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The Effect of Gum Arabic Encapsulated with Rosemary Extract on the Quality of Minced Silver Carp and *Eschershia coli* Inoculated in the Mince

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ABSTRACT: Quality of minced silver carp (*Hypophthalmicthysmolitrix*) with gum arabic encapsulated with 1 and 1.5%(w/w) and rosemary extract (RE) (1 and 1.5% w/w) stored at 4˚C was studied chemically (peroxide value and total volatile nitrogen) and microbiologically (total viable count, psychrotrophic count) analyses. The efficacy of the encapsulated extract to control the population of *Eschershia coli* inoculated in silver carp minces was also investigated. Minced silver carp samples treated with gum arabic encapsulated with 1.5% and samples containing rosemary extract significantly showed ($P < 0.05$) lower peroxide value and total volatile nitrogen content compared to the control during the storage period. Microbial deterioration of the minced samples was restarted by RE as reflected by lower total viable count and psychrotrophic counts in the samples while the lowest counts was observed at 1.5% encapsulated RE. Although encapsulated RE at 1.5% could reduce the population of *E.coli* on compared to the control and unencapsulated RE treated samples, but it could not control the population of *E. coli* or reduce it below the acceptable level (<2 log CFU/g). Generally, gum arabic encapsulation could help to obtain higher antimicrobial activity in minced fish.

Keywords: *Encapsulation, Fish Mince, Gum Arabic, Rosemary Extract*.

Introduction

Fish mince can be used as raw material to produce many varieties of seafood products. It is an intermediate ready product for preparation of different highly acceptable ready to serve seafood products like frozen surimi, frozen mince block, extruded product, imitated products etc (Solomakos *et al*., 2008).

However, like other fish products, fish mince is highly susceptible to both chemical and microbiological deterioration and spoilage. This is related to its high water activity, neutral pH, relatively large quantities of free amino acids, the presence of autolytic enzymes and high percent of unsaturated fatty acids (Duan *et al*., 2010). One of the most commonly employed methods for seafood preservation is cold storage which combined with the addition of antimicrobial and antioxidant additives especially of synthetic origin to extend refrigerated storage time. However, the increasing number of consumers demands the use of natural products as an alternative to food containing preservatives, as the safety of synthetic additives has been called into questioned (Solomakos *et al*., 2008).

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To answer this demand, the food industry has focused on the use of natural preservatives, such as tocopherols, various spices and herbs, vegetable extracts and ascorbic acid. The antioxidant and antimicrobial properties of spices and herbs are attributed to their phenolic contents (Safari *et al.*, 2018). Many studies reported the effectiveness of these additives in retarding lipid oxidation and microbial deterioration in food products. In this regard, extracts from plants in the rosemary family, *Rosmarinusofficinalis* L. are considered to have the most effective antioxidant properties (Waszkowiak, 2008) among the wide range of herbs and spices tested (Baratta *et al*., 1998; Bicchi *et al*., 2000; Wijeratne & Cuppett, 2007). In addition, rosemary can be effective against Gram-positive food pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus* as well as Gram-negative pathogens, such as *Escherichia coli* (Ture *et al*., 2008; Abdollahi *et al*., 2012a, b). Also, Smith-Palmer *et al*. (1998) and Hammer *et al*. (1999) reported that rosemary (*R. officinalis*), sage (*Salvia sclarea*), thyme (*Thymus vulgaris*), were known as the most active against strains of *E. coli* which is recognized as an important cause of food borne disease. It can cause hemorrhagic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura, and can result in death (Karmali, 1989).

However, most natural active compounds like plant extract and essential oils are biologically instable, poorly soluble in water and they distribute poorly to target sites. In recent years, some novel strategies have been introduced in order to improve their stability and their bioavailability, among which is the use of liposomal encapsulation (Shoji and Nakashima, 2004). Encapsulation decreases reactivity with the environment (water, oxygen, light), reduces the evaporation or the transfer rate of the active compounds to the outside environment. It also promotes their handling ability, the bioavailability and half-life of the compound (Javadian *et al*., 2017), masks their unpleasant taste and increase dilution to achieve a uniform distribution in the food products when used in very small amounts (Liolios *et al*., 2009). Some studies (Gortzi *et al*., 2007) have also showed that encapsulation can improve antimicrobial activity of compounds and maintains the stability of antimicrobials over prolonged periods of time.

