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Onion Peel Extract as a Natural Antimicrobial Against Foodborne Pathogens in Kefir

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Abstract

This study investigated the antimicrobial effect of onion peel extract on Listeria monocytogenes and Bacillus cereus in kefir, as well as its impact on lactic acid bacteria (LAB). Kefir samples were artificially inoculated with different initial concentrations of L. monocytogenes (3 and 5 log CFU/mL) and B. cereus (5 and 7 log CFU/mL). Onion peel extract was applied at concentrations of 3% and 5% (v/v), followed by short-term incubation at 25 °C. Microbial populations were enumerated using selective media, and statistical analyses were performed using one-way ANOVA.

The addition of onion peel extract resulted in a significant reduction of both pathogens in kefir samples ($p < 0.05$). L. monocytogenes counts were reduced from initial levels of 3–5 log CFU/mL to 2.05–2.24 log CFU/mL following treatment with both extract concentrations. Similarly, B. cereus populations decreased from 5.02 and 7.00 log CFU/mL to ranges of 4.18–4.34 log CFU/mL and 4.05–4.48 log CFU/mL, respectively, depending on extract concentration and initial inoculum level.

In contrast, LAB populations remained high, ranging between 8.18 and 9.59 log CFU/mL in extract-treated samples, and were not significantly different from control kefir in several treatments ($p > 0.05$). No clear dose-dependent difference was observed between 3% and 5% onion peel extract. These results demonstrate that onion peel extract exhibits selective antimicrobial activity, effectively inhibiting foodborne pathogens while preserving beneficial kefir microflora, supporting its potential application as a natural preservative in fermented dairy products.

Keywords**:** onion peel extract; kefir; *Listeria monocytogenes*; *Bacillus cereus*

1. Introduction

Fermented dairy products have been consumed worldwide for centuries due to their nutritional value, extended shelf life, and health-promoting properties. Among these products, kefir is distinguished by its complex microbial ecosystem, which includes lactic acid bacteria (LAB), acetic acid bacteria, and yeasts, contributing to its probiotic potential and functional characteristics [1, 2]. The presence of LAB in kefir plays a crucial role in improving gut health, inhibiting pathogenic microorganisms, and enhancing food safety through the production of organic acids, bacteriocins, and other antimicrobial metabolites [3].

Despite its antimicrobial nature, kefir may still be susceptible to contamination by foodborne pathogens during production, handling, or storage. *Bacillus cereus* is of particular concern in dairy products due to its spore-forming ability, resistance to adverse environmental conditions, and capacity to produce toxins that cause food poisoning [4]. The survival and growth of *L.monocytogenes* and *B. cereus* in fermented foods pose a

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significant challenge to food safety, especially when contamination occurs post-fermentation [5].

In recent years, increasing consumer demand for minimally processed foods with clean labels has encouraged the food industry to seek natural alternatives to synthetic preservatives. Plant-derived extracts rich in phenolic compounds have attracted considerable attention due to their antimicrobial and antioxidant properties [6]. Among these, onion (*Allium cepa L.*) and its by-products have been widely studied as sources of bioactive compounds, particularly flavonoids such as quercetin, which exhibit strong antibacterial activity against Gram-positive and Gram-negative bacteria [7, 8].

Onion peel, a major by-product of onion processing, is often discarded as waste despite its high concentration of phenolic compounds compared to edible onion tissues. Several studies have demonstrated that onion peel extracts possess significant antimicrobial activity against foodborne pathogens, including *B. cereus, Listeria monocytogenes*, and *Staphylococcus aureus* [9, 10]. The valorization of onion peel not only contributes to sustainable food processing but also provides an opportunity to develop natural antimicrobial agents for food preservation.

However, an important consideration in the application of plant extracts in fermented foods is their potential impact on beneficial microorganisms. While many plant-derived antimicrobials effectively inhibit pathogens, they may also negatively affect probiotic or starter cultures, thereby compromising the functional quality of fermented products [11]. Therefore, it is essential to evaluate both the antimicrobial efficacy against pathogens and the compatibility of these extracts with LAB populations.

