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THE ROLE OF HAND PEELING AND PLANT INGREDIENTS IN CONTROLLING MICROBIAL CONTAMINATION DURING REFRIGERATED STORAGE OF *LITOPENAEUS VANNAMEI*

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Abstract: Fresh shrimp are highly perishable, so in addition to chemical and physical methods, natural preservation methods are also applied to prolong their shelf life. A trial was carried out to assess the efficacy of hand peeling and the addition of garlic (*Allium sativum*) and chilli pepper (*Capsicum annum*) as natural conserving agents in the control of microbiological contamination in *Litopenaeus vannamei* shrimp during cold storage. The highest level of *Staphylococcus aureus* was found after 5 days of storage in unpeeled shrimps without additives (3.32 log cfu·g^{-1} = colony forming units per gram). There was a statistically significant effect (p <0.05) of all the factors tested, that is, the day of testing, the preparation method and the additive used on the average number of *S. aureus* in shrimp. The average number of *Vibrio parahaemolyticus* in the garlic-only and garlic and chilli samples was very similar, at 2.30 and 2.38 log cfu·g^{-1}, respectively. The highest average number of psychrotrophs was recorded in samples with garlic and chilli: 4.41–5.65 log cfu·g^{-1}, 5 days after purchase. The addition of plant additives used in this work inhibits the fungi in peeled shrimp. The composition of the ingredients used does not provide complete protection against spoilage, but to a greater extent, it improves the shelf life of the hand-peeled shrimp.

Key words: *Litopenaeus vannamei,* shrimp, hand peeling, *Allium sativum* (garlic), *Capsicum annum* (chilli pepper), microorganisms, quality

INTRODUCTION

Shrimps are among the products that are especially valued all over the world. In 2020, white-legged shrimp (*Litopenaeus vannamei*) was the most produced species, reaching 5.8 million tonnes [1]. *L. vannamei* has a high nutritional and protein content (16.8–17.6%). Shrimps contain essential omega-3 and omega-6 unsaturated fatty acids, minerals, and vitamins that positively affect human health [2]. Fresh shrimp are products prone to spoilage due to microorganism growth, lipid oxidation, and blackening (melanose) during post-harvest processing [3, 4, 5]. Shrimp contain a high proportion of nonprotein nitrogen compounds, which are easily metabolised by microorganisms and lead to more rapid spoilage [6, 7]. Certain groups of microorganisms (*Shewanella* spp., *Aeromonas* spp., *Enterobacteriaceae* spp., *Pseudomonas* spp., *Vibrio* spp.) are responsible for negative organoleptic properties such as unpleasant ammonia-like off-taste and fishy flavours [8, 9]. There are many methods by which

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shrimp are protected from quality losses during the storage process. The most common treatments to prevent the undesirable effects of this loss on the physical, chemical, and sensory properties of shrimp include the addition of preservatives (commonly 0.6% benzoic acid and 0.6% sorbic acid), irradiation, modified atmosphere packaging, ice cooling, freezing and high-pressure treatment. To extend shrimp shelf life, the cephalothorax and head are removed and glazed with ice to prevent undesirable quality changes and oxidation during storage [3, 5, 6, 10]. However, despite the application of the methods mentioned above, shrimp are still susceptible to microorganism growth, leading to a loss of quality in the final product. Shrimp available on the Polish market (Table 1) are usually sold frozen, pickled, blanched (whole) or raw (whole, defrosted).

Table 1. Shrimp types most frequently available on the Polish market

Shrimp type	Latin name	Origin
Whiteleg	*Litopenaeus vannamei/ Penaeus vannamei*	Bangladesh, Ecuador, India, Indonesia, Costa Rica, Thailand, Vietnam
Cocktail	Metapenaeus dobsoni	India
Cocktail	*Metapenaeus monoceros*	Bangladesh
Cocktail	Solenocera crassicornis	Western Indian Ocean
Argentine red	*Pleoticus muelleri*	Argentina
Tiger	*Penaeus monodon*	Ecuador, Thailand, Bangladesh, Vietnam
Banana	*Penaeus merguiensis*	Vietnam
Northern	Pandalus borealis	waters off Greenland

Source: own elaboration.

