Effect of EDTA on Physical and Sensory Properties of Pacific White Shrimp (*Litopenaeus vannamei*) during Ice Storage

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Abstract

Fresh white shrimp were soaked in different concentrations of EDTA and soaking periods and stored on ice. The following treatments were used: A (control samples), ED-32 (30 mM EDTA for 20 min), ED-33 (30 mM EDTA for 30 min), ED-52 (50 mM EDTA for 20 min) and ED-53 (50 mM EDTA for 30 min). Quality evaluation of product treated with EDTA was conducted using physical and sensory analysis. Untreated white shrimp sample showed lower shear force and higher cooking loss for the whole storage time compared to ED-32, ED-33, ED-52 and ED-53 samples, with the latter showing a significant decreased at the end of the storage period. Interestingly, the presence of ED-53 white shrimp sample resulted in the lowest drip loss and the highest shear force compared to all other samples. Based on sensory score, the shelf-life of the soaked ED-53 shrimp stored at 4 °C was approximately 7 days, while the soaked ED-52, ED-33 and ED-32 were 6 days compared to the control's (C) shelf-life was only 5 days.

Keywords : Quality, Pacific white shrimp, Soaking, EDTA, Deterioration.

1. Introduction

Shrimps are of economic importance worldwide by an extremely good source of protein, yet are very low in fat and calories, making them a very healthy choice of food. Additionally, shrimp flesh consists of highly unsaturated fatty acids such as eicosapentaenoic (C20:5n3, EPA) and docosahexaenoic (C22:6n3, DHA) acids, which are considered as essential for the human diet [1]. Nowadays Thailand is the world's leading shrimp-farming country. Pacific white shrimp, *Litopenaeus vannamei* (Boone,1931) is the most important shrimp for aquaculture [2-3].

Despite their delicacy, shrimps are highly perishable and postmortem changes occur rapidly compared with fish. The neutral pH, high water activity, high content of free amino acids and other soluble non-nitrogenous substances [4-5], which partly contribute to the desirable, delicate sweet taste of shrimp with a limited shelf-life. Its shelf life and wholesomeness during refrigerated storage and shipping is greatly influenced by microbial spoilage, chemical changes and melanosis (discoloration) [6-7].The majority of shrimps purchased by consumers are cooked and chilled [8]. The market value of shrimp is predominately based on the first issue is the visual appearance of their body colour. The appearance of the product and the resulting quality implications play a significant role

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in maintaining a high consumer acceptance. Unfavorable color change associated with melanosis on the surface of shrimp products has been of great concern to food processors. Discoloration in shrimp, called melanosis or blackspot is a natural post-mortem process originated by the polymerization of phenols into insoluble black pigments, the melanins by polyphenoloxidase (also called phenoloxidase) followed by non-enzymatic polymerization of the quinones, giving rise to dark pigments of high molecular weight [9-10]. The second problem limiting market value of shrimp is the deterioration of acceptable texture. Unwanted textural changes such as muscle softening are often seen and endogenous proteolytic enzymes are played an important role in this process. Different proteolytic systems exist within a muscular cell. Calpain system and the cathepsins, in synergy, are suggested to be responsible for post mortem muscle protein degradation [11].

To increase shelf-life and reduce melanosis, also delay soft texture were shrimp processing factory needed. Ethylenediamine tetraacetic acid (EDTA) is a chelating agent permitted for use in the food industry as a chemical preservative and has been approved for use as food additive by the United States Food and Drug Administration. The World Health Organization (WHO) and the FDA have independently assessed the health safety of EDTA and have set the acceptable daily intake of EDTA at 2.5 mg/kg/person/day or roughly 150 mg/day for the calcium disodium salt. [10]. The added EDTA food grade suppresses the melanosis and proteolytic activity by chelation [12-13]. Nevertheless, no information regarding the use of EDTA in preventing melanosis formation and maintain texture in Pacific white shrimp has been reported. Thus, the aims of this investigation were to study the effect of EDTA on Pacific white shrimp quality as well as to monitor physical quality and sensory properties of Pacific white shrimp during ice storage.

