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Survival of Major Food Pathogens in Natural Zeolite (Clinoptilolite) at Different Ratios and in Chicken Wings After Dipping

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Abstract: The aim of this study was to determine the viability of *Salmonella* Typhimurium and *Listeria monocytogenes* in solutions prepared with readily available natural zeolite and in chicken wings decontaminated with these solutions. To determine the effect of zeolite on pathogen viability, solutions of different concentrations (5%, 10%, 25%) were prepared and contaminated. Their numbers were then determined at different times (2, 6, and 24 hours) during storage at 4 °C. To determine the effect of zeolite on the viability of pathogens in chicken wings, contaminated chicken wings were immersed in zeolite solutions prepared at three different concentrations (5%, 10%, 25%) for two different times (1.5 min, 3 min) and their numbers were determined. According to the results of this study, the number of *S.* Typhimurium decreased by approximately 2.5 $\log_{10}$, and the number of *L. monocytogenes* decreased by approximately 1.4 $\log_{10}$ in zeolite solutions. The number of pathogens was significantly reduced in decontaminated chicken wings ($P \leq 0.05$). In addition, increasing the concentration of zeolite and changing the time had a significant effect on the number of *S.* Typhimurium ($P \leq 0.05$). In conclusion, zeolite was found to be antimicrobial against *S.* Typhimurium and *L. monocytogenes* and has the potential to be used in the decontamination of poultry meat. It is envisaged that zeolite may be a natural alternative to ensure food safety in the near future. To this end, zeolite should be extensively investigated in other potential food applications.

Keywords: *Chicken wings, Clinoptilolite, Listeria monocytogenes, Salmonella Typhimurium, Zeolite.*

Farklı Oranlardaki Doğal Zeolitde (Klinoptilolit) ve Daldırma Sonrası Tavuk Kanatlarda Önemli Gıda Patojenlerin Yaşam Kabiliyetleri

Özet: Bu çalışmanın amacı kolaylıkla temin edilebilen doğal zeolit ile hazırlanmış solüsyonlarda ve bu solüsyonlar ile dekontamine edilmiş tavuk kanatlarında *Salmonella* Typhimurium ve *Listeria monocytogenes'in* yaşam kabiliyetlerini belirlemektir. Zeolitin, patojenlerin yaşam kabiliyeti üzerine etkisini belirlemek için farklı konsantrasyonlarda (%5, %10, %25) solüsyonlar hazırlanıp kontamine edildi. Daha sonra 4 °C'de muhafaza boyunca farklı sürelerde (2., 6., 24. saat) sayıları belirlendi. Zeolitin, tavuk kanatlarında patojenlerin yaşam kabiliyetine etkisinin belirlemesinde ise kontamine edilmiş tavuk kanatları üç farklı konsantrasyonda (%5, %10, %25) hazırlanan zeolit solüsyonlarına iki farklı sürede (1,5 ve 3 dk) daldırma işlemi yapılıp sayısı belirlendi. Bu çalışmanın sonuçlarına göre, zeolit solüsyonlarında *S.* Typhimurium sayısı yaklaşık 2,5 $\log_{10}$ azaldığını, *L. monocytogenes* sayısında ise yaklaşık 1,4 $\log_{10}$ azaldığı saptandı. Dekontamine edilmiş tavuk kanatlarında patojenlerin sayısı önemli ölçüde azaldığı saptandı ($P \leq 0,05$). Ayrıca zeolit konsantrasyonunun arttırılması ve sürenin değişimi *S.* Typhimurium sayısı üzerinde önemli etkisinin olduğu tespit edildi ($P \leq 0,05$). Sonuç olarak zeolitin *S.* Typhimurium ve *L. monocytogenes'*e karşı antimikrobiyal etkisinin olduğu ve kanatlı etlerinin dekontaminasyonunda kullanım potansiyeli olduğu ortaya konuldu. Yakın gelecekte gıda güvenliğinin sağlanması için zeolit doğal bir alternatif olabileceği öngürülmektedir. Bunun için zeolitin diğer potansiyel gıda uygulamaları içinde kapsamlı bir şekilde araştırılması gerekmektedir.

