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Microbial quality monitoring of caviar in processing plants of northern Iran

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Abstract

Food safety and caviar quality are of paramount importance for ensuring consumer protection and preserving the caviar industry. The present study aimed to investigate total microbial count, total fungal count, total coliform count, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* detection in processed caviar samples from six extraction and processing centers in the northern region of the country. This descriptive-analytical cross-sectional study was conducted over three years from 2020 to 2022. In this study, 205 caviar samples were examined, of which total microbial count exceeded the standard permissible limit in 12 samples (5.85%), total fungal count in 57 samples (27.8%), and total coliform count in 13 samples (6.3%). Furthermore, none of the samples were contaminated with *E. coli*, *Salmonella*, or *S. aureus*. Low standards of personal hygiene were identified as the primary factor contributing to coliform contamination. Additionally, due to the resistance of various fungi to brine used in processed caviar and the airborne transmission of fungal spores, fungal contamination was found to be more prevalent than other types of contamination in these samples. Therefore, improving sanitary conditions and implementing Hazard Analysis and Critical Control Point (HACCP) principles in caviar extraction and processing facilities could minimize such contamination. Nevertheless, based on the obtained results, further control measures are deemed necessary to reduce fungal contamination more effectively.

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Introduction

Contamination, particularly by pathogenic bacteria, poses a considerable risk to both public health and the economic viability of caviar production. Bacteria constitute the primary agents of biological spoilage. Among various microorganisms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* have been identified as potential pathogens capable of contaminating caviar and causing foodborne illnesses; furthermore, these bacteria play a significant role in protein hydrolysis (Altuğ and Bayrak, 2003).

Recent bacteriological analyses confirm the presence of various bacteria in different caviar samples, a matter that may raise concerns regarding food safety (Altuğ and Bayrak, 2003). Fahim *et al*. (2018) investigated microbial spoilage indices in processed beluga caviar stored at −2°C for 20 days. Their results demonstrated that mesophilic bacteria, coliforms, and psychrotrophs decreased significantly with increasing storage duration (Fahim *et al.,* 2018). In another study examining changes in psychrotrophic bacteria in Iranian sturgeon caviar during processing, 60 samples were analyzed and stored at −3°C for six months. The findings indicated that psychrotrophic bacteria increased in processed caviar and subsequently in caviar stored at −3°C; however, contamination levels remained within standard permissible limits (Santi *et al.,* 2019). Given the limited data available on the quality of processed caviar in Iran, the present study aimed to evaluate the microbiological quality of processed caviar in the country's northern region.

Materials and Methods

Study design and sampling procedure

This descriptive-analytical cross-sectional study was conducted over three years from 2020 to 2022. A total of 205 processed caviar samples from six extraction and processing centers in the northern region of the country were examined for six types of contamination: total microbial count, total fungal count, *E. coli* count, coliform count, *S. aureus* count, and *Salmonella* detection. A total of 43, 62, and 100 samples were collected in 2020, 2021, and 2022, respectively. Samples were randomly selected from different production batches at each processing facility using batch identification records. All production batches available during the sampling period had an equal probability of selection. Sampling was conducted with regular frequency, at least twice weekly, from the designated centers, and the collected samples were dispatched to the laboratory. All samples were transported under refrigerated conditions (4°C) and analyzed within 24 hours of collection. These centers were located in the cities of Babolsar (Mirud), Izdeh, Nashtarud, Anzali, Rasht (Hasanabad), and Gorgan. The samples examined in this study consisted of processed caviar with a salt content of three to four percent. The microbiological contamination levels of these products were evaluated in accordance with the international ISO 17025 standard and CODEX STAN 291–2010. All samples were transported to the laboratory under refrigerated conditions (4°C) and subjected to microbiological analysis within 24 hours of collection. In accordance with the Iranian national standard, the microbiological characteristics of caviar must conform to those presented in Table 1.

Table 1. Microbiological characteristics of caviar according to Iranian national standard No. 186 (Iran National Standard Organization No. 186, 2016).

