ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Effects of Vacuum and Modified Atmosphere Packaging on Physicochemical and Microbiological Quality of Cooked Squid Stuffed with Sticky Rice During Refrigerated Storage

Kamontip Kanpairo[1*], Suteera Srisuk[1], and Pharadee Phalachai[1]

[1] Faculty of Science Technology and Agriculture, Yala Rajabhat University, Yala, 95000, Thailand
* Correspondence: kamontip.k@yru.ac.th

Citation:
Kanpairo, K.; Srisuk, S.; Phalachai, P. Effects of vacuum and modified atmosphere packaging on physicochemical and microbiological quality of cooked squid stuffed with sticky rice during refrigerated storage. *ASEAN J. Sci. Tech. Report.* **2026**, *29*(12), e263971. https://doi.org/10.55164/ajstr.v29i12.263971

Article history:
Received: February 27, 2026
Revised: June 26, 2026
Accepted: August 16, 2026
Available online: November 9, 2026

Publisher's Note:
This article is published and distributed under the terms of the Thaksin University

Abstract: Stuffed squid with sticky rice (*Tupa Sutong*) is a traditional seafood product from southern Thailand with limited refrigerated shelf life because of rapid microbial spoilage and quality deterioration. This study evaluated the effects of atmospheric packaging (AP), vacuum packaging (VP), and modified atmosphere packaging (MAP; 50% O_2, 25% CO_2, and 25% N_2) on the physicochemical and microbiological quality of cooked stuffed squid during refrigerated storage (4 ± 2 °C) for 24 days. Changes in hardness, pH, total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), and total viable count (TVC) were determined throughout storage. Storage time significantly affected all quality parameters ($p \leq 0.05$). Samples stored under AP deteriorated most rapidly, showing faster increases in TVB-N, TMA-N, TBA, and TVC, accompanied by a greater reduction in texture quality. Both VP and MAP delayed quality deterioration by slowing microbial growth and chemical changes during storage. Based on the microbiological acceptability limit, the shelf life of the product was 12, 18, and 21 days under AP, MAP, and VP, respectively. Among the packaging systems evaluated, VP provided the greatest extension of refrigerated shelf life while maintaining the physicochemical and microbiological quality of cooked stuffed squid with sticky rice. These findings provide useful information for selecting appropriate packaging systems for the commercial production and distribution of this traditional seafood product.

Keywords: Squid; sticky rice; modified atmosphere packaging; quality change; microbial analysis

1. Introduction

Cooked squid stuffed with sticky rice, locally known as *Tupa Sutong* (or *Tapa Sutong*), is a traditional ready-to-eat seafood product originating from the southern border provinces of Thailand [1]. Similar to the Japanese product *Ikameshi*, it consists of squid filled with glutinous rice and cooked until tender. Unlike *Ikameshi*, however, Tupa Sutong is prepared with coconut milk, which contributes a rich flavor and desirable texture but also increases its susceptibility to quality deterioration during storage [2]. Consequently, the product is generally prepared for immediate consumption or local distribution because of its limited shelf life. The deterioration of Tupa Sutong during refrigerated storage is influenced by the combined characteristics of its three major components. Squid muscle is highly susceptible to microbial spoilage and

enzymatic protein degradation, resulting in the accumulation of volatile nitrogen compounds and undesirable odors [3]. The glutinous rice filling undergoes starch retrogradation during chilled storage, leading to increased firmness and reduced eating quality, while the lipid fraction in coconut milk is prone to oxidation, producing secondary oxidation products that adversely affect flavor and overall acceptability [4]. These deterioration mechanisms occur simultaneously and may interact with one another, resulting in quality changes that are more complex than those observed in single-component seafood products.

Packaging technologies that limit oxygen exposure are widely used to delay spoilage in refrigerated seafood. Vacuum packaging reduces oxygen availability, thereby suppressing the growth of aerobic spoilage microorganisms and slowing oxidative reactions. Modified atmosphere packaging (MAP), particularly systems containing carbon dioxide, further inhibits microbial growth by extending the lag phase and reducing microbial metabolic activity [5]. Nevertheless, the effectiveness of MAP depends on several factors, including gas composition, packaging material, storage temperature, and the physicochemical characteristics of the food matrix [6]. Previous studies have demonstrated the effectiveness of vacuum packaging and MAP for extending the shelf life of seafood products such as fish fillets, shellfish, and cooked seafood [7–8]. However, these studies have primarily focused on single-component muscle foods. Information remains limited for composite ready-to-eat products containing seafood together with starch- and lipid-rich ingredients. In products such as Tupa Sutong, deterioration is expected to be governed by concurrent microbial growth, protein degradation, lipid oxidation, and starch retrogradation. Interactions among these processes may alter spoilage patterns and reduce storage stability in ways that cannot be predicted from studies on seafood alone [9]. Consequently, the applicability of packaging strategies developed for conventional seafood products to this traditional composite food remains unclear.

Therefore, this study evaluated the effects of atmospheric packaging (AP), vacuum packaging (VP), and modified atmosphere packaging (MAP) on the physicochemical, microbiological, and textural quality of cooked squid stuffed with sticky rice during refrigerated storage. By investigating quality changes in a seafood–rice–coconut milk matrix, this study provides new information on the preservation of traditional composite ready-to-eat seafood products and offers practical guidance for shelf-life determination and commercial distribution.

2. Materials and Methods

2.1 Materials

2.1.1 Preparation of Stuffed Squid with Sticky Rice (Tupa Sutong)

The production of stuffed squid with sticky rice (Tupa Sutong) followed a traditional recipe consisting of 36.80% fresh squid (*Uroteuthis chinensis*), 33.20% commercial pasteurized coconut milk, 11.00% short-grain glutinous rice (*Oryza sativa* L. var. *glutinosa*), 18.00% commercial palm sugar, and 1.00% refined iodized salt. The preparation started by cleaning fresh squid (approximately 8–9 cm in mantle length, purchased from a local market in Yala Province, Thailand) by removing the internal organs and eyes, washing thoroughly with potable water, and then storing at 4°C until use. To prepare the glutinous rice, the grains were washed three times and soaked in distilled water for 1 h, followed by soaking in a portion of coconut milk for exactly 20 min at ambient temperature. The soaked glutinous rice was then stuffed into the squid mantle up to two-thirds of its capacity to allow for expansion, and a wooden toothpick was used to secure the opening. For the cooking process, the remaining coconut milk, palm sugar, and salt were combined in a stainless-steel pot and heated at 100°C for 5 min. The stuffed squid was then added and simmered at an operational heating temperature of 100°C for 10 min, ensuring the core temperature of the stuffed squid reached at least 75°C. Afterward, the squid was temporarily removed and set aside. The remaining sauce was continuously simmered at 100°C for approximately 5–10 min until it thickened to a final total soluble solids content of 65 ± 2°Brix. Finally, the cooked squid was coated and mixed thoroughly with the thickened sauce, allowed to cool rapidly to room temperature (25 ± 2°C), and immediately packaged for further treatment.