Thus, the present study is aimed to investigate the effect of encapsulated and unencapsulated rosemary extract on the quality of minced silver carp during storage at refrigeration condition (4 ± 1°C).

Materials and Methods

Rosmarinus officinalis was purchased from a local market. The solvent (ethanol) were added to the powdered rosemary in ratio of 1: 10 and the resulting mixtures were shaken overnight to extract thyme's phenol compounds. After 24 h, the extracts were filtered through Whatman No. 42 filter paper to separate thyme particles. The solvents were completely evaporated in an oven at 40°C. Finally, they were placed in a refrigerator. *Escherichia coli* were purchased from the Iranian Research Organization for Science and Technology (Persian Type Culture Collection (1399, PTCC, Tehran, Iran). Gum arabic was obtained from Sigma-Aldrich Chemical Co., USA. All other chemicals were analytical grade and were cibrained from Merch chemical of company (Germany).

- *Encapsulation by spray drying*

Encapsulation of the rosemary extract with gum arabic was carried out according to the method described by Beristain (2001) with some modifications. Solutions of gum arabic were prepared by dissolving 300 g (w/w) of gum arabic powder in distilled water. The obtained solutions were heated at

60˚C with constant stirring for 20 min, covered and left overnight at room temperature. Rosemary extract was added to the hydrated gum solution at extract: the ratio of 1:4 (extract to gum w/w) with respect to gum solids. The mixture was homogenized with a Ultra-Turrax T50 homogenizer (IKA-WERKE Works Inc., Wilmington, NC, USA) at 7000 rpm for 15 min and fed to a Buchii Mini Spray Dryer model 190 (BuchiiLaboratoriums-Technik AG., Flawil, Switzerland). Drying condition for inlet air temperature was 105˚C and for outlet air temperature was 108˚C with a feeding rate of 5.6 ml/min.

- *Fish sample preparation and storage condition*

Fresh silver carp, varying from 1000 ± 100 g in weight were purchased from a public market alive and transferred to Caspian Sea Ecology Research Center in (Iran) in sealed foamed polystyrene boxes containing flaked ice. The fish were gutted, skinned, filleted, and washed by hand. The fish muscle was minced twice. Minced fish were randomly assigned into five batches (100±10 g minced fish in each group). The first batch was prepared without rosemary extract (control batch), four of the batches processed with unencapsulated and encapsulated rosemary extract as following; batch 2 (1% unencapsulated RE), batch 3 (1.5% unencapsulated RE), batch 4 (1% encapsulated RE), batch 5 (1.5% encapsulated RE). For *E.coli* analysis, minced samples were inoculated with 1×10^3cfu/g of *E.coli*. The encapsulated and unencapsulated rosemary extract were added according to the above mentioned treatments. Finally, all samples stored at 4 ± 1 °C for 15 days. Chemical and microbiological analyses were performed at three day intervals to determine the overall quality of the minces.

- *Chemical analyses*

The peroxide value (PV) of the samples was determined in the total lipid extracts according to the method of Pearson (Egan *et al*., 1997).

The total volatile basic nitrogen (TVB-N) of the samples was determined by the micro-diffusion method as described by Goulas and Kontominas (2005).

- *Microbiological analysis*

Bacteriological counts were determined by placing a 10 g of the mince sample in 90 ml of physiological serum, and homogenizing with a stomacher. Total viable count (TVC) and total psychrotrophic count (PTC) of the samples were determined by the pour plate method, using plate count agar (PCA, Merk, Darmstadt, Germany). The inoculated plates were incubated at 37°C for 2 days for total viable counts, and at 10 °C for 7 days for psychrotrophilic counts.