The shelf life of defrosted shrimp is short (it is recommended to consume them within 2 days of delivery), so besides chemical and physical methods, natural preservation methods are also being applied to maintain high-quality and fresh shrimp. Natural compounds of plant origin are very popular in the conservation of seafood. Their strong antimicrobial and antioxidant activity offers great potential for the food industry [11]. The aim of the study was to investigate the effects of hand peeling and the addition of garlic (*Allium sativum*) and chilli pepper (*Capsicum annum*) on the microbiological contamination of vacuum-packed *L. vannamei* white shrimp during cold storage.

MATERIAL AND METHODS

Test material

The test material consisted of thawed white shrimp (*Litopenaeus vannamei*) purchased in the MAKRO store immediately after delivery to the store. *L. vannamei* originated from the *Southeast Pacific* (Area 87) – Ecuador. In the MAKRO, the products were stored in Styrofoam containers filled with ice under controlled temperature conditions of 0 to 2°C. The shrimp were transported to the microbiological laboratory in the packaging mentioned above to ensure the continuity of the “cold distribution chain”. After being transported (less than 15 minutes) to a research laboratory, the samples were analysed.

Method of shrimp preparation

After *L. vannamei* was delivered to the laboratory (approx. 1.5 kg), a portion of the shrimp (approx. 100 g) was discarded for microbiological testing before storage, while the rest was divided into two parts. Half of *L. vannamei* were peeled by hand under sterile conditions (with sterile gloves and in sterile boxes to minimise cross-contamination between the shell and meat tissue) [6], while in the second half, the tail was left with the intestine (Fig. 1). The division of shrimp into peeled and unpeeled was carried out to compare the spoilage rate. The removal of the cephalothorax, which contains organs rich in autolysis enzymes, should increase the storage quality of shrimp.