In this context, the present study aimed to investigate the effect of onion peel extract at different concentrations on *L.monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 11778 and lactic acid bacteria in kefir artificially contaminated with varying levels of the pathogen. By assessing the selective antimicrobial activity of onion peel extract, this study seeks to contribute to the development of natural, sustainable, and consumer-friendly preservation strategies for fermented dairy products.

2. Materials and methods

2.1. Materials

Commercial plain kefir was obtained from a local market in Konya, Türkiye and stored at 4±1°C until use. *Listeria monocytogenes* ATCC 7644 and *Bacillus cereus* ATCC 11778 (foodborne reference strains) was obtained from a culture collection and maintained at −18°C in glycerol stocks until analysis. Onion peels (*Allium cepa L.*) were collected from fresh onions, washed thoroughly with distilled water, and air-dried prior to extraction. All microbiological media, including Oxford agar, Mannitol Egg Yolk Polymyxin (MYP) agar, and de Man–Rogosa–Sharpe (MRS) agar, were purchased from a commercial supplier and prepared according to the manufacturer's instructions.

2.2. Methods

2.2.1. Preparation of onion peel extract

Dry peels separated from yellow onions were cleaned up by washing with tap water. A 3 g and 5 g of peel was boiled in 100 mL water (3% and 5%, v/v), separetely for 10 minutes. The mixture was cooled down to a 25ºC of temperature and then subjected to a filtration through a Whatman no: 1 paper. A 10 mL of kefır was added with 0.0 and 1 mL of extract (1 mL) [12].

2.2.2. *Preparation of bacterial inocula*

Listeria monocytogenes and *Bacillus cereus* were activated by transferring a loopful of culture into nutrient broth and incubating at 37°C for 18–24 h. *L.monocytogenes* was adjusted to a concentration of 3 log cfu/mL and 5 log cfu/mL; *B.cereus* was adjusted to a concentration of 5 log cfu/mL and 7 log cfu/mL.

2.2.3. Experimental design and kefir inoculation

For each experimental group, 10 mL of kefir was aseptically transferred into sterile tubes. Kefir samples were separately inoculated with 1 mL of *L.monocytogenes* suspension at either 3 or 5 log CFU/mL. The inoculated samples were incubated at 25 °C for 20 min to allow bacterial adaptation.

Following this initial incubation, 1 mL of onion peel extract at either 3% or 5% concentration was added to the respective samples. The mixtures were gently homogenized and further incubated at 25°C for 40 min. Control samples included kefir without pathogen inoculation and kefir

inoculated with *L.monocytoegenes* but without onion peel extract.

The same procedures were repeated for *B.cereus* at concentration of 5 or 7 log CFU/mL.

The experimental groups were defined as follows, K; kefir control, K-3-L; kefir + 3 log CFU/mL *L.monocytogenes*, K-5-L; kefir + 5 log CFU/mL *L.monocytogenes*, K-3-L-3S; kefir + 3 log CFU/mL *L.monocytogenes* + 3% onion peel extract, K-3-L-5S; kefir + 3 log CFU/mL *L.monocytogenes* + 5% onion peel extract, K-5-L-3S; kefir + 5 log CFU/mL *L. monocytogenes* + 3% onion peel extract, K-5-L-5S; kefir + 5 log CFU/mL *L. monocytogenes* + 5 % onion peel extract; K-5-B; kefir + 5 log CFU/mL *B.cereus*, K-7-B; kefir + 7 log CFU/mL *B.cereus*, K-5-B-3S; kefir + 5 log CFU/mL *B.cereus* + 3% onion peel extract, K-5-B-5S; kefir + 5 log CFU/mL *B.cereus* + 5% onion peel extract, K-7-B-3S; kefir + 7 log CFU/mL *B.cereus* + 3% onion peel extract, K-7-B-5S; kefir + 7 log CFU/mL *B.cereus* + 5% onion peel extract

2.2.4. Microbiological analysis

At the end of the incubation period, serial decimal dilutions were prepared using sterile peptone water. *L.monocytogenes* and *Bacillus cereus* enumeration were performed by spread-plating appropriate dilutions onto Oxfor agar and MYP agar, respectively, followed by incubation at 37 °C for 24–48 h. Presumptive *L.monocytogenes* and *B. cereus* colonies were counted and expressed as log CFU/mL.

