



Application of Orange Peel Extract as a Natural Preservative for Refrigerated Nobashi Shrimp

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Abstract

Pacific white shrimp, *Litopenaeus vannamei*, is one of the most extensively cultured shrimp species worldwide and is highly susceptible to biochemical and microbial deterioration during refrigerated storage. The present study aimed to evaluate the effectiveness of orange peel extract (OPE) as a natural preservative for improving the quality and shelf life of coated Nobashi shrimp under refrigerated conditions ($\leq 4^\circ\text{C}$). Orange peel extract was prepared from dried orange peels by methanolic extraction (1:10 w/v) followed by concentration using a rotary evaporator. Stretched Nobashi shrimp were treated with 3.5% OPE, 150 ppm Butylated Hydroxytoluene (BHT), and distilled water as the control, packed in polypropylene pouches, and stored under refrigeration for 15 days. Quality changes were assessed through biochemical, microbiological, sensory, colour, and texture analyses. OPE-treated samples exhibited significantly lower microbial counts (6.6 log CFU/g on Day 15) than the control samples (7.4 log CFU/g), while the BHT-treated samples showed the lowest microbial count (3.9 log CFU/g). Lower peroxide value (1.10 meq O_2/kg fat) and TBARS value (1.10 mg malondialdehyde/kg meat) were also observed in OPE-treated samples compared to the control, demonstrating reduced lipid oxidation. Total volatile base nitrogen (TVBN) values of OPE-

treated shrimp remained within acceptable limits up to the 12th day of storage, while control samples exceeded the permissible limit earlier. Sensory evaluation revealed better overall sensory acceptability than the BHT-treated shrimp while maintaining comparable texture characteristics. The study demonstrates that orange peel extract can serve as an effective natural preservative for coated Nobashi shrimp, extending refrigerated shelf life by approximately 3 days compared to untreated control while maintaining acceptable biochemical, microbial, and sensory quality.

Keywords: *Litopenaeus vannamei*, refrigeration, shrimp, citrus fruit peel, nobashi

Introduction

Shrimp is a prominent choice among aquatic food commodities, widely favoured and consumed, renowned for its significant nutritional value and palatability, particularly in industrialised nations (Wang, Lei, Ma, Yuan, & Sun, 2018). *Litopenaeus vannamei*, the predominant species among cultivated shrimp, has seen a substantial increase in production, reaching 815,745 metric tonnes according to recent reports (MPEDA, 2021). This species constitutes approximately 52.9% of the total global crustacean yield, as reported by MPEDA (2021). The emergence of coated shrimp products presents a burgeoning category of delectable and nutritious ready-to-eat or ready-to-fry items, highly sought after in both export and upscale domestic markets (Venugopal, 2006). Various coated shrimp varieties such as peeled and deveined, butterfly-cut, round tail-on, and stretched shrimp have gained popular-

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ity over time and offer enhanced convenience for consumers (Venugopal, 2006).

The product, widely recognised as 'Nobashi' in Japan, is a popular variant of stretched shrimp, prepared by applying controlled mechanical force using stretching devices. This innovative processing technique not only enhances the shrimp's aesthetic appeal but also addresses consumer preferences by preventing curling during cooking, thereby maintaining its unique shape post-cooking. The potential of battered and breaded stretched shrimp as a value-added offering holds promise for urban domestic markets, presenting an opportunity for increased consumer demand and premium pricing (Annamalai, Lakshmi, Sivam, & Rao, 2018).

Citrus fruits, with an annual production exceeding 100 million tonnes, constitute the world's largest fruit sector (Shehata et al., 2021). *Citrus sinensis*, belonging to the family Rutaceae, is a widely cultivated and economically important species of citrus fruit. The industrial utilisation of oranges generates significant fruit waste, including peels, seeds, and pulp, posing an environmental challenge if not managed properly. Synthetic food additives have historically played a crucial role in the food industry, offering benefits such as enhancing oxidative stability, prolonging shelf life, and imparting specific flavours. Nevertheless, concerns have arisen regarding potential health risks linked with consuming foods containing synthetic antioxidants (Xu et al., 2021). Consequently, there has been a concerted effort in research to explore natural antioxidants derived from plants or animals as viable alternatives to synthetic counterparts. These natural antioxidants and flavourings have the potential to impact the texture and sensory attributes of food products (Nikoo & Gavlighi, 2022).

In this context, the present study aims to explore the efficacy of orange peel extract as a preservative and compare its performance with the widely used industrial preservative, Butylated Hydroxytoluene (BHT). Orange peel extract represents a valuable reservoir of bioactive compounds, particularly polyphenols, renowned for their antioxidant and antibacterial properties.