2. Materials and methods

2.1 Chemicals and preparation of EDTA solution.

Ethylenediamine tetraacetic acid was purchased from Wako Chemical Industries (Tokyo, Japan), $C_{10}H_{14}N_2O_8.Na_2.2H_2O$, 99.5% purity, food grade. A stock solution of 500 mM concentration was prepared by diluting 186.15 g in 1 L distilled water. A final concentration of 30 and 50 mM EDTA solution were prepared from the stock solution. The amount of EDTA added to the treated shrimps was 0.28 g/kg.

2.2 Shrimp collection and preparation

Pacific white shrimps (*L. vannamei*) with the size of 100–110 shrimps/kg were purchased from shrimp farm at Chachoengsao, Thailand. The shrimps were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Aquatic Science, Burapha University within 1.3-2.0 h. Upon arrival, shrimps were washed in cold water and stored on ice until used (not more than 4 h).

2.3 Preparation of shrimps soaked in EDTA solute

Whole fresh Pacific white shrimps (head-on) were soaked in the different of EDTA concentration and soaking time as 30 mM EDTA for 20 min (ED-32), 30 mM EDTA for 30 min (ED-33), 50 mM EDTA for 20 min (ED-52) and 50 mM EDTA for 30 min (ED-53) at a shrimp/solution ratio of 1:2 (w/v) at 4^{o}C. Treated shrimps were drained on the screen for 5 min at 4^{o}C. Shrimps without any treatment were used as the control. All samples were stored in LDPE bag (23×16 cm^2) (Chonburi, Thailand) before stored in polystyrene boxes containing ice using a shrimp/ice ratio of 1:2 (w/w). To maintain shrimp/ice ratio, the molten ice was removed and the same amount of ice was added. Samples (80 shrimps) were taken for each treatment

everyday up to 8 days for physical and sensorial analysis.

2.4 Effect of EDTA on physical quality of Pacific white shrimp during ice storage.

The clay tiles were removed every day. The periphytic was scrubbed from the clay tiles by toothbrush, and thenfrom time to time, distilled water was poured over the tiles using a pipet. The solution obtained was filled up to 100 ml. From this solution, 30 ml aliquots were taken and filtered for chlorophyll a and c measurement according to trichromatic method [1]

2.4.1 Determination of cooking loss.

Cooking loss was measured by weighing the whole shrimp before and after pre-cooking. Shrimps were pre-cooked by submerging the samples in boiling water (70°C) until the core temperature of the second segment of shrimp reached 70°C for 50 sec, immediately cooled in ice water for 1 min and drained at 4 °C for 5 min. The 'pre-cooked shrimps' were weighed. Cooking loss was calculated using the following equations: Cooking loss (%) = ((A–B)/A) × 100

Where A is the weight before pre-cooking and B is the weight after pre-cooking, followed by cooling in ice water.

2.4.2 Shear strength.

Shear strength was determined on the axis of muscle fibers at the second segment of shrimp for peeled raw sample and peeled pre-cooked sample of similar size and weight. The sample was placed on base and used Warner-Blatzler shear blade. A computer-controlled TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY) was used, with a cell load of 50 N at a setting of 2 mm/sec. Results were average of 10 samples and were expressed as Newtons per gram of muscle at the point of maximum load before sample breaking.

2.4.3 Colour

The second segment of shrimp for peeled raw sample and peeled pre-cooked sample colour were measured by Hunterlab colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) and reported in CIE system. L∗, a∗ and b∗ parameters indicate lightness, redness/greenness and yellowness/blueness, respectively [14]. Results were expressed as the mean of ten measurements.