Anahtar Kelimeler: *Klinoptilolit, Listeria monocytogenes, Salmonella Typhimurium, Tavuk kanat, Zeolit.*

Introduction

Poultry meat has a very important place in human nutrition due to its protein content, which is rich in essential amino acids, B-complex vitamins and unsaturated fatty acids, as well as low fat and cholesterol content (Güngören et al., 2023; Keykhosravy et al., 2020; Mehdizadeh and Langroodi, 2019). However, contaminated poultry meat can deteriorate rapidly due to the creation of a favourable environment for microbial growth, such as water activity and high pH (Silva et al., 2018). Therefore, the presence of possible pathogens and spoilage microorganisms in these products causes health problems and economic losses in the poultry industry (İncili et al., 2020). Poultry meat and meat products appear to be responsible for a significant proportion of foodborne illness caused by these pathogens, and the use of antimicrobials is therefore important to reduce the risk of these pathogens and protect human health. Many methods have been tried to improve the microbial and chemical quality of poultry meat and meat products. A wide range of decontamination methods are available for chemical decontamination in the meat and meat products industry (Özbay and Sarıçoban, 2014). However, as these products still pose a risk in terms of shelf life and public health, interest in natural preservatives and additives has recently increased (Aydemir and Arslan, 2023).

Zeolites, commonly found in nature, are microporous crystalline aluminosilicates consisting of AlO_4 and SiO_4 tetrahedral units and are used in various industries (Huwei et al., 2021). Zeolites are edible, biocompatible and most likely non-toxic substances, but they have several special properties, such as molecular sieve structure, ionic exchangeability and water absorbency. These properties allow them to be used in various applications in different fields. (Hecht et al., 2011; Papaioannou et al., 2002).

Zeolites (cinoptilolite) have been used as additives in food and feed for several years due to their ability to adsorb toxins produced by moulds and parasites, fungal mycotoxins, ammonium ions and heavy metals (Deshmukh et al., 2023; Singh and Kumar, 2023; Tzia and Zorpas, 2012; Villa et al., 2022). In addition, the use of zeolites in food packaging materials can prevent spoilage reactions by selectively absorbing oxygen, thereby increasing the shelf life of the product (Kombaya-Touckia-Linin et al., 2019; Lu et al., 2017). They can also be used for odour absorption in the packaging of food products that are sensitive to odours and generate bad odours, or absorb odours from the environment (Sharma et al., 2023). Another important feature of zeolites, their antimicrobial effect that prevents the growth of harmful microorganisms, has been studied by various researchers (Janićijević et al., 2020; Prabhu and Devaraju, 2018; Sánchez et al., 2017; Soysal et al., 2015; Tunç and Duman, 2011). This demonstrates the potential of zeolites as antimicrobial agents.

The potential food applications of zeolites are that they are classified as generally recognised as safe (GRAS) substances by the United States Food and Drug Administration (USFDA, 2015) and the European Food Safety Authority (EFSA, 2011). These substances, which have been approved by organisations, have been widely researched for medical applications or environmental remediation, but have been less studied and researched for food applications. (Eroğlu et al., 2017 ; Lopes et al., 2021). Therefore, it was concluded that more research on zeolites is needed in various fields related to food science and technology.

The aim of this study was to determine the viability of *Salmonella* Typhimurium and *Listeria monocytogenes* in solutions prepared with readily available natural zeolite and in chicken wings decontaminated with these solutions.

Materials and Methods

Ethics Committee: This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees.

Preparation of inoculum: For the preparation of the inoculum, reference strains of *Salmonella* Typhimurium (NCTC 74, 12416, and ATCC 14028) and *Listeria monocytogenes* (N 7144, RSKK 474, and 476) were employed. The method used by İncili et al. (2020) was applied. An inoculum of approximately 6.0 $\log_{10}$/mL was used for the survival assay of pathogens in zeolite and for chicken wing samples.

Zeolite supply and preparation: Zeolite (Nanokar, Türkiye) was supplied by purchase. Zeolite was prepared at 5%, 10% and 25% concentrations. Sterile distilled water was used to prepare different zeolite concentrations and mixed until the zeolite dissolved.

Preparation of chicken wings: On the day of the experiment, fresh chicken wings were purchased from a local market in Şanlıurfa and brought to the laboratory in the cold chain, and the experiments were performed as soon as possible. To all samples, 0.5 mL of diluted bacterial cocktail was added by spreading it on all surfaces of the samples and allowed to adhere for 15 minutes at room temperature. The samples were then randomly selected and divided into ten groups: control (no treatment), three different zeolite concentrations (5%, 10% and 25%) and two different immersion times (1, 5 and 3 min). The groups were marinated by the immersion method. To obtain the concentrations of zeolite used in the study, the zeolite was diluted with sterile distilled water and shaken well to mix it. The ratio of immersion liquid to meat sample was 2:1 and immersion procedures were performed in sterile glass jars.

