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Microbiological assessment of dry oat (*Avena sativa* L.) seeds before and after maceration

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ABSTRACT

The aim of this study was to differentiate surface-associated microflora and microorganisms resistant to washing in dry oat (*Avena sativa* L.) seeds and to evaluate the effect of 24 h maceration in sterile distilled water on microbial release. Commercial seed samples were processed aseptically in the microbiology laboratory. Two experimental phases were applied: (i) pre-maceration, where seeds were briefly rinsed in sterile distilled water and the suspension was analyzed for surface-associated microorganisms, and (ii) post-maceration, where seeds were soaked for 24 h, allowing the release of microorganisms resistant to washing and associated with protected seed-coat niches. Suspensions were filtered through 0.45 μm membranes and cultured on selective and differential media: nutrient agar for heterotrophs, m-Endo agar for total coliforms, m-FC agar for fecal coliforms, Salmonella–Shigella agar for enteric pathogens, and potato dextrose agar (PDA) for yeasts and molds. Results showed that before maceration, the highest microbial load was fecal coliforms (244 CFU/100 mL), followed by heterotrophs and total coliforms (39 CFU/100 mL), while no yeasts or molds were detected. After maceration, microbial counts decreased by 41% for fecal coliforms, 90% for *Salmonella*/*Shigella*, and more than 90% for heterotrophs. These findings indicate that dry oat seeds may harbor potentially harmful microorganisms not only on the surface but also tightly associated with protected structures of the seed coat. The internal presence of microorganisms has important implications for agriculture, as it may adversely affect seed germination and early plant development in the field, thereby reducing growth quality and productivity. Maceration combined with membrane filtration represents a simple and effective approach to detect these risks, with direct relevance for seed quality control and food safety.

Introduction

Cereals represent one of the most important groups of agricultural crops, as they are the main source of energy and food for more than half of the world's population. Their consumption is closely related to food security, economic development, and public health. Among them, oats (*Avena sativa* L.) have gained particular importance over the past decades, not only as a staple food but also for their functional and bioactive values. Oats differ from other cereals due to their rich chemical composition in soluble fibers, especially β-glucans, which contribute to lowering cholesterol and regulating blood glucose levels (Whitehead et al. 2014). In addition, they contain proteins of high biological value, unsaturated lipids, and a wide spectrum of bioactive compounds, including avenanthramides, which show strong antioxidant and anti-inflammatory activity (Peterson 2001). From an agricultural point of view, oats are a crop resistant to cold and humid conditions, making them suitable for regions where wheat or maize provide lower yields. They are widely used as food for humans and animals, as well as in the pharmaceutical and cosmetic industries due to their bioactive composition (Butt et al. 2008). From the perspective of public health, the World Health Organization (WHO 2023a) and the European Food Safety Authority (EFSA 2011) have emphasized the importance of regular consumption of oat β-glucans for the prevention of cardiovascular diseases and type 2 diabetes. Consequently, the global demand for oat-based products has increased significantly, turning oats from a crop once considered modest into a "superfood", highly valued in modern markets (EFSA 2011). The importance of oats is not limited to the nutritional aspect. They also play an essential role in the economy, public health, and industrial innovation. Furthermore, their agricultural sustainability due to lower requirements for chemical inputs makes them a favorable crop for biodiversity conservation and more ecological production systems. In this research, oat seeds represent not only carriers of genetic information and productive potential of plants but also a small microscopic ecosystem rich in microorganisms. These microorganisms, including bacteria, fungi, and yeasts, are known as the seed microflora and play an important role in the plant life cycle and in the final quality of agricultural production. Many of them perform beneficial functions: some bacteria of the genera *Pseudomonas* and *Bacillus* act as plant probiotic agents by colonizing the seed surface and later the root system, creating a biological shield against soil pathogens (Nelson 2018). However, seed microflora are not always favorable. Seeds often carry pathogenic microorganisms that remain latent during storage and become active after germination, one of the main routes for the spread of diseases in agricultural crops. Among them, fungi of the genera *Fusarium*, *Alternaria*, and *Aspergillus* are the most problematic, as they can produce phytotoxic secondary metabolites that negatively affect the initial metabolism of seeds, impair germination, and reduce seedling vigor. These fungi are known to produce toxins such as trichothecenes, fumonisins, aflatoxins, and alternariol, which interfere with key metabolic processes during early seed development (Desjardins 2006; Logrieco et al. 2009; Munkvold 2009).