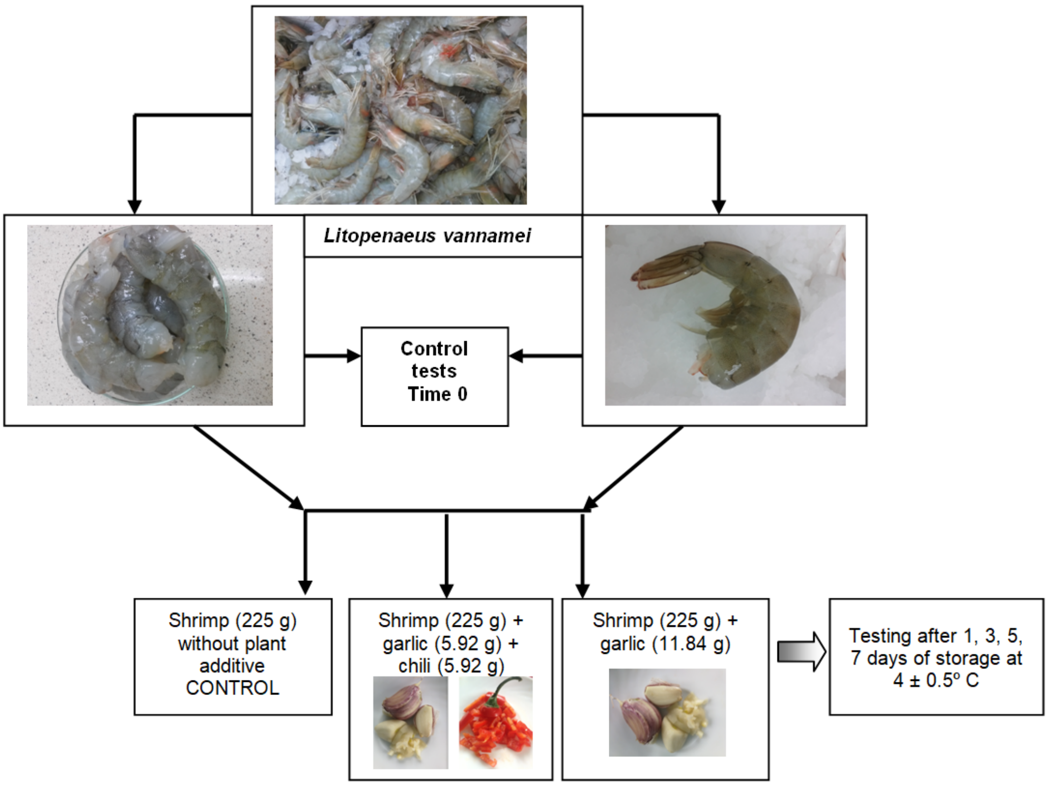


Figure 1. Test diagram

Source: own elaboration.

METHOD OF PLANT PREPARATION

As an accompaniment, vegetable ingredients: garlic (*Allium sativum*) and chilli peppers *(Capsicum annuum*), which are served most often in the kitchen, are used as additives to shrimp dishes. Garlic of the Harnaś variety and Cyklon red hot chilli pepper from Poland were used for the research. In addition to being very well suited to this type of seafood, they have antibacterial and antifungal properties [12, 13, 14, 15]. The ingredients were washed for 15 minutes in sterile distilled water to remove impurities from the surface and then crushed with a Grind & Chop Transa Electronics electric grinder (ARCOTECH, Opole, Poland). Cutting or crushing releases the enzyme alliinase, which converts alliin to active allicin [16]. Allicin is the main bioactive component in garlic products [17]. Before rinsing and crushing, the garlic husks and the small kernels of the chilli were removed. The plant components produced in this way were used for further experiments.

THE COURSE OF THE EXPERIMENT

Each 100 g of peeled and unpeeled *L. vannamei* was microbiologically examined before storage, while the remainder was divided according to the diagram shown in Figure 1. In sterile PE/PA bags intended for vacuum packaging of food, appropriate amounts of garlic and chilli peppers (to obtain a 5% addition) were added to 225 g of shrimp (popular single packs available in Poland) according to the diagram (in addition to the control tests); (Fig. 1). The contents were thoroughly and carefully mixed so that the shrimp were evenly covered with plant additives, then packed in PA/PE bags and vacuum welded using a CAS CVP-350/MS vacuum packing machine (Poland). The prepared samples were refrigerated for 7 days at a temperature of 4 ±0.5°C.

MICROBIOLOGICAL ASSAYS

In a chamber with laminar airflow, 20 g of the product was withdrawn and then homogenised with 180 mL of Ringer liquid with the use of a Stomacher Lab-Blender 400 (Seward, Worthing, UK). To determine the presence of *Vibrio parahaemolyticus*, 25 g of the product was collected and then homogenised with 225 mL of alkaline peptone water. In the products studied, the following values were marked:

- psychrotrophic microbes, according to PN-ISO-17410:2004 [18],
- *Staphylococcus aureus,* according to PN-EN ISO 6888–1:2001/A1:2004 [19],
- fungi (moulds and yeasts), according to PN-ISO 21527–1:2009 [20],
- *V. parahaemolyticus,* according to ISO 8914:1990 [21].

Microbiological tests were carried out by sowing additional dilutions of 1 mL on the bottom of a sterile plate and then pouring a liquefied and cooled solid medium (approximately 15 mL) over. Microbiological analyses were carried out immediately after delivery to the laboratory and after 1, 3 and 5 days of cold storage (Fig. 1). Due to the unsuitable organoleptic characteristics (mucous surface, discolouration and darkening, rotten odour) of the shrimp stored for 7 days (Fig. 2), no microbiological tests were performed.

