Short communication:

Influence of encapsulated pomegranate peel extract on the chemical and microbial quality of silver carp (*Hypophthalmichthys molitrix* Val. 1844) fillet during refrigerating storage

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Introduction

Fish meat plays an important role in supplement of human protein necessity (Rahimzade *et al.*, 2019).

Fish products deteriorate rapidly as a result of high water activity, neutral pH, relatively large quantities of free amino acids, presence of autolytic enzymes and high percent of unsaturated fatty acids (Duan *et al.*, 2010). This problem and the increasing request for high quality fresh seafood has intensified the search for technologies that favor fresh fish preservation. Different methods have been used for extending fish products shelf life (Rostamzad *et al.*, 2010).

One of the most commonly used methods for fish preservation is cold storage. Nevertheless, it does not sufficiently prohibit the quality deterioration of fish (Jeon *et al.*, 2002) but it can be improved using

antimicrobial and antioxidant compounds.

Moreover, there is an increasing demand for natural antimicrobial and antioxidant preservatives because of the concern about safety of synthetic materials due to possible carcinogenic effects (Ozogul et al., 2010). During last decade, significant interest has been focused on the natural preservatives like plants extract and essential oil as an alternative to synthetic materials. Thus, the determination of the antioxidant and antimicrobial capacity of spices and their derivate in foods is being given greater importance by researchers and those involved in the agro-food industry (Viuda-Martos et al., 2010). Among pomegranate obtained has popularity in recent years due to its multi-functionality and nutritional benefits in the human diet. Besides pomegranate fruit potential for reducing disease risk (Aviram et al., 2000), its

peel which constitutes about 50% of the total fruit weight (Dahham et al., 2010), and it is often discarded as by-products contain higher amounts of polyphenol compounds than the fruit juice, and it shows stronger biological activities (Guleria and Kumar, 2006). In many studies it has been reported that pomegranate peel extracts possess a wide range of biological actions including anti-cancer properties (Kulkarni et al., 2005), antimicrobial properties (Al-Zoreky, 2009), apoptotic and anti-genotoxic activities (Kirilenko et al., 1978), anti-tyrosinase activity (De et al., 1999), anti-inflammatory and anti-diabetic properties (Dahham et al., 2010; Shuhua et al., 2010). Biological activity of the fruit peel extracts is mainly related to their polyphenol compounds such as ellagic tannins, anthocyanins, flavonols. catechin. procyanidins, ellagic acid and gallic acid (Dahham et al., 2010; Fazeli et al. 2011). Several studies have also reported the efficacy of pomegranate juice, seed and peel extracts in meatbased products preservation (Naveena et al., 2008; Devatkal et al., 2010; Kanatt et al., 2010; Topuz et al., 2015; Vaithiyanathan et al., 2011; Zarei et al., 2015).

However, unfortunately, most natural active compounds are biologically instable, poorly soluble in water and they distribute poorly in the 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 handling ability, their the bioavailability and half-life of the compound (Fang and Bhandari, 2010), their unpleasant taste and masks increase dilution to achieve a uniform distribution in the food products when used in a 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 maintain the stability antimicrobials over prolonged periods of time.

Thus, the present study was aimed to investigate the effects encapsulated and un-encapsulated pomegranate peel extract on the quality of silver carp fillets and the possible efficacy of liposomal encapsulation in the improvement of its antimicrobial and antioxidant activities during the preservation of the fillets at 4 °C.

Materials and methods

Materials

Pomegranate fruits were purchased from a local market. Fennel has also purchased from local market. All other chemicals were analytical grade and purchased from Merck Co., Germany.

Preparation of pomegranate peel extract and liposomes

The pomegranates were manually peeled and the peels immediately have been dried at 60 °C for 24 h. The dried

peels were powdered and were extracted with 100 ml of methanol for 24 h according to Paari *et al.* (2011). The extracts having filtered, and were re-extracted with same solvent and concentrated under reduced pressure through rotator evaporator. Liposome has obtained from Sigma-Aldrich Chemical Co., USA.