Pathogens survival experiment in zeolite: To evaluate the antibacterial effect of zeolite, approximately 6.0 $\log_{10}$/mL *Salmonella* Typhimurium and *Listeria monocytogenes* were added to three different zeolite concentrations (5%, 10% and 25%). The number of pathogens was determined immediately after inoculation and after 2, 6 and 24 hours of incubation at 4 °C. The experiment was performed in triplicate.

Microbiological analyses: Each marinated meat sample (25 ± 1 g) was collected under aseptic conditions and transferred to sterile sampling bags. Next, 225 mL of 0.1%

peptone water (PW) was added to the sampling bags, and the mixture was homogenized using a stomacher (BagMixer Interscience, France) for 3 minutes. For the detection of *L. monocytogenes*, Oxford agar (Biokar, France) was used, while xylose-lysine-deoxycholate agar (XLD agar) (Biokar, France) was employed for *S.* Typhimurium. The XLD and Oxford plates were incubated at 37±1 °C for 24 hours, and the number of colonies with specific morphology was recorded.

pH analyses: The pH of the chicken wing samples was measured using a pH meter (HI 11310, Hanna Instruments, USA). The fluid (rinse fluid) remaining in the sample bags after microbiological analysis of the chicken wing samples was used for pH analyses.

Statistical analyses: Microbial counts and pH values of the samples were subjected to statistical analysis. Microbiological data were logarithmically transformed for statistical analysis. The general linear model (GLM) was used for statistical analysis. In the GLM procedure, zeolite concentrations (5%, 10% and 25%) and immersion times (1, 5 and 3 min) were considered as fixed effects and replications as random effects. Multiple comparisons were made using the Tukey test (P≤0.05). In this study, all data were obtained from three independent replicates and results are presented as mean ± standard error of the mean.

Results

pH value: The average pH values of chicken wings samples at 4 °C are depicted in Table 1. There was no difference between the groups in terms of pH (P≥ 0.05).

Table 1. pH values of chicken wings (Mean ± SE).

Concentration	Time	pH
Control		6.67±0.28
5%	1.5 min.	6.63±0.01
	3 min.	6.44±0.06
10%	1.5 min.	6.61±0.07
	3 min.	6.53±0.07
25%	1.5 min.	6.53±0.12
	3 min.	6.58±0.02
Statistics	C	$P \geq 0.05$
	T	$P \geq 0.05$
	CxT	$P \geq 0.05$

C: Concentration, **T:**Time

Pathogens survival experiment at zeolite concentrations: The number of *S.* Typhimurium decreased by approximately 2.5 $\log_{10}$ (Figure 1), while the number of *L. monocytogenes* decreased by approximately 1.4 $\log_{10}$ (Figure 2). Although increasing the zeolite concentration had an insignificant effect on reducing the number of bacteria (P ≥ 0.05), time had a significant effect on the number of *S.* Typhimurium (P ≤ 0.05).

Survival of pathogens in chicken wings after zeolite decontamination: Compared to the control group, the number of *S.* Typhimurium was significantly reduced in the samples of chicken wings immersed in zeolite (Figure 3). The highest decrease was observed at 10% and 25% concentrations after 3 min. *S.* Typhimurium counts decreased by 0.86, 1.20 and 1.28 $\log_{10}$ after 3 min. decontamination at 5%, 10% and 25% concentrations, respectively. Concentration time interaction was not significant for *S.* Typhimurium counts. Compared to the control group, the number of *L. monocytogenes* was significantly reduced in the samples of chicken wings immersed in zeolite (Figure 4). *L. monocytogenes* counts decreased by 1.31, 1.46 and 1.59 $\log_{10}$ after 3 min. decontamination at 5%, 10% and 25% concentrations, respectively. Concentration time interaction was not significant for *L. monocytogenes* counts.

Discussion

The unique structural properties of zeolite provide them with adsorptive, ion exchange and molecular sieving properties (Dikić, 2021). Due to their existing properties, they can have an antimicrobial effect. In the present study, zeolite was found to significantly reduce the number of *S.* Typhimurium and *L. monocytogenes* in both the survival experiment and chicken wing meat (P≤0.05). There is previous evidence that zeolite can be used as an antimicrobial agent (Uchida et al., 1992; Mallek, 2012; Pajnik et al., 2020). Although zeolite has been used as an antimicrobial agent, to the best of our knowledge, no research on the decontamination of pathogens in chicken meat has been found (Villa et al., 2022). Mallek et al. (2012) reported that the addition of zeolite (0.5 or 1% a/a) to chicken diets resulted in a significant (P≤0.05) reduction in total culturable microbial levels and also resulted in improved organoleptic quality of meat.

