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Effects of Nisin Treatment on the Shelf Life of Ready-to-Eat Roasted Shrimp (*Penaeus vannamei*)

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Abstract: The microbiological, biochemical, and physicochemical changes of ready-to-eat shrimps (*Penaeus vannamei*) with high water content, subjected to nisin treatment in combination of hurdle technology, were investigated. The ready-to-eat shrimps were processed by boiling, drying, treatment with nisin solution, seasoning, and roasting, followed by vacuum packaging, sterilization, and storage at room temperature (25°C). The results showed that the samples treated with nisin in combination with other hurdles resulted in a significant decrease in bacterial counts (*Bacillus cereus* and native microflora) compared to the control samples. Additionally, the nisin-treated samples possessed better biochemical and physicochemical properties, as well as better sensory patterns. According to the safety guidelines for roasted shrimp (SC/T 3305-2003), the shelf life of ready-to-eat shrimp with 48–53% moisture content was extended by nisin application at concentrations of 60 and 100 mg/kg of nisin; specifically, ready-to-eat shrimp maintained good quality from 4–6 days up to 6–12 and 8–14 days corresponding to 60 and 100 mg/kg of nisin treatments, respectively. Nisin treatment combined with hurdle technology in the production of ready-to-eat shrimp provides a highly valued product in China.

Keywords: Natural Antimicrobials, Hurdle Technology, Ready-to-Eat Shrimp, Shelf Life, Spoilage Organisms

1. Introduction

Current consumer demand for safe, high quality food prepared without chemical preservatives but with a long shelf life requires new preservation techniques [1, 2]. Hurdle technology aims to reduce pathogenic and spoilage organisms while improving total nutritional quality by applying combinations of hurdles [3, 4]. The most important hurdles used for food preservation are temperature (high or low), water activity (aw), acidity (pH), redox potential (Eh), preservatives, and competitive microorganisms. The hurdle effect is of fundamental importance for the preservation of foods. Such technologies meet both industrial and consumer demands for improved quality including organoleptic and nutritional value, and sustained microbiological safety [5].

China is one of the largest shrimp farming countries in the world. Ready-to-eat shrimp is a novel domestic product that is convenient for the consumer [6]. The characteristics of ready-to-eat shrimp products are low pH (pH > 5.0) and high water activity (aw > 0.9), which make this product highly susceptible to spoilage and pathogenic bacteria. The development of an effective treatment method to prepare a shelf-stable and microbiologically-safe product of high quality is necessary to meet consumer demand. Bacillus cereus, which has thermal resistant strains, is a dominant organism in the spoilage process of ready-to-eat shrimp [7] and other food commodities such as pastry products, meats, other seafoods, and rice [8, 9]. Its spores are difficult to kill using typical hurdles [10], so antimicrobial natural compounds make an attractive alternative method to inhibit *Bacillus cereus* colonization.

Nisin produced by *Lactococcus lactis*, is a small, natural, heat-stable peptide comprised of 34 amino acids [11]. The use of this compound as a food preservative was approved by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 1969 and by the FDA in 1988 [12]. Nisin is a commercially available

bacteriocin and has been used as a food preservative since the 1940s in more than 50 countries [13]. It is in the generally recognized as safe (GRAS) category of food additives and is an effective bactericidal agent against gram-positive bacteria (GPB) including *Lactococcus* spp., *Listeria* spp., *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Lactobacillus* spp., *Mycobacterium* spp. and also spore-forming bacteria [14]. Nisin has a dual mode of action by blocking cell wall biosynthesis and inducing pore formation in the cell membranes of GPB, leading to the leakage of intracellular compounds and disruption of proton motive force [1, 15]. Many researchers have demonstrated its potential as a biopreservative to control pathogenic and spoilage bacteria in food products [15].

Water content and sterilization conditions are essential preservation hurdles in the processing of ready-to-eat shrimp. In a previous study, water content and secondary sterilization methods were optimized for ready-to-eat shrimp [16]. These optimized conditions did not completely kill spore-former strains of *B. cereus*. In this study, we investigated the effects of nisin treatment in combination with the water activity and sterilization hurdles to determine their effects on shelf life and sensory properties of ready-to-eat shrimp.