Such seed-borne infections affect yield, quality, and food safety. Contaminated products from infected seeds may enter the food chain, causing severe health consequences. For this reason, international organizations such as the Food and Agriculture Organization of the United Nations (FAO) and WHO have set strict limits for the presence of mycotoxins in food products, emphasizing the importance of control starting from the seed stage (FAO n.d.; WHO 2023b). The process of seed colonization by microorganisms is among the most fascinating mechanisms in plant ecology, as it represents the first point of contact between microflora and a biological system that will later develop into a complete plant. This colonization occurs in two main ways: superficial and endophytic. In superficial colonization, microorganisms reside on the seed coat or its outer layers and are able to survive in dry and nutrient-poor conditions due to protective mechanisms against oxidative stress. Some of them become active immediately after seed soaking, releasing hydrolytic enzymes or substances that stimulate germination (Shade et al. 2017). Endophytic colonization is more complex, as microorganisms penetrate the inner tissues of the seed through the micropyle or microscopic injuries during seed formation. Once inside, they may settle in the endosperm or embryo, forming a close association with the future root system. This symbiosis is often beneficial: endophytes may produce hormones such as auxins and cytokinins that stimulate root development or secondary metabolites that increase resistance to biotic and abiotic stress (Truyens et al. 2015). However, confirmation of true endophytic colonization requires rigorous surface sterilization protocols prior to seed grinding, which were not applied in the present study. Infection of seeds by pathogenic microorganisms is one of the most serious challenges in agriculture, as it affects the most critical phase of the plant life cycle, the germination process. Infected seeds often show low germination rates, delayed seedling emergence, and weakness in root and hypocotyl structures. This reduces field uniformity and makes the plant more sensitive to biotic and abiotic stress (Gebeyaw 2020). A direct consequence is the loss of seed vitality. Pathogens such as *Fusarium* spp. or *Alternaria* spp. degrade the nutrient reserves of the endosperm and damage the embryo, causing early seedling death or symptoms such as root rot and necrosis (Munkvold 2009). Some fungi generate phytotoxic toxins that negatively affect the initial metabolism of the seed. These infections can significantly reduce yield, since plants developed from infected seeds often have weaker root systems and limited growth, remaining a continuous source of infection in the field (Dell'Olmo et al. 2023; Uwineza et al. 2024). From the food safety point of view, infected seeds present a great risk because the pathogens frequently produce mycotoxins such as aflatoxins, trichothecenes, and fumonisins – substances with carcinogenic and immunosuppressive effects that pose a serious threat to public health (Desjardins et al. 2007). Despite the legal limits set by international authorities, their practical monitoring remains challenging, especially in developing countries. Recent studies have demonstrated that microbial growth, inhibition, and community stability are strongly influenced by equilibrium processes governed by environmental conditions and substrate availability. Such equilibrium-driven interactions determine the balance between microbial persistence and suppression under different treatment conditions, providing an important conceptual framework for understanding changes in seed-associated microflora (Tenno et al. 2018).

The control of seed infections and the assurance of seed quality require the use of modern methods that enable early detection and effective reduction of microbial load. One of the earliest and still widely used methods is the maceration of seeds under sterile conditions, followed by cultivation on nutrient media. This method allows the identification of microorganisms through the formation of colonies and their morphological characteristics. Although simple and low-cost, the method is time-consuming and has limitations in distinguishing morphologically similar pathogens (Agarwal and Sinclair 1997). Another approach is the use of membrane filters, where the seed extract is filtered and the trapped microorganisms are then cultured on selective media. This method increases the efficiency of detecting pathogens present in low numbers and is particularly useful for microorganisms that are not uniformly distributed within the seed (ISTA 2018). The counting of colony-forming units (CFU) remains the classical quantitative method, which provides a clear idea of the microbial load of seeds and serves as an indicator of potential risks. However, it does not distinguish between pathogenic, harmless, or beneficial microorganisms, limiting its use in precise diagnostics (Nelson 2018). Modern techniques include molecular analyses such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), which enable specific and rapid detection of certain pathogens, even at very low levels, providing results within a few hours (Selcuk et al. 2008; Pecchia et al. 2019). In recent years, high-throughput sequencing approaches, such as Illumina-based analyses, have been increasingly applied to investigate microbial community structure, growth dynamics, and inhibition mechanisms under different environmental and processing conditions, providing a deeper understanding of microbial interactions beyond culture-based methods (Kallistova et al. 2021). Based on the literature and the importance of seed microflora for plant health and food safety, the objective of this research is to analyze the presence and diversity of microorganisms in oat (*Avena sativa*) seeds. Our study focuses on identifying the main microbial groups associated with seed quality and safety, including total heterotrophic bacteria, total coliform bacteria, fecal coliforms, as well as dangerous pathogenic microorganisms such as *Salmonella* and *Shigella*. Particular attention has also been given to analyzing the presence of fungi, including yeasts and molds, which are often associated with reduced seed vitality and the production of mycotoxins. The final goal of this study is to evaluate the level of microbial load in oat seeds and the potential impact of these microorganisms on food safety. The results obtained aim to contribute to a deeper understanding of the role of seed microflora, the identification of possible sources of contamination, and the creation of a foundation for safer practices of seed storage and utilization in agriculture and the food industry. This study provides a clear overview of the hygienic-microbiological condition of *Avena sativa* seeds, aiming to strengthen preventive measures for consumer protection.

Materials and methods

Selection of plant material

For this study, oat seeds (*Avena sativa*) were used, obtained from a cereal storage facility importing grain from abroad. The selected samples were packaged and analyzed at the Microbiology Laboratory of the Department of Biology, Faculty of Mathematical and Natural Sciences, University of Prishtina, where standard microbiological analyses were performed, including the enumeration of total coliforms, fecal coliforms, *Salmonella*/*Shigella*, heterotrophic bacteria, and fungi, using selective culture media. After sampling, seed samples were stored in their original packaging at room temperature (20–22 °C), and microbiological analyses were performed within 24–48 h after sampling.