Materials and Methods

Fresh orange peels were collected from local juice shops in Vashi, Navi Mumbai, Maharashtra, sorted, thoroughly washed to remove adhering dirt and

impurities, and drained. The cleaned peels were dried in a ventilated hot-air oven at 40–45 °C for 3 days until a constant weight was achieved, ground into a fine powder, and stored in airtight containers until further use. Orange peel powder was extracted with methanol as the extraction solvent following the method described by Feng (2022) with slight modifications. Briefly, 40 g of orange peel powder was mixed with 400 mL of methanol (1:10, w/v) and extracted at 50 °C for 60 min with continuous shaking. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a Rotary Flash Evaporator (Heidolph Instruments, Germany) at 40 °C to remove the extraction solvent. The concentrated extract was further dried to obtain the crude orange peel extract. Subsequently, the dried extract was incorporated into the edible coating formulation prepared in sterile distilled water, following the approach described by Charoenphun, Rajasekaran, Palanisamy, and Venkatachalam (2023), before application to the shrimp samples.

Fresh *L. vennamei* shrimp specimens were obtained from a shrimp freezing facility located near Vashi, Navi Mumbai. Morphometric measurements were recorded, followed by the dressing process to remove the head and shell while retaining the last carapace and tail fans.

The shrimps were then stretched using a 'CIFT-shrimp stretching mould', and the change in length during stretching was documented. After each step, the shrimps were washed with potable water. The resulting stretched shrimps (Nobashi) were subsequently subjected to the treatment process.

The Nobashi sample was divided into three equal portions by weight. One portion was designated as the control and was treated with double-distilled water (DDW). The second portion was exposed to a 150 ppm solution (Hosseini et al., 2014; Lee et al., 2023) of Butylated Hydroxytoluene (BHT), while the third portion was treated with a 3.5% solution of Orange Peel Extract (OPE) in distilled water. Based on preliminary investigations, a 3.5% citrus fruit extract concentration was selected because it provided the most effective preservation among the concentrations evaluated.

The treatment process involved immersing the Nobashi shrimp in the respective solution at a 1:1 ratio. This treatment was conducted for a duration of 30 minutes, after which the excess solution was

drained. Subsequently, the samples were packed in food-grade polypropylene (PP) bags with a thickness of 51 μm and stored under refrigerated conditions ($<4^\circ\text{C}$) for 15 days, and analysed at predetermined intervals (0, 3, 6, 9, 12 and 15 days).

Proximate composition of stretched shrimps before and after the treatment was carried out. Parameters evaluated were moisture (Hot Air Oven, Thermo), crude protein (Kjeldahl Instrument; Soren & Co.), crude fat, total ash (Muffle Furnace, Pathak Electric Works) and carbohydrate (AOAC, 2002). Protein content was determined from nitrogen content estimated by the Micro-Kjeldahl method by factor multiplication. The crude fat content was determined using the solvent extraction method with a Soxhlet apparatus. The carbohydrate content was calculated by subtracting the cumulative total of all other measured parameters from 100. Total volatile base nitrogen (TVB-N) was evaluated using the micro-diffusion analysis technique as described by Beatty and Gibbons (1937). pH was estimated by homogenising the sample with distilled water using a pH meter (Eutech Instruments, Malaysia). The level of oxidation during the storage study was determined by Peroxide Value (PV) as described by Jacob (1958), and Thiobarbituric acid value was determined by the method described by Tarladgis, Watts, Younathan, and Dugan (1960).

The colour analysis of the fried Nobashi shrimp was conducted using a HunterLab instrument (Virginia, USA) following the methodology outlined by Beriain, Gómez, Petri, Insausti, and Sarriés (2011). The parameters measured included the lightness value 'L*', the redness or greenness value 'a*', and the yellowness/blueness value 'b*'. Additionally, the colour difference from the control sample was determined.

Texture profile analysis (TPA) was performed using a texture analyzer TVT-6700 (Perten Instruments) following the method prescribed by Kaewudom, Benjakul, and Kijroongrojana (2013) with slight modifications. Every sample was subjected to multiple compressions. The parameters evaluated included gumminess, chewiness, springiness, cohesiveness, and stringiness. Analysis was performed in triplicate, and the average value of the treatment was determined.

