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EFFECTS OF MANUAL PEELING ON *LITOPENAEUS VANNAMEI* MICROBIOLOGICAL CONTAMINATION

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Abstract: The aim of the study was to assess the effects of manual peeling on microbiological contamination of whiteleg shrimp *Litopenaeus vannamei* in cold storage. The test material was whiteleg shrimps (*Litopenaeus vannamei*), raw, which were kept in cold storage for 72 hours after peeling. The count of psychrotrophic microbes, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and fungi was determined in the test products. After 72 hours of storage, a higher growth of microbes was shown in the peeled shrimps, as compared to unpeeled ones. After 24 hours of storage, a significantly higher count of *S. aureus* and psychrotrophic bacteria was observed in the unpeeled shrimp group. Peeling shrimps before the storage process led to a lower contamination with the microflora in question.

Keywords: *Litopenaeus vannamei,* shrimps, microbiological contamination, manual peeling, cold storage.

1. INTRODUCTION

Fish and shellfish are particularly valuable for their high content of protein and unsaturated fatty acids. In recent years, the consumption of fish and shellfish per capita in Poland has slightly increased from 12.92 kg in 2017 to 13.33 kg in 2020 [Wysoczyńska 2021]. ANMEI (Agriculture and Nutrition Management Economy Institute) data indicates that consumption of shellfish alone has increased as well, having now reached 0.35 kg per capita (an increase of 14%). Shrimps are the most popular among consumers, with their consumption per capita increasing in 2019 to 0.19 kg [Grzegórska-Szpyt 2019].

The literature data indicates that the total world production of farm-grown sea shrimp has increased by 86% during the last 10 years [Emerenciano et al. 2022]. Globally, shrimp production reached 5 million tons in 2020, and it is predicted to reach 5.5 million tons in 2021 [Herianto et al. 2022]. Countries from East and Southeast Asia (83.4% production) and Latin America (16.3%) have the greatest share in the production of *Litopenaeus vannamei*, with 83.1% of the production, and *Penaeus monodon* shrimps [Emerenciano et al. 2022]. The whiteleg shrimp

(*L. vannamei*) is among the most popular crustaceans in the world, and one of the most economically important marine aquaculture species grown. It is consumed in considerable quantities in North America, Latin America, Europe and Asian countries [Wei et al. 2014; Tawade et al. 2019].

The shrimps most commonly sold in Poland are summarised in Table 1.

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 study

Shrimps are an important source of protein, rich in essential amino acids, i.e. lysine, methionine, cysteine, threonine, and tryptophan. The fats found in shrimps contain essential omega-3 and omega-6 unsaturated fatty acids, which provide health benefits, such as proper brain functioning as well as preventing diabetes and coronary artery disease. Shrimps contain mineral ingredients (copper, zinc, manganese, iron, chromium) and vitamins (choline, cobalamin) that have a positive effect on the human body [Priyadarshini et al. 2015; Tawade et al. 2019; Frey 2022].

Table 2 shows a typical chemical composition of *Litopenaeus vannamei*.

Table 2. Typical chemical composition of *Litopenaeus vannamei*

Component	Content [%]
Protein	16.8–17.6
Fat	1.3–1.5
Water	73.2–77.9
Ash	1.4–1.9
Carbohydrates	1.5–2.4

Source: prepared based on Tawade et al. [2019].

Shrimps are susceptible to quick spoiling due to their high content of water (Tab. 2) and non-protein nitrogen compounds. Contamination of these crustaceans with *Vibrio* spp., *Salmonella* spp., *Staphylococcus* spp. bacteria, fecal streptococci, and coli bacteria may result from improper hygiene conditions maintained during processing, preservation and storage. There are multiple methods of extending the shelf life of harvested shrimps, such as irradiation, packing in modified atmospheres, freezing, high-pressure processing, and adding preservatives (usually 0.6% benzoic acid and 0.6% sorbic acid). Unfortunately, these methods are not perfect, as they can cause undesired changes in the physical, chemical or sensory properties of the product. The most common way of preservation is cooling with ice generated from various sources, such as the water supply network, ozonated water, and electrolysed water. In spite of using these methods, shrimps remain susceptible to microorganism growth, leading to degraded end product quality [Broekaert et al. 2013; Talukder et al. 2019; Herianto et al. 2022]. On the Polish market, raw shrimps are not available. It is therefore necessary to seek effective methods of prolonging the microbiological shelf life of the available raw (thawed) *Litopenaeus vannamei*. The aim of the study was to assess the effects of manual peeling on microbiological contamination of whiteleg shrimp *Litopenaeus vannamei* in cold storage.