Preparation of nutrient media

For the isolation and identification of microorganisms, selective media were prepared according to international standards (ISO 4833-1:2013; Salfinger and Tortorello 2015). All media were prepared following standard formulations and sterilization procedures when required.

Heterotrophic bacteria: standard peptone–agar medium (pH 7.4 ± 0.2), sterilized in an autoclave at 121 °C for 15 minutes.

Fecal coliforms: a selective medium containing proteose peptone, tryptose, lactose, and bile salts, with methylene blue as an indicator, prepared according to standard formulations. All components were of analytical grade and used at concentrations recommended by the manufacturer.

Total coliforms: lauryl sulfate agar with basic fuchsin as an indicator.

Salmonella/Shigella: deoxycholate citrate agar, prepared without autoclaving to avoid denaturation of selective salts.

Fungi (yeasts and molds): malt extract agar, sterilized in an autoclave at 121 °C for 15 minutes, which represents a standard and sufficient sterilization procedure for fungal culture media. After preparation, all media were poured into sterile Petri dishes (Ø 6 cm and Ø 10 cm) and allowed to solidify under aseptic conditions.

Preparation of samples and isolation of microorganisms

From each sample, 10 g of dry oat seeds were weighed and transferred into a sterile Erlenmeyer flask containing 100 mL of sterilized water. After shaking for 2 h using a Startech apparatus, 10 mL of the resulting suspension was taken and serially diluted (1:9) with sterile water to a final concentration of 10^{-3}.

Alternative procedure (for microorganisms resistant to washing)

10 g of seeds were ground in a sterile porcelain mortar with 50 mL of sterilized water to obtain a homogeneous mixture (milk-like emulsion). From this extract, 10 mL was taken and diluted in the same way to 10^{-3}. No chemical surface sterilization (e.g., ethanol or sodium hypochlorite) was applied prior to maceration or grinding; therefore, this procedure does not allow discrimination between true endophytic microorganisms and tightly attached or protected surface-associated microflora.

Membrane filtration method

For colony enumeration, the membrane filtration (MF) method was applied according to Salfinger and Tortorello (2015) and EPA (2023).

Advantec filters (Ø 47 mm; pore size 0.45 µm) were used.

- From each dilution, 10 mL of suspension was filtered using a Sartorius apparatus.
- Filters were then transferred to the appropriate media and incubated under the following conditions: heterotrophic bacteria, total coliforms, fecal coliforms, *Salmonella*/*Shigella*: 37 °C for 48 h.
- Fungi (yeasts and molds): room temperature for 7 days.

After incubation, the developed colonies were counted on Petri plates, and results were expressed as colony forming units (CFU/mL or CFU/g). Counts were based on the average number of colonies from two parallel plates for each dilution.

Calculation of colony numbers

The total number of colonies was calculated according to the following equation:

$$\mathrm{TCN} = \left(\frac{\mathrm{NC} \times \mathrm{DF} \times 100}{V}\right), \tag{1}$$

where:

- NC = number of counted colonies per plate,
- DF = dilution factor,
- V = volume of the filtered sample (mL).

Results were reported as CFU per 100 mL of suspension or per 1 g of dry seed.

Microbial contamination index

To evaluate the overall contamination level of the seeds, the microbial contamination index (MCI) was calculated as the ratio of total heterotrophic colonies to the sum of all other identified microbial groups:

$$\mathrm{MCI} = \left(\frac{\text{CFU of heterotrophic bacteria}}{\text{CFU of total coliforms} + \text{CFU of fecal coliforms} + \text{CFU}\frac{Salmonella}{Shigella} + \text{CFU fungi}}\right). \tag{2}$$

This index provides a comparative indicator of the dominance of non-pathogenic microflora over potentially pathogenic microorganisms and can be used as an additional parameter to assess the microbiological quality of oat seeds.

Statistical analysis

All experimental data were analyzed using GraphPad Prism version 10. Microbiological results were expressed as colony-forming units per gram (CFU/g) and tested for normality prior to statistical comparisons. Differences in microbial counts between samples analyzed before and after maceration were evaluated using a paired t-test ($n = 3$), as the same samples were measured under both conditions. Percentage and logarithmic ($\log_{10}$) reductions were calculated to describe the efficiency of the maceration treatment for each microbial group, including total coliforms, fecal coliforms, *Salmonella*/*Shigella*, heterotrophic bacteria, and fungi.

Results were expressed as mean ± standard deviation (SD), and differences were considered statistically significant at $p < 0.05$. The MCI was calculated to assess changes in the relative proportion of non-pathogenic to potentially pathogenic microorganisms before and after maceration.

To explore potential relationships among microbial indicators, Pearson correlation coefficients (r) were calculated between the bacterial groups: total coliforms (TC), fecal coliforms (FC), *Salmonella*/*Shigella* (SS), heterotrophic bacteria (HB) and fungal counts (F). Correlation strength was interpreted as weak ($r < 0.3$), moderate ($0.3 \leq r < 0.7$), or strong ($r \geq 0.7$).

