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Antioxidant Effect of Orange Peel Extract on Chemical Quality, Sensory Properties, and Black Spots of Farmed White Shrimp

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ABSTRACT

Background: Black spots are a major problem in commercial shrimp species and can have negative effects on shrimps' appearance, quality, shelf life, economic value, and product acceptance by consumers. This study was conducted to investigate the effect of orange peel extract on chemical and sensory qualities as well as black spots on *Litopenaeus vannamei* species of white farmed shrimp. **Methods**: Samples included treated shrimps at concentration of 150 g, orange peel extract for 30 minutes, and control shrimps. After storage for 10 days at 1 ± 4 °C, the samples' chemical and sensory evaluations were performed with an interval of 5 days. **Results:** pH factors, peroxide value, and total volatile network (TVN) of treated samples were significantly lower compared to those of the control samples ($P < 0.05$). There was no significant difference in the moisture content. Black spots did not appear in the treated sample until the end of refrigerated storage, but melanosis appeared in control shrimp 5 days after storage. **Conclusion**: The results showed that because of having antioxidant and antimicrobial activity, orange peel extract improved shrimps' chemical and sensory qualities and reduced their black spots in the refrigerator temperature.

Key words: *Orange peel extract; White shrimp; Antioxidants; Black spot.*

Introduction

Shrimps' quality is an important issue for product acceptance by consumers. So, in order to have successful marketing the product features should be considered and the most appropriate technology should be chosen to produce this product (Gujja and Finger-Stich, 1996, Hall, 2004). In this regard, proper operation of farmed shrimp by applying optimal methods of shrimp transportation and processing as well as considering changes after harvest is particularly important in Iran. Nowadays, frozen, blocked, cooked shrimp salad, individual quick frizzing, and farmed shrimps are available in the world's markets. Frozen shrimp due to its long storage period has high commercial value and is in a high demand. The most important quality changes that occur during long storage of frozen shrimp in fridge include loss of color, fat oxidation, protein denaturation, and formation of ice crystals (Erickson, 1997, Gonçalves and Junior, 2009). Black spot or melanosis is insoluble black pigment (melanin) in the inner shells of shrimp that is

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related to enzymatic oxidation of the phenol precursors. This is an enzymatic reaction that occurs in the outer shell of shrimp body. The enzyme causing this reaction is polyphenol oxidase; an internal enzyme which activity is not associated with spoilage bacteria. Maintenance at high temperature as well as exposure to the sun and oxygen play important roles in occurrence of black spots (Gonçalves and Junior, 2009). Black spots are a major problem in commercial shrimp species and can have negative effects on shrimp's appearance, quality, shelf life, market-friendliness, economic value, and product acceptance by consumers (Flores and Crawford, 1973). Now days, sodium metabisulphite is used to prevent formation of black spots, this combination is a synthetic and allergen antioxidant that releases sulfur dioxide during the process and in some cases labors' death has been reported due to its inhalation. In addition, since it remains in shrimp's tissue and due to its side effects, legal bans are still applied in many countries on use of this compound. Therefore, finding a natural, safe, and convenient alternative to preserve shrimp is one of the food industry professionals' concerns. Among the specialists' achievements, using herbal and natural antioxidant compounds instead of industrial and chemical elements can be mentioned (Gökoğlu, 2004, Iyengar *et al.*, 1991).