Characteristics	Maximum permissible limit
Total microbial count (in each gram of sample)	10^4 CFU/g
Coliforms (in each gram)	10
E. coli (in each gram)	Negative
S. aureus coagulase-positive (in each gram)	Negative
Salmonella (in 25 grams of sample)	Negative
Clostridium sulfite-reducing anaerobes (in each gram)	Negative
Listeria monocytogenes (in each gram)	Negative
Yeast and mold (in each gram)	10^2 CFU/g

Total microbial count

Samples were subjected to serial decimal dilution in sterile peptone water (0.1% w/v). A 100-µl aliquot of each appropriately diluted sample was aseptically spread-plated onto the surface of Plate Count Agar (PCA; Merck, Darmstadt, Germany). Inoculated plates were incubated aerobically at 37 ± 1°C for 48 ± 2 hours. Following incubation, colonies were enumerated using a colony counter, and the results were expressed as colony-forming units per gram (CFU/g). Only plates containing 30–300 colonies

were included in the calculations to ensure statistical reliability (Alak *et al.,* 2021).

S. aureus enumeration

Samples were subjected to serial decimal dilution in sterile peptone water (0.1% w/v). A 100-µl aliquot of each appropriately diluted sample was aseptically spread-plated onto the surface of Baird-Parker Agar (BPA; Sigma-Aldrich, Steinheim, Germany) supplemented with egg yolk tellurite emulsion in duplicate. Inoculated plates were incubated aerobically at 37 ± 1°C for 24–48 hours. Following incubation, presumptive *S. aureus* colonies exhibiting characteristic morphology (jet-black, shiny, convex, 1–1.5 mm in diameter, surrounded by a clear zone with an opaque halo due to lecithinase activity) were enumerated. Colonies with atypical morphology were subjected to confirmation via the coagulase test. The final results were expressed as colony-forming units per gram (CFU/g) of caviar (Saklani *et al.,* 2020).

Salmonella detection

For *Salmonella* identification and confirmation, 25 g of each sample was homogenized with 225 ml of buffered peptone water (BPW) using a filter-based stomacher with pulsatile action. Following homogenization, pre-enrichment was performed by incubating the samples in BPW at 35–37°C for 18–24 hours. Subsequently, the pre-enriched cultures were transferred to selenite cystine broth (SCB) and incubated at 35°C for 24 hours (selective enrichment). Following selective enrichment, colonies with presumptive *Salmonella* morphology were identified using standard laboratory methods and protocols based on the latest available technical procedures (Altuğ and Bayrak, 2003).

Total coliform count and *E. coli* enumeration

The multiple-tube fermentation technique was employed in accordance with ISO 4831:2006 (MPN method). Initially, 25 g of each sample was aseptically weighed and homogenized with 225 ml of sterile buffered peptone water (BPW; 0.1% w/v) using a stomacher for 60–120 seconds to obtain a homogeneous primary suspension (10^{-1} dilution). Serial decimal dilutions were subsequently prepared in sterile physiological saline (0.85% w/v NaCl). From each dilution, 1 ml aliquots were inoculated into lauryl tryptose broth (LTB; Merck, Darmstadt, Germany) containing inverted Durham tubes. The inoculated tubes were incubated at 37 ± 1°C for 24 ± 2 hours and examined for gas production (≥ 10% displacement in the Durham tube). Tubes exhibiting positive gas formation were subjected to confirmatory testing: 0.1 ml of culture was transferred to brilliant green bile lactose broth (BGBLB) and incubated at 37 ± 1°C for 48 ± 2 hours. Gas production in BGBLB confirmed the presence of total coliforms. For *E. coli* confirmation, positive LTB cultures were simultaneously inoculated into EC broth and incubated at 44 ± 0.5°C for 24 ± 2 hours. Gas production in EC broth at 44°C, combined with indole positivity from tryptone water incubated at 44 ± 0.5°C, confirmed thermotolerant *E. coli*. The Most Probable Number (MPN) of total coliforms and confirmed *E. coli* was calculated from the combination of positive tubes using standard MPN tables and expressed as MPN per gram (MPN/g) of caviar (Jelodar and Safari, 2006).

Total fungal count

For total fungal count enumeration, 25 g of each sample was accurately weighed and homogenized with 225 ml of sterile peptone water (0.1% w/v) to achieve a 1:10 dilution. Serial decimal dilutions were subsequently prepared. A 1-ml aliquot of each appropriately diluted sample was transferred using a sterile pipette onto Rose Bengal Chloramphenicol Agar (RB-CA; Merck, Darmstadt, Germany), a selective medium specifically formulated for fungal isolation. The antibiotic chloramphenicol was incorporated into this medium to suppress bacterial proliferation, thereby ensuring selective conditions for fungal growth. The inoculated Petri dishes were incubated in an inverted position at 25 ± 1°C for 5 days. During the incubation period, plates were examined daily to monitor fungal development. Following the 5-day incubation, observations were recorded, and fungal colonies were enumerated, calculated, and reported as colony-forming units per gram (CFU/g) of caviar (Jelodar and Safari, 2006).

