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The Effect of Free and Encapsulated forms of *Quercus persica* Extract on the Quality of Minced Kilka (*Clupeonella cultriventris caspia*)

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ABSTRACT: *Quercus persica* has a natural antimicrobial and antioxidant properties that are effective on pathogens and retard lipid oxidation. The aim of this study is using two concentrations of free and encapsulated forms (with two covers: maltodextrin + carrageenan and gelatin + carrageenan) of *Q. persica* extract (0.5% and 1%) in order to improve the shelf life of minced kilka at the refrigeration temperature. Chemical tests (Peroxide value, total volatile nitrogen, *thiobarbituric acid*, and free fatty acid) and microbial parameters (total viable count and psychrotrophic count) were carried out. Minced Kilka samples treated with encapsulated form of extract at the concentration of 1% showed significantly lower chemical changes as compared to other treatments during the storage period. The results of microbial examination indicated at different period (0, 4, 8, 12 and 16 days) that the treatments containing encapsulated form of extract showed improvements, however of the twelve days all parameters were outside of the standard range (7 log CFU/g). The results of this study indicated that the encapsulation of 1% showed better results as compared to other concentration employed and improved the shelf life of kilka.

Keywords: *Encapsulation, Kilka Fish, Quercus persica, Shelf Life*.

Introduction

Common Kilka (*Clupeonella cultriventris*) is one of the most abundant fish species in the Caspian Sea. The frequency of common Kilka has increased and reached 28,200 ton in 2014. This fish has a special place in the food basket of Iranian households. Innate talent oxidation and changes in the color and texture of the fish caused the limited period of storage and approximately 25% of aquatic primary production has been lost due to the microbial and chemical spoilage (Bagheri *et al*., 2016). There is a growing trend for using natural preservatives with antibacterial and antioxidant activity to improve the quality, increase the shelf life and prevent economic losses. Different essential oils and plant extracts such as *Zataria multiflora*, turmeric, shallot, thyme, garlic, laurel, rosemary, clove, sage, mint and oregano has been used to increase the shelf life of refrigerated fish and delay the microbial and chemical

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spoilage (Zakipour Rahimabadi *et al*., 2013; Pezeshk *et al*., 2011; Mexis *et al*., 2009).

Quercus persica is a kind of trees that belongs to the genus Quercus. The distribution range of this tree in Iran includes a large region of forest in the north-west of Iran especially the Zagros Mountains (Saffarzadeh *et al*., 1999). *Q. persica* is regarded as anti-viral, antibacterial, antifungal and antioxidant. This plant is also useful for treatment of anemia and diarrhea. *Q. persica* contains compounds such as tannins, carotenoid, *tocopherol*, thymoquinone, beta-cyanine, and t-anethole with antioxidant properties (Saffarzadeh *et al*., 1999). Studies carried out have indicated the antimicrobial activity of *Q. persica* against some microorganisms namely *Klebsiella pneumonia*, *Escherichia coli*, Staphylococcus aureus and *Pseudomonas aeruginosa* (Teimouri *et al*., 2004).

Encapsulation is one of the newest technologies that has been emerged in the food industry to produce materials with high quality and increase the shelf-life (Beyki *et al*., 2014). *One of* the advantages of this technique is the protection of foods against oxidation during storage to prevent undesirable odor, and taste and loss of nutritional value in the products (Krishnaswamy *et al*., 2012). Some studies (Gortzi *et al*., 2007; Alipour Mazandrani *et al*., 2016) have shown that encapsulation can improve the antimicrobial activity of compounds and maintain the stability of antimicrobials over prolonged periods of time.

Given the economic and nutritional value of kilka fish and temporary maintenance methods, it seems to be essential to investigate the quality and shelf life of kilka fish in during refrigeration (Bagheri *et al*., 2016). In the current study, the antimicrobial and antioxidant activities of *Q. persica* extract in free and encapsulated forms have been studied in order to increase the shelf-life of *C. cultriventris* during storage at 4° C.

