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Research Article

The effect of organic matter based decontamination technique on *E. coli* inhibition in shrimp

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ABSTRACT

As a result of the increasing world population, people are looking for new sources of nutrition. Alternative seafood, such as crabs, mussels, and shrimps, have gained interest recently as a source of nutrients in addition to traditional seafoods. This study aimed to develop new strategies for reducing *Escherichia coli* count in shrimp. In our study, the effects of nisin, lactic acid, acetic acid and their combinations were investigated in shrimp contaminated with *E coli*. At the end of the study, a statistical difference was found between the effects of single and combined use of all substances ($p<0.05$). In addition, it was observed that lactic acid was the most effective with a decrease of 1.92 CFU (Colony Forming Units)/mL in single use, while acetic acid and nisin had a good synergistic effect with a decrease of 2.2 CFU/mL in combined use.

INTRODUCTION

The aquaculture industry is thought to be a globally expanding field that is effective in supplying human food demands (Macusi et al., 2022). Considering the information in the TUIK 2021 data, it is shown that 5,204 tons of shrimp are caught in our country (Anonymous, 2021). In working societies, consumers are increasingly buying frozen, ready-to-cook goods like fish fillets and shrimp (Arpa et al., 2020). As in the production of all foodstuffs, it is of great importance to produce and consume seafood in a safe and hygienic manner (Yazıcı and Mazlum, 2019). Shrimp meat is easily digested and easily spoiled food with high economic value. The risk of contamination for organisms developing in contaminated habitats rises when water resources and oceans are polluted. Additionally, bacteria may become resistant to antibiotics when they are used for illness prevention and accelerating aquaculture development (Yazıcı and Mazlum, 2019).

One of the most important issues that the food industry focuses on is food quality and safety. Microbial contamination is a major issue, especially about public health. Microbial contamination in seafoods can occur from primary sources as well as secondary sources such as processing and storage conditions (Öztürk and Altınok, 2014). *E. coli* is a bacterium that can be found in environments where shrimp are reared and can cause diseases (Okonko et al., 2008). According to Codex Microbiological Criteria Regulation, 25 g sample should not contain *E. coli* O:157 H:7. (Annex-3) (TGK, 2011).

To preserve freshness and lengthen shelf life of shrimp, various antibacterial substances have been used (Nirmal and Benjakul, 2012; Alparslan et al., 2019). Due to consumer concerns, it is evident that research focuses on organic materials and natural resources. A natural antibiotic called nisin is used to stop bacterial contamination of food (Yehia et al., 2022). Nisin is a widely used food additive in the food industry, as it is generally considered to be safe (GRAS) (FDA, 1988). In general, nisin, which affects Gram (+) bacteria more, has little effect on Gram negative bacteria (Pabon et al., 2021). In addition, it is emphasized that when combined with substances such as lactate, citrate and EDTA, it is also effective against Gram (-) bacteria (Khalafalla et al., 2016). Nisin has been used for bacterial inhibition in dairy products, meat products and canned foods (Li et al., 2022). It has been emphasized that the application of nisin (500 IU/mL) and lactic acid (1.5%) in red meat reduces the number of E. coli (De Martinez et al., 2002).

The use of lactic acid at a rate of 1-3% against pathogenic bacteria in meat, vegetables and fruits is GRAS (Wang et al., 2021). However, it is emphasized that long-term (>30 minutes) use of high concentrations of lactic acid (>2%) in seafood should be avoided so that sensory properties are not adversely affected (Wang et al., 2013). However, studies using 3% lactic acid experimentally were conducted (Shirazinejad et al., 2010; Terzi and Güçlükoğlu, 2010). Although there are studies and recommendations for the use of lactic acid in red meat and poultry for its protective effect, there are very few studies on seafood, especially shrimp (Shirazinejad et al., 2010).