2. Materials and Methods

2.1. Bacterial Strains and Growth Medium

B. cereus DH8003 was previously isolated from ready-to-eat roasted shrimp and stored in 13% glycerol at −80°C. The bacteria were grown in brain heart infusion medium (BHI; Oxoid, Basingstoke, England) at 30°C.

2.2. Nisin Solution Preparation and Antimicrobial Activity Assay

A nisin solution was prepared in sterile distilled water (1 mg/mL) and stored at −18°C prior to use. The antimicrobial activity of nisin was determined using a microtiter plate assay as previously described [17]. The bacteriocin unit was defined as the amount of nisin required to inhibit 50% of the indicator strain *B. cereus* DH8005 in the assay. The minimum inhibition concentration (MIC) was defined as the concentration of nisin required to inhibit 50% of the indicator strain at OD600 of 0.4–0.5.

2.3. Preparation and Treatment of Ready-to-Eat Roasted Shrimp

Frozen shrimps (*Penaeus vannamei*) were obtained and transferred on ice to the lab within 6 h. The shrimps used in the experiments were selected to be approximately 20 g. They were thawed in running tap water, beheaded, peeled, removed their gonads and washed with tap water. The shrimps were then cooked in boiling water for 15 min, and then placed in ice-cold water for 10 min. Subsequently, the shrimps were maintained at room temperature until the water was drained off. The shrimps were weighed and divided into four groups. The control group received no nisin treatment, while the other three groups were sprayed with nisin solution to final concentrations of 20, 60, and 100 mg/kg shrimp. In addition, sucrose (11.2%, w/w), NaCl (1.9%, w/w), and monosodium glutamate (0.9%) were added as seasonings to all groups. The seasoned shrimps were mixed well and maintained at 4°C for 16 h. During the seasoned period, the shrimps were stirred every 3 h to distribute the seasoning equally. The shrimp were then spread evenly on a tray and dried in an oven at 180°C for 20 minutes. The shrimps were sealed in sterile plastic bags/aseptic bags and kept at 4°C for 24 h to equilibrate the water contents. After water equilibration, they were roasted at 170°C on a baking tray until they reached the optimal temperatures to result in three different water contents (48%, 51%, and 53%). Each nisin treatment group now is divided into water content three groups (Table 1). After cooling at room temperature for 15 min, the roasted shrimps were immediately vacuum-sealed individually into sterile flexible packages and then kept at 4°C for 48 h. The packaged shrimps were sterilized at 90°C for 40 min and cooled in running water for 20 min. The vacuum-sealed products with intact packages were selected and stored at 25°C. Biochemical, microbiological, and pH measurements, and sensory evaluations of the ready-to-eat shrimp were performed at 0, 2, 4, 6, 8, 10, 12, 14, and 16 days.

Table 1. *Summary of moisture contents and concentration of nisin in 12 groups.*

Group name	Moisture contents (%)	Concentration of nisin (mg/kg)
0-48	48	0
0-51	51	0
0-53	53	0
20-48	48	20
20-51	51	20
20-53	53	20
60-48	48	60
60-51	51	60
60-53	53	60
100-48	48	100
100-51	51	100
100-53	53	100

a "A"-"B", the number "A" stands for final concentration of nisin in roast shrimps, and the number "B" stands for moisture content of roasted shrimp

2.4. Microbial Analysis

Ready-to-eat shrimp samples (10 g) were aseptically trimmed and then transferred into a sterile stomacher bag with 90 mL of 0.85% saline. The mixture was homogenized in a stomacher (Lab Blender Stomacher 400, Seward, Mo, USA) for 2 min. Serial 10-fold dilutions of the bacterial suspensions were prepared with 0.85% saline. Each dilution was inoculated in triplicate onto the indicated agar media. Total viable counts (TVCs) were measured using the spread plate method on BHI agar plates. Mannitol yolk polymyxin B agar plates (MYP, Shanghai reagent providing and research center, Shanghai, PR China) were utilized for *Bacillus* species detection and enumeration. All plate counts were performed in triplicate after incubation at 37°C for 24 h.