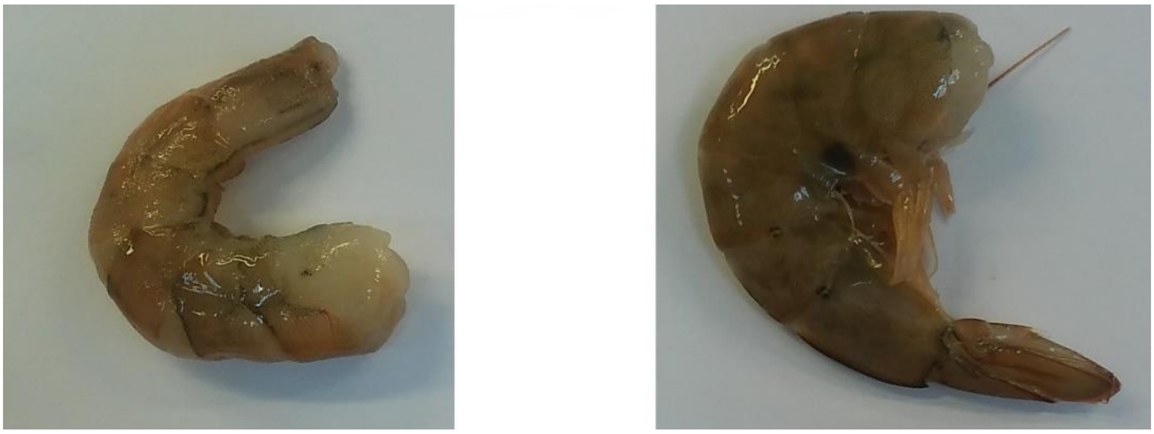


Figure 2. Shrimp (peeled and unpeeled) after 7-day storage

Source: own elaboration.

After incubation, the number of microorganisms was determined according to PN-EN ISO 7218:2008 [22]. Analyses were performed in three independent replicates.

STATISTICAL ANALYSIS

Data were logarithmically transformed (for mould, log cfu·g^{-1} + 1 transformation due to 'clean' samples without mould), and baseline values for location and variability were calculated for both the entire sample and subgroups based on the test day and sample preparation. A two-factor model (preparation, additives) of ANOVA with repeated measurements was used for the statistical analysis. The assumption of homogeneity of variance in the subgroups was tested with the Levene test. The Newman–Keuls post-hoc test was used to investigate the significance of the differences between subgroups. In addition, bifactorial ANOVA was used to determine the effect of the investigated factors on the content of the investigated microorganisms in shrimp on the fifth day after purchase.

RESULTS

The quality and safety of shrimp may be directly related to non-compliance with good seafood hygiene practices and contact with contaminated work surfaces, tables, and unwashed knives [23]. Table 2 shows shrimp contamination on the day of purchase: *S. aureus* with an average number of 1.35 log cfu·g^{-1} (with a minimum value of 1.00 and a maximum value of 1.85 log cfu·g^{-1}); *V. parahaemolyticus* – the mean number was 1.71 (1.48–1.95) log cfu·g^{-1}. Furthermore, the following were detected in the samples: psychrotrophic bacteria – 3.48 (2.99–4.23) log cfu·g^{-1}; mould – 1.36 (1.00–1.60) log cfu·g^{-1} +1 and yeast – 2.15 (1.70–2.58) log cfu·g^{-1}. The microorganism content of the product was slightly variable – V_x was no more than 36%.

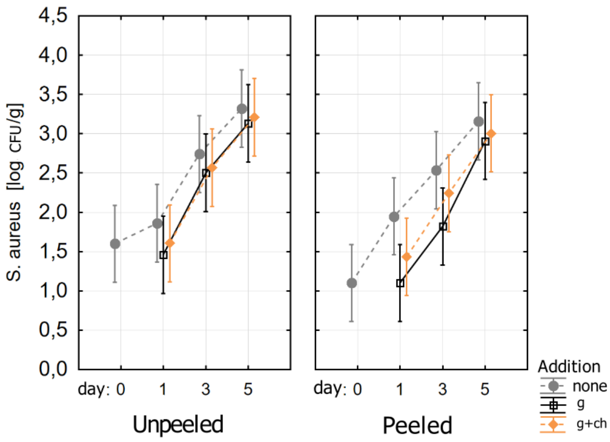
Table 2. Contaminating microbe count in shrimp on the day of purchase