Lactic acid bacteria (LAB) were enumerated by plating suitable dilutions onto MRS agar and incubating at 37°C for 48 h under anaerobic conditions. Colony counts were recorded and expressed as log CFU/mL.

2.2.5. Statistical analysis

All experiments were conducted in triplicate (n = 3). Results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. When significant differences were detected, Tukey's multiple comparison test was applied. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussions

Figure 1 presents the effects of onion peel extract at 3% and 5% concentrations on *Listeria monocytogenes* and lactic acid bacteria (LAB) populations in kefir samples inoculated with different initial pathogen loads. The results clearly demonstrate that onion peel extract exhibits a selective antimicrobial effect, significantly reducing *L. monocytogenes* counts while maintaining or even enhancing LAB populations.

In the control kefir sample (K), LAB levels were high (9.62 ± 0.01 log CFU/mL), reflecting the natural microbiological richness of kefir, while *L. monocytogenes* was not detected. Upon inoculation with *L. monocytogenes* at 3 and 5 log CFU/mL (K-3-L and K-5-L), a statistically significant decrease in LAB counts was observed ($p < 0.05$), accompanied by the expected increase in pathogen load.

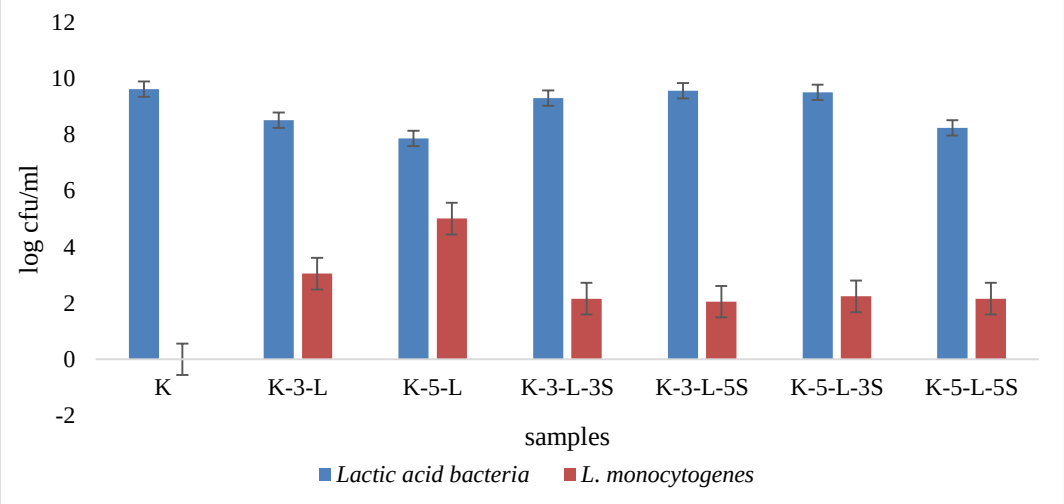

Figure 1. Effects of onion peel extract on *Listeria monocytogenes* and lactic acid bacteria (LAB)

This inverse relationship suggests competitive interactions between kefir microbiota and the pathogen, consistent with previous findings on pathogen-induced stress in fermented dairy ecosystems [1, 2].