Organoleptic evaluation of coated and fried Nobashi was carried out according to a nine-point 'hedonic scale' (where '9' indicates like extremely and '1'

indicates dislike extremely) reported by Amerine, Roessler, and Ough (1965). Organoleptic quality of Nobashi as a function of various treatments and refrigerated storage period was evaluated by trained panellists (6 no.). The stretched shrimps were fried in hot oil over low heat until golden-brown colour, after which they underwent further analysis. The quality attributes, such as appearance, colour, odour, flavour, texture, tenderness, chewiness, juiciness and overall sensory score, were observed.

The microbiological quality of Coated Nobashi (control, OPE and BHT-treated) stored under refrigerated conditions was determined by total plate count (TPC) as per AOAC (2002). 10 g of the sample was serially diluted in PBS and spread-plated on total plate count (TPC) agar in triplicate. After 48 hours of incubation at 30°C , colonies were counted and represented as log CFU/g.

Shelf-life was determined primarily based on the microbiological acceptability limit of TPC of 6 log CFU/g, which is widely recognised as the upper acceptable limit for chilled seafood products. The microbiological findings were corroborated with sensory evaluation and biochemical quality parameters (TVBN, TBARS, and pH) to assess the overall quality and acceptability of the product during refrigerated storage.

All samples were collected and analysed in triplicate to ensure the reliability and reproducibility of the experimental data. The results from these replicates were statistically expressed as the mean value with the corresponding standard deviation (mean $\pm$ SD), representing the variability within the data set.

Results and Discussion

The average total length of the *L. Vannamei* used for the study was 16.70 ± 0.7393 cm. The mean weight of the shrimp was 28.55 ± 3.4199 g. The average increase in stretched length of shrimp was 3.68 ± 0.5641 cm.

The proximate composition of untreated (raw) shrimp and the processed Nobashi samples subjected to different treatments is presented in Table 1. Moisture and fat contents differed significantly ($p < 0.05$) among the treatment groups, as indicated by the superscript letters. The ash content of the untreated (raw) shrimp was significantly higher than that of all processed samples, whereas no significant differences ($p < 0.05$) were observed

Table 1. Proximate composition of untreated and treated Nobashi shrimp

Proximate	Moisture	Ash	Fat	Protein	Carbohydrate
Raw	77.99±0.10 ^a	1.21±0.01 ^a	0.87±0.01 ^a	18.63±0.00 ^a	1.31±0.10 ^a
Control	80.32±0.04 ^b	0.92±0.01 ^b	0.79±0.01 ^b	17.74±0.30 ^b	0.23±0.27 ^a
BHT	78.11±0.23 ^c	0.89±0.01 ^b	0.30±0.00 ^c	18.69±0.28 ^c	2.00±0.52 ^b
OPE	79.91±0.12 ^b	0.88±0.01 ^b	0.41±0.12 ^d	17.89±0.00 ^{ab}	0.90±0.11 ^a

Values are expressed as mean ± standard deviation (n = 3). Different superscript lowercase letters indicate significant differences among treatments ($p < 0.05$)

among the control, BHT, and OPE-treated samples. Protein content showed minor but significant variation among treatments, with the OPE-treated samples exhibiting values comparable to both the untreated and control samples. Carbohydrate content did not differ significantly ($p > 0.05$) among the untreated, control, and OPE-treated samples; however, the BHT-treated samples showed a significantly higher carbohydrate content. The statistical comparisons are indicated by different superscript letters in Table 1.

The pH measurements showed a consistent increase throughout the storage duration, gradually transitioning towards a marginally alkaline state by the conclusion of the storage period (Table 2). Metabolic processes, including the generation of total volatile basic nitrogen (TVBN) and trimethylamine (TMA) within shrimp, contribute to the pH elevation during storage (Okpala, Choo, & Dykes, 2014). Although the pH values observed in our investigation were slightly lower than those reported by Khaledian and Basiri (2022), they were comparable to those documented by Alparslan et al. (2016). As storage duration increased, the accumulation of alkaline compounds such as ammonia and metabolic byproducts from spoilage bacteria further contributed to the observed increase in pH (Raj, Raju, Lakshmisha, & Jagpal, 2018).

