J Package Technol Res (2017) 1:101–112
https://doi.org/10.1007/s41783-017-0013-2

CrossMark

RESEARCH ARTICLE

Combination Effect of Phosphate and Vacuum Packaging on Quality Parameters of Refrigerated *Aurigequula fasciata* Fillets

Lida Nobakht Ghalati[1] · Ainaz Khodanazary[1] · Seyyed Mehdi Hosseini[1] · Soheila Matroodi[2]

Received: 29 April 2017 / Accepted: 10 August 2017 / Published online: 23 August 2017
© Indian Institute of Packaging 2017

Abstract The effect of vacuum packaging on the quality changes of *Aurigequula fasciata* fillets treated with or without phosphate during refrigerated storage of 16 days was investigated. The results showed that the fillets treated with polyphosphate significantly maintained tissue hardness when compared to the control. The polyphosphate especially sodium tripolyphosphate (STPP) treatment also inhibited the microbiological and chemical properties throughout the storage times. Sulfhydryl (SH) content of fish actomyosin increased in fillets storage at refrigerator. Pretreatment with tetra sodium pyrophosphate resulted in the retarded protein denaturation as evidenced by the reduced changes in sulfhydryl content during the extended storage. Therefore, Pretreatment by soaking the *A. fasciata* fillet in STPP prior to storage was more effective in improving the quality properties during refrigerated storage when compared with other samples.

Keywords *Aurigequula fasciata* · Vacuum packaging · Phosphate · Shelf-life

✉ Ainaz Khodanazary
khodanazary@yahoo.com

[1] Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

[2] Department of Marine Biology, Faculty of Marine Sciences, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

Introduction

Aurigequula fasciata is one of the marine fish species in the Persian Gulf, which due to its high nutritional quality and excellent sensory properties, is preferred by the customers in the south of Iran. *A. fasciata* is generally consumed fresh, in whole or filleted form, in Iran. Because this species is consumed domestically, it is very important to extend its shelf life, which is normally quite limited when kept refrigerated. Vacuum packaging (VP) is one of the methods of the natural preservation in order to delay the degradation and maintain the quality of the products longer [37]. Although VP is widely used as a supplement to ice or refrigeration to decrease the supply of oxygen to the aerobic bacteria in the flesh to extend the shelf life of product, loss in water-holding capacity of fish stored under VP generally occurs [2]. So, phosphate compounds have been used in seafood products to improve the functionality before VP, especially to increase the water holding capacity [16]. Addition of phosphates to fishery products inhibited the growth of bacteria in stored fish in refrigerator and retarded the oxidation of unsaturated fatty acids in fish muscle [27]. Increase water retention ability by the phosphates is achieved by muscle fiber expansion caused by electrostatic repulsions, which allows more water to be immobilized for the myofibril lattices [34]. The antimicrobial effectiveness of various orthophosphates, pyrophosphates and polyphosphates was determined by changes in pH and the ability to chelate metal ions essential for bacteria metabolism [31]. Phosphates may prevent lipid oxidation through the chelating of prooxidative metal ions [28]. The effectiveness of phosphates on functional properties and water holding capacity of fish products depends on the type of phosphates, the amount used, and the specific food products [23]. Phosphates activity may be

because of efforts on pH and ionic strength and specific interactions of phosphates anions with divalent captions and myofibrillar proteins [49]. Through the shelf life of *A. fasciata* could be extended by VP, drip was produced. This problem causes unacceptability of seafood. Therefore the use of phosphate in combination with VP would provide an effective means to improve the quality of seafood products. Thus the objective of this study was to determine the combinative effect of phosphate, VP and air packaging on the shelf life of *A. fasciata* fillets by monitoring microbiological, chemical and sensory changes throughout the storage at 4 °C [4].

Materials and Methods

Fish Preparation

Aurigequula fasciata with an average weight of 600 g were caught with beach seine in the Persian Gulf, Khorramshahr, Iran. Fish were placed in crushed ice with a fish/ice ratio of 1:3 (w/w) and transported to the fish processing laboratory of Khorramshahr University of Marine Science and Technology within 1–2 h after catching. They were then washed with tap water, filleted and cut into slices with a thickness of 1 cm.

Phosphate Pretreatments

Sodium tripolyphosphate (STPP), tetra sodium pyrophosphate (TSPP) and mixture of tetra sodium pyrophosphate and STPP (1% TSPP + 1% STPP) were individually dissolved in the distilled water to obtain a final concentration of 2 g/100 ml. *A. fasciata* slices were soaked in five volume of the solution (4 °C) for 10 min and drained for 10 min at 4 °C. Control samples were soaked in the distilled water and drained under the same condition. After draining, the slices weighing approximately 500 g were packed. Fish samples with and without phosphate pretreatments were packed in vacuum bags and in air, respectively. All packs maintained at refrigerator and were taken for microbiological, chemical, color, textural and sensory analyses every 4 days for up to 16 days.

Microbiological Analysis

The samples (25 g) were placed in a stomacher bag containing 225 ml of 0.85% saline water. After mixing for 1 min in a stomacher blender, further serial dilution was done using the same diluent. Thereafter, 0.1 ml of appropriate dilution was used for microbiological analysis by spread plate method. The media and condition used were: Plate Count Agar (PCA, Merck, Denmark, Germany) incubated for psychrotrophilic bacteria count at 4 °C for 10 days and for total viable count at 30 °C for 24–48 h [38].

The pH Measurement

The pH measurement was carried out using a Metrohm model 713 pH meter. Fish muscle (2 g) was homogenized thoroughly with 10 ml of distilled water and the homogenate was subjected to pH determination according to the method of Masniyom et al. [27].

Determination of Total Volatile Base (TVB)

TVB muscle was determined according to the method proposed by Goulas and Kontominas [9]. Ten grams of meat was homogenized with 2 g MgO and 300 ml distilled, and seven drops of anti-foam and some boiling stones were added. The blend was heated for 45 min until the volume of boric acid solution reached 150 ml. Boric acid containing methyl red reagent, which initially due to its acidity was red, gradually became alkali and turned green. Finally, the solution obtained from the accumulation of distillation gases by 0.1 N sulfuric acid to reach the onion skin color was titrated.

Determination of Thiobarbituric Acid (TBA)

Thiobarbituric acid (TBA) was determined according to the method proposed by Siripatrawan and Noipha [43]. Ten grams of meat were homogenized for 2 min with 97.5 ml distilled water and 2.5 ml 4 N HCl solution, and then three drops of anti-foam and some boiling stones were added. The blend was distilled until a 50 ml of TBA reagent (0.02 M 2-thiobarbituric acid in 90% acetic acid) were blended and heated in a boiling water bath for 35 min. After cooling under running water for 1 min, the absorbance was measured at 538 nm against a blank, which was 5 ml of distilled water with 5 ml TBA reagent.

Determination of Free Fatty Acid (FFA)

The free fatty acid content was determined in the lipid extract by Woyewoda's method. Results were expressed in % of oleic acid [53].

Determination of Water Holding Capacity (WHC)

Each minced meat sample (10 g) and 15 ml of 0.06 M NaCl solution were added into a 15 ml centrifuge tube and mixed with a vortex mixer for 1 min the tube was then refrigerated at 4 °C for 15 min before being centrifuged at 4 °C and 3000*g* for 15 min according to the method of Zhuang et al. [54].

Preparation of Actomyosin

0.5 g (0.5–1 cm of tissue) of muscle tissue from fish (be sure to avoid the fat) and transfer it to the appropriately labeled microfuge tube containing Laemmli sample buffer. The microtube flick 15 times with your finger to mix the fish tissue into the sample buffer. Alternatively, the sample can be vortexed for a few seconds. The samples incubate for 5 min at room temperature to extract and solubilize the proteins. The buffer containing the extracted proteins pipet into a new 1.5 mL screw cap tube. Fish protein samples boil, as well as the purified actin and myosin samples. Additionally, protein standards (ladder) boil to denature the proteins in preparation for electrophoresis.