2. MATERIAL AND METHODS

2.1. Test material

The test material was whiteleg shrimps (*Litopenaeus vannamei*), raw (thawed) purchased at a MAKRO chain store after they were delivered to the store by the supplier, in accordance with an ATP agreement [Umowa ATP]. The shrimps came from south-eastern Pacific Ocean (FAO code 87), from Ecuador. At the store, the products were stored in foamed polystyrene packages filled with ice, under controlled temperature conditions, between 0 and +2ºC. The shrimps were transported from the store to the microbiology laboratory in the above-mentioned packaging, allowing the "cold distribution chain" continuity to be maintained. The transport time was about 15 minutes. The samples were analysed immediately after reaching the laboratory.

2.2. Method of shrimp preparation

After the shrimps were delivered to the laboratory (approx. 1.5 kg), some of them (approx. 100 g) were set aside for microbiological testing before the storage process, while the rest were divided into 2 parts. Half of the *Litopenaeus vannamei* were manually peeled in sterile conditions (wearing sterile gloves and working in a sterile box, so as to minimise cross-contamination between the shell and the meat tissue)

[Broekaert et al. 2013], while the other part were left with the tail and intestine (Fig. 1).

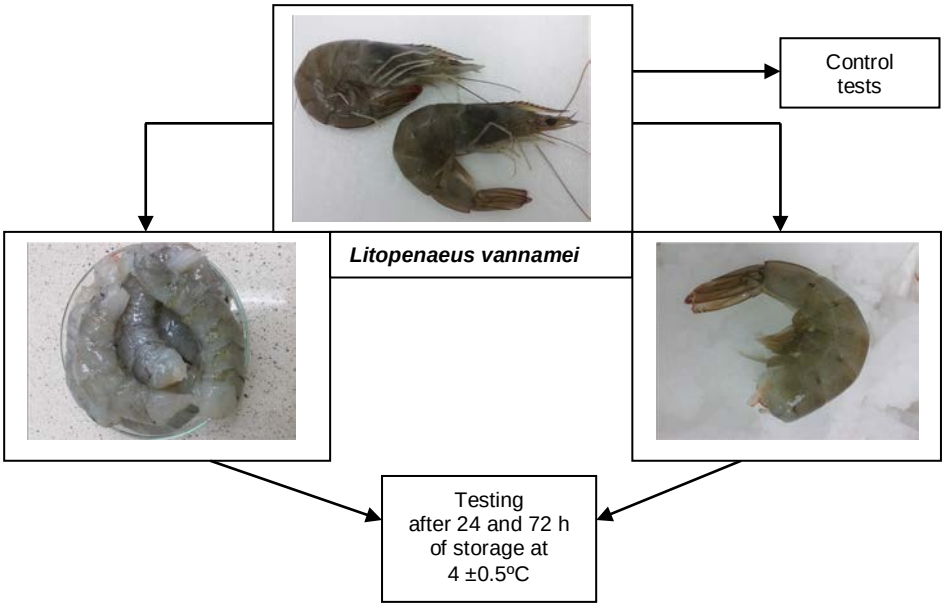

Fig. 1. Test diagram

Source: own study.

Both the peeled and unpeeled shrimps, locked in sterile plastic containers, were stored under cold storage conditions (at 4 ±0.5ºC) for 3 days. The storage time was consistent with the fish and shellfish importer's recommendations.

2.3. Microbiological assays

In a laminar flow chamber, 20 g of product was taken and homogenised with 180 ml Ringer's liquid using a Stomacher Lab-Blender 400 (Seward, Worthing, UK). The microbiological assays were performed by transferring 1 ml of consecutive dilutions to the bottom of a sterile dish, then covering with a liquefied and cooled solid medium (approx. 15 ml). The following counts were determined in the test products:

- psychrotrophic microbes, as per PN-ISO-17410:2004;
- *Staphylococcus aureus*, as per PN-EN ISO 6888–1:2001/A1:2004;
- *Vibrio parahaemolyticus*, as per PN-ISO 8914:2002;
- fungi (moulds and yeasts), as per PN-ISO 21527–1:2009.

The test shrimps were stored for 7 days, but due to their inadequate organoleptic properties (slimy surface, unusual colour, rotting odour), the microbiological assays were performed immediately after the material was delivered to the laboratory, and after 24 and 72 h of cold storage (Fig. 1). Following the incubation, the microbial count was determined, as per PN-EN ISO 7218*:*2008. The assays were performed in three independent repetitions.

