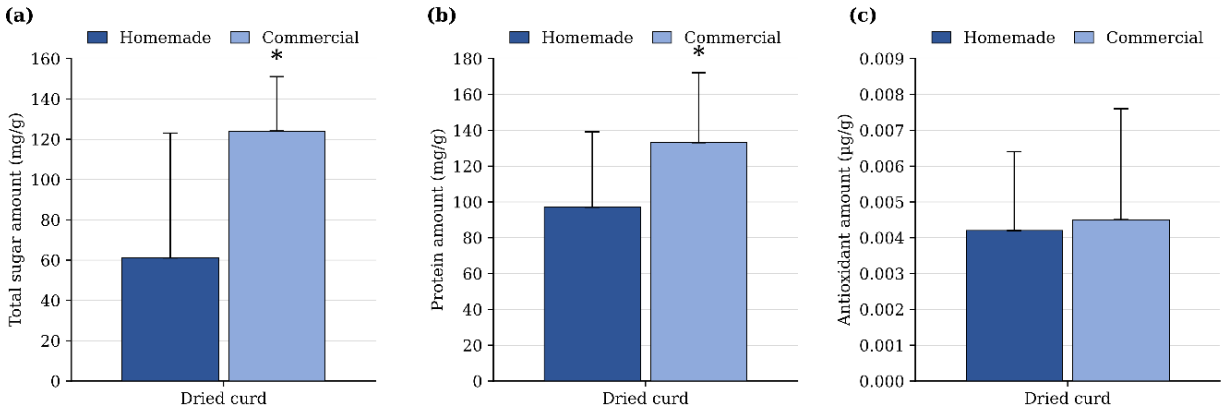


Figure 5 - (a) Total sugar, (b) protein and (c) antioxidant activity of homemade (n = 20) and commercial (n = 9) dried curd. Values are mean ± SD; asterisks indicate significant differences ($p < 0.05$, Student's t-test).

dried curd may reflect the addition of sugar during industrial production. More than half of the samples had protein contents below the minimum specified in MNS 4230:2005. Reference methods for protein in dairy products are based on Kjeldahl nitrogen determination [33], whereas the Lowry method used here depends on protein solubility and on the content of reactive amino acid residues; the comparison with the standard should therefore be interpreted with caution. The decreases in measured protein (34–46%) and total sugar (35–51%) at 3 and 5 kGy were larger than would be expected from the literature, as doses up to 10 kGy are generally considered to have minimal effects on the nutritional value of foods [13]. For example, gamma irradiation of an artisanal hard cheese at up to 5 kGy reduced aerobic colony counts by 77% and eliminated *Listeria monocytogenes*, while protein, fat and other physicochemical parameters remained within the acceptable

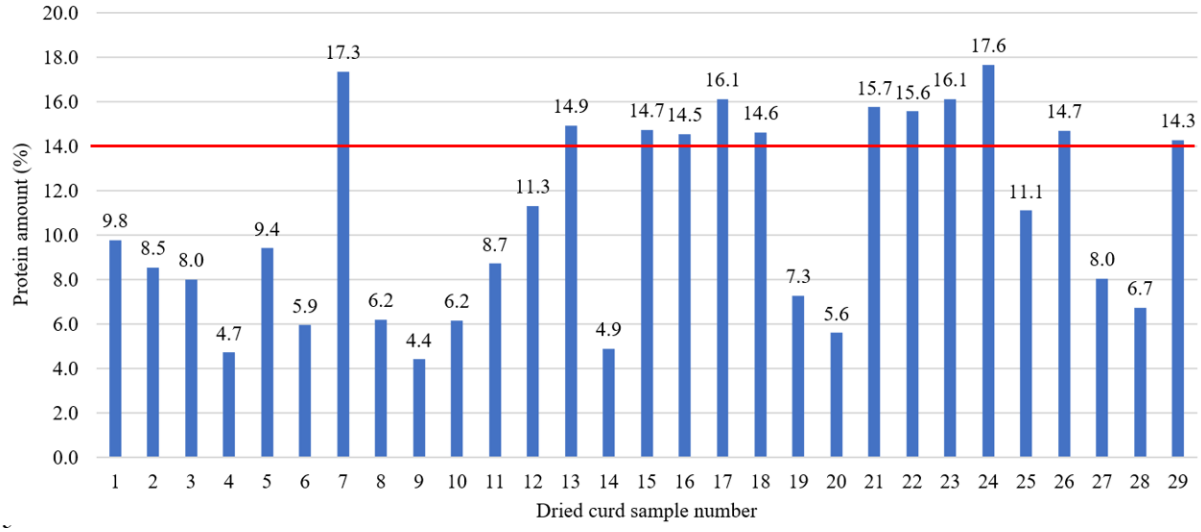


Figure 6 - Protein content of individual dried curd samples (n = 29). The red line indicates the minimum protein content of 14% required by MNS 4230:2005.

