Influence of combined vacuum packaging and pomegranate peel extract on shelf life and overall quality of pacific white shrimp (*Peneous vannamei*) during refrigerated storage

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Summary

The present work was carried out to study the effect of vacuum packaging and pomegranate peel extract (PE) treatments on the quality of pacific white shrimp (*Peneous vannamei*) during refrigerated storage. Changes in pH, thiobarbituric acid (TBA), total volatile base nitrogen (TVB-N), trimethylamine (TMA), aerobic plate counts, psychrophilic, lactic acid bacteria and enterobacteriaceae counts, melanosis and sensory characteristics were investigated in up to 10 days of storage at 4°C. Microbial growth of vacuum packed and PE treated (1 or 2%) shrimps were retarded during storage time in comparison with the control group ($P<0.05$). Furthermore, use of PE in combination with vacuum packaging enhanced the antimicrobial effect of vacuum packaging against the bacterial populations. Vacuum packaging demonstrated a significant reduction in TVB-N, TMA and TBA content of shrimps during refrigerated storage. However, use of vacuum packaging and PE in combination did not significantly improve chemical parameters of the samples. Hence, vacuum packaging is considered as an effective method to extend the shelf-life of shrimp and, when combined with PE, can dramatically improve the overall likeness and microbial quality of the product.

Key words: Vacuum packaging, Pomegranate peel extract, Pacific white shrimp, *Peneous vannamei*

Introduction

Crustaceans are widely consumed all over the world because of their delicacy and nutritional value. Shrimp undergo rapid spoilage faster than finfish due to the high free amino acid content and soft texture (Muruganantham *et al*., 2006). Rapid microbial spoilage during postmortem storage is a serious problem in shrimp processing (Gokoglu and Yerlikaya, 2008). Free amino acids and other soluble non-nitrogenous substances in shrimp serve as nutrients for microbial growth (Zeng *et al*., 2005).

Melanosis is triggered by a biochemical mechanism essentially involving oxidation of phenols to quinines by polyphenoloxidases (PPO). This is followed by non enzymatic polymerization, giving rise to pigments of high molecular weight and very dark or black coloring (Benjakul *et al*., 2005). Even though the presence of black spots seems to be harmless to consumers, it drastically reduces the product's market value and the consumer's acceptability, leading to considerable financial loss (Montero *et al*., 2001). Apart from melanosis and microbial spoilage, lipid oxidation associated with physicochemical changes, which leads to off-flavors and loss in freshness and quality brings about considerable market loss in shrimp industry (Decker and Hultin, 1990).

Nowadays, natural antioxidant and antimicrobial compounds, especially of plant origin, have received increasing attention as food additives (Gokoglu and Yerlikaya, 2008). Pomegranate (*Punica granatum*), recently described as nature's power fruit (Abdel Moneim *et al*., 2011) is widely cultivated in the Mediterranean region. Pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a high antioxidant and antimicrobial activity (Abdel Moneim *et al*., 2011; Yehia *et al*., 2011).

Vacuum packaging is one of the preservative packaging methods which can greatly enhance the shelf life and overall quality of muscle foods for a long time (Sahoo and Kumar, 2005). The process involves removal of air from the packaging, thus extending the viable shelf-life of many foods (Rajesh *et al*., 2002).

However, no information regarding the use of pomegranate peels and/or in combination with vacuum packaging to prevent melanosis or extending shelf life of shrimp is available. The aim of this study was to investigate the combined effect of pomegranate PE and vacuum packaging on melanosis and quality changes of pacific white shrimp (*Peneous vannamei*) during

refrigerated storage.

Materials and Methods

Extraction of effective ingredient from pomegranate peels

Pomegranate (Rabbab variety) was obtained from a garden in Shiraz, Iran. The fruits peels were dried in an oven at 50°C until a constant weight, and grounded to powder. Peel powder was dissolved in methanol (1:20 w/v) and then extracted in incubator with shaker at a speed of 200 vib/min at 40°C for 4 h and kept overnight at room temperature. The extract was filtered and subjected to rotary evaporator at 40°C under reduced pressure to remove the solvent and obtain crude extract (Basiri *et al*., 2013).

