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ORIGINAL ARTICLE

Combined effects of lactoperoxidase system-whey protein coating and modified atmosphere packaging on the microbiological, chemical and sensory attributes of Pike-Perch fillets

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Abstract The present study aimed to evaluate the efficacy of lactoperoxidase system-whey protein coating and modified atmosphere packaging (60% CO_2, 30% N_2, 10% O_2) combination (LPOS + WPS + MAP) on the microbiological, chemical and sensory specifications of Pike-Perch (*Sander Lucioperca*, Linnaeus 1758) fillets. The highest bacterial count was observed in the fish fillets packaged with whey protein coating solutions (WPS) in compare with the other groups. Combination of WPS + LPOS and MAP packaging could significantly inhibit bacterial growth. Total volatile basic nitrogen (TVB-N), as a quality index of flesh, had strong correlation (r = 0.98–0.99) with microbial load, so that the highest and the lowest TVB-N values were observed in WPS and WPS + LPOS + MAP batches, respectively. Assessments of thiobarbituric acid reactive substances index showed that incorporation of LPOS with WPS or MAP did not have remarkable effect on lipid oxidation, but combined effect of MAP and WPS + LPOS on reducing fat oxidation was significant. The pH values in WPS + LPOS, WPS + MAP and WPS + LPOS + MAP were significantly lower than WPS. Sensory evaluations indicated that LPOS + WPS + MAP kept Pike-Perch fillets at high sensory acceptability for at least 16 days in refrigerated temperature. In conclusion, combination of MAP and WPS + LPOS showed synergistic effects on shelf-life extension of Pike-Perch fillets under refrigerated storage.

Keywords Whey protein · Modified atmosphere packaging · Lactoperoxidase system · Pike-Perch · Shelf-life

Introduction

Hurdle technology has been defined as integration of preservative methods to improve the microbial stability and augmenting sensory qualities and their nutritional and economic properties (Leistner 2000). Modified atmosphere packaging (MAP) is a kind of food packaging method with shelf life extension potential in seafood (Kostaki et al. 2009). Typical gases were usually used in mixtures for MAP application including oxygen, nitrogen and carbon dioxide (Kostaki et al. 2009; Messina et al. 2015). Carbon dioxide acts as an antimicrobial agent, and it also inhibits the growth of microorganisms during the logarithmic phase and extends the lag phase. Oxygen promotes the growth of aerobic bacteria and oxidative rancidity in fatty fish and it can strictly inhibit the growth of anaerobic microorganisms. Nitrogen is an inactive and tasteless gas which can delay oxidative rancidity and prevent the growth of aerobic microorganisms by displacing oxygen in the package (Mastromatteo et al. 2010). Active packaging refers to another form of packaging with antioxidant, antimicrobial, oxygen scavenging, flavoring and other properties (Quintavalla and Vicini 2002). Using whey protein incorporated with antimicrobial agents such as lactoperoxidase (LPO) is a novel suggested active packaging (Shokri et al. 2015;

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Yener et al. 2009). Whey protein, as a cheese manufacturing by-product, can be used as a biodegradable and edible film for packaging, with an excellent oxygen, aroma and oil barrier properties, after heating and adding a plasticizer (Gennadios 2002). LPO is an enzyme in milk, saliva and tear secreted from mammary, salivary and lacrimal glands of mammals. This enzyme shows bactericidal and bacteriostatic effects on gram negative and gram positive bacteria, respectively. LPO was also approved to have antifungal and antiviral activities. (Yener et al. 2009). LPO catalyzes the oxidation of thiocyanate (SCN^-) and produces antimicrobial intermediates such as hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN).These compounds can inhibit bacterial growth by the oxidation of sulphydryl (^-SH) groups in bacterial enzyme systems and proteins (Cissé et al. 2012). Integration of different methods of packaging with natural preservatives can be investigated as an appropriate food storage idea. Since consumer's are interesting to using minimum processed foodstuffs containing bio-preservatives for extended food shelf-life, thus this study was conducted to determine the effects of MAP combined with whey protein coating incorporated with lactoperoxidase system (LPOS) which consists of the LPO enzyme, thiocyanate and hydrogen peroxide in maintaining shelf stability of Pike-Perch (*Sander Lucioperca*, Linnaeus 1758) fillets stored under refrigeration (4 °C).