Materials and Methods

- *Extract preparation*

500 grams of *acorn fruit* ***(****Quercus persica****)*** were collected from the Chaharmahal and Bakhtiari province, dried and powdered plants were extracted with ethanol 80% (1:4) using Soxhlet apparatus at 60 °C for 6 h. The extract was filtered and concentrated under vacuum at 40 °C using a rotary evaporator (Buchi EL 141, Switzerland). The extract was stored in a dark bottle at 4 °C required (Ghaderi Ghahfarokhi *et al*., 2011).

- *Encapsulation of Quercus persica*

Nanoparticles of acorn fruit extract were prepared by using the method recommended by Beristain *et al*. (2001). Solutions of maltodextrin, carrageenan, and gelatin were prepared by dissolving each of the polymers in distilled water separately. Then they were mixed with 50:50 ratio (maltodextrin+ carrageenan (M+C), and gelatin +carrageenan (G+C)). The solutions were stirred with a magnetic stirrer, covered and left overnight at room temperature. *Q. persica* extract was added to each of the solutions in a ratio of 1:4. The mixture was homogenized with an Ultra-Turrax T50 homogenizer (IKA-WERKE Works Inc., Wilmington, NC, USA) at 7000 rpm for 15 min and fed to a Buchi Mini Spray Dryer model 190 (BuchiiLaboratoriums-Technik AG., Flawil, Switzerland). Drying condition carried out at inlet air temperature of 105˚C and outlet air temperature of 108˚C with a feeding rate of 5.6 ml/min.

The powder was kept at 4 ˚C until required. Final approval of encapsulation of Q. persica extract was carried out by particle size analysis and the morphology of dry free and extract-loaded nanoparticles was confirmed with a scanning electron microscope (SEM).

- *Fish samples preparation and storage condition*

The samples of Kilka (*Clupeonella cultriventris caspia*) with approximate weight of 7 g were obtained from a local market. Fish were iced and transported to the Caspian Sea Ecology Research Center in Iran. Then washed by hand, and minced twice. Minced fish samples were divided into 7 treatments (100 ± 10 g minced fish in each group) as follow: control samples without acorn fruit extract, E 0.5%: treatment samples in acorn fruit extract 0.5% (0.5% v/v), E 1%: treatment samples in acorn fruit extract 1% (1% v/v), M+C 0.5%: treatment samples in maltodextrin+ carrageenan incorporated with 0.5% E extract, M+C 1%: treatment samples in maltodextrin+ carrageenan incorporated with 1% E (1% v/v), G+C 0.5%: treatment samples in gelatin + carrageenan incorporated with 0.5% E (0.5% v/v) and G+C 1%: treatment samples in gelatin + carrageenan incorporated with 1% E (1% v/v). Finally, all the samples were placed in individual freezer bags and stored at 4 °C for 16 days. Sampling was carried out at predetermined time intervals of 0, 4, 8, 12 and 16 days, for evaluation of chemical and microbiological analysis to determine the overall quality of fish (Zakipour Rahimabadi *et al*., 2013). All measurements were carried out in triplicate order.

- *Chemical analysis*

Peroxide value (PV) was measured according to the AOAC (2005) method. Results were expressed as milliequivalent peroxide per kg of sample:

$$PV(meq / kg) = (S \times N)/W \times 1000$$

Where: S is the volume of titration (ml), N is the normality of sodium thiosulfate solution (N =0.01), and W is the weight of the sample (kg).

The thiobarbituric acid (TBA) assay was determined according to the method described by Egan *et al.* (1997). TBA value was expressed as milligram malonaldehyde (MDA) equivalents per kilogram of fish muscle.

Free fatty acid (FFA), was measured according to the method described by AOAC (2005). This method is based on a complex formation between the acid group of FFA and cupric acetate in the presence of pyridine at pH = 6.1. Results were expressed as grams of FFA per 100 g of lipids.

Total volatile basic nitrogen (TVB-N) was determined according to the method described by Goulas and Kontominas (2005). Results were expressed in mg N /100 g of fish

- *Microbiological analysis*

Ten grams of fish samples were aseptically removed from the trays and homogenized for 1 min in a stomacher (VRN-200, Taiwan R.O.C) containing 90 ml of physiological saline solution (0.85% NaCl) (Merck, Darmstadt, Germany). After resuscitation (for 30 min at 25 °C) further decimal serial dilutions were prepared from this homogenate in the same sterile diluent. The appropriate dilutions were subsequently used for enumeration and differentiation of microorganisms and particular microbial genera in the samples, at each of the predetermined time intervals, during refrigerated storage (Ibrahim Sallam *et al*., 2007).