2.1.2 Packaging Conditions

Cooked stuffed squid with sticky rice (100 g per package) was packed in laminated high-barrier pouches consisting of 15-μm oriented nylon (ONy) and 80-μm linear low-density polyethylene (LLDPE) (6 × 9 inches; water vapor transmission rate < 5 g/m²/day; oxygen transmission rate < 30 cm³/m²/day/atm). Three packaging treatments were evaluated: atmospheric packaging (AP; ambient air), vacuum packaging (VP), and modified atmosphere packaging (MAP; 50% O_2, 25% CO_2, 25% N_2) [10].

2.2 Methods

2.2.1 Textural Analyses

Texture Profile Analysis (TPA) of the cooked samples was performed using a texture analyzer (Model TA.XT2). Samples of stuffed squid with sticky rice were prepared in dimensions of 3 × 3 × 1.5 cm. The operating parameters were set at a cross-head speed of 2 mm/s with a 25 kg load cell. Hardness (N) was recorded, and the results were reported as the mean of at least nine replicates per treatment.

2.2.2 Determination of pH value

The pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 10.0. The pH measurement was performed by weighing a 10 g sample of stuffed squid and homogenizing it with 90 mL of distilled water (achieving a sample-to-water ratio of 1:9, w/v) using homogenizer at a speed of 10,000 rpm for 2 min. The pH measurement was then performed by immersing the electrode directly into the resulting suspension. All measurements were carried out in triplicate per treatment package at each storage interval to ensure precision, and the mean values were reported.

2.2.3 Determination of TVB-N

The total volatile basic nitrogen (TVB-N) content was determined using the modified Conway micro-diffusion method as described by [11]. For reagent preparation, the 2% (w/v) boric acid absorbing solution was prepared by dissolving 5.0 g of boric acid in 100 mL of 95% ethanol, mixing it with 350 mL of distilled water and 5 mL of a mixed indicator (0.066% methyl red and 0.033% bromocresol green in ethanol), titrating with 40% sodium hydroxide until a faint reddish hue appeared, and finally adjusting the volume to 500 mL with 95% ethanol. Other required reagents included a 10% (w/v) trichloroacetic acid (TCA) solution, a 50% (w/v) potassium carbonate (K_2CO_3) solution, and a standardized 0.01 N hydrochloric acid (HCl) solution. For sample extraction, a 10 g sample was homogenized with 90 mL of distilled water for 2 min, and a 5 mL aliquot of this homogenate was subsequently mixed with an equal volume of 10% TCA solution. After allowing the mixture to stand for 15 min, it was filtered through Whatman No. 1 filter paper to obtain a clear filtrate for diffusion analysis. The micro-diffusion process was performed by introducing 1.0 mL of the 2% boric acid absorbing solution containing the mixed indicator into the inner chamber of a Conway cell unit. Concurrently, 1.0 mL of the filtered sample extract and 1.0 mL of the 50% K_2CO_3 solution were added to the outer chamber. The cell was immediately sealed with a greased glass plate and incubated at 37 °C for 120 min. A blank test was prepared simultaneously using 10% TCA instead of the sample extract. Following incubation, the volatile bases trapped in the inner chamber were titrated with the 0.01 N HCl solution until the green color reverted to a pale pink endpoint.

The TVB-N content, expressed as milligrams per 100 grams of sample (mg/100g), was calculated using the equation:

$$\text{TVB-N}\left(\frac{mg\ \%}{100\text{g}}\right) = \frac{\text{N x (a}-\text{b) x f x c x d}}{\text{s}} \text{ x } 100$$

where a and b represent the volumes of HCl (mL) consumed for the sample and blank titrations, respectively; N is the normality of the standard HCl solution (0.01 N); f is the correction factor of the HCl solution; c is the atomic weight of nitrogen (14.007 meq/mL); d is the total dilution factor of the sample extract; and *s* is the initial sample weight (g).

2.2.4 Determination of TMA-N

The Trimethylamine Nitrogen (TMA-N) content was determined using the Conway micro-diffusion method as described by [12]. Briefly, the sample was extracted with 5% trichloroacetic acid (TCA). The extract

was then reacted with neutralized formaldehyde in a Conway dish to fix the ammonia, allowing only TMA-N to be released under alkaline conditions. After incubation, the TMA-N absorbed in the boric acid solution was titrated with standard hydrochloric acid (HCl). The results were expressed as mg N/100g of sample.

2.2.5 Determination of TBA value

Determination of TBA value [13]. TBA (mg malondialdehyde (MDA)/kg) analysis was conducted to determine the lipid oxidation of all test groups. The distillation process was conducted by weighing 10 g of the sample and then homogenizing it with 97.5 mL distilled water and 2.5 mL 4 N HCl. 5 mL was taken from the collected distillate and put into tubes. 5 mL of reactive was added on top of it. The sample tubes were kept in a 90 °C water bath for 35 min. After cooling, the absorbance of the mixture was measured at 532 nm.

2.2.6 Total viable count (TVC)

Determination of total bacterial counts. For total bacterial counts, 0.1 mL of relevant dilutions was inoculated onto sterile nutrient agar plates and spread on the surface using a sterile bent glass rod. Inoculated plates were incubated at 37 °C for 48 h before colonies were counted and reported as colony-forming units/g (CFU/g).

