

Chemical and antimicrobial properties of silver carp surimi enriched by Thyme leaves extract

R. Farahmandfar^{*1}, R. Safari², F. Ahmadi Vavsari², T. Bakhshandeh²

Received: 2018.03.10 Accepted: 2018.10.24

Abstract

In this study, effect of Thyme (*Thymus vulgaris*) ultrasonic extract on the quality of *Hypophthalmichthys* molitrix surimi was evaluated. For this purpose, the leaves of *Thymus vulgaris* were dried, grinded and soaked in methanol (1:10 w/v) for 30 min at 45°C and sonicated at 30 kHz for 15 min at 40°C. The extract was suspended by Dimethyl sulphoxide and mixed with surimi (0.4 and 0.8% w/w). Then, the analysis of chemical (free fatty acid, peroxide value, Thiobarbituric acid and total volatile base nitrogen) and microbial (mesophilic and psychrotrophic viable count) properties of the samples were done at specific intervals after zero, 4, 8, 12 and 16 days of storage at 2 °C. Results of chemical and microbial analysis showed that 0.8% concentration of *T. vulgaris* could increase the shelf life of *Hypophthalmichthys molitrix* surimi and there is significant difference between control and treated samples. Moreover, the results could be claimed that the *T. vulgaris* due to marvelous antioxidant and antimicrobial component such as thymol (52.17%), ρ -cymene (14.42%), carvacrol (9.11%) and γ - terpinene (4.45%) has significant effect on preventing the *Hypophthalmichthys molitrix* surimi oxidation and microbial growth. The results also showed ultrasound was the effective way to extract the *Thymus vulgaris* beneficial compounds.

Keywords: Antioxidant; Fish; Shelf life; Surimi, Thyme

Introduction

Rancidity caused by oxidation of fish lipids is one of the major problems encountered in fish processing, producing off-flavor and reducing their nutritional value (M. Asnaashari, Farhoosh, & Sharif, 2014). Many efforts have been carried out for supplying fresh fish according to consumer's demand (Farhoosh *et al.*, 2016).

The use of natural antioxidants is one of interest to processing industry (Asnaashari *et al.*, 2015; Eshghi *et al.*, 2014). Many natural compounds have been extracted and used as antimicrobial agents in food preservation like essential oils and extracts from herbal plants acting against food spoilage and suppressing a wide variety of pathogens (Asnaashari *et al.*,

2015; Asnaashari et al., 2016; Farahmandfar, et al., 2015). Thyme (Thymus vulgaris), belongs to Lamiaceae family (Gandomi et al., 2009) is extensively used as a flavor ingredient in a wide variety of food in Iran. This plant possesses carvacrol, thymol as main phenolic compounds and p-cymene as main nonphenolic compounds (Sharififar et al., 2007). Its antioxidant property is due to free radical production chain with one hydrogen atom to break up fat oxide and subsequent delay that able to inhibit linoleic acid oxidation (Asnaashari et al., 2015). Thyme has also antibacterial effect and could be used as an antibacterial preservative in hamburger or other meat products (Sharafati Chaleshtori, et al., 2013).

Production of fish protein ingredients such as surimi, minced fish and fish protein isolate is common (Farahmandfar *et al.*, 2016; Ruusunen & Puolanne, 2005; Shaviklo & Johannsson, 2006). Surimi contains about 76% water, 15% protein, 6.85% carbohydrate, 0.9% fat and 0.03% cholesterol (Chaijan *et al.*, 2004; Jin *et al.*, 2007). Suitable properties of surimi such as light color, bland odor, low fat

^{1.} Assistant Professor, Department of Food Science and Technology, Sari Agricultural Sciences & Natural Resources University (SANRU), Sari, Iran.

^{2.} PhD Student, Department of Food Science and Technology, Sari Agricultural Sciences & Natural Resources University (SANRU), Sari, Iran.