Materials and methods

Materials

Whey protein (80% protein) was purchased from DMV Co. (Veghel, Netherlands). Lactoperoxidase (LPO) (120 U/mg, Sigma-Aldrich), glucose oxidase (GO; Sigma-Aldrich) (235 U/mg), D-(α)-glucose (Glu, Sigma-Aldrich), potassium thiocyanate (KSCN, Bioserae, France) and H_2O_2 (Merck, Germany) were also prepared. Glycerol (Merck, Germany) was also used as plasticizer for coating flexibility.

LPOS preparation

In this study, the concentration ratio of the LPO, GO, Glu, KSCN, and H_2O_2 for LPOS, was 1.00; 0.35; 108.70; 1.09 and 2.17, respectively. The concentration ratios were adjusted on the basis of 15.5 mg LPO. The components were dissolved separately in 50-mL phosphate buffer (pH 6.2, Sigma-Aldrich) (Shokri et al. 2015; Cissé et al. 2012; Min et al. 2005). The obtained solution was incubated at 23 ± 2 °C for 24 h, under shaking at 160 rev min^{-1} to increase its antimicrobial activity (Min et al. 2007).

Preparation of fish fillets

Pike-Perch (*Sander Lucioperca*, Linnaeus 1758) with an average weight of 500 ± 15 g was purchased at a local market at September 2015. The fish were transferred in insulated foam polystyrene box in ice to the laboratory. They were then de-headed, eviscerated and filleted by hand and finally washed by tap water. Two fillets were obtained from each fish and stored at 4 °C for 16 days after treatment.

Whey protein solutions preparation and treatment of samples

Whey protein solutions (WPS) were prepared as described by Min et al. (2005). Ten grams of whey protein isolate (WPI) were mixed with 100 mL of distilled water by gradual stirring the WPI in distilled water for 30 min at ambient temperature. To be WPS with plasticizer, glycerol, equal to WPI weight, was added and mixed. This solution then was stirred and heated by a hot stirrer at 90 °C until the dispersions became clear. The prepared WPS was divided into two parts; a part with 2.5% (v/v) LPOS and the other part without LPOS. The fillets were randomly divided into two groups, and each group was immersed and coated by WPS and WPS + LPOS twice each 60 s. The ratio of samples to the coating solution was 1:2. After drainage, each of these groups was divided into two groups that each of them was packaged by MAP, as would be stated (Kostaki et al. 2009). Eventually, the groups were WPS, WPS + LPOS, WPS + MAP and WPS + LPOS + MAP. All groups were stored at 4 ± 1 °C for 16 days.

MAP packaging

The samples were packaged in polyethylene/polyamide bag with 85 µm thickness, using a Boxer 42 vacuum packaging machine (HENKLMAN, Netherlands) and a gas mixture machine (CRYOTECH Eng., M05, UK) for a mixture of 60% CO_2, 30% N_2, 10% O_2 and the bags were immediately sealed. The final gas mixture/fish fillets ratio was about 2:1 (v/w) for MAP conditions.

Microbial assessment

Ten grams of fillets were aseptically taken and mixed with 90 mL of 0.1% peptone water and also homogenized with a stomacher (STOMACHER®, UK) for 1 min, to determine bacterial count. Further dilutions were prepared by this dilution. For bacterial count, 0.1 ml of each dilution was spread on the surface of appropriate media. Bacterial load was expressed as log CFU/g. For determination of total viable counts (TVC), Nutrient Agar (Merck, Darmstadt,

Germany) were used and counting was done after incubation at 37 °C for 48 h. *Shewanella putrefaciens* and *Pseudomonas fluorescens,* as fish specific spoilage organisms (SSO) (Xu et al. 2012), were enumerated on Iron Agar LYNGBY (Laboratorios Conda, Madrid, Spain) after incubation at 30 °C for 3–4 days, which had been identified by black and white colonies, respectively. Determination of Psychrotrophic bacteria was performed by using King Agar (Merck, Darmstadt, Germany) after incubation at 21 °C for 48 h.