2.2.7 Data analysis

All experiments were performed in triplicate using three independent sample preparations. Data are presented as mean ± standard deviation. Statistical analysis was performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Differences among treatments and storage periods were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Statistical significance was accepted at $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Packaging Condition on Quality Change of Cooked Squid Stuffed with Sticky Rice Product

3.1.1 Textural Analyses

Figure 1 shows the changes in hardness of cooked squid stuffed with sticky rice (*Tupa Sutong*) packaged under atmospheric packaging (AP), vacuum packaging (VP), and modified atmosphere packaging (MAP; 50% O_2, 25% CO_2, 25% N_2) during refrigerated storage (4 ± 2 °C). At the beginning of storage, hardness did not differ significantly among treatments ($p > 0.05$), indicating that all samples had comparable texture immediately after cooking. However, storage time and packaging condition significantly affected hardness throughout refrigerated storage ($p \leq 0.05$).

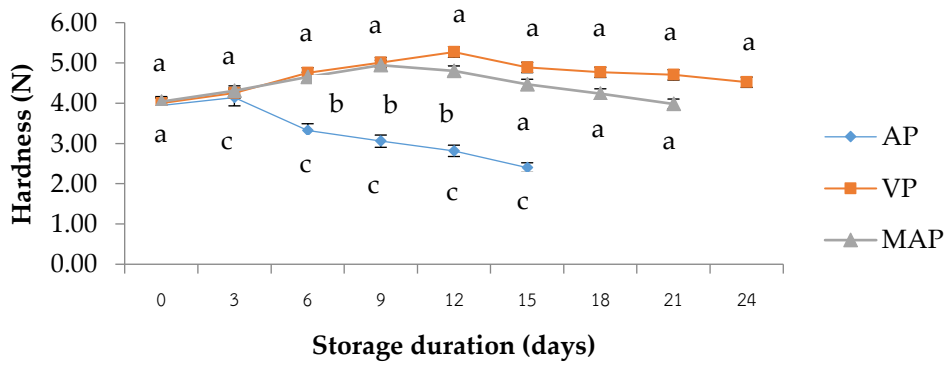


Figure 1. Changes in the hardness (N) of cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 °C for 24 days. Data are presented as mean ± SD ($n = 3$). [a–c] Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

In the AP treatment, hardness increased slightly during the first three days of storage before declining markedly thereafter, reaching 2.40 ± 0.30 N on day 15. The initial increase was most likely associated with starch retrogradation in the glutinous rice, which commonly occurs during chilled storage and temporarily

increases product firmness. As storage progressed, the marked decrease in hardness coincided with rapid microbial spoilage and quality deterioration. Proteolytic enzymes produced by endogenous enzymes and spoilage microorganisms degrade myofibrillar proteins, weaken the muscle structure, and reduce the mechanical strength of squid tissue [14]. At the same time, structural changes in the rice matrix further contributed to the overall softening of the composite product. In contrast, VP maintained product firmness throughout storage, with hardness gradually increasing to 4.52 ± 0.62 N on day 24. The greater hardness observed under VP may be attributed to reduced moisture mobility and the close contact between the packaging film and the product, which limited structural deformation during storage. Oxygen removal also delayed microbial growth and protein degradation, thereby preserving the integrity of the squid muscle. After day 24, hardness decreased slightly, which corresponded with the gradual increase in microbial activity observed during the late stage of storage. Samples packaged under MAP exhibited the most stable textural profile throughout refrigerated storage. Hardness changed only slightly, from 4.04 ± 0.40 N at day 0 to 3.98 ± 0.85 N after 21 days, indicating minimal structural deterioration. The relatively stable texture may be explained by the inhibitory effect of CO_2 on spoilage microorganisms, which reduced proteolytic activity and delayed degradation of the squid muscle proteins. In addition, limiting microbial growth helped maintain the structural integrity of both the squid mantle and the glutinous rice matrix during storage. Although starch retrogradation likely contributed to the slight increase in hardness during the early storage period, its effect was offset by the slower rate of protein degradation under MAP [15]. Overall, the results demonstrate that packaging atmosphere played an important role in maintaining the texture of cooked squid stuffed with sticky rice during refrigerated storage. AP resulted in rapid softening because of accelerated microbial and enzymatic degradation, whereas VP and MAP better preserved product firmness by slowing protein degradation and delaying microbial spoilage. Among the packaging systems evaluated, MAP provided the most stable texture throughout storage, while VP produced the highest hardness values during the later storage period.

3.1.2 Determination of pH value

The changes in pH of cooked squid stuffed with sticky rice (*Tupa Sutong*) packaged under atmospheric packaging (AP), vacuum packaging (VP), and modified atmosphere packaging (MAP) during refrigerated storage (4 ± 2 °C) are presented in Figure 2. The initial pH values were similar among treatments, ranging from 6.43 ± 0.22 to 6.45 ± 0.15 ($p > 0.05$). Both storage time and packaging conditions significantly affected pH during refrigerated storage ($p \leq 0.05$).

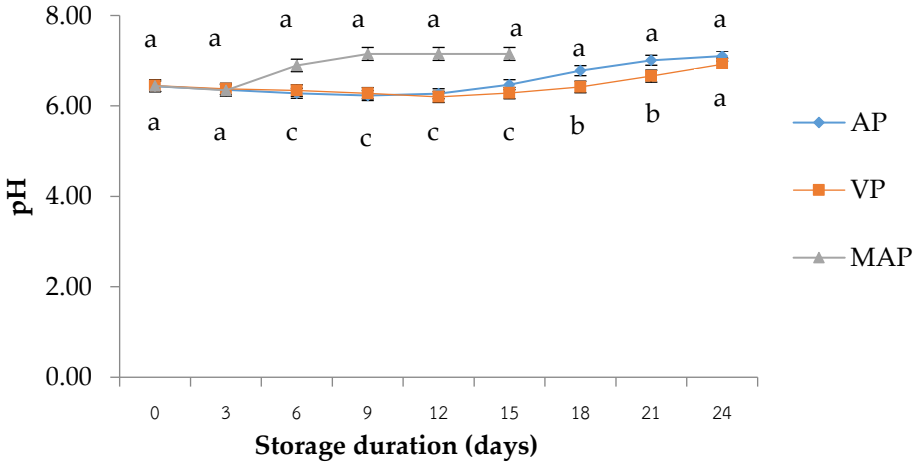


Figure 2. Changes in the pH value of cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 °C for 24 days. Data are presented as mean ± SD ($n = 3$). $^{a–c}$ Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