In order to prepare *E. coli*, the thawed culture (0.1 mL) was transferred to 10 mL of BHI broth and grown in a shaker incubator at 37 °C for 24 h. A second transfer of 0.1 mL of culture into 10 mL of BHI broth was grown in a shaker incubator at 37 °C for 24 h to the end of the exponential phase of growth. Subsequently, this appropriately diluted culture was used for the inoculation of silver carp mince in order to obtain a target inoculum of 10^3 CFU/g of *E. coli*. For counting the bacteria, 5 g of minced fish were homogenized with 45 ml of physiological serum. Serial dilutions were made, and counts of *E. coli* were determined by spreading 0.1 ml of serial dilutions on CHRO Magar^{tm} STEC to screen *E. coli*. The plates were incubated at 37°C for 24 h, and the surviving cell population was counted (Kim *et al*., 2014). All counts were expressed as log colony-forming units (CFU/g)and performed in triplicate (Ojagh *et al*., 2010).

- *Statistical analysis*

One-way ANOVA was used and mean comparison was performed by Duncans' new multiple range test. Statistical analysis was prepared using the SPSS statistical software, (release 18.0) for Windows (SPSS Inc. Chicago, IL). All data are presented as mean ±SD. Significant differences were considered at the 95% confidence level ($P < 0.05$).

Results and Discussion

- *Chemical changes*

Changes in PV of silver carp mince during refrigeration storage are shown in Fig. 1. The initial amount of PV was about 0.88 mEq O_2/kg which in different treatments increased until the day 12^{th} and then decreased. At the day 12, the maximum and minimum PV were related to control (6.03 mEq O_2/kg) and 1.5% encapsulated rosemary extract (3.67 mEq O_2/kg), respectively. Generally, the PV of the samples treated with unencapsulated and encapsulated rosemary extracts was significantly ($p < 0.05$) lower than the control during the storage period. These results can be attributed to the well-known antioxidant activity of rosemary which is mainly attributed to its polyphenol contents. As explained by Safari *et al*. (2018) phenolic antioxidants do not function as oxygen absorbers; rather, they prohibit the formation of fatty acid free radicals, which do react with or absorb oxygen in the autoxidation process. This performance postpones the onset of the autoxidative process in fat or oil (Abdollahi *et al*., 2014). This result was in agreement with those reported by Tironi *et al*. (2010), and Tironi *et al*. (2009) about the antioxidant properties of rosemary extract as an effective means of controlling lipid oxidation in minced sea salmon.

In addition, minced silver carp samples treated with gum arabic encapsulated rosemary extract showed significantly lower PV content on compared to the control and minced silver carp containing unencapsulated extract during the storage period ($p < 0.05$). This may show the potential of encapsulation to improve the antioxidant activity of the rosemary extract during application in minced silver carp by prolonging its availability. As mentioned before, encapsulation decreases the reactivity of bioactive compound with the environment (water, oxygen, light), reduces the evaporation or the transfer rate of the active compounds to the outside environment. It also promotes their handling ability, the bioavailability and half-life of the compound (Fang and Bhandari, 2010; Donsì *et al*., 2011). Evidence of encapsulation improving the bioactivity and bioavailability of polyphenols has been reported by a number of researchers (Javadian *et al*., 2017). For instance, Belščak-Cvitanović *et al*. (2011) reported higher total phenol content and antioxidant capacity of raspberry leaf, hawthorn, ground ivy, yarrow, nettle and olive leaf extracts after encapsulation in alginate–chitosan micro beads.

Figure 2 indicates changes in TVB-N values of minced silver carp samples during refrigerated storage. According to Leroi *et al*. (1998), fish muscle with a level of 30 mg TVB-N per 100 g is usually regarded as spoiled. The initial TVB-N value of the minced silver carp samples was in average about 10.14 mg/100 g which revealed the good quality of the fresh minced samples in that, freshwater fish muscle has 10–20 mg/100 g TVB-N after harvesting (Alçiçek, 2011). The value of TVB-N increased progressively with the time of storage for all mince samples. The TVB-N values of the samples in our study exceeded the maximum level by day 9 for control (31.06 mg/100 g) and by day 10 and 11 for minced sample containing 1% and 1.5% unencapsulated rosemary extract. The treatment containing 1.5% encapsulated rosemary extract (30.50 mg/100 g) reached the maximum by day 12.