Chemical analysis

Determination of pH

The pH values were measured by using a pH meter (MetrohmHerisau®, Switzerland) at ambient temperature. Five grams of each sample was homogenized for 30 s in 25 mL of distilled water and pH of this solution was determined.

Analysis of thiobarbituric acid reactive substances (TBARS)

Fish muscle (10 g) was homogenized with 97.5 mL distilled water and 2.5 mL of 4 N HCl solution for 2 min, and then some boiling stones and four drop of silicon, as antifoam, were added and distilled until obtaining 50 ml solution. Five milliliters of thiobarbituric acid (TBA) reagent (0.02 M 2-TBA in 90% acetic acid) and 5 mL of the distillate were mixed and heated in a boiling water bath for 35 min and after that, right away cooling under piped water for 1 min. For determination of TBARS index, the absorbance of this mixture was measured at 538 nm against a blank (5 mL of distilled water with 5 mL of TBA reagent). Results were expressed as mg of malondialdehyde/kg fish muscle (Etemadian et al. 2011).

Total volatile basic nitrogen (TVB-N)

For determination of TVB-N, ten grams of fish muscle were blended with 50 mL of distilled water by a Moulinex mixer (Moulinex, France). The mixture was transferred into a 500 mL round bottom flask and after addition of 200 mL of distilled water, 2 g of MgO and then one drop of silicon, as antifoam, was distilled. The distillation recipient was a flask containing 20 mL of 3% aqueous solution of boric acid with an indicator (0.1 g of methyl red and 0.1 g of methylene blue in 100 mL of ethanol). After the collection of 125 mL of distillate, the boric acid solution was titrated with a 0.1 N hydrochloric acid (HCl) solution. The HCl consumption was used for the TVB-N value (mg/100 g fish sample) determination (Goulas and Kontominas 2005).

Sensory analyses

The sensory assessments were performed by a 12 semi-trained panel (from the food hygiene students and laboratory staff) for evaluation the sensory characteristics such as color, odor, texture and overall acceptability of fish fillets. Sensory attributes were scored from 1 to 9 according to a scoring table which described by Erkan (2012). According to the scoring tables, limits of unacceptability, moderate acceptability and high acceptability were indicated the scores 1–3.9, 4–6.9 and 7–9, respectively.

Statistical analyses

Analyses of experimental data were performed with SPSS software (version 18.0 for Window, SPSS, Inc., Chicago, IL, USA). All results were expressed as mean ± standard deviation (n = 3). Data were initially checked for normality by using Shapiro–Wilk's. The homogeneity of variance was tested using the Levene's test. Data was subjected to Duncan's post hoc test and Kruskal–Wallis test to evaluate the significance of differences among mean values. Significance level of differences was set at 5%.

Results and discussion

Microbiological analysis

Bacterial growth curve in Pike-Perch fillets, during 16 days of refrigeration storage (4 °C), is shown in Fig. 1a–d. As it is evident in Fig. 1, bacterial number was significantly ($P < 0.05$) increased during the storage time in all batches and the WPS + LPOS + MAP batch had the lowest bacterial count in all sampling days, which verified the inhibitory effect of lactoperoxidase system (LPOS) and MAP packaging at all bacterial growth curves. The initial TVC was 4.71 log CFU/g in Pike-Perch fillets. This amount exceeded to 7 log CFU/g, the upper acceptable limit for raw fish (Ojagh et al. 2010), in WPS, MAP + LPOS and WPS + MAP in 8, 12 and 16 days of storage, respectively. While TVC of WPS + LPOS + -MAP batch were still less than 7 log CFU/g at the end of the storage period. The main responsible bacterial group for fish flesh spoilage in cold storage conditions are psychrotrophic bacteria (Sallam 2007). In our study the initial number of psychrotrophic bacteria in Pike-Perch fillets was 3.74 log CFU/g. As well as TVC, psychrotrophic bacteria values were lower than 7 log CFU/g in WPS + LPOS + -MAP group until the end of 16 days storage. The psychrotrophic count of WPS + LPOS and WPS + MAP at the