A slight decrease in pH was observed in all treatments during the first three days of storage. This reduction is commonly associated with post-mortem glycolysis and the production of organic acids during

the early stage of storage. In addition, the initial growth of psychrotrophic lactic acid bacteria may contribute to acid production through the metabolism of soluble carbohydrates present in the glutinous rice, resulting in a temporary decline in pH [16]. After the initial decrease, pH increased progressively in all treatments, although the rate of increase depended on the packaging method. The AP samples exhibited the most rapid increase, reaching 7.60 ± 0.50 by day 12. This increase corresponded with accelerated microbial growth and protein degradation observed during storage. Proteolytic activity of endogenous enzymes together with spoilage microorganisms promotes the formation of alkaline compounds, including ammonia, trimethylamine (TMA), and other volatile basic nitrogen compounds, increasing pH [17]. The rapid rise in pH under AP is therefore consistent with the higher TVB-N and microbial counts observed in this treatment, indicating faster quality deterioration under aerobic conditions. In contrast, VP and MAP significantly delayed the increase in pH ($p \leq 0.05$), with final values of 6.92 ± 0.24 and 7.10 ± 0.40, respectively, after 24 days of storage. The slower increase in VP is mainly attributed to oxygen removal, which limits the growth of aerobic spoilage microorganisms and consequently reduces protein degradation. A similar trend was observed in MAP, where the presence of CO_2 further inhibited microbial growth. Dissolved CO_2 forms carbonic acid, producing a mild acidifying effect while suppressing the growth and metabolic activity of spoilage bacteria. As a result, the formation of alkaline metabolites occurred more slowly than in AP. Overall, the pH changes observed in this study reflected the balance between acid production during the early stage of storage and the subsequent accumulation of alkaline compounds generated through microbial and enzymatic degradation [18]. The lower pH values maintained under VP and MAP were consistent with their reduced microbial growth and lower TVB-N values, demonstrating that both packaging systems effectively delayed chemical and microbiological spoilage during refrigerated storage.

3.1.3 Determination of TVB-N

Total volatile basic nitrogen (TVB-N) is a widely accepted indicator of seafood freshness because it reflects the accumulation of volatile nitrogenous compounds, including ammonia, trimethylamine (TMA), and dimethylamine (DMA), generated during protein degradation by endogenous enzymes and spoilage microorganisms. As shown in Figure 3, both storage time and packaging conditions significantly influenced TVB-N values ($p \leq 0.05$). The initial TVB-N value was 3.50 ± 0.52 mg/100 g and did not differ among treatments.

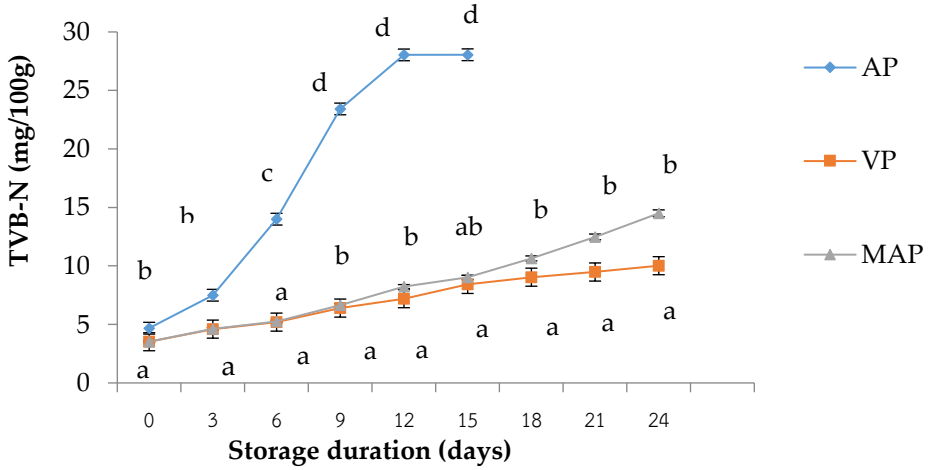


Figure 3. Changes in the TVB-N values of cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 °C for 24 days. Data are presented as mean ± SD ($n = 3$). [a–d] Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

TVB-N increased progressively throughout refrigerated storage under all packaging conditions, although the rate of increase varied markedly with the packaging method. The AP samples exhibited the fastest increase, reaching 28.04 ± 0.80 mg/100 g on day 15, which approached the commonly accepted spoilage limit for seafood products (approximately 30 mg/100 g) [19]. This increase corresponded with visible signs of

spoilage, including slime formation on the squid surface and the development of ammonia-like off-odors. The rapid accumulation of TVB-N under AP is consistent with the unrestricted growth of aerobic spoilage microorganisms, which accelerate proteolysis and the subsequent production of volatile basic compounds. Proteolytic degradation of squid muscle proteins generates peptides and free amino acids that are further metabolized into ammonia, TMA, and other volatile amines, resulting in a progressive increase in TVB-N [20]. The composite nature of Tupa Sutong, which contains squid, glutinous rice, and coconut milk, may further support microbial growth by providing readily available nutrients, thereby accelerating protein degradation during storage. In contrast, VP and MAP significantly delayed the increase in TVB-N ($p \leq 0.05$), reaching only 10.02 ± 0.90 and 14.50 ± 1.02 mg/100 g, respectively, after 24 days of storage. The slower increase observed under VP was associated with oxygen removal, which restricted the growth of aerobic spoilage microorganisms and reduced protein degradation. A similar effect was observed under MAP, where dissolved CO_2 inhibited microbial growth and metabolic activity, thereby slowing the formation of volatile basic nitrogen compounds [21]. Although TVB-N increased gradually throughout storage, the values remained substantially lower than those observed under AP. The TVB-N results were consistent with the microbiological findings. Samples stored under VP and MAP exhibited lower microbial counts and a slower increase in TVB-N than those stored under AP, indicating that microbial activity played a major role in protein degradation during refrigerated storage. These findings also agree with the pH changes observed in this study, where the accumulation of volatile basic compounds was accompanied by a progressive increase in pH during the later stages of storage [22]. Collectively, these results demonstrate that controlling oxygen availability effectively delayed microbial spoilage, reduced protein degradation, and prolonged the chemical stability of cooked squid stuffed with sticky rice during refrigerated storage.