Wastes from juice and concentrate factories contain lots of natural antioxidants. Among these wastes, citrus, tomatoes, apples, and grapes' wastes can be mentioned which contain large amounts of phenolic compounds (Kang *et al.*, 2006). Citrus are important sources of bioactive compounds with antioxidant effects including ascorbic acid, flavonoids, phenolic compounds, and pectin. flavanones, flavones, and flavonols are three flavonoid compounds in citrus. The main flavonoid compounds existing in different species of citrus are *hesperidin*, *narirutin*, *naringin*, and *eriocitrin* (Hegazy and Ibrahium, 2012). The antioxidant and antimicrobial properties of citrus peel have been reported in several studies. Ghasemi et al. compared 13 varieties of citrus and announced that citrus' peels have more amounts of phenol and flavonoid than fruits' pulp (Ghasemnezhad *et al.*, 2012). Lee et al. also reported that pomegranate peel has higher amounts of phenol and flavonoid than its pulp (Li *et al.*, 2006). Since the main tasks of phenolic and flavonoid compounds are protection of plants against ultraviolet radiation, insects, and diseases, they also have an important role in attracting pollinating insects and their high density is understandable in epidermal layer (Agusti *et al.*, 2003). Furthermore, because light is effective in phenolic and flavonoid compounds' biosynthesis and in fact, since these materials have a protective role against light, particularly short wavelength, these compounds are more in the peel section (Angell, 2004). Considering the antioxidant and antimicrobial properties of orange peel, this worthless byproduct of the food processing plants can be used to improve foods' quality and shelf life. Moreover, economic loss of foods, such as shrimp which are susceptible to oxidation and microbial spoilage can be reduced. In this regard, this study was conducted to investigate the effect of orange peel extract on quality of chemical and sensory properties as well as formation of black spots on the farmed white shrimp in refrigerator temperature.

Materials and Methods

In this study, 12 kg of farmed white shrimp of Litopenaeus vannamei species produced in shrimp farms of Bushehr province was bought from Tehran fishery (the average weight of each shrimp was 120 g). Then, samples were transferred to the laboratory in cold boxes under sanitary conditions while covered by ice in ratio of 2 to 1. Shrimps were immediately washed with cold and clean urban water (4 °C). This study was conducted as a treatment and repeated three times. Treatment included processed shrimp, orange peel extract, and shrimp without antioxidants (control sample) which were then categorized randomly in two groups of control and treatment. Treatment samples were placed into orange peel extract with a concentration of 150 g to a ratio of 2 to 1 for 30 minutes. After this step, shrimps were packed in quantities of 250 g by polyethylene plastic; they

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were later transferred and kept in refrigerator with cryogenic temperature of 1 ± 4 °C. Later, Chemical and sensory experiments were conducted to investigate the quality of treatment and control frozen samples for 10 days with an interval of 5 days. Sampling was carried out randomly for these experiments. Thomson orange peels were dried at 70 °C for 72 hours in an oven for extraction. Then, they were powdered and 100 grams of their powder was placed in soxhlet extractor with petroleum ether solvent (at 60 °C for 6 hours) and the fat was removed. Defatted orange peel powder was extracted in soxhlet for 8 hours with 200 ml of ethanol. The extract was condensed under vacuum condition at temperature lower than 40 °C. The obtained crude extract was lyophilized. To prepare the orange peel extract solution, 50 g of this extract was diluted in a liter of water (Ghasemi *et al.*, 2009). Chemical tests for treatment and frozen control samples, such as measuring pH with Digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd. Tokyo, Japan) and measuring the humidity by dried oven were conducted (Namsanguan *et al.*, 2004).

Measurement of total volatile network (TVN): To measure TVN, 10 g uniformed shrimp sample, 2 g magnesium oxide, 300 ml water, and a few pieces of boiling stones were added to the kjeldahl distillation balloon. In an Erlenmeyer flask (as the cooling section of distiller) 25 cm^2 of 2% boric acid and a few drops of methyl red reagent were added. Distillation apparatus was attached and the distillation flask contents were heated. After 25 minutes of heating, the inner side of cooler was washed by distilled water and distilled solution was titrated with 0.1N sulfuric acid. The amount of sulfuric acid was multiplied by 14 and amount of volatile nitrogen was calculated in mg per 100 g of shrimp (COBB *et al.*, 1973).

Measurement of peroxide value (PV): To measure the PV, samples were dissolved in 50 ml of glacial acetic acid and isooctane, then, 5.0 ml of saturated potassium iodide solution was added to it. Erlenmeyer flask was closed and stirred with magnetic stirrer so that no severe rotational movement was created and no air was drawn into the solution. The released iodine was immediately titrated with a few ml of 0.01N standard sodium thiosulfate, so that the solution's color turned from yellow-orange to pale yellow. After adding 5.0 ml of starch solution, purple color was created which was then stopped by continuation of titration and titration sustainable colorlessness. The amount of PV is calculated in terms of meq and that of active oxygen per kg is calculated by the following formula: (Wills, 1966)

$$PV = \frac{(V-V_0)\times C_{thio}\times C_{stand}\times 1000}{m}$$

Where: V is the volume of standard solution of sodium thiosulfate, 0.01 normal used for the test in ml, $\boldsymbol{V_0}$ is the volume of standard solution of sodium thiosulfate, 0.01 normal used as control in ml, $\boldsymbol{C_{stand}}$ is the approximate concentration of standard sodium thiosulfate, 0.01 normal solution in moles per liter, and m is the mass in g.