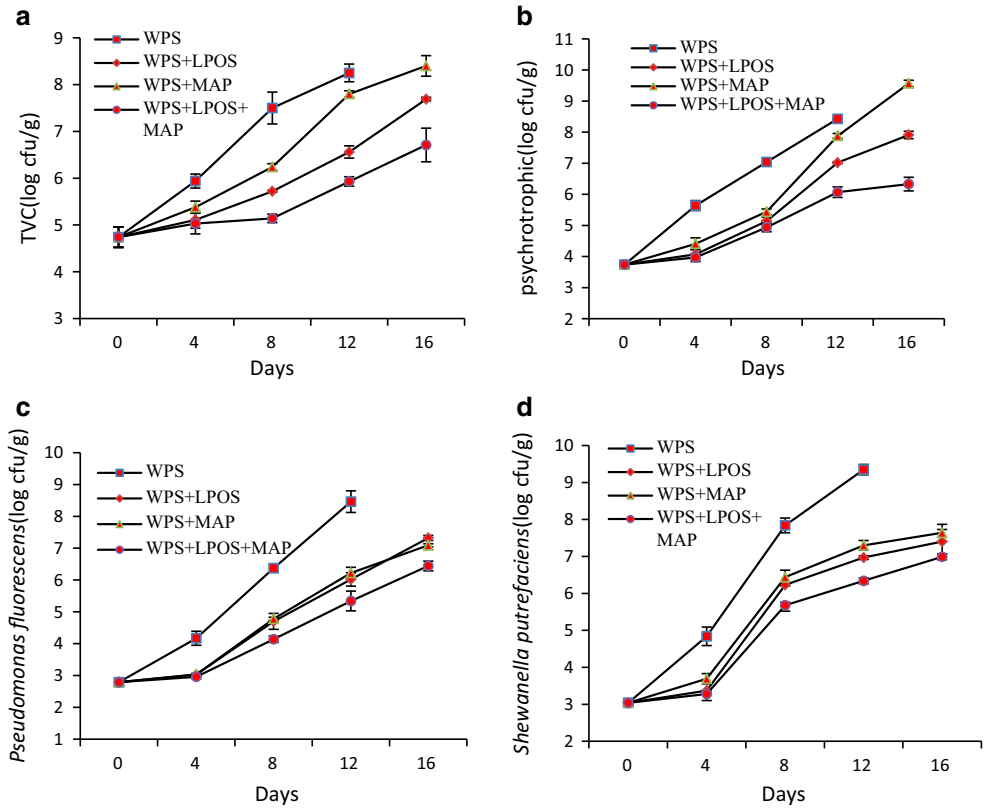

Fig. 1 Bacteriological changes of Pike-Perch fillet during 16 days of refrigerated storage (4 °C). Values were expressed on basis the mean ± SD (n = 3)