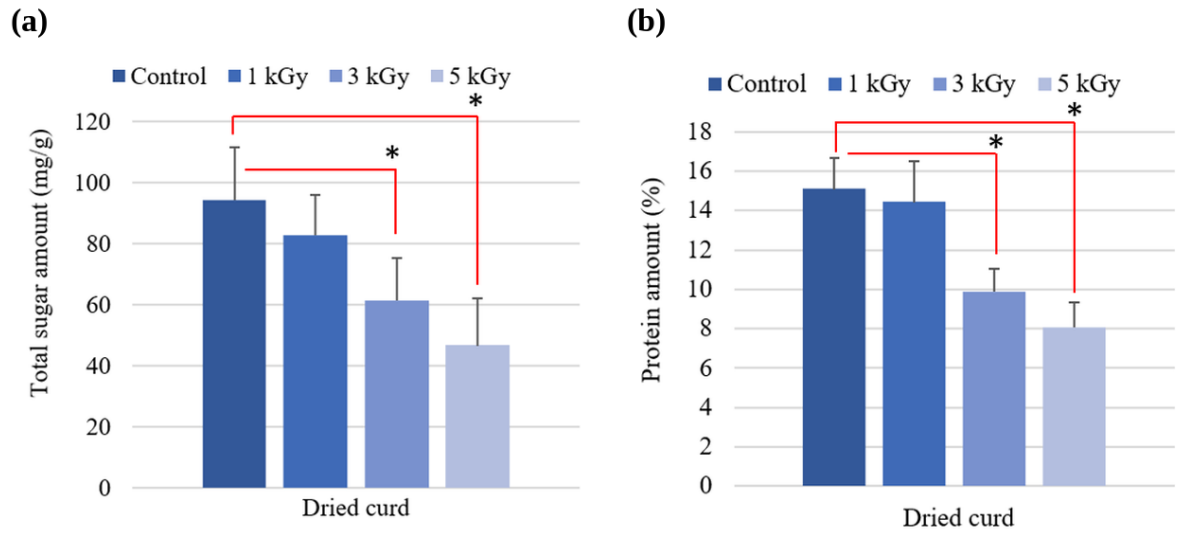


Figure 7 - Effect of X-ray irradiation (1, 3 and 5 kGy) on (a) total sugar and (b) protein content of dried curd. Values are mean ± SD; asterisks indicate significant differences from the control ($p < 0.05$).

ranges for hard cheese, although color and texture changed significantly [34]. These decreases are more likely to reflect radiation-induced cross-linking and aggregation of proteins, which reduce their solubility and reactivity in colorimetric assays, changes in carbohydrate structure and possible sample changes during the long exposure times, rather than an actual loss of nutrients. Confirmation with reference methods is therefore needed.

Selection of the irradiation dose and limitations of the study

Although 5 kGy produced the greatest reduction in AFM1 and bacteria, it was also associated with the largest decreases in the measured compositional parameters. Under the present experimental conditions, 3 kGy therefore provided the best compromise between AFM1 and microbial reduction and compositional changes. This should not be interpreted as a universally optimal dose, but rather as a practical compromise within the spe-

cific conditions of this study. Importantly, none of the tested doses reduced AFM1 in the contaminated curd below 50 ng/kg. X-ray irradiation should therefore be regarded as a complementary measure, whereas preventing AFB1 contamination of animal feed remains the primary strategy for controlling AFM1 in dairy products.

This study has several limitations. The number of samples, particularly milk samples, was limited, and the irradiation experiments were performed on only three dried curd samples. The thickness of the dried curd layer and the dose uniformity within the sample were not measured, although samples were manually spread to form a visually uniform layer. AFM1 quantification was based on ELISA, which is suitable for screening but should be confirmed by HPLC with fluorescence detection or LC-MS/MS, and the toxicity of degradation products was not evaluated. In addition, AFM1 concentrations in milk were close to the detection limit of the ELISA kit. Moisture content was not determined, protein was measured by the Lowry rather than the Kjeldahl method, and molds were identified by morphology only. Finally, sensory properties, texture and storage stability of irradiated curd were not assessed. Despite these limitations, the study provides the first data on AFM1 in Mongolian dried curd and preliminary evidence on the potential of X-ray irradiation for reducing AFM1 and microbial load in traditional dried dairy products.

Conclusion

This study aimed to assess AFM1 contamination of traditional Mongolian dried curd and milk by competitive ELISA and to evaluate X-ray irradiation at 1, 3 and 5 kGy as a non-thermal method for reducing AFM1 and microbial load. AFM1 was detected in all sample types. The mean AFM1 level of commercial dried curd (37.6 ng/kg) was below the maximum level of 50 ng/kg, although 2 of 9 samples exceeded this value, whereas 80% of homemade samples exceeded it. AFM1 levels in household and commercial milk were low (mean $\leq$ 5.7 ng/L). These results indicate a food safety concern associated with homemade dried curd and highlight the need for regular monitoring and for control of AFB1 in dairy feed.

X-ray irradiation reduced AFM1 in dried curd and milk in a dose-dependent manner and markedly reduced the bacterial load of dried curd. Among the doses tested, 3 kGy provided the best compromise between AFM1 reduction (14.4%), microbial reduction (92%) and changes in the measured compositional parameters; however, none of the tested doses reduced AFM1 in contaminated curd below 50 ng/kg. Further studies should confirm these findings by chromatographic methods, evaluate the toxicity of degradation products, and assess the sensory quality and storage stability of irradiated dried curd. In practice, X-ray irradiators, which do not require a radioactive source, could be used by dairy producers and food-control laboratories as a post-processing step to improve the microbial safety and shelf life of dried curd, provided that the dose is optimized for product quality.

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