- *Total viable aerobic bacterial count (TVAC)*

Total viable aerobic bacterial count (TVAC) were determined by inoculating 0.1 ml of the sample homogenate onto triplicate sterile plates of dried Tryptic Soy Agar (Merck, Darmstadt, Germany) using the surface spread technique, then the plates were incubated for 48 h at 25 °C (Ibrahim Sallam *et al*., 2007). All counts were expressed as log CFU/g and performed in triplicate.

- *Psychrotrophic count*

Psychrotrophic counts (PTC) were measured by inoculating 0.1 ml of the sample homogenate onto duplicate sterile plates of dried Tryptic Soy Agar (Merck, Darmstadt, Germany) using the surface spread technique, then the plates were incubated at 7 °C for 10 days (Ibrahim Sallam *et al*., 2007).

- *Statistical analysis*

The obtained data were subjected to one-way analysis of variance using SPSS statistical software, release 19.0. Duncan's new multiple range tests were performed to determine the significant differences of the means at the 5% probability level (P< 0.05).

Results and Discussion

- *Particle size analysis and SEM*

Figure 1 shows SEM micrographs of encapsulated *Q. persica* extract. Based on the obtained results, the average particle size and density of the encapsulated form was 114.5 nm and 89.2 percent, respectively.

- *Chemical changes*

The effects of encapsulated and free Q. persica extract on the changes in the PV of the minced Kilka are shown in Figure 2. The initial value of PV was very low (0.84 - 0.94 meq O_2 /Kg). The PV values of the samples significantly increased ($p<0.05$) with storage time, until day 12. This increase may be due to the high production rate of hydroperoxide than the rate of decomposition (Bahram *et al*., 2016). Then the PV value decreased until the end of storage time. Significant differences ($p<0.05$) were observed in the PV value between the control and other treatments. Generally, the lowest PV values were observed in encapsulated Q. persica *extract at 1%* ($p<0.05$). These results could be attributed to the antioxidant activity of Q. persica**,** as reported in previous studies by Ebrahimi *et al*. (2012) and Ghaderi Ghahfarokhi *et al*. (2018). Some main components of Q. persica**,** like tannin, phenolic compounds, gallic acid, and ellagic acid have shown antioxidant and antimicrobial activities (Ebrahimi *et al*., 2012; Ghaderi Ghahfarokhi *et al*., 2011). These results were compatible with reports of Ghaderi Ghahfarokhi *et al*. (2012) about the antioxidant activities of methanolic extracts of Quercus on sunflower oil. They showed that Quercus extract could retard the oxidation of sunflower oil at 70°c more efficiently than BHA and BHT due to the antioxidant activity of Quercus extract.

The results of Ravichandran *et al*. (2014), indicated that maltodextrin, guar gum, gum Arabic, pectin and xanthan gum can be used as encapsulating agents. Also, Akdeniz *et al*. (2017) showed the use of maltodextrin and gum arabic had a significant effect on the encapsulation efficiency of onion skin phenolic compounds.