3.1.4 Determination of TMA-N

Trimethylamine nitrogen (TMA-N) is a widely used indicator of spoilage in marine fish and cephalopods because it reflects the microbial reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA), which is responsible for the characteristic fishy odor of spoiled seafood. As shown in Figure 4, storage time and packaging conditions significantly affected TMA-N values ($p \leq 0.05$).

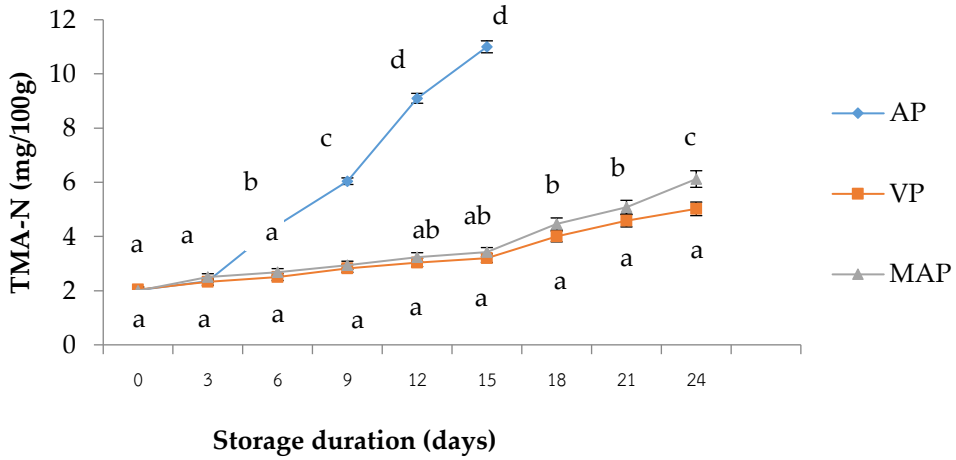


Figure 4. Changes in the TMA-N values of cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 °C for 24 days. Data are presented as mean ± SD ($n = 3$). [a–d] Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

The initial TMA-N value of cooked squid stuffed with sticky rice was 2.02 ± 0.52 mg/100 g for all treatments. TMA-N increased throughout refrigerated storage under all packaging conditions, although the rate of increase differed among treatments. The AP samples showed the most rapid increase, reaching 11.00 ± 0.90 mg/100 g on day 15, exceeding the commonly accepted sensory rejection limit of approximately 10 mg/100 g. This increase corresponded with the development of spoilage characteristics observed during storage, including off-odors and increased microbial counts [23]. The rapid accumulation of TMA-N under AP is

consistent with the unrestricted growth of spoilage microorganisms capable of reducing TMAO to TMA, thereby accelerating quality deterioration. In contrast, VP and MAP significantly slowed the accumulation of TMA-N ($p \leq 0.05$), with values of 5.02 ± 0.64 and 6.12 ± 0.80 mg/100 g, respectively, after 24 days of storage. The lower TMA-N values observed under VP are mainly associated with oxygen removal, which suppresses the growth of aerobic spoilage microorganisms. A similar trend was observed under MAP, where the presence of CO_2 inhibited microbial growth and delayed spoilage, resulting in a slower increase in TMA-N [24]. Although TMA-N increased gradually during storage, both VP and MAP maintained values below the sensory rejection limit throughout the storage period. The TMA-N results were consistent with the TVC and TVB-N findings, which also showed slower microbial proliferation and protein degradation under VP and MAP than under AP. These results indicate that reducing oxygen availability and controlling microbial growth effectively delayed the formation of TMA and preserved the freshness of cooked squid stuffed with sticky rice during refrigerated storage.

3.1.5 Determination of TBA value

Thiobarbituric acid (TBA) value is widely used as an indicator of secondary lipid oxidation because it reflects the accumulation of malondialdehyde (MDA), a major oxidation product formed during lipid degradation. As shown in Figure 5, both storage time and packaging conditions significantly affected TBA values ($p \leq 0.05$). The initial TBA values ranged from 0.50 ± 0.32 to 0.54 ± 0.50 mg MDA/kg, with no significant differences among treatments at the beginning of storage.

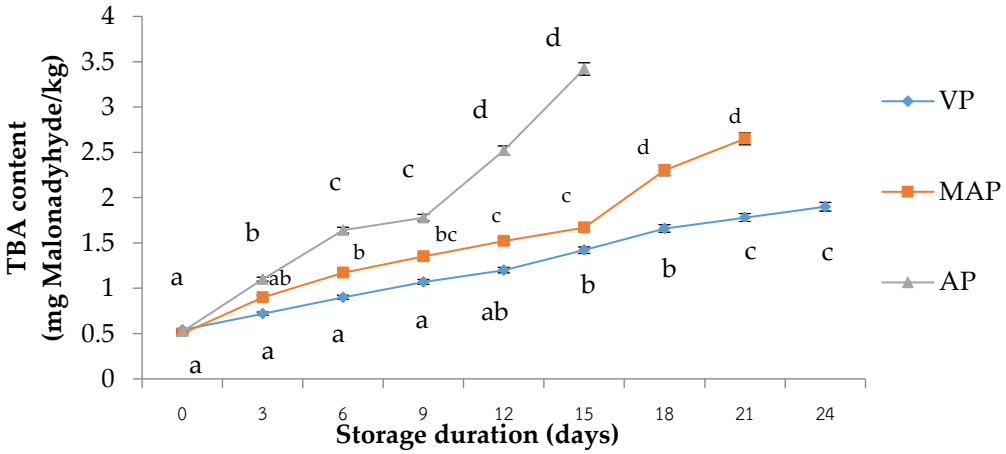


Figure 5. Changes in the TBA content of cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 °C for 24 days. Data are presented as mean ± SD ($n = 3$). [a–d] Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

TBA values increased progressively throughout refrigerated storage under all packaging conditions, indicating continuous lipid oxidation. The AP samples exhibited the fastest increase, rising from 0.52 ± 0.50 to 3.42 ± 0.80 mg MDA/kg by day 15. This value exceeded the threshold commonly associated with the development of rancid odors in seafood products. The rapid increase in TBA under AP reflects the greater availability of oxygen, which promotes lipid oxidation during storage. In addition, the progression of microbial spoilage may accelerate lipid degradation through the production of oxidative metabolites and lipolytic enzymes. Lower TBA values were observed under MAP and VP throughout storage. In the MAP treatment, TBA increased from 0.50 ± 0.32 to 2.65 ± 0.92 mg MDA/kg after 21 days, whereas VP showed the lowest value (1.90 ± 1.04 mg MDA/kg) after 24 days. The slower increase under VP is mainly attributed to oxygen removal, which limits oxidative reactions and delays lipid degradation. Although the MAP system contained 50% O_2, the presence of 25% CO_2 inhibited microbial growth and reduced spoilage activity, resulting in a slower increase in TBA compared with AP. Similar effects of modified atmosphere and vacuum packaging on lipid oxidation have been reported in refrigerated seafood products [25].