^{(*} Corresponding author: r.farahmandfar@sanru.ac.ir) DOI: 10.22067/ifstrj.v0i0.71323

content and high in myofibrillar proteins as well as considerable functional characteristics due to the unique gelling property of its concentrated proteins, make surimi as a perfect functional ingredient to make up new food products (Lanier *et al.*, 2013).

recent In years, ultrasound-assisted maceration (UAM) has received considerable attention for the recovery of different compounds from various sources. This technique is attractive because of its simplicity and low equipment cost and temperature (avoid thermal damage), high efficiency and saving time of extraction compared with solvent extraction. It has been suggested that improvement of solvent extraction from material by ultrasound is due to mainly the mechanical effects of acoustic cavitation, which enhances mass transfer and solvent penetration into the plant material by disrupting the cell walls (Farahmandfar et al., 2015)

The aim of this study was to use of ultrasound-assisted maceration for extraction of *T. vulgaris* and its effect on quality properties of *Hypophthalmichthys molitrix* surimi after 0, 4, 8, 12 and 16 days of storage at 2° C.

Materials and Methods

Materials

T. vulgaris wild leaves were collected from Mazandaran (north of Iran), and dried in oven (Behdad, Iran) then leaves were powdered by mixer (Panasonic, MK-G20NR). 5 Silver carp (Hypophthalmichthys molitrix) were purchased from warm water fish farm in Mazandaran (north of province Iran). Average characteristics of fish were male, 1 year, 40 cm length, 10 cm width and 1.25 kilograms. They were transferred to laboratory under cool chain and stored at 2°C for 4h for further processing. All chemical reagents used for experiments were analytical grade.

Ultrasound-assisted extraction

Crushed dried *T. vulgaris* leaves were added to appropriate methanol (1:10 w/v) in a sample bottle. The leaves were extracted in a

shaking water bath for 30 min at 45°C. The mixture was sonicated with an ultrasonic probe system (Branson, 8510R-Mt, Canada) at 30 kHz for 15 min at 40°C. The extracts were filtered through Whatman No.1. The organic solvents were concentrated to near dryness using rotary evaporator bath (Buchi EL 141, Switzerland) under reduced pressure (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004).

GC-MS analysis

The GC-MS was carried out on an Agilent model 5975C USA mass spectrometry operating in the ionizing energy mode at 70 eV, combined with the GC. The separation was done on a 30 m \times 0.25 mm column coated with $0.25 \ \mu m$ HP5-MS. The analytical conditions were as follows: carrier gas, He, 1.3 mL/min in the constant flow mode; injector temperature, 250 °C; injection volume, 1 μ L; split ratio, 15:1; temperature program, 2 min at 40 °C, raised at 3 °C/min to 180 °C, raised at 10 °C/min to 280 °C; transfer line to MSD, 280 °C; MSD, 170 °C. The ionization energy was 70 eV. The range m/z 40-300 was scanned at a rate of 0.52 scans/s. A mixture of the nalkanes (C_9 - C_{30}) was analyzed under the same conditions to calculate the retention indices. The compounds were identified according to their mass spectra and their retention indices (Sparkman, 2005).

Sample preparation

The fish were eviscerated, deboned and washed prior to be minced by a meat grinder (Panasonic, MK-G20NR), then the minced fish fillets were processed to surimi. The process involved three washing steps by cold water $(10\pm1^{\circ}C)$ for removing water soluble proteins and then dewatered using cloth filtration. In each step, the ratio of water to the minced meat was 3:1 (v/w). During each washing step, the mixture was stirred for 5 min. For more dewatering, at the third step, 0.2% NaCl was added to the mixture (Moosavi-Nasab et al., 2005). The extract was suspended by Dimethyl sulphoxide (DMSO) and two concentrations of ultrasound-assisted extraction of T. vulgaris (0.4 and 0.8%) were

added to surimi. The surimi was packaged in 100 g containers without open air exposure and evaluated in triplicate for microbial and chemical properties after zero, 4, 8, 12 and 16 days of storage at $2\pm1^{\circ}$ C. Control experiment was surimi untreated without any *T. vulgaris* extract.

Chemical analyses

Determination of free fatty acids (FFA)

The lipid extraction was performed using the ratio of 20 parts of 2:1 dichloromethane/methanol to 1 part of tissue. A weak salt solution (e.g., 0.66% NaCl) was then added to achieve a final ratio of 8:4:3 dichloromethane/methanol/water including the water contained within the tissue. The dichloromethane phase was concentrated in a rotary evaporator to 20 mL at ambient temperature. The flask was totally covered with a black material to avoid light influence. The lipid extract was deposited in tubes. All of the solvent was evaporated with nitrogen, and 3 mL of cyclohexane was added, followed by 1.0 mL of cupric acetate-pyridine reagent with agitation of the biphasic system for 30 s. After centrifugation at 2000g for 10 min, the upper layer was read at 710 nm (Bernárdez et al., 2005).

