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Effect of whey protein concentrate coating enriched with spearmint essential oil on oxidation and microbial spoilage of minced common kilka during refrigerated storage

Shabnam Nobahar[1], *Shabnam Haghighat Khajavi*[1*] *and Reza Safari*[2]

[1] Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Caspian Sea Ecology Research Center, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Mazandaran, Iran

***Corresponding author**:
Shabnam Haghighat Khajavi
sh.khajavi@srbiau.ac.ir

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ABSTRACT

The present study was conducted to estimate the effect of edible whey protein concentrate (WPC)-based coating incorporated with spearmint (*Mentha spicata* L.) essential oil (SEO) at concentrations of 0.5 and 1% w/v on quality and shelf-life extension of the minced common kilka during refrigerated storage for 20 days. For evaluating the antimicrobial and antioxidant effects of this coating as natural food preservative, all samples were tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas fluorescens* and *Escherichia coli* recognized as important food pathogens and spoilage bacteria in fish. The antimicrobial effects of SEO and WPC+SEO were investigated and this inhibitory effect reduced psychrotrophic bacteria counts in the treated samples during storage period. All the samples were also analyzed for chemical parameters. The results obtained from 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, peroxide value, and thiobarbituric acid values showed higher antioxidant activity for the samples treated with SEO and WPC+SEO. For WPC+1% w/v SEO coating, only pH was slightly increased from 6.9 on day 0 to 7.1 on day 20 of cold storage, while pH of the control sample was increased to 7.6 on day 20. According to the microbial and chemical results, WPC+1% w/v SEO-treated samples represented significantly higher quality.

Keywords: antioxidant activity; edible coating; essential oil; *Mentha spicata*; *Clupeonella cultriventris*

1. INTRODUCTION

Plants have significant benefits due to high amounts of bioactive constituents and polyphenolic compounds. Essential oils are substances that can be obtained from extraction of different parts of a plant and perform antioxidant and antimicrobial effects due to the existence of these functional compounds (Shahidi and Ambigaipalan, 2015; Wetwitayaklung et al., 2009). Application of essential oils in food preservation is limited due to their high costs of production, intense flavor, and their toxicity. For reducing amount of essential oil and maintaining efficacy at the same time, these compounds can be applied with edible coatings. *Mentha spicata,* a flowering plant belongs to Lamiaceae family (Lansdown, 2014), is widely cultivated in many places of the world and is used as spices and herbal medicine. Its volatile

essential oil has antimicrobial and antioxidant properties (Sacchetti et al., 2005). *M. spicata* is a traditional herb that has been utilized in cuisines and foods as a flavoring agent especially in recipes of the minced kilka balls cooked in northern provinces of Iran.

Edible coatings and films enriched with antioxidant or antimicrobial compounds (e.g., essential oils and extracts) generally used for covering foods to delay and inhibit bacterial growth on surface of foods, prevent oxidation and extend their shelf-life (Pereira et al., 2018; Shokri et al., 2015; Dehghani et al., 2018). Several studies have revealed that antimicrobial activity of *M. spicata* essential oil might be attributed to high contents of polyphenolic compounds such as carvone, *cis*-carveol, 1,8-cineole and limonene, which having antimicrobial, antiviral, and other medicinal properties (Jirovetz et al., 2002; Sivropoulou et al., 1995; Watanabe et al., 2018; Hussain et al., 2010; Stanley, 2007). It can be used to improve film properties of WPC-based coatings due to their hydrophobic characteristics (Da Veiga et al., 2019; Galus and Kadzinska, 2019).

Edible coatings are applied onto food surfaces by immersing the food into edible solution and draining it to form coating. Among the protein coatings used in industry, whey protein biodegradable edible-based coatings are widely used because of their low-cost, abundance, and availability (Schmid and Müller, 2019). Several studies also indicated the antimicrobial activities of whey protein concentrate edible films incorporated with different essential oils (Catarino et al., 2017; Bahram et al., 2014).

Common kilka is one of the most important species of Clupeidae family widely found in northwestern Black, Azov, and Caspian seas (Freyhof and Kottelat, 2008). It has small size and high-fat content (Pazhouhanmehr et al., 2016). Shelf-life of this fish is short due to breakdown of protein by protease enzymes, production of volatile nitrogen and pH changes, lipid oxidation, or deterioration caused by bacteria or endogenous enzymes (Bagheri et al., 2016). Like other fish species, common kilka can be affected by lipid oxidation (Pazhouhanmehr et al., 2016). Oxygen is an important storage factor influencing lipid oxidation and fish and fishery products appearance through causing discoloration, off-odor, and off-flavor. Whey proteins are highly nutritious and produce transparent, flexible water-soluble coatings and films with excellent oxygen and aroma barrier properties (Pérez-Gago and Krochta, 2002).