2.5. Biochemical and pH Analyses

Total volatile basic nitrogen (TVB-N) was determined according to method GB 5009.228-2016 (National food safety standard determination of total volatile basic nitrogen in foods, semi-micro determination), and the results were expressed as mg N/100 g of ready-to-eat shrimp. The pH values of the stomacher bag supernatants were measured by pH electrode (Weiye Pty. Ltd., shanghai, PR China) at room temperature after samples were plated.

2.6. Sensory Evaluation

The sensory evaluations of both the control and nisin-treated samples were conducted by six trained panelists from the Food Science faculty in accordance with the methods of the China National Standard (SC/T 3305-2003) (Ministry of Health 2003). Scores were assigned based on the color, odor, surface viscosity, flexibility, and clarity of ready-to-eat shrimp using a 10-point hedonic scale (Table 2). A sensory score of 20 was considered the cutoff for acceptance.

Table 2. *Sensory evaluation scale of roasted shrimp.*

Parameter	Scale		
	10	**5**	**0**
Appearance	No water on surface of shrimp	Little water surface of shrimp	Lot of water surface of shrimp
Colour	Even; glossy; light red colour;	General even; dull in colour	Uneven; Dim; Dark red colour;
Smell	Rich roasted shrimp odor	Poor roasted shrimp odor; little unpleasant odor	No roasted shrimp odor; Unpleasant odor
Texture	High elasticity	Low elasticity; Loose	No elasticity; Loose; Soft

2.7. Statistical Analysis

All experiments were performed in triplicate. Values are expressed as the mean ± standard deviation. TVCs were recorded and expressed as log (CFU/g) before performing ANOVA (one-way). All calculations were performed using the statistical analysis software SPSS 13.0. Statistical significance was defined as $p < 0.05$.

3. Results and Discussion

3.1. Minimum Inhibition Concentration of Nisin

In our study, nisin exhibited antimicrobial activity against *B. cereus*, and its MIC was 100 ± 34 mg/L for *B. cereus*. He et al. found that the MIC of nisin was 156 mg/L for *B. subtilis* [11, 17].

3.2. Moisture Content and Sensory Characteristics of Ready-to-Eat Shrimp

Water content is an effective hurdle in the food safety industry. Studies have found a positive correlation between aw and high moisture content [16]. Thus, aw is used as an alternative hurdle to water content. Reducing aw below 0.90 can partially inhibit the growth of spoilage bacteria in perishable food [6]. However, the treatments used to lower water activity can cause a loss of color, texture, and flavor in the product. These properties directly impact the sensory properties and consumer reception of the products.

In our study, The results showed that moisture content of ready-to-eat shrimp between 45% and 55% results in a product with a good taste property. In addition, the optimal sterilization condition was heating at 90°C for 30 min, which did not affect the moisture content of ready-to-eat shrimp. Therefore, we used these parameters in the whole process.

3.3. Effects of Nisin on Microbial Growth in Ready-To-Eat Shrimp

Microbial growth is major cause of spoilage in seafood. Inhibiting the growth of microorganisms with the addition of natural additives can prolong the shelf life of seafood. Table 3 shows the TVCs of ready-to-eat shrimp after storage at 25°C following different nisin treatments. All experimental groups exhibited reduced growth compared to the control group based on aerobic plate counts. According to the standard SC/T 3305-2003 (roast shrimp) (Ministry Of Health 2003), ready-to-eat shrimp typically possess unacceptable sensory properties when TVCs reach 4.5 log CFU/g. We used this TVC standard to predict shelf life of the shrimp products. The shelf lives of the control groups (0 mg/kg nisin) with 48%, 51%, and 53% water content were 6, 6, and 4 days, respectively. Shelf lives in the 20 mg/kg nisin experimental groups with 48%, 51%, and 53% water content were successfully extended to approximately 7 d, 7 d, and 6 d, respectively. This was further increased in the 60 mg/kg nisin groups 48%, 51%, and 53% water content to 12 d, 8 d, and 6 d, respectively. The 100 mg/kg nisin experimental groups 48%, 51%, and 53% water content had shelf lives of 14 d, 10 d, and 8 d, respectively. Differences in TVCs between the control and 20 mg nisin/kg shrimp-treated groups at the testing points were not significant. However, bacterial growth was more effectively inhibited in the 60 and 100 mg nisin/kg shrimp treatment groups compared to the 20 mg nisin/kg shrimp treatment group after day 4.