Measurement of peroxide value (PV)

Peroxide value (PV) was measured according to the AOAC method (Willian, 2000). Three samples (each sample 5 g) were weighed in a 250-mL glass and heated in a water bath at 60 °C for 3 min, then thoroughly agitated for 3 min with 30 mL acetic acidchloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman No. 1. Saturated potassium iodide solution (0.5 mL) was added to the filtrate, which was transferred into titrator equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/L). PV calculated and expressed was as milliequivalent of oxygen per kg of sample:

$$PV = \left(\frac{S \times N}{W}\right) \times 1000 \tag{1}$$

Where: S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N=0.01), and W the sample weight (kg) (Namulema *et al.*, 1999).

Determination of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid value was determined colorimetrically (Pezeshk et al., 2011). A portion (200 mg) of sample was weighed into a 25 ml volumetric flask. An aliquot (1 ml) of 1-butanol was added to dissolve the sample. A portion (5.0 ml) of the mixture was added into 5 ml of TBA reagent. The test tubes were vortexed and placed in a water bath at 95°C for 120 min, then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde equivalents/kg of tissue) was obtained by the formula.

$$TBA = \frac{50 \times (As - Ab)}{200} \tag{2}$$

Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was determined by distillation after the addition of magnesium oxide to fillet sample (Willian, 2000). The distillate was collected in an Erlenmeyer containing 3% aqueous solution of boric acid and mixed indicator from dissolution of 0.1 g of methyl red and 0.05 g of methylene blue to 100 ml of ethanol 96%. Finally, the boric acid solution was titrated with a 0.1 N hydrochloric acid solution (Namulema *et al.*, 1999).

Microbiological analyses

Ten grams of fish fillet samples (3 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) in triplicate. 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 in the samples, at each of

the pre-determined time intervals, during refrigerated storage. Mesophilic and psychrotrophic viable count (MVC and PVC) were determined by inoculating 0.1 mL of the sample homogenate onto triplicate sterile plates of dried Tryptic Soy Agar (Liofilchem, Italy) using the surface spread technique, then the plates were incubated for 48 h at 35 °C and for 10 days at 2°C for MVC and PVC, respectively. All counts were expressed as log CFU/g (Amin, 2012).

Statistical analysis

The obtained data were subjected to oneway analysis of variance using SPSS statistical software, release 18.0. Duncan's new multiple range test was performed to determine the significant differences of the means at the 5% probability level (P<0.05).

Results and Discussion

GC-MS analysis of thyme extract

According to GC-MS analysis, the main phenolic and non-phenolic active compounds in Thyme UAM extract were as: thymol (52.17%), ρ-cymene (14.42%), carvacrol (9.11%), y- terpinene (4.45%), a- terpineol (1.25%), sabinene hydrate (1.56%), linalool (7.32%) and geraniol (1.78%). Thymol, pcymene, carvacrol, γ - terpinene, α - terpineol, sabinene hydrate, linalool and geraniol were the most important compounds in Thymus. In comparison with other studies, recovery of different compounds in conventional method (solvent extraction) were more than UAE technique, however there was not any significant difference (Chizzola et al., 2008). In the present experiments, quantitative changes observed in the contents of yterpinene, p-cymene, carvacrol and thymol can be attributed to their localization in the biosynthetic (Kowalski pathway & Wawrzykowski, 2009). The increase of γ terpinene concentration may result from the UAM influences on the shift of chemical balance through the conversion of thymol, carvacrol, and *p*-cymene in the biosynthetic pathway in the direction of γ -terpinene synthesis. Researchers found a reduction of the content of important components in tea extract after ultrasonically assisted extraction (Xia, *et al.*, 2006). The quantity of these compounds can be also varied due to harvesting season, plant age, soil, climate, geographical sources and herb drying method (Bagamboula *et al.*, 2004; Shafiee & Javidnia, 1997).

