Effect of sodium ascorbate and sodium tripolyphosphate treatments on the quality and shelf life of snail meat during chilled storage for commercial application in Thailand

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Summary

This research examined how sodium ascorbate, sodium tripolyphosphate (STPP) and their mixture influence the preservation of snail meat stored at 4 °C for a week, with a focus on commercial use in Thailand. Four treatments were applied to the snail meat: soaking in sodium ascorbate, STPP, a combination of both and a control group. Various quality parameters were analysed. The most effective treatment was the combination of 25.0 $g \cdot l^{-1}$ sodium ascorbate and 25.0 $g \cdot l^{-1}$ STPP, which enhanced pH stability, minimized K-values, total volatile basic nitrogen and *p*-anisidine values, reduced microbial growth and maintained the amino acid profile. Additionally, this treatment lowered biogenic amines and certain acids, indicating the suppression of enzymatic and microbial activities. This study suggested that the combined treatment could be a viable procedure for maintaining the quality and shelf life of snail meat in cold storage, presenting significant contribution to Thailand's seafood industry and the development of efficient preservation methods for seafood.

Keywords

snail meat; sodium ascorbate; sodium tripolyphosphate; quality

Seafood is a vital component of the global food supply, providing essential nutrients, and contributing to food security as well as economic growth in many countries. Snail meat is a popular seafood product in several regions, including Thailand [1], where it is highly valued for its unique taste and nutritional properties. However, like other seafood products, snail meat is highly perishable due to its high water content, the specific activities of enzymes such as proteases, lipases or phospholipases, and its susceptibility to microbial spoilage [2]. Therefore, it is important to develop effective preservation techniques to maintain the quality and extend the shelf life of snail meat during storage, transportation and commercial application.

Sodium ascorbate and sodium tripolyphosphate (STPP) are commonly used additives in the seafood industry due to their antioxidant and antimicrobial properties, respectively. Sodium ascorbate, a salt of ascorbic acid (vitamin C), is known to slow down oxidation processes, thereby delaying discoloration and rancidity in seafood products. STPP, on the other hand, is a chelating agent that can sequester metal ions, inhibit microbial growth and enhance the water-holding capacity of seafood products. The combined use of sodium ascorbate and STPP has the potential to synergistically improve the overall quality and shelf life of seafood products, including snail meat, during chilled storage.

To date, limited research has been conducted on the effectiveness of sodium ascorbate, STPP and their combination in preserving snail meat quality during storage. The present study, therefore, aimed to investigate the effects of these treatments on the quality and shelf life of snail meat during 4 °C storage for seven days, with a focus on commercial application in Thailand. Quality parameters, including pH, K-value, total volatile basic nitrogen (*TVB-N*), *p*-anisidine value, microbiological parameters, amino acid composi-

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tion, biogenic amines content, together with malic, succinic and lactic acid contents, were assessed to evaluate the effectiveness of the treatments. The findings of this study will contribute to the development of effective preservation techniques for snail meat and other seafood products, providing valuable insights for the seafood industry in Thailand and other countries. The improved understanding of the synergistic effects of sodium ascorbate and STPP in preserving seafood quality will also promote the sustainable utilization of seafood resources and enhance the economic value of the industry.

MATERIALS AND METHODS

Samples

Live spotted babylon snails (*Babylonia areolata*), weighing 8.0–10.0 g each, were sourced from a farm in Petchburie, Thailand, in January 2022 and transported to the laboratory within 6 h. The live, whole-shelled snails were immediately washed with tap water and then kept in flaked ice at a ratio of 1.0 kg of snails to 2.0 kg of ice for 30 min until their demise. After this, the shells were broken to separate the meat. The snail meat was further divided into four groups: control (marked as C), treated with 50.0 g·l^{-1} sodium ascorbate (marked as A), treated with 50.0 g·l^{-1} STPP (marked as B) and treated with a combination of 25.0 g·l^{-1} STPP and 25.0 g·l^{-1} sodium ascorbate group (marked as AB). After 30 min in the respective solutions, the snails were drained and split into 2.5 kg lots. Then they were stored in polypropylene boxes at 4 ± 1 °C for a week.

pH value determination

The pH values of the snail meat were gauged using a modified method of Susanto et al. [3]. The meat was homogenized, diluted with distilled water (1.0 g of meat per 10.0 ml water), and the pH value was measured using a pH meter from Toko Chemical Laboratory (Tokyo, Japan) equipped with a glass electrode, specifically designed for accurate pH measurement in semi-solid or homogenized samples.

K-value determination

This study adapted a method from Veciana-Nogues et al. [4] and Chotimarkorn et al. [2] to evaluate compounds related to adenosine triphosphate (ATP) and compute the K-value. The process involved homogenizing a snail meat sample of 10.0 g in 15.0 ml of 0.60 mol·l^{-1} perchloric acid ($HClO_4$) at 0 °C, followed by centrifugation at 1 500 ×g for 10 min. The supernatant was neutralized to pH 6.5 using 0.1 mol·l^{-1} potassium hydroxide (KOH) and set aside at 4 °C for 30 min. After filtering out potassium perchlorate ($KClO_4$) through a 0.2 μm cellulose acetate membrane syringe filter, the filtrate was preserved at –80 °C. The ensuing analysis employed reversed-phase high-performance liquid chromatography (RP-HPLC) with a UV-Vis detector (Agilent Technologies, Palo Alto, California, USA), a Hypersil ODS column (4.0 mm × 250.0 mm, particle size 5.0 μm; Agilent Technologies) and an Agilent 1100 series instrument (Agilent Technologies). A 0.1 mol·l^{-1} phosphate buffer containing 0.04 mol·l^{-1} KH_2PO_4 and 0.06 mol·l^{-1} K_2HPO_4 was used as the mobile phase, with a flow rate of 0.75 ml·min^{-1}. Quantification and identification of ATP-related compounds were based on peak areas, standard curves and distinct relative retention times. Lastly, the K-values were computed using the formula of Saito et al. [5] and expressed in percent:

$$K = \frac{(HxR + Hx)}{P + (HxR + Hx)} \times 100 \quad (1)$$

$$P = ATP + ADP + AMP + IMP \quad (2)$$

where *HxR, Hx, ATP, ADP, AMP* and *IMP* represent the contents of inosine, hypoxanthine, ATP, adenosine diphosphate, adenosine monophosphate and inosine monophosphate, respectively.

Total volatile base nitrogen

TVB-N quantification was carried out using the method of Woyewoda et al. [6]. A 10.0 g sample was mixed with 300.0 ml of distilled water, transferred into a 1 000.0 ml flask with 2.0 g of magnesium oxide (MgO) and heated for 25 min in a distillation apparatus. The condensate was collected in a flask containing 20.0 g·l^{-1} boric acid solution and titrated using 0.05 mol·l^{-1} sulfuric acid (H_2SO_4). The endpoint was identified by a colour match with a reference chart. *TVB-N* values were computed using Eq. 3 and expressed as milligrams per kilogram:

$$TVB\text{-}N = \frac{(V_1 - V_2) \times N \times 14.007 \times 10}{w_0} \quad (3)$$

where V_1 and V_2 are volumes of 0.05 mol·l^{-1} H_2SO_4 used in sample and blank titrations, respectively; N is the normality of the H_2SO_4 solution; 14.007 is nitrogen's atomic weight; w_0 is the original sample's weight in grams.

p-Anisidine value

The total lipid content from the snail

meat was extracted using a mixture of chloroform and methanol, following the method of FOLCH et al. [7]. This extraction process was conducted at a controlled room temperature, specifically maintained within the range of 20–25 °C. Subsequently, the solvent was evaporated at 40 °C using an N-N Series rotary evaporator (Eyela, Tokyo, Japan). The extracted lipids were then stored at –20 °C under nitrogen gas to prevent oxidation. The lipids were stored for a maximum duration of 2 weeks, ensuring their stability and reliability of subsequent analysis. The *p*-anisidine value (*p-AV*) was determined as per AOCS Cd 18-90 method [8]. An amount of 100 mg of the oil was dissolved in iso-octane and its absorbance was measured at 350 nm. Then, *p*-anisidine was added and absorbance was read again. *p-AV* was calculated as:

$$p\text{-}AV = 25 \times \frac{1.2 \times (A_2 - A_1)}{w} \quad (4)$$

where A_1 and A_2 are the absorbances before and after adding *p*-anisidine, respectively, w is the sample weight.

Microbiological analysis

Following Harrigan and McCance's method [9], live mesophilic and psychrophilic bacteria counts were gauged using the pour plate method with plate count agar (Difco Laboratories, Detroit, Michigan, USA). A 10.0 g sample of snail meat was mixed with 90.0 ml of 0.1 g·l^{-1} peptone water, homogenized and then diluted. The resulting samples were pipetted onto agar plates and incubated at 30 °C for 48 h for mesophilic bacteria and at 5 °C for 72 h for psychrophilic bacteria counts.

Amino acids determination

Free amino acids were measured following AQUINO et al. [10] with enhancements by CHOTIMARKORN et al. [2], utilizing *o*-phthaldehyde (OPA) and 2-mercaptoethanol for derivatization and high-performance liquid chromatography (HPLC) with a fluorescence detector. A 10 g sample was homogenized with 15.0 ml of 0.60 mol·l^{-1} perchloric acid ($HClO_4$), then centrifuged and the supernatant was filtered through 0.2 μm pore cellulose acetate membrane syringe filter. The filtrate was stored at –80 °C. The solution was maintained under these conditions for a maximum duration of 2 weeks, ensuring its stability. In the derivatization stage, the supernatant was combined with OPA and 2-mercaptoethanol solution. The mixture was then left to react for 1 min, allowing for complete derivatization, and then analysed by HPLC using a Shimadzu 6A system with a Shimadzu RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). Wavelengths of 340 nm (excitation) and 440 nm (emission) were used for most amino acid derivatives, but 425 nm (excitation) and 450 nm (emission) were applied for cysteine and proline.

Biogenic amines determination

The method of ÖZOGUL et al. [11] modified by CHOTIMARKORN et al. [2] was employed for biogenic amines quantification by HPLC. Briefly, each sample was homogenized in 10.0 g·l^{-1} trichloroacetic acid (TCA), filtered and then derivatized using sodium hydroxide and benzoyl chloride. The derivative was mixed with sodium chloride and extracted with diethyl ether twice. The evaporated ether extracts were reconstituted in acetonitrile for HPLC. Analysis was performed on a Shimadzu 6A device using a Spherisorb 5 Si C18 column (4.6 mm × 250.0 mm, particle size 5.0 μm; Phenomenex, Torrance, California, USA). The detection was done at 254 nm using a diode array detector model SPD-M20A (Shimadzu). The mobile phase was an acetonitrile-water gradient solution. Biogenic amines quantification was done based on peak areas and corresponding standard curves.