B. cereus is a spoilage organism and is ubiquitous in food. It is difficult to completely sterilize mildly processed food due to this organism. Table 4 shows the effects of different nisin concentrations on *B. cereus* growth in ready-to-eat shrimp during storage at 25°C. The number of *B. cereus* in ready-to-eat roasted shrimp was inversely correlated with the shelf life of the product. The Bacillus plate counts correlated closely with the TVCs. *B. cereus* accounted for approximately 90% of the total aerobic plate counts in ready-to-eat shrimp stored at 25°C at the end of the shelf life (Tables 3 and 4), indicating that it plays a role as the dominant spoilage organism in this food product. These results correlate with a previous study by Wang et al. that reported *B. cereus* comprised approximately 60% of all

bacteria at the end of the shelf life in ready-to-eat roasted shrimp stored at 36 ± 1°C [7].

Table 3. *TVCs of aerobic microbes of ready-to-eat shrimp following nisin treatment during storage at 25°C.*

Name	Storage period (days)							
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**
0-48	0.00±0.00a	0.00±0.00a	3.67±0.02b	4.75±0.01c	5.12±0.00d			
0-51	0.00±0.00a	1.69±0.12b	4.31±0.01c	5.26±0.33d	5.93±0.02e			
0-53	0.00±0.00a	2.95±0.07b	4.78±0.03c	5.46±0.19d				
20-48	0.00±0.00a	0.00±0.00a	3.17±0.12b	4.48±0.03c	4.74±0.44cd	5.16±0.03d		
20-51	0.00±0.00a	1.48±0.00b	3.43±0.00c	4.47±0.24d	4.96±0.01e			
20-53	0.00±0.00a	2.78±0.00b	3.93±0.03c	4.57±0.04d	5.04±0.14e			
60-48	0.00±0.00a	0.00±0.00a	0.00±0.00a	2.60±0.02b	3.98±0.01c	4.30±0.01d	4.78±0.03e	5.00±0.02f
60-51	0.00±0.00a	0.00±0.00a	2.90±0.04b	3.84±0.00c	4.74±0.02d	5.97±0.03e		
60-53	0.00±0.00a	0.00±0.00a	3.78±0.03b	4.94±0.40c	5.29±0.00c			
100-48	0.00±0.00a	0.00±0.00a	0.00±0.00a	2.78±0.03b	3.18±0.02c	3.88±0.03d	4.48±0.01e	4.95±0.00f
100-51	0.00±0.00a	0.00±0.00a	0.00±0.00a	3.90±0.02b	4.25±0.03c	4.93±0.01d	5.07±0.02e	
100-53	0.00±0.00a	0.00±0.00a	3.93±0.01b	4.39±0.02c	5.02±0.02d	5.89±0.31e		

Values are means ± standard deviations of three replicates experiments. Mean values expressed in log CFU g^{-1}
Mean values in the same row with the same letter are not significantly ($P > 0.05$)
Corresponding numbers at the end of shelf life are in bold.