Total volatile basic nitrogen (TVBN), a well-established parameter for evaluating spoilage in fish and fish-derived products, is a critical quality marker for shrimp (Wang et al., 2018). Initially, all three samples displayed comparable TVBN levels during the early stages of storage. However, TVBN increased in all treatments during storage, with the highest values observed in the control, followed by the OPE-treated samples, whereas the BHT-treated samples showed the lowest increase (Table 2). The increase in TVBN levels is likely attributable to

heightened microbial presence and endogenous enzyme activity (Annamalai et al., 2018). However, the difference between the OPE-treated and BHT-treated samples was negligible. Previous studies have established an acceptable threshold for TVBN levels for human consumption at 30-35 mg N/100 g (European Commission, 1995). While the initial TVBN values were lower than those reported by Khaledian and Basiri (2022) but higher than those obtained by Alparslan et al. (2016), significant differences in TVBN levels were observed in each treatment group as the storage duration progressed. The lower TVBN range observed during the initial storage days for the treatment groups may be attributed to their inhibitory effects on spoilage microbes and enzymes that accelerate product deterioration. Conversely, the increase in TVBN values during later storage stages may be linked to the degradation of protein and non-protein compounds induced by microbial activity.

The initial average Total Plate Count (TPC) was 2 log CFU/g in the control, OPE-treated, and BHT-treated Nobashi samples. All three treatment groups exhibited a significant reduction in TPC up to the third day of refrigerated storage. Thereafter, the average TPC increased gradually in all samples, reaching 6 log CFU/g on Day 12 in the control samples and on Day 15 in the OPE-treated samples (Table 2). The TPC of the BHT-treated samples remained below 6 log CFU/g throughout the storage period. These results indicate that OPE treatment extended the microbiological shelf life of refrigerated Nobashi by approximately three days compared with the control. Although psychrotrophic bacteria are recognised as the predominant spoilage microorganisms during refrigerated storage, only TPC was determined in the present study as an indicator of overall microbiological quality. Future investigations incorporating psychrotrophic bacterial counts, in addition to TPC, would provide a

more comprehensive assessment of microbial spoilage during chilled storage.

The initial sensory score of the control group was higher, followed by OPE and BHT. The BHT-treated samples exhibited a comparatively harder texture and lower flavour acceptability throughout storage (Table 2). This may explain the lower scores observed for the BHT-treated sample from the beginning. OPE-treated shrimp consistently received higher overall acceptability scores than the BHT-treated samples throughout refrigerated storage. Tenderness and juiciness of control and OPE-treated shrimps were good compared to BHT-treated shrimp. The sensory analysis showed a similar decrease when compared with the findings from Rezaei, Shekarforoush, Hosseinzadeh, and

Basiri (2021). The higher sensorial acceptance for the OPE group might be due to the favourable impact of orange peel extract on the sensorial criterion of the shrimp. Similar observations were reported by Alparslan et al. (2016), who observed the positive impact of orange leaf essential oil on the sensorial attributes of the shrimps.

Colour is one of the primary quality attributes that influence consumers' first impression and purchase decision, making it an important indicator of freshness and overall product quality. In the present study, the L^* , a^* , b^* , and ΔE values were monitored to evaluate the colour stability of Nobashi shrimp during refrigerated storage (Fig. 1). The OPE-treated samples consistently exhibited slightly lower L^* values than the control and BHT-treated samples,

Table 2. Changes in pH, TVBN, TPC and overall acceptability of Nobashi shrimp during refrigerated storage

	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day
pH	6.59±0.008 (Control)	6.25±0.12 (Control)	6.66±0.05 (Control)	7.16±0.05 (Control)	7.65±0.03 (Control)	7.68±0.03 (Control)
	6.52±0.08 (BHT)	6.52±0.09 (BHT)	6.53±0.08 (BHT)	6.56±0.03 (BHT)	7.37±0.04 (BHT)	7.56±0.02 (BHT)
	6.47±0.06 (OPE)	6.53±0.06 (OPE)	6.59±0.03 (OPE)	6.65±0.05 (OPE)	7.44±0.03 (OPE)	7.6±0.03 (OPE)
TVBN (mg N/100 g)	12.5±1.09 (Control)	17.8±1.06 (Control)	19.6±0.76 (Control)	29.2±0.32 (Control)	42±0.31 (Control)	59.3±0.76 (Control)
	10.6±0.67 (BHT)	13.9±0.69 (BHT)	16.2±0.31 (BHT)	28±0.36 (BHT)	28±0.72 (BHT)	33.3±0.19 (BHT)
	10.2±0.58 (OPE)	15.7±0.74 (OPE)	18.9±0.22 (OPE)	26.6±0.18 (OPE)	30.8±0.27 (OPE)	39.3±1.09 (OPE)
TPC (log CFU/g)	2.2±0.08 (Control)	1.2±0.17 (Control)	3.7±0.31 (Control)	5.8±0.24 (Control)	6.9±0.38 (Control)	7.4±0.14 (Control)
	2.1±0.14 (OPE)	1.1±0.05 (OPE)	2.5±0.27 (OPE)	3.4±0.36 (OPE)	5.2±0.4 (OPE)	6.6±0.41 (OPE)
	2±0.18 (BHT)	1.1±0.22 (BHT)	1.9±0.34 (BHT)	2.4±0.19 (BHT)	3.1±0.32 (BHT)	3.9±0.22 (BHT)
Overall Acceptability	8.8±0.04 (Control)	8±0.1 (Control)	7±0.36 (Control)	6.2±0.26 (Control)	4.5±0.14 (Control)	4±0.23 (Control)
	7.6±0.14 (BHT)	6.5±0.33 (BHT)	5.5±0.17 (BHT)	5±0.31 (BHT)	4±0.15 (BHT)	3.5±0.32 (BHT)
	8.7±0.22 (OPE)	8.3±0.18 (OPE)	7.5±0.27 (OPE)	6.5±0.09 (OPE)	5.8±0.26 (OPE)	5±0.05 (OPE)