Graphical representations, including bar plots, radar charts, and comparative log-scale plots, were generated using GraphPad Prism to visualize relative changes in microbial counts, percentage reductions, and compliance with international microbiological standards (CXC 75-2015; ISO 6579-1:2017; ISO 21528-1:2017; EFSA 2022).

Results and discussion

In this study, microbiological analyses of *Avena sativa* seeds were carried out with two main objectives: first, to determine the initial microbial load before treatment, and second, to assess the effect of maceration on its reduction. Several key indicators were selected for this purpose: TC, FC (*Escherichia coli*), pathogens of the SS group, HB, and F. These indicators are recognized as the most commonly used parameters for evaluating the safety and hygiene of cereal grains according to international standards. Codex Alimentarius (hereafter Codex) and ISO specify that some microorganisms, such as *Salmonella* and *E. coli*, must be completely absent, while others, such as HB or F, are acceptable only within defined limits. Data for these indicators before and after maceration are presented in Table 1.

The results showed that the microbial load of the oat (*Avena sativa*) seeds before treatment was relatively high, particularly for FC (244 CFU/g) and SS (29 CFU/g). Both of these groups are considered critical indicators of food safety. According to international standards (Codex/ISO), *E. coli* should be completely absent in 1 g of a sample, while *Salmonella* must be absent in 25 g. This indicates that the analyzed samples may have undergone fecal contamination or exposure to pathogens during cultivation or storage.

After maceration treatment, a noticeable decrease in microbial load was observed, especially for TC, approximately 94.9% reduction, and HB, approximately 92.3% reduction. However, for FC and SS, although a decrease occurred, their levels remained above acceptable limits: FC decreased to 144 CFU/g, but the standard requires complete absence, while SS decreased to 6 CFU/g, although the standard requires complete absence in 25 g. This finding suggests that maceration alone is not sufficient to eliminate these pathogens, and additional decontamination methods are necessary.

A positive finding was the absence of fungi, both before and after maceration. This is important because cereals are often susceptible to fungal contamination and the potential production of mycotoxins. The absence of fungi suggests that the samples were stored under relatively good conditions, with limited humidity and minimal exposure time. Overall, the data clearly indicate that maceration effectively reduced the general bacterial load (TC and HB), but it was not adequate for the complete removal of critical pathogens such as FC and SS. Therefore, additional technological measures are required to meet microbiological safety standards. Alternative decontamination

Table 1. Microbiological profile of oat (*Avena sativa*) seeds before and after maceration

Code	Before (CFU/g)	After (CFU/g)	Absolute reduction	Relative reduction (%)	Ratio before/after	$\log_{10}$ reduction ($\log_{10}$ before/after)	Standard	Risk level
TC	39	2	37	94.87	19.50	1.29	$\le 10^2$ CFU/g (according to ISO 4833-1:2013)	Low
FC	244	144	100	40.98	1.69	0.23	0/1 g (according to ISO 21528-1:2017, Codex)	Elevated
SS	29	6	23	79.31	4.83	0.68	0/25 g (according to ISO 6579-1:2017, Codex)	Very high
HB	39	3	36	92.31	13.00	1.11	$\le 10^4$–10^5 CFU/g (according to ISO 4833-1:2013)	Non-pathogenic
F	0	0	0	0.00	1.00	–	$\le 10^2$–10^3 CFU/g (according to EFSA 2022)	None

TC – total coliforms, FC – fecal coliforms (*Escherichia coli*), SS – *Salmonella/Shigella*, HB – heterotrophic bacteria, F – fungi. Standards: ISO 21528-1:2017 (*Enterobacteriaceae/E. coli*), ISO 6579-1:2017 (*Salmonella* spp.), CXC 75-2015 (Codex Alimentarius), EFSA (2022). The values for TC, HB, and F are based on indicative hygienic limits for cereals and plant-based products. Differences between before and after maceration were statistically significant (paired *t*-test, $p < 0.05$–0.01). Statistical significance is indicated in Figs 1 and 2. Risk categories are used as descriptive indicators based on relative microbial load and presence of safety-relevant microorganisms and do not represent absolute or regulatory risk classifications.

approaches, such as UV irradiation, ozonation, or mild oxidative washing, have been proposed for dry cereals and could be evaluated in future studies as complementary steps to maceration for improved pathogen control. To visualize the changes before and after treatment, a two-panel figure was used: microbial counts (Fig. 1a) and the MCI (Fig. 1b).

Figure 1 illustrates the effect of maceration on the microbial profile of *Avena sativa* seeds. Figure 1a presents the absolute counts (CFU/g) for each indicator: TC, FC (*E. coli*), SS, HB, and F, while Fig. 1b summarizes the same data as the MCI, as described in the *Materials and methods* section. The corresponding numerical data are provided in Table 1. In Fig. 1a, a clear decrease is observed in TC and HB after maceration. This pattern is typical of a treatment that acts primarily on the surface: weakly attached microorganisms are removed through washing and agitation,