Sensory experiments: They were carried out to examine the quality of treatment and control samples by using raw shrimps' qualitative rating table (with 10 points and 4 quality levels). The studied parameters are displayed in **Table 1** that include melanosis, meat, eyes, cephalothorax and tail, smell, legs, skin and tentacles, as well as apparent color. Sensory evaluation was conducted through quality index method (QIM) scoring within 5 days and at certain times with three repetitions (Zeng *et al.*, 2005). The QIM is a promising method for quick and reliable assessment of the freshness of seafood. It is expected to become the leading reference method for the assessment of fresh fish within the European community (Hyldig and Green-Petersen, 2005).

Data analysis: Experiments were performed with 3 repetitions in all stages and the obtained results were statistically analyzed by SPSS software and through repeated measure test. Tukey's test was further used to express significance of results at level of P-value < 0.05. Results were finally reported with mean± standard deviation.

Results

Results of chemical factors related to treated shrimps immersed in 50 g/L of orange peel extract

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and control shrimps during storage at refrigerator temperature (1 ± 4 °C) including moisture, pH, TVN, and peroxide are shown in **Table 2**. The least amount of moisture was 74.62% related to control sample on the 10th day while the highest value, i.e., 76.37%, was related to the treated sample on the first day. The pH in the treatment sample had the lowest amount (6.57) on the first day and the highest value (8.38) on the 10th day. The peroxide and TVN values had an increasing trend but were lower in treated samples than the control cases. The lowest amounts of peroxide and TVN on the first day for the treated samples were respectively 0.98 (Meq/kg) and 23.22 (mg/100 g) while the highest amounts on the 10th day for the control samples were 7.48 (Me/ kg) 48.7 and 44.69 (mg/100 g), respectively.

Sensory properties (color, odor, texture, and overall acceptability): Sensory and qualitative studies on the treatment and control samples of shrimp during storage at the temperature of 1 ± 4 °C with an assessment of apparent color, smell, texture, eyes, cephalothorax, tail, legs, and shell are represented in **Table 3**. On the first day both the treatment and control samples had good quality, but after five days of maintenance, dark spots appeared on the control samples. On the 10th day control shrimps spoiled while the treated samples received an acceptable score until the end of storage period.

Table 1. Sensory And Quality Evaluation Of Raw Shrimp

Feature score	10-9-8	7-6-5	4-3-2	1
Color (Apparent)	Colorless, transparent, with no dark color	Colorless, slightly transparent, emergence of darkness signs, dark brown cephalothorax	Dark / black cephalothorax, black caudal fins, some black lines in the shell	Completely black (Cephalothorax, caudal fins, and shells)
Cephalothorax/ Tail	Cephalothorax and tail are stiff and completely attached	Cephalothorax and tail have little attachment and are peeled easily, natural laxity, some tails and cephalothorax are peeled	Cephalothorax and tail are attached but they are not so stiff, laxity has started in some cases	Most cephalothorax and tails are peeled
Legs, Shells, Tentacles	Complete, stiff	Complete, legs and tentacles have less stiffness (peeled easily)	Legs and tentacles start to peel, they are kept in box	Most tentacles and legs are peeled and some shells are also peeled
Eyes	Transparent, stiff	Reduced transparency, a little dark	Color reduction, some eyes are peeled (Search in maintenance box)	Most eyes are peeled
Smell	Smells like seaweeds, sea water smell, pleasant	No smell	Mild smell of fish	Strong nauseous smell of Ammonia and sulfides
Meat (Texture, Color, Vessel)	Firm, juicy, white, transparent, stiff vessel, resistant	Reduced stiffness, soft, white opaque, the vessel is still complete but less resistant, there is no darkness	Emergence of darkness in cephalothorax flesh, vessels' self-digestion has started	Darkness (meat, cephalothorax, tail), existence of some yellow and green colors in tail flesh, tearing of vessels

According to this Table, if the final quality score is 4, the sample's quality is marginally acceptable. If this score is less than 4, the sample is unacceptable. If the final score is 8 to 10, sample has a very good quality. If the final score is 5-7, the sample is acceptable.