Values are expressed as mean ± standard deviation (n = 3). Control = untreated Nobashi shrimp; BHT = butylated hydroxytoluene-treated Nobashi shrimp; OPE = orange peel extract-treated Nobashi shrimp. TVBN = total volatile basic nitrogen; TPC = total plate count. Overall acceptability was evaluated using a nine-point hedonic scale, where 9 = like extremely and 1 = dislike extremely

indicating a relatively darker appearance. Such changes in lightness are likely associated with variations in surface moisture, protein denaturation, and structural changes in the muscle, which alter the light-scattering properties of the shrimp tissue during storage.

Among the treatments, the BHT-coated samples showed comparatively higher a^* values than the control and OPE-treated samples. Since shrimp muscle contains negligible amounts of myoglobin, changes in redness cannot be attributed to myoglobin oxidation as in red meat. Instead, these changes are more likely related to alterations in carotenoid pigments, particularly astaxanthin, as well as protein denaturation and oxidative changes that occur during storage. Oxidation of lipids and proteins can modify pigment–protein interactions, thereby affecting the stability and distribution of astaxanthin and consequently influencing the a^* values. Likewise, the b^* values were lowest in the control samples, followed by values in the OPE and BHT-treated samples. The gradual increase in b^* values during storage may be attributed to lipid oxidation, protein degradation, and changes in carotenoid pigments, thereby enhancing the yellowness of the shrimp muscle (Rezaei et al., 2021).

The overall colour difference (ΔE), which integrates changes in L^* , a^* , and b^* values, provides a useful measure of the extent of colour deterioration during storage. In the present study, ΔE values increased gradually with storage time, indicating progressive changes in the product appearance. Higher ΔE values reflect greater colour deterioration, whereas lower values indicate better colour stability. Among the treatments, BHT-treated samples exhibited relatively higher ΔE values towards the end of storage, suggesting more pronounced colour changes. In contrast, the control and OPE-treated samples maintained comparatively lower to moderate ΔE values, indicating better preservation of the original colour. The improved colour stability observed in the OPE-treated samples may be attributed to the antioxidant compounds present in orange peel extract, which likely retarded oxidative degradation of carotenoid pigments and other colour-related constituents.

The L^* values obtained in the present study were slightly lower than those reported by Rezaei et al. (2021), who evaluated CMC coatings incorporated with clove oil nanoemulsion, and by Khazaei,

Esmaili, and Emam-Djomeh (2016), who studied basil seed gum and thymol-based edible coatings for shrimp preservation. However, the a^* and b^* values were comparatively higher than those reported in these studies. Overall, the observed changes in colour parameters during refrigerated storage can be explained by progressive lipid oxidation, protein denaturation, and alterations in carotenoid pigments, particularly astaxanthin, which collectively contribute to the gradual deterioration of shrimp colour (Li et al., 2024).

The peroxide value (PV) serves as a quantitative indicator of the primary products resulting from lipid oxidation, predominantly in the form of hydroperoxides. Initial observations revealed higher PV values in both the BHT-treated and control groups. However, as storage duration progressed, the change in PV values for the OPE-treated and control shrimps exceeded those of the BHT-treated shrimps. Remarkably, neither treated group surpassed the acceptability threshold during the storage period, whereas the control group exceeded the 20 meq O_2/kg of fat limit by the 14th day of storage (Fig. 2). The lower increase in PV observed in the OPE and BHT groups, which received treatment with orange peel extract and BHT possessing antioxidant properties, compared to the control group, may be attributed to the antioxidant effects of these treatments. OPE-treated shrimps exhibited a lower PV when compared to shrimps coated with thymol (Khazaei et al., 2016).