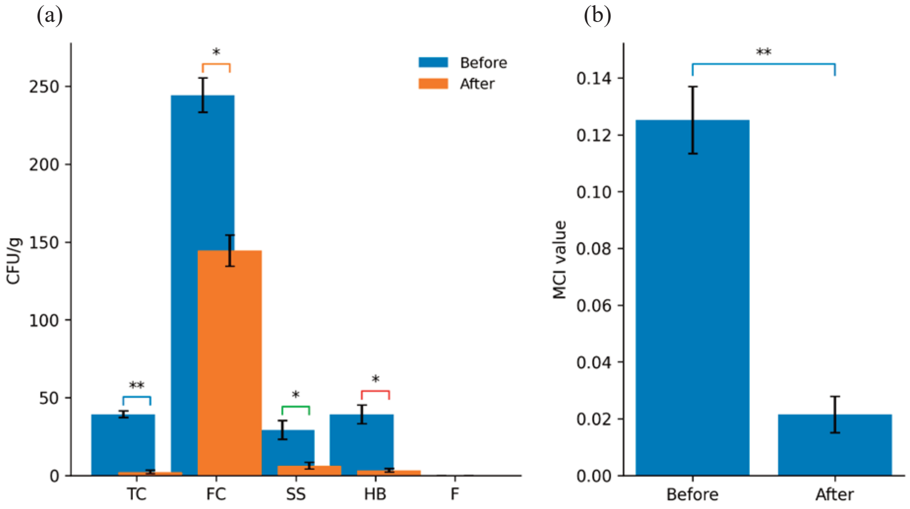

Fig. 1. Microbiological counts (CFU/g) of *Avena sativa* seeds before and after maceration (a) and the corresponding values of the microbial contamination index (MCI) before and after treatment (b). Values are expressed as mean ± SD ($n = 3$). Statistical significance between before and after maceration was assessed using a paired *t*-test ($*p < 0.05$, $**p < 0.01$). Pearson correlation analysis was applied in an exploratory manner to examine linear relationships between selected microbial indicators. The resulting correlation coefficients (*r*) did not materially affect the interpretation of the overall microbial profile, which is primarily driven by before/after comparisons, $\log_{10}$ reductions, and presence/absence criteria. Therefore, Pearson correlation is not emphasized as a central analytical outcome in this study.

resulting in a reduction of approximately 1 $\log_{10}$. This indicates a marked improvement in hygienic background, where environmental and saprophytic microflora are largely reduced without achieving sterilization.

For FC and SS, a more resistant behavior is observed. Although these indicators also decreased after treatment, they remained detectable. This suggests their localization within micro-niches on the seed coat, stronger surface adhesion, or the presence of biofilm-like structures that make them less sensitive to a single washing step. It should be emphasized that the persistence of microorganisms after maceration reflects resistance to washing and possible association with protected seed-coat micro-niches or biofilm-like structures, rather than confirmed endophytic colonization, as no chemical surface sterilization was applied prior to maceration or grinding. Since Codex and ISO regulatory criteria require complete absence of *E. coli* in 1 g and *Salmonella* in 25 g of a sample, their persistence after maceration indicates that maceration alone is insufficient to meet food safety standards. The fungal indicator F remained unchanged: no growth was detected either before or after maceration. This aligns with storage under dry and controlled humidity conditions. It should be emphasized that only colony counts were reported; mycotoxins were not analyzed, so no conclusions can be drawn regarding their potential risks.

Figure 1b (MCI) summarizes the overall picture. The index drops sharply after maceration, reflecting a general reduction in microbial load dominated by TC and HB. However, a lower MCI represents hygienic improvement, not necessarily regulatory compliance for enteric pathogens such as FC and SS. When considered together with Fig. 1a, both panels show a clear contrast: the general microbial background decreases significantly, while critical safety components remain present. Overall, Fig. 1 demonstrates that maceration functions as a preliminary step for reducing total bacterial load. To achieve the “zero tolerance” requirements for *E. coli* and *Salmonella*, an additional bactericidal or bacteriostatic step (e.g., mild heat treatment, controlled acidification, or low-dose oxidative washing) would be necessary, evaluated using the same microbiological indicators as in this study. This two-step approach background reduction through maceration followed by pathogen inactivation aligns with best practices in cereal processing and food safety standards. MCI was selected to provide a single, integrative measure of treatment effect across all the indicators (TC, FC, SS, HB, F). Equation (1) normalizes each indicator before calculation to prevent groups with higher absolute counts (e.g., TC or HB) from dominating the result. This makes before/after and between-batch comparisons more consistent and interpretable. In our data, MCI decreased markedly after maceration (Fig. 1b), consistent with reductions in TC and HB shown in Fig. 1a; however, the index did not reach zero because FC and SS remained detectable. Thus, MCI reflects hygienic improvement but not full compliance with “zero” standards for *E. coli* and *Salmonella*. Practically, this indicates that maceration can serve as a first step, while MCI helps compare and optimize subsequent decontamination processes (e.g., time/temperature, seed-to-water ratio, or alternative treatments) in future experiments.

