Shelf-life extension of pre-cooked shrimp (*Litopenaeus vannamei*) by oregano essential oil during refrigerated storage

Bayu Kusuma[1] and Savaminee Teerawut[1]*

[1] *Department of Aquatic Science, Faculty of Science, Burapha University*

Abstract

The present study evaluated the use of alginate–based coating with or without addition of oregano essential oil (OEO), as an antimicrobial treatment for shelf-life extension of pre-cooked shrimp stored under refrigeration for a period of 16 days. Four different treatments were tested: TC, control sample, TA, alginate–based coating and T05, T10, coated samples with alginate–based coating incorporated with 0.5% OEO and 1.0% OEO (v/v), respectively. With regard to the chemical freshness indices determined, total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) values of OEO treated pre-cooked shrimp samples were significantly lower compared to control samples during the entire refrigerated storage period. Based primarily on sensory evaluation (odor), the use of T10 and T05 extended the shelf-life of pre-cooked shrimp by 12 and 14 days, respectively, compared to 8 days in TC treatment samples, extending the shelf-life of T10 and T05 by 2 and 4 days, respectively,

Keywords : Shrimps / Oregano Essential Oil / Coating / Shelf-life

***Corresponding author.** E-mail: sawamin@buu.ac.th

1. Introduction

Prawn and shrimp are the most important products from aquaculture: more than 3.7 million tons were produced in 2010 with a value of more than 16 billion US dollars. Moreover, *Litopenaeus vannamei*, also called Pacific white shrimp, is the species most reared with about 2.7 million tons in 2010 representing 11 billion US dollars on its own (FAO Fisheries Statistics, 2012). The current market trend is for the processing of value-added products like cooked shrimp, which is very popular and widely sold in supermarkets as a chilled ready-to-eat product. Shrimp is a perishable product. Its shelf-life and wholesomeness during refrigerated storage and shipping is greatly influenced by both enzymatic and microbiological changes. During the last several years, reliable methods have been developed to extend the shelf-life of shrimp and to avoid health hazards for consumers (Al-Dagal and Bazaraa, 1999). Such preservation methods include cold storage in ice (Shamshad *et al*., 1990). The growing consumer demand for food without chemical preservatives has focused efforts towards research on natural antimicrobials and antioxidants. Thus, investigation in an attempt to extend the shelf-life of seafood without adversely affecting its quality and safety is more interest. Recently, a biopreservation method involving essential oils (EOs) has been successfully used to extend the shelf-life of seafood products (Abdollahzadeh, *et al*., 2014) and edible coating from alginate has also been applied on seafood products during storage for retarding its shelf-life too (Lu, *et al*., 2010). EOs are liquid, volatile, limpid, and rarely coloured. Lipids are soluble organic solvents as well as soluble in themselves. They can be synthesized by all plant organs (Bakkali, *et al*., 2008) and are categorised as Generally Recognised as Safe (GRAS) (López *et al*., 2007). EOs also display an antimicrobial role by enzyme systems and genetic material of bacteria, interfering with and destabilizing the operation of the phospholipid bilayer of the cell membrane (Solomakos *et al*., 2008). Carvacrol and thymol are the principal constituents of oregano essential oil (Burt, 2004). Its antimicrobial properties have been demonstrated in numerous studies (Ozkalp *et al*., 2010, and Gómez *et al*., 2010). Alginate, isolated from brown algae, is a salt of alginic acid. It is used as an edible coating because of its unique colloidal properties and its ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations, like calcium (Rhim, 2004). Alginate coating containing antimicrobial agents such as nisin had been studied (Jin and Zhang, 2008). Oregano oil incorporated in alginate–based coating has not been studied.

Considering the above, the aim of this study was to investigate the effect of oregano essential oil loaded active coating on the shelf-life of pre-cooked shrimps. In particular, quality decay of the investigated product was assessed by monitoring the following quality sub-indices: chemical and sensorial.

2. Materials and methods

2.1 Shrimps

Pacific white shrimps (*L. vannamei*) at a weight of 60-80 shrimp/kg were harvested fresh from farms located in Chachengsao, Thailand. Shrimps were distributed uniformly in the styrene foam boxes between layers of ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the laboratory within approximately 2 h. Upon arrival, shrimp were washed in cold water and stored in ice until cooked (not longer than 2 h). Whole Pacific white shrimp (head-on) was pre-cooked by submerging the samples in boiling water (100 °C) until the core temperature of the second segment of shrimp reached 75°C. To measure the core temperature, the thermo-couple (Union, Kowloon, Hong Kong) was inserted into the middle of the second segment of abdomen. The samples were cooled rapidly in iced water for 1 min and then the shrimp samples were drained for 5 min at 4 °C. The shrimps were manually deshelled, rinsed. The samples were referred to as 'pre-cooked shrimp'.