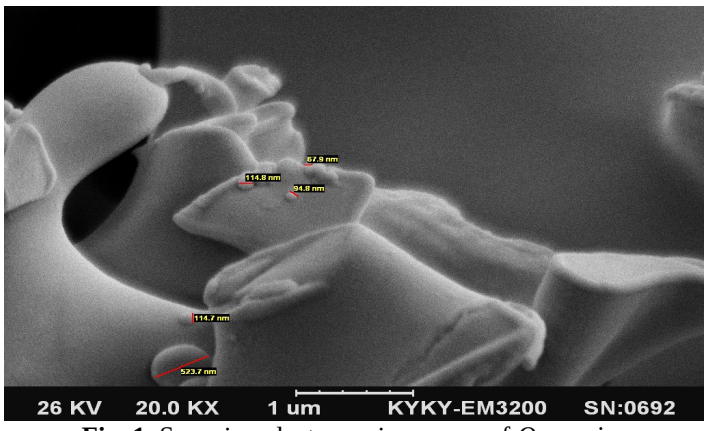

Fig. 1. Scanning electron microscopy of *Q. persica*

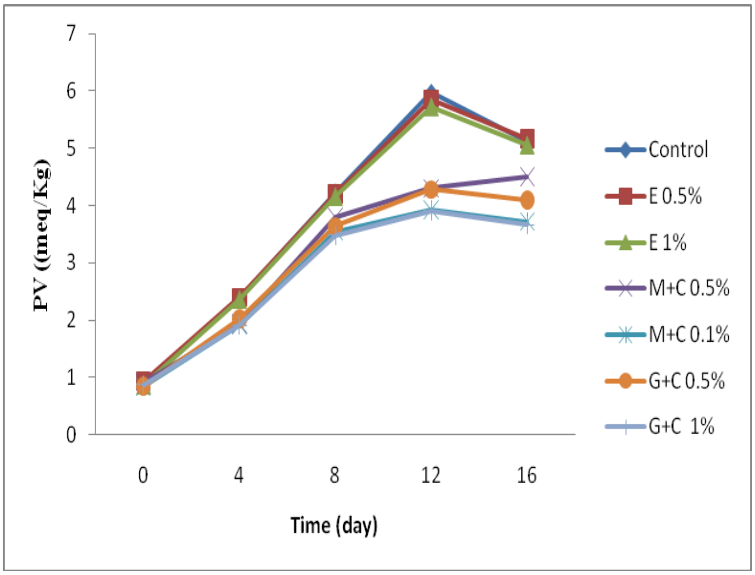

Fig. 2. Changes in PV values of fish samples during refrigerated storage.
(control: Without E, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extract with maltodextrin+ carrageenan solution (1% v/v), G+C 0.5%: encapsulated extract with gelatin + carrageenan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

The results of Bagheri *et al*. (2016) about the effect of encapsulated and unencapsulated fennel extract on minced common kilka indicated that encapsulated fennel extract had higher antioxidant effect on lipid oxidation due to improving the bioactivity and bioavailability of polyphenols.

TBA is an indicator for the assessment of the degree of lipid oxidation (Ariaii *et al*., 2014). In Figure 3 the TBA values for the different treatments are presented. The initial TBA values ranged from 0.53 (mg malonaldehyde/kg of fish) in samples with free extract, 0.54 in samples with encapsulated extract to 0.63 in control. TBA values of the control and other samples increased with storage time; by the end of the storage period (day 16). The increase in TBA values during storage time is probably due to partial dehydration of mince and the increased oxidation of unsaturated fatty acids (Ariaii *et al*., 2014; Javadian *et al*., 2017). However, samples with encapsulated forms of Q. persica (1% G+C) showed lower TBA values of 3.23 mg malonaldehyde/kg of fish in comparison with control and free extract, that attained a higher level of 5.02 and 4.48 mg malonaldehyde/kg of fish, respectively ($p<0.05$). These results could be attributed to the antioxidant activity of Q. persica as explained by Ebrahimi *et al*. (2012) and Ghaderi Ghahfarokhi *et al*. (2012). This observation also coincides with those of other researchers (Gortzi *et al*., 2007; Alipour Mazandrani *et al.*, 2016), who reported that encapsulation can improve the antioxidant activity of extract and maintains the stability of antioxidant over prolonged periods of time.

The FFA values in different treatments during the period of 16 days are presented in Figure 4. According to the results, the amount of FFA in all treatments was increased with the passage of time. Similar results were reported by Ariaii *et al*. (2014), who showed that the FFA values in silver carp (*Hypophthalmicthys molitrix*) increased during the refrigerated storage. FFA values increased from 0.45% to 4% in control,

whereas in samples with free and encapsulated extract the values were lower than the control during the storage period ($p<0.05$). The lower FFA value in the samples with free and encapsulated extract of Q. persica is probably due to the influence of extract on meat enzymes and their activities (Ariaii *et al*., 2014).