Determination of Total Sulfhydryl Content

One milliliter of actomyosin (0.4 g/ml) solution was added to 9 ml of Tris–HCl buffer (0.2 M), pH 6.8, containing urea (8 M), SDS (2 g/100 ml) and EDTA (10 mM). To a 4 ml aliquot of the mixture, 0.4 ml of DTNB (0.1 g/100 ml) solution were added. The mixture was incubated at 40 °C for 25 min and the absorbance was measured at 412 nm with a spectrophotometer. A blank was prepared by replacing the sample with KCl using the molar extinction coefficient of 13,600/M/cm and was expressed as mol/10^5 g protein [3, 27].

SDS–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to monitor the polymerization of the modified proteins. The cuttlefish paste was solubilised in 5% SDS (1:9, w/v) and dissolved in sample buffer with and without b-mercaptoethanol. SDS-PAGE was performed using 4% stacking gels and 10% running gels [21].

Color Measurements

A Minolta Chroma Meter CR400 (Minolta, Osaka, Japan) was used for color measurements. Colors were expressed as CIELab coordinates. In this system, L* represents the color lightness on a 0–100 point scale from black to white; a* is the position between red (+) and green (−); and b* is the position between yellow (+) and blue (−). The color intensity is expressed by a chroma value (C^*_{ab}), while hue (H°_{ab}) corresponds to the name of the color as found in its pure state on the spectrum. These values were calculated according to the formulae:

$$C^*_{ab} = \left(a^{*2} + b^{*2}\right)^{1/2} \text{and } H^0_{ab} = \arctan(b^*/a^*)$$

Texture Measurements

Textural profile analysis (TPA) were performed using an LFRA-4500 texture analyser (Brookfield Engineering Laboratories, inc., Middleboro, MA) equipped with a 4.5-kg load cell and texture Pro Lite V1.0 software. Samples removed from the dorsal part of the fish of size 3.5 × 3.5 × 0.7 cm were compressed perpendicularly using a 20-mm diameter cylindrical probe. The testing conditions were two consecutive cycles at 25% compression, cross-head movement at a constant speed of 0.8 mm/s, and a trigger point of 22.5 g. Texture variables (hardness, cohesiveness, springiness, chewiness, adhesiveness and gumminess) were calculated as described by Kilinc et al. [16] and Hernandez et al. [13].

Sensory Evaluation

Samples were prepared by steaming for 60 min at 80 °C. Salt (1.5%) was added. The cooked samples were evaluated by 17 panelists from the Department of seafood processing with the ages of 23–28 (14 females and 3 males), using the 5-point hedonic scales where 5: like extremely; 3: neither like or nor dislike; 1: dislike extremely. Panelists were regular consumers of fish and had no allergies to fish. All panelists were asked to evaluate for odor and flavor.

Statistical Analyses

Significant differences between means were determined by one-way analysis of variance (ANOVA) using SPSS16.0 for Windows (SPSS Inc., Chicago, IL, USA). Duncan's test was used to compare the means. A significance level of $p < 0.05$ was used.

Results and Discussion

Effect of Polyphosphate Pretreatment on Microbiological Changes

Variations in the value of total viable counts (TVC) of phosphate pretreatment in combination with VP during the refrigerated storage are presented in Table 1. The initial TVC in the control fillet was 3.13 $\log_{10}$ CFU/g, it was 3.37, 3.11, 2.66 and 2.77 $\log_{10}$ CFU/g for VP, TSSP + VP, STTP + VP and TSSP + STTP + VP, respectively. The number of bacteria in fresh fillet with high-quality vary from 3 to 4 $\log_{10}$ CFU/g [42]. TVC of *A. fasciata* fillets stored in air increased rapidly and was generally higher than other treatments ($P < 0.05$). Among all treatments,

Table 1 Changes in total viable count (TVC) and psychrotrophilic bacteria counts (PTC) in *Aurigequula fasciata* fillets stored under different condition during refrigerated storage

Days of storage	0	4	8	12	16
TVC					
Control	3.13 ± 0.40^{dA}	5.29 ± 0.39^{cA}	6.70 ± 0.36^{cA}	9.71 ± 0.91^{bA}	13.03 ± 0.70^{aA}
VP	3.37 ± 0.57^{dA}	3.97 ± 0.26^{dAB}	5.94 ± 0.30^{cAB}	7.83 ± 0.35^{bB}	11.44 ± 0.43^{aB}
TSPP + VP	3.11 ± 0.25^{dA}	3.44 ± 0.16^{dB}	4.81 ± 0.29^{cC}	6.22 ± 0.11^{bB}	7.23 ± 0.06^{aC}
STPP + VP	2.66 ± 0.25^{cA}	3.29 ± 0.52^{cB}	4.55 ± 0.36^{bC}	6.29 ± 0.48^{aB}	6.96 ± 0.31^{aC}
TSPP + STPP + VP	2.77 ± 0.39^{cA}	3.60 ± 0.63^{cB}	5.29 ± 0.06^{bBC}	6.60 ± 0.09^{aB}	6.86 ± 0.31^{aC}
PTC					
Control	3.66 ± 0.60^{dA}	4.41 ± 0.26^{dAB}	6.01 ± 0.33^{cA}	7.39 ± 0.29^{bA}	8.80 ± 0.37^{aA}
VP	3.69 ± 0.32^{dA}	4.14 ± 0.08^{dAB}	5.14 ± 0.09^{cB}	5.94 ± 0.11^{bB}	6.84 ± 0.34^{aB}
TSPP + VP	3.83 ± 0.16^{dA}	4.52 ± 0.14^{cAB}	4.91 ± 0.32^{cB}	6.00 ± 0.17^{bB}	6.84 ± 0.04^{aB}
STPP + VP	3.86 ± 0.14^{cA}	3.81 ± 0.29^{cB}	4.89 ± 0.32^{bB}	5.68 ± 0.23^{bB}	6.86 ± 0.22^{aB}
TSPP + STPP + VP	3.82 ± 0.39^{dA}	4.58 ± 0.19^{cdA}	4.97 ± 0.20^{bcB}	5.84 ± 0.29^{bB}	7.16 ± 0.31^{aB}

Mean values and standard errors from the three replicates are presented

The different capital letters in same columns within the same storage time indicate the significant differences ($P < 0.05$)

The different small letters in same rows within the same treatment indicate the significant differences ($P < 0.05$)

sample pretreated with phosphate and kept under VP had the lowest TVC. The effectiveness of phosphates as antimicrobial agents in flesh depends on the type of phosphate, the amount used, specific food product and conditions under which they are used [45]. TVC of samples pretreated with phosphate and kept under VP increased more slowly than those stored under VP without phosphate pretreatment, indicating that phosphate might show the synergistic effect on the retardation of bacterial growth in the sample kept under VP.

It is well recognized that gram-negative psychrophilic or psychrotrophilic organisms are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperature [10, 38]. Psychrotrophilic bacteria counts (PTC) of *A. fasciata* fillet treated with and without phosphate under VP under 16 days of refrigerated storage in comparison with the control are shown in Table 1. The initial PTC of the fish samples ranges from 3.66 to 3.86 $\log_{10}$ CFU/g, indicating the good quality of the fish used in this study. PTC of all samples increased with increasing time of storage at refrigerator ($P < 0.05$). Higher counts of psychrophilic bacteria were also observed in samples kept in air, compared with those stored under VP and treated with polyphosphate ($P < 0.05$). After 16 days of storage, no marked difference in PTC of samples pretreated with different phosphate types was observed. The results indicated that pretreatment by soaking the *A. fasciata* fillets in polyphosphate solution prior to storage was more effective in reducing microbial numbers compared with control samples. This results was in accordance with Masniyom et al. [27] who found that seabass slices treated with phosphate had lowered PTC, compared with the control during refrigerated storage of 21 days. Phosphate might show synergistics effect with VP on inhibition of PTC in *A. fasciata* [5]. reported that STTP in combined with VP reduced PTC in *Rutilus frisii kutum* fillets.