Shrimp collection and preparation

Pacific white shrimp (*Peneous vannamei*) with the size of 30-35 shrimps/kg were purchased from a shrimp farm (Bandar-Abbas, Iran). Immediately after harvesting, the shrimps were dipped into liquid nitrogen before being transported to the laboratory. Upon arrival, shrimps were defrosted, washed in cold water and stored on ice flake until further use (less than 1 h). The shrimps were then divided into four portions and treated as follows:

Group 1 (Control): No treatment followed by packaging in the polystyrene trays and wrapped by polyolefin stretch film.

Group 2 (Vac.): No treatment followed by vacuum packaging (using a D-44866 machine, Germany) in polyethylene bag (thickness: 85 μ, density: 1.1 g/ml).

Group 3 (Vac. + 1% PE): Immersed in 1% pomegranate peel extract solution at a shrimp/solution ratio of (1:1, w/v) at 4°C for 15 min., drained on the screen for 1 min and vacuum packed as described above.

Group 4 (Vac. + 2% PE): Immersed in 2% pomegranate peel extract solution at a shrimp/solution ratio of (1:1, w/v) at 4°C for 15 min, drained on the screen for 1 min and vacuum packed as described above.

All samples were stored at 4°C and subjected to microbial, chemical and sensorial analysis every 2 days for up to 10 days.

Microbiological analysis

Bacteriological analysis included total mesophilic bacteria, total psychrophiles, total lactic and enterobacteriaceae count was performed following the method of Nirmal and Benjakul (2009). Twenty Five g of aseptically grounded shrimps were diluted into 225 ml of normal saline and homogenized using a stomacher bag. A tenfold serial dilution was subsequently prepared. Appropriate dilutions were poured onto plate count agar (Merck, Germany). The plates were then incubated at 37°C for 2 days and at 7°C for 10 days for mesophilic and psychrotorophic bacteria, respectively. Pour plate on MRS-agar (Merck, Germany) was also performed for the enumeration of lactic acid bacteria following incubation at 35°C for 48 h. Most probable number (MPN) technique was employed for the enumeration of enterobacteriaceae using MacConkey broth (containing 1% glucose). Tubes were incubated at 37°C for 24 h.

pH measurement

Grounded whole shrimps (2 g) were homogenized with 10 ml of deionized water for 1 min. using a homogenizer (DI18B, Germany). The homogenate was kept at room temperature for 10 min. pH was recorded using a pH-meter (CG824, Germany).

Determination of total volatile base nitrogen (TVB-N) and trimethylamine (TMA) content

TVB-N was calculated using steam distillation in the minced shrimp tissue, followed by titration (AOAC, 2002a). The TVB-N content was expressed in mg N/100 g shrimp tissue.

TMA was determined using the Dyer method as described in the AOAC (2002b). Volatile bases were extracted with trichloroacetic acid. Bases other than TMA were fixed with formaldehyde. Toluene (Merck) was used to extract the TMA from a basic medium and then reacted with picric acid to yield a colored picrate salt which was spectrophotometrically (Jenway, UK) analysed. Results were expressed in milligrams of TMA per 100 mg of sample.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in the samples was determined following the method of Benjakul and Bauer (2001), with some modifications. One g of grounded shrimps was mixed with 9 ml 0.25 N HCl solution containing 0.375% TBA (Sigma-Aldrich, USA) and 15% TCA (Merck, Germany). The mixture was heated in boiling water for 10 min, followed by cooling with running water. The mixture was centrifuged at 3500 rpm for 15 min. The supernatant was collected, and the absorbance was read at 532 nm using a spectrophotometer. TBARS was calculated from the standard curve of malonaldehyde (0-2 mg/kg) (Merck, Germany) and expressed as mg malonaldehyde per kg of sample.