The observed increase in TBA is also related to the composition of the product. Squid muscle contains polyunsaturated fatty acids that are susceptible to oxidative deterioration during refrigerated storage. In addition, the inclusion of coconut milk introduces an additional lipid fraction that may contribute to oxidation as storage progresses. Consequently, the combined effects of product composition and oxygen availability influenced the rate of lipid oxidation under different packaging conditions. Overall, VP provided the greatest protection against lipid oxidation, followed by MAP, whereas AP showed the fastest deterioration. These findings were consistent with the chemical and microbiological results obtained in this study. Samples stored under VP and MAP exhibited lower TBA, TVB-N, and microbial counts than those stored under AP, demonstrating that oxygen reduction effectively delayed both lipid oxidation and overall quality deterioration during refrigerated storage.

3.1.6 Total viable count (TVC)

The changes in total viable count (TVC) of cooked squid stuffed with sticky rice under atmospheric packaging (AP), modified atmosphere packaging (MAP), and vacuum packaging (VP) during refrigerated storage are presented in Table 1. According to the microbiological standard for cooked seafood products, the acceptable limit for TVC is 1.0×10^6 CFU/g. The initial microbial loads of all treatments were low, ranging from 1.1×10^2 to 1.3×10^2 CFU/g, indicating good hygienic quality of the freshly prepared product. Microbial growth increased progressively throughout storage in all packaging systems, although the rate of increase differed significantly among treatments. The AP samples exhibited the fastest microbial proliferation, reaching 1.1×10^6 CFU/g on day 15, which exceeded the microbiological limit. At this stage, visible slime formation and pronounced off-odors were observed, indicating microbial spoilage. Consequently, the shelf life of AP samples was limited to 12 days. In contrast, microbial growth was significantly delayed under MAP and VP conditions. The TVC of MAP samples exceeded the acceptable limit on day 21 (4.2×10^6 CFU/g), resulting in an estimated shelf life of 18 days, whereas VP samples reached 1.5×10^6 CFU/g only on day 24, corresponding to a shelf life of 21 days. These findings demonstrate that both MAP and VP effectively delayed microbial spoilage compared with atmospheric packaging, with vacuum packaging providing the greatest extension of refrigerated shelf life.

The slower microbial growth observed in MAP can be attributed primarily to the antimicrobial activity of carbon dioxide (CO_2). Carbon dioxide dissolves in the aqueous phase of the food to form carbonic acid, thereby lowering the surface pH and inhibiting the growth of many aerobic spoilage microorganisms. In addition, elevated CO_2 concentrations increase the lag phase and reduce the growth rate of spoilage bacteria by interfering with cellular metabolism and enzyme activity [26]. Nevertheless, the antimicrobial effect gradually diminished during prolonged storage because CO_2 diffusion through the packaging film and microbial metabolism reduced the effective gas concentration within the package [27]. Vacuum packaging provided even greater microbial stability because oxygen was almost completely removed from the package, thereby suppressing the growth of aerobic spoilage microorganisms while simultaneously reducing oxidative deterioration [28]. Under refrigerated conditions, the combined effects of oxygen depletion and low temperature further limited microbial proliferation, resulting in the longest shelf life among all treatments. The superior performance of VP observed in this study is consistent with the lower levels of TVB-N, TMA-N, and lipid oxidation reported during storage, suggesting that microbial spoilage and chemical deterioration occurred simultaneously and collectively determined product shelf life. The present findings agree with those reported by **[**29], who demonstrated that vacuum packaging was more effective than modified atmosphere packaging in preserving the quality of gray triggerfish (*Balistes capriscus*) fillets stored at 4 °C, extending shelf life to 15 days by reducing microbial growth, hydrolytic reactions, and lipid oxidation. Although the shelf life obtained in the present study was longer (21 days), the difference is likely attributable to variations in raw materials, product formulation, cooking process, and packaging conditions. Nevertheless, both studies confirm that oxygen reduction through vacuum packaging is an effective strategy for delaying microbial spoilage and extending the refrigerated shelf life of seafood products.

Table 1. Total viable count in cooked squid stuffed with sticky rice (Tupa Sutong) under different packaging conditions during chilled storage at 4 ± 2 ℃ for 24 days.

Experimental set	Storage duration (days)	Total viable count (cfu/g)
Atmospheric packaging	0	$1.3x10^2 \pm 0.0^a$
	3	$2.8\times10^3 \pm 0.04^{ab}$
	6	$1.5\times10^4 \pm 0.12^b$
	9	$7.5\times10^4 \pm 0.05^b$
	12	$6.5\times10^5 \pm 0.14^c$
	15	$1.1\times10^6 \pm 0.08^c$
	18	-
	21	-
	24	-
Modified atmosphere packaging	0	$1.3\times10^2 \pm 0.05^a$
	3	$2.5\times10^3 \pm 0.03^{ab}$
	6	$6.4\times10^3 \pm 0.12^{ab}$
	9	$2.2\times10^4 \pm 0.00^b$
	12	$7.5\times10^4 \pm 0.08^b$
	15	$5.4\times10^5 \pm 0.48^c$
	18	$8.0\times10^5 \pm 0.18^c$
	21	$4.2\times10^6 \pm 0.0^c$
	24	-
Vacuum packaging	0	$1.3\times10^2 \pm 0.0^a$
	3	$4.2\times10^2 \pm 0.02^a$
	6	$4.3\times10^3 \pm 0.08^{ab}$
	9	$8.3\times10^3 \pm 0.22^{ab}$
	12	$1.2\times10^4 \pm 0.06^{ab}$
	15	$7.5\times10^4 \pm 0.12^b$
	18	$1.5\times10^5 \pm 0.14^c$
	21	$7.5\times10^5 \pm 0.04^c$
	24	$1.5\times10^6 \pm 0.10^c$