A microbiological acceptability limit is 7 log CFU/g for fresh water and marine species that is fit for human consumption [14]. By the day 12 of storage, control samples exceeded the value of 7 log CFU/g for TVC and PTC, which was regarded as the upper acceptability limit for raw fish [14] while that of all samples pretreated with phosphate and kept under VP did not achieve this count to the end of 16 days storage time. Polyphosphate may suppress the growth of bacteria by the chelation of metal ions in cell membranes which leads to cation deficiency with resulting loss of membrane integrity and inhibition of normal cell division [51]. Other mechanisms include effect of pH, increase in ionic strength, interactions with cell walls and membranes, and interference with various transport functions [40, 19]. Other researchers have proposed that enzyme inhibition may be responsible for the antimicrobial mechanism [52].

Effect of Polyphosphate Pretreatment on Chemical Analysis

Changes in TBA Content

TBA index is a widely used indicator for the assessment of degree of lipid oxidation of product, some of which have unpleasant flavors and odors [38, 15]. TBA values of the different treatments during storage are presented in

Table 2 Chemical changes of *Aurigequula fasciata* fillets treated with polyphosphates during refrigerated storage

Days of storage	0	4	8	12	16
TBA (mg MDA/kg muscle)					
Control	0.23 ± 0.06^{bA}	1.25 ± 0.23^{aA}	1.25 ± 0.10^{aA}	0.99 ± 0.33^{aA}	1.32 ± 0.06^{aA}
VP	0.44 ± 0.17^{cA}	1.25 ± 0.15^{aA}	1.33 ± 0.13^{aA}	0.78 ± 0.03^{bcAB}	1.05 ± 0.09^{abAB}
TSPP + VP	0.27 ± 0.14^{bA}	0.63 ± 0.14^{abA}	0.86 ± 0.21^{aAB}	0.70 ± 0.21^{abAB}	0.79 ± 0.09^{abBC}
STPP + VP	0.65 ± 0.02^{aA}	0.63 ± 0.30^{aA}	0.41 ± 0.07^{aB}	0.22 ± 0.06^{aB}	0.61 ± 0.15^{aC}
TSPP + STPP + VP	0.58 ± 0.21^{aA}	0.92 ± 0.50^{aA}	0.98 ± 0.27^{aAB}	0.55 ± 0.05^{aAB}	0.83 ± 0.11^{aBC}
FFA (% oleic acid)					
Control	0.94 ± 0.49^{bA}	1.66 ± 0.37^{bA}	0.95 ± 0.15^{bB}	5.23 ± 1.79^{aA}	5.94 ± 0.44^{aA}
VP	0.70 ± 0.07^{cA}	1.42 ± 0.08^{bcA}	1.27 ± 0.34^{bcB}	4.07 ± 1.22^{aAB}	3.24 ± 0.88^{abB}
TSPP + VP	1.03 ± 0.15^{bA}	0.89 ± 0.18^{bA}	2.24 ± 0.38^{aA}	1.31 ± 0.19^{abB}	1.90 ± 0.51^{abB}
STPP + VP	1.06 ± 0.08^{aA}	1.30 ± 0.22^{aA}	0.79 ± 0.19^{aB}	1.91 ± 0.34^{aB}	1.78 ± 0.56^{aB}
TSPP + STPP + VP	0.89 ± 0.02^{aA}	1.35 ± 0.31^{aA}	1.06 ± 0.14^{aB}	0.91 ± 0.16^{aB}	1.24 ± 0.50^{aB}
pH					
Control	6.18 ± 0.06^{bA}	6.92 ± 0.03^{aA}	6.66 ± 0.18^{abA}	6.68 ± 0.27^{abA}	7.30 ± 0.28^{aA}
VP	6.18 ± 0.06^{cA}	6.55 ± 0.15^{bcAB}	6.85 ± 0.08^{abA}	6.45 ± 0.17^{bcA}	7.23 ± 0.31^{aAB}
TSPP + VP	6.18 ± 0.06^{bA}	6.61 ± 0.05^{aAB}	6.12 ± 0.06^{bB}	7.72 ± 0.09^{aA}	6.64 ± 0.11^{aAB}
STPP + VP	6.18 ± 0.06^{aA}	6.21 ± 0.14^{aB}	6.56 ± 0.14^{aAB}	6.35 ± 0.08^{aA}	6.52 ± 0.20^{aB}
TSPP + STPP + VP	6.18 ± 0.06^{aA}	6.40 ± 0.29^{aAB}	6.47 ± 0.20^{aAB}	6.23 ± 0.11^{aA}	6.55 ± 0.12^{aAB}
TVBN (mg N/100 g muscle)					
Control	14.00 ± 0.00^{cA}	19.13 ± 4.45^{cA}	36.86 ± 0.93^{bA}	49.93 ± 6.58^{bA}	120.46 ± 7.21^{aA}
VP	14.93 ± 0.46^{cA}	16.80 ± 2.13^{cA}	32.46 ± 3.42^{bcAB}	38.33 ± 2.53^{bAB}	70.93 ± 11.66^{aB}
TSPP + VP	18.20 ± 3.52^{bA}	20.06 ± 0.93^{bA}	21.46 ± 0.46^{bC}	20.06 ± 1.68^{bC}	56.33 ± 18.75^{aB}
STPP + VP	14.46 ± 0.46^{bA}	23.33 ± 6.17^{bA}	26.46 ± 1.62^{bBC}	27.06 ± 4.14^{bBC}	48.53 ± 5.19^{aB}
TSPP + STPP + VP	15.86 ± 1.86^{cA}	25.66 ± 2.33^{bA}	28.93 ± 4.87^{bABC}	21.93 ± .93^{bcC}	53.20 ± 2.80^{aB}
WHC (%)					
Control	85.33 ± 0.33^{aA}	68.33 ± 0.33^{cD}	70.33 ± 0.33^{bD}	62.00 ± 0.57^{dC}	52.33 ± 0.33^{eA}
VP	85.33 ± 0.33^{aA}	68.33 ± 0.033^{cD}	70.33 ± 0.33^{bD}	61.33 ± 0.33^{dC}	52.33 ± 0.33^{eA}
TSPP + VP	85.33 ± 0.33^{aA}	81.33 ± 0.33^{bB}	77.33 ± 0.33^{cC}	70.33 ± 0.33^{dB}	50.33 ± 0.33^{eB}
STPP + VP	85.33 ± 0.33^{aA}	83.33 ± 0.33^{bA}	80.66 ± 0.66^{cB}	72.33 ± 0.33^{dB}	54.00 ± 0.57^{eA}
TSPP + STPP + VP	85.33 ± 0.33^{aA}	78.33 ± 0.33^{bC}	84.00 ± 0.57^{aA}	78.66 ± 1.85^{bA}	44.66 ± 0.88^{cC}
SH (mol/105 g protein)					
Control	1.65 ± 0.48^{aA}	0.44 ± 0.00^{bB}	0.30 ± 0.01^{bC}	0.20 ± 0.01^{dD}	0.87 ± 0.02^{bC}
VP	1.46 ± 0.59^{aA}	0.55 ± 0.03^{abB}	0.37 ± 0.02^{bC}	1.06 ± 0.02^{abB}	0.98 ± 0.07^{abC}
TSPP + VP	1.91 ± 0.32^{bA}	1.54 ± 0.24^{bA}	0.09 ± 0.01^{cD}	0.44 ± 0.03^{cC}	2.98 ± 0.43^{aA}
STPP + VP	1.53 ± 0.22^{aA}	1.19 ± 0.00^{bA}	0.94 ± 0.02^{bB}	0.13 ± 0.00^{cD}	0.93 ± 0.01^{bC}
TSPP + STPP + VP	1.90 ± 0.15^{aA}	1.47 ± 0.02^{bA}	1.84 ± 0.06^{aA}	1.26 ± 0.02^{bA}	1.94 ± 0.08^{aB}

Mean values and standard errors from the three replicates are presented

The different capital letters in same columns within the same storage time indicate the significant differences ($P < 0.05$)

The different small letters in same rows within the same treatment indicate the significant differences ($P < 0.05$)