The addition of onion peel extract significantly altered this dynamic. In all extract-treated groups, *L. monocytogenes* counts were reduced by approximately 0.8–3 log units compared to their respective untreated counterparts. Notably, both 3% and 5% onion peel extract treatments resulted in *L. monocytogenes* counts clustering around ~2.1–2.2 log CFU/mL, regardless of the initial inoculum level. This indicates a strong bacteriostatic or bactericidal effect that is relatively independent of initial pathogen concentration.

Simultaneously, LAB populations were restored to levels comparable to, or higher than, the control group. For example, LAB counts in K-3-L-5S (9.56 ± 0.06 log CFU/mL) and K-5-L-3S (9.50 ± 0.04 log CFU/mL) were statistically similar to the control kefir. These findings suggest that onion peel extract does not exert inhibitory effects on beneficial kefir microbiota; on the contrary, it may exert a protective or prebiotic-like effect.

This selective antimicrobial activity is in agreement with earlier studies reporting that onion peel is rich in flavonoids, particularly quercetin and its derivatives, which disrupt pathogenic bacterial cell membranes while having limited impact on LAB [13, 14].

Similar outcomes were reported by Dini *et al*. [15], who demonstrated that onion-derived phenolic compounds effectively inhibited *L. monocytogenes* without suppressing probiotic strains.

The absence of a clear dose-dependent difference between 3% and 5% extract concentrations suggests that 3% onion peel extract may be sufficient to achieve antimicrobial efficacy, which is advantageous from both technological and sensory perspectives. Comparable saturation effects have been observed in studies evaluating plant-based antimicrobials in fermented dairy products [16].

Figure 2 presents the effect of onion peel extract at two different concentrations (3% and 5%) on *Bacillus cereus* and lactic acid bacteria (LAB) populations in kefir samples artificially contaminated with different initial pathogen loads. The results demonstrate that onion peel extract exhibits a significant antimicrobial effect against *Bacillus cereus* while largely preserving the viability of beneficial lactic acid bacteria.

In the control kefir sample (K), LAB counts were high (9.61 log CFU/mL), confirming the rich probiotic microflora characteristic of kefir, while *B. cereus* was not detected. In samples inoculated with *B. cereus* at 5 and 7 log CFU/mL (K-5-B and K-7-B), pathogen counts increased as expected, reaching 5.02 and 7.00 log CFU/mL, respectively.

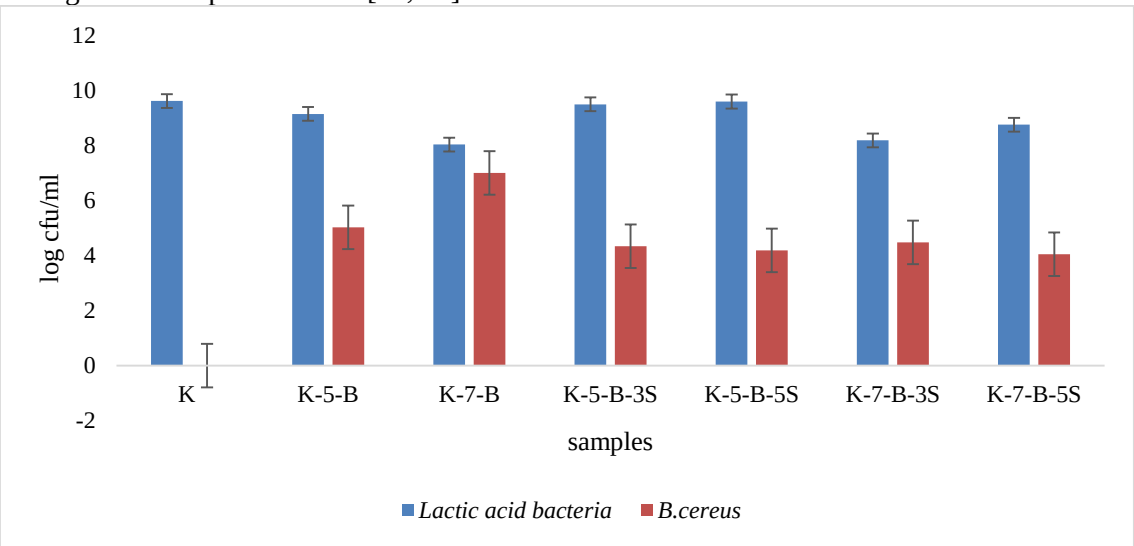

Figure 2. Effects of onion peel extract on *B.cereus* and lactic acid bacteria (LAB)