Since, lipid oxidation and microbial spoilage are among reactions that can reduce shelf-life of fish and fish products, this study was conducted to enhance storage stability and quality of the minced common kilka meat by application of WPC-based coating enriched with spearmint essential oil stored in refrigerator.

2. MATERIALS AND METHODS

2.1 Materials and bacterial strains

Aerial parts of spearmint (*M. spicata* L.) were collected in April, 2018 from Mazandaran province, Iran. Fresh common kilka (*Clupeonella cultriventris caspia*) was obtained in June, 2018 from Babolsar pier (Babolsar, Mazandaran province, Iran). WPC (80% protein) was purchased from the DMV (Amersfoort, Netherlands). Glycerol, barium sulfate, 1-butanol, potassium iodide, thiosulfate, octanal, magnesium oxide, boric acid, sulfuric acid, chloroform, methanol, thiobarbituric acid reagent, methyl red reagent, starch reagent, tryptone soya agar (TSA), and DeMan-Rogosa-Sharpe agar (MRSA) were purchased from Merck (Darmstadt, Germany). *Staphylococcus aureus* (PTCC 1112), *Pseudomonas fluorescens* (PTCC 1181), and *Escherichia coli* (PTCC 1330) were collected from the Industrial Microorganism Collection Center (Tehran, Iran).

2.2 Preparation of spearmint essential oil

For preparing spearmint essential oil (SEO), aerial parts of the plant were air-dried at an ambient temperature and were ground before extraction. The essential oil from 150 g of *M. Spicata* plant was obtained by hydrodistillation for 3 h using a Clevenger-type apparatus at atmospheric pressure (Baser and Buchbaure, 2010). The obtained essential oil was dehydrated by anhydrous sodium sulfate and was kept in dark air-tight glass container at 4°C under nitrogen gas atmosphere until further use.

2.3 Preparation of the treated fish samples

For preparing the common kilka fish samples, immediately after being caught freshly, fish were transferred in ice to laboratory, and washed. Their skin, head, and bone were separated; they were filleted and washed with tap water. Then, fish fillets were minced using a meat mincer (Bosch MFW-1501, Germany) and were divided into 50 g rounded balls under laminar flow hood. Two concentrations, including 0.5 and 1% w/v SEO, were prepared. For preparation of WPC-based coating solution, 5% w/v WPC solution was dissolved in distilled water under agitation for 20 min at an ambient temperature. Then, glycerol 0.13% w/w in WPC was added as a plasticizer and the solution was incubated in a water bath at 90°C for 20 min. The solution was then, cooled to 25°C, and SEO was added at different concentrations of 0.1, 0.2, 0.3, 0.5 and 1% w/w in WPC+SEO and homogenized (Ultra-Turrax T25-IKA-Werke, Staufen, Germany) at 13000 rpm for 3 min. Samples containing 50 g minced fish meatballs were assigned into control group (uncoated), treated with 0.5% w/v SEO, treated with 1% w/v SEO, treated with WPC, treated with WPC+0.5% w/v SEO, and treated with WPC+1% w/v SEO. In order to prepare coated sample, the fish meatballs were dipped in different coating solutions for 2 min and were allowed to drain. All the samples were prepared in triplicate, packed in polyethylene air-tight bags, and stored in refrigerator (4 ± 1°C) for 20 days. Chemical and microbial examinations were done at 5-day intervals to measure quality of the minced common kilka meat.

2.4 Assessing antioxidant activity of spearmint essential oil by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA) assay

Antioxidant activity of the *M. spicata* essential oil was assessed according to the method proposed by Miliauskas et al. (2004) with some modifications. The stock solution of DPPH in methanol was prepared at a concentration of 6×10^{-5} mol/L. Various concentrations of 0.1, 0.2, 0.3, 0.5, and 1% w/v SEO were prepared with 96% methanol and according to the DPPH-RSA method; 100 µL of each of solution was added and mixed with 1.5 mL of DPPH stock solution. Subsequently, the mixture was shaken vigorously and kept in the dark at room temperature for 30 min. The mixture was then placed in an UV-Vis spectrophotometer and its absorbance was measured at 517 nm (Shimadzu UV-1800, Kyoto, Japan). Methanol was used as a blank. DPPH-RSA in the samples was calculated according to Equation (1), where, A_{Blank} and A_{Sample} are the

blank and sample absorption, respectively. The test was performed in triplicate for each sample.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{Blank}-A_{sample}}{A_{Blank}} \times 100 \quad (1)$$