2.4. Statistical analysis

The data were transformed and then the basic position and variability measures were calculated both for the sample as a whole, and subdivided by day of testing and specimen processing method. The data were processed using the Statistica software (StatSoft, Inc.).

3. TEST RESULTS

The test product contamination results on the purchase day are shown in Table 3. Directly following the purchase, the shrimps were characterised by the highest level of psychrotrophic bacteria (3.48 log cfu/g), while *S. aureus* and moulds were the least prevalent (Tab. 3). The analysed microbe count in *Litopenaeus vannamei* was characterised by low variability (< 25%) (Tab.3).

Table 3. Contaminating microbe count in the whole *Litopenaeus vannamei* on the day of purchase [log cfu/g]

Microbe type	T	Min.	Max.	SD	Vx [%]
S. aureus	1.35	1.00	1.85	0.32	23.7
V. parahaemolyticus	1.71	1.48	1.95	0.18	10.5
Psychrotrophs	3.48	2.99	4.23	0.50	14.4
Moulds	1.36	1.00	1.60	0.28	20.6
Yeasts	2.15	1.70	2.58	0.33	15.3

M – arithmetic average, Min. – minimum, Max. – maximum, SD – standard deviation, Vx [%] – variability index.

Source: own study.

After the first day of storage, a lower count of the analysed bacteria and fungi was found in the peeled shrimps, compared to the unpeeled shrimps (Tab. 4). A significantly higher count of *S. aureus* and psychrotrophic bacteria was observed in the unpeeled shrimp group ($p < 0.05$). The *V. parahaemolyticus* and psychrotrophic bacteria counts in the crustaceans analysed showed low variability (Vx no greater than 10%).

Table 4. Contaminating microbe count in peeled and unpeeled *Litopenaeus vannamei* after 24 h of storage [log cfu/g]

Microbe type	M Sp	M Su	t	p	Vx Sp	Vx Su
	[log cfu/g]				[%]	
S. aureus	1.10	1.60	-3.15	**0.034**	15.4	13.1
V. parahaemolyticus	1.62	1.88	-1.33	0.253	9.2	9.6
Psychrotrophs	3.03	3.89	-3.78	**0.019**	2.6	9.2
Moulds	1.16	1.56	-2.44	0.071	23.3	4.5
Yeasts	2.06	2.25	-0.63	0.563	18.0	15.1

M – arithmetic mean, Vx – variability index, t – t-Student test result, p – significance level, Sp – peeled shrimps, Su - unpeeled shrimps.

Source: own study.

After 72 h of *Litopenaeus vannamei* cold storage, no significant differences between the counts of microorganisms were present in the tested shellfish (Tab. 5, $p > 0.05$). A higher growth of microbes was shown in the peeled crustaceans, as compared to the unpeeled ones. The highest difference in growth (about 0.3 logarithmic cycle) was noted for *S. aureus* and psychrotrophic bacteria, while the lowest was observed for yeasts and moulds (Tabs. 4 and 5). Similarly to after 24 h of storage, the *V. parahaemolyticus* count in the analysed shrimps showed little variation, while the mould count was characterised by average variability (Tab. 5).

Table 5. Contaminating microbe count in peeled and unpeeled *Litopenaeus vannamei* after 72 h of storage [log cfu/g]

Microbe type	M Sp	M Su	t	p	Vx Sp	Vx Su
	[log cfu/g]				[%]	
S. aureus	2.54	2.74	-0.44	0.682	21.2	21.5
V. parahaemolyticus	3.12	3.23	-0.69	0.526	6.1	6.8
Psychrotrophs	5.34	5.90	-0.76	0.489	20.0	12.2
Moulds	2.49	2.81	-0.489	0.650	32.5	28.1
Yeasts	4.17	4.37	-0.25	0.808	22.3	21.3

M – arithmetic mean, Vx – variability index, t – t-Student test result, p – significance level, Sp – peeled shrimps, Su - unpeeled shrimps

Source: own study.