The results of the present study showed that zeolite exhibited antimicrobial activity against Gram-positive (*L. monocytogenes*) and Gram-negative (*S.* Typhimurium) bacteria. The high ion exchange capacity of zeolite enhanced this antimicrobial property. It has been recommended that zeolite should be combined with ions such as zinc oxide (ZnO) ions, and silver (Ag +) ions for a higher antimicrobial effect (Dutta and Wang, 2019; Wang et al., 2019). In particular, Ag + doped zeolite systems have been reported to exhibit broad-spectrum antimicrobial activity against both Gram-negative (*Escherichia coli* and *S.* Typhimurium) and positive (*L. monocytogenes*, Staphylococcus aureus) bacteria (Janićijević et al., 2020; Sánchez et al., 2017).

It is known that S. Typhimurium can grow optimally at a pH range of 6.5-7.5 and can survive between pH 4.5 and 9.0, while L. monocytogenes is highly resistant to low temperature and pH (İncili et al., 2021). The pH values of the zeolite concentrations used in the study are at values where bacteria can survive (Table 1). Therefore, we believe that pH has no effect on the decrease in bacterial numbers. Its believe that the main reduction in bacterial numbers is due to the high ion exchange and absorption properties of the zeolite This is because it has been reported that clinoptilolite can adsorb bacteria and thus cause a decrease in bacterial numbers (Prasai et al., 2017). It is emphasised that the rough

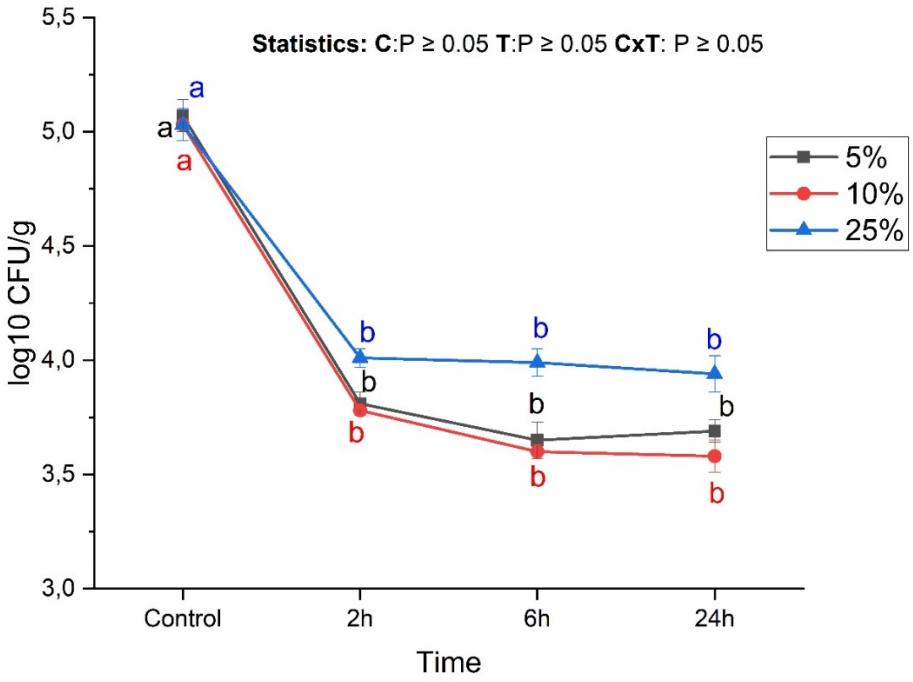

Figure 1. Survival of *Listeria monocytogenes* in zeolite at 4 °C for 24 hours (log10 CFU/g±SE). [a-b]: The mean values with different letters among the sampling hour are significantly different (P≤0.05). **C:** Concentration, **T:** Time.