2.5 Chemical analysis

2.5.1 Proximate composition

The minced meat of common kilka was analyzed for protein, lipid, moisture, and ash contents using a standard method (AOAC, 2005). According to this method, for determination of moisture content, 2 g of sample was placed in an aluminum dish and dried in an oven at 105°C until reaching a constant weight. Total lipid in the sample was extracted by Soxhlet method. The total crude protein in raw materials was measured by Kjeldahl method using the conversion factor of 6.25. Ash content was determined by ashing the pre-dried sample at 600°C until formation of white ash.

2.5.2 Determination of pH

The pH values of the samples were determined after homogenizing 5 g of the sample in 45 mL of distilled water using homogenizer (Ultra-Turrax T25-IKA-Werke) at 3000 rpm for 30 s by the calibrated digital pH meter at an ambient temperature.

2.5.3 Determination of peroxide value

Peroxide value (PV) was measured based on meq peroxide per 1000 kg of fat in fish samples according to the method proposed by Bahram et al. (2016). PV was expressed in Equation (2), where V is the volume (mL) of sodium thiosulphate; N is normality of this solution, and W is the weight (g) of fish oil.

$$PV = \frac{V \times N \times 1000}{W} \quad (2)$$

2.5.4 Determination of thiobarbituric acid

Thiobarbituric acid (TBA) value was estimated using colorimetric method (Kirk and Sawyer, 1991). According to this method, 200 mg of the minced fish meat was placed in 25 mL volumetric flask and filled with 1-butanol. Then, 5 mL of this mixture was transferred to the capped test tube, where it was mixed with 5 mL of TBA reagent. The test tubes were capped and placed in a water bath at 95°C for 2 h. Absorbance of samples at 538 nm was detected using spectrophotometer (Shimadzu UV-1800) against 5 mL of distilled water using 5 mL of TBA reagent as a blank. TBA values were expressed as mg of malonaldehyde (MDA) per kg of sample and determined according to Equation (3), where Abs_{Blank} and Abs_{Sample} are the blank and sample absorption, respectively.

$$TBA = \frac{(Abs_{Sample}-Abs_{Blank}) \times 50}{200} \quad (3)$$

2.5.5 Determination of total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) value was determined according to the method suggested by Antonacopoulos and Vyncke (1989). For this purpose, 10 g of the minced fish sample was put in a suitable container and was homogenized with 100 mL of 6% perchloric acid solution for 2 min. Then, the sample was filtered and alkalized with 20% sodium hydroxide solution. Steam distillation was performed by Kjeldahl apparatus after addition of magnesium oxide. Next, distillate was collected in a flask containing 10 mL of 2% boric acid and mixed indicator (solution containing 0.1 g of methylene blue in 10 mL of ethanol). Volatile base components were absorbed by aqueous acid solution and color turned into green. Then, boric acid solution was titrated with 0.1 mol/L of hydrochloric acid solution. When complete neutralization was achieved, color turned into pink using only one drop of hydrochloric acid. Afterwards, TVB-N value was expressed as mg of nitrogen per 100 g of fish sample. All the analyses were performed in triplicate.

2.6 Antimicrobial effects

2.6.1 Microbiological analysis

To determine microbial loads of the minced fish meat during storage time, 25 g of each of the treated samples was transferred into sterile sampling bags separately, diluted with 225 mL of sterilized 0.1% peptone water. Then, samples were homogenized at 230 rpm for 2 min using a stomacher type pulverizer (Stomacher 400 Circulator, Seward, London, UK). Serial dilutions of the homogenized samples were prepared. For enumeration of total viable counts (TVC), lactic acid bacteria (LAB), and psychotropic bacteria count (PTC), 0.1 mL from each of the prepared dilutions was spread. For specifying TVC and PTC of the samples, the TSA was used. The number of colony forming units for TVC was counted after incubating the plates at 37°C for 2 days. For enumeration of PTC colonies, plates were incubated at 4°C for 10 days. LAB count was determined using the MRSA and then, the samples were incubated at 30°C for 2 days (Khanipour et al., 2020).