Concurrently, LAB populations decreased significantly ($p < 0.05$), suggesting a competitive or inhibitory interaction between *B. cereus* and the native kefir microflora. Similar reductions in LAB viability in the presence of foodborne pathogens have been reported previously in fermented dairy systems [7].

The addition of onion peel extract resulted in a marked reduction in *B. cereus* counts across all treated groups. In the K-5-B samples, *B. cereus* levels decreased from 5.02 log CFU/mL to 4.34 and 4.18 log CFU/mL following treatment with 3% and 5% onion peel extract, respectively. A comparable trend was observed in samples inoculated with 7 log CFU/mL *B. cereus*, where pathogen levels were reduced to approximately 4.05–4.48 log CFU/mL. These reductions were statistically significant ($p < 0.05$) and indicate that onion peel extract exerts a dose-dependent antimicrobial activity.

The antimicrobial efficacy of onion peel extract can be attributed to its high content of phenolic compounds, particularly quercetin and its derivatives, which are known to disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with nucleic acid synthesis [9, 18]. Previous studies have reported similar inhibitory effects of onion by-product extracts against *B. cereus* and other Gram-positive pathogens in food matrices [7, 10], supporting the findings of the present study.

Importantly, LAB counts in onion peel extract-treated samples remained relatively high, ranging between 8.18 and 9.59 log CFU/mL. In several cases (K-5-B-3S and K-5-B-5S), LAB populations were not significantly different from the control group ($p > 0.05$). This selective antimicrobial behavior is particularly advantageous for fermented foods, as it indicates that onion peel extract can suppress pathogenic bacteria without adversely affecting beneficial starter or probiotic cultures. Similar selective effects have been observed for plant-derived phenolic extracts in fermented dairy products [11, 19].

4. Conclusion

The present study demonstrated that onion peel extract exhibits a pronounced and selective antimicrobial effect against *Listeria monocytogenes* and *Bacillus cereus* in kefir, while preserving the viability of lactic acid bacteria. The results clearly indicate that both 3% and 5% concentrations of onion peel extract significantly reduced pathogen loads in artificially contaminated kefir samples, regardless of the initial inoculum level. In contrast, LAB populations remained stable or were restored to levels comparable to the control group, highlighting the compatibility of the extract with the native kefir microbiota.

The antimicrobial activity observed against *L. monocytogenes* and *B. cereus* can be attributed to the high phenolic content of onion peel, particularly flavonoids such as quercetin and its derivatives, which are known to interfere with bacterial cell membranes and metabolic processes. Importantly, the absence of a pronounced dose-dependent difference between 3% and 5% extract concentrations suggests that lower concentrations may be sufficient to achieve microbial safety, offering advantages in terms of cost, sensory acceptance, and technological applicability.

From a food safety perspective, the ability of onion peel extract to suppress pathogenic bacteria without compromising beneficial lactic acid bacteria is particularly significant for fermented dairy products such as kefir, where probiotic viability is a key quality attribute. These findings support the potential use of onion peel extract as a natural antimicrobial ingredient aligned with clean-label and sustainable food production trends.

In addition to its antimicrobial efficacy, the valorization of onion peel as a functional food ingredient contributes to waste reduction and circular economy approaches in the food industry. Overall, this study provides experimental evidence that onion peel extract can serve as an effective and selective natural preservative in fermented dairy systems. Further studies focusing on sensory properties, shelf-life extension, and the identification of specific bioactive compounds are recommended to facilitate its industrial application.

Compliance with ethics requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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