8, 12 and 16 days were significantly ($P < 0.05$) lower than WPS treatment in same days. With regard to the *Shewanella putrefaciens* and *Pseudomonas fluorescens* as fish specific spoilage organisms, the initial number of them in day 0 was 3.4 and 2.79 log CFU/g, respectively. As shown in Fig. 1c, d, similar to TVC and psychotropic bacteria, the lowest and the highest number of *Shewanella putrefaciens* and *Pseudomonas fluorescens*, were observed in WPS + LPOS + MAP and WPS, during 16 days storage, respectively. Number of *Pseudomonas fluorescens* reached the value of 7 log cfu/g in 12th day for WPS and 16th day for both WPS + LPOS and WPS + MAP treatments. On the other hand, number of *Shewanella putrefaciens* for WPS, WPS + LPOS and WPS + MAP exceeded the value of upper acceptable microbiological limit for fish (7 log cfu/g) on days 8, 16 and 12 of storage, respectively. Based on our assessment from the *Shewanella putrefaciens* and *Pseudomonas fluorescens* counting, there was no significant difference ($P > 0.05$) between the WPS + LPOS and WPS + MAP batches in all sampling days. The reported TVC, psychrotrophic bacteria, *Shewanella putrefaciens* and *Pseudomonas fluorescens* number for the WPS + LPOS were in agreement with those reported by Shokri et al. (2015) for the inhibitory effect of LPOS incorporated with WPS on bacterial growth in rainbow trout fillets. LPOS by producing intermediate products, with antimicrobial properties, such as hypothiocyanite ($OSCN^-$) and hypothiocyanous acid (HOSCN) can oxidize essential sulphydryl groups in bacterial enzymes and proteins and hence prevent bacterial growth (Kamau et al. 1990). As well as our observations, similar results about the inhibiting the growth of bacteria in stored fish flesh under MAP conditions have been reported by others (Yesudhason et al. 2014; Kostaki et al. 2009; Fernández et al. 2009). The suggested mechanism to prevent the growth of bacteria in MAP packaging is inhibiting the growth of aerobic bacteria by reducing oxygen levels and replacing it with nitrogen and inhibits the growth of microorganisms during the logarithmic phase which extends the lag phase by carbon dioxide (Mastromatteo et al. 2010). On the basis our findings, combination of whey protein coating incorporated with LPOS to MAP packaging may had been synergistic effect on the growth of bacteria.

Chemical analysis

Assessment of total volatile basic nitrogen index

Changes in total volatile basic nitrogen (TVB-N) content, a fish spoilage index (Goulas and Kontominas 2005), of

Pike-Perch fillets are shown in Fig. 2. TVB-N mainly includes trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds which are produced by microbial and enzyme activity in fish flesh (Huss 1995). The statistical analysis of TVB-N revealed strong correlation (r = 0.98–0.99) between the microbial load and the amount of TVB-N in Pike-Perch fillets. The initial TVB-N value of 10.96 mg N/100 g was similar to the values reported for fish flesh (between 5 and 20) by Huss (1988). TVB-N has an increasing trend during 16 days cold storage in all treatments and the highest and the lowest value were observed in WPS and WPS + LPOS + MAP, respectively. TVB-N amount in WPS, WPS + LPOS and WPS +MAP batches exceeded from the proposed upper acceptability limit of 30–35 mg N/100 g (Kykkidou et al. 2009; Huss 1988) on days 8, 16 and 12, respectively, whereas for WPS + LPOS + MAP was 25.81 mg N/100 g at the end of 16 days storage. It is noteworthy that the statistical analysis of the TVB-N data showed significant differences ($P < 0.05$) between all batches in all sampling days. The TVB-N is produced mainly by bacterial decomposition of protein and non-protein nitrogenous compounds in fish flesh. These differences can be attributed to the antimicrobial effect of LPOS and MAP condition, which subsequently less TVB-N is formed by bacterial activity. Similar results about the effectiveness of MAP to inhibit the formation of TVB-N have been reported by Kostaki et al. (2009) and Goulas and Kontominas (2005). The TVB-N data obtained in the LPOS containing batches in this study are in agreement with those reported by Shokri et al. (2015), who noted WPS incorporated with LPOS can delay the formation of TVB-N in rainbow trout fillets.

Assessment of thiobarbituric acid reactive substances values

Thiobarbituric acid reactive substances (TBARS) value considered as an index for lipid oxidation which shows malondialdehyde (MDA) content as secondary product of lipid oxidation. MDA has formed through hydroperoxides, which are the primary reaction product of polyunsaturated fatty acids with oxygen (Fernández et al. 1997). As shown in Fig. 3, at the beginning of the storage period, TBARS value in Pike-Perch fillets was 0.6 mg MDA/kg. Amount of TBARS has an increasing trend at beginning of the storage until day 4 and also after day 8 until end of storage in all batches, which can be due to accumulation of secondary lipid oxidation products (Pezeshk et al. 2011), but TBARS values were decreased between days 4 and 8, which could be attributed to breakdown of the MDA, because of tertiary degradation (Ehsani et al. 2014). This observation was in agreement with previous reports (Bensid et al. 2014; Kostaki et al. 2009; Goulas and Kontominas 2005). Overall the highest TBARS value was belonging to WPS, however, no differences ($P > 0.05$) were observed throughout all sampling days between this treatment and WPS + LPOS group. On the other hand, a statistically significant difference ($P < 0.05$) was observed in TBARS values of the WPS + MAP and WPS + LPOS + MAP compared to other groups. This may probably be related to the reducing oxygen levels in MAP packaging, since oxygen plays a key role in lipid oxidation. Our observation is in agreement with Kostaki et al. (2009) and Goulas and Kontominas (2005). It is important to note that despite of non-significant differences ($P > 0.05$) between WPS + MAP and WPS + LPOS + MAP batches for TBARS, but the sensory evaluations indicated at the point of sensory rejection of WPS + MAP (16th day) the