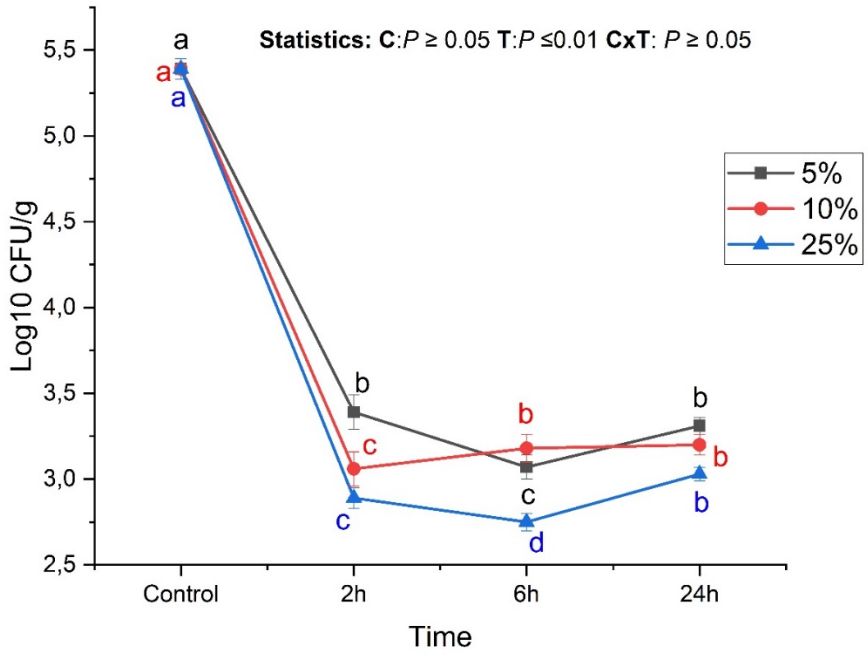

Figure 2. Survival of *Salmonella* Typhimurium in zeolite at 4 °C for 24 hours (log10 CFU/g±SE). [a-d]: The mean values with different letters among the sampling hour are significantly different (P≤0.05). **C:** Concentration, **T:**Time.

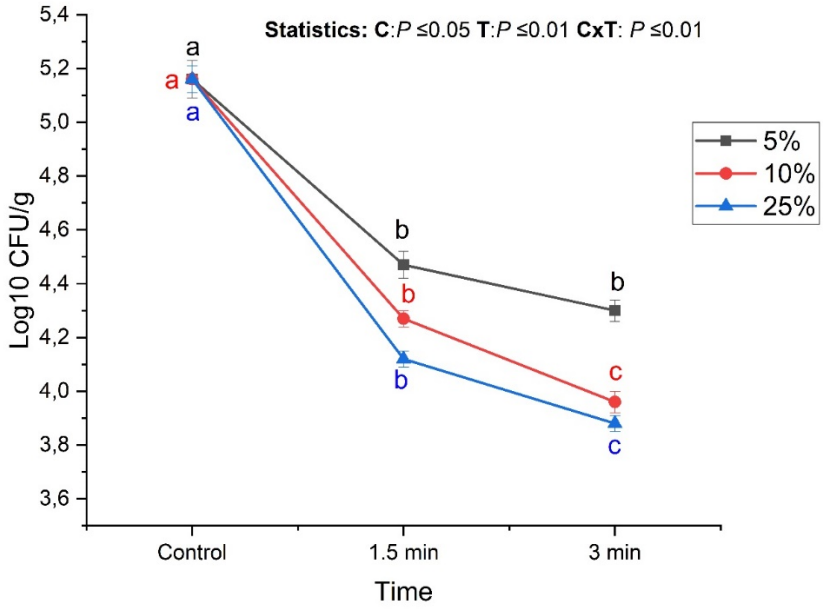

Figure 3. Mean *Salmonella* Typhimurium counts (log10 CFU/g±SE) in chicken wings at different zeolite concentrations at different times. a-c: The mean values with different letters among the sampling hour are significantly different (P≤0.05). **C:** Concentration, **T:**Time.