2.5 Effect of EDTA soaking on sensory properties of Pacific white shrimp during ice storage.

2.5.1 Sensory evaluation.

At day 0 and 7 of storage, the control and shrimps soaked in EDTA solution were boiled at 70°C for 50 sec., drained at room temperature for 15 min. The pre-cooked samples were evaluated by 15 panelists from the Department of Aquatic science with the ages of 20–25, using the 9-point hedonic scale, where 9: like extremely; 7: like moderately; 5: neither like or nor dislike; 3: dislike moderately; 1: dislike extremely [15]. Panelists were regular consumers of shrimp and had no allergies to shrimp. All panelists were asked to evaluate for appearance, odour, taste, texture and overall likeness (total acceptance). Samples were presented peeled in plates coded with three-digit random numbers.

2.6 Statistical analyses

All experiments were performed in triplicate. A factorial in completely randomized design (CRD) was used for physical quality and randomized completely block design (RCBD) was used for sensory evaluation. Analysis of variance (ANOVA) was performed and means comparisons were done by Duncan's multiple range tests [16]. Analysis was performed using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL, USA).

3. Results and Discussion

3.1 Effect of EDTA soaking on physical quality of Pacific white shrimp during ice storage

3.1.1 Cooking loss

As shown in Figure 1, there were significant differences in cooking loss between storage time for all EDTA soaking condition and cooking loss increased with an increased in the storage time. Increasing trend of cooking loss during ice storage caused by enzymatic activity. As proteins denature and water is liberated from the tissue and this water needs to reach the surface to be ‘‘lost’’ [17]. Whilst cooking losses showed significant differences amongst the differentiate of EDTA soaking condition. Cooking losses were lowest in ED-53, whilst those of ED-52, ED-33 and ED-32 were the highest, respectively. The increase in water loss volume indicated loss of water-holding capacity of the muscle. Thus, the higher cooking loss samples may also be construed as weakening of the myofibril lattices due to protein degradation by proteolytic activity. EDTA soaking suppresses the proteolytic activity by chelation [12, 13, 17].

3.1.2 Shear strength

Significant effect on shear strength in EDTA soaking shrimp was observed. Shear strength decreased in control and treated samples during storage time as shown in Figure 2. Reduction of shear strength is due to deterioration by proteolytic activity on protein muscle [18]. The decrease in shear force suggested loss in integrity of muscle fibers, leading to the weakening of muscle [19]. It was most likely that the destruction of muscle fibers of the control sample was more pronounced, compared with sample treated with EDTA. The degradation of muscle tissue caused by hepatopancreatic enzymes started from the perimysium, endomysium, the Z line and the H zones with concurrent degradation of the connective fibers and myofibrillar proteins [20].

During 1–7 days of storage, shear force of the ED-53 sample was the highest, when compared with other samples ($p \leq 0.05$). The highest shear force of ED-53 sample kept in ice for a long time might be caused by EDTA inhibited muscle proteolytic enzyme and hepatopancreatic enzymes, which caused the destruction of muscle fibers and connective tissues. Similar result was observed in haddock (*Melanogrammus aeglefinus*) fillets treated with EDTA solution [21].

3.1.3 Colour

Changes in colour parameters L* (lightness), a* (redness) and b* (yellowness) of raw Pacific white shrimp (*L. vannamei*) during chilled storage are indicated in Figure. 3. L*, a* and b* values decreased with longer storage time in all treatments, which caused by the development of melanosis during the storage in ice. Melanosis occurs in shellfish during storage as a result of the action of polyphenol oxidase (PPO) and phenol oxidase (PO) on tyrosine or its derivatives, such as tyramine, to form melanin [22]. A decrease in L* value may be considered as indicative of browning. Raw Pacific white shrimp soaked with EDTA (ED-32, ED-33, ED-52 and ED-53) presented significantly higher lightness than control samples after treatment. The lightness of ED-53 sample was also the highest during 7 days of chilled storage. In all cases, redness (a*) and yellowness (b*) tended to decrease during storage, meaning that samples changed colour to greenish and more blue. It is important to emphasise that ED-53 sample showed significantly the highest values during 7 days of storage. Melanosis inhibited by EDTA which may chelated with copper on copper-containing o-diphenoloxidase caused enzyme inhibition. Melanosis as a result of the action of polyphenol oxidase (PPO) and phenol oxidase (PO) were decreased.