2.2 Materials

Food grade sodium alginate (Yantai Xinwang Seaweed Co., Ltd., Shandong, China) was used as a polysaccharide-based edible coating. Tween (Sigma–Aldrich, Argentina) was added as an emulsifier. Calcium chloride (Quzhou Menjie Chemicals Co., Ltd, Shandong, China) was used for gel forming and cross-linking reactions. Food grade oregano essential oil (OEO) (Changsha Winner Bio-Tech., Co., Ltd (Hunan, China) obtained by steam distillation used as a natural antimicrobial agent in the alginate-based edible coating formulation.

2.3 Preparation of the coating-forming solutions and treatments

An alginate-based edible coating formulation was prepared by dissolving sodium alginate 0.5% (w/v) powder in distilled water while heating with stirring on a hot plate at 80 °C until the mixture became clear. Different concentrations (0.5% and 1.0%, v/v) of oregano essential oil (OEO) were then incorporated into the alginate-based edible coating formulation. The overall volume for each formulation was 500 mL and this included alginate, OEO with the remainder distilled water. All formulations were mixed in homogenizer (Homogeniser PowerGen 125, Fisherbrand., UK) for 5 min at 24,500 rpm to form emulsions. For a cross-linking reaction necessary for gel formation, a 1% (w/v) calcium chloride solution was prepared.

2.4 Coating treatment and storage of pre-cooked shrimp

The pre-cooked shrimps were immersed in the OEO alginate-based formulations at a shrimp/solution ratio of 1:4 (w/v) for 5 sec at 4 °C and excess coating materials were allowed to drip off for 1 min at 4 °C. The treated shrimps were then dipped in calcium chloride solution for 1 min. The samples were then air-dried at 4 °C for 10 min. Then ten shrimp of each treatment were placed on a polystyrene tray. The tray containing samples were inserted in LDPE bag (23×16 cm^2) (Chonburi, Thailand), and were stored at 4 ± 1^{O}C. All treatments are listed as follows: TC: Pre-cooked shrimp, TA: Pre-cooked shrimp coated alginate-based formulations, T05: Pre-cooked shrimp coated with 0.5% OEO alginate-based formulations and T10: Pre-cooked shrimp coated with 1.0% OEO alginate-based formulations. Determinations of chemical analysis and sensory evaluation were carried out every 2 days up to 16 days. Total number of bags used was 18 for each treatment.

2.5 Samples analyses

2.5.1 Chemical analysis

Total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) were measured in triplicate, using the method described by Conway and Byrne (1933) on a 60 g portion of shrimp.

2.5.2 Sensory evaluation

Sensory evaluation of pre-cooked shrimp treated with OEO was performed by using a twenty member untrained panel. Panelists were recruited among students and staff of the department. Treated pre-cooked shrimp samples were wrapped in aluminium foil individually and cooked in a steam-cooker (IM-LP-864, Imarflex, Thailand) for 5 min, and served warm to panelists for sensory evaluation. Each sample was coded with 3 digits random numbers. The panelists were scored the sensory appearance, odour, taste and overall acceptability attributes by using a 9-point hedonic scale, where 1 refers to unacceptable and 9 refers to very acceptable, while over score of 5 was defined as the odor attribute, whereas the sample was classified as unacceptable after development of the first off-odor (Botta, 1995). The odor attribute was used as the decisive parameter in a related study of OEO on the shelf-life of sea bream (Goulas and Kontominas, 2007)

2.6 Statistical analyses

Statistical evaluations of the chemical and sensory analysis were made by using a SPSS package (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL, USA). A one-way ANOVA (analysis of variance) method followed by Duncan's multiple range tests was used to evaluate the significant difference ($p < 0.05$) among the different OEO treatments.

3. Results and discussion

3.1. Changes in chemical quality

TVBN may be considered as a quality index for fish. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Ruiz-Capillas and Moral, 2005). TVBN represents the sum of ammonia, dimethylamine (DMA), trimethylamine (TMA) and other basic volatile nitrogenous compounds. DMA and TMA were the degradation products of trimethylamine oxide (TMAO), a fish typical molecule which has an important role in osmoregulation. DMA is mostly produced by endogenous enzymes and TMA is generated by bacterial enzymes (Zeisel *et al*., 1985). OEO alginate–based coating has a significant effect on TVBN in pre-cooked shrimp during chill storage. Changes in TVBN content of control and incorporation of 0.5% and 1.0% (v/v) OEO and alginate-based coated samples stored in chill storage are shown in Fig. 1(a). Initial TVBN values were 14.27, 14.53, 12.34 and 10.23 mg N/100 g in TC, TA, T05 and T10 samples, respectively in agreement with literature data (Gimenez *et al*., 2002). During storage TVBN values were found to be increasing in all samples. TVBN values were the lowest