Microbe type	*M*	–95% PU	+95% *PU*	Min.	Max.	*SD*	V_x [%]
S. aureus log cfu·$^{g–}$1	1.35	1.01	1.69	1.00	1.85	0.32	35.6
V. parahaemolyticus log cfu·$^{g–}$1	1.71	1.52	1.90	1.48	1.95	0.18	10.5
Psychrotrophs log cfu$^{·g}$–1	3.48	2.95	4.01	2.99	4.23	0.50	14.5
Moulds log cfu·g$^{–1}$+1	1.36	1.06	1.66	1.00	1.60	0.28	20.9
Yeasts log jtk · g$^{–1}$	2.15	1.80	2.51	1.70	2.58	0.33	15.5

Explanatory notes: *M* – arithmetic average, *PU* – confidence interval, Min. – minimum, Max. – maximum, *SD* – standard deviation, V_x [%] – variability index.

Source: own elaboration.

There was a statistically significant effect (p <0.05) of all factors tested, i.e. the day of the test, the preparation method and the additive used on the average number of *S. aureus* in shrimp. Interactions between factors were found to be statistically insignificant. The average count of *S. aureus* 24 hours after purchase was 1.57 log cfu·g$^{–1}$ with a 95% confidence interval of 1.39–1.75 log cfu·g$^{–1}$, and it statistically increased to 2.40 (2.15–2.66) log cfu·g$^{–1}$ after 3 days of storage (p <0.001). The average number of *S. aureus* in unpeeled shrimp was 2.12 (1.98–2.27) log cfu·g$^{–1}$, statistically significantly higher than that of peeled shrimp, 1.85 (1.70–1.99) log cfu·g$^{–1}$ (p <0.05). The average number of *S. aureus* in samples with garlic alone and with garlic and chilli was similar (Fig. 3). Statistically significant differences were observed between the group without additives and the samples with additives (p <0.05). The highest level of *S. aureus* was found after 5 days of storage in unpeeled shrimps without any additives (3.32 log cfu·g$^{–1}$), while the lowest level was found in peeled shrimp with only garlic (2.91 log cfu·g$^{–1}$), (Fig. 3).

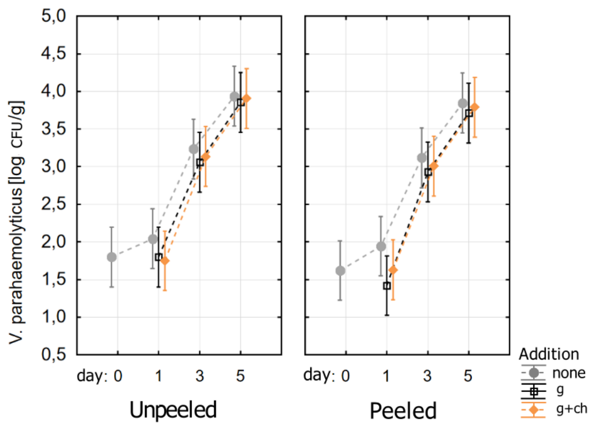


Explanatory notes: g – garlic, g + ch – garlic with chilli

Figure 3. Changes in the number of *S. aureus* depending on the day of testing, the preparation method, and the additive used

Source: own elaboration.

A statistically significant effect of the study day was observed on the average number of *V. parahaemolyticus* in shrimp. The remaining factors and interactions between them were found to be statistically insignificant. In shrimp tested 24 hours after purchase, the average number of *V. parahaemolyticus* was 1.76 (1.53–1.99) log cfu·g$^{–1}$; it increased statistically significantly 3 days after purchase to 3.08 (2.98–3.18) log cfu·g$^{–1}$ (p <0.001). In unpeeled shrimp, *V. parahaemolyticus* was recorded at an average level of 2.50 (2.28–2.73) log cfu·g$^{–1}$, slightly higher than in peeled shrimp (2.34 (2.11–2.57) log cfu·g$^{–1}$). When analysing the effect of additives on the content of *V. parahaemolyticus* in shrimp, the highest number of 2.58 (2.31–2.86) log cfu·g$^{–1}$ was found in samples without additives. The average number of *V. parahaemolyticus* in the garlic-only and garlic and chilli samples was very similar, at 2.30 (2.02–2.58) and 2.38 (2.10–2.66) log cfu·g$^{–1}$, respectively. The differences between the averages were not significant. Detailed changes in the number of *V. parahaemolyticus* according to the date and preparation of the test are shown in Figure 4.