Liposome, as a carrier for pomegranate peel extract, has been produced according to the method described by Gortzi et al. (2006) with some modifications. Liposome mixture has dissolved in chloroform/methanol (3/1) in a round bottom flask and the organic solvent has removed by a rotary evaporator until a thin film layer has formed on the walls. Pomegranate peel extract was also dissolved dichloromethane/methanol (2/1) mixed with liposome mixture (4/1 ratio, liposome/extract) and the solvents have evaporated under nitrogen steam. The produced lipid film was dissolved in 2 ml of phosphate buffer (10 mM, pH 7) and vortexed for 15 min at 35 °C. The obtained suspension was allowed to hydrate for 2 h in the dark at room temperature and then centrifuged at 6500 rpm at 4 °C. Finally, multilamellar lipid vesicles were obtained by freeze-drying. freeze-drying The process was as follows: (1) freezing at -50 °C for 8 h; (2) primary drying -50°C for 48 h; and (3) secondary drying at 25 °C for 24 h.

Treatment of silver carp fillets by fennel extract

36 live silver carps with an average weight of 1000±100 g were purchased

from a local aquaculture farm. They were transported to the laboratory within an hour in sealed foamed polystyrene boxes containing flaked ice. Then, the fish were gutted, skinned, filleted (100±10 g), and washed up by tap water in a laboratory. Furthermore, a ranking test previously carried out comparing fish samples with encapsulated and un-encapsulated pure pomegranate peel extract at different concentrations showed significantly lower acceptability of the samples incorporating 1.25 or 1.5% encapsulated and un-encapsulated pure peel pomegranate extract compared to the rest (1% or lower) (data not shown). After these results, the encapsulated pure pomegranate peel extract concentration of 0.5% and 1% were chosen as optimal for following study of fish preservation. 15 from each treatment randomly-selected-and divided to one of five treatments as presented in the following:

C: control, without treatment

PPE 0.3: treatment with 0.5% pure pomegranate peel extract

F 0.5: treatment with 1% pure pomegranate peel extract

FE 0.3: treatment with 0.5% encapsulated pomegranate peel extract FE 0.5: treatment with 1% encapsulated pomegranate peel extract

Different concentrations of pomegranate peel extracts having been sprayed on the fillets by syringe. After packaging all samples in polyethylene dishes with cellophane blanket, they were stored at 4±1 °C for subsequent quality assessment. Chemical and

microbiological analyses were performed at 3-day intervals to determine the overall quality of the fish for 15 days.

Chemical analysis

The total volatile basic nitrogen (TVB-N)

TVB-N of the silver carp samples were measured by the micro-diffusion method as described by Goulas and Kontominas (2005). The values were reported in mgN 100g⁻¹ of fish. Measurements were repeated three times for studying repeatability.

Evaluation of lipid oxidation

The colorimetric method described by Kirk and Sawyer (1991) has used to measure the thiobarbituric acid (TBA) value in fish fillets for secondary lipid oxidation products evaluation. All measurements were repeated three times as mentioned above.

Microbiological analysis

The pour plate method was used to determine total viable count (TVC) and total psychrotrophic count (TPC). 10 g of the fish minced sample was aseptically taken and homogenized in 90 ml of sterile 85% NaCl solution with a blender (HBM-400B, HBM Biomed, Tianjin, China) at room temperature. Appropriate dilutions were serially prepared and then 1 ml of each was spread onto plate count agar media (Merck, Darmstadt, Germany). The prepared plates were incubated at 37 °C for 2 days for TVC and at 10 °C for 7 for TPC. All counts were days

expressed as log colony-forming units (CFU) g⁻¹ and performed in triplicate.