Malic, succinic and lactic acids determination

Quantification of certain organic acids was conducted using a method that blended the extraction technique of WONGSO and YAMANAKA [12], the HPLC analytical protocol of AGUIAR et al. [13] and the adjustments made by CHOTIMARKORN et al. [2]. Briefly, a 5.0 g sample was homogenized with 15.0 ml of 60.0 g·l^{-1} perchloric acid for 1 min at 0 °C. After centrifugation, the supernatant was neutralized with potassium hydroxide and diluted to a total volume of 25.0 ml using distilled water. The solution was filtered through a 0.2 μm pore cellulose acetate membrane syringe filter and stored at –80 °C. The solution was maintained under these conditions for a maximum duration of 2 weeks, ensuring its stability. HPLC was performed using an Aminex HPX-87H ion-exchange column (7.8 mm × 300.0 mm, particle size 5.0 μm; Bio-Rad Laboratories, Hercules, California, USA) and a UV-Vis detector model SPD-10AV (Shimadzu) set at 210 nm. The mobile phase of 0.005 mol·l^{-1} sulfuric acid at pH 2.1 was applied at a flow of 0.75 ml·min^{-1}. Succinic, malic and lactic acids were quantified based on peak areas and corresponding standard curves.

RESULTS AND DISCUSSION

pH

Changes in pH reflect alterations in the quality and freshness of stored seafood, with rising pH often signifying spoilage and bacterial growth [14]. The snail meat initially had a pH of 6.38 ± 0.04 at the start of 4 °C storage. The pattern of pH change in snail meat during 7 days of storage is presented in Fig. 1. From the second day of storage, the control group had significantly higher pH than other groups ($p < 0.05$). After 7 days of storage, the pH values for the control group, the group soaked in 50.0 g·l^{-1} sodium ascorbate, the group in 50.0 g·l^{-1} STPP and the group in a combination of 25.0 g·l^{-1} sodium ascorbate with 25.0 g·l^{-1} STPP, were 7.01 ± 0.08, 6.69 ± 0.07, 6.66 ± 0.07, and 6.61 ± 0.06 respectively. The control group showed a higher pH, apparently due to the absence of antimicrobial or antioxidant agents to counteract processes increasing pH. Rising pH levels in stored seafood often indicate spoilage and bacterial growth, impacting its freshness and quality. STPP disrupts bacterial cell membranes, causing cell content leakage, membrane potential loss and, eventually, bacterial cell death [15]. Sodium ascorbate, an ascorbic acid salt, exhibits antimicrobial properties due to its antioxidant activity, creating a less favorable environment for bacterial growth [16]. These traits contribute to the lower pH in treated snail meat samples compared to the control during 4 °C storage. Treatment with sodium ascorbate, STPP and their combination effectively blocked the increase in pH levels in snail meat during chilled storage, thus delaying spoilage and bacterial growth. This quality improvement stems from the synergistic effects of the antioxidant activity of sodium ascorbate and antimicrobial properties of STPP.

K-value

The K-value, measuring ATP degradation postmortem, is a vital metric for evaluating seafood freshness and quality, with higher K-values indicating more spoilage and lower ones suggesting better preservation. Nonetheless, acceptable K-value ranges can differ based on the specific seafood, storage circumstances and market requirements. This study explored the K-value of snail meat during a 7-day chilled storage at 4 °C (Fig. 2). The initial K-value was 2.8 ± 0.1 % and it generally rose during storage, in line with previous reports of CHOTIMARKORN et al. [2] on snail meat during iced storage. After a week's storage, the K-value for both the control and snail meat immersed in 50.0 g·l^{-1} sodium ascorbate solution rose significantly from day 2 ($p < 0.05$), reaching 39.1 ± 2.0 % and 35.5 ± 2.0 %, respectively. In contrast, snail meat treated with 50.0 g·l^{-1} STPP solution or a 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP mix had significantly lower K-values at the same storage duration ($p < 0.05$), showing values of 27.7 ± 2.2 % and 25.1 ± 1.9 %, respectively, after seven days. The control and sodium ascorbate-treated snail meat had a faster K-value increase compared to the samples treated with STPP or the sodium ascorbate-STPP combination. This result indicated that sodium ascorbate alone may not adequately maintain snail meat quality during refrigerated storage. Despite

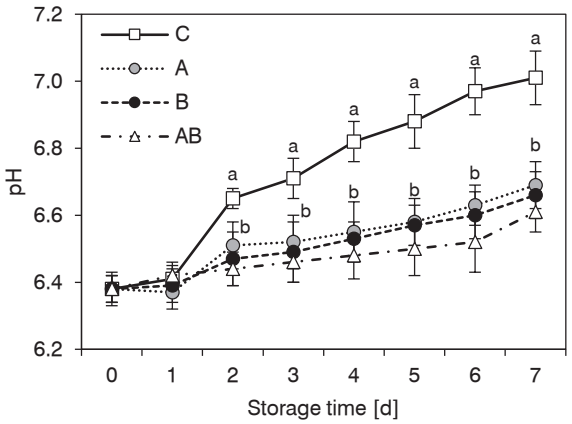

Fig. 1. Variation in pH of snail meat over seven days.

C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

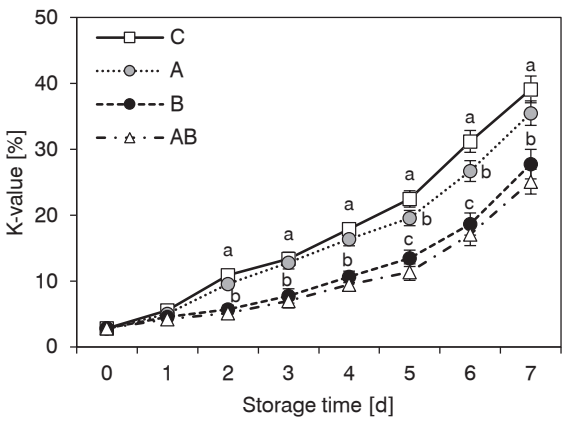

Fig. 2. K-value variation in snail meat over seven days.