Melanosis assessment and sensory evaluation

Melanosis of the shrimps was evaluated through visual inspection by twelve panelists. Panelists scored melanosis according to a scale from 1 to 4 as follows:

where,
1: Complete absence of black spots
2: A few small spots on the carapace
3: Considerable spotting on the carapace
4: Substantial spotting over the entire shrimp (Montero *et al*., 2004)

Whole shrimps were boiled in water for 3 min and evaluated by 12 panelists, using the 4-point scale,

where,
4: Like extremely
3: Like moderately
2: Neither like or nor dislike

1: Dislike

Panelists were asked to evaluate the color, odor, flavor and overall likeness of the samples.

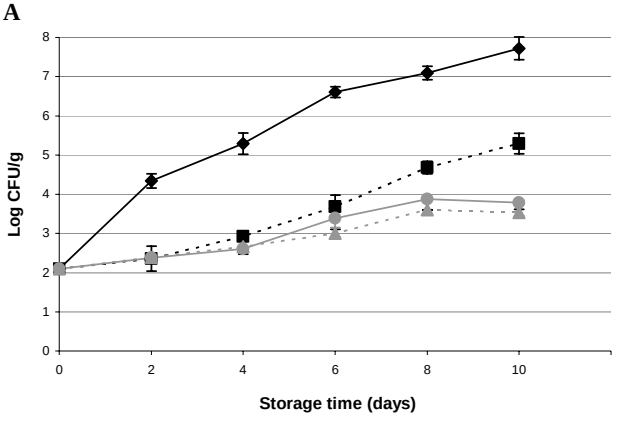
Statistical analysis

All experiments were performed in triplicate. Analysis of variance was performed by repeated ANOVA and means comparisons were done by Duncan's multiple range tests. Non-parametric data were analysed using Kruskal-Wallis test. P-values less than 0.05 were considered statistically significant. Analysis was performed using a SPSS package (SPSS 16 for windows, SPSS Inc., Chicago, IL, USA).

Results

Effect of PE and vacuum packaging on microbiological changes of white shrimp during storage

Changes in mesophilic, psychrophilic, LAB and enterobacteriaceae counts in the treatment groups during storage are respectively shown in Figs. 1A-D. APC was increased significantly during the storage period ($P<0.05$). In the control group, APC was increased from log 2.1/g in fresh shrimp to 7.66 ± 0.28 log CFU/g following 10 days of storage. During the same period, the log CFU/g in group 2 (Vac.), group 3 (Vac. + 1% PE) and group 4 (Vac. + 2% PE) were 5.29 ± 0.18, 3.53 ± 0.09 and 3.79 ± 0.05, respectively. It was observed that APC in the vacuum packaged samples were lowered compared to the control samples. Treated shrimps with different concentrations of PE, showed lower APC in comparison with the control group from day 8 to day 10 of storage ($P<0.05$). At the end of storage (day 10), psychrophilic count of the control, Vac., Vac. + 1% PE and Vac. + 2% PE were 7.50 ± 0.30, 5.56 ± 0.16, 3.64 ± 0.39 and 3.50 ± 0.20 log CFU/g, respectively. Psychrophilic counts in vacuum samples were lower in comparison to the control ($P<0.05$). Vacuum packaging in combination with PE was more effective in reduction of psychrophilic counts. As PE concentration increased, the antibacterial effect against psychrophils did not significantly increase ($P>0.05$). After 10 days of storage, vacuum groups showed lower LAB and enterobactericeae count than the control group ($P<0.05$).