Statistical analysis

The differences among all measurements were evaluated by oneway analysis of variance (ANOVA). Duncan's multiple range tests were used to compare the means to identify which were significantly groups different from other groups. Significance was defined at p < 0.05. All data are presented as mean ±SD.

Results and discussion

Changes in total volatile basic nitrogen (TVB-N)

TVB-N is widely studied as an indicator of deterioration of fish muscles and measures the compounds composed of ammonia and primary, secondary and tertiary amines (Fan et al., 2008; Abdollahi et al., 2014). Variations in TVB-N values for silver carp fillets are summarized in Figure 1. According to Leroi et al. (1998), fish flesh with a level of 30 mg TVB-N per 100 g is usually regarded as spoiled. The initial TVB-N value of the silver carp fillets was 10.09 mg 100g⁻¹ which showed the good quality of the fresh samples in that, and freshwater fish muscle has 10-20 mg 100g⁻¹ TVB-N after harvesting (Alçiçek, 2011). Figure 1 showed the value of TVB-N increased progressively with the time of storage for all fish samples. However, TVB-N content of the samples treated with PPE was significantly lower than the control during the storage period (p<0.05). TVB-N content of the control samples reached 31.47 mg 100g⁻¹ while by day

12 while it was 28.20 in samples treated with 1% pomegranate encapsulated extract. The TVB-N values of the samples exceeded the maximum level by day 9 for control and by day 12 for samples treated with 0.5 and 1% PPE. Lower TVB-N content in the fillets treated with encapsulated pomegranate peel extract may be related to the antibacterial activity of the extract. Antibacterial compounds like plant extracts can reduce TVB-N production due to the decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks et al., 1980). Dahham (2010)described the antibacterial activities of pomegranate peel extract

(rind), seed extract, juice and whole fruit on the selected bacteria. According to their results, the peel extract showed highest antimicrobial compared to other extracts. Opara et al. (2009) and Al-Zoreky et al. (2009) also reported appreciable antimicrobial activity for pomegranate peel extract against selected strains of bacteria and pathogenic fungi. Gokoglu et al. (2009) studied the effects of pomegranate sauce on the quality of marinated anchovy during refrigerated storage. Similarly, they have found lower TVB-N values for samples in pomegranate sauce samples compared to those in sunflower oil.

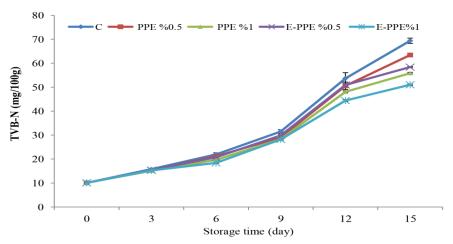


Figure 1: Changes in total volatile base nitrogen (mg N_2 100g⁻¹) value of silver carp fillets during storage.

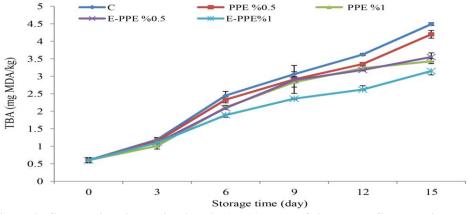


Figure 2: Changes in thiobarbituric acid (TBA) value of silver carp fillets during storage.

Table 1: Changes in total viable count (TVC) and total psychrotrophic count (TPC) of silver carp fillets during storage.