Table 4. *Numbers (log CFU g^{-1}) of Bacillus cereus of ready-to-eat shrimp following nisin treatment during storage at 25°C.*

Name	Storage period (days)							
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**
0-48	0.00±0.00a	0.00±0.00a	3.37±0.04b	4.60±0.03c	4.76±0.01d			
0-51	0.00±0.00a	1.47±0.10b	4.19±0.02c	5.04±0.17d	5.71±0.01e			
0-53	0.00±0.00a	2.69±0.12b	4.72±0.32c	5.45±0.03d				
20-48	0.00±0.00a	0.00±0.00a	3.13±0.01b	4.22±0.03c	4.54±0.00d	5.02±0.01e		
20-51	0.00±0.00a	1.00±0.00b	3.37±0.01c	4.29±0.01d	4.73±0.01e			
20-53	0.00±0.00a	2.40±0.01b	3.76±0.01c	4.27±0.27d	4.78±0.01e			
60-48	0.00±0.00a	0.00±0.00a	0.00±0.00a	2.00±0.08b	3.74±0.01c	4.12±0.16d	4.56±0.03e	4.65±0.11e
60-51	0.00±0.00a	0.00±0.00a	2.48±0.02b	3.68±0.00c	4.59±0.02d	5.68±0.11e		
60-53	0.00±0.00a	0.00±0.00a	3.22±0.02b	4.88±0.01c	4.96±0.08c			
100-48	0.00±0.00a	0.00±0.00a	0.00±0.00a	2.54±0.00b	3.57±0.00c	3.74±0.02d	4.00±0.04e	4.75±0.13f
100-51	0.00±0.00a	0.00±0.00a	0.00±0.00a	3.48±0.02b	4.00±0.08c	4.66±0.16d	4.81±0.12d	
100-53	0.00±0.00a	0.00±0.00a	3.63±0.00b	4.25±0.03c	4.74±0.01d	5.69±0.19e		

Values are means ± standard deviations of three replicates experiments. Mean values expressed in log CFU g^{-1}
Mean values in the same row with the same letter are not significantly ($P > 0.05$)
Corresponding numbers at the end of shelf life are in bold.

In our study, we added nisin to ready-to-eat roasted shrimp to inhibit the growth of bacteria with the goal of extending the shelf lives of products with high moisture contents. Our results predicted that an extension of up to 8 days is possible. Thus, we were able to extend the shelf life and sensory properties of ready-to-eat shrimp, a food with a high moisture content, by treatment with nisin.

3.4. pH Changes

pH can be used as an indicator of protein and nucleotide degradation during storage of shrimp. The increase in pH during storage is due to the release of volatile alkali substances produced by microorganisms [18, 19]. The changes in pH of ready-to-eat shrimp following nisin treatment during storage at 25°C are listed in Table 5. The initial pH for all groups was 6.72–6.82; this pH and high water content are optimal for microbial growth. During storage, the pH of other groups increased slightly. Therefore, the amount of nisin combined with the water contents was in good agreement with the formulations. During the 14 days of storage, the pH of the 0-53 control group showed a slight decline. The most significant change in pH was observed in the 20-53 group, which increased by 0.54 (from 6.74 to 7.28). The pH value in the 100-48 group changed slightly from 6.79 to 6.97. During storage, the pH values of the experimental groups increased slowly and fluctuated in a narrow range compared to the control groups except for the 0-53 control group. The decrease in pH of the 53% water content control group could be attributed to fat oxidation and decomposition or microbial metabolism, which can produce some acidic substances and lead to a decrease in pH. Considering the slow growth of spoilage microorganisms that metabolize ammonium and other volatile nitrogenous compounds, nisin effectively inhibited spoilage bacteria indirectly, extending the shelf lives of the products. Similar applications of nisin against spoilage bacteria have been reported [1].

Table 5. *Change in pH of ready-to-eat shrimp following nisin treatment during storage at 25°C.*