Statistical analysis

Descriptive results for the enumerated bacteria, total microbial count, and total fungal count were expressed as median and interquartile range (IQR), minimum, and maximum values. The presence of *Salmonella* was reported as a relative

frequency percentage. Data were entered into SPSS statistical software, and heat maps were generated to illustrate temporal trends across the months and years under investigation. The distribution of microbial count data was evaluated using the Shapiro–Wilk normality test. As the data violated the assumption of normality, non-parametric statistical analyses were conducted. Consequently, the Kruskal–Wallis test was applied to evaluate differences among years, and the Mann–Whitney U test was used for pairwise comparisons when necessary. Additionally, one-sample Wilcoxon signed-rank tests were conducted to compare microbial contamination levels against the maximum permissible limits specified in the relevant standards. All statistical analyses were performed at a significance level of $p < 0.05$ using SPSS version 26 (IBM Corp., Armonk, NY, USA).

Results

Total microbial count (TMC) in caviar

The maximum permissible limit for TMC according to Iranian National Standard No. 186 is 10^4 CFU/g (4 log CFU/g). Among the 205 caviar samples examined, 12 samples (5.85%) exceeded the standard permissible limit for TMC. As presented in Table 2, no statistically significant difference was observed in median contamination levels across the three study years ($p = 0.37$). The highest contamination level was recorded in 2022, with a median value of 6×10^2 CFU/g.

Table 2. Median total microbial count ($\times 10^3$ CFU/g) in caviar from extraction and processing centers in the northern region by study year.

Year	No. of sample	Median	Interquartile range	Minimum	Maximum	No. (%) Exceeding Standard Limit
2020	43	0.40	0.1-2	0	42.0	4 (9.30)
2021	62	0.55	0.175-3	0	23.0	3 (4.83)
2022	100	0.60	0.2-1.55	0	97.0	5 (5.00)
Total	205	0.6	0.2-2.15	0	97.0	12 (5.85)

*$p < 0.05$ was considered statistically significant. The maximum permissible standard limit was 10^4 CFU/g. *p-value for overall difference among three years (Kruskal-Wallis test) = 0.37

As illustrated in Fig. 1, the highest mean total microbial contamination (1.74×10^4 CFU/g) was observed in October 2020. In 2021, the peak contamination occurred in July with a mean value of 1.15×10^4 CFU/g. In 2022, the highest mean total microbial contamination was recorded in both April and October, at 8.63×10^3 CFU/g and 8.50×10^3 CFU/g, respectively.

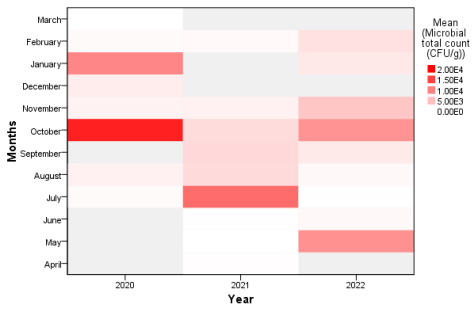


Fig. 1. Heat map of total microbial count in caviar from extraction and processing centers in the northern region by month and year of study. The color gradient from white to red indicates total microbial count ranging from 0 to 2×10^4 CFU/g.

The maximum permissible limit for total fungal count according to Iranian National Standard No. 186 is 10^2 CFU/g (2 log CFU/g). Among the 205 caviar samples examined, 57 samples (27.8%) exceeded the standard permissible limit for total fungal count. The median total fungal count was not higher than the standard permissible limit (10^2 CFU/g), and this difference was not statistically significant ($p = 0.75$). The highest contamination level was recorded in 2022, with a median value of 1×10^2 CFU/g (Table 3). This value was significantly different from those reported in the other study years ($p = 0.001$).

Table 3. Median total fungal count (× 10^2 CFU/g) in caviar from extraction and processing centers in the northern region by study year.