[a–c] Different lowercase letters within the same storage duration indicate significant differences between packaging methods ($p \leq 0.05$)

4. Conclusions

This study investigated the effects of atmospheric packaging (AP), modified atmosphere packaging (MAP; 50% O_2, 25% CO_2, and 25% N_2), and vacuum packaging (VP) on the physicochemical and microbiological quality of cooked squid stuffed with sticky rice during refrigerated storage (4 ± 2°C) for 30 days. This traditional ready-to-eat seafood product contains squid, sticky rice, and coconut milk, a complex food matrix that is highly susceptible to simultaneous protein degradation, lipid oxidation, and microbial spoilage. Storage time and packaging conditions significantly affected pH, total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), and total viable count (TVC) ($p \leq 0.05$). Products packaged in laminated nylon/LLDPE (15/80 μm) bags exhibited shelf lives of 12, 18, and 21 days under AP, MAP, and VP, respectively. Vacuum packaging was the most effective treatment in delaying oxidative and microbial deterioration by limiting oxygen exposure, while MAP also improved product stability through the antimicrobial effect of CO_2. These findings demonstrate that oxygen-controlled packaging effectively extends the shelf life of cooked squid stuffed with sticky rice and provide practical guidance for the commercial production and refrigerated distribution of traditional ready-to-eat seafood products, while serving as a basis for further development of advanced shelf-life extension technologies.

5. Acknowledgments

The authors gratefully acknowledge the financial support from Yala Rajabhat University, Thailand.

Author Contributions: Conceptualization, K.K., S.S., and P.P.; methodology K.K., S.S., and P.P.; software, K.K.; validation, K.K., and P.P; formal analysis, K.K.; investigation, K.K., and S.S.; resources, K.K., and S.S.; data curation, K.K., S.S., and P.P.; writing—original draft preparation, K.K., writing—review and editing, K.K.; visualization, K.K.; project administration, K.K.; funding acquisition, K.K..

Funding: This work was financially supported by Yala Rajabhat University, Thailand.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

[1] Kheokao, J.; Nopsuwan, R.; Phovijit, P.; Pornsuksawat, S.; Plianrung, S. Tracing the acculturation of food in Thailand's three southern border provinces. *Asia-Pacific J. Compassion Stud.* **2022**, *7*(1), 29–39. https://doi.org/10.13140/RG.2.2.31398.33600

[2] Plankensteiner, S. L.; Faxholm, P. L.; Olsen, K.; Mouritsen, O. G.; Frøst, M. B. Physicochemical characterization of sous vide cooked squid (*Loligo forbesii* and *Loligo vulgaris*) and the relationship to selected sensory properties and hedonic response. *Int. J. Gastron. Food Sci.* **2021**, *23*, 100298. https://doi.org/ 10.1016/j.ijgfs.2020.100298

[3] Manoj, D.; Auddy, I.; Nimbkar, S.; Chittibabu, S.; Shanmugasundaram, S. Development of co-immobilized enzymes amperometric biosensor for the determination of triglycerides in coconut milk. *Int. Food Res. J.* **2020**, *27*(5), 875–882. .

[4] Gokoglu, N.; Ceylan, A. Improving the Physicochemical and Textural Properties of Squid (*Loligo vulgaris*) Muscle by Sous-Vide Cooking in Different Time-Temperature Combinations. *J. Aquat. Food Prod. Technol.* **2022**, *31*(8), 872–881. https://doi.org/10.1080/10498850.2022.2108360

[5] Moreira, L. B.; Antunes, R.; Cotas, J.; Martins, N.; Costa, N.; Noronha, J. P.; Mata, P.; Diniz, M. Modified Atmosphere Packaging (MAP) for Seaweed Conservation: Impact on Physicochemical Characteristics and Microbiological Activity. *Foods* **2023**, *12*(14), 2736. https://doi.org/10.3390/foods12142736

[6] Chen, X.; Zhao, J.; Zhu, L.; Luo, X.; Mao, Y.; Hopkins, D.; Zhang, Y.; Dong, P. Effect of modified atmosphere packaging on shelf life and bacterial community of roast duck meat. *Food Res. Int.* **2020**, *137*, 109645. https://doi.org/10.1016/j.foodres.2020.109645

[7] Koukou, L.; Devitt, T. D.; Dalgaard, P. Extensive growth and growth boundary model for non-proteolytic *Clostridium botulinum* – Evaluation and validation with MAP and smoked foods. *Food Microbiol.* **2022**, *102*, 103931. https://doi.org/10.1016/j.fm.2021.103931

[8] Hauzoukim, S. S.; Mohanty, B. Modified atmosphere packaging of fish and fishery products: A review. *J. Entomol. Zool. Stud.* **2020**, *8*(4), 651–659.

[9] Bassey, A. P.; Chen, Y.; Zhu, Z.; Odeyemi, O. A.; Gao, T.; Olusola, O. O.; Ye, K.; Li, C.; Zhou, G. Evaluation of spoilage indexes and bacterial community dynamics of modified atmosphere packaged super-chilled pork loins. *Food Control* **2021**, *130*, 108383. https://doi.org/10.1016/j.foodcont.2021.108383

[10] Ameer, A.; Seleshe, S.; Kang, S. K. Effect of Modified Atmosphere Packaging Varying in CO_2 and N2 Composition on Quality Characteristics of Dry Fermented Sausage during Refrigeration Storage. *Food Sci. Anim. Resour.* **2022**, *42*(4), 639–654. https://doi.org/10.5851/kosfa.2022.e27

[11] Yang, Z.; Liu, S.; Sun, Q.; Zheng, O.; Wei, S.; Xia, Q.; Ji, H.; Deng, C.; Hao, J.; Xu, J. Insight into muscle quality of golden pompano (*Trachinotus ovatus*) frozen with liquid nitrogen at different temperatures. *Food Chem.* **2022**, *374*, 131737. https://doi.org/10.1016/j.foodchem.2021.131737