C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

sodium ascorbate's antioxidative qualities that can reduce oxidative stress and slow down the lipid oxidation [17], it might not directly hinder the enzymatic and microbial activities leading to ATP degradation and spoilage. On the other hand, using 50.0 g·l^{-1} STPP solution or a mixture of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP led to a slower increase in K-value, suggesting improved snail meat preservation during storage. STPP, a recognized food additive, has antimicrobial properties by chelating metal ions [18], which can restrain microbial growth and activity of various enzymes. The combination of sodium ascorbate and STPP seems to offer a synergistic effect where the antioxidative qualities of sodium ascorbate and the antimicrobial properties of STPP collectively slow down ATP degradation and spoilage processes. Many fish species deem a K-value below 20.0–30.0 % as fresh quality [19], with values between 30.0–50.0 % indicating early spoilage stages and those over 80 % pointing to severe quality degradation [20]. As per Fig. 2, by day 5 and day 6 for the control group and 50.0 g·l^{-1} sodium ascorbate-soaked snail meat, the K-value exceeded 30 % during 4 °C storage. However, snail meat soaked in 50.0 g·l^{-1} STPP solution or in the mixture of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP kept the K-value under 30 % even by day 7 of storage. This implied that STPP and the combined sodium ascorbate-STPP treatments effectively curtailed the K-value in snail meat during refrigerated storage. Reduced K-values indicate slower ATP degradation, better preserved seafood product quality and freshness. Sodium ascorbate's antimicrobial and antioxidative properties and STPP's indirect antimicrobial property contribute to these treatments' effectiveness. Lower K-values in snail meat treated with 50.0 g·l^{-1} STPP and the mixed 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP treatment were attributable to the synergistic effects of sodium ascorbate's antioxidative and STPP's antimicrobial properties. The rapid K-value surge from the second storage day across all treatments underscored the significance of efficient preservation methods in maintaining seafood quality and freshness. The results demonstrated that the sodium ascorbate and STPP combination is especially effective in lowering K-values, thus better preserving snail meat quality during chilled storage.

Total volatile basic nitrogen

TVB-N compounds, primarily formed through degradation of proteins and breakdown of other nitrogenous materials in seafood, have a pronounced effect on seafood flavour [21]. Thus, it is imperative to monitor *TVB-N* levels to characterize the freshness and quality of seafood over time. Initially, the snail meat had a *TVB-N* content of 15.41 ± 3.82 mg·kg^{-1}. Over a 7-day storage at 4 °C, there was a clear increase in *TVB-N* content (Fig. 3), paralleling findings by Zeng et al. [22] and Malga et al. [23] on various seafood stored under iced conditions. Our findings also correlate with previously observed trends in iced storage [2]. This study built upon our previous knowledge of post-mortem changes in farmed spotted babylon snails during iced storage.

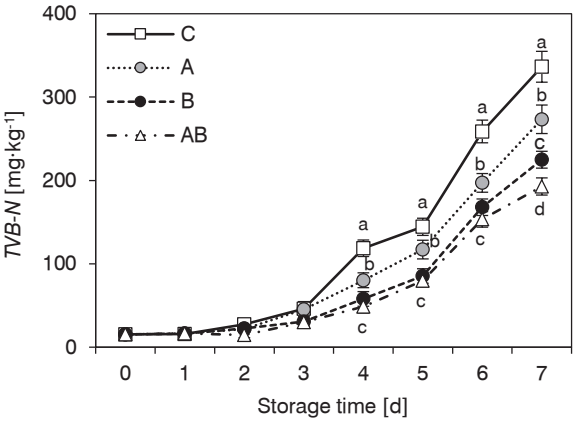

Fig. 3. Total volatile basic nitrogen variation in snail meat over seven days.

TVB-N – total volatile base nitrogen, C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

To our knowledge, while numerous studies have examined *TVB-N* changes in seafood during iced storage, there is a scarcity of literature on studies exploring the direct influence of sodium ascorbate and STPP on *TVB-N* levels. This underscores the unique and crucial nature of our research. From day 4 onward, we noted a significant increase in *TVB-N* in the control group and in snail meat treated with 50.0 g·l^{-1} sodium ascorbate solution ($p < 0.05$). This was especially remarkable when compared to meat treated with 50.0 g·l^{-1} STPP solution or a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP. The elevated *TVB-N* values in the control and exclusively sodium ascorbate-treated groups can be attributed to higher microbial activity and protein degradation, both of which lead to volatile amine production. The antimicrobial attributes of sodium ascorbate and STPP potentially aid in curtailing *TVB-N* content by suppressing microbial activity that spurs protein degradation.

After 7 days of storage at 4 °C, *TVB-N* content in the control group and the various treated groups (50.0 g·l^{-1} sodium ascorbate, 50.0 g·l^{-1} STPP and a combination of 25.0 g·l^{-1} sodium ascorbate with 25.0 g·l^{-1} STPP) were recorded as 336.43 ± 18.53 mg·kg^{-1}, 273.43 ± 17.11 mg·kg^{-1}, 224.73 ± 9.92 mg·kg^{-1}, and 192.82 ± 10.34 mg·kg^{-1}, respectively.