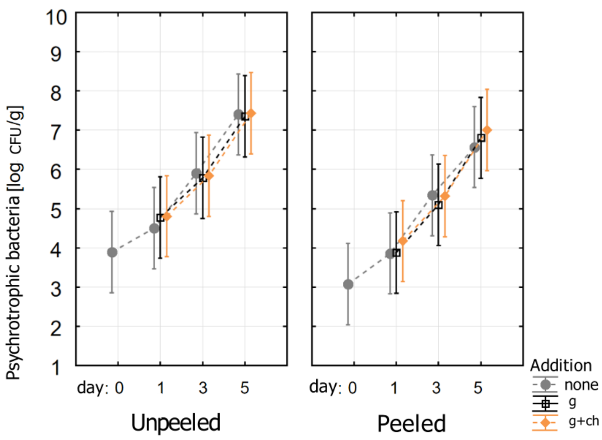


Explanatory notes: g – garlic, g + ch – garlic with chilli.

Figure 4. Changes in the number of *V. parahaemolyticus* depending on the day of testing, the preparation method, and the additive used

Source: own elaboration.

As in *V. parahaemolyticus*, a statistically significant effect on the average number of psychrotrophic bacteria was observed on the study day. The remaining factors and interactions between them were found to be statistically insignificant. The average number of psychrotrophic bacteria 24 hours after purchase was 3.27 (3.02–3.52) log cfu·g^{-1}, and 3 days after purchase, it statistically increased to 4.17 (3.70–4.64) log cfu·g^{-1} (p <0.001). The average number of these microorganisms in unpeeled shrimp was 5.27 (4.76–5.77) log cfu·g^{-1}, slightly higher than in peeled shrimp (4.61 (4.10–5.12) log cfu·g^{-1}). The highest average number of psychrotrophs was recorded in samples with garlic and chilli: 5.03 (4.41–5.65) log cfu·g^{-1}. The average number of these bacteria in the samples with only garlic and without additives was very similar, 4.88 (4.26–5.50) and 4.90 (4.28–5.52) log cfu·g^{-1}, respectively. The differences between the averages were not statistically significant. On day 5, the level of psychrotrophic bacteria in the peeled shrimp was almost 1 logarithmic cycle higher than in the peeled shrimp (Fig. 5).

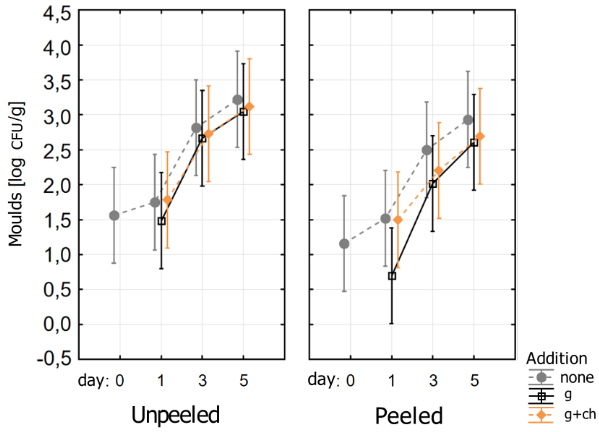


Explanatory notes: g – garlic, g + ch – garlic with chilli.

Figure 5. Changes in the number of psychrotrophic bacteria depending on the day of testing, the preparation method, and the additive used

Source: own elaboration.