A similar trend was observed in EDTA treated samples of pre-cooked Pacific white shrimp (*L. vannamei*) as shown in Figure 4.

All colour attributes (L*, a* and b*) decreased with longer storage time in all treatments. Pacific white shrimp colour turn to dark and greenish along the storage time. Colour of EDTA treated samples were increased with increasing EDTA concentration and soaking periods.

The lightness, redness and yellowness of ED-53 sample was also the highest during 7 days of chilled storage. Apart from melanosis shrimp colour changes also occur because of lipid oxidation, which degrades carotenoid pigment astaxanthin [23-24]. EDTA suppresses the non-heme iron-catalyzed lipid oxidation by chelation [25]. Similar results have been reported in deepwater pink shrimp (*Parapenaeus longirostris*) treated with EDTA mixed solution [26].

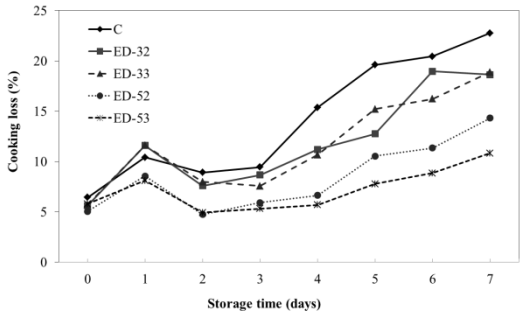
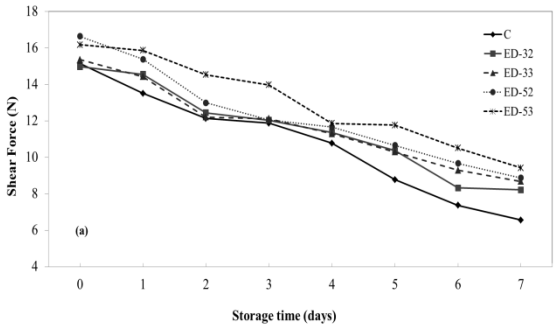


Fig .1. Changes in cooking loss of Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.



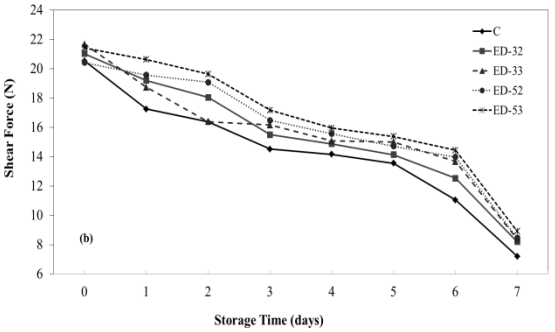
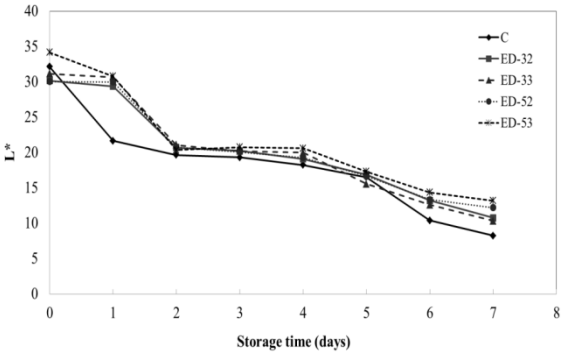
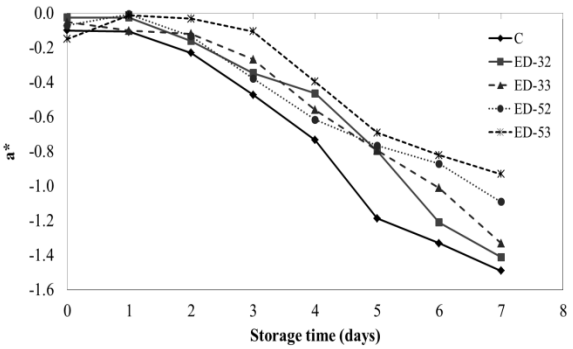


Fig .2. Changes in shear strength of (a) raw Pacific white shrimp (*L. vannamei*) (b) pre-cooked Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.