4. DISCUSSION

Peeling the shrimps before the storage process led to a lower contamination with the microflora in question. The *S. aureus* count in the test shrimps was 1.1–2.54 log cfu/g and 1.6–2.74 log cfu/g for the peeled and unpeeled crustaceans, respectively. Amin, Ahemd and Ahmed [2021] in their research obtained higher results for *S. aureus* (2.9 ±2.3 log cfu/g). However, they found a slightly lower (by 1%) percentage of results that exceeded the upper limit of 3 log cfu/g, in accordance with the recommendations of the Egyptian Standard for staphylococcus count in crustaceans [Amin, Ahemd and Ahmed 2021]. Talukder et al. [2019] in their analysis of fresh shrimps found staphylococci at a level of 2.21– 2.49 log cfu/g. It was a similar count as for peeled shrimps, but those after 72 h storage. Unpeeled *Litopenaeus vannamei* after 24 h storage had a similar count of *S. aureus* (Tab. 4) as the fresh shrimps (1.6 –2.05 log cfu/g) tested by Tawade et al. [2019]. The literature data indicates that people with a cold or sore throat could have enterotoxic strains of staphylococcus on their hands and then transfer them to food [Amin, Ahemd and Ahmed 2021]. Gram-negative psychrotrophic microorganisms *Moraxella*, *Aeromonas*, *Pseudomonas*, and *Acinetobacter*, which cause spoiling and have proteolytic properties, are the main group of organisms that develop in crustaceans [Premaratne, Nip and Moy 1986]. Premaratne, Nip and Moy [1986] showed that psychrotrophic bacteria count on day 0 and 4 of shrimp storage at 5°C remained at the same level (6.2 log cfu/g). This count was almost twice as high as that obtained in this study for the control sample and after 24 h of storage (Tabs. 3 and 4). Ever after 3 days of storage, the psychrotrophic bacteria count did not exceed 6 log cfu/g. Leitão and Rios [2000] obtained an average psychrotrophic bacteria count of 5.2 log cfu/g. It was a similar result as that obtained in this study for peeled *Litopenaeus vannamei* after 3 days of storage (Tab. 5).

According to the International Commission on Microbiological Specifications for Foods, fresh shellfish show psychrotrophic bacteria at levels from 3 to 7 log cfu/g. Above 7 log cfu/g, food begins to show organoleptic signs of spoiling (changes in odour, colour and texture) [Leitão and Rios 2000]. The test results of Dabade et al. [2016] showed a similar level of *Pseudomonas* spp. and *Vibrio* spp. bacteria contamination in their test shrimps, at a level of 3.6–4.5 log cfu/g. In this study, the highest count of *V. parahaemolyticus* was found for unpeeled shrimps (3.23 log cfu/g) stored for 72 h, although this was still lower than the results of Dabade et al. [2016]. The *Vibrio* spp. species are among bacteria that very frequently inhabit the digestive tract of shrimps. Consumption of crustaceans not subjected to proper heat treatment can cause food poisoning [Dabade et al. 2016]. Talukder et al. [2019] in their analysed fresh shrimps found the presence of *Vibrio* spp. at the level of 2.06–2.11 log cfu/g, which is slightly higher (by approx. 0.4 logarithmic cycle) from the count obtained in the authors' own study (Tab. 3). Based on the research conducted, large quantities of fungi were found after 72 h of storage (1.5 times more

yeasts than moulds). Kukułowicz [2012] found mould and yeast counts in fresh shrimps at a level of 0.91 and 3.03 log cfu/g, respectively. Compared to the present study, it was about 0.25 logarithmic cycle less moulds and about 1 logarithmic cycle less yeasts. Crustacean contamination with fungi may have been caused by unhygienic environment conditions, poor handling and improper processing. In studying shrimp, Ahmed, ALameed and Khalaf [2015] demonstrated considerable quantities of moulds in them, primarily *Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger*, which are toxigenic, producing mycotoxins, and have a pathogenic effect on humans, damaging the liver and kidneys, which leads to death. Additionally, they found the presence of *Cladosporium herborum Rhizopus spp., Saccharomyces cerevisiae*, as well as *Candida kefyer, Candida tropicalis, Candida gullimodii* and *Cryptococcus neoformans* yeasts. *Candida* can cause systemic infections, particularly in patients with lowered immunity.

5. CONCLUSIONS

1. After 24 h of storage, a significantly higher count of *S. aureus* and psychrotrophic bacteria was observed in the unpeeled shrimp group.
2. After 72 h of *Litopenaeus vannamei* cold storage, there were no significant differences between the counts of microorganisms present in the peeled and unpeeled shrimps.
3. At the end of storage, a higher growth of microbes was shown in the peeled shrimps, as compared to the unpeeled ones.
4. The test shrimps, when stored for 3 days, were characterised by high counts of fungi, which can have an adverse effect on the human body.
5. To avoid food poisoning, shrimps must undergo a suitable method of heat treatment.

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