Table 2. The initial TBA values ranged from 0.23 mg malondialdehyde/kg sample in samples stored in air to 0.65 mg malondialdehyde/kg samples in samples pre-treated with STPP and stored under VP. TBA values was increased in all samples when the storage time increased ($P < 0.05$). Higher increase in TBA value was observed in *A. fasciata* fillets stored in air and VP. An increase of TBA values in VP may be due to denaturation of muscle proteins because of carbonic acid formed of muscle during storage in refrigerator, leading to the release of free haem iron, a potential pro-oxidant in the muscle system. The increase in TBA value in air also was due to interacting lipids with air oxygen [6] and may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids [17]. However, phosphate pretreatment resulted in lower TBA values of samples kept under VP to some

extent. Samples pretreated with STPP tended to have lower TBA values, compared with that TSPP and mixture of STPP + TSPP pretreated during refrigerated storage. Polyphosphates are stronger chelators and antioxidants than mono- and diphosphates (Food lipids). Polyphosphate-metal complexes are stronger than pyrophosphate complexes which are stronger than orthophosphate complexes [50]. Lipid in fish muscle typically has a high percentage on polyunsaturated fatty acids and considered to be susceptible to oxidation during storage compared with saturated fatty acids [46]. Thus, phosphate, especially STPP pretreatment, potentially retard the oxidation in *A. fasciata* fillets through the chelation of pro-oxidant metal ions [44, 27]. Shahidi et al. [39] stated that phosphates are reasonably effective when compared with other chelators. It has been proposed that the TBA value of 1–2 mg malondialdehyde/kg muscle is an acceptable limit [41]. In the current study, TBA values of all samples were lower than such proposed limits throughout the 16 days storage period.

Changes in FFA Content

It is well known that hydrolysis of glycerol-fatty acid esters is one important change that occurs in fish muscle lipids with the release of FFA which catalyzed by lipase and phospholipase. The initial FFA value was from 0.70 to 1.06% of oleic acid. A gradual increase in FFA formation in all samples was observed, but samples stored under VP with phosphate pretreatment, decelerated developing process of FFA production during storage of refrigerated (Table 2) due to the action of lipase and phospholipase on phospholipids and triglycerides [36]. FFA value of control samples was higher than treated samples, significantly ($p < 0.05$). At the end of the storage, FFA value was 5.94, 3.24, 1.90, 1.78 and 1.24% of oleic acid for control, VP, TSSP + VP, STTP + VP and TSSP + STTP + VP, respectively. A separate effect on lipids attributed to TSPP and STPP is one of saponification of free fatty acids. Alkaline phosphates interacted with free fatty acids in meat emulsions and thus contributed to fat emulsification.

Changes in pH Content

Changes in pH of *A. fasciata* muscle as affected by storage air and phosphate pretreatment during storage are presented in Table 2. The pH of the muscle on day 0 was 6.18. During storage, pH of samples pretreatment with phosphate such as control samples, an increase in pH was observed throughout the storage time ($P < 0.05$), presumably due to accumulation of basic compounds generated from both autolytic processed by endogenous enzymes and microbial enzymatic actions [33] although it could also be associated with the increase in bacterial counts especially psychrotrophilic bacteria counts (Table 1). This results was in accordance with Masniyom et al. [27] and Etemadian et al. [6]. The sharp increase in pH was observed in the samples stored in air to end day. Among all phosphates used, STTP retreatment resulted in a lower pH of sample, compared with TSPP and TSPP + STPP. Thus, the lowered increased in pH value of phosphate pretreatment samples was more likely related with the lower microbial growth in the sample during the refrigerated storage. Moreover, phosphates are employed in food processing for a variety of function as pH stabilization and acidification. Ludorff and Meyer [26] showed that the acceptable upper limit for the pH of fish is 6.8–7. In this study, the pH values of samples stored under VP with phosphate pretreatment did not exceed this limit.

Changes in TVBN Content

TVBN content of all samples was showed in Table 2. At day 0, TVBN content of all samples was found between 14.00 and 18.20 mg N/100 g muscle. Generally, control samples had the higher TVBN, compared with samples pretreated with phosphates throughout the storage. TVBN, products of bacterial spoilages (*Aeromonas* spp., Enterobacteriaceae, *P. phosphoreum*, *Shewanella putrifaciens* and *Vibrio* spp.) which were capable of reducing TMAO to TMA, and decarboxylating amino acid to biogenic amines, is widely used as an index to assess the keeping quality and shelf life of seafood products [11, 25]. A level of 25 mg N/100 g muscle has been considered the highest acceptable level [17] and above 25–30 mg N/100 g muscle indicate that fish and fish products are decomposed and inedible [6]. For all of the samples except samples stored in air and VP, TVBN level was less 30 mg N/100 g muscle after 12 days, indicating that the fillets of fish maintained at a good quality during storage. From the result, it was found that TVBN content in the sample correlated well with the increase in microbial load and pH, especially the sample stored in air. Thus, inhibitory effect of phosphate against microbial growth could retard or lower the production of microbial degradation products.

Changes in WHC

Water-holding capacity or water-binding ability of food product is one the most important quality parameters that affects both profitability and quality because it affects the weight change during storage and the juiciness and tenderness of the muscle [35, 54]. WHC of *A. fasciata* fillets kept under different conditions are shown in Table 2. WHC of all samples decreased as the storage times increased. Decreases in WHC with storage time may originate from

proteolytic activity in the muscle during storage. The initial WHC of all samples was 85.33% that with increasing storage time in samples stored under VP with phosphate pretreatment especially STTP reached 54.00% and in samples stored in air and VP without phosphate were 52.33%. Increased WHC of samples stored in VP with phosphate may be related to the lower myosin denaturation of fish samples and the protective effect of the phosphate, due to interact with the positive charges of the protein molecule to increase the net negative charges, resulting in the increased WHC [18, 48]. Moreover, cryoprotectants, act as water binders, could also bind water by hydrogen bonding [24]. The phosphate split actomyosin into its component proteins and converted them from a gel to sole state. On the other hand, the lower microbiological degradation of fish fillets treated by phosphate also led to a higher WHC.

Changes in Sulfhydryl (SH) Content

The functional and textural characteristics of seafood depend mainly on myofibrillar proteins and actomyosin, which is the main protein in myofibrils [32]. Changes in the composition of actomyosin result in changes to the fractional groups, such as sulfhydryl groups and hydrophobic groups, and physicochemical properties such as ATP activity [12]. The changes in total SH content of actomyosin extracted from *A. fasciata* fillets under different conditions are shown in Table 2. In all fish samples, the values of SH decreased gradually, followed by an increase over the 12-days refrigerated storage in all of the samples. The reduction of SH content may be explained by the denaturation and aggregation of muscle proteins as a result of cysteine thiol group oxidation, located at the catalytic center of the myosin head, or disulfide interchanges, leading to the formation of disulfide bonds [3, 12].The reduction in the SH content in samples stored in air might be due to the sulfhydryl groups forming cross-linkages or the exposed sulfhydryl groups in protein interacting with additives or small molecular weight compounds in the water soluble protein fraction [22]. From the results, it was suggested that SH groups in *A. fasciata* muscle underwent oxidation to the highest extend when kept in air, especially as the storage time increased. The rate of oxidation was lower in sample stored under VP with and without phosphate pretreatment. After 18 days of storage, samples stored under VP pretreated with TSSP contained the highest SH content. From the result, phosphate pretreatment in combination with VP could retard the oxidation of SH group in muscle proteins, which might be associated with the denaturation of muscle proteins [3]. Masniyom et al. [27] and Etemadian et al. [6] noted that the combination of cryoprotectants and packaging could clearly prevent a decrease in SH contents. Kong et al. [20] demonstrated that the addition of cryoprotectants could serve as a protective barrier against oxidation initiators, so it can reduce the increase in carbonyl contents and the decrease in SH contents. In refrigerated fish, oxidation of sulfhydryl groups and the increase in TVBN was retard by the addition of phosphate with cryoprotective effect and this was coincidental with the decreased disulfide bond formation [27].