Chemical analyses of surimi Free Fatty Acids (FFA)

Free Fatty Acids in fish muscle develop undesirable flavors and tissue damage by a combination of muscle protein. They also accelerate the degeneration and loss of product quality and increase of fat oxidation (Sayyari & Farahmandfar, 2016). The results of FFA in different treatments of Hypophthalmichthys molitrix surimi were shown in Table 1. The initial FFA value in all samples at first was 0.41 - 0.51 oleic acid. According to the results, the amount of FFA in all treatments was increased with the passage of time and reached 4.17, 2.43, and 2.11% oleic acid for control, 0.4 and 0.8% thyme, respectively. Similar results were reported by (Zolfaghari et al., 2011) study. Although the amount of FFA was increased during storage, but this increase was not regular. The time and concentration had a significant effect on the formation of FFA during storage. The results showed that in the higher concentrations of thyme, formation of FFA was less compared to the other treatments.

 Table 1. FFA formation in Hypophthalmichthys molitrix surimi samples including 0.4% and 0.8% ultrasound thyme extracted during storage

	dui ing stor	" 5°	
Storage time (days)	Control	0.4% Extract	0.8% Extract
zero	0.51 ± 0.02^{aE}	0.41 ± 0.02^{aE}	0.45 ± 0.05^{aE}
4 1.21 ± 0.02^{aD}		0.64 ± 0.04^{bD}	0.52 ± 0.07^{bD}
8	2.35 ± 0.04^{aC}	1.29 ± 0.04^{bC}	0.95±0.03°C
12	3.12±0.03 ^{aB}	1.85 ± 0.07^{cB}	1.43 ± 0.08^{dB}
16 4.17±0.12 ^{aA}		2.43 ± 0.05^{cA}	2.11 ± 0.08^{dA}

Means with the same small letters in a raw and capital letters in a column were not significantly different (P < 0.05).

Peroxide value (PV)

PV values in treated and control samples of *Hypophthalmichthys molitrix* surimi are shown in Figure 1. The initial PV value in all samples at the beginning of storage period was 0.83-0.86 meqO₂/kg. PV values after 16 days of

storage period were 5.13, 4.24 and 4.13 meqO₂/kg for control, 0.4 and 0.8% of thyme UAM extracts, respectively. Significant lower PV value was observed for treated samples during the storage period at 4°C (P<0.05).



Fig. 1. Hydroperoxide formation (PV) in Hypophthalmichthys molitrix surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

The PV value is an index of lipid oxidation measuring primary oxidation products (Farhoosh et al., 2016). Fish are very susceptible to both microbiological and chemical deteriorations, due to their chemical composition (Goulas & Kontominas, 2007). Storage of food products is accompanied by oxidation of unsaturated fatty acids. This process is important in seafood due to higher poly-unsaturated fatty acid content. Degradation of products formed during oxidation of unsaturated fatty acids is followed by the formation of low-molecular-weight volatile compounds, which accounts for foreign shades of odor and flavor (Misharina & Polshkov, 2005).

The primary value of PV in *Hypophthalmichthys molitrix* surimi was lower than raw and unwashed compared samples of *Hypophthalmichthys molitrix* reported by Asgharzadeh, Shabanpour, Aubourg, and Hosseini (2010). The results of present study indicate that the UAM is effective in retarding

the production of primary lipid oxidation. Similar results, conventional maceration, were obtained by Mexis, Chouliara, and Kontominas (2009) and Ojagh, Rezaei, Razavi, and Hosseini (2010). The major protective effect of UAM thyme extract is owed to its carvacrol, thymol content (Mexis *et al.*, 2009). Similar consequences were expressed by Ojagh *et al.* (2010).

Thiobarbituric acid reactive substances (TBARS)

TBA value in treated and control samples of *Hypophthalmichthys molitrix* surimi are shown in Table 2. The initial TBA value in all samples at first was 0.55-0.65 mg of malonaldehyde/kg fat. TBA value increased with time of storage at 2°C for all treatments and reached 3.65, 3.22, and 2.87 for control, 0.4 and 0.8% thyme, respectively. Significant lower of TBA value was observed for treated samples and 0.8% of thyme during the storage period at 4°C (P<0.05). TBA values of control and 0.4% samples reached the maximal

recomm	nended lin	nit (l	Lakshn	nanan,	2000)	for
	values					
malonal	ldehyde/kg	g of t	issue)	at 8 th	of stora	age,

while for 0.8% treatment, TBA content was still lower than upper acceptability limit.