The general consensus places the *TVB-N* threshold at 300 mg·kg^{-1} [24], denoting seafood freshness. Our observations highlight the potential of sodium ascorbate and STPP treatments, either in isolation or synergistically, to impede the increase in *TVB-N* content in snail meat over its storage. This suggests they can help maintain the flavour quality of snail meat during refrigerated storage by inhibiting the emergence of volatile compounds that negatively affect flavour.

p-Anisidine value

The *p*-anisidine value is crucial for assessing secondary lipid oxidation in stored seafood, a key factor in evaluating the efficiency of preservation methods. It reflects the emergence of off-flavours, off-odours, and formation of potentially harmful compounds, thereby affecting sensory quality, nutrition and safety. This metric dominantly indicates aldehydes responsible for rancid odours and off-flavours originating in oxidized lipids [25].

Initially, the snail meat presented a *p*-anisidine value of 0.21 ± 0.05. As depicted in Fig. 4, this value remained relatively constant for the first four days of storage at 4 °C across all treatments ($p > 0.05$). However, from the fourth day onward, notable increase was apparent, especially in the control group and the group treated with 50.0 g·l^{-1} STPP solution, when compared to the 50.0 g·l^{-1} sodium ascorbate and combined 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP solution groups ($p < 0.05$). By the end of the seven-day storage period at 4 °C, the *p*-anisidine values for the control and treated groups were 12.44 ± 0.58, 8.24 ± 0.59, 9.87 ± 0.64 and 7.63 ± 0.64, respectively.

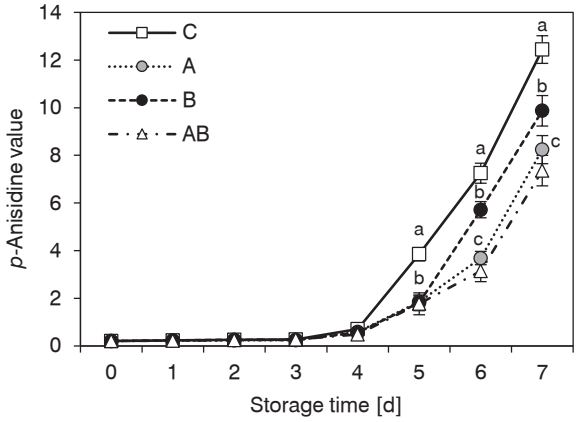

Fig. 4. *p*-Anisidine value variation in snail meat over seven days.

C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

While the observed increase in the *p*-anisidine value during the latter half of the iced storage was similar to the results of the study by Chotimarkorn et al. [26], various factors impacting lipid oxidation during chilled storage deserved consideration. Apart from well-known factors like oxygen exposure and temperature, other intricacies such as enzymatic activity, microbial actions and specific pro-oxidants play important roles [27]. For example, studies by Tenyang et al. [28] and Yehia et al. [29] underscored the vulnerability of certain seafoods to lipid oxidation at cold storage mainly due to oxygen exposure.

In terms of preservation strategies, sodium ascorbate stands out for its antioxidative capabilities, delaying lipid oxidation through free radical scavenging, metal ion chelation and lipid hydroperoxide reduction. This ensures prolonged quality retention of the snail meat. On the other hand, STPP is commonly used in seafood for its water retention and pH modulation benefits, effective binding of divalent metal ions like Ca^{2+} and Mg^{2+}, which could intensify lipid oxidation in the absence of STPP.

Microbiological analysis

This study examined the microbiological quality of seafood, focusing on psychrophilic and mesophilic bacteria in snail meat stored at 4 °C for 7 days [30]. These bacteria are indicators of seafood spoilage, reduction of food quality and threat to the health of consumers. Observing their population changes may provide information on the effectiveness of preservation methods. Sodium ascorbate and STPP, known for their antimicrobial properties, were used as treatments to study their impact on bacterial growth. Initial bacteria counts were 2.30 ± 0.12 log CFU·g^{-1} (psychrophilic) and 3.45 ± 0.13 log CFU·g^{-1} (mesophilic), which tended to rise over time, which was similar to previous findings at iced storage [2].

During the first two days, all treatments showed stable levels ($p > 0.05$) of psychrophilic bacteria (Fig. 5A). However, after this period, control and snail meat in 50.0 g·l^{-1} sodium ascorbate solution exhibited a significant increase in psychrophilic

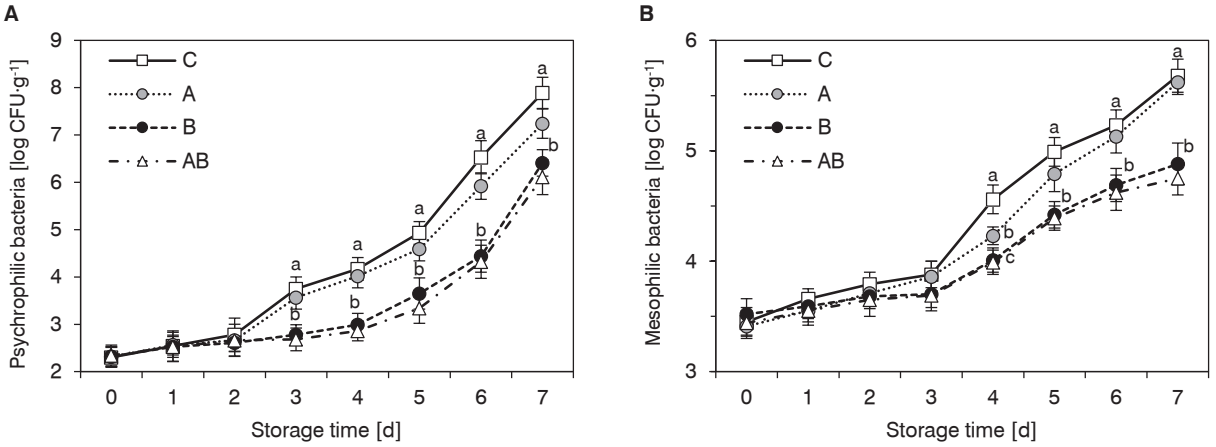

Fig. 5. Variation in levels of psychrophilic and mesophilic bacteria in snail meat over seven days.