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Table 2. Results of chemical factors changes in treatment and control shrimps

Feature time	Peroxide (meq/kg)		TVN (mg/100 g)		pH		Moisture	
Type	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Day 0	0.03^{a}± 0.98	0.04^{a}±1.06	1.2^{a}±23.22	1.29^{a}±23.25	0.26^{a}±6.57	0.29^{a}±7.28	3.05^{b}±76.37	1.29a±75.24
Day5	0.2^{b}±2.2	0.26^{b}±5.5	1.37^{a}±24.32	1.9^{b}±33.5	0.29^{b}±7.48	0.31^{b}±8.09	1.7^{b}±74.46	3.01a±75.39
Day 10	0.38^{c}±4.66	0.21^{c}±7.48	1.57^{b}±29.53	2.1^{c}±44.69	0.31^{b}±7.72	0.32^{b}±8.38	3.03^{b}±74.94	3.03a±74.62

Dissimilar letters in a column indicate significant differences ($P < 0.05$).

Table 3. Results of sensory and quality tests from treatment and control shrimps

Feature time	Apparent color		Cephalothorax/ tail		Legs, shells, tentacles		Eyes		Smell		texture, color,) (vessel	
Type	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Day 0	9.2 ± 0.6^{a}	9.1 ± 0.7^{a}	9.0 ± 0.6^{a}	9.1 ± 0.5^{a}	9.2 ± 0.5^{a}	9.3 ± 0.4^{a}	9.1 ± 0.6^{a}	9.3 ± 0.6^{a}	9.4 ± 0.6^{a}	9.2 ± 0.5^{a}	9.1 ± 0.6^{a}	9.0 ± 0.4^{a}
Day5	7.3 ± 0.7^{b}	2.2 ± 0.5^{b}	7.2 ± 0.6^{b}	2.0 ± 0.4^{b}	7.2 ± 0.8^{b}	2.8 ± 0.4^{b}	7.3 ± 0.9^{b}	2.0 ± 0.7^{b}	7.5 ± 0.6^{b}	1.9 ± 0.8^{b}	7.8 ± 0.5^{b}	2.2 ± 0.7^{b}
Day 10	6.1 ± 0.5^{c}	1.7 ± 0.6^{c}	6.3 ± 0.5^{c}	1.6 ± 0.5^{c}	6.1 ± 0.7^{c}	1.8 ± 0.6^{c}	6.3 ± 0.7^{c}	1.7 ± 0.7^{c}	6.2 ± 0.6^{c}	1.3 ± 0.8^{c}	6.5 ± 0.7^{c}	1.6 ± 0.8^{c}

Dissimilar letters in a column indicate significant differences ($P < 0.05$).

Discussion

The results showed no significant difference in the moisture content between control and treatment samples. The amount of pH in the treatment sample decreased in comparison with the control sample. Production of volatile bases by spoilage bacteria activities and alkalinity of these compositions increased the amount of pH during storage period. The amount of pH in the treated samples was significantly lower than the control samples during storage. The total amount of volatile nitrogenous bases is the chemical index to measure microbial spoilage. On the first day, the amount of TVN had no difference in the treatment and control samples, but increase of TVN was significant in control samples compared with the treatment cases during storage. Lin et al. reported that orange peel extract significantly reduces the microbial content of surfaces in contact with food (Lin *et al.*, 2010). In another report Mandalary et al. found similar results with measuring antimicrobial activities of different varieties of citrus peel extract (Mandalari *et al.*, 2007). Chantafun et al. reported in their study that limonene, beta-pinene, and gamma-tripiene are the main compounds in orange peel extract that have an effective and deterrent effect on the growth of micro-organisms (Chanthaphon *et al.*, 2008). The amount of peroxide on the first day was not significantly different between treatment and control samples. During refrigerated storage, the peroxide value was significantly lower in the treated samples than the control ones. Orange peel is rich in phenolic compounds. The antioxidant activity of phenolic compounds in extracts of orange peel, because of their oxidation-reduction features and chemical structure can play an important role in neutralizing free radicals, surrounding metals, transmitting and suppressing singlet and triplet oxygen molecules by changing the location or analysis of peroxides (Bocco *et al.*, 1998). Hasani et al. studied the effect of orange peel extract and butyl hydroxy toluene (BHT) antioxidant on the quality of carp fillet during storage for 16 days at refrigerator temperature. They reported that samples treated with orange peel extract in a concentration of higher than 1% had the lowest rate of oxidation. Samples treated with extract of orange peel had acceptable chemical and sensory quality until the end of