Shrimp lipids exhibit a notable degree of unsaturation with multiple double bonds in fatty acids, rendering

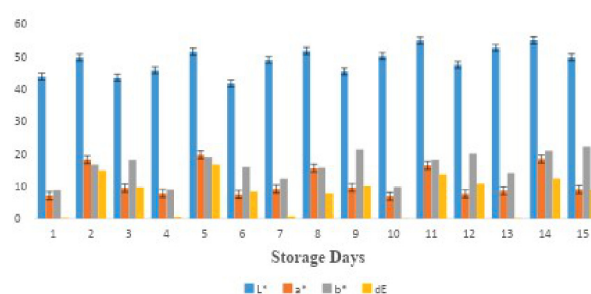


Fig. 1. Changes in colour parameters (L^* , a^* , b^* , and ΔE) of Control, BHT-treated, and OPE-treated Nobashi during refrigerated storage. Control = untreated Nobashi; BHT = Nobashi treated with butylated hydroxytoluene; OPE = Nobashi treated with orange peel extract. Values are expressed as mean $\pm$ standard deviation (SD) ($n = 3$). Error bars represent standard deviation

them highly susceptible to molecular oxygen. Additionally, lipid oxidation generates off-flavour compounds, initiating initial quality deterioration, consequently reducing nutritional quality and altering texture and colour. Peroxides represent intermediate products produced during the early stages of lipid oxidation, followed by the formation of aldehydes and ketones as final products of oxidation in food products (Minh, Nhi, Lap, Van Phong, & Kieu, 2019).

The onset of lipid oxidation from unsaturated lipids is accepted as a primary factor contributing to the deterioration of quality attributes such as flavour, colour, nutritional value, and safety. Malondialdehyde (MDA) is commonly used to assess lipid oxidation and is typically determined by the TBA method. Additionally, elevated MDA levels are linked to rancidity in sensory evaluations (Franklin & Mitchell, 2019). The rise in TBARS values is linked to the generation of secondary lipid oxidation byproducts, particularly aldehydes, formed from the breakdown of polyunsaturated fatty acids. TBA values ranging from 1 to 2 mg MDA/kg in fish muscle may lead to undesirable taste and odour (Farajzadeh, Motamedzadegan, Shahidi, & Hamzeh, 2016), thereby reducing the shrimp's shelf life (Zhang, Deng, & Wang, 2015). In the present study, it was observed that for the control group, the TBARS value reached above 1 mg malondialdehyde/kg meat by the 9th day of storage, whereas in the treatment group reached above 1 mg

malondaldehyde/kg meat by the 12th day (OPE) and 15th day (BHT) (Fig. 3). The reduction in TBARS values observed during the later stages of storage may be attributed to the degradation of malondaldehyde by spoilage microorganisms (El Asuoty, Fayza, & Abou-Arab, 2023). Lower TBARS levels in OPE- and BHT-treated samples suggest their role in reducing lipid oxidation in shrimp during refrigerated storage. Various studies indicate that certain natural plant extracts delay lipid oxidation. For instance, white leg shrimp treated with green tea extract exhibited reduced TBARS content after 12 days of iced storage (Nirmal & Benjakul, 2012). Moreover, antioxidant-rich plant coatings, such as thymol and basil seed gum (Khazaei et al., 2016), as well as green tea extract and quince seed mucilage (Noshad, Nasehi, & Anvar, 2017), offer protection against lipid oxidation, enhancing stability.

Texture profile analysis (TPA) revealed a progressive decline in the measured textural parameters of Nobashi shrimp during refrigerated storage (Table 3). Overall, gumminess, chewiness, stringiness, springiness, and cohesiveness decreased with increasing storage duration across all treatment groups. The control and OPE-treated samples generally exhibited comparable trends, whereas the BHT-treated samples showed comparatively lower values for several texture parameters from the beginning of storage.