in T10, whilst those of T05, TA and TC were higher, respectively. The lowest TVBN of T10 sample might be caused by the higher oregano essential oil concentration was more inhibited the microbial growth and the enzyme production of bacteria, which caused the spoilage. TA samples were found to be higher TVBN value than TC may be due to the preventing microbe contamination (Yu *et al*., 2008). Similar result was observed in Mediterranean octopus (*Octopus vulgaris*) treated with oregano essential oil 0.2 and 0.4% (v/w) and vacuum packed (Atrea *et al*., 2010). Increasing trend of TVBN content during chill storage may be due to the endogenous enzymatic activity (Ruiz-Capillas and Moral, 2005). Limit of acceptability of TVBN content in freshly caught fish is typically between 5 and 20 mg N/100 g, and TVBN content of 30–35 mg N/100 g is usually regarded as the limit of acceptability for fish (Huss, 1988). It has been suggested that the TVBN content is affected by species, season, harvesting area, age and sex of fish (Kilinc and Cakli, 2005). In TC, TA and T05 samples TVBN exceed before 8th, 10th and 12th day of storage respectively, whereas in T10 the limits were attained on 14th day of storage. During storage, TVBN values in OEO treated samples were lower than control and the results agree with Atrea *et al*. (2010). The preservative action of OEO can be attributed mainly to the antibacterial properties of this aromatic plant and more specifically to its phenolic constituents: carvacrol and thymol (Burt, 2004). An important characteristic of these phenolic components is their hydrophobicity, which enables their partition within the lipids of the bacterial cell membrane and mitochondria, interfering with the phospholipid bilayer. Such activity disturbs cell structures, which causes an increased permeability and loss of cellular constituents (Mejlholm and Dalgaard, 2002).

TMAN values of pre-cooked shrimps are presented in Fig 1 (b). The content of TMA-N was from 3.48 to 4.85 mg/100 g in pre-cooked shrimp and OEO treated sample. As noticed for TMAN values of control were found to be higher than in the other sample. The increase in TMAN values was observed throughout the storage time. TMA is produced by the decomposition of TMAO, mainly because of microorganism-mediated spoilage and enzymatic activity, and TMAN is the major component responsible for the fishy off-flavors in spoiled seafoods (Fu *et al*., 2007).

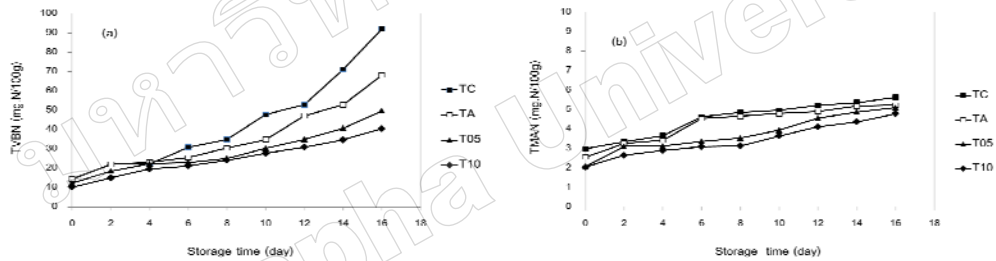

Fig. 1. The effect of OEO alginate–based coating on (a) TVBN and (b) TMAN values of pre-cooked shrimp during refrigerated storage at 4 ± 1^{O}C: uncoated (TC; ■) alginate-based coated (TA; □), 0.5% OEO incorporated alginate-based coated (T05; ▲) and 1.0% OEO incorporated alginate-based coated (T10; ♦).Bars represent the standard deviation (n = 3).

At the last day (days 16) the formation of TMAN value in TC when compared with those treated samples at TA, T05 and T10, which attained much higher value and the TMAN value in TC was 5.67 mg/100 g while TA, T05 and T10 were 5.23, 5.10 and 4.80 mg/100 g, respectively. OEOs, its phenolic constituents as carvacrol and thymol can degrade the cell wall, disturb the phospholipid bilayer of the cytoplasmic membrane, and damage the membrane proteins leading to increased permeability of the cell membrane and loss of cellular constituents. (Burt, 2004, and Teerawut, in press). This was consistent with the behavior OEO treated sea bream (*Sparus aurata*) as shown in report of Goulas and Kontominas (2007). With respect to TMAN, 5 mg N/100 g was considered the limit of acceptability for freshness of *L. vannamei* shrimps quality (Odilichukwu *et al*., 2014). Our results indicated that the TMAN of TC, TA, T05 and T10 samples were not acceptable after days 10, 12, 14 and 16, respectively. Odilichukwu *et al*. (2014) and Huang *et al*. (2012) suggested that the TMAN value 5 mg N/100 g of Pacific white shrimp muscle at the upper limit of acceptability while according to Connell (1995) the acceptable limit of TMAN was assigned as 10-15 mg N/100 g for fish muscle. The amounts of TMAN in fish are considered by many workers

as the most promising indices of quality deterioration of seafood during storage (Krzymien & Elias, 1990). TMAN content varies with species, season and type of storage (Ababouch *et al*., 1996).