2.7 Statistical analysis

The experiment was performed in triplicate for each specific storage time and all values were reported as mean±standard deviation (SD) by Microsoft Excel. Data were analyzed by one-way analysis of variance (ANOVA) method using the SPSS software (SPSS version 22.0 for Windows, SPSS INC., Chicago, IL, USA). Means were compared using the Duncan's multiple range test to evaluate any significant difference between different treatments at a significant level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity

3.1.1 DPPH-RSA assay

Evaluation of antioxidant properties of SEO can be carried out by the DPPH assay as a routine colorimetric method. Antioxidant activity is directly related to free radical consumption. The higher the free radical scavenging, the greater the antioxidant activity and the lower the IC_{50}. According to the results presented in Table 1, the increase in antioxidant activity was concentration-dependent and the *M. spicata* essential oil exhibited considerable free radical scavenging activity with the IC_{50} value of 0.44% w/v. This considerable antioxidant activity might be related to the existence of different terpenoids and the major phenolic compounds in the *M. spicata* L. plant, especially carvone, *cis*-carveol, and limonene in SEO, largely depending on stage of harvest, seasonal and environmental factors, and geographical sources as well as different methods for extraction of the volatile components. The essential oil extracted from *M. spicata* cultivated in different countries such as Egypt, Pakistan, Brazil, Ethiopia, Turkey, and Iran have been reported to be genetically different. Several authors have indicated the presence of carvone, *cis*-carveol,

1,8-cineole, carvacrol, menthone, and limonene as the major components in SEO (Abootalebian et al., 2016; Scherer et al., 2013; Kačániová et al., 2017; Anwar et al., 2019; Gharib and Teixeira da Silva, 2013). The obtained results were in agreement with those of the studies by Ben Haj Yahia et al. (2019) and Saba and Anwar (2018), who reported that the SEO had significant antioxidant properties. Kanatt et al. (2007) reported antioxidant activity of *M. spicata* aqueous extract during radiation processing of lamb and confirmed that it was able to retard lipid oxidation and give a pleasing aroma and flavor to the meat.

Table 1. Antioxidant activity of SEO

Concentration (% w/v)	DPPH-RSA (%)
0.1	16.45±0.33
0.2	23.79±0.25
0.3	48.21±0.40
0.5	67.35±0.62
1	85.42±0.12

Note: Values are mean±SD (n=3)

3.2 Microbial analyses

3.2.1 TVC

Changes in the TVC value during the refrigerated storage is presented in Figure 1.

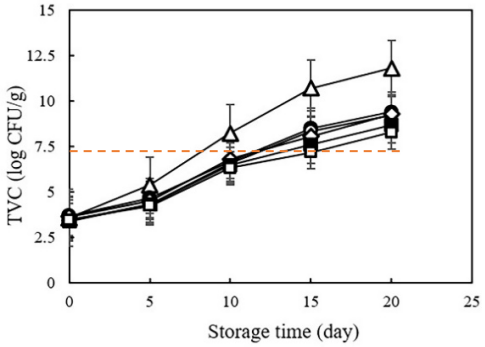

Figure 1. Changes in TVC during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

The initial TVC of the minced kilka fish sample was about 3.5 $\log_{10}$ CFU/g. Based on the international commission on microbiological specifications for foods (ICMSF) statement, the maximum recommended limit (MRL) of TVC in raw fish is equal to 7 $\log_{10}$ CFU/g (ICMSF, 1986). According to Figure 1, TVC of all the samples was gradually increased during storage period. The obtained results showed no significant difference between TVC of the treated samples by day 10 of refrigerated storage. TVC of the control sample reached the maximum standard limit (7 $\log_{10}$ CFU/g) earlier, compared to the other samples before day 10. Based on these results, samples treated with essential oil and those treated with WPC (without essential oil) maintained quality of the minced fish samples up to day 11. However, quality of WPC+SEO-treated samples remained acceptable by day 10 of refrigerated storage, indicating that progress in microbial growth for all the treated samples was slower than that in the control sample. From day 10 to 20, increasing trends of TVC in the samples treated with WPC+SEO were significantly lower in comparison with other samples. By the end of storage period, the maximum level of inhibition was observed for WPC+1% w/v SEO-coated samples. Our results were in good agreement with those obtained by Jalali et al. (2016), which reported that application of clove essential oil on sliver carp fillets reduces microbial loads during refrigerated storage, and Alparslan and Baygar (2017) that declared orange peel essential oil represents a significant inhibitory effect on microbial loads of shrimp samples compared to the control samples.

3.2.2 PTC

Psychrotrophic organisms have a significant role in deterioration of fishery products by producing off-odors as a result of joining units of strains in fishs' myomere at low temperature (Albertos et al., 2019). Changes in PTC during storage period are shown in Figure 2.