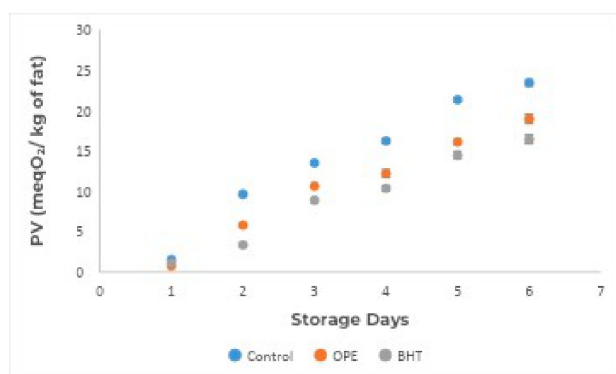


Fig. 2. Changes in peroxide value (PV) of Control, BHT-treated, and OPE-treated Nobashi during refrigerated storage. Control = untreated Nobashi; BHT = Nobashi treated with butylated hydroxytoluene; OPE = Nobashi treated with orange peel extract. Values are expressed as mean $\pm$ SD ($n = 3$). Error bars indicate standard deviation

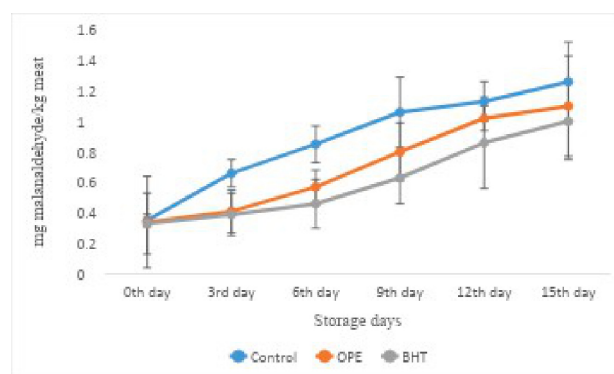


Fig. 3. Changes in thiobarbituric acid reactive substances (TBARS) of Control, BHT-treated, and OPE-treated Nobashi during refrigerated storage. Control = untreated Nobashi; BHT = Nobashi treated with butylated hydroxytoluene; OPE = Nobashi treated with orange peel extract. Values are expressed as mean $\pm$ SD ($n = 3$). Error bars represent standard deviation

Among the measured parameters, chewiness showed a pronounced reduction during storage. In the control samples, chewiness decreased from 270 ± 0.16 on Day 0 to 12 ± 0.12 on Day 15. Similarly, the values decreased from 235 ± 0.07 to 7 ± 0.10 in OPE-treated samples and from 105 ± 0.11 to 5 ± 0.11 in BHT-treated samples. The progressive reduction in chewiness indicates a gradual loss of the resistance of the shrimp muscle to mastication during storage. Such changes may be associated with structural modifications of the muscle matrix, including alterations in myofibrillar proteins, moisture redistribution, and weakening of muscle fibres.

Gumminess also decreased progressively throughout refrigerated storage. The control samples showed a reduction from 14 ± 0.06 on Day 0 to 4 ± 0.13 on Day 15, while OPE-treated samples decreased from 12 ± 0.13 to 3 ± 0.11 and BHT-treated samples from 12 ± 0.16 to 3 ± 0.11 . The decrease in gumminess indicates a gradual reduction in the firmness and compactness of the shrimp muscle during storage.

A gradual reduction in stringiness was observed in all treatment groups. The values decreased from 5 ± 0.22 to 2 ± 0.16 in the control samples, from 5 ± 0.16 to 2 ± 0.17 in OPE-treated samples, and from 4 ± 0.15 to 2 ± 0.21 in BHT-treated samples. The reduction may indicate progressive weakening of the muscle structure during refrigerated storage.

Springiness also declined markedly during storage. The control samples decreased from 22 ± 0.21 on Day 0 to 3 ± 0.14 on Day 15, while OPE-treated samples decreased from 22 ± 0.16 to 2 ± 0.31 and BHT-treated samples from 18 ± 0.04 to 2 ± 0.22 . The reduction in springiness indicates a gradual loss of elastic recovery of the shrimp muscle following compression. Such changes may be associated with alterations in muscle proteins and disruption of the muscle fibre network during storage. Comparable changes in springiness during refrigerated storage have been reported by Wang et al. (2018) and Khaledian and Basiri (2022).

Table 3. Changes in the textural properties of Nobashi shrimp during refrigerated storage