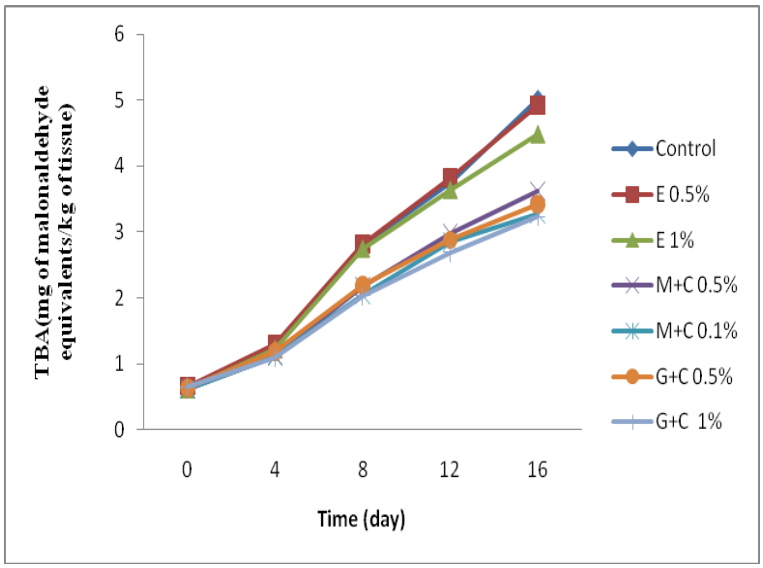

Fig. 3. Changes in TBA values of fish samples during refrigerated storage.
(control: Without extract, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extract with maltodextrin+ carrageenan solution (1% v/v), G+C 0.5%: encapsulated extract with gelatin + carrageenan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

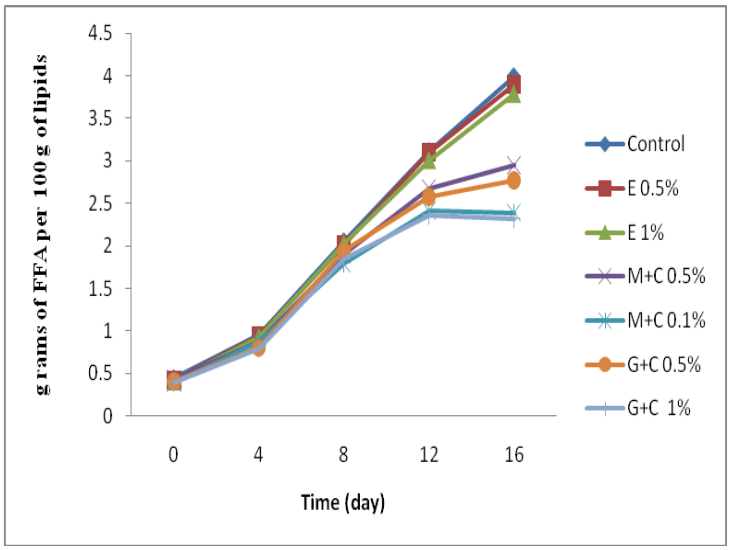

Fig. 4. Changes in FFA of fish samples during refrigerated storage.
(control: Without extract, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extract with maltodextrin+ carrageenan solution (1% v/v), G+C 0.5%: encapsulated extract with gelatin + carginan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

The TVB-N values of treatments during storage are shown in Figure 5. The initial TVB-N value (mg/100 g) of the minced Kilka was 10.29 mg/100g which showed the good quality of fresh samples. According to Alçiçek (2011), a level of 10-20 mg TVB_N/100 g of freshwater fish muscle after harvesting is usually regarded as good quality. This value increased with storage time ($p< 0.05$). At the end of storage time (day 16), samples with encapsulated forms of Q. persica ***(***1% M+C and G+C***)*** reached a significantly ($P < 0.05$) lower TVB-N value of 52.53 and 52.98 in comparison with the samples with free extract (E 1%) and control, which attained higher levels of 65.53 and 67.86 respectively. *These levels* were allocated in standard range ($P<0.05$). TVN-B is an indicator of seafood deterioration that is produced mainly by bacterial decomposition and endogenous enzymes of fish muscle (Bahram *et al*., 2016). The increase in TVN-B value in fish muscle, during storage at 4°C, is probably due to amino acid deamination by bacteria (Ariaii *et al*., 2014). The lower TVN-B value in samples with encapsulated extract of Q. persica may be due to the antibacterial effect of Q. persica on proteolytic bacteria that could inhibit the growth of PTC (Figure 6) and TVC (Figure 7) in mince.