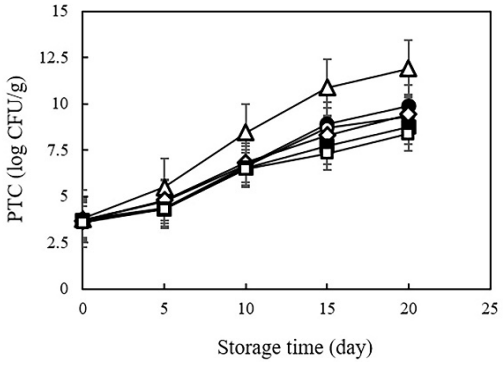

Figure 2. Changes in PTC during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

The initial population of psychrotrophic bacteria of the samples was found to be around 3.7 log CFU/g. According to the obtained results, growing trends of the psychrotrophic bacteria count showed an increasing pattern during 20 days of storage (Figure 2). From day 0 to 10 of storage, PTC had a similar increasing trend with no significant difference in all the treated samples. Thereafter, PTC increased significantly for the samples treated only with SEO and WPC separately in comparison with those treated with WPC incorporated with SEO coating. From day 10 to 20 of storage, PTC of the control sample was significantly higher than that of the treated samples. In other words, growth rate of psychrotrophic bacteria in the treated samples was lower than the control sample. The obtained results showed that the most effective treatment for PTC growth inhibition was WPC+1% w/v SEO during storage period. Similarly, the previous results have also shown the maximum level of inhibition of TVC for growth of the psychrotrophic bacteria by the end of storage time, indicating that the WPC+1% w/v SEO-coated samples had the highest inhibition level. In agreement with current study, Ehsani et al. (2020) found that fish burgers coated with chitosan containing sage essential oil show an inhibitory effect on population of psychrotrophic bacteria. Feng et al. (2016) reported that the fish fillets coated with tea polyphenols and fish gelatin maintain acceptable quality better than the control group. Shokri et al. (2015) mentioned that the PTC is significantly lower in rainbow trout fillets treated with lactoperoxidase system-whey protein coating than the control samples. These results

indicated that the WPC-based coating enriched with SEO can be considered as potentially effective method to prolong shelf-life and increase quality of the minced fish samples because it can react as an oxygen barrier compound and inhibit oxidation of fatty acids in fish.

3.2.3 LAB

LAB as Gram-positive bacteria can produce lactic acid by fermentative metabolism and also are among healthy fish natural microflora (Banerjee and Ray, 2017). Several studies have demonstrated that the LAB are able to produce some off-odors in fish products and also reduce pH value of these products due to formation of lactic acid (Li et al., 2019; Chen et al., 2016). These bacteria are among harmless bacteriocin-producing strains, which may prevent growth of other harmful microorganisms. Certain species of LAB have been shown to reduce spoilage and exert inhibitory activity against spoilage microbiota (Chaillou et al., 2014). Changes in the LAB count during storage are shown in Figure 3.

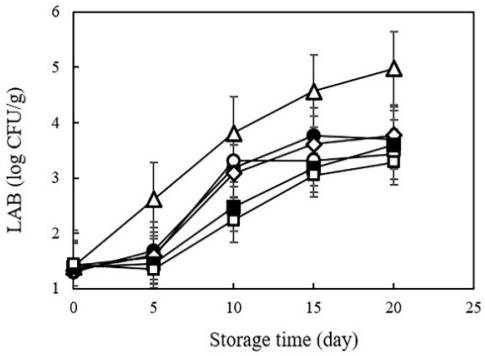

Figure 3. Changes in LAB during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

The initial population of LAB in the samples was around 1.4 log CFU/g, but the LAB count increased during storage period. Based on the results shown in Figure 3, the increase in LAB count was significant for control sample. However, there were no significant difference between the LAB counts of all the treated samples on day 5 and their initial counts. From day 5 to 10, the LAB count showed increasing trend for all the samples. A significant increase was observed in the LAB count for 1% w/v SEO-treated sample only on day 5 to 10 of storage period. Thereafter, there was no significant increase in the LAB count for this sample by the end of storage. According to the obtained results, the WPC+SEO treatments significantly lowered population of LAB in the samples, compared to the control sample by day 20 of storage period. Among all the treatments, the WPC+1% w/v SEO treatment had the highest ability in limiting the LAB growth. Accordingly, growth rate of LAB decreased in the treated samples, which was due to application of SEO incorporated with WPC-based coatings on the treated samples. Regarding the LAB, similar results have been also obtained for the fresh frankfurter sausage coated with a combination of turmeric, starch, and gelatin coatings stored at 5°C for 20 days (Tosati et al., 2017).