Storage Days	Gumminess	Chewiness	Stringiness	Springiness	Cohesiveness
0 th day (Control)	14 ± 0.06	270 ± 0.16	5 ± 0.22	22 ± 0.21	13 ± 0.11
0 th day (OPE)	12 ± 0.13	235 ± 0.07	5 ± 0.16	22 ± 0.16	6 ± 0.15
0 th day (BHT)	12 ± 0.16	105 ± 0.11	4 ± 0.15	18 ± 0.04	5 ± 0.17
3 rd day (Control)	11 ± 0.10	105 ± 0.15	4 ± 0.13	12 ± 0.11	5 ± 0.10
3 rd day (OPE)	10 ± 0.08	85 ± 0.22	4 ± 0.17	12 ± 0.16	5 ± 0.18
3 rd day (BHT)	10 ± 0.14	75 ± 0.16	3 ± 0.06	11 ± 0.10	5 ± 0.15
6 th day (Control)	9 ± 0.16	55 ± 0.14	3 ± 0.09	10 ± 0.23	5 ± 0.24
6 th day (OPE)	9 ± 0.06	45 ± 0.13	3 ± 0.20	10 ± 0.20	5 ± 0.16
6 th day (BHT)	7 ± 0.04	25 ± 0.30	3 ± 0.12	8 ± 0.19	4 ± 0.13
9 th day (Control)	6 ± 0.09	30 ± 0.13	3 ± 0.16	5 ± 0.14	4 ± 0.09
9 th day (OPE)	6 ± 0.11	25 ± 0.22	2 ± 0.22	5 ± 0.17	4 ± 0.15
9 th day (BHT)	5 ± 0.25	17 ± 0.17	2 ± 0.05	4 ± 0.20	4 ± 0.16
12 th day (Control)	5 ± 0.23	16 ± 0.20	2 ± 0.08	3 ± 0.25	3 ± 0.22
12 th day (OPE)	4 ± 0.09	12 ± 0.15	2 ± 0.13	3 ± 0.23	3 ± 0.07
12 th day (BHT)	3 ± 0.23	10 ± 0.21	2 ± 0.14	2 ± 0.18	3 ± 0.10
15 th day (Control)	4 ± 0.13	12 ± 0.12	2 ± 0.16	3 ± 0.14	2 ± 0.11
15 th day (OPE)	3 ± 0.11	7 ± 0.10	2 ± 0.17	2 ± 0.31	2 ± 0.11
15 th day (BHT)	3 ± 0.11	5 ± 0.11	2 ± 0.21	2 ± 0.22	2 ± 0.11

Values are expressed as mean $\pm$ standard deviation ($n = 3$). Control = untreated Nobashi shrimp; BHT = butylated hydroxytoluene-treated Nobashi shrimp; OPE = orange peel extract-treated Nobashi shrimp. Gumminess, chewiness, stringiness, springiness and cohesiveness were determined by texture profile analysis. Units are as reported by the texture analyzer/software

Cohesiveness also declined during storage. The control samples decreased from 13 ± 0.11 on Day 0 to 2 ± 0.11 on Day 15, whereas OPE-treated samples decreased from 6 ± 0.15 to 2 ± 0.11 and BHT-treated samples from 5 ± 0.17 to 2 ± 0.11 . The reduction in cohesiveness indicates a progressive decrease in the internal bonding characteristics of the shrimp muscle matrix during storage.

Overall, the progressive deterioration in textural properties during refrigerated storage may be associated with modifications of myofibrillar and stromal proteins, together with the action of endogenous proteolytic enzymes and microbial activity. These processes can contribute to degradation of muscle proteins, disruption of muscle fibre structure, and changes in water-holding characteristics, resulting in gradual softening and loss of structural integrity of shrimp muscle. The observed changes are consistent with the textural deterioration previously reported during refrigerated storage of shrimp (Zhang et al., 2020).

In the present study, the end of shelf life was defined primarily by the microbiological acceptability limit of 6 log CFU/g TVC, together with the observed deterioration in sensory attributes and biochemical quality indices. Based on these criteria, the control samples reached the rejection limit on Day 12, whereas the OPE-treated samples remained microbiologically acceptable until Day 15, thereby extending the refrigerated shelf life by approximately three days.

The present study demonstrated that both BHT and orange peel extract (OPE) coatings effectively retarded quality deterioration and extended the refrigerated shelf life of Nobashi shrimp. Shelf life was determined primarily based on the microbiological acceptability limit of 6 log CFU/g Total Plate Count (TPC), supported by sensory evaluation and biochemical quality indices (TVBN, TBARS, and pH). Based on these criteria, the control samples reached the microbiological rejection limit on Day 12, whereas the OPE-treated samples remained acceptable until Day 15, thereby extending the refrigerated shelf life by approximately three days. Although the BHT-treated samples exhibited superior control of microbial growth and lipid oxidation, the OPE-treated samples maintained better sensory acceptability while extending refrigerated shelf life by approximately three days compared with the control. As a natural antioxidant, orange peel extract

represents a promising alternative to synthetic preservatives for enhancing the quality, safety, and storage stability of refrigerated Nobashi shrimp.

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