Table 2- TBA value changes in <i>Hypophthalmichthys molitrix</i> surimi samples including 0.4% and 0.8% ultrasound thyme
extracted during storage

Storage time (days)	Control	0.4% Extract	0.8% Extract
zero	0.55±0.01 ^{aE}	0.65±0.05 ^{aE}	0.61±0.03 ^{aE}
4	1.85 ± 0.05^{aD}	1.32 ± 0.02^{bD}	1.25 ± 0.04^{bD}
8	2.65±0.12 ^{aC}	2.25±0.11 ^{bC}	1.84±0.01 ^{cC}
12	3.25±0.17 ^{aB}	2.56 ± 0.02^{bB}	2.29±0.04 ^{cB}
16	3.65±0.23 ^{aA}	3.22±0.06 ^{bA}	2.87±0.03cA

Means with the same small letters in a raw and capital letters in a column were not significantly different (P < 0.05).

Thiobarbituric acid (TBA) is a common indicator for the assessment of degree of lipid oxidation (Manju *et al.*, 2007). Results of Pezeshk *et al.* (2011) indicated that the effect of the turmeric extract and shallot extract on the rainbow trout samples decreased the TBA value and increased the shelf life of fish sample during the refrigerated storage. Similar results have been reported by Mexis *et al.* (2009) during storage of oregano essential oiltreated rainbow trout fillets at 2°C.

Total volatile basic nitrogen (TVB-N)

TVB-N values in treated and control samples of *Hypophthalmichthys molitrix* surimi are presented in Fig. 2. The initial TVB-N value in all samples at first was 11.22-11.27 mg N/100g. TVB-N values increased progressively with time of storage at 2°C for all treatments and reached to 65.7, 53.98 and 38.13 for control, 0.4 and 0.8% thyme UAM extracts, respectively. However, significant lower TVB-N value was observed for treated samples during the storage period at 2°C (P<0.05). TVB-N values of control and 0.4% samples reached the upper acceptability limit set by the EU (European-Union, 1995) for TVB-N values of fish (30 mg/ 100 g of fish flesh) at day 12 of storage, while for 0.8% treatment, TVB-N content was still lower than upper acceptability limit.



Fig. 2. TVB-N value changes in Hypophthalmichthys molitrix surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

TVB-N content in fish muscle is not only different among species but also is variable in same species due to age, sex, season and environment (Razavi Shirazi, 2007). Some researchers concluded that TVB-N was not a good quality index for fish (Mexis et al., 2009), but it could be used as a quality index. Because increasing TVB-N content of fish samples during storage is directly related to the activity of spoilage bacteria and endogenous enzymes (Goulas & Kontominas, 2007; Özogul, Polat, & Özogul, 2004). TVB-N is composed of different compounds including ammonia, methylamine, dimethylamine as well as trimethylamine (Razavi Shirazi, 2007) which are produced by spoilage bacteria and endogenous enzymes.

The lower TVB-N content in samples treated by thyme UAM extract may be attributed to the antibacterial properties of phenolic compounds such as carvacrol, thymol and linalool. Low levels of TVB-N in treated samples can be attributed to either decreased bacterial population or reduced capacity of bacteria for oxidative deamination of nonprotein nitrogen compounds or both (Manju *et al.*, 2007), which was due to the effect of thyme UAM extract. Similar results have been reported by Fahimdezhban, *et al.*, (2014) regarding to Zataria multiflora and Rosemarinus officinalis extracts on quality of minced frozen Hypophthalmichthys molitrix.

Bacteriological analysis of surimi

Changes in MVC and PVC of control and two concentrations of thyme UAM in *Hypophthalmichthys molitrix* surimi, during the refrigerated storage, are shown in Table 3. The initial MVC and PVC (log CFU/g) of samples were from 3.28 to 3.48 and from 3.24 to 3.34 ranges, respectively. By the day 12 of storage, however, MVC and PVC in 