Effect of Polyphosphate Pretreatment on Color Changes

The appearance of food products is an important parameter to consumer, both from the point of view of acceptability and preference. Surface color is influenced by both muscle structure characteristics and pigment concentrations [8]. Color values including lightness (L*) coordinate, redness (a*) value and blueness (b*) value of control and sample stored under VP with and without phosphate pretreatment during storage 4 °C are shown in Table 3. The a* value of all samples gradually decreased during the refrigerated storage. On the other hand, L* and b* values of *A. fasciata* fillets increased as storage time increased ($P < 0.05$), reflecting an evolution toward lightness and grey-blue tones respectively. The chroma (color intensity) (C^*_{ab}) value decreased with time in storage, indicating a reduction in color intensity. No significant changes were seen for hue of polyphosphate pretreatment stored under VP over storage time. Color loss in fish fillets during storage might be attributed to the lipid oxidation, oxidation of proteins with haemo groups (haemoglobin and myoglobin), non-enzymatic browning reactions between lipid oxidation products and the amine groups in proteins, and microbial spoilage [43]. The greatest rate of decrease in a* value was found in control sample. The highest increase in L* and b* values was also observed in control sample. From the result, degree of changes in color caused by VP could be lowered with phosphate pretreatment, especially dipping fillets in STPP of *A. fasciata*. The phosphates have been reported by a number of investigators to improve the color of fish flesh [27, 16]. The beneficial effect of polyphosphates on flesh color also has been documented [16, 7]. STTP reduced myoglobin oxidation in refrigerated fish. However, it should not be constructed that STPP has not direct effect on meat pigment. In fact, the binding of polyphosphates to heme has been shown [29].

Effect of Polyphosphate Pretreatment on Textural Changes

The texture of fish is considered an important quality attribute for fish products and seafood processing industries' palatability. In the present study, hardness was found to be

Table 3 Changes in color of *Aurigequula fasciata* fillets treated with polyphosphates during refrigerated storage

Days of storage	0	4	8	12	16
a*					
Control	−0.63 ± 0.01aA	−0.77 ± 0.00abA	−0.20 ± 0.51aA	−0.82 ± 0.03abA	−1.50 ± 0.18bC
VP	−0.63 ± 0.01aA	−0.88 ± 0.02bA	−0.92 ± 0.00bAB	−1.01 ± 0.01cBC	−0.91 ± 0.00bA
TSPP + VP	−0.63 ± 0.01aA	−0.89 ± 0.01bA	−0.89 ± 0.01bAB	−0.99 ± 0.02cB	−1.06 ± 0.02dAB
STPP + VP	−0.63 ± 0.01aA	−0.94 ± 0.01bA	−0.95 ± 0.01bAB	−0.95 ± 0.01bB	−1.09 ± 0.02cAB
TSPP + STPP + VP	−0.63 ± 0.01aA	−0.58 ± 0.26aA	−1.00 ± 0.01bB	−1.11 ± 0.05bC	−1.21 ± 0.01bB
b*					
Control	−3.51 ± 0.09dA	−3.15 ± 0.02cA	−2.25 ± 0.06bA	−2.14 ± 0.01bA	−0.87 ± 0.05aA
VP	−3.51 ± 0.09dA	−3.31 ± 0.02dB	−2.88 ± 0.04cB	−2.54 ± 0.07bB	−1.64 ± 0.12aB
TSPP + VP	−3.51 ± 0.09cA	−3.40 ± 0.09cBC	−2.90 ± 0.13bB	−2.65 ± 0.07bB	−2.65 ± 0.07aC
STPP + VP	−3.51 ± 0.09cA	−3.54 ± 0.04cC	−3.20 ± 0.06bC	−3.02 ± 0.05bC	−2.55 ± 0.15aC
TSPP + STPP + VP	−3.51 ± 0.09dA	−3.43 ± 0.02cdBC	−3.20 ± 0.06bcC	−3.04 ± 0.07bC	−2.45 ± 0.09aC
L*					
Control	58.99 ± 1.19aA	49.24 ± 0.56bC	45.56 ± 0.82cC	40.68 ± 0.38dB	37.04 ± 0.30eD
VP	58.99 ± 1.19aA	51.96 ± 0.85bB	50.94 ± 0.15bB	42.70 ± 0.64cB	40.86 ± 0.16cC
TSPP + VP	58.99 ± 1.19aA	55.08 ± 1.12bA	52.26 ± 0.58bB	46.70 ± 1.25cA	43.09 ± 0.98dB
STPP + VP	58.99 ± 1.19aA	56.14 ± 0.59bA	54.62 ± 0.46bA	48.42 ± 1.17cA	45.43 ± 0.10dA
TSPP + STPP + VP	58.99 ± 1.19aA	52.30 ± 0.54bB	50.62 ± 0.31bB	43.31 ± 0.66cB	40.67 ± 0.25dC
C^*_{ab}					
Control	3.56 ± 0.09aA	3.24 ± 0.02bC	2.37 ± 0.06cC	2.29 ± 0.00cC	1.73 ± 0.18dB
VP	3.56 ± 0.09aA	3.41 ± 0.03aB	3.05 ± 0.04bB	2.77 ± 0.07cB	2.04 ± 0.08dB
TSPP + VP	3.56 ± 0.09aA	3.52 ± 0.08aAB	3.05 ± 0.12bB	2.81 ± 0.07bcB	2.59 ± 0.05cA
STPP + VP	3.56 ± 0.09abA	3.64 ± 0.04aA	3.32 ± 0.06bcA	3.18 ± 0.05cA	2.70 ± 0.14dA
TSPP + STPP + VP	3.56 ± 0.09aA	3.53 ± 0.02aAB	3.31 ± 0.05abA	3.19 ± 0.06bA	2.66 ± 0.09cA
$H°_{ab}$					
Control	1.32 ± 0.07abA	1.32 ± 0.00aA	1.24 ± 0.00abB	1.19 ± 0.01bAB	0.52 ± 0.03cD
VP	1.32 ± 0.07aA	1.39 ± 0.07aA	1.22 ± 0.01abB	1.08 ± 0.07bcB	0.92 ± 0.04cC
TSPP + VP	1.32 ± 0.07aA	1.29 ± 0.00aA	1.24 ± 0.01aB	1.21 ± 0.00abA	1.13 ± 0.00bB
STPP + VP	1.32 ± 0.07aA	1.32 ± 0.00aA	1.28 ± 0.00aA	1.24 ± 0.00aA	1.22 ± 0.01aA
TSPP + STPP + VP	1.32 ± 0.07aA	1.31 ± 0.00aA	1.29 ± 0.00aA	1.25 ± 0.01abA	1.15 ± 0.01bAB

Mean values and standard errors from the three replicates are presented

The different capital letters in same columns within the same storage time indicate the significant differences ($P < 0.05$)

The different small letters in same rows within the same treatment indicate the significant differences ($P < 0.05$)

decreased in all the samples during storage ($P < 0.05$). Texture analyses results during storage for *A. fasciata* fillets are shown in Table 4. Hardness was found to be decreased in all the samples during storage ($P < 0.05$). In samples treated with polyphosphates and control, hardness at first was 23.14 N, but gradually over time, hardness of fillet fell in all samples. This result is in accordance with those obtained by Alasalvar et al. [1] using *Sparus aurata* fillet, Kilinc et al. [16] using *Oncorhynchus mykiss* fillet, Hernandez et al. [13] using *Argyrosomus regius* fillet and Etemadian et al. [5] on *Rutilus frisii kutum* studies. Reduction in fillet hardness is probably due to the weakened endomysium as part of the connective tissue and also due to degradation in the Z-line of myofibrils [27] and is also due to increase in microbial load, that make the muscle softer and less elastic. Our results indicated that the denaturation (aggregation and/or hydrolysis) that could occur in the myofibrillar protein of *A. fasciata* fillets during the storage period affected its texture in accordance with the hardness, gumminess and chewiness data. At the end of storage, the hardness of the treated samples with phosphate was significantly higher than that of the control sample ($P < 0.05$). It was obvious that the texture of the polyphosphate treated fillets was better kept than that of the control sample during storage, showing that these TSPP and STPP somehow could reduce the action of endogenous enzymes and microbial activity in fillets (Table 1), the former consisted of collagens, cathepsins, and calpains, resulting the degradation of proteins, and the later thereafter was accelerated along with the breakdown of protein.