Through Fig. 2, these dynamics are further visualized, showing both the before/after counts (bars) and the relative reduction (%) for each indicator, based on the same data as Table 1. This combined representation facilitates interpretation of treatment effects even when initial microbial loads differ across groups. The trend is consistent with Fig. 1: TC and HB exhibit the strongest reductions (~95% and ~92%), typical of surface-associated or loosely bound microflora, which are removed efficiently by washing and agitation. SS also shows a notable reduction (~79%), while FC (*E. coli*) shows a smaller decrease (~41%), indicating higher resistance to a single water-based treatment. For F, the reduction line reaches 0% because no colonies were detected either before or after treatment, thus reflecting absence, not lack of effect. The percentage line highlights a practical insight: relative reduction in TC and HB is high even when absolute counts differ between indicators. Conversely, the FC profile clearly shows the limitation of maceration as a standalone process – it lowers microbial load but does not render samples “undetectable.” This distinction is crucial for safety interpretation: improvement in background hygiene (TC/HB) does not automatically equate to compliance with *E. coli/Salmonella* safety standards. In this context, Fig. 2 reveals the heterogeneous response among microbial groups: the background indicators (TC, HB) are efficiently reduced by maceration, whereas the critical safety indicators (FC, SS) persist and require an additional step (e.g., extended maceration time, mild heat treatment, acidification, or low-dose oxidative rinse). Together, Figs 1 and 2 provide a practical framework for process optimization,

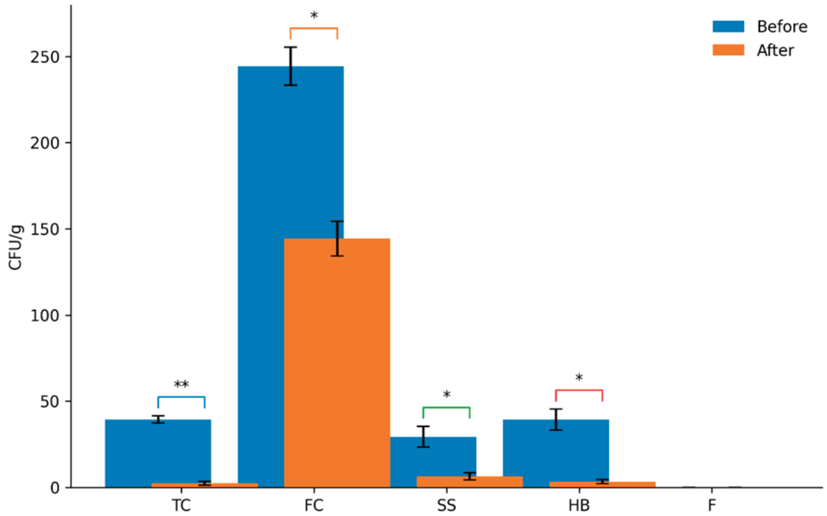

Fig. 2. Microbiological counts (CFU/g) of *Avena sativa* seeds before and after maceration. Values are expressed as mean ± SD ($n = 3$). Statistical significance between before and after maceration was assessed using a paired t-test ($*p < 0.05$, $**p < 0.01$).

showing that maceration serves as a preparatory step to reduce general microbial load, while the second phase should directly target FC and SS to achieve full “zero” compliance.

In Fig. 3, it is evident that for HB and F, our values are consistent with or below the global standards, whereas for FC and SS, there remains a significant discrepancy compared to food safety requirements. This clear contrast indicates that improvements can only be achieved through the application of additional treatment methods and by enhancing storage and transport conditions.

In this study, the microbiological results were expressed as CFU per 100 mL of *Avena sativa* seed suspension, reflecting a methodological approach based on maceration. This form of expression is important for transparency, as unlike the direct analysis of 1 g of dry material, the microbial load here was evaluated in the liquid extract obtained during treatment.

Regarding the applied standards for FC and SS, the mandatory criteria defined by Codex and ISO were applied, which require complete absence in 25 g of a sample, making the “zero tolerance” standard non-negotiable for cereal products. For HB, it should be noted that there is no universal binding international limit; therefore, a reference threshold of $\leq 10^3$ CFU/g was adopted based on scientific literature describing average microbial loads in stored cereals, thus providing a reasonable framework for data interpretation.

The absence of fungi, although not regulated by a specific CFU standard, represents a positive indicator of proper seed storage conditions, minimizing the risk of mycotoxin production.

Overall, this comparison highlights that despite the substantial reductions achieved after maceration, most microbial indicators remain above the permissible limits for cereals. This implies

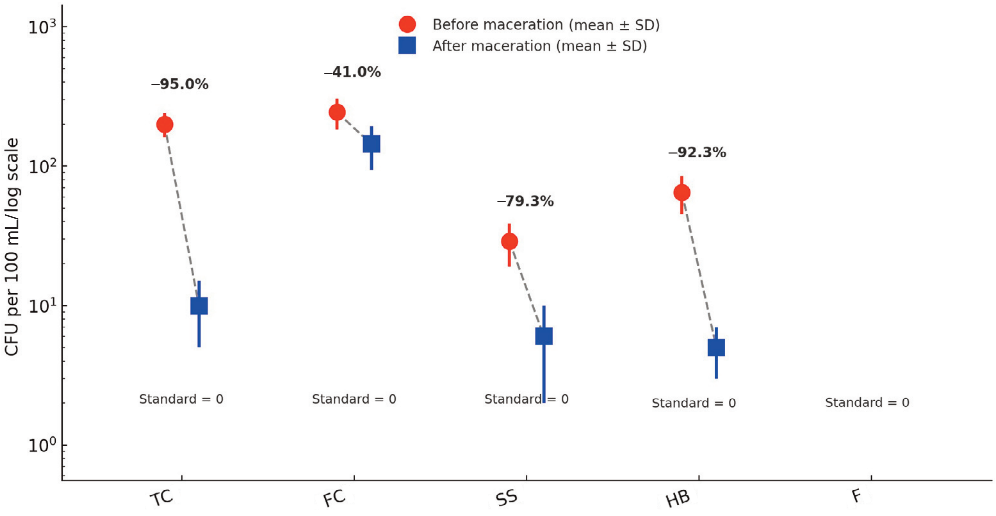

Fig. 3. Comparison of microbiological indicators before and after maceration in relation to international standards.

that the maceration process alone is insufficient to ensure food safety compliance according to international standards.