A – psychrophilic bacteria, B – mesophilic bacteria.
C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

bacteria compared to meat in 50.0 g·l^{-1} STPP solution or in the combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP ($p < 0.05$). After seven days, the respective values for psychrophilic bacteria were 7.89 ± 0.33 log CFU·g^{-1} (control), 7.24 ± 0.31 log CFU·g^{-1} (50.0 g·l^{-1} sodium ascorbate), 6.41 ± 0.28 log CFU·g^{-1} (50.0 g·l^{-1} STPP), and 6.11 ± 0.37 log CFU·g^{-1} (combined solution).

In the case of mesophilic bacteria, levels remained stable ($p > 0.05$) for three days (Fig. 5B), followed by a significant rise in the control and 50.0 g·l^{-1} sodium ascorbate-treated groups. After seven days, the respective values for mesophilic bacteria were 5.68 ± 0.14 log CFU·g^{-1} (control), 5.65 ± 0.11 log CFU·g^{-1} (50.0 g·l^{-1} sodium ascorbate), 4.88 ± 0.19 log CFU·g^{-1} (50.0 g·l^{-1} STPP), and 4.70 ± 0.15 log CFU·g^{-1} (combined solution).

In previous research [31], the safe microbiological limit of 6 log CFU·g^{-1} was set for fish. Our study found a 6-day shelf life for psychrophilic bacteria in control samples and those treated with 50.0 g·l^{-1} sodium ascorbate. However, the shelf life increased to 7 days with 50.0 g·l^{-1} STPP or a 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP combination. All treatments maintained mesophilic bacteria under the acceptable limit after a week. The disparity in the time for significant bacterial growth could be attributed to the unique growth properties and environmental preferences of the bacterial groups. Psychrophilic bacteria, growing comparatively faster at lower temperatures, may surge before mesophilic bacteria, which favour moderate temperatures. Different bacterial growth rates may account for the varied timing of their significant increase. The differences in bacterial growth between control and treated groups can be linked to the antimicrobial action of sodium ascorbate and STPP. Sodium ascorbate, an antioxidant, curbs reactive oxygen species production, while STPP, a polyphosphate, sequesters metal ions and compromises bacterial membrane integrity. Their combination exerts a synergistic effect, resulting in lower bacterial counts than individual treatments due to their complementary bacterial metabolism and cell structure targets. These findings underscore the potential of sodium ascorbate and STPP as preservatives to enhance snail meat shelf life during cold storage. Their ability to control bacterial growth, namely psychrophilic and mesophilic bacteria, could help sustain safety and quality of snail meat as well as safety and quality of other seafood during refrigeration.

Amino acids composition

Tab. 1 presents data on changes in the amino acid composition of snail meat over a one-week storage period at 4 °C. Initially, leucine and lysine dominated the essential amino acids, with contents of 1 185.31 ± 112.11 mg·kg^{-1} and 1 546.11 ± 147.21 mg·kg^{-1}, respectively. Among the non-essential amino acids, glutamic acid, glycine and arginine stood out with contents of 4 256.64 ± 257.22mg·kg^{-1},1 064.11 ± 102.81mg·kg^{-1}, and 3 568.83 ± 222.82 mg·kg^{-1}, respectively. These findings are consistent with previously published data [32].

By the end of the seven-day storage, there was a noticeable decrease in specific essential (lysine and histidine) and non-essential (glutamic acid, aspartic acid, glycine, arginine and tyrosine) amino

Tab. 1. Amino acid profile of snail meat after being stored for seven days.