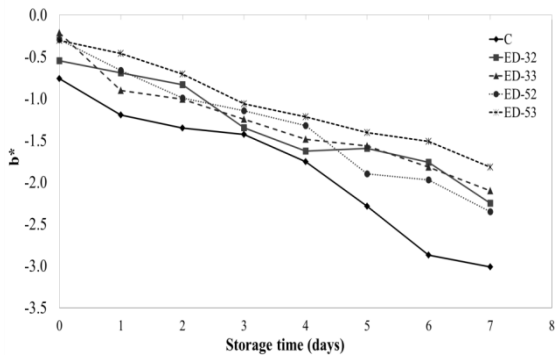


Fig .3. Changes in colour of raw Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.

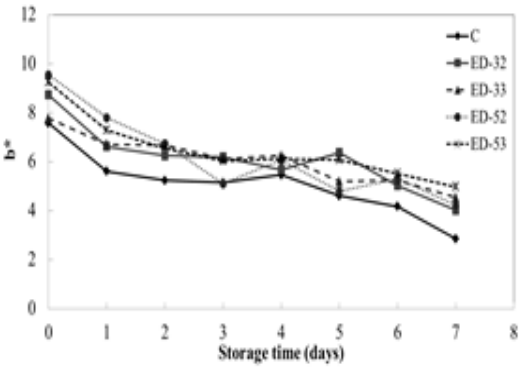
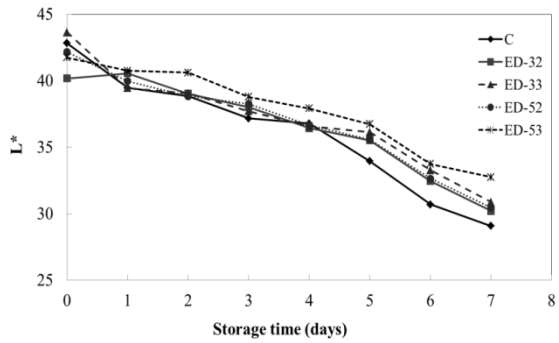
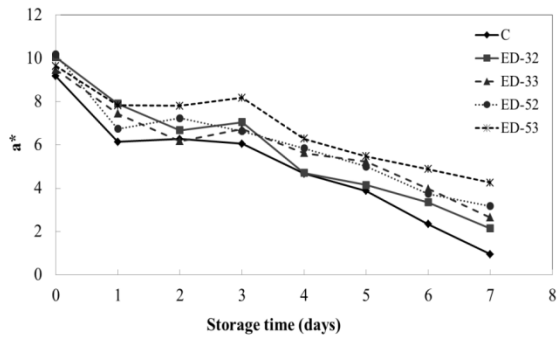


Fig.4. Changes in colour of pre-cooked Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.





3.2 Effect of EDTA on sensory properties of Pacific white shrimp during ice storage

3.2.1 Sensory evaluation

Figure 5 and 6 shows all of the organoleptic parameters (appearance, colour, odor, taste and overall) was significantly affected by EDTA soaking. Immediately after EDTA soaking (day 0), the panelists detected no difference ($p > 0.05$) between all treatment for every quality attributes. While, for all samples the panelists detected significant difference ($p \leq 0.05$) in sensory attributes by storage periods. As expected, the scores of each attribute were highest on day 0.

Appearance likeness scores of raw shrimp were decreased by storage times because increasing of blackening of raw shrimp then spread along the exoskeleton during further storage and was manifest mainly in the zones where the cuticle segments are joined to the pleopods. These results were in agreement with the study of brown shrimps (*Farfantepenaeus aztecus*) [27] in which the development of melanosis following 3 days refrigerated storage. A similar trend was observed in pre-cooked shrimp.