3.3 Proximate composition

The moisture, fat, protein, and ash contents (wet basis) were equal to 75.72±0.24, 2.4±0.04, 18.79±0.56, and 2.59±0.03%, respectively for the common kilka. Fat content of this fish may have various values due to environmental conditions, fishing season, sexual variation, size, and age of the fish. Variation in fat content may change shelf- life of the common kilka due to its effect on oxidation rate.

3.4 Chemical analysis

3.4.1 Determination of pH

The pH value is one of important indicators of fish freshness. Changes in pH value might be related to production of metabolites from bacteria, including basic nitrogen and lactic acid during bacterial growth. In addition, activity of bacterial enzymes may lead to destruction of amino acids and also an increase in the amount of free fatty acids, which can influence pH of samples during storage (Li et al., 2013). Changes in pH values of the samples during storage time are shown in Figure 4.

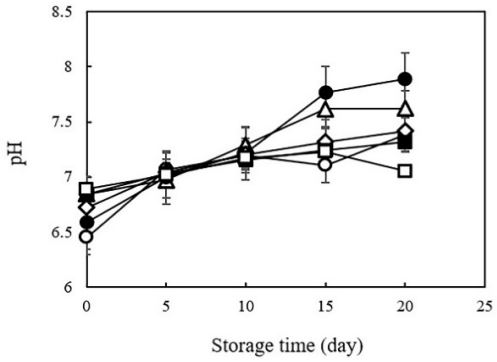

Figure 4. Changes in pH value during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

The initial pH of the minced common kilka meat was equal to 6.8. For the control sample, pH value was increased significantly during cold storage and reached to 7.2-7.6 until day 20. This low pH inhibited growth of H_2S-producing bacteria (*P. fluorescens*) by increasing hydrophobic nature of SEO (rich in carvone and *cis*-carveol) and disintegrating outer membrane of Gram-negative bacteria, leading to cell membrane permeability and death. However, it was observed that from day 10, pH of the samples treated with 0.5 and 1% w/v essential oil, WPC-based coating without SEO and WPC+0.5% w/v SEO was increased gradually during storage. Whereas, pH of WPC+1% w/v SEO-treated samples was equal to 6.9 and was increased to 7.0-7.2 by day 15 of storage and was decreased to 7.0 on day 20. This drop could be attributed to formation of metabolites such as organic acids by the LAB, since these harmless bacterial strains playing a protective role against pathogenic microorganisms in fish appeared to become predominant by the end of storage and were not affected by the WPC+SEO antimicrobial coating. Similarly, López de lacey et al. (2012) also reported that pH value of hake (*Merluccius merluccius*) fishs muscles stored by gelatin coatings and films incorporated with LAB was slightly decreased during the chilled storage.

Accordingly, pH of WPC+1% w/v SEO-treated samples was significantly (p<0.05) lower, compared to the control sample, indicating that incorporation of WPC with higher concentration of SEO to coating significantly inhibited microbial count and reduced formation of alkali compounds such as ammonia and trimethylamine (Hebard et al., 1982) and prolonged shelf-life of the minced flesh of common kilka. Hydrophobicity of essential oil is increased at lower pH, resulting in better penetration of antimicrobial components through bacterial cell membrane, influencing linkage of the cell contents and consequently, causing death (Juven et al., 1994; Shahbazi and Shavisi, 2016).

3.4.2 PV

Rancidity development in fish and seafood can be monitored by determining the amount of PV and formation of malondialdehyde. Therefore, PV is one of the most important and effective parameters to investigate quality of food products with high-fat content by measuring the primary products of oxidative degradation (De Marchi et al., 2018). Fish contains high amount of fatty acid and in this regard, it is very prone to lipid oxidation and deterioration causing unpleasant odors and reducing quality acceptance (Miyashita et al., 2018). PVs of the samples measured during storage period are presented in Figure 5.