However, TVB-N content of the minced silver carp samples containing encapsulated and unencapsulated rosemary extract was significantly lower than the control during the storage period ($p < 0.05$). Lower TVB-N content in the minced silver carp treated with rosemary extract may be related to the antibacterial activity of rosemary. Antibacterial compounds like plant extracts can decrease TVB-N production in fish products due to the decreased capacity of bacteria for oxidative deamination of nonprotein nitrogen compounds or both (Banks *et al*., 1980). Several authors have also reported lower TVB-N content in fish samples treated with rosemary extract (Ozogul *et al*., 2010) and other plant extracts like tea polyphenols (Fan *et al*., 2008) during refrigerated storage. Vareltzis *et al*. (1997) also reported lower TVB-N content in filleted and minced frozen horse mackerel and hake.

Moreover, minced silver carp samples containing 1.5% arbaic gum encapsulated rosemary extract showed significantly lower TVB-N content on compared to the minced samples treated with unencapsulated rosemary extract during the storage period ($p < 0.05$). This may also reveal the enhanced antimicrobial activity of the rosemary extract after encapsulation or the better protection of the extract functionality during the processing or storage period. Similarly, Gortzi *et al*. (2006) and Gortzi *et al*., 2007) showed that after encapsulation in liposome, the antimicrobial activity of *Thymus* ssp. And *O. dictamnus* extracts proved to be higher than those of the same extracts in pure form.

- *Changes in total viable and psychrotrophic counts*

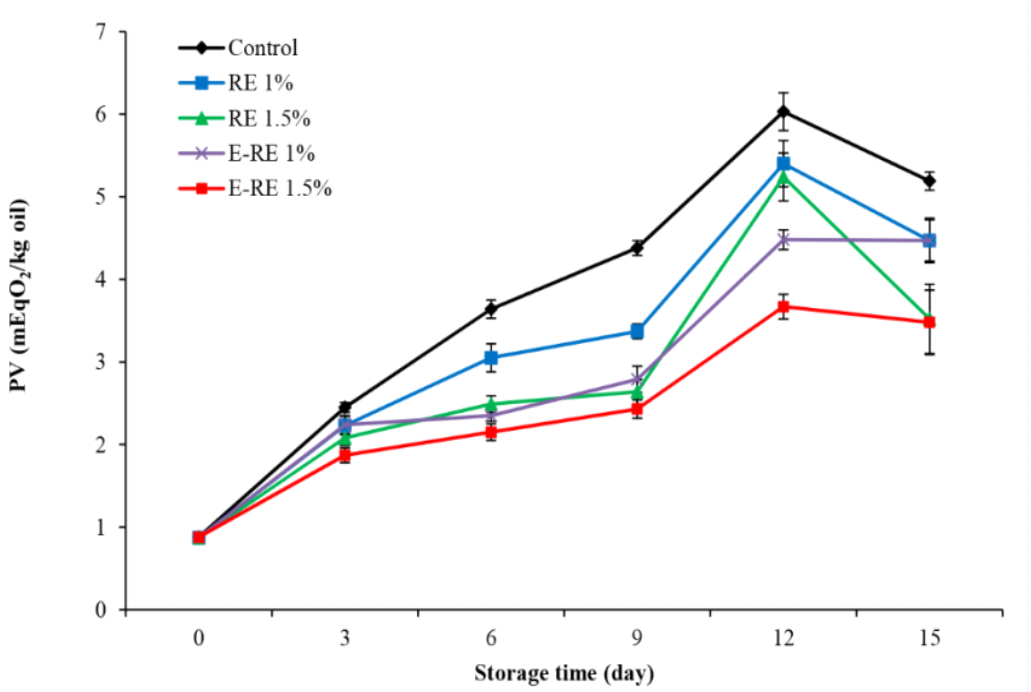

Fig. 1. Changes in the proxide value (PV) of minced silver carp during refrigerated storage. (control: Without rosmary extract, RE 1%: 1% unencapsulated rosemary extract, RE 1.5%: 1.5% unencapsulated rosemary extract, E-RE 1%: 1% encapsulated rosemary extract, E-RE 1.5%: 1.5% encapsulated rosemary extract).