The tests carried out showed a statistically significant effect of the test day and the preparation method on the average mould count in shrimp. The effects of additives and interactions between the factors investigated were not statistically significant. The average mould count 24 hours after purchase was 1.45 (0.08–1.62) log cfu·g^{-1}+1. However, 3 days after purchase, it increased statistically significantly to 2.49 (2.10–2.88) log cfu·g^{-1}+1 (p <0.001). The mean mould formation in unpeeled shrimp (2.20 (1.88–2.52) log cfu·g^{-1}+1) was significantly higher than in peeled shrimp (1.74 [1.42–2.06] log cfu·g^{-1}+1). The highest average mould count was recorded in samples without additives and in those with garlic and chilli: 2.14 (1.75–2.54) and 2.05 (1.66–2.44) log cfu·g^{-1}+1, respectively. The average mould count in samples with only garlic added was 1.71 (1.32–2.11) log cfu·g^{-1}+1. Differences between mean values were not statistically significant. On the fifth day of storage, the mould count in the unpeeled shrimp was similar, regardless of the additive used. On the other hand, in peeled shrimp to which chilli and garlic and garlic alone were added, the mould count was lower by approximately a third of a logarithmic cycle compared to the shrimp without additions (Fig. 6).

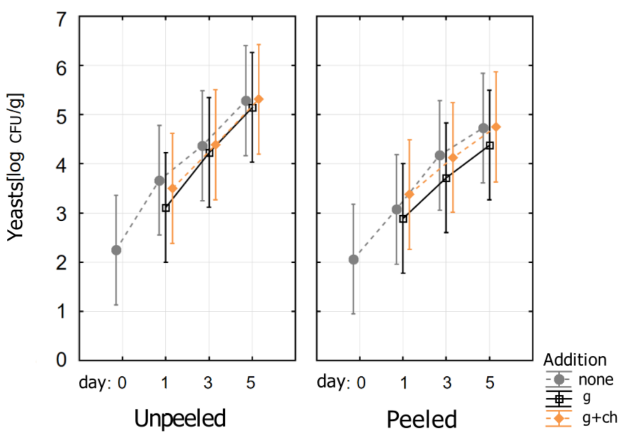


Explanatory notes: g – garlic, g + ch – garlic with chilli.

Figure 6. Changes in the number of moulds depending on the day of testing, the preparation method, and the additive used

Source: own elaboration.

The studies showed a statistically significant effect of the study day on the average yeast count in shrimp. Other factors and interactions between factors were found to be statistically insignificant. The average yeast count 24 hours after purchase was 3.27 (3.02–3.52) log cfu·g^{-1}, and 3 days after purchase, it statistically increased to 4.17 (3.70–4.64) log cfu·g^{-1} (p <0.001). The average yeast count in unpeeled shrimp (3.88 (3.41–4.35) log cfu·g^{-1}) was significantly higher than in peeled shrimp (3.56 (3.09–4.03) log cfu·g^{-1}). The highest average yeast count (such as in moulds) was recorded in samples without additives and in those with garlic and chilli: 3.82 (3.25–4.40) and 3.85 (3.27–4.42) log cfu·g^{-1}, respectively. On the other hand, the average yeast count in samples with only garlic was 3.49 (2.91–4.06) log cfu·g^{-1}. The differences between the mean values were not statistically significant. On day 5, the yeast content in the unpeeled shrimp was approximately 0.6 logarithmic cycles higher than in the peeled shrimp (Fig. 7).



Explanatory notes: g – garlic, g + ch – garlic with chilli.

Figure 7. Changes in the yeast count depending on the day of testing, the preparation method, and the additive used

Source: own elaboration.