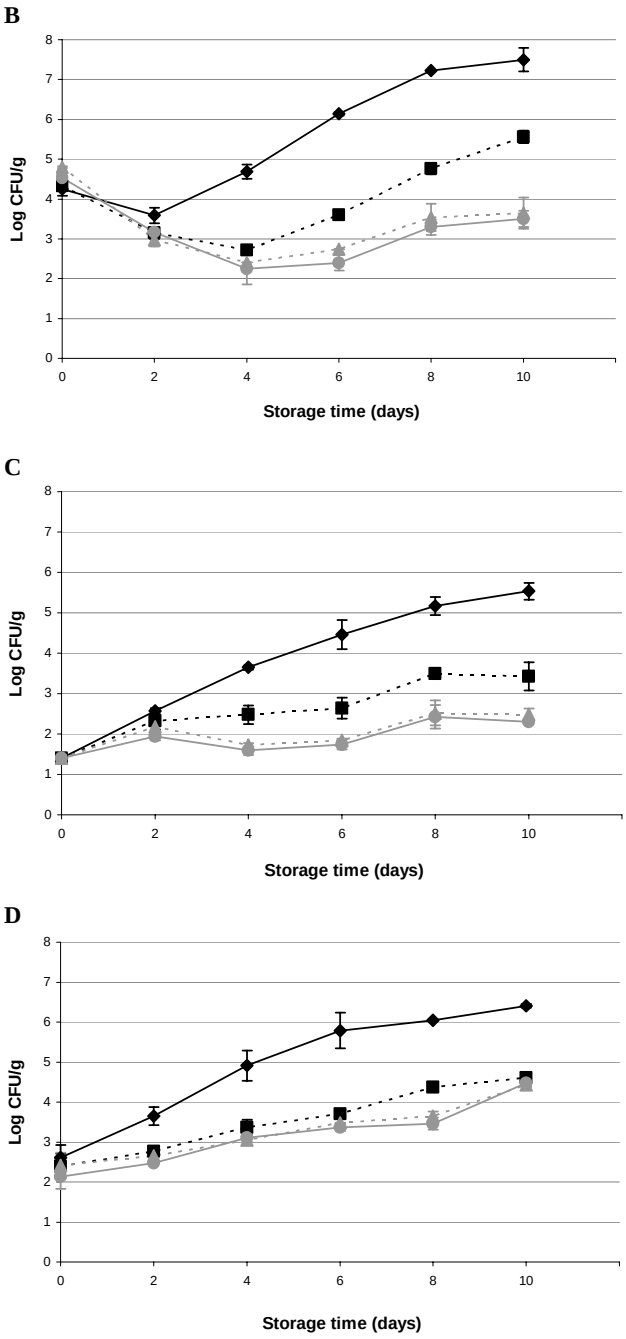


Fig. 1: Aerobic plate count (A), psychrophilic (B), lactic acid bacteria (C), and enterobacteriaceae (D). Count of shrimps treated with pomegranate peel extract at two levels during refrigerated storage. ♦: Control, ■: Vacuum control, ▲: Vacuum + 1% pomegranate peel extract, and ●: Vacuum + 2% pomegranate peel extract

Vacuum packaging in combination with PE, was more effective against LAB than the vacuum alone ($P<0.05$). However, differences in PE concentrations did not significantly affect LAB and enterobactericeae count ($P>0.05$).

Effect of PE and vacuum packaging on pH of white shrimp during storage

pH of the shrimps in the control and treatment groups at day 0 was 7.22 ($P>0.05$). As the storage time

increased, pH of all shrimps increased (P<0.05). At the end of storage period, the lowest and highest pH was obtained in the control (7.43 ± 0.09) and Vac. + 2% PE (7.85 ± 0.00) groups, respectively (P<0.05) (Fig. 2). However, pH of the Vac. and Vac. + 1% PE did not show significant differences compared with the Vac. group (P>0.05).