3.2 Sensory evaluation

Sensory analysis is very important, especially for coatings incorporated with OEO. These components may impact on the sensory attributes such as taste and odor of coated seafood product (Goulas and Kontominas, 2007). In the present study, sensory evaluation based on appearance, odor, taste and overall acceptability scores of coated and uncoated samples was carried out every 2 days of storage at 4°C (Fig. 2).

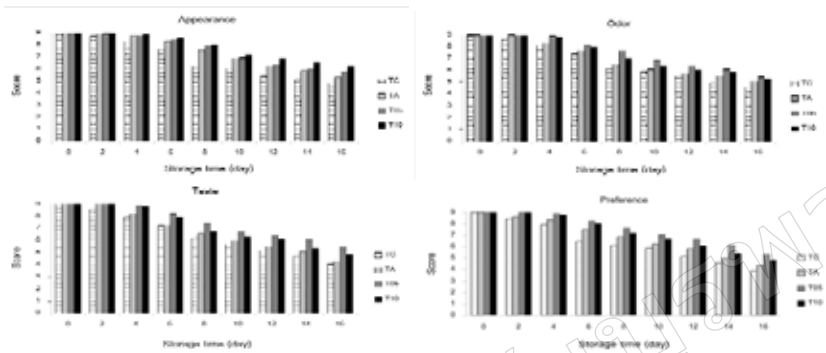

Fig. 2. Sensory evaluation of pre-cooked shrimp coated with oregano essential oil at 0.5% (TO5), 1.0% (T10) and alginate–based (TA) and uncoated (TC) during refrigerated storage at 4 ± 1°C. Attribute odor score limit 6.

As the storage time increased, a continuous increase in deterioration. Shrimp is a highly perishable product and its shelf-life under refrigerated storage is limited due to its biological composition (Gram and Dalgaard, 2002). The main cause of deterioration is the activity of typical spoilage seafood microorganisms, provoking loss of essentially fatty acids, fat-soluble vitamins and protein functionality, production of biogenic amines, and formation of off odors. Also the high content of free amino acids and other soluble non-nitrogenous substances, which partly contribute to the desirable, delicate sweet taste of shrimp, which are decrease by spoilage. The results obtained indicated that all OEO coated sample had significantly ($p<0.05$) higher sensory score as compare to TA and TC after the first two days of storage. Every sensory attributes score of all samples showed a similar pattern of decreasing acceptability. The reason behind such the inhibitory effect of OEO against the microbial growth could retard or lower the production of micro degradation products, which cased for an unpleasant “fishy” odor and the softening texture (Shakila *et al*., 2013). However the higher OEO concentration at 1% imparted both a little bitter taste and strong odor to the sample, which was lower sensorial score than 0.5% OEO sample. Thus odor and taste were both sensitive attributes for assessing the quality of pre-cooked shrimp. At this point it should be noted, that the presence oregano oil in T05 and T10 samples produced a distinct but sensorial acceptable pleasant odor, well received by the panelists (Alcicek, 2011). As determined by sensory analysis (odor acceptance) data the observed shelf-life of pre-cooked shrimp was the longest for T05 (14 days) followed by T10 (12 days), TA (10 days) and control (TC) samples (8 days). Results obtained in the present study were in agreement with those of Frangos *et al*. (2010), who studied the effects of oregano oil and vacuum-packaging on sensory characteristics of trout fillets. They observed that the sensorial score was significantly ($p < 0.05$) reduced by 0.2% oregano essential oil and the higher OEO concentration (0.4%) had a negative effect on odor and taste of coated trout fillets.

4. Conclusions

Results obtained in this study showed that among all OEO coated solution, the alginate-based edible coating incorporated with 0.5% and 1.0% (w/v) OEO significantly ($p < 0.05$) reduced TVBN and TMAN while maintained the sensory characteristics of pre-cooked shrimp during low temperature storage. Thus, it can be concluded that alginate-based edible coating formulation incorporated with 0.5% and 1.0% (w/v) OEO has potential to extend the shelf-life and maintain the quality of pre-cooked shrimp.

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