DISCUSSION

Pathogenic bacteria in food can reach high levels without causing a noticeable change in odour or taste [23]. The use of plant extracts has been shown to prolong the shelf life of foods, including fish and fish products [24]. The presence of *S. aureus* in food is considered a potential hazard that may lead to food poisoning [8, 25]. The highest *S.aureus* level was found after 5 days of storage in unpeeled shrimp without additives (3.32 log cfu·g^{-1}), exceeding the limits for *S. aureus* laid down in EU Directive 2073/2005 [26]. The authors propose that the main active ingredient in the antimicrobial garlic extract is allicin, which can penetrate the cell wall and influence the cytoplasmic components and enzymes [27, 28]. Other authors' results have shown that the antibacterial activity of garlic is entirely dependent on allicin, which is three times more effective against Gram-positive bacteria than against Gram-negative bacteria [25]. This study shows a lower effect of garlic on psychrotrophic bacteria and *V. parahaemolyticus* than on *S. aureus* (Fig. 2–4). Mozaffari Nejad et al. [25] observed a significant effect of garlic extract on the growth of *S. aureus* in hamburgers. The number of psychrotrophic bacteria detected in this study after 3 days of storage was slightly lower than that of Premaratne et al. [29], but after two more days, the concentration of these bacteria exceeded 7 log cfu·g^{-1}. Products containing more than 7 log cfu·g^{-1} of psychrotrophic bacteria exhibit organoleptic perishability (off-flavour including fruity, stale, bitter, putrid, and rancid, as well as changes in odour, colour, and texture) [31, 32], which means that the fish product is spoiled [32]. The organoleptic changes observed in shrimps after 7 days of storage (unpleasant odour, changes in texture); (Fig. 2) were characteristic of the microbial degradation of amino acids. Fungi can flourish in both raw and processed foods, even if environmental conditions are unfavourable for most bacteria [33, 9]. References in the literature confirm the antifungal properties of garlic and chilli [34, 35]. The addition of garlic alone or garlic and chilli used in this work has an inhibitory effect on the fungi in the hand-peeled shrimp. Kim et al. [16] showed the activity of freeze-dried garlic against yeast, which develops in stored kimchi. After 5 days of storage, the results showed a yeast content higher than that of mould by approximately 2 logarithmic cycles. However, moulds in raw or processed seafood cause a change in the flavour, texture, odour, and nutrient quality, as well as the formation of mycotoxins [9]. Mycotoxins are very stable and, above all, heat-resistant, so they remain in food during processing and storage, leading to a serious food safety problem [33, 36]. Cruz da Silva et al. [37] observed in a study on *L. vannamei* that the 146 isolated fungi included 46 species, of which the genera *Aspergillus*, *Penicillium,* and *Fusarium* were the most dominant species. In hand-peeled shrimps to which garlic was added, less yeast and mould formation was observed on day 5 of refrigerated storage, which is confirmed by the reports by Liu *et al*. [38] on the inhibitory effect of chopped garlic added to minced meat. The addition of garlic (5 or 10%) to raw meat reduced the number of microorganisms in the range of the total number of aerobic mesophilic bacteria, yeasts and moulds [38]. *V. parahaemolyticus* is a bacterium that causes food poisoning from eating raw or lightly cooked seafood. Leaving the food product for 2–3 hours at room temperature can result in the growth of *V. parahaemolyticus* from 10^2–10^3 cfu·g^{-1} to or more than 10^5 cfu·g^{-1} [39]. According to Takoundjou *et al.* [39] to induce a disease with a 100% probability, an exposure value of 10^6 cfu/meal is required, while in our study, the maximum level (2,1·10^4 cfu·g^{-1}) of *V.parahaemoliticus* was observed in 5-day storage of unpeeled shrimp. Basil, clove, garlic, horseradish, marjoram, oregano, rosemary, and thyme have been shown to have antibacterial activity against *V. parahaemolyticus* at 30°C, but at low temperatures, the herbs show little antibacterial effect [40]. It is possible that the storage temperature (4°C) used in this study reduced the activity of the herbs used. Although combinations of several spices have a greater antibacterial effect than single spices [38, 41], no such association was found for the combination of chilli and garlic in these studies. Studies by different authors have confirmed the potential of herbs as a substitute for synthetic additives used to prevent oxidation and degradation of food quality, especially fish and fish products [24, 25].

CONCLUSIONS

At the end of storage, slightly higher growth of microbes was shown in the unpeeled shrimps, as compared to the hand-peeled ones. The composition of the ingredients used does not provide complete protection against spoilage but does, to a greater extent, improve the shelf life of hand-peeled shrimp. With the use of a 5% garlic additive, the shelf life of peeled shrimp in refrigerated conditions can be extended from 2 to 5 days.

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