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Acetic acid is produced at the level of 15 million tons/year in the world and it is used commercially in various sectors such as chemistry, textile, food and beverage. It has been used as food additive, food preservative, antimicrobial agent, flavor and flavoring, acidifier, edible packaging material and artificial ripening. Acetic acid, an organic acid, is approved for use in the GRAS category for inhibition of pathogen such as *Salmonella, Escherichia coli* in poultry, pork and red meat (Wali and Abed, 2019). It is stated to be effective against both Gram (-) and Gram (+) bacteria (Deshmukh and Manyar, 2020). Acetic acid has been used in different doses for decontamination in many foodstuffs (Yao et al., 2021; Wang et al., 2021; Sallam et al., 2020).

The study was aimed to reduce the microbial load of shrimps using nisin, lactic acid, acetic acid and their combinations and provide the food safety. Thus, the risk of *E. coli* that may occur in both frozen and ready-to-eat packaged shrimp was tried to be minimized and it was aimed to contribute to the researches in this field.

MATERIALS and METHODS

Materials

E. coli ATCC 25922 strain used in the experimental contamination of shrimp was obtained from the Department of Food Hygiene and Technology, Burdur Mehmet Akif Ersoy University. Shrimps (Balık Dünyası, Turkey) were purchased from local markets. Nisin (Sigma-Aldrich, Germany), Lactic Acid (Merck, Germany), Acetic Acid (Merck, Germany), Sorbitol MacConkey Agar (SMAC-Merck, Germany), Tryptic Soy Broth (TSB-Merck, Germany), Buffered Peptone Water (TPW- Biokar, France) was used.

Experimental Design

Research consisted in the evaluation of nine working groups as follows: K1 (negative control group), K2 (positive control group 10^8 CFU/mL), D1- was immersed in 0.016 mg/mL nisin solution for 20 minutes (Rodpan et al. 2022); D2- was immersed in 3% (v/v) lactic acid solution for 10 minutes (Khalafalla et al., 2016); D3- was immersed in 1% (v/v) acetic acid solution for 10 minutes (Rodpan et al. 2022); D4- was immersed in 0.016 mg/mL nisin solution for 20 minutes and then in lactic acid 3% (v/v) solution for 10 minutes; D5- was placed in a 0.016 mg/mL nisin solution for 20 minutes, followed by a 1% (v/v) acetic acid solution for 10 minutes; D6- was immersed in 3% (v/v) lactic acid solution for 10 minutes followed by 1% (v/v) acetic acid solution for 10 minutes; and D7- was immersed in 0.016 mg/mL nisin solution for 20 minutes, then in lactic acid 3% (v/v) solution for 10 minutes and finally in acetic acid 1% (v/v) solution for 10 minutes.

Preparation of the Inoculum

30 µl of *E. coli* ATCC 25922 strain was added to 10 mL Tryptic soy broth and incubated for 18 hours at 37 °C. At the end of the incubation period, the tubes were centrifuged at 5000 rpm for 5 min (Eppendorf Centrifuge 5810 R, Merck, USA) and the pellet and supernatant were separated. Pellets were dissolved in 1 mL of sterile 0.1% Peptone Water (PW) and then centrifuged. After the second centrifugation, the supernatants were removed and the pellets were dissolved in 1 mL sterile PW again and the inoculum was prepared (Dikici et al., 2013). The bacterial concentration in the contamination liquid was adjusted to 0.5 mcFarland (1.2×10^8 CFU/mL) in 200 mL of TSB. Sterile glass jars were used for all contamination and decontamination solutions.

Contamination Process

The shrimps used in the research were left to completly thaw at +4 °C. Three shrimps, each 3.3 ± 0.3 g, were taken and contaminated by immersion in inoculum solution. At this stage, the contamination process was completed by mixing the shrimps with sterile drumsticks at 30 rpm for 15 min. The shrimps, whose contamination time was completed, were taken into sterile containers and kept at room temperature for 15 min to allow the bacteria to adhere.

Decontamination Solution Control

In order to determine whether there is contamination in the solutions prepared for decontamination, serial dilutions of the liquids were made after all the procedures and inoculated on SMAC agar, and typical colonies were counted as a result of incubation.