Name	Storage period (days)								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
0-48	6.77±0.03a	6.78±0.00a	6.84±0.03a	6.89±0.06a	6.96±0.03b	7.04±0.06b	7.09±0.03c	7.18±0.01d	7.23±0.00d
0-51	6.76±0.01a	6.89±0.01ab	6.90±0.06ab	7.00±0.01b	7.09±0.04b	7.09±0.01c	7.12±0.03c	7.18±0.00c	7.25±0.00c
0-53	6.79±0.00a	6.82±0.04a	6.90±0.04a	7.15±0.06ab	7.28±0.03ab	7.34±0.03cd	7.18±0.00d	6.98±0.00cd	6.85±0.01bc
20-48	6.76±0.03ab	6.77±0.03ab	6.78±0.01a	6.79±0.01ab	6.86±0.04ab	6.99±0.03b	7.03±0.01c	7.09±0.04d	7.13±0.03d
20-51	6.79±0.03a	6.90±0.04ab	6.88±0.00abc	6.92±0.01abc	7.04±0.06bc	7.09±0.03c	7.16±0.01d	7.20±0.03de	7.26±0.03e
20-53	6.74±0.01a	6.90±0.01ab	6.89±0.01ab	6.90±0.04ab	7.09±0.00ab	7.12±0.01bc	7.19±0.01cd	7.25±0.06cd	7.28±0.06d
60-48	6.79±0.04a	6.81±0.01a	6.80±0.04ab	6.87±0.01a	6.94±0.00ab	7.01±0.00bc	7.02±0.00cd	7.09±0.01d	7.15±0.00d
60-51	6.72±0.03a	6.75±0.03a	6.88±0.01a	6.96±0.06ab	6.99±0.01bc	7.10±0.03cd	7.18±0.03de	7.20±0.00ef	7.23±0.01f
60-53	6.80±0.01a	6.90±0.04a	6.91±0.03a	6.82±0.01ab	7.00±0.03a	7.09±0.00ab	7.13±0.03bc	7.23±0.03c	7.29±0.01c
100-48	6.79±0.04ab	6.77±0.00ab	6.82±0.04a	6.81±0.00ab	6.89±0.03ab	6.91±0.03bc	6.92±0.01bc	6.95±0.01c	6.97±0.00c
100-51	6.82±0.04a	6.86±0.01ab	6.88±0.03ab	6.96±0.04ab	6.97±0.03bc	7.01±0.03cd	7.03±0.03d	7.10±0.03d	7.14±0.01d
100-53	6.76±0.00a	6.95±0.04ab	6.96±0.01ab	7.00±0.03b	6.92±0.03b	7.14±0.01bc	7.19±0.04bc	7.23±0.01cd	7.26±0.01d

Values are means ± standard deviations of three replicates experiments.
Mean values in the same row with the same letter are not significantly (P > 0.05)
Corresponding numbers at the end of shelf life are in bold.

3.5. Total Volatile Basic Nitrogen of Ready-to-Eat Shrimp

TVB-N is a product of microbial spoilage from the metabolism of protein or non-protein nitrogen to trimethylamine, dimethylamine, and ammonia. It is often used as an index to evaluate the quality and shelf life of seafood products [11]. When the TVB-N values reach 30 mg/100 g, the products are considered unacceptable or at the end of their shelf life according to the standard SC/T 3305-2003 (roasted shrimp) (Ministry of Health 2003). Table 6 shows the TVB-N values of ready-to-eat shrimp during storage at 25°C. The initial TVB-N values for all groups were 6.98–8.92 mg/100 g. The low TVB-N values correlated with the low initial TVC, and indicate the high initial quality of the products. Relatively high TVB-N values have been reported in frozen shrimp (17.97–21.63 mg/100 g) [20]. When the bacterial count reached approximately 3 log CFU/g, the TVB-N values started to significantly increase. TVB-N values reached 18.20–28.52 mg/100 g at the end of the shelf life. The TVB-N values were 22.00 ± 0.76 mg/100 g for group 20-48 on day 8, 22.92 ± 1.30 mg/100 g for group 60-48 on day 12, and 20.86 ± 1.02 mg/100 g for group 100-48 on day 14. In the 20-51, 60-51, and 100-51 groups, the TVB-N values were 24.36 ± 1.87, 23.88 ± 1.67, and 18.20 ± 1.81 mg/100 g, respectively, at the end of the shelf life. In the 20-53, 60-53, and 100-53 groups, the TVB-N values were 28.52 ± 2.63, 24.20 ± 0.96, and 22.40 ± 1.96 mg/100 g, respectively, at the end of the 16-days storage period. Over the storage period, the TVB-N values of the control ready-to-eat shrimp were higher than the nisin treated shrimp on the same days. The differential increase in TVB-N values at the end of the shelf life of different batches could have been due to nitrogen production, which corresponded to the growth of bacteria and pH values in our study. Due to the antimicrobial activity of nisin, higher concentrations of nisin correlated with lower TVB-N values in groups with the same moisture content.