Year	No. of sample	Median	Interquartile range	Minimum	Maximum	No. (%) Exceeding Standard Limit
2020	43	0.0[a]	0.0 - 0.0	0	0	0 (0.00)
2021	62	0.05[a]	0.0 - 1.25	0	40.0	15 (24.19)
2022	100	1.0[b]	0.0 - 3.0	0	42.0	42 (42.00)
Total	205	0.0	0.0 - 2.0	0	42.0	57 (27.80)

As illustrated in Fig. 2, the highest mean total fungal contamination (2.10 × 10^3 CFU/g) was observed in July 2021 in caviar samples collected from processing facilities in the northern region. In 2022, peak mean total fungal contamination occurred in September (8.77 × 10^2 CFU/g), December (7.57 × 10^2 CFU/g), and November (6.75 × 10^2 CFU/g).

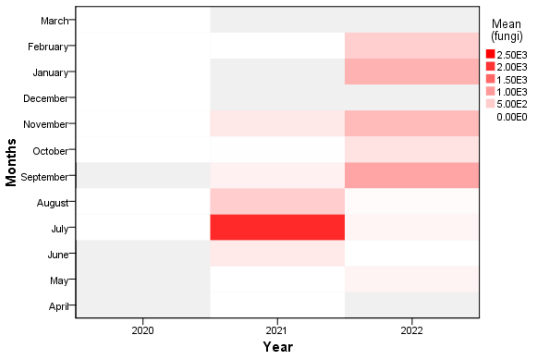


Fig. 2. Heat map of total fungal count in caviar from extraction and processing centers in the northern region by month and year of study. The color gradient from white to red indicates total fungal count ranging from 0 to 2.50 × 10^3 CFU/g.

Table 4. Median total coliform count (MPN/g) in caviar from extraction and processing centers in the northern region by study year.

Year	No. of Sample	Median	Interquartile range	Minimum	Maximum	No. (%) Exceeding Standard Limit
2020	43	0	0 - 0	0	0	0 (0.00)
2021	62	0	0 - 4	0	23.0	5 (8.06)
2022	100	0	0 - 0	0	23.0	8 (8.00)
Total	205	0	0 - 0	0	23.0	13 (6.30)

p < 0.05 was considered statistically significant. The maximum permissible standard limit was 10 MPN/g.p-value for overall difference among three years (Kruskal-Wallis test) = 0.60

Of the 205 caviar samples examined, 13 samples (6.30%) exceeded the standard permissible limit for total coliform count (10 MPN/g). As shown in Table 4, no statistically significant difference was observed in median total coliform counts across the study years (p = 0.60).

As illustrated in Fig. 3, the highest mean total coliform contamination (11.5 MPN/g) was observed in November 2021 in caviar samples collected from processing facilities in the northern region. In 2022, peak mean total coliform contamination occurred in April (19.5 MPN/g) and August (8.42 MPN/g). Furthermore, none of the caviar samples examined during the study years were contaminated with *E. coli*, *Salmonella*, or *S. aureus* (Table 4).

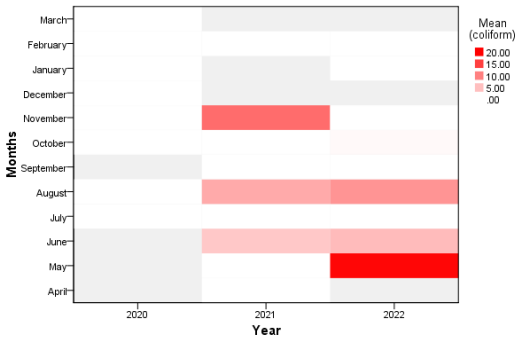


Fig. 3. Heat map of total coliform count in caviar from extraction and processing centers in the northern region by month and year of study. The color gradient from white to red indicates total coliform count ranging from 0 to 20 MPN/g.

Discussion

Chemical and microbial contamination of caviar is influenced by various factors, including fish species, caviar quality grade, processing method, type and concentration of preservatives used, and storage conditions. Furthermore, temperature in processing facilities affects bacterial growth kinetics. In recent years, sturgeon aquaculture has expanded in northern Iran; however, suboptimal farm conditions, intensive fish rearing, increased stress, and elevated harvesting frequency have heightened the risk of bacterial infection. The present study was conducted over three years (2020–2022) on 205 caviar samples from six extraction and processing centers in the northern region. Based on the results obtained, the predominant contaminants observed were coliform and fungal agents.