Microbiological Analysis

In the negative control group (K1), microbiological analyzes of untreated shrimps were performed to investigate the presence of *E. coli*. In the positive control group (K2), only contamination was performed and microbiological analyzes were performed to determine the bacterial load in the inoculum. All groups were immediately evaluated for microbiological analysis after decontamination. The prawns were taken into a sterile stomacher bag, 90 mL of BPW was added and homogenized in the stomacher (Nr 140/420 IUL) for 2 min. Serial dilutions were prepared from the homogenized samples and the respective dilutions were parallelly plated onto SMAC agar. The petri dishes were incubated at 37 °C for 24 hours, and at the end of the period, typical colonies were counted. All procedures were performed in 3 replications and the results were calculated as $\log_{10}$ CFU/mL.

Statistical Analysis

The study was carried out in 3 parallels and Minitab® 19.1.1 (64-bit) (USA) package program was used to evaluate the data. ANOVA test was applied to the data first and Duncan multiple comparison test was applied to the parameters found statistically significant ($P<0.05$) in the ANOVA test. Data are given in the study as mean ± standard deviation.

RESULTS

Microbiological Analysis and Statistical Results

The decontamination effect of organic acids and their combinations on the inhibition of *E. coli* is shown in Table 1. As a result of the present study, the effect of all organic acids and their combinations on *E. coli* was found to be statistically significant ($P<0.05$). D1 group was found to be the group with the lowest decrease in *E. coli* counts with approximately 0.85 log CFU/mL compared to the control group. The highest decrease in *E. coli* counts was observed in the D5 group with 2.2 log CFU/mL (P<0.05). Considering the inhibition effect of all organic acids alone (D1, D2, D3), it was found that the D7 group containing all of them was more effective (P<0.05). While no contamination was observed in lactic acid and acetic acid solutions in plating made from decontamination solutions, 4.03 log CFU/mL *E. coli* was detected in nisin solution.

Table 1. *E. coli* Decontamination Results

GROUPS	K2	D1	D2	D3	D4	D5	D6	D7
Count Results (log_{10} CFU/mL)	5.53± 0.04[a]	4.68± 0.09[b]	3.61± 0.06[ef]	4.14± 0.08[c]	3.98± 0.07[d]	3.33 ± 0.07[g]	3.70± 0.04[e]	3.55± 0.10[f]
Count Differences (log_{10} CFU/mL)	-	0.85	1.92	1.39	1.55	2.2	1.83	1.98

[abcdefg]: Means in the same row with different superscripts are statistically different (P<0.05).

Table 2. Bacterial Load in Post-Treatment Decontamination Solutions

Decontamination solutions	Count Results (log_{10} CFU/mL)
Nisin	4.03
Lactic acid	Not detected
Asetic asic	Not detected

less permeable to nisin. Similar to the results of our study, it was revealed that the groups treated with nisin and lactic acid were more effective in reducing *E. coli* than the other treated groups, and therefore lactic acid increased the effect of nisin against *E. coli.*

Yehia et al. (2022), it was reported that nisin alone had a bacteriostatic effect on methicillin-resistant *S. aureus*, but did not show the same effect on *S. aureus* ATCC 25923. It has been found that the combination of nisin and reuterin has a bactericidal effect on both microorganisms, and combination of nisin and reuterin can produce a more active effect against both microorganisms. Rodpan et al. (2022) evaluated the inhibitory effects of acetic and propionic acids in combination with nisin in preventing meat and potato spoilage caused by many pathogens. It was found that the synergism of nisin, acetic acid and propionic acids showed a synergistic effect in bacteria such as *E. faecalis, P. aeruginosa, S. Typhimirium* and *E. coli*, which were tested using fractional inhibitory concentration indices, as in our study.

DISCUSSION

In the present study, the microbiological quality of frozen shrimp contaminated with *E. coli* was investigated to examine the antimicrobial activity of lactic acid and acetic acid and nisin as organic decontamination agents. Sultana et al. (2021) investigated the total coliform amount in shrimp farms in winter and summer seasons in Khulna district of Bangladesh and determined enterovirulent groups in their study. As a result of the research, coliform was detected in all farms, while *E. coli* was found in 55% of the farms. Monte et al. (2019) recently reported the emergence of *E. coli* carrying clinically relevant resistance genes in seabirds, wild fish and bivalves, highlighting an urgent need for monitoring of marine environments. In another study, *E. coli* was also isolated from shrimp farm wastewater in Ramanathapuram, India (Chinnadurai et al., 2018). These investigations, with similar results, confirm that *E. coli* is a potential public health pathogen in shrimp production.