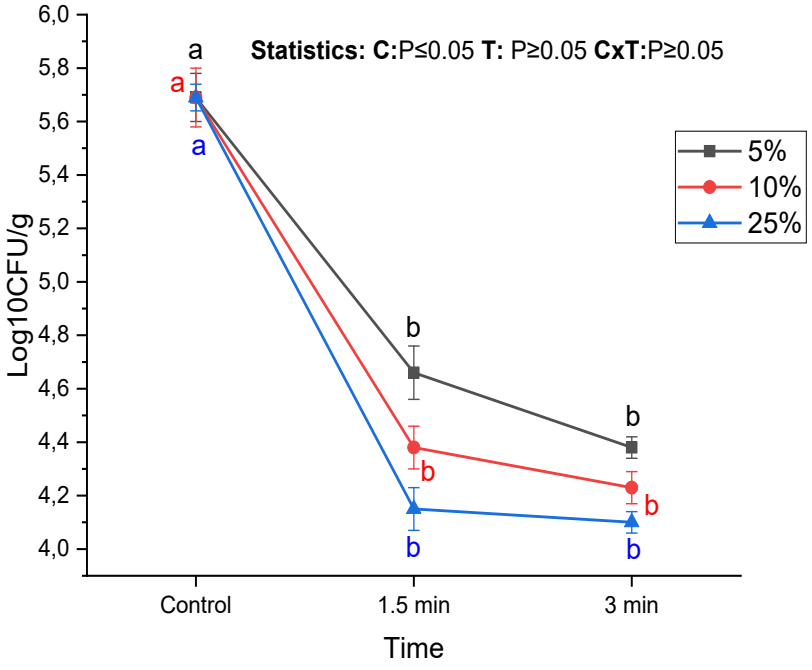

Figure 4. Mean *Listeria monocytogenes* counts (log10 CFU/g±SE) in chicken wings at different zeolite concentrations at different times. a-c: The mean values with different letters among the sampling hour are significantly different (P≤0.05). **C:** Concentration, **T:**Time.

surfaces of clinoptilolite particles provide a better microenvironment for the adsorption of bacteria (Hrenovic et al., 2005). An in vitro study by Wu et al. (2013) reported that *Escherichia coli* and *S.* Typhimurium were adsorbed by clinoptilolite (Wu et al., 2013). In addition, we believe that the different levels of *S.* Typhimurium and *L. monocytogenes* bacteria in zeolite solutions are due to the different effects of zeolite on gram-negative and gram-positive bacteria and,

most importantly, the different adsorption properties of zeolite for each bacterium. In fact, clinoptilolite has been reported to adsorb selectively on bacterial species (Prasai et al., 2017).

In zeolite solutions, the number of *S.* Typhimurium decreased by 2.5 $\log_{10}$ (Figure 1) and the number of *L. monocytogenes* decreased by approximately 1.4 $\log_{10}$ (Figure 2). For chicken wing decontamination, the numbers of *S.* Typhimurium and *L. monocytogenes* decreased by 1.28 (Figure 3) and 1.59 $\log_{10}$ (Figure 4), respectively. The greater reduction in *S.* Typhimurium in the zeolite solution can be explained by the fact that bacteria firmly attached to the wing meat can be protected from the effects of antibacterial agents (İncili et al., 2020). The opposite situation for *L. monocytogenes* can be explained by the fact that the bacteria cannot fully adhere to the wing meat after contamination and also that the bacteria enter the stationary phase in the zeolite solution.

In the zeolite solution, the number of *S.* Typhimurium decreased significantly after 2 and 6 hours, but not after 24 hours (Figure 2), while the number of *L. monocytogenes* decreased significantly after 2 hours, but not after 24 hours. This may be explained by the fact that bacteria develop resistance to the antimicrobial mechanisms of zeolite after a certain period of time. Indeed, Wang et al. (2019) showed that environmental conditions such as exposure to acids can harm bacteria. However, bacteria that are not fatally injured can enter the stationary phase and/or regain the ability to regrow. Bacteria have also been found to be highly adaptable to environmental conditions over time (Chung et al., 2018). In chicken wings decontaminated with zeolite solution, *S*. Typhimurium continued to decrease at 10% and 25% concentrations with time (Figure 3). However, the interaction of concentration and time was not effective for *L. monocytogenes* (Figure 4). This may be explained by the slowing of growth of *L. monocytogenes* against antimicrobial mechanisms and entry into stationary phase (İncili et al., 2020).

As a result, zeolite was shown to be antimicrobial against *S.* Typhimurium and *L. monocytogenes* and has a potential for use in the decontamination of poultry meat. Due to the high ion exchange properties of zeolite, modified zeolites can have very different, perhaps more potent, effects on pathogens. Therefore, zeolite can be combined with modification for stronger bactericidal effects. It is envisaged that zeolite may be a natural alternative for food safety in the near future. To this end, zeolite should be extensively investigated in other potential food applications

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Similarity Rate

We declare that the similarity rate of the article is 12% as stated in the report uploaded to the system.

Explanation

Some data from this study was presented as an abstract at the 10th Veterinary Food Hygiene Congress on 25-27 April 2024.

Author Contributions

Motivation / Concept: MEA, MNG, ES
Design: MEA
Control/Supervision: MEA
Data Collection and / or Processing: MEA, MNG
Analysis and / or Interpretation: MEA, MNG
Literature Review: MEA, ES
Writing the Article: MEA, ES
Critical Review: MEA

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