Sample	C	C	A	B	AB
Storage time	Day 0	Day 7			
Amino acids	Content [mg·kg^{-1}]				
Essential amino acids					
Leucine	1 185.31 ± 112.11	1 145.62 ± 108.51	1 166.95 ± 123.66	1 139.75 ± 104.81	1 157.72 ± 130.91
Lysine	1 546.11 ± 147.21 ^{a}	1 024.42 ± 100.84 ^{c}	1 053.94 ± 78.73 ^{c}	1 256.93 ± 10221 ^{b}	1 321.12 ± 121.42 ^{ab}
Threonine	309.43 ± 25.31	312.22 ± 31.13	324.31 ± 31.92	311.71 ± 26.42	308.51 ± 20.92
Isoleucine	498.84 ± 19.94	486.71 ± 23.46	502.64 ± 34.51	470.92 ± 17.81	510.23 ± 29.93
Histidine	812.48 ± 30.73 ^{a}	612.39 ± 24.52 ^{c}	614.96 ± 22.12 ^{c}	698.72 ± 43.46 ^{b}	726.43 ± 36.71 ^{b}
Methionine	402.84 ± 30.72	398.82 ± 23.31	412.53 ± 36.54	405.85 ± 37.14	421.44 ± 43.21
Non-essential amino acids					
Glutamic acid	4 256.64 ± 257.22 ^{a}	3 082.21 ± 178.64 ^{c}	3 155.16 ± 165.84 ^{c}	3 648.23 ± 234.55 ^{b}	3 894.66 ± 210.88 ^{ab}
Aspartic acid	867.12 ± 49.93 ^{a}	453.62 ± 38.72 ^{c}	470.33 ± 56.72 ^{c}	646.75 ± 48.14 ^{b}	656.56 ± 37.83 ^{b}
Glycine	1 064.11 ± 102.81 ^{a}	656.16 ± 62.13 ^{c}	699.28 ± 51.14 ^{c}	864.94 ± 57.63 ^{b}	856.12 ± 36.84 ^{b}
Arginine	3 568.83 ± 222.82 ^{a}	1 886.45 ± 141.19 ^{c}	1 962.27 ± 124.25 ^{c}	2 325.83 ± 194.16 ^{b}	2 651.77 ± 184.64 ^{b}
Proline	462.94 ± 24.33	474.56 ± 19.92	459.23 ± 25.12	483.18 ± 38.26	459.76 ± 26.72
Alanine	1 487.76 ± 96.78	1 503.64 ± 102.31	1 514.66 ± 121.75	1 496.86 ± 103.13	1 496.76 ± 99.91
Serine	862.45 ± 76.29	877.72 ± 60.91	886.92 ± 57.84	855.94 ± 46.91	866.74 ± 50.91
Tyrosine	786.57 ± 53.52 ^{a}	386.52 ± 31.45 ^{c}	402.53 ± 38.97 ^{c}	624.76 ± 44.33 ^{b}	657.86 ± 53.21 ^{b}

Data are expressed as mean ± standard deviation from three experiments (n = 3), different lowercase letters in superscript indicate significant differences ($p < 0.05$) among specific amino acids.
C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

acids. Though microbial activity and proteolysis by endogenous enzymes might drive protein degradation [33], it is important to understand that amino acids might not degrade at the same rate. Oxidative processes, for example, might particularly impact amino acids like histidine and tyrosine, while other amino acids may show resistance or lesser susceptibility to degradation [34]. The effect of cold storage on amino acid dynamics in seafood is an area of interest.

After a week at 4 °C, the levels of amino acids such as lysine, histidine, glutamic acid, aspartic acid, glycine, arginine or tyrosine were significantly lower in both the control and the snail meat treated with 50.0 g·l^{-1} sodium ascorbate compared to those treated with 50.0 g·l^{-1} STPP or the combined 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} STPP ($p < 0.05$). This decline indicated that the treatment with only sodium ascorbate might not effectively inhibit microbes and/or enzymes, which contribute to protein and amino acid degradation. On the other hand, treatments with 50.0 g·l^{-1} STPP or its combination with sodium ascorbate may provide synergistic protection against protein breakdown and amino acid degradation.

A reduction in amino acids can impact the nutritional and sensory attributes of the meat [35]. A notable decrease in glutamic acid might alter the umami taste of the meat. Moreover, the degradation of certain amino acids could affect the structural integrity of the proteins, leading to changes in texture, tenderness and mouthfeel. Thus, maintaining the integrity of proteins and levels of amino acids is important for sustaining the quality of snail meat during storage. Our research suggested that sodium ascorbate and STPP have the potential to preserve the amino acid content and the overall quality of snail meat during refrigeration.

Biogenic amines

Understanding the formation of biogenic amines in snail meat during storage is crucial for ensuring food safety and quality. This section examined biogenic amine formation during a seven-day storage period at 4 °C, with the findings presented in Tab. 2. Initially, no agmatine, cadaverine, histamine, putrescine or tyramine were detected in snail meat. However, as storage progressed, levels of these biogenic amines rose across all treatment groups, in line with trends reported in previous studies [2]. After seven

days, the control group and snail meat soaked in 50.0 g·l^{-1} sodium ascorbate showed significantly higher agmatine and putrescine levels than the treatment groups, which included 50.0 g·l^{-1} STPP solution and a combination of 25.0 g·l^{-1} sodium ascorbate with 25.0 g·l^{-1} STPP solution ($p < 0.05$). The control group also exhibited significant increase in cadaverine, histamine and tyramine levels ($p < 0.05$). Biogenic amine formation during storage is known to be associated with amino acid decarboxylation, involving leucine, lysine, glutamic acid, aspartic acid, glycine, arginine, and tyrosine [36]. Changes in amino acid content could influence biogenic amine levels in snail meat, potentially affecting the product's overall quality, safety and consumer acceptability. High levels of biogenic amines in food products can pose health risks, in particular to sensitive individuals [37], emphasizing the importance of studying their formation in snail meat. Factors such as specific microorganisms, pH, temperature, water activity and free amino acid availability can affect biogenic amine formation. Controlling these factors is vital for ensuring quality and safety of snail meat and of other seafood products during storage. Preservation treatments, such as with sodium ascorbate, STPP or their combination, can apparently mitigate amino acid decarboxylation and the formation of biogenic amines. These treatments may inhibit proteolytic enzymes and microbial activity responsible for protein degradation, helping maintain the amino acid content and overall quality of snail meat during refrigerated storage.

Malic, succinic and lactic acids contents

The changes in the contents of malic, succinic, and lactic acids in snail meat during storage at 4 °C for seven days are summarized in Tab. 3. After seven days, the control group and snail meat soaked in 50.0 g·l^{-1} sodium ascorbate solution showed significantly higher malic, succinic and lactic acid contents compared to the treatment groups, which included snail meat soaked in 50.0 g·l^{-1} STPP solution and in a combination of 25.0 g·l^{-1} sodium ascorbate with 25.0 g·l^{-1} STPP solution ($p < 0.05$). During storage, especially under anaerobic conditions, glucose contained in the snail meat is converted into lactic acid via anaerobic glycolysis. Anaerobic glycolysis is a metabolic process that occurs in the absence

Tab. 2. Content of biogenic amines in snail meat after seven days of storage.