Table 4 Texture changes of *Aurigequula fasciata* fillets treated with polyphosphates during refrigerated storage

Days of storage	0	4	8	12	16
Hardness (N)					
Control	23.14 ± 0.42^{aA}	20.32 ± 0.60^{Bb}	20.40 ± 1.54^{bA}	15.00 ± 0.23^{cC}	14.23 ± 0.63^{cC}
VP	23.14 ± 0.42^{aA}	22.48 ± 0.60^{abA}	21.39 ± 0.57^{bA}	18.31 ± 0.03^{cB}	16.72 ± 0.32^{dB}
TSPP + VP	23.14 ± 0.42^{aA}	22.05 ± 0.32^{abAB}	21.82 ± 0.36^{bA}	19.27 ± 0.51^{cAB}	18.73 ± 0.26^{cA}
STPP + VP	23.14 ± 0.42^{aA}	22.21 ± 0.71^{abAB}	21.21 ± 0.57^{bcA}	19.68 ± 0.22^{cdA}	19.38 ± 0.54^{dA}
TSPP + STPP + VP	23.14 ± 0.42^{aA}	22.47 ± 0.60^{bA}	20.83 ± 0.39^{cA}	18.95 ± 0.30^{dAB}	18.72 ± 0.30^{dA}
Cohesiveness					
Control	0.47 ± 0.02^{abA}	0.49 ± 0.00^{aA}	0.43 ± 0.00^{bcB}	0.42 ± 0.00^{cA}	0.41 ± 0.00^{cB}
VP	0.47 ± 0.02^{abA}	0.49 ± 0.01^{aA}	0.44 ± 0.00^{abAB}	0.45 ± 0.01^{abA}	0.42 ± 0.00^{bAB}
TSPP + VP	0.47 ± 0.02^{abA}	0.49 ± 0.00^{aA}	0.45 ± 0.01^{abcAB}	0.43 ± 0.00^{bcA}	0.42 ± 0.00^{cAB}
STPP + VP	0.47 ± 0.02^{abA}	0.48 ± 0.01^{aA}	0.46 ± 0.00^{abAB}	0.43 ± 0.01^{bA}	0.43 ± 0.00^{bA}
TSPP + STPP + VP	0.47 ± 0.02^{aA}	0.46 ± 0.02^{aA}	0.47 ± 0.00^{aA}	0.43 ± 0.00^{aA}	0.42 ± 0.00^{aAB}
Springiness (mm)					
Control	1.28 ± 0.09^{aA}	1.13 ± 0.04^{abB}	1.08 ± 0.04^{bcB}	0.95 ± 0.01^{cdB}	0.91 ± 0.00^{dA}
VP	1.28 ± 0.09^{aA}	1.18 ± 0.01^{abAB}	1.14 ± 0.01^{abAB}	1.05 ± 0.02^{bcA}	0.95 ± 0.01^{cA}
TSPP + VP	1.28 ± 0.09^{aA}	1.28 ± 0.02^{aA}	1.15 ± 0.01^{abAB}	1.06 ± 0.02^{bcA}	0.95 ± 0.01^{cA}
STPP + VP	1.28 ± 0.09^{aA}	1.27 ± 0.03^{aA}	1.18 ± 0.01^{abA}	1.08 ± 0.01^{abA}	0.73 ± 0.30^{bA}
TSPP + STPP + VP	1.28 ± 0.09^{aA}	1.21 ± 0.02^{abAB}	1.15 ± 0.01^{abAB}	1.06 ± 0.02^{bcA}	0.95 ± 0.01^{cA}
Chewiness (N mm)					
Control	14.14 ± 1.60^{aA}	11.39 ± 0.87^{abB}	9.64 ± 0.83^{bB}	6.09 ± 0.13^{cB}	5.34 ± 0.20^{cA}
VP	14.14 ± 1.60^{aA}	13.06 ± 0.46^{abAB}	10.86 ± 0.14^{bcAB}	8.64 ± 0.39^{cdA}	6.74 ± 0.28^{dA}
TSPP + VP	14.14 ± 1.60^{aA}	13.82 ± 0.19^{abA}	11.39 ± 0.29^{abA}	8.86 ± 0.51^{cA}	7.55 ± 0.24^{cA}
STPP + VP	14.14 ± 1.60^{aA}	13.69 ± 0.41^{abA}	11.67 ± 0.43^{abA}	9.19 ± 0.30^{bcA}	6.22 ± 2.64^{cA}
TSPP + STPP + VP	14.14 ± 1.60^{aA}	12.76 ± 0.89^{abAB}	11.28 ± 0.20^{bcA}	8.69 ± 0.12^{cdA}	7.55 ± 0.21^{dA}
Adhesiveness (N)					
Control	-1.29 ± 0.12^{cA}	-0.93 ± 0.00^{bA}	-0.84 ± 0.01^{bB}	-0.76 ± 0.01^{abB}	-0.64 ± 0.02^{aB}
VP	-1.29 ± 0.12^{cA}	-0.79 ± 0.01^{bA}	-0.69 ± 0.05^{abA}	-0.54 ± 0.01^{aA}	-0.51 ± 0.00^{aA}
TSPP + VP	-1.29 ± 0.12^{bA}	-0.20 ± 0.51^{aA}	-0.68 ± 0.01^{abA}	-0.58 ± 0.03^{abA}	-0.52 ± 0.00^{abA}
STPP + VP	-1.29 ± 0.12^{cA}	-0.75 ± 0.01^{bA}	-0.64 ± 0.01^{abA}	-0.57 ± 0.02^{abA}	-0.53 ± 0.01^{aA}
TSPP + STPP + VP	-1.29 ± 0.12^{cA}	-0.76 ± 0.00^{bA}	-0.70 ± 0.01^{abA}	-0.57 ± 0.02^{abA}	-0.54 ± 0.02^{aA}
Gumminess (N)					
Control	11.02 ± 0.62^{aA}	10.03 ± 0.41^{abA}	8.90 ± 0.69^{bA}	6.40 ± 0.15^{cB}	5.87 ± 0.26^{cC}
VP	11.02 ± 0.62^{aA}	11.00 ± 0.27^{aA}	9.47 ± 0.14^{bA}	8.23 ± 0.30^{cA}	7.07 ± 0.20^{dB}
TSPP + VP	11.02 ± 0.62^{aA}	10.80 ± 0.28^{aA}	9.88 ± 0.33^{aA}	8.35 ± 0.32^{bA}	7.92 ± 0.17^{bA}
STPP + VP	11.02 ± 0.62^{aA}	10.80 ± 0.36^{aA}	9.89 ± 0.36^{aA}	8.45 ± 0.16^{bA}	8.33 ± 0.30^{bA}
TSPP + STPP + VP	11.02 ± 0.62^{aA}	10.47 ± 0.54^{aA}	9.79 ± 0.29^{aA}	8.20 ± 0.06^{bA}	7.92 ± 0.10^{bA}

Mean values and standard errors from the three replicates are presented

The different capital letters in same columns within the same storage time indicate the significant differences ($P < 0.05$)

The different small letters in same rows within the same treatment indicate the significant differences ($P < 0.05$)

For hardness, cohesiveness, springiness, chewiness, adhesiveness and gumminess of *A. fasciata* fillets, there were no significant differences ($P > 0.05$) between the type of phosphates. However, there were significant differences between fillets treated with polyphosphates, specially STTP and TSPP treatments and the control sample from the eighth day until the end of storage period in chewiness and gumminess.