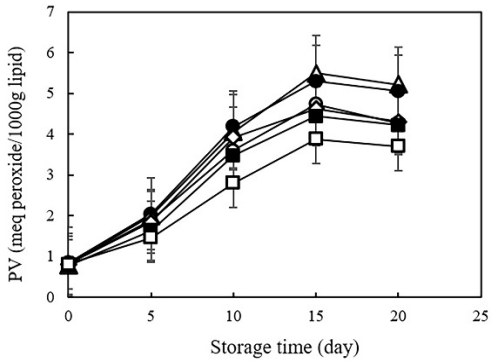

Figure 5. Changes in PV during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

Results showed that PV of all the samples was increased significantly with time throughout storage period (p<0.05) and the initial PV of 0.8 meq/kg was observed for the samples. There were no significant difference (p>0.05) between PV for the control sample and the samples treated with 0.5% w/v SEO. However, PV of 1% w/v SEO, WPC (without SEO) and WPC+SEO-treated samples was lower, compared to control sample and remained acceptable by the end of storage, which is in accordance with the Codex Alimentarius Commission standard for fish oils, stating the maximum acceptable level of 5 meq/kg (Codex, 2017). The minimum increase in PV between the samples was equal to 3.70 meq/kg for WPC+1% w/v SEO treatment. These results are similar to those obtained by Alparslan and Baygar (2017), who reported that application of chitosan film enriched with orange peel essential oil reduced PV of deep-water pink shrimp. The results indicated that WPC-based coating enriched with 1% w/v SEO was effective in retarding production of PV in the minced common kilka stored in refrigerator, which might be explained by antioxidant and oxygen barrier action of WPC+SEO coating inhibiting lipid oxidation.

3.4.3 TBA

TBA as one of the methods commonly used to determine concentration of malondialdehyde is also known as the secondary product of lipid oxidation of fatty acid caused by endogenous enzymes of spoilage bacteria and indicates the fish deterioration (Gram and Huss, 1996). Malondialdehyde usually builds up to a certain point of time and then, it is decreased due to breakdown to various products. Changes in TBA values for different samples are shown in Figure 6.

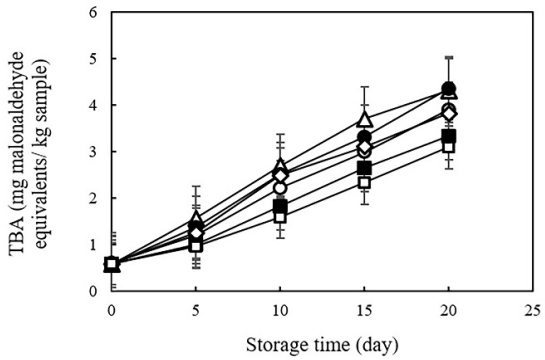

Figure 6. Changes in TBA during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

As indicated in Figure 6, TBA values were found to be 0.58 mg MA/kg for all the samples in the beginning of the experiment. Afterwards, increasing trend of TBA value in all the samples had similar pattern or no significant change during the refrigerated storage. This increase in TBA value indicated oxidation of lipids and formation of malondialdehyde as the secondary oxidation products. However, no decrease was observed in TBA values during storage period, indicating that breakdown of MA to tertiary lipid oxidation products had not occurred. The obtained results indicated that TBA in the WPC+SEO-treated samples was significantly lower than the control sample during storage period (p<0.05). The maximum TBA value for acceptable reported quality was equal to 5 mg MA/kg in fish products (Lemus-Mondaca et al., 2018; Kilinc et al., 2009). Thus, by the end of storage time, quality of WPC+SEO-treated samples remained significantly higher than SEO-treated, WPC-treated (without SEO), and control samples (p<0.05). Higher TBA value was found for the sample treated with 0.5% SEO (4.36 mg MA/kg) by the end of storage period. In agreement with our results, Vital et al. (2018) described how TBA value is reduced in tilapia fillets covered with alginate-based coating enriched with ginger and oregano essential oil during 14 days of storage period. Dolea et al. (2018) also found that TBA value in burgers made of salmon and seaweeds coated with thyme and oregano essential oil becomes lower than the samples without essential oil during storage period. SEO incorporated with WPC-based coating exhibited both antioxidant and antimicrobial activities. The antimicrobial and antioxidant effects of polyphenolic compounds in SEO are attributed to distraction of cell wall lipopolysaccharides in Gram-negative bacteria and the

increase in membrane permeability for both Gram-positive and Gram-negative tested foodborne pathogens and spoilage strains, which influenced their cell metabolism and resulted in death. However, on the other hand, WPC-based coating exerted hydrophobic, oxygen, and CO_2 barrier effect on the treated samples and inhibited oxidation of fatty acid in fish. Accordingly, trend of changes in PV and TBA values for the minced common kilka treated with WPC+1% w/v SEO indicated that the primary and secondary decomposition were substantially inhibited.