Sample	C	C	A	B	AB
Storage time	Day 0	Day 7			
Biogenic amines	Content [mg·kg^{-1}]				
Agmatine	0^{c}	221.92 ± 31.21^{a}	216.32 ± 28.83^{a}	164.33 ± 19.92^{b}	148.16 ± 12.44^{b}
Cadaverine	0^{e}	115.13 ± 11.41^{a}	110.74 ± 9.81^{b}	87.82 ± 6.41^{c}	63.38 ± 7.51^{d}
Histamine	0^{d}	10.22 ± 0.43^{a}	8.65 ± 0.25^{b}	5.33 ± 0.27^{c}	5.09 ± 0.25^{c}
Putrescine	0^{c}	137.73 ± 10.84^{a}	125.24 ± 10.32^{a}	94.41 ± 3.12^{b}	89.98 ± 2.74^{b}
Tyramine	0^{d}	26.16 ± 1.84^{a}	20.77 ± 2.21^{b}	10.21 ± 0.72^{c}	11.36 ± 0.94^{c}

Data, given as mean ± standard deviation from three experiments ($n = 3$), different lowercase letters in superscript indicate significant differences ($p < 0.05$) among specific biogenic amines.
C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

Tab. 3. Content of malic, succinic and lactic acids in snail meat after seven days of storage.

Sample	C	C	A	B	AB
Storage time	Day 0	Day 7			
Acids	Content [mg·kg^{-1}]				
Malic acid	8.95 ± 0.22^{c}	19.76 ± 0.42^{a}	18.98 ± 0.54^{a}	15.22 ± 0.54^{b}	15.09 ± 0.41^{b}
Succinic acid	45.57 ± 2.11^{c}	64.56 ± 3.13^{a}	62.46 ± 2.81^{a}	59.91 ± 1.45^{a}	53.46 ± 2.53^{b}
Lactic acid	7.26 ± 0.33^{d}	23.46 ± 0.54^{a}	21.62 ± 0.43^{b}	16.82 ± 0.35^{c}	16.13 ± 0.47^{c}

Data, represented as mean ± standard deviation from three experiments ($n = 3$), different lowercase letters in superscript indicate significant differences ($p < 0.05$) among specific organic acids.
C – control, A – samples treated with 50.0 g·l^{-1} sodium ascorbate, B – samples treated with 50.0 g·l^{-1} sodium tripolyphosphate, AB – samples treated with a combination of 25.0 g·l^{-1} sodium ascorbate and 25.0 g·l^{-1} sodium tripolyphosphate.

of oxygen, converting glucose into lactic acid. It is relevant in post-mortem changes in seafood products [38], including snail meat. This process contributes to the increase in lactic acid levels observed during chilled storage [39]. In addition to anaerobic glycolysis, the increase in malic, succinic and lactic acid contents can be attributed to microbial metabolism and endogenous enzyme activity, as previously discussed. The higher levels of these organic acids in the control group and snail meat soaked in 50.0 $g \cdot l^{-1}$ sodium ascorbate solution compared to other treatment groups indicated that sodium ascorbate alone might not be sufficient to effectively inhibit anaerobic glycolysis, microbial metabolism and endogenous enzyme activity. On the other hand, the treatments involving 50.0 $g \cdot l^{-1}$ STPP solution and the combination of 25.0 $g \cdot l^{-1}$ sodium ascorbate with 25.0 $g \cdot l^{-1}$ STPP solution resulted in lower malic, succinic and lactic acid contents in the snail meat. The antimicrobial properties of STPP and the antioxidant properties of sodium ascorbate likely contributed to this outcome by inhibiting the processes that lead to increased acid production, including anaerobic glycolysis.

CONCLUSIONS

This study comprehensively investigated the effects of various treatments on the quality of snail meat during storage at 4 °C for seven days. The treatments included soaking snail meat in 50.0 $g \cdot l^{-1}$ sodium ascorbate solution, 50.0 $g \cdot l^{-1}$ STPP solution, a combination of 25.0 $g \cdot l^{-1}$ sodium ascorbate with 25.0 $g \cdot l^{-1}$ STPP solution, and a control group without any treatment. The quality parameters assessed were pH, K-value, *TVB-N*, *p*-anisidine value, microbiological analysis, amino acid composition, biogenic amine content and malic, succinic and lactic acid contents. The results demonstrated that the treatment involving a combination of 25.0 $g \cdot l^{-1}$ sodium ascorbate and 25.0 $g \cdot l^{-1}$ STPP solution was the most effective in preserving the overall quality of snail meat during chilled storage. This treatment led to a better maintenance of pH, lower K-values, reduced *TVB-N* and *p*-anisidine values, lower microbial counts and a more stable amino acid composition compared to the control group and the other two treatments. Additionally, the combined treatment resulted in lower contents of biogenic amines and malic, succinic and lactic acids, indicating effective inhibition of enzymatic and microbial processes. These findings suggest that the combination of 25.0 $g \cdot l^{-1}$ sodium ascorbate and 25.0 $g \cdot l^{-1}$ STPP solution as a soaking treatment is a promising procedure for preserving the quality and extending the shelf life of snail meat during chilled storage.

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