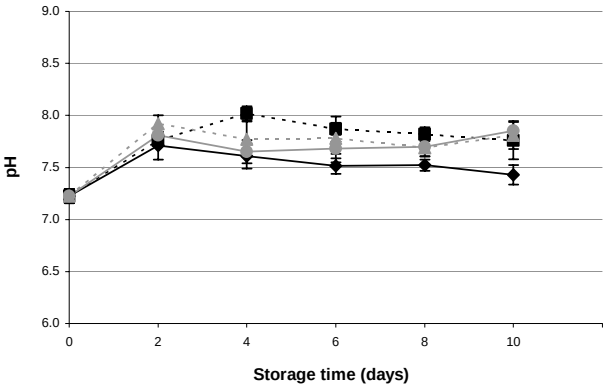


Fig. 2: pH of shrimps treated with pomegranate peel extract at two levels during refrigerated storage. ♦: Control, ■: Vacuum control, ▲: Vacuum + 1% pomegranate peel extract, and ●: Vacuum + 2% pomegranate peel extract

Effect of PE and vacuum packaging on TVB-N and TMA of white shrimp during storage

TVB-N and TMA contents of shrimps in different experimental groups are shown in Fig. 3. The initial value of TVB-N was 32.67 mg/100 in fresh shrimp flesh. In the control group, the TVB-N values continuously increased and reached 165.2 mg/100 on the 10th day of storage. The increase in TVB-N content of Vac., Vac. + 1% and 2% PE samples was gradual and reached 62.53 mg/100, 72.1 mg/100 and 58.8 mg/100, respectively at the end of 10 days of refrigerated storage. TVB-N content of the control group was significantly higher than treatment groups from day 6 to the end of the storage time (P<0.05) (Fig. 3A). Although according to our previous study (Basiri *et al*., 2013) use of pomegranate peel extract in shrimp preservation can reduce TVBN production during 10 days of refrigerated storage, the current study showed that use of PE in combination with vacuum packaging did not have an additional effect on TVB-N content of vacuum shrimps. The correlation coefficient between TVB-N content, and APC, psychrophilic, lactic acid bacteria and enterobacteriaceae counts of the shrimps stored at 4°C were 0.88, 0.96, 0.92 and 0.90, respectively, which were highly significant (P<0.001). TMA content at the beginning of refrigerated storage was 0.025 mg/100 g shrimp. At the end of storage, TMA content of the control, Vac., Vac. + 1% and 2% PE were 0.251, 0.042, 0.05 and 0.038 mg/100 g, respectively (Fig. 3B). The values were increased continuously in the control samples throughout the storage for 10 days (P<0.05). Vacuum packaging, resulted in a considerable reduction in TMA, in comparison with the control (P<0.05). The correlation coefficient between TMA content, and APC, psychrophilic, lactic acid bacteria and enterobacteriaceae counts of the shrimps were 0.72, 0.91, 0.8 and 0.79, respectively (P≤0.002).