Effect of Polyphosphate Pretreatment on Sensory Changes

Fresh *A. fasciata* fillets were generally considered to possess very high acceptability. Sensory attributes of fish were divided into 2 elements, whose preference levels were scored from 1 to 5, the higher preference level, the higher element score. All samples started with score of 5. Upon

Table 5 Sensory changes of *Aurigequula fasciata* fillets treated with polyphosphates during refrigerated storage

Days of storage	0	4	8	12	18
Odor					
Control	5.00 ± 0.00^{aA}	4.42 ± 0.29^{abA}	4.00 ± 0.21^{bB}	2.00 ± 0.30^{cB}	1.14 ± 0.14^{dB}
VP	5.00 ± 0.00^{aA}	4.75 ± 0.16^{aA}	4.25 ± 0.16^{bAB}	3.87 ± 0.22^{bA}	2.50 ± 0.18^{cA}
TSPP + VP	5.00 ± 0.00^{aA}	4.85 ± 0.14^{aA}	4.71 ± 0.18^{aA}	3.64 ± 0.41^{bA}	2.64 ± 0.41^{cA}
STPP + VP	5.00 ± 0.00^{aA}	4.85 ± 0.14^{aA}	4.57 ± 0.20^{abAB}	3.85 ± 0.45^{bA}	2.85 ± 0.45^{cA}
TSPP + STPP + VP	5.00 ± 0.00^{aA}	4.85 ± 0.14^{aAs}	4.57 ± 0.20^{aAB}	3.71 ± 0.28^{bA}	2.71 ± 0.28^{cA}
Flavor					
Control	5.00 ± 0.00^{aA}	4.71 ± 0.18^{aA}	3.71 ± 0.28^{aB}	2.92 ± 0.35^{bA}	1.57 ± 0.20^{cB}
VP	5.00 ± 0.00^{aA}	4.78 ± 0.14^{aA}	4.57 ± 0.20^{aA}	3.57 ± 0.29^{bA}	2.64 ± 0.17^{cA}
TSPP + VP	5.00 ± 0.00^{aA}	4.85 ± 0.14^{aA}	4.14 ± 0.14^{bAB}	3.35 ± 0.28^{cA}	2.35 ± 0.28^{dA}
STPP + VP	5.00 ± 0.00^{aA}	4.71 ± 0.18^{aA}	4.71 ± 0.18^{aA}	3.85 ± 0.28^{bA}	2.85 ± 0.28^{cA}
TSPP + STPP + VP	5.00 ± 0.00^{aA}	4.71 ± 0.18^{aA}	4.54 ± 0.19^{aA}	3.64 ± 0.28^{bA}	2.64 ± 0.28^{cA}

Mean values and standard errors from the three replicates are presented

The different capital letters in same columns within the same storage time indicate the significant differences ($P < 0.05$)

The different small letters in same rows within the same treatment indicate the significant differences ($P < 0.05$)

the judgment made by the members of a sensory panel, odor and flavor were given unacceptable scores by the 16th day. On the other hand, the sensory scores of fillets treated with polyphosphate stored under VP were in the range of 2.35–2.85, after 16 days of storage. Among treatments, the highest score was recorded for the samples treated with polyphosphate. The result of the sensory evaluation was correlated with the microbial and chemical analyses (Tables 1, 2). The results of the sensory evaluation (odor and flavor) of cooked *A. fasciata* fillets are presented in Table 5. The sensory evaluation results showed that odor and flavor scores decreased with increasing storage time. For control samples, the deterioration occurred after 4 days of storage as evidenced by strong fishy and putrid odor. Also the deterioration in favor occurred after 8 days during storage at refrigerator. Odor and taste showed a similar pattern of decreasing acceptability. The antioxidant and antimicrobial effects of polyphosphate has been shown to prolong the shelf life of fish by 16 days as compared to the control sample. The result suggested that the polyphosphate was more effective in maintaining the quality the *A. fasciata* fillets.

Effect of Phosphate Pretreatment on SDS-PAGE Pattern

Changes in fish protein affect the functional and textural characteristics [47], so such changes and the reduction of myofibrillar proteins during storage in refrigerator is evidence of proteolytic activity in the muscle [3]. Major

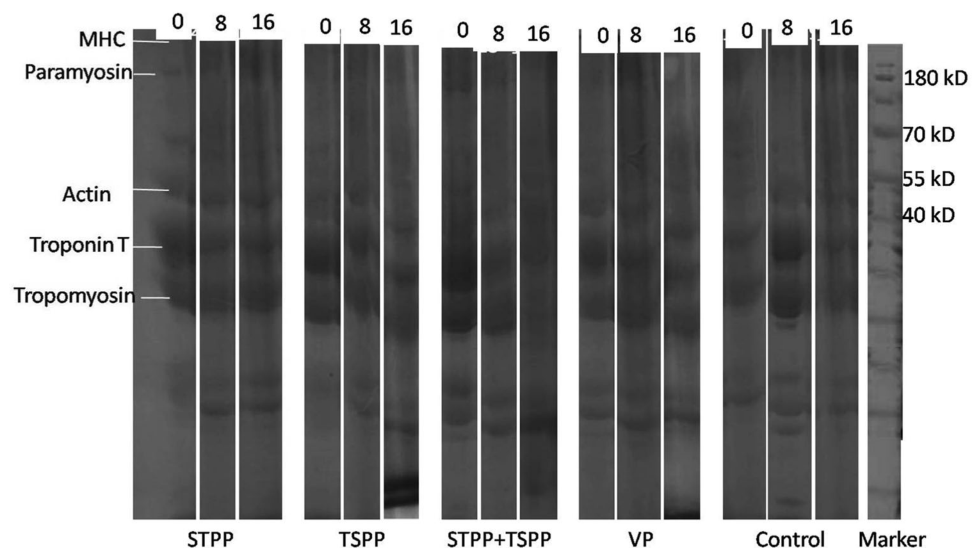

Fig. 1 Electrophoresis patterns of SDS-PAGE gels of actomyosin from *Aurigequula fasciata* fillets during refrigerated storage. *MHC* Myosin heavy chain

proteins of muscle are myosin and actin, which contribute to most of the functional properties [30]. Thus, a reduction in myofibrillar proteins during storage are evidence of the proteolytic activity in the muscle. As shown in Fig. 1, five major protein bands, which correspond to myosin heavy chain (MHC), paramyosin, actin, troponin T and tropomyosin bands, were observed in the *A. fasciata* muscle. The band intensity of these proteins in all of the treatments decreased with increased storage time. After 16 days of storage, the protein band intensities of MHC, paramyosin, actin, troponin T and tropomyosin in the samples treated with phosphate was markedly higher than those obtained for the samples without phosphate. The molecules of phosphate may bind to or associate with protein molecules at one of the functional groups through either ionic bonds or hydrogen bonds. Thus, each protein molecule is coated by the phosphate, and this coating inhibits proteolytic changes. Etemadian et al. [6] showed that significant difference in protein band intensity of myosin, actin and tropomyosin *Rutilus frisii kutum* among treatment were not observed until the end of storage.

Conclusion

The TBA and FFA values remained significantly lower, compared to control, over the same period of storage in the polyphosphate treatments. These results indicated that the rancidity of lipids was retard in the treated fish. Thus, flavor and odor were improved in samples treated with polyphosphate. The results of the present study revealed that there is a direct relationship between fat oxidation and the loss of color in refrigerated *A. fasciata* fillets. Addition of polyphosphate especially STPP to vacuum- packed *A. fasciata* fillets has a profound effect on sensory quality, TVBN value, pH, WHC and SH and microbiological growth, and improve textural qualities. Thus, treatments with polyphosphates are an alternative way to improve the shelf life of fish.