Decreasing of odor scores for raw and pre-cooked shrimp by storage

periods could be related to the presence of some volatile compounds produced by bacteria deterioration, including trimethylamine already known to be responsible for strong fishy and ammonia-like off-odours. Lipid oxidation leads to unpleasant odour, rancid taste and discolouration [28-30].

Hence, the decrease in taste likeness scores of pre-cooked shrimp could in some part be due to the oxidation of lipids. The products of fatty acid oxidation produce off-flavours and odours usually described as rancid [30]. Lipid oxidation in muscle system is initiated at the membrane level in the phospholipid fractions as a free-radical autocatalytic chain mechanism in which prooxidants interact with unsaturated fatty acids specially shrimp muscle consists of highly unsaturated fatty acids (HUFA) such as eicosapentaenoic (20:5n3, EPA) and docosahexaenoic (22:6n3, DHA) acids [31]. The resulting of interaction is the generation of free radicals and propagation of the oxidative chain [32].

Texture likeness scores of pre-cooked shrimp were decreased by storage periods because protein in shrimp muscle was deteriorated by proteolytic activity, leading to the weakening of muscle [18-19]. During 1-8 days of storage, sensory scores for raw and pre-cooked shrimp in ED-53 sample was the highest, when compared with other samples ($p \leq 0.05$). The highest sensory score of ED-53 sample kept in ice for a long time might be caused by chelate divalent cations involved in stabilizing the myofibrillar protein structure and consequently making the proteins unsusceptible to proteolytic degradation. Also the soaked EDTA suppresses the non-heme iron-catalyzed lipid oxidation by chelation. Therefore, in the present study the overall attribute (total acceptance) was used for the sensory evaluation of pre-cooked white shrimp and determination of shelf-life. Based on sensory analysis, the control's (C) shelf-life was only 5 days, ED-52, ED-33 and ED-32 extended the shelf-life of white shrimp by 1 day while treatments ED-53 by 2 days (Figure 6) because it is noteworthy that soaked EDTA (ED-53) imparted desirable and pleasant odor, taste in cooked white shrimp samples until day 7 of storage, attributes well appreciated by the panellists in agreement to results reported for fresh northern snakehead (*Chana argus*) fillets coated with EDTA mixed solution were increased the overall sensory score compared with control [33].