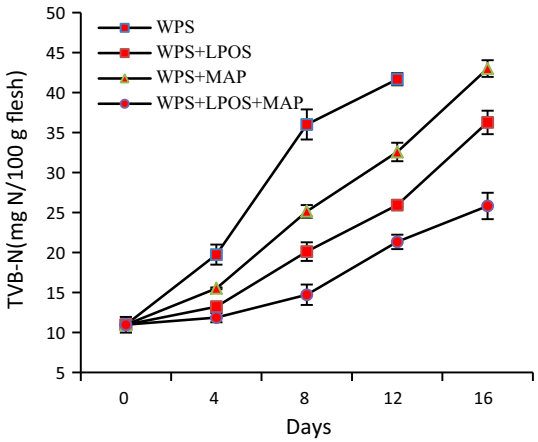

Fig. 2 Changes in total volatile basic nitrogen (TVB-N) of Pike-Perch fillet during 16 days of refrigerated storage (4 °C). Values were expressed on basis the mean ± SD (n = 3)

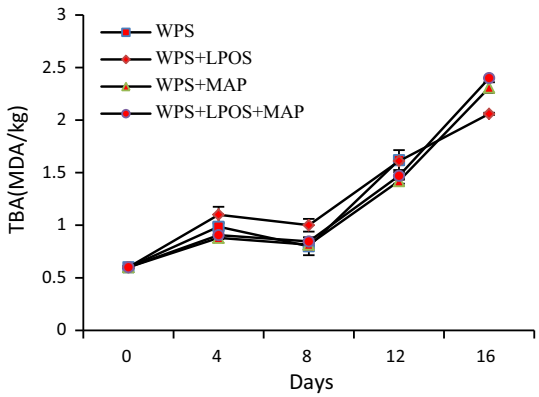

Fig. 3 Changes in thiobarbituric acid reactive substances (TBARS) of Pike-Perch fillet during 16 days of refrigerated storage (4 °C). Values were expressed on basis the mean ± SD (n = 3)

WPS + LPOS + MAP has a high acceptability. As a consequence of these results, it can be concluded that the TBARS values may not give actual rate of lipid oxidation, since MDA can interact with nucleosides, nucleic acid, proteins, amino acids and other ingredients of fish flesh (Aubourg 1993). There have been similar suggestions by Shokri et al. (2015), Bensid et al. (2014) and Kostaki et al. (2009). On the basis of results obtained in present study, LPOS has not significant effect ($P > 0.05$) on TBARS values in LPOS + WPS compared with the WPS, which was in agreement with those reported by Shokri et al. (2015) and Jasour et al. (2015). It has been suggested that TBARS value in perfect quality and good quality fish flesh should be less than 3 and 5 mg MDA/kg, respectively (Kilinc et al. 2009). In the present study, TBARS values for all treatments were lower than 3 mg MDA/kg (maximum 2.31). This could be related to low Pike-Perch fillets fat content (3.6%) (Kostaki et al. 2009).