Table 6. *Changes in TVB-N of ready-to-eat shrimp following nisin treatment during storage at 25°C.*

Name	Storage period (days)							
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**
0-48	7.58±0.71a	8.02±0.88a	10.68±1.24b	15.76±1.24c	23.20±0.51d			
0-51	7.88±0.76a	10.02±2.88a	18.48±2.26b	25.12±1.61c	29.08±0.57c			
0-53	8.00±1.44a	13.24±2.21a	23.84±1.87b	29.40±2.15c				
20-48	8.36±0.65a	9.38±1.47a	13.80±0.99b	18.76±1.05c	22.00±0.76d	26.06±0.54e		
20-51	7.48±0.51a	13.00±0.62b	18.20±1.05c	19.88±0.40c	24.36±1.87d			
20-53	8.72±0.48a	18.48±0.74b	25.20±0.96c	28.52±2.63cd	32.20±2.46d			
60-48	7.80±1.53a	7.76±0.99a	8.52±1.44a	11.84±2.60a	15.88±0.17b	19.88±1.70c	22.92±1.30cd	25.92±2.49d
60-51	7.24±0.31a	8.34±0.91a	10.24±0.65a	16.44±1.58b	23.88±1.67c	28.16±1.78d		
60-53	7.72±0.99a	7.24±1.05a	17.44±1.24b	24.20±0.96c	30.92±1.27d			
100-48	6.98±1.64a	7.68±1.39ab	7.92±0.51ab	10.08±1.44bc	11.72±0.06cd	13.36±1.41d	17.30±0.82e	20.86±1.02f
100-51	8.14±2.55a	8.02±1.87a	8.76±0.76a	11.36±0.88a	16.52±0.68b	18.20±1.81b	25.20±2.80c	
100-53	8.92±1.22a	9.22±0.91a	11.48±1.41ab	13.72±0.71b	22.40±1.16c	27.68±1.47d		

Values are means ± standard deviations of three replicates experiments. Mean values expressed in mg/100g.
Mean values in the same row with the same letter are not significantly (P > 0.05)
Corresponding numbers at the end of shelf life in bold.

3.6. Sensory Properties

Sensory evaluation quickly and directly measures the product qualities important to consumer acceptance. Table 7 shows the sensory scores of ready-to-eat shrimps during storage at 25°C. The sensory properties of all experimental groups obtained higher scores than the control group (Table 7). The sensory scores in the experimental groups slowly

declined within 16 d. When sensory scores of an experimental group dropped below the borderline value, the aerobic bacteria plate count was ≥ 4.5 log CFU/g. The sensory score of the 0-48 control group reached 20 on 6 d, whereas the 100-48 experimental group scores remained in the acceptable range up to 14 d.