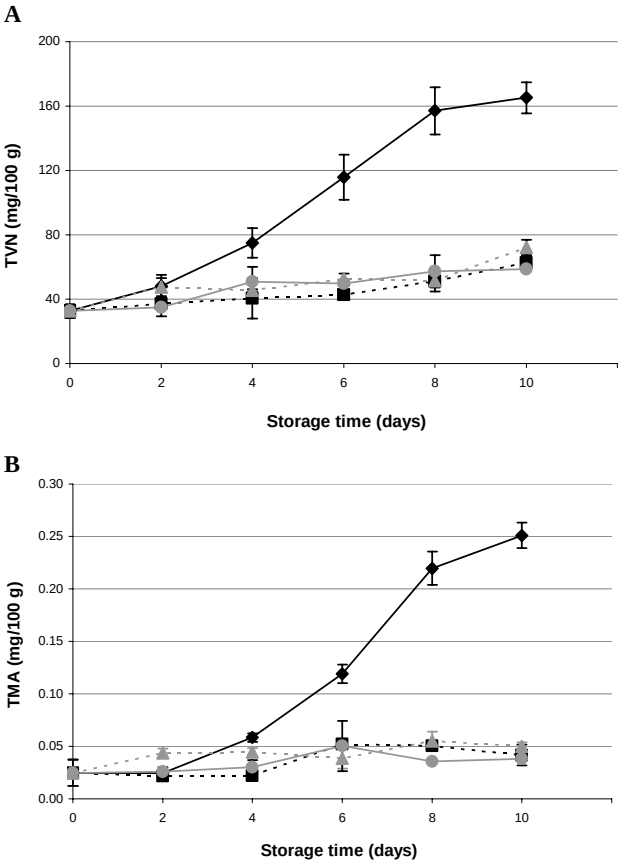


Fig. 3: TVB-N (A) and TMA (B) content of shrimps treated with pomegranate peel extract at two levels during refrigerated storage. ♦: Control, ■: Vacuum control, ▲: Vacuum + 1% pomegranate peel extract, and ●: Vacuum + 2% pomegranate peel extract

Thiobarbituric acid reactive substances (TBARS) value

TBARS value of control group was raised as the storage time increased (P<0.05). TBARS value of the control group was significantly higher than treatment groups from day 2 to the end of the storage time (P<0.05) (Fig. 4). In combination, use of PE and vacuum packaging did not have an additional effect on TBARS value.

Changes in melanosis and sensory properties of shrimp during storage

Melanosis score of control and treatment groups during 10 days of refrigeration is shown in Fig. 5. At day 0, all samples showed a negligible melanosis score. Upon increasing the storage time, melanosis score in the control group was raised (P<0.05). From days 4 to the end of storage time melanosis scores of treated shrimps were significantly lower than the control group (P<0.05). During 10 days of storage, the formation of melanosis was lowest in Vac. + 2% PE, followed by those vacuum treated with 1% PE and Vac. control, respectively Vac. +

2% PE groups showed the best appearance as compared to others at the last day of storage, while the severe melanosis was found in the control samples.

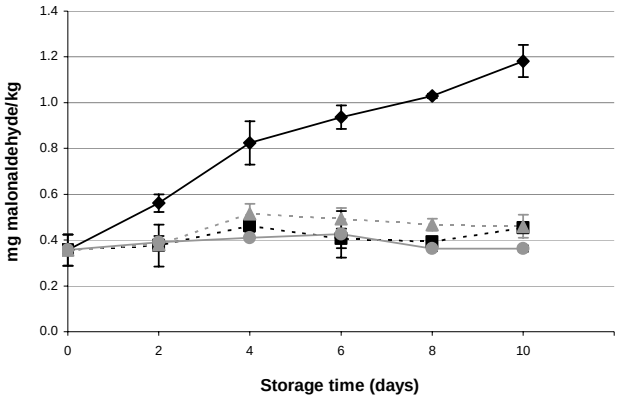


Fig. 4: TBA content of shrimps treated with pomegranate peel extract at two levels during refrigerated storage. ♦: Control, ■: Vacuum control, ▲: Vacuum + 1% pomegranate peel extract, and ●: Vacuum + 2% pomegranate peel extract

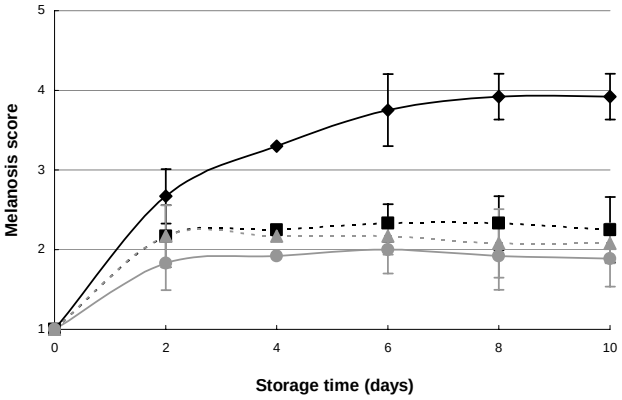


Fig. 5: Melanosis score of shrimps with and without treatments during refrigerated storage. Where, 1: Complete absence of black spots, 2: A few small spots on the carapace, 3: Considerable spotting on the carapace, and 4: Substantial spotting over the entire shrimp. ♦: Control, ■: Vacuum control, ▲: Vacuum + 1% pomegranate peel extract, and ●: Vacuum + 2% pomegranate peel extract