References

1. Alasalvar C, Taylor KD, Öksüz A, Garthwaite T, Alexis MN, Grigorakis K (2001) Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. Food Chem 72:33–40
2. Ayala MD, Santaella M, Martínez G, Periago MJ, Blanco A, Vázquez JM, Albors OL (2011) Muscle tissue structure and flesh texture in gilthead sea bream *Sparus aurata* L., fillets preserved by refrigeration and by vacuum packaging. LWT Food Sci Technol 44:1098–1106
3. Benjakul S, Seymour TA, Morrissey MT, An H (1997) Physicochemical changes in pacific whiting muscle proteins during iced storage. J Food Sci 62:729–733
4. Debevere J, Boskou G (1996) Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. J Food Microbiol 31:221–229
5. Etemadian Y, Shabanpour B, Sadeghi Mahoonak AR, Shabani A, Alami M (2011) Cryoprotective effects of polyphosphates on *Rutilus kutum kutum* fillets during ice storage. Food Chem 129:1544–1551
6. Etemadian Y, Shabanpour B, Sadeghi Mahoonak A, Shabani A (2012) Combination effect of phosphate and vacuum packaging on quality parameters of *Rutilus frisii kutum* fillets in ice. Food Res Int 45:9–16
7. Etemadian Y, Shabanpour B, Sadeghi Mahoonak A, Shabani A (2013) Effects of phosphate dip treatment on quality of Rutilus frisii kutum fillets during ice storage. J Aquat Food Prod Technol 22:449–459
8. Ginés R, Valimrsdottir T, Sveinsdottir K, Thorarense H (2004) Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). Food Qual Prefer 15:177–185
9. Goulas AE, Kontominas MG (2005) Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. Food Chem 93:511–520
10. Gram L, Huss H (1996) Microbiological spoilage of fish and fish products. Food Microbiol 33:121–137
11. Gram L, Dalgaard P (2002) Fish spoilage bacteria—problems and solutions. Curr Opin Biotechnol 13:262–266
12. Hayakawa S, Nakai S (1985) Contribution of hydrophobicity, net charge and sulfhydryl groups to thermal properties of ovalbumin. Can Inst Food Sci Technol J 18:290–295
13. Hernandez MD, Lopez MB, Alvarez A, Ferrandini E, Garcia Garcia B, Garrido MD (2009) Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem 114:237–245
14. ICMSF (1986) Microorganisms in foods. The international commission on microbiological specifications for foods of the international union of biological societies. Blackwell Scientific Publications, Oxford
15. Jeon CO, Kamil YVA, Shahidi F (2002) Chitosan as an edible invisible film for quality preservation of Herring and Atlantic Cod. J Agric Food Chem 50:5167–5178
16. Kilinc B, Cakil S, Csdun A, Sen B (2009) Effect of phosphate dip treatments on chemical, microbiological, color, textural, and sensory changes of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage. J Food Product Technol 18:108–119
17. Kilincceker O, Dogan IS, Kucukoner E (2009) Effect of edible coatings on the quality of frozen fish fillets. LWT Food Sci Technol 42:868–873
18. Knipe CL (2004) Use of phosphates in meat products. September 30 to October 2, Meat industry research conference, Nashville
19. Knabel SJ, Walker HW, Hartman PA (1991) Inhibition of *Aspergillus flavus* and selected Gram-positive bacteria by chelation of essential metal cations by polyphosphates. J Food Prot 54:360–365
20. Kong B, Guo Y, Xia X, Liu Q, Li Y, Chen H (2013) Cryoprotectants reduce protein oxidation and structure deterioration induced by freeze- thaw cycles in common carp (*Cyprinus carpio*) surimi. Food Biophys 8:104–111
21. Laemmli UK (1970) Cleavage of structural proteins during a assembly of head of bacteriophage T4. Nature 227:680–685
22. Leelapongwattana K, Benjakul S, Visessanguan W, Howell NK (2005) Physicochemical and biochemical changes during frozen storage of minced flesh of lizardfish (*Saurida* micropectoralis). Food Chem 90:141–150
23. Lindsay RC (1996) Food additives. In: Fennema OR (ed) Food chemistry. Marcel Dekker, NewYork, pp 767–823

24. Liu Q, Kong B, Han J, Chen Q, He X (2014) Effects of superchilling and cryoprotectants on the quality of common carp (*Cyprinus carpio*) surimi: microbial growth oxidation, and physicochemical properties. LWT Food Sci Technol 57:165–171
25. Lopez-Caballero ME, Goncalves A, Nunes ML (2002) Effect of CO2/O2-containing modified atmospheres on packed deepwater pink shrimp (*Parapenaeus* longirostris). Eur Food Res Technol 214:192–197
26. Ludorff W, Meyer V (1973) Fische und fischerzeugnisse. Paul parey verlag, Berlin, pp 95–111, 176–269
27. Masniyom P, Soottawat B, Visessanguan W (2005) Combination effect of phosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices. J Food Sci Technol 38:745–756
28. Matlock RG, Terrel RN, Savell JW, Rhee KS, Dutson TR (1984) Factors affecting properties of raw-frozen pork sausage patties made with various NaCl/phosphate combination. J Food Sci 49:1363–1366
29. Mikkelsen A, Jun H, Skibsted LH (1992) Effects of polyphosphates on reactions of metmyoglobin related to oxidative changes in meat products. Zeitschrift für Lebensmittel- Untersuchung und- Forschung 194:317–321
30. Mohan M, Ramachandran D, Sankar TV, Anandan R (2008) Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*). J Food Chem 106:451–457
31. Molins RA (1991) Antimicrobial uses of food preservatives. Phosphate in food. CRC Press Inc, Boca Raton
32. Montecchia CL, Roura SI, Roldan H, Perezborla O, Crupkin M (1997) Biochemical and physicochemical properties of actomyosin from frozen pre and post-spawned hake. J Food Sci 62:491–495
33. Nirmal NP, Benjakul S (2011) Retardation of quality changes of Pacific White shrimp by green tea extract treatment and modified atmosphere packaging during refrigerated storage. Int J Food Microbiol 149:247–253
34. Offer G, Trinick T (1983) On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. J Meat Sci 8:245–281
35. Olsson GB, Ofstad R, Lodemelc JB, Olsen RL (2003) Changes in waterholding capacity of halibut muscle during cold storage. Lebensmittel- Wissenschaft und -Technologie 36:771–778
36. Rostamzad H, Shabanpour B, Shabani A, Shahiri H (2011) Enhancement of the storage quality of frozen Persian sturgeon fillets by using of ascorbic acid. Int Food Res J 18:109–116
37. Sahoo J, Kumar N (2005) Quality of vacuum packaged muscle foods stored under frozen conditions: a review. J Food Sci Technol 42:209–213
38. Sallam KI (2007) Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control 18:566–575
39. Shahidi F, Rubin LJ, Diosady LL, Kassam N, Li Sui Fong JC (1986) Effect of sequestering agents on lipid oxidation in cooked meats. Food Chem 21:145
40. Shelef LA, Wang Z-L, Udeogu AC (1990) Growth of *Staphylococcus aureus* and enterotoxin A production in foods containing polyphosphates. J Food Saf 10:201–208
41. Shakila R, Jeyasekaran G, Vijayalakshmi S (2005) Effect of vacuum packaging on the quality characteristics of seer fish (*Scomberomorus commersonii*) chunks during refrigerated storage. J Food Sci Technol 42:438–443
42. Sikorski ZE, Kolakowska A, Burt JR (1990) Post harvest biochemical and microbial changes seafood. In: Sikorski ZE (ed) Resources nutritional composition and preservation. CRC Press Inc, Boca Raton, pp 55–75
43. Siripatrawan U, Noipha S (2012) Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocoll 27:102–108
44. Smith LA, Simmons SL, McKeith FK, Bechtel PL, Brady PL (1984) Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. J Food Sci 49:1636–1639
45. Sofos JN (1986) Use of phosphate in low-sodium meat products. J Food Technol 40(3):52–69
46. Stammen K, Gerdes D, Caporaso F (1990) Modified atmosphere packaging of seafood. Crit Rev Food Sci Nutr 29:301–331
47. Thippeswamy K, Ammu K, Joseph J (2002) Biochemical changes during iced storage of indian milk fish (*Chanos chanos*). J Food Sci Technol 39:144–148
48. Trout GR, Schmidt GR (1984) Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. J Food Sci 49:687–694
49. Thorarinsdottir KA, Gudmundsdottir G, Arason S, Thorkelsson G, Kristbergsson K (2004) Effects of added salt, phosphates, and proteins on the chemical and physicochemical characteristics of frozen cod (*Gadus morhua*) fillets. J Food Sci 69:144–152
50. Van Wazer JR, Callis CF (1958) Metal complexing by phosphates. Chem Rev 58:1011–1046
51. Wagner MK (1986) Phosphates as antibotulinal agents in cured meats: a review. J Food Prot 49:482–487
52. Wanger MK, Busta FF (1985) Inhibition of Clostridium botulinum 52A toxicity and protease activity by sodium acid pyrophosphate in meat systems. Appl Environ Microbiol 50:16–20
53. Woyewoda AD, Shaw SJ, Ke PJ, Burns BG (1986) Recommended laboratory methods for assessment of fish quality. Can Techn Rep Fish Aquat Sci 1448:143–149
54. Zhuang H, Savage EM, Smith DP, Berrang ME (2008) Effect of dry-air chilling on Warner-Bratzler shear force and water-holding capacity of broiler breast meat deboned four hours postmortem. J Food Poult Sci 7(8):743–748