Attributes	Treatment	Storage period (days)					
		0	3	6	9	12	15
TVC (log ₁₀ cfu g ⁻¹)	С	4.28 ± 0.07^{a}	5.27 ± 0.04^{a}	7.47 ± 0.03^{a}	9.67 ± 0.05^{a}	10.53 ± 0.17^{a}	10.94 ± 0.02^{a}
	PPE 0.5%	4.28 ± 0.07^{a}	5.03 ± 0.06^{b}	5.87 ± 0.06^{b}	6.66 ± 0.09^{b}	7.83 ± 0.02^{b}	8.67 ± 0.03^{c}
	PPE 1%	4.28 ± 0.07^{a}	4.91 ± 0.03^{c}	5.79 ± 0.06^{bd}	6.70 ± 0.02^{b}	7.78 ± 0.04^{b}	8.77 ± 0.02^{b}
	EPPE 0.5%	4.28 ± 0.07^{a}	4.94 ± 0.02^{c}	5.78 ± 0.01^{bd}	6.69 ± 0.06^{b}	7.76 ± 0.03^{b}	8.68 ± 0.04^{c}
	EPPE 1%	4.28 ± 0.07^{a}	4.77 ± 0.05^{d}	5.68 ± 0.06^{c}	6.61 ± 0.02^{c}	7.66 ± 0.01^{b}	8.75 ± 0.01^{b}
TPC (log ₁₀ cfu g ⁻¹)	C	4.16 ± 0.01^{a}	5.40 ± 0.03^{a}	7.67 ± 0.06^{a}	9.77 ± 0.01^{a}	10.68 ± 0.04^{b}	10.98 ± 0.0^{a}
	PPE 0.5%	4.16 ± 0.01^{a}	5.27 ± 0.11^{a}	5.88 ± 0.01^{b}	6.64 ± 0.10^{c}	7.77 ± 0.04^{b}	8.70 ± 0.04^{c}
	PPE 1%	4.16 ± 0.01^{a}	5.03 ± 0.06^{b}	5.87 ± 0.04^{b}	6.79 ± 0.01^{b}	7.80 ± 0.04^{b}	8.79 ± 0.02^{b}
	EPPE 0.5%	4.16 ± 0.01^{a}	5.05 ± 0.07^{b}	5.85 ± 0.04^{b}	6.71 ± 0.05^{b}	7.79 ± 0.02^{b}	8.71 ± 0.04^{c}
	EPPE 1%	4.16 ± 0.01^{a}	5.87 ± 0.04^{c}	5.84 ± 0.01^{b}	6.73 ± 0.02^{b}	7.69 ± 0.01^{c}	8.78 ± 0.01^{b}

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

In other hand, samples treated with liposomal encapsulated pomegranate peel extract showed significantly lower TVB-N content compared to the control and fillets treated with pure extract during the storage period (p<0.05). This observation may be explained by the enhanced antimicrobial activity of the extract after encapsulation or better protection of their functionality during the processing or storage period. Gortzi et al. (2007) also reported that after encapsulation liposome, in antimicrobial activity of Origanum dictamnus extracts proved to be higher than those of the same extracts in pure form.

Lipid oxidation

Changes in thiobarbituric acid (TBA) values has been used to exhibit the degree of lipid oxidation as second stage auto-oxidation during chilled storage of silver carp fillets (Fig. 2). Presents the TBA values of different treatment groups during the storage period. As shown, the initial value of TBA was around 0.6 mg MDA kg⁻¹, close to the value reported for silver carp by Fan *et al.* (2009). The TBA

value of the silver carp fillets increased through the whole storage period, especially in the control samples (reached to 4.29 mg MDA kg⁻¹) which shows secondary lipid oxidation in the samples. However, the TBA value of the samples treated with pomegranate peel extract (reached to 3.43 and 3.25 mg MDA kg⁻¹ in PPE 1% and EPPE 1%, respectively) was significantly lower than the control during the storage, indicating the pomegranate peel extract could be effective in reducing lipid oxidation. Other authors have also been reported strong antioxidant properties for the ethanol extracts of pomegranate peel during in vitro studies (Negi and Jayaprakasha, 2003; Kanatt et al., 2010; Fazeli et al. 2011) which was explained by their high phenolic content. It has been well confirmed that phenolic compounds are able to donate a hydrogen atom to the radicals, thus stopping propagation chain reaction during lipid oxidation process (Singh et al. 2006). Kanatt et al. (2010) studied the effect of 0.1 and 0.5% pomegranate peel extract on the oxidative stability of chicken products and found significantly lower TBA content in the samples treated with PPE. O"zen et al. (2011) results showed that the formation of lipid hydro peroxides and thiobarbituric acidreactive substances was significantly inhibited by pomegranate seed extract addition when compared with control in mackerel. Similarly, minced addition of pomegranate peel extract to goat fish (Paari et al., 2011), halibut fillets (Ünalan and Korel, 2011), silver carp (Zarei et al., 2015) and anchovy fish oil (Topuz et al., 2015) could prohibit lipid oxidation and formation during refrigerated storage.