3.4.4 TVB-N

TVB-N is a physicochemical index used to investigate quality of seafood and fishery products. Changes in TVB-N of the samples are presented in Figure 7.

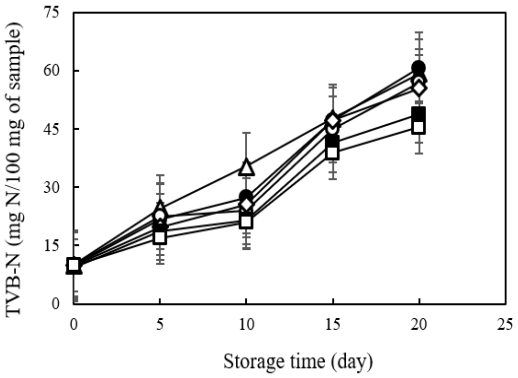

Figure 7. Changes in TVB-N during refrigerated storage

Note: Control (△), SEO 0.5% (●), SEO 1% (○), WPC (◇), WPC+SEO 0.5% (■), WPC+SEO 1% (□)

The initial TVB-N value of the samples ranged from 9.83 to 9.98 mg/100 g for WPC+0.5% w/v SEO-treated samples and 1% w/v SEO-treated samples, respectively. From day 5, TVB-N concentrations were significantly (p<0.05) increased due to formation of ammonia, trimethylamine, and dimethylamine in all the samples during storage period as a result of activities of spoilage bacteria and endogenous enzymes. However, the increase in TVB-N value was significantly higher for control sample than the treated samples (p<0.05). The recommended acceptable limit of TVB-N value ranges between 30-35 mg/100g for cold-stored fish (Mohan et al., 2019). According to this limit, quality of all the treated samples remained acceptable by days 13 and 14, whereas the control sample showed good quality only for days 8 and 9 of storage. According to these results, quality of the treated samples remained acceptable more than the control sample, which was due to the effect of SEO in combination with WPC in reducing activity of spoilage bacteria and endogenous enzymes. The results obtained from measuring TVB-N content of the samples showed no significant difference between SEO- (without coating), WPC- (without SEO) treated and control samples from day 15 to day 20. However, it was significantly different for WPC+SEO-treated samples by the end of storage time. Accordingly, WPC-based coating incorporated with SEO reduced and inhibited activity of the studied bacterial strains and led to delay in formation of TVB. This inhibition could be due to hydrophobicity of the essential oil and its polyphenols penetrating lipids of the bacterial cell membrane, damaging cytoplasmic membrane permeability, disrupting membrane transport function, interfering with generation of cellular adenosine triphosphate and disrupting proton motive force, which in turn inhibits growth and results in a decrease in bacterial count and death (Sikkema et al., 1994). These results are similar to those found by Gómez-Estaca et al. (2010) who stated that gelation-chitosan-clove films delay or even prevent both growth of pathogenic and spoilage bacteria and formation of total volatile nitrogen during the chilled storage of fish. Shokri et al. (2015) indicated that application of lactoperoxidase system-whey protein coating on rainbow trout fillets reduces formation of TVB-N due to its antimicrobial activity.

4. CONCLUSION

The use of essential oils as natural antimicrobial and antioxidant agents incorporated in coatings and films has received a great deal of interest in the field of food preservation. Therefore, in the current research, edible coating was developed by incorporation of carvone-rich *M. spicata* essential oil with WPC solution. According to our results, the oxygenated compounds and polyphenols of SEO at higher concentration of 1% w/v incorporated with WPC-based coating as an oxygen barrier matrix that helped to slow release of essential oil showed significant slowing down of microbial growth by their lag-phase extension and reduction of growth rate and led to retardation or inhibition of growth of *Pseudomonas fluorescens*, *Escherichia coli, and Staphylococcus aureus* as representatives of Gram-negative and Gram-positive pathogenic and spoilage bacteria.

According to the results, WPC+1% SEO treatment delayed or even prevented both growth of microorganisms and formation of total volatile nitrogen, and pH changes without any effect on lipid oxidation by reducing PV and TBA, and represented a considerable antioxidant effect, decreasing enzymatic spoilage, and preventing deterioration in the minced kilka during cold storage compared to control and WPC-free SEO samples.

Therefore, this edible coating, WPC+1% SEO treatment could provide an extended shelf-life, safety, and quality by better performance to inactivate post-processing contaminations on cold-stored ready to eat foods, such as the minced kilka meat and could be potentially used as a promising natural food preservative.

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