In this study, a marked reduction was observed in TC (39 → 2 CFU/g, ≈1.29 log$_{10}$) and HB (39 → 3 CFU/g, ≈1.11 log$_{10}$) after maceration. It should be noted that the relatively high microbial counts observed before maceration reflect the actual microbiological status of the analyzed market samples and indicate that the starting material may have been grossly contaminated. This profile agrees with the findings of Liu et al. (2022) and Acuff et al. (2023) for low-moisture foods, where water-based or wetting treatments mainly remove loosely attached surface microflora and typically yield approximately one log-unit reduction, without a complete bactericidal effect.

Comparable treatment-dependent patterns of microbial inhibition and selective survival have been reported in recent studies, indicating that processing steps may act as selective pressures shaping microbial communities rather than exerting a uniform inactivation effect. Nikitina et al. (2023) demonstrated that different microbial groups exhibit variable resistance and adaptive responses depending on environmental stressors and substrate interactions, which is consistent with the selective reductions observed in the present study following maceration.

Similar background reductions in microbial load have also been reported in other dry cereals. Rose et al. (2012) described that physical cleaning steps before milling in the wheat chain result in approximately a one-log reduction of the bacterial load, which is consistent with the magnitude observed here for TC and HB. Likewise, the review by Podolak et al. (2010) emphasized that, in low-moisture matrices, physical interventions lead to limited reductions on the order of 1 log CFU/g, consistent with our observations for non-pathogenic indicators. For *Avena sativa*, Anthero et al. (2019) modeled maceration as a two-phase process: a rapid capillary uptake in the outer layers followed by a slower diffusion phase toward the core. The data fit well with Peleg's model and Fick's second law, supporting the expectation that surface microflora (TC and HB) would be greatly reduced while the process would not act as a full-scale inactivation treatment. In simple terms, maceration "washes" the outer layer but does not eliminate microorganisms that are resistant to washing or associated with protected seed-coat niches.

In our data, *E. coli* decreased from 244 to 144 CFU/g (≈0.23 log$_{10}$) and *Salmonella/ Shigella* from 29 to 6 CFU/g (≈0.68 log$_{10}$); both remained detectable. This same pattern is widely described for dry matrices: Liu et al. (2022) and Acuff et al. (2023) note that enteric pathogens in low-moisture foods (LMF) (e.g., *Salmonella* spp., *E. coli*) can persist for long periods and are not eliminated by mild water-based interventions. In wheat flour, Forghani et al. (2019) demonstrated long-term survival of *Salmonella enterica* and enterohemorrhagic *E. coli* (EHEC), with thermal-death curves indicating enhanced resistance under dry conditions. Hence, the persistence observed in our samples is typical for cereals and other LMF.

International standards require the complete absence of these pathogens: ISO 6579-1:2017 mandates absence of *Salmonella* spp. in 25 g, ISO 21528-1:2017 describes detection of *Enterobacteriaceae* (with *E. coli* as fecal indicator) in enriched test portions, and CXC 75-2015 for LMF clearly frames pathogen criteria as "presence/absence" in the final product. Within this framework, the post-maceration presence of *E. coli* and/or *Salmonella/Shigella* fails to meet the "absence" requirement, consistent with previous LMF reviews. Beyond *Avena sativa*, studies on wheat flour confirm similar persistence of *Salmonella* spp. and *E. coli* after mild processing steps, for example, the studies by Forghani et al. (2019) on survival and thermal inactivation in flour and by Podolak et al. (2010) on sources and risk factors in LMF. These works reinforce the idea that approximately 1 log reductions in overall counts do not equate to pathogen absence. In practical terms, reviews of the wheat-processing chain (Rose et al. 2012) note that pre-milling physical cleaning produces visible microbial reductions but not guaranteed eliminations. In the present study, fungi were not detected in either stage (before or after maceration), indicating storage under adequately dry and non-favorable conditions. Nevertheless, EFSA (2011, 2017) reports frequent detection of T-2/HT-2 toxins (trichothecene mycotoxins) and deoxynivalenol (DON) in European oat surveys; thus, the absence of fungal colonies in culture does not automatically imply absence of mycotoxins along the production chain. The most recent EFSA (2022) update continues to discuss exposure to T-2/HT-2 in cereals, maintaining oats among matrices of ongoing concern. Most cereal-related literature reports data as CFU/g (dry matter) or as presence/absence according to ISO standards. Similarly, the interpretation in this study focuses on the before/after trend showing strong decreases in total coliforms and heterotrophic bacteria but residual presence of *E. coli* and *Salmonella*/*Shigella*. This approach is consistent with LMF reviews, which emphasize evaluating trends and presence/absence for pathogens rather than direct numerical equivalence among analytical protocols. In our data, MCI decreased sharply after maceration because total coliforms and heterotrophic bacteria were substantially reduced; however, the index did not reach its minimum value because *E. coli* and *Salmonella*/*Shigella* remained detectable. This reflects the overall condition: a clear improvement in the microbial profile but not complete compliance with key safety indicators – an interpretation fully aligned with the ISO 6579-1, ISO 21528-1, and CXC 75-2015 frameworks. While culture-based CFU analysis provides a robust initial hygiene and safety assessment, it does not capture the full complexity of seed-associated microbiota. Therefore, the present results should be interpreted within the limits of culture-dependent methods. Higher-resolution approaches, such as high-throughput sequencing (e.g., Illumina-based analyses), could complement future studies by providing deeper insight into microbial community structure, selective survival, and community dynamics under maceration and related treatments.