This may be also due to the better protection of Q. persica after encapsulation that caused it to show good functionality during storage. Similar results have been reported by Javadian *et al*. (2017) about liposomal encapsulated thyme extract on silver carp mince during refrigerated storage.

Q. persica has been shown to have antibacterial activities. Tanin is one of the major components of Q. persica *with antimicrobial effect* (Ebrahimi *et al*., 2012***).*** Ebrahimi *et al*. (2012) reported the antibacterial effect of *Quercus persica* on E. coli. Borjian-Borujeni *et al*. (2016) showed that the extract of *Quercus branti* due to having high tannins has the antimicrobial, bactericidal and inhibitory effect on *L. monocytogenes* and *E. faecalis.*

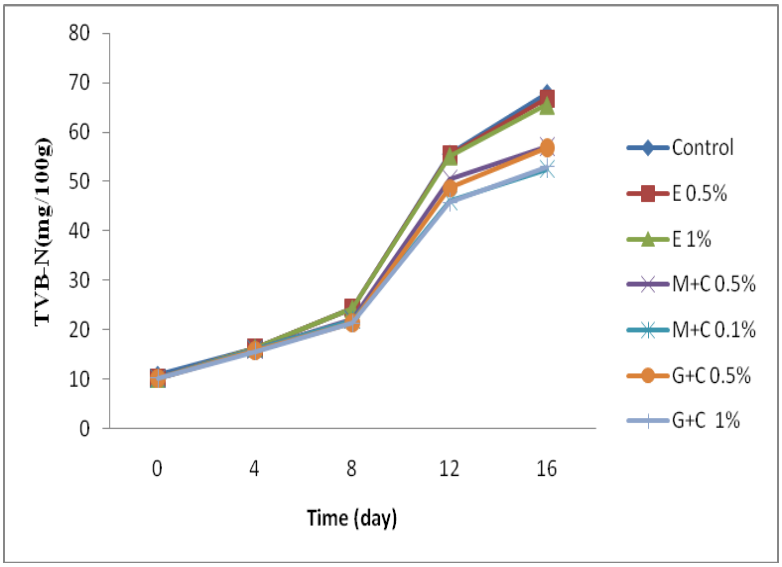

Fig. 5. Changes in TVB-N values of fish samples during refrigerated storage.
(control: Without extract, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extractwith maltodextrin+ carrageenan solution (1% v/v) , G+C 0.5%: encapsulated extract with gelatin + carrageenan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

- *Microbiological changes*

The results of the psychrotrophic count are shown in Figure 6. The results indicated that PTC in control samples on day 8, in free form on day 12 and in the encapsulated form on day 16 reached the standard limit. Maximally recommended limit of PTC is 7 log CFU/g in raw fish (ICMSF, 1986). The difference of PTC in the control and other samples increased with storage time and significant differences were seen in free and encapsulated samples in comparison with the control group ($P< 0.05$). These results show good antibacterial activities of Q. persica *and indicate that it could control the growth of* PTC. Encapsulation is a good process for improvement of antimicrobial properties of plant extract (Ebrahimi *et al*., 2012; Ghaderi Ghahfarokhi *et al*., 2012***)***. In the present study encapsulated samples indicated lower PTC during storage time ($P<0.05$). This observation is in agreement with that reported by Javadian *et al.* (2017) about liposomal encapsulated thyme extract on silver carp mince during refrigerated storage.

Variations in the value of total aerobic viable count (TVAC) during the refrigerated storage are presented in Figure 7. The initial TVAC (log CFU/g) in kilka mince ranged from 3.72 in free form, 3.56 in the encapsulated form to 3.48 in control. The increase of TVAC in the fish flesh during storage has been demonstrated by Fan *et al*., (2008). Control, free and encapsulated samples attained a count of 7.25 log CFU/g at 8 day, 7.30 log CFU/g at 12 day and 7.63 log CFU/g at 16 day which is higher than the maximal recommended limit of 7 log CFU/g for TVAC in raw fish (ICMSF, 1986). This result coincides with our observation about PTC. These results were compatible with those reported by Bagheri *et al*. (2016) and Javadian *et al*. (2017) about increasing of TVC in fish mince during storage time. They also reported that plant extract due to having antibacterial activities could inhibit the growth of TVC in fish mince.