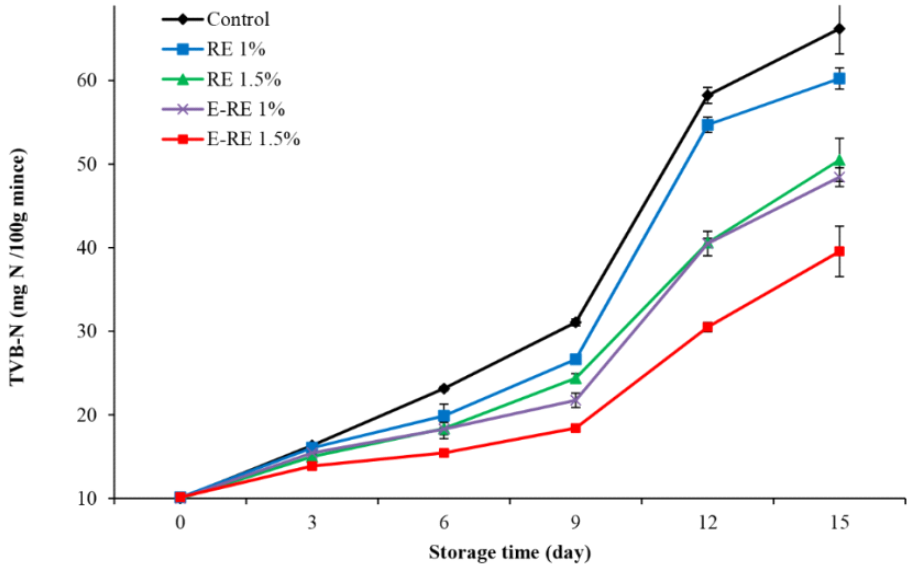

Fig. 2. Changes in the total volatile basic nitrogen (TVB-N) of minced silver carp during refrigerated storage**.** (control: Without rosmary extract, RE 1%: 1% unencapsulated rosemary extract, RE 1.5%: 1.5% unencapsulated rosemary extract, E-RE 1%: 1% encapsulated rosemary extract, E-RE 1.5%: 1.5% encapsulated rosemary extract).

Figure 3 (a and b) shows the variations in total viable (TVC) and psychrotrophic counts (TPC) for the treated and untreated fish mince. The initial TVC and TPC of the silver carp minced samples were respectively 3.66 and 3.55 log CFU/g. It shows the high quality of samples used in the present study (ICMSF, 1986). However, the initial microbial load of freshwater fish may vary depending on the water conditions and temperature in the range of 2-6 log10 CFU/g. Both the TVC and TPC of all treatments increased gradually but the value increased faster for the control. Both the mesophilic and psychrophilic bacteria of control (6.65 and 6.47 log10CFU/g, respectively) exceeded the maximum acceptable limit of 6 log10 CFU/g (ICMSF, 1986) for freshwater and marine fish at the day 6th. As can be observed, all minced samples treated with encapsulated and unencapsulated rosemary extracts significantly inhibited the growth of mesophilic bacteria on compared to the control during the storage period.

These results showed antimicrobial properties of rosemary extract as a well-known source of phenol diterpenes, such as carnosic acid, carnosolrosmanol, isorosmanol and rosmarinic acid known to exert antimicrobial activity (Kykkidou *et al*., 2009). Ozogul *et al*. (2010) also reported that rosemary extract at 1 and 2% could inhibit microbial deterioration of sardine fillets and improve their shelf life for 3 to 6 days, respectively.

Moreover, during the whole storge period, minced silver carp sample containg 1.5% of encapsulated rosemary extracts showed significantly ($P < 0.05$) lower TVC and TPC on compared to the control and unencapsulated RE. The improvement of the antimicrobial activity of natural plant extracts and essential oils when encapsulated into liposomal delivery systems was also reported by others (Gortzi *et al*., 2006; Gortzi *et al*., 2007; Liolios *et al*., 2009; Donsì *et al*., 2011). The encapsulation of eugenol and carvacrol into nanometric surfactant micelles also resulted in improved antimicrobial activity (Gaysinsky *et al*., 2005). A possible explanation is that the use of gum arabic, in order to deliver bioactive agents like plant extract can provide the

necessary protection against their oxidation, while the incorporation of food antimicrobials could aid in the protection of food products against the growth of spoilage and pathogenic microorganisms (Taylor and Davidson, 2005).