[12] Zhao, C. C.; Eun, J. B. Characterization of volatile compounds and physicochemical properties of hongeo using headspace solid-phase microextraction and gas chromatography-mass spectrometry during fermentation. *Food Biosci.* **2021**, *44*, 101379. https://doi.org/10.1016/j.fbio.2021.101379

[13] Khodanazary, A. Effects of Carboxymethyl Chitosan/Pectin Coating Containing Free and Nanoliposome *Mentha piperita* Essential Oil on the Shelf Life of Shrimp During Ice Storage. *Food Sci. Nutr.* **2025**, *13*(1), 70184. https://doi.org/10.1002/fsn3.70184

[14] Kandeepan, G.; Tahseen, A. Modified Atmosphere Packaging (MAP) of Meat and Meat Products: A Review. *J. Packag. Technol. Res.* **2022**, *6* (3), 137–148. https://doi.org/10.1007/s41783-022-00139-2

[15] Das, K. C.; Alice, E. J.; Hossain, M. A.; Mehbub, M. F.; Islam, M. T. Effects of vacuum and modified atmosphere packaging on the shelf life of Rohu fish (*Labeo rohita*) stored at refrigerated temperature (4 °C). *Int. Food Res. J.* **2021**, *28*(3), 586–593. https://doi.org/10.47836/ifrj.28.3.18

[16] Li, N.; Zhang, Y.; Wu, Q.; Gu, Q.; Chen, M.; Zhang, Y.; Sun, X.; Zhang, J. High-throughput sequencing analysis of bacterial community composition and quality characteristics in refrigerated pork during storage. *Food Microbiol.* **2019**, *83*, 86–94. https://doi.org/10.1016/j.fm.2019.04.013

[17] Prabhakar, P. K.; Srivasta, P. P.; Pathak, S. S.; Das, K. Mathematical Modeling of Total Volatile Basic Nitrogen and Microbial Biomass in Stored Rohu (*Labeo rohita*) Fish. *Front. Sustain. Food Syst.* **2021**, *5*, 669473. https://doi.org/10.3389/fsufs.2021.669473

[18] Sai-Ut, S.; Indriani, S.; Srisakultiew, N.; Kingwascharapong, P.; Rawdkuen, S.; Kaewprachu, P.; Jung, Y. H.; Zhang, W.; Pongsetkul, J. Microbial dynamics and quality of goat meat under elevated CO2 in high-O2 modified atmosphere packaging during cold storage. *Food Control* **2026**, *179*, 111577. https://doi.org/10.1016/j.foodcont.2025.111577

[19] Kontominas, M. G.; Badeka, A. V.; Kosma, I. S.; Nathanailides, C. I. Recent Developments in Spoilage Packaging Technologies. *Foods* **2021**, *10*(5), 940. https://doi.org/10.3390/foods10050940

[20] Suprapto, H.; Kumalaningsih, S.; Wignyanto, W.; Santoso, I.; Sucipto, S. Chemical and microbial changes of stingray fish (*Trygon sephen*) during soaking in salt solution before smoking process. *Food Res.* **2022**, *6*(5), 68–75.

[21] Milijasevic, J. B.; Milijasevic, M.; Lilic, S.; Djinovic, S. J.; Nastasijevic, I.; Geric, T. Effect of Vacuum and Modified Atmosphere Packaging on the Shelf Life and Quality of Gutted Rainbow Trout (*Oncorhynchus mykiss*) during Refrigerated Storage. *Foods* **2023**, *12*(16), 3015. https://doi.org/10.3390/foods12163015

[22] Bekhit, A. A.; Holman, B.; Giteru, S.; Hopkins, D. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage. *Trends Food Sci. Technol.* **2021**, *109*, 280–302. https://doi.org/10.1016/j.tifs.2021.01.006

[23] Nurjanah, N.; Suwandi, R.; Hidayat, T.; Окторомпа, V. Chemical composition and amino acid profile of fresh and steamed cobia (*Rachycentron canadum* L.). *Food ScienTech J.* **2020**, *2*(1), 12–19. https://doi.org/10.33512/fsj.v2i1.8136

[24] Rizzo, M. Measurement of Malondialdehyde as a Biomarker of Lipid Oxidation in Fish. *Am. J. Anal. Chem.* **2024**, *15*(9), 303–332 https://doi.org/10.4236/ajac.2024.159020

[25] Nauman, K.; Jaspal, M. H.; Asghar, B.; Manzoor, A.; Akhtar, K. H.; Ali, U.; Ali, S.; Nasir, J.; Sohaib, M.; Badar, I. H. Effect of Different Packaging Atmosphere on Microbiological Shelf Life, Physicochemical Attributes, and Sensory Characteristics of Chilled Poultry Fillets. *Food Sci. Anim. Resour.* **2022**, *42*(1), 153–174. https://doi.org/10.5851/kosfa.2021.e71

[26] Jaberi, R.; Kaban, G.; Kaya, M. Effects of vacuum and high oxygen modified atmosphere packaging on physicochemical and microbiological properties of minced water buffalo meat. *Asian-Australas. J. Anim. Sci.* **2019**, *32*(3), 421–429. https://doi.org/10.5713/ajas.18.0391

[27] Moradinezhad, F.; Dorostkar, M. Effect of Vacuum and Modified Atmosphere Packaging on the Quality Attributes and Sensory Evaluation of Fresh Jujube Fruit. *Int. J. Fruit Sci.* **2021**, *21*(1), 82–94. https://doi.org/10.1080/15538362.2020.1858470

[28] Parlapani, F. F.; Michailidou, S.; Anagnostopoulos, D. A.; Koromilas, S.; Kiosa, K.; Pasentsis, K.; Psomopoulos, F.; Argiriou, A.; Haroutounian, S. A.; Boziaris, I. S. Bacterial communities and potential spoilage markers of whole blue crab (*Callinectes sapidus*) stored under commercial simulated conditions. *Food Microbiol.* **2019**, *82*, 325–333. https://doi.org/10.1016/j.fm.2019.03.011

[29] Esteves, E.; Guerra, L.; Aníbal, J. Effects of Vacuum and Modified Atmosphere Packaging on the Quality and Shelf-Life of Gray Triggerfish (*Balistes capriscus*) Fillets. *Foods* **2021**, *10*(2), 250. https://doi.org/10.3390/foods10020250