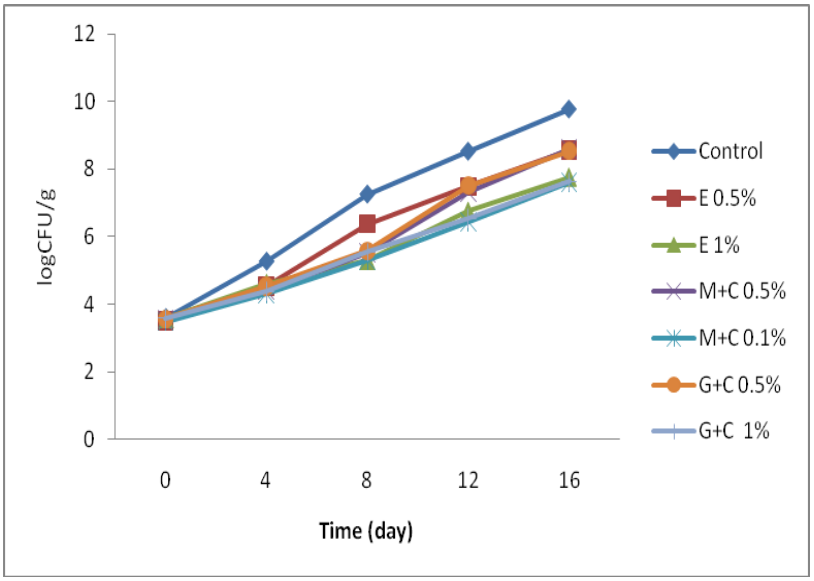

Fig. 6. Changes in PTC of fish samples during refrigerated storage.
(control: Without extract, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extract with maltodextrin+ carrageenan solution (1% v/v), G+C 0.5%: encapsulated extract with gelatin + carrageenan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

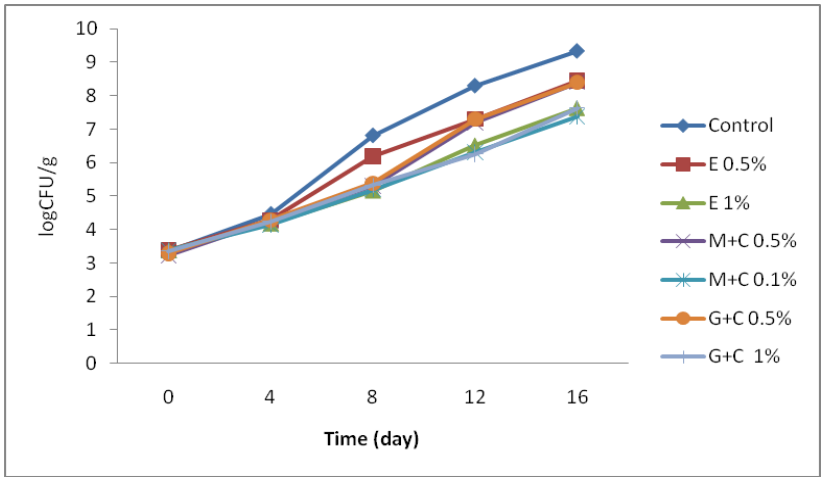

Fig. 7. Changes in TVAC of fish samples during refrigerated storage.
(control: Without extract, E 0.5%: 0.5% unencapsulated *Q. persica* extract, E 1%: 1% unencapsulated *Q. persica* extract, M+C 0.5%: encapsulated extract with maltodextrin+ carrageenan solution (0.5% v/v), M+C 1%: encapsulated extract with maltodextrin+ carrageenan solution (1% v/v), G+C 0.5%: encapsulated extract with gelatin + carrageenan solution (0.5% v/v), G+C 1%: encapsulated extract with gelatin + carrageenan solution (1% v/v).

Conclusion

The effect of free and encapsulated *Quercus persica* extract on the quality of minced common Kilka was studied. The results showed that *Q. persica* extract could improve the quality of Kilka minced. However, the encapsulated form of the extract could act better than free form, especially at 1% concentration. Chemical and Microbial analysis revealed a significant reduction in PV, TBA, FFA, TVB-N, PTC and TVAC in encapsulated form of the extract.

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