Changes in sensory properties of shrimps stored under different conditions are presented in Table 1. Appearance, odor, texture and color of shrimps were evaluated at day 0 and 10 of refrigerated storage. There were no significant differences for all attributes values among all samples at day 0 ($P>0.05$). At day 10, all attributes were higher in treatment groups in comparison with the control group ($P<0.05$). The higher scores for color, texture and overall likeness were found in vacuum shrimps treated with 2% PE ($P<0.05$). Nevertheless, no significant differences in shrimp likeness were found among Vac. and Vac. + 1% PE groups ($P>0.05$).

Discussion

According to our previous work (Basiri *et al*., 2013), methanolic extract of pomegranate peel showed an antimicrobial effect on the microorganism counts during refrigerated storage. Vacuum packaging under chilled conditions has proved very effective in extending the shelf life of perishable foods, such as fresh meat and meat products (Church and Parsons, 1995). Vacuum packaging with the restriction of oxygen supply has an antimicrobial effect on aerobic mesophils and psychrophilic bacteria involved in the microbial spoilage of refrigerated foods. However, selective pressure conditions for the proliferation of anaerobic and microaerophils may arise (Perez-Alonso *et al*., 2004; Fontana *et al*., 2006). Lactic acid bacteria (LAB) are the main spoilage organisms associated with chilled vacuum packaged fresh meat products. Shortly after vacuum packaging of meat, LAB populations are usually below the routine detection limit but they increase during storage (Fontana *et al*., 2006). The slow growth of lactic acid bacteria on the vacuum packed shrimp in the initial stages of storage was probably due to the high initial pH of the samples as well as growth dominance by facultative anaerobic biota (enterobacteriaceae). The results are supported by Babji *et al*. (2000) on the vacuum packed minced goat meat. During refrigerated storage, pH of shrimps was increased. The increase in pH is associated with the accumulation of basic compounds, such as TVB-N and TMA, which was mainly resulted from microbial action (Lopez-Caballero *et al*., 2007).

TVB-N and TMA are products of bacterial spoilage and the content is often used as an index in assessing the shelf life and storage quality of seafood products

Table 1: Effect of vacuum packaging and pomegranate peel extract on likeness score of shrimp (*Peneous vannamei*) during refrigerated storage

Storage time (days)	Treatments	Appearance	Odor	Texture	Color	Color after cooking
0	Control	4.0 ± 0.00^{a}	3.5 ± 0.71^{a}	4.0 ± 0.00^{a}	3.5 ± 0.25^{a}	4.0 ± 0.00^{a}
	Vac.	4.0 ± 0.52^{a}	3.0 ± 0.54^{a}	4.0 ± 0.30^{a}	4.0 ± 0.52^{a}	4.0 ± 0.52^{a}
	Vac. + 1% PE	4.0 ± 0.54^{a}	3.0 ± 0.52^{a}	3.8 ± 0.52^{a}	4.0 ± 0.43^{a}	4.0 ± 0.24^{a}
	Vac. + 2% PE	4.0 ± 0.45^{a}	3.5 ± 0.60^{a}	4.0 ± 0.24^{a}	3.5 ± 0.61^{a}	4.0 ± 0.30^{a}
10	Control	1.0 ± 0.00^{a}	1.5 ± 0.52^{a}	2.3 ± 0.87^{a}	1.0 ± 0.00^{a}	1.3 ± 0.45^{a}
	Vac.	2.8 ± 0.45^{b}	2.9 ± 0.67^{b}	3.0 ± 0.00^{b}	2.8 ± 0.45^{b}	3.2 ± 0.58^{b}
	Vac. + 1% PE	2.7 ± 0.65^{b}	2.8 ± 0.72^{b}	3.1 ± 0.67^{b}	3.0 ± 0.60^{b}	3.3 ± 0.87^{b}
	Vac. + 2% PE	3.3 ± 0.49^{bc}	3.4 ± 0.52^{b}	3.3 ± 0.49^{bc}	3.6 ± 0.52^{bc}	3.4 ± 0.52^{b}