In the evaluation of TMC, 12 samples (5.9%) exceeded 10^4 CFU/g. A study by Altuğ and Bayrak (2003) on caviar samples from Iran and Russia reported TMC values of 2×10^5 CFU/g in 68 examined samples. In 2006, Jelodar and Safari (2006) collected 144 caviar samples and 936 environmental samples from four processing facilities in Iran between April 2003 and December 2004. Samples were obtained from raw and processed caviar, harvesting equipment, water, processing room air, and workers' hands. The microbiological parameters examined included total microbial count, *E. coli*, coliforms, *Clostridium botulinum*, *Listeria*, *S. aureus*, yeast, and mold. Their results demonstrated that *C. botulinum*, *Listeria*, *S. aureus*, and *E. coli* were absent in all samples, while TMC in processed caviar exceeded that in raw caviar, with values greater than 10^4 CFU/g.

In the present study, the highest number of contaminated samples was observed in 2020 (9.30%); however, no statistically significant difference was detected among the study years. The peak microbial contamination in 2022, with a median value of 6×10^2 CFU/g. This study further demonstrated that the highest mean TMC (1.74×10^4 CFU/g) in caviar samples from northern processing facilities occurred in October 2020. In the subsequent year, the highest mean contamination was observed in July 2021 (1.15×10^4 CFU/g), and in 2022 in April (8.63×10^3 CFU/g) and October (8.50×10^3 CFU/g).

Fungal contamination of fish eggs represents a prevalent problem in aquaculture. Researchers have endeavored to mitigate this issue through the application of disinfectants and the improvement of water quality. One fungal species responsible for egg contamination is *Saprolegnia*, which causes severe mortality in sturgeon eggs, with reported death rates in Iranian ship sturgeon eggs ranging from 7% to 22% (Kayiş *et al.,* 2017). Post-infection management and assurance of adequate microbial water quality and sufficient aeration are critical for controlling this contamination. The precise identification and removal of infected eggs constitute another effective strategy. Additionally, the use of nanosilver, T-chloramine, and hydrogen peroxide has been recommended for prevention of fungal contamination in sturgeon eggs. In the present study, 57 samples (27.8%) exceeded the standard permissible limit for total fungal count. Jelodar and Safari (2006), examining 144 caviar samples and 936 environmental samples from four processing facilities in Mazandaran Province between April 2003 and December 2004, reported fungal contamination levels of 3×10^2 CFU/g and 4.4×10^4 CFU/g in raw and processed caviar, respectively. The extensive fungal contamination appears attributable to favorable temperatures (18–20°C), high humidity in processing rooms (70–75%), and the abundance of fungal spores in the air. Razavilar *et al*. (2001) also reported elevated fungal contamination in various caviar samples, further noting that fungal presence was evident on most caviar processing equipment. Inadequate storage conditions and insufficient equipment washing, resulting in residual caviar grains on surfaces, constitute the principal factors

contributing to fungal contamination. Altuğ and Bayrak (2003), examining 68 caviar samples, reported fungal counts ranging from 10^5 to 10^6 CFU/g.

The presence of coliforms, which serve as indicator microorganisms, demonstrates that the product was processed under substandard hygienic conditions (ICMSF, 1978). In the present study, 192 of 205 examined samples (93.7%) contained less than 10 MPN/g coliforms, while 13 samples (6.3%) exceeded this threshold. Altuğ and Bayrak (2003) identified coliforms in 29.41% (20 of 68) of their samples. The mean coliform count across their 68 samples ranged from 10 CFU/g to 2.4×10^3 CFU/g, with a mean of 8×10^2 CFU/g. Razavilar *et al*. (2001) reported that coliform contamination in caviar was not at acceptable standard levels; furthermore, fecal coliforms such as *E. coli* were detected in certain samples, likely attributable to the use of untreated water, resulting in poor sanitary conditions at the processing site. Coliform contamination (6.3% of samples) suggests possible lapses in hygienic handling, as coliforms are indicator organisms for fecal or environmental contamination. However, direct assessment of personnel hygiene, equipment contamination, or environmental sources was not performed in the present study

Through the implementation of microbiological control measures and adherence to Hazard Analysis Critical Control Point (HACCP) principles at all stages of caviar production, extraction, packaging, and storage, microbial contamination can be effectively prevented, consumer health risks mitigated, and a product of optimal quality and sanitary safety delivered to the market. Key microbiological control strategies during caviar extraction and processing include: the utilization of purified and potable water throughout all processing stages; strict compliance with personal hygiene and workplace sanitation protocols by personnel; application of appropriate thermal processes to reduce microbial load; packaging of caviar under hygienic and sterile conditions; and maintenance of caviar at appropriate temperatures with controlled humidity levels.

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Conflict of Interest

The authors declare no conflicts of interest.

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