Khalafalla et al. (2016) investigated the effect of nisin, lactic acid and their combinations on the shelf life and microbiological quality of chicken breasts. In their results, similar to the results of our study, a significant difference was found between the control and treated groups, except for the group treated with nisin on 0. day of storage in coliforms. In addition, the groups treated with lactic acid (1 and 2%) were found to have a higher reduction in coliform counts than the group treated with nisin. On the other hand, the results showed that nisin alone was less effective on coliforms as their cell walls were

De Martinez et al. (2002) stated that the mixture of nisin and lactic acid provided the highest decrease in total coliform and *E. coli* in beef carcasses in their study on red meats. It has also been reported that this mixture can reduce the total bacterial load by 2 log CFU/g. Mustapha et al. (2002) investigated the antimicrobial effect of 2% low molecular weight polylactic acid, 2% lactic acid, 200 IU nisin and their combinations on raw meat contaminated with *E. coli* O157:H7. Contrary to our study, it was observed that nisin did not increase its antimicrobial effect in combination with acids.

Shirazinejad et al. (2010) evaluated the antimicrobial activity of lactic acid against *V. cholerae, V. parahaemolyticus, S. Enteritidis* and *E. coli* O157:H7 for different durations in a study on fresh raw shrimp. After treatment, 10 min of 3% lactic acid treatment was found to be appropriate in reducing pathogenic bacteria and being organoleptic acceptable. A 10-minute 3% lactic acid treatment has been shown to provide a 2.30 log CFU/

mL reduction in *E. coli*. It is seen that the results obtained are clearly similar and in direct proportion with the decrease of 1.92 log CFU/mL after 10 min of 2% lactic acid treatment as in our study.

Sallam et al. (2020) investigated the effect of lactic acid, acetic acid and trisodium phosphate spray on the microbiological population of cattle carcass surfaces slaughtered in a conventional slaughterhouse in Egypt. It provided complete inhibition of enterococcal growth with lactic acid and acetic acid sprays, and trisodium phosphate was found to be more effective. Hashemi et al. (2021), the effects of relative humidity and temperature on the effectiveness of acetic acid and two different essential oils against pathogens in the vapor phase were investigated. It was determined that the initial population of *B. cereus* (8.1 log CFU/g) was 4.3, 3.9 and it was determined that it decreased to 3.3 log CFU/g.

CONCLUSION

In recent years, we have seen that the consumption of alternative seafood as well as basic seafood has increased and become popular. However, in recent studies, the contamination of marine microflora and consequently the contamination of almost all seafood reveals a frequently encountered situation. In addition, it poses a threat to public health with inadequate sanitation procedures. Considering synthetic preservatives and their negative effects in order to eliminate these problems, natural organic substances have started to be preferred more as preservatives. In cases where the antimicrobial activity of organic agents such as lactic acid and acetic acid is used without impairing the orgonaleptic properties of the foodstuff, and in cases where the effectiveness of nisin alone is not sufficient, new combinations have been tried. It is shown as promising new decontaminant agents that such combinations provide a synergetic effect by eliminating the negative or inadequate conditions in the use of products one by one.

DECLARATIONS

Ethics Approval

Ethics committee approval is not required since humans/animals were not used in our study.

Conflict of Interest

Authors do not have any conflict of interests to disclose nor do they endorse the use of any product/technology/service over the other.

Consent for Publication

Not applicable.

Competing Interest

The authors declare that they have no competing interests

Author contribution

Idea, concept and design: HY, ZP

Data collection and analysis: HY, ZP

Drafting of the manuscript: HY, ZP

Critical review: HY, ZP

Data Availability

Not applicable.

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