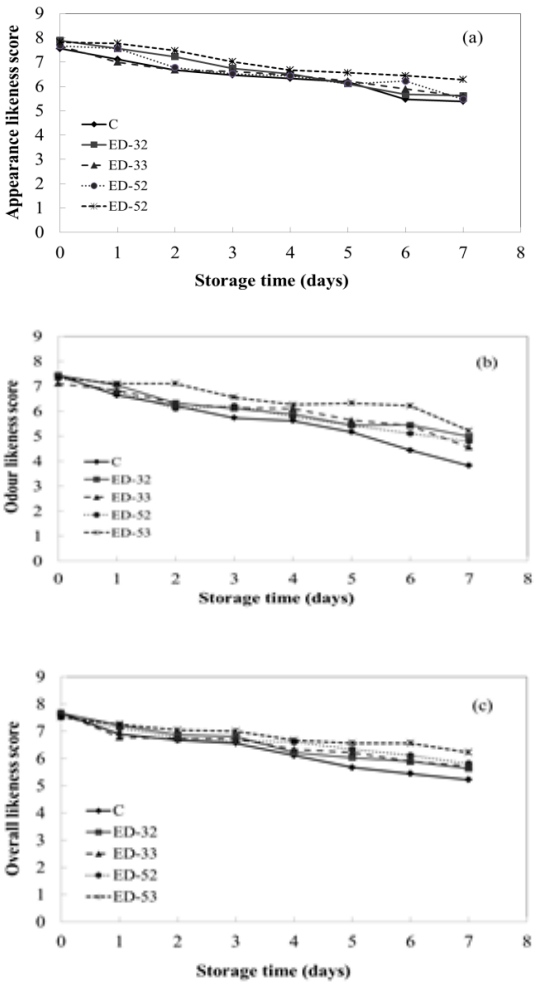
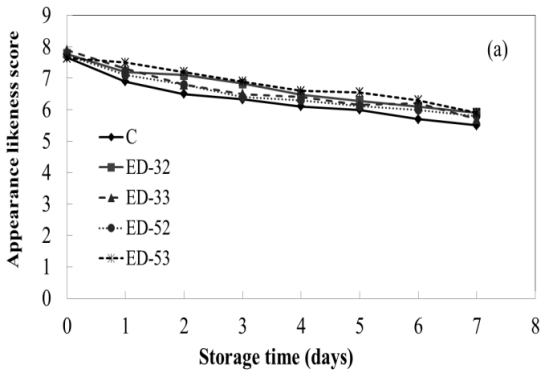
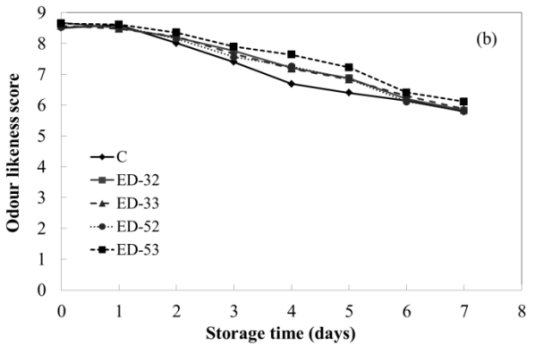
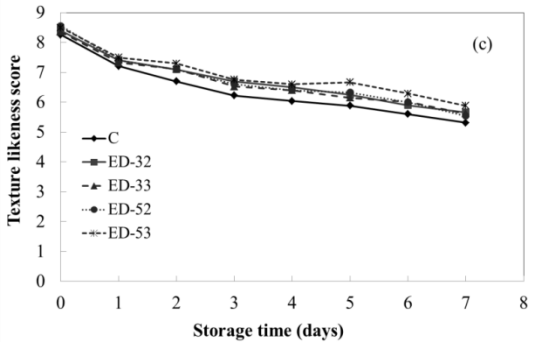
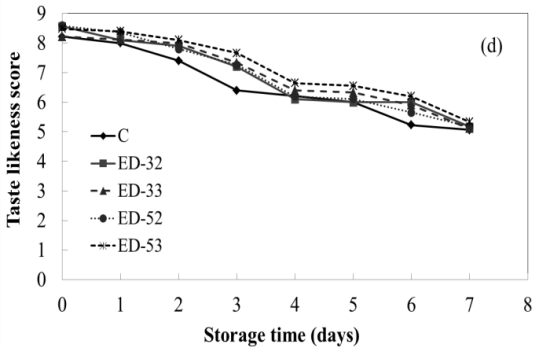


Fig. 5. Changes in sensory score of raw Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.









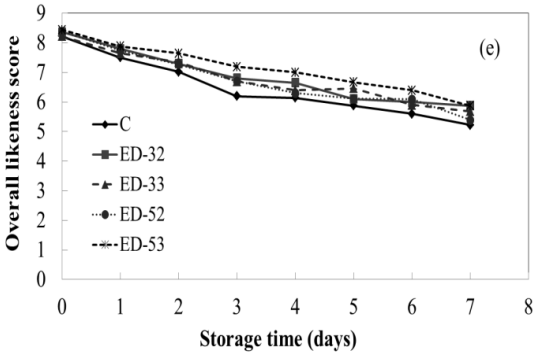


Fig .6. Changes in sensory score of pre-cooked Pacific white shrimp (*L. vannamei*) in control and EDTA treated samples during ice storage.

4. Conclusions

Based on physical and sensory analyses data, the shelf-life of the soaked ED-53 (50 mM EDTA for 30 min) shrimp stored at 4 °C was approximately 7 days, while the soaked ED-52, ED-33 and ED-32 were 6 days compared to white shrimps without any treatment was 5 days. Present results support the hypothesis that the physical quality and sensory properties of Pacific white shrimp be retarded by EDTA soaked.

5. Acknowledgements

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6. References

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