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maintenance period, while control samples only had four days of shelf-life (Hasani and Javadian, 2016). In evaluating the sensory and qualitative properties, treated shrimps received significantly higher scores. A significant difference was observed between the treated and control samples for sensory factors, including the skin, flesh and eyes, smell, cephalothorax and tail, legs, shells, and tentacles. Treated shrimp with orange peel extract had good color, meat, texture, and smell quality until the end of shelf life in the refrigerator. In addition, the tentacles of these shrimps were attached to their heads until the end of shelf life and enjoyed a high strength. Their eyes were also attached to heads until the end of storage period. In the processed samples, orange peel extract stuck to the shrimp surface and prevented shrimps from bad smell and corruption. Melanosis was not observed in treated samples until the end of the storage period, while it appeared in control cases after five days. Oxygen removal is one of the mechanisms to prevent melanosis, because polyphenol oxidase enzyme uses molecular oxygen as co-substrate. This enzyme, in *met* state ([Cu (II) Cu (II)]) reacts with molecular oxygen and forms polyphenol oxidase in *oxy* state ([$Cu(II)$–$O2^{2-}$–$Cu(II)$]), which is prone to catalytic reactions of mono-di-phenol. This enzyme in matt type (mono-phenol oxidase or tyrosinase) catalyzes hydroxylation of mono phenols (tyrosine) into di-phenol and causes incidence of melanosis in the shrimp. D-phenolic substrate oxidation to Quonones is catalyzed by D-phenol oxidase enzyme in the presence of oxygen, which produces melanin and black pigments affected by auto-oxidation and polymerization (Sánchez-Alonso *et al.*, 2007, Solomon *et al.*, 1996). Orange peel extract removes oxygen by covering the product surface and having antioxidant properties, it also prevents from enzyme activity and discoloration. The achieved results from this study are consistent with reports provided by many researchers. Seifzade et al. studied the effect of grape seed extract on chemical and sensory quality as well as black spots of Western white shrimp in freezing conditions. They reported significant decrease of TVN and trimethylamine factors in treated samples. Sensory properties had high qualities by the end of treated samples' shelf life (Seifzade *et al.*, 2013). Black spots were observed in control samples after 20 days of storage in freezing conditions but treated samples remained without melanosis until the end of the storage period. In another study, Seifzade et al. investigated the antioxidant effects of catechins in shrimp breeding (Seifzade *et al.*, 2014). The results of this study showed that 0.2% and 0.3% of catechin extract significantly reduced the peroxide value, pH, Thiobarbituric acid, and TVN during freezing conditions compared with control cases. No black spots were observed in samples containing catechins by the end of storage in fridge, while melanosis appeared in the control sample, over a period of less than a month. Begay et al. reported that a 5% of orange peel extract improves chemical quality indicators including pH, peroxide, Thiobarbituric acid, TVN, as well as sensory quality of carp fillets during refrigerated storage significantly (Alibeigi *et al.*, 2013). Liu et al. also examined the effect of orange peel extract along with calcium alginate coating on the quality of white shrimp. The findings of this study showed that orange peel extract significantly reduces microbial load of shrimps as a result of decrease in TVN and pH (Liu *et al.*, 2016).

Conclusions

Results of the current study represented that orange peel extract improves the chemical quality and sensory characteristics of shrimps during storage at refrigerator temperature by lowering the pH, peroxide value, and TVN. Due to its antibacterial and antioxidant activity, orange peel extract can be used as a natural preservative and alternative to chemical compounds to increase shrimps' shelf life and prevent black spots.

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Authors' contribution

Vakili Sh designed the paper and analyzed the data; Yasini - Ardakani SA conducted the study and wrote the manuscript; all authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflict of interests.

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