samples treated Likely, liposomal encapsulated pomegranate peel extract showed significantly lower TBA content compared to the control and fillets treated with pure extract during the storage period (p<0.05). This may show the potential of liposomal encapsulation to improve antioxidant activity of the pomegranate peel extract during application on the fish fillet by prolonging its availability. As mentioned before, encapsulation decreases 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 liposomes improving the bioactivity and bioavailability of polyphenols has reported by a number researchers (Fang and Bhandari, 2010). For example, Gortzi et al. (2007) reported higher antioxidant activity of O. dictamnus extracts after encapsulation in liposome.

Changes in total viable and psychrotrophic counts

The changes in total viable counts (TVC) with the storage period for the treated and untreated silver carp fillets are summarized in Table 1. The initial TVC of the samples was low (3.44 log₁₀cfu g⁻¹), indicating the high quality of fish fillets used in this study (ICMSF, 1986). TVC of all samples increased with storage time and the value of control increased faster and exceeded the maximum 10⁶ log₁₀cfu g⁻¹ after 6 days and reached to 10.94 Log CFU g⁻¹ This acceptability limit of 10⁶ CFU g-1 has been recommended for fresh fish (ICMSF, 1986). TVC of the fillets treated with pomegranate peel extract increased gradually and reached to 8.67, 8.77, 8.68, and 8.75 log_{10v}CFU g⁻¹ for PPE 0.5, PPE 1, EPPE 0.5 and EPPE 1, respectively, at the end of storage period. As a result, treatments significantly inhibited (about 2 Log) the growth of mesophilic bacteria in silver carp compared with the control samples during the storage period. A similar trend was also observed about psychrotrophic counts in all treatments (Table 1). The lower TVC and TPC observed in samples treated with pomegranate peel extract can be related to the antibacterial activity of the extract. Dahham (2010) described the antibacterial activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria. According to their results, the peel extract showed highest antimicrobial activity compared to other extracts. Opara et al. (2009) and Al-Zoreky et al. (2009) also have reported appreciable antimicrobial activity for pomegranate peel extract against selected strains of bacteria and pathogenic fungi. Our results coincide with those reported by Naveena et al. (2008) and Vaithiyanathan et al. (2011) showed using pomegranate which (Punica granatum) rind powder extract and fruit juice phenolic solution could inhibit the growth of microorganism in chicken patties and chicken meat, respectively, during refrigeration storage. Similar observations have been reported by Zarei et al. (2015) about carp filler treated with silver pomegranate peel extract combined with chitosan nanoparticles.

Furthermore, in the present study, the lowest TVC and TPC have been observed in the samples treated with encapsulated pomegranate peel extract (Table 1). The improvement of the antimicrobial activity of natural plant and essential oils extracts encapsulated into liposomal delivery systems has 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).

The effects of encapsulated and unencapsulated pomegranate peel extract on the quality of refrigerated silver carp fillet has studied. Results have showed that the extract could reduce chemical deterioration and lipid oxidation in the fillets compared to the control, as reflected with lower TVBN and TBA values. Also, pomegranate peel extract reduced TVC of the fillets about 2 Log10CFU g⁻¹ compared with control. Moreover, the efficacy of the extract was improved with liposomal encapsulation.

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