- Inhibition the growth of E. coli in minced fish

The effects of encapsulated and unencapsulated rosemary extract on *E. coli* inoculated in silver carp mince are presented in Table 1. As can be observed, the initial population of *E. coli* in the control group increased rapidly during the storage period. Although the sample treated with encapsulated and unencapsulated rosemary

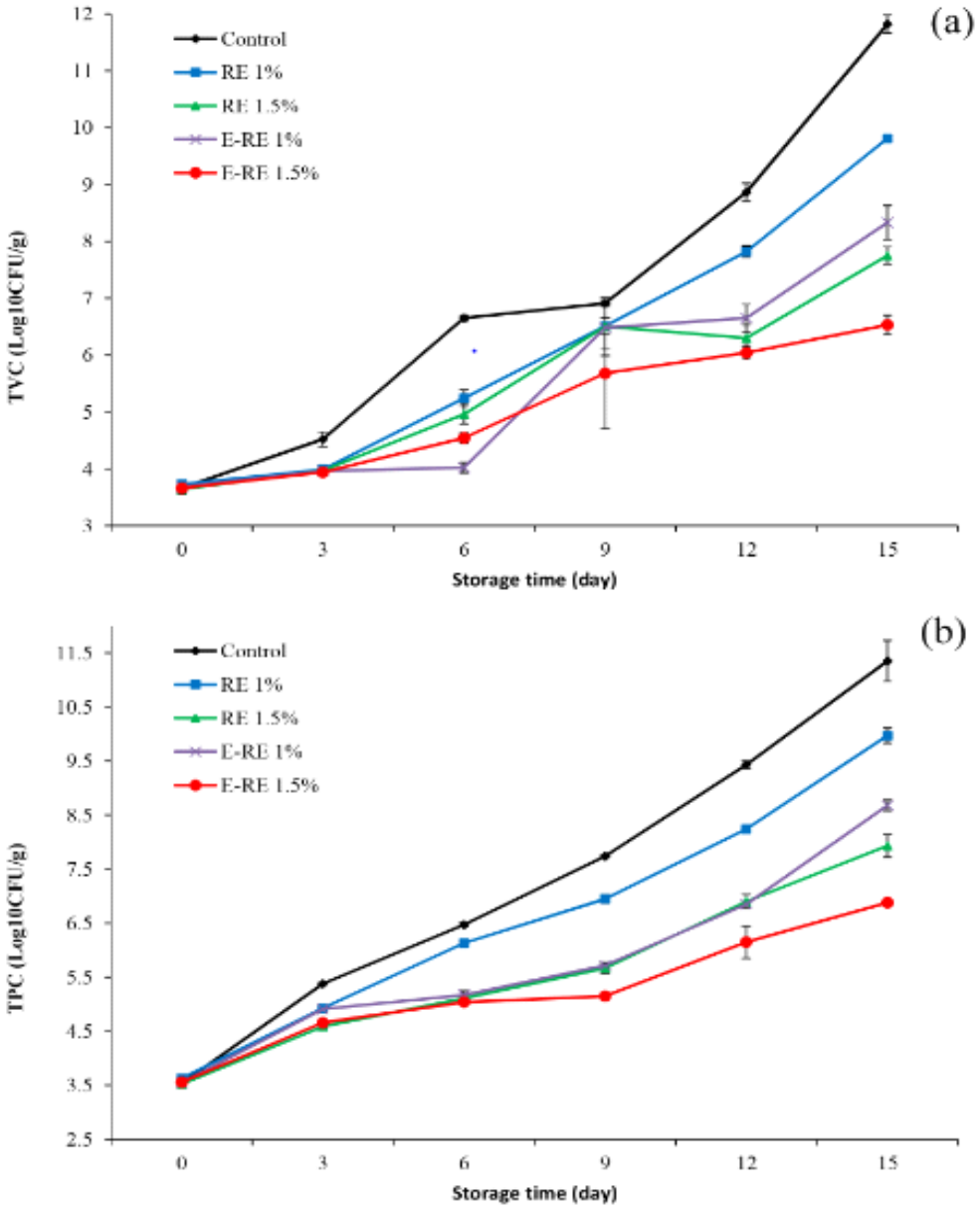

Fig. 3. Changes in (a) total viable count (TVC) and (b) total psychrotrophic count (TPC) of minced silver carp during refrigerated storage. (control: Without rosmary extract, RE 1%: 1% unencapsulated rosemary extract, RE 1.5%: 1.5% unencapsulated rosemary extract, E-RE 1%: 1% encapsulated rosemary extract, E-RE 1.5%: 1.5% encapsulated rosemary extract).