Assessment of pH

The pH changes of Pike-Perch fillets stored under refrigerated temperature for 16 days are shown in Fig. 4. The initial pH of samples at the first day of storage was 6.88. The pH decreased until day 4 and thereafter had an increasing trend in all samples until the end of storage period. Since the carbohydrate content of fish is very low, production of lactic acid cannot have significant impact on the postmortem reduction of pH (Gram and Huss 1996). The reduction of pH is probably related to dissolution of CO_2 in the fish fillets (Ordóñez et al. 2000). Campos et al. (2005) showed increased pH in fish flesh may be attributed to utilization of amino acids and other low molecular weight compounds by fish spoilage bacteria and production of alkaline ammonia components such as ammonia and trimethylamine. The statistical analysis showed a significant increase ($P < 0.05$) in pH values of WPS compared to other groups, which may be due to dissolution of CO_2 in flesh and production of carbonic acid (Ordóñez et al. 2000) and also antibacterial effect of CO_2 in MAP condition, resulting lower formation of basic amines (Kostaki et al. 2009). Similar results were reported by Latou et al. (2014), Kostaki et al. (2009) and Goulas and Kontominas (2005). Samples content LPOS (WPS + LPOS + MAP and WPS + LPOS) has lower increase in pH compared to the others due to the antimicrobial effect of LPOS. These results are in agreement with Shokri et al. (2015), who reported that LPOS via inhibition of bacterial growth cause lower increase in pH compared with the control group in rainbow trout fillets.

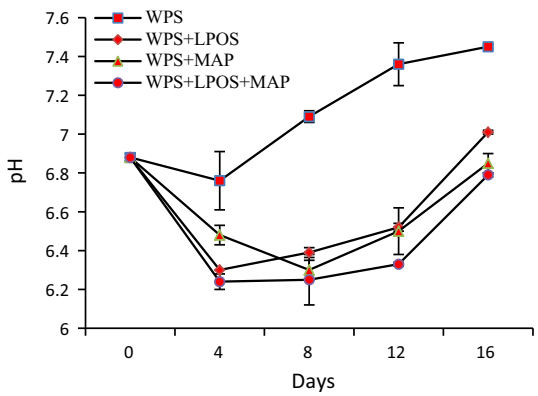

Fig. 4 Changes in pH of Pike-Perch fillet during 16 days of refrigeration storage (4 °C). Values were expressed on basis the mean ± SD (n = 3)

Assessment of sensory attributes

In the present study, odor, color, texture and overall acceptability were used for evaluation of sensory attributes of Pike-Perch fillets. Results showed that the sensory attributes were decreased gradually in all treatments during the storage time (Table 1). Statistical analysis of sensory data showed a good correlation between the sensory analysis and the microbial and TVB-N results. Using a sensory score of 3.9 < as the limits of unacceptability, it was found that WPS batch was rejected at 12th day for all parameters while at the same time WPS + LPOS, WPS + MAP and WPS + MAP + LPOS batches were moderate, moderate and high acceptable, respectively. As shown in Table 1, a statistically significant difference ($P < 0.05$) was observed in sensory attributes of WPS + MAP + LPOS compared to other treatments. It is noteworthy that WPS + LPOS had significant upper scores ($P < 0.05$) as compared WPS + MAP in terms of texture and overall acceptability, which may be attributed to the drip loss of fillets under MAP packaging. As reported by Sivertsvik et al. (2002), the CO_2 dissolution in the aqueous phase of fish fillet, can lead to fall in fish flesh water-holding capacity as a result of reduction in pH values in MAP conditions. The evaluation of sensory attributes indicated that LPOS + WPS + MAP keeps Pike-Perch fillets at high acceptability for at least 16 days storage in refrigerated temperature, while this level of acceptability in WPS,WPS + LPOS and WPS + MAP occurred after 4, 12 and 8 days of storage, respectively. In agreement with our results, Shokri et al. (2015) and Jasour et al. (2015) reported that LPOS in combination with coating materials could significantly extend the shelf-life of fish fillets under refrigerated storage at the point of sensory quality. Our observations also are in agreement with the results reported previously about MAP influence on the shelf-life extension of fish flesh (Kostaki et al. 2009; Goulas and Kontominas 2005).