Table 7. *Sensory scores of ready-to-eat shrimp during storage at 25°C.*

Name	Storage period (days)							
	0	2	4	6	8	10	12	14
0-48	39.83±0.41^{g}	35.67±2.07^{f}	26.33±1.97^{e}	17.33±2.07^{d}	11.67±2.16^{c}	3.00±2.10^{b}	0.00±0.00^{a}	0.00±0.00^{a}
0-51	40.00±0.00^{g}	31.50±1.38^{f}	23.33±2.94^{e}	18.83±1.47^{d}	10.50±1.52^{c}	3.33±1.97^{b}	0.00±0.00^{a}	0.00±0.00^{a}
0-53	40.00±0.00^{f}	29.67±2.07^{e}	18.83±1.47^{d}	11.33±1.63^{c}	3.50±1.22^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
20-48	39.67±0.52^{g}	38.50±2.07^{g}	35.50±1.87^{f}	28.50±1.76^{e}	18.83±1.47^{d}	12.50±1.52^{c}	3.33±2.16^{b}	0.00±0.00^{a}
20-51	39.83±0.41^{g}	34.67±1.63^{f}	27.17±1.83^{e}	18.50±1.38^{d}	11.67±2.16^{c}	5.83±1.17^{b}	0.00±0.00^{a}	0.00±0.00^{a}
20-53	40.00±0.00^{f}	31.33±1.21^{e}	23.00±2.37^{d}	15.50±1.38^{c}	3.83±1.60^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
60-48	40.00±0.00^{f}	39.83±0.41^{f}	39.83±0.41^{f}	34.50±1.87^{e}	29.50±2.07^{d}	24.17±1.94^{c}	20.00±1.90^{b}	16.33±1.51^{a}
60-51	40.00±0.00^{g}	40.00±0.00^{g}	35.83±1.47^{f}	28.67±1.97^{e}	19.83±1.72^{d}	12.33±1.75^{c}	4.50±1.87^{b}	0.00±0.00^{a}
60-53	40.00±0.00^{g}	32.00±1.90^{f}	27.17±1.83^{e}	19.00±1.26^{d}	11.83±1.94^{c}	4.00±1.79^{b}	0.00±0.00^{a}	0.00±0.00^{a}
100-48	39.83±0.41^{f}	39.83±0.41^{f}	39.33±0.82^{f}	37.50±1.38^{e}	32.67±2.25^{d}	28.33±1.86^{c}	24.17±1.94^{b}	19.00±1.90^{a}
100-51	40.00±0.00^{g}	40.00±0.00^{g}	37.17±2.14^{f}	31.83±1.33^{e}	27.17±1.60^{d}	17.67±1.86^{c}	12.5±1.52^{b}	4.17±1.47^{a}
100-53	40.00±0.00^{g}	39.67±0.82^{g}	36.83±2.40^{f}	30.83±1.47^{e}	21.33±1.97^{d}	16.33±1.75^{c}	3.67±1.86^{b}	0.00±0.00^{a}

Mean values in the same row with the same letter are not significantly (P > 0.05)

Spoilage microorganisms can produce volatile compounds which affect the odor of the products. The greater retention of smell property in the experimental groups compared to the control groups is due to nisin inhibiting the growth of spoilage microorganisms. In contrast, the color in all groups was not affected by nisin or water content. Peeling procedures after shrimps are cooked can prevent the loss of astaxanthin during preparation. Roasted shrimp with high water content in general show good texture properties and maintained high texture sensory scores. Similarly, most seafood products become less elastic when proteins denature during heating. In general, treating ready-to-eat roasted shrimp with nisin can extend their shelf life, and their maintain good taste and texture qualities.

4. Conclusion

Many food preservation techniques have been used to control microbial growth. However, some of these techniques adversely affect the organoleptic properties of foods and reduce consumer acceptance [5], which are not acceptable in the processing of high-moisture seafoods. The high content of water and nutrients in seafood make them conducive to spoilage by microorganisms. Therefore, novel preservation methods are needed to combat this difficult combination of properties. In our study, nisin combined with reduced moisture contentresulted in the inhibition of TVCs, particularly *B. cereus*, in ready-to-eat shrimp stored at room temperature (25°C). Throughout the experiments, the pH and TVB-N values of shrimp surfaces increased slightly. According to the standard of SC/T 3305-2003 (Ministry of Health 2003), we demonstrated that nisin treatment can extend the shelf life of ready-to-eat shrimp up to an additional 8 days.

A limitation of using nisin in food preservation applications is its spectrum of antimicrobial activity against GPB [21]. Therefore, nisin in combination with other preservatives that target gram negative bacteria would produce better outcomes than nisin alone [11, 13]. Further studies are required to establish the proper combination of nisin and other natural antimicrobial agents to control bacterial growth and increase the shelf life of ready-to-eat shrimp. In addition, water-activity-lowering agents can also be applied to ready-to-eat shrimp. In the future, hurdle technology can be applied to high-moisture aquatic products (aw > 0.9) to maintain their quality and extend their shelf lives.

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