Conclusion

In our study, we aimed to emphasize the importance of safety during the storage and preservation of cereal seeds. As a case study, we took oat (*Avena sativa*) seeds. Through a simple "before/after" maceration protocol, we assessed the microbiological status of the packaged oat seeds on the market. The analysis before maceration showed a broad bacterial presence. Maceration was used to evaluate whether contamination was limited to surface-associated microflora or involved microorganisms resistant to washing and associated with protected seed structures. As a physical process, maceration washed the outer layers and substantially reduced the overall bacterial load but did not eliminate the enteric risk. Numerically, maceration reduced total coliforms from 39 to 2 CFU/g (≈95%, 1.29 $\log_{10}$ reduction) and heterotrophic bacteria from 39 to 3 CFU/g (≈92%, 1.11 $\log_{10}$ reduction), while fecal coliforms decreased from 244 to 144 CFU/g (≈41%) and *Salmonella*/*Shigella* from 29 to 6 CFU/g (≈79%), remaining detectable. Fecal markers and enteric pathogens remained detectable, which indicates that a single wetting displaces the "free" microorganisms from the surface, whereas the micro-niches of the seed coat and stronger adhesions remain unaffected. These remaining microorganisms should not be interpreted as confirmed endophytic microflora, as no chemical surface sterilization was applied prior to maceration or grinding. The use of seeds with this profile, without further evaluation, carries a real risk of transferring contamination to the sowing environment or to the stages of food processing. The absence of fungal growth in both phases is consistent with dry storage but does not allow a judgment on mycotoxins (they were not measured). The microbial contamination index (MCI) reflected this analysis; it decreased with the reduction of background indicators, but remained sensitive to the presence of safety indicators and does not replace presence/absence criteria. Since our study is based on the maceration protocol, comparisons with the literature should be read as profiles and trends, not as a numerical equivalence across different matrices. Essentially, maceration improves seed cleanliness by reducing surface-associated microflora but does not guarantee microbiological safety or confirm endophytic colonization. This paper reflects the real status of the samples on the market and sets the basis for further assessments of compliance with the standard criteria of pathogen presence/absence.

Data availability statement

The data sets used and/or analyzed during the current study are available from the corresponding author at reasonable request.

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Kaeraseemnete mikrobioloogiline ohutus ja leotamisprotokolli mõju

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Uuringus hinnati turul müüdavate kaeraseemnete (*Avena sativa* L.) mikrobioloogilist seisundit, et rõhutada nende ohutu käitlemise tähtsust. Kasutati enne ja pärast leotamist tehtud katsetel põhinevat tooteohutuse hindamise protokolli, et eristada viljaseemnete pinnasaastet sisemisest saastest.

Tulemused näitasid, et teraviljaseemnete leotamine vähendas märgatavalt seemnete üldist bakteriaalset saastet, kuid ei taganud täielikku tooteohutust.

Üldindikaatorid: kolilaadsete bakterite arv vähenes leotamisel u 95% ja heterotroofsete bakterite arv u 92%.

Ohutusriskid: patogeenid ja fekaalsed saasteindikaatorid jäid seemnetes endiselt tuvastatavaks – fekaalsete kolilaadsete bakterite arv vähenes 41% ja *Salmonella/Shigella* suhteline arvukus 79%.

Uuring tõestab, et teraviljaseemnete ühekordne leotamine eemaldab neilt vaid pinnal asetsevad mikroobid, kuid seemnekesta kihid kaitsevad enteraalseid patogeene teraviljas endiselt. Kuna keemilist pinnasteriliseerimist uuringus ei kasutatud, ei saa teraviljale jäänud mikroorganisme pidada kinnitunud endofüütideks ega teha sellekohaseid järeldusi mikrobioloogilise saaste kohta.

Seemnete leotamine parandab nende mikrobioloogilist puhtust, kuid ei taga mikrobioloogilise saaste suhtes täielikku ohutust. Sellise saasteprofiiliga seemnete kasutamine on riskantne nii külvikeskkonnale kui ka toiduainetööstusele. Uuring näitab jaemüügis olevate teraviljade tegelikku seisundit ja on aluseks edasistele vastavushindamistele.