Table 1 Sensory scores of Pike-Perch fillet during 16 days of refrigeration storage (4 °C)

Sensory parameter	Treatment	Storage time (days)				
		0	4	8	12	16
Odour	WPS	8.87 ± 0.25^{a}	7.08 ± 0.38^{c}	5.16 ± 0.28^{c}	2.75 ± 0.25^{c}	NE
	WPS + LPOS	8.87 ± 0.25^{a}	8.35 ± 0.13^{a}	6.91 ± 0.14^{b}	6.25 ± 0.25^{b}	4.91 ± 0.14^{c}
	WPS + MAP	8.95 ± 0.05^{a}	7.88 ± 0.12^{b}	7.16 ± 0.14^{b}	6.50 ± 0.25^{b}	5.41 ± 0.28^{b}
	WPS + LPOS + MAP	8.90 ± 0.10^{a}	8.61 ± 0.12^{a}	8.00 ± 0.25^{a}	7.83 ± 0.14^{a}	7.00 ± 0.25^{a}
Colour	WPS	8.81 ± 0.07^{b}	7.08 ± 0.38^{b}	5.25 ± 0.25^{c}	2.08 ± 0.38^{c}	NE
	WPS + LPOS	8.91 ± 0.07^{ab}	8.33 ± 0.14^{a}	7.33 ± 0.14^{b}	6.58 ± 0.14^{b}	5.35 ± 0.13^{b}
	WPS + MAP	8.96 ± 0.05^{a}	8.09 ± 0.16^{a}	6.91 ± 0.38^{b}	6.41 ± 0.14^{b}	5.83 ± 0.38^{b}
	WPS + LPOS + MAP	8.95 ± 0.05^{a}	8.50 ± 0.25^{a}	7.91 ± 0.14^{a}	7.66 ± 0.28^{a}	7.08 ± 0.52^{a}
Texture	WPS	8.95 ± 0.08^{a}	7.08 ± 0.52^{b}	5.58 ± 0.62^{c}	2.16 ± 0.28^{d}	NE
	WPS + LPOS	9.00 ± 0.00^{a}	8.61 ± 0.12^{a}	8.00 ± 0.25^{a}	7.25 ± 0.25^{c}	6.41 ± 0.28^{c}
	WPS + MAP	8.97 ± 0.02^{a}	8.16 ± 0.14^{a}	7.25 ± 0.25^{b}	6.12 ± 0.12^{b}	4.91 ± 0.62^{b}
	WPS + LPOS + MAP	8.98 ± 0.02^{a}	8.58 ± 0.14^{a}	8.35 ± 0.13^{a}	8.08 ± 0.14^{a}	7.21 ± 0.20^{a}
Overall acceptability	WPS	8.86 ± 0.05^{a}	7.38 ± 0.12^{c}	5.16 ± 0.28^{c}	1.66 ± 0.28^{c}	NE
	WPS + LPOS	8.86 ± 0.07^{a}	8.11 ± 0.12^{b}	7.41 ± 0.14^{b}	6.58 ± 0.28^{b}	5.25 ± 0.25^{c}
	WPS + MAP	8.90 ± 0.10^{a}	7.98 ± 0.02^{b}	6.91 ± 0.28^{b}	6.08 ± 0.14^{b}	3.75 ± 0.25^{b}
	WPS + LPOS + MAP	8.91 ± 0.03^{a}	8.50 ± 0.25^{a}	8.00 ± 0.20^{a}	7.50 ± 0.25^{a}	7.08 ± 0.14^{a}

Scores followed by different lower case superindex letters correspond to statistically significant differences ($p < 0.05$) at same column. Scores are given as mean ± SD

Scale from 9 to 0 (limits of unacceptability, moderate acceptability and high acceptability indicated the scores 1–3.9, 4–6.9 and 7–9, respectively)

NE not evaluated

Conclusion

The results of this study showed that LPOS + WPS + MAP inhibited microbial growth, reduced the TBARS values and TVB-N formation and could also maintain sensory attributes at high acceptability for at least 16 days under refrigerated storage. Based on the results of present study, it can be concluded that these methods may have a synergistic effect on shelf life extension of Pike-Perch fillets under cold storage condition.

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