Values are mean ± SD (n = 12). Vac.: Vacuum control, Vac. + 1% PE: Vacuum + 1% pomegranate peel extract, Vac. + 2% PE: Vacuum + 2% pomegranate peel extract. Different letters in the same column within the same storage time indicate significant differences ($P<0.05$)

(Connell, 1990). In our study, the correlation coefficient between TVBN content and microbial count of shrimp was high, which confirmed the important role of microbial count on TVBN content of samples. The lower TVB-N content of treatment groups in comparison with the control group might be due to the inhibitory effect of vacuum packaging on spoilage bacteria and proteolytic enzymes.

Since the correlation between microbial count and TMA content of shrimps was highly significant, the content of TMA was significantly affected by the bacterial count of the samples. In our study, vacuum packed shrimps showed lower TMA content during refrigerated storage. Similar to these findings, Ozogul *et al*. (2004) found that TMA content of sardines (*Sardina pilchardus*) after 15 days of storage at 4°C was lower in vacuum stored samples than those stored in air. It is also possible that some of the basic compound (TVB-N and TMA) produced in the chilled shrimp could be originated from endogenous or bacterial enzymatic activities or in a combination of both (Kawai, 1996).

Tissue membrane of crustacean contains high levels of polyunsaturated fatty acid and the damage of tissues during processing can induce lipid oxidation. In this study, vacuum packed shrimps had lower TBARS contents. It seems that vacuum packaging with limited oxygen retards the oxidative process of the polyunsaturated fatty acids of shrimp. The increase in TBARS content of the control group suggested that fatty acids in shrimp muscle underwent oxidation during storage, in which malonaldehyde was formed. Similar to our results, it was reported that minimum TBARS values were recorded in trout fillet packaged with MAP (100% CO_2) and vacuum (Arashisar *et al*., 2004). Nam and Ahn (2003) have reported that vacuum-packaged meat was more resistant to lipid oxidation than aerobically packaged meat.

Enzymatic oxidation (melanosis) in shrimp was retarded in PE treated and vacuum packed shrimps. It seems that this effect is due to PE and vacuum packaging inhibitory effect on polyphenoloxidase enzyme. Pomegranate peel extract has been shown to be rich in polyphenols (Basiri *et al*., 2013). Chang (2009) consider polyphenols to be the primary category of inhibitors of tyrosinase (poly phenol oxidase). Vacuum packaging with limited oxygen level, decrease the amount of oxidation. Reddy and Patange (2011) reported that combined effect of controlled atmosphere and melanosis inhibitors delayed black spot development as compared to the shrimps stored in ice alone.

The higher sensorial scores of treated samples were mostly associated with the lower microbial load in those samples, in comparison with the control. Therefore, the vacuum packaging of PE treated shrimps could improve the sensory properties of shrimps during storage time, which was most likely associated with the lowered melanosis.

Methanolic extracts of pomegranate peel act as antimicrobial agents, improving shrimp quality and prolonging shelf life. Vacuum packaging has a similar effect, delaying microbial growth and chemical deterioration of shrimp during cold storage. Therefore, the combined use of anti-melanotic treatment and vacuum packaging delays both biochemical deterioration and the occurrence of melanosis in pacific white shrimp.

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