extract showed that the population of *E. coli* significantly ($P < 0.05$) is lowered than the control, they could not inhibit its growth completely during the storage period. However, encapsulated rosemary extract at 1.5% could significantly reduce the population of *E. coli* on compared to the other samples. However, none of the treatments could not reduce its population below the acceptable level (<2) until the end of storage period. Last studies (Bozin*et al*., 2006; Abdollahi*et al*., 2012a) have also reported that rosemary could have good antibacterial effect against *E. coli*as. As mentioned before, antimicrobial characteristics of rosemary are mainly attributed to phenol diterpenes, such as carnosic acid, carnosol, rosmanol, isorosmanol, and rosmarinic acid (Bozin *et al*., 2006; Türe *et al*., 2008). In other hand, encapsulated rosemary extract at 1.5% could reduce the population of *E. coli* significantly during the storage period on compared to other treatments. This coincides with our observation about the TVC and TPC of minced silver carp which revealed the efficacy of gum arabic encapsulation in improving the antimicrobial properties of rosemary extract. Similarly, Liolios *et al*. (2009) reported enhanced antimicrobial activities of thymol and carvacrol after the encapsulation against 12 bacteria including *E. coli*. Donsì *et al*. (2011) also investigated the effect of nanoencapsulation on the antimicrobial activity of terpenes against three different classes of microorganisms (*Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *Escherichia coli*). Their results showed that the increase of the antimicrobial activity depend on the formulation and mean diameter of the delivery systems as well as on the microorganism's class.

Conclusion

The abblity of encapsultaed and unencapsulated rosemary extract in controlling the quality of minced silver carp and *E. coli* inoculated in the mince was studied. Both encapsultaed and unencapsulated rosemary could improve the quality of the mince by reducing PV, TVB-N and microbial changes during the storage period. However, the encapsulated extract could act significantly better than unencapsulated rosemary extract in all the treatments studied, especially at 1.5% RE. However, it could not control the population *E. coli* or reduce it below the acceptable level (<2). Gum arabic encapsulation could help to obtain higher antimicrobial activity in lower rosemary extract concentrations in minced fish.

Table 1. Changes in *E. coli* count (Log10 CFU/g) in minced silver carp samples during storage. (control: Without rosmary extract, RE 1%: 1% unencapsulated rosemary extract, RE 1.5%: 1.5% unencapsulated rosemary extract, E-RE 1%: 1% encapsulated rosemary extract, E-RE 1.5%: 1.5% encapsulated rosemary extract)

Treatment	Storage period (days)					
	0	3	6	9	12	15
Control	3.24 ± 0.16^{a}	4.85 ± 0.04^{a}	6.16 ± 0.12^{a}	7.18 ± 0.27^{a}	8.68 ± 0.12^{a}	9.70 ± 0.13^{a}
RE 1%	3.29 ± 0.03^{a}	4.76 ± 0.09^{ab}	5.16 ± 0.06^{b}	5.80 ± 0.11^{b}	6.62 ± 0.15^{b}	7.20 ± 0.03^{b}
RE 1.5%	3.28 ± 0.10^{a}	4.73 ± 0.04^{b}	5.15 ± 0.08^{b}	5.40 ± 0.08^{c}	6.36 ± 0.54^{b}	6.87 ± 0.03^{c}
E-RE 1%	3.32 ± 0.04^{a}	4.55 ± 0.08^{c}	5.18 ± 0.05^{b}	5.42 ± 0.05^{c}	6.33 ± 0.11^{b}	6.78 ± 0.07^{c}
E-RE 1.5%	3.38 ± 0.07^{a}	3.80 ± 0.06^{d}	4.04 ± 0.04^{c}	4.19 ± 0.09^{d}	4.57 ± 0.06^{c}	4.25 ± 0.15^{d}

^{a,b,c} Different small letters in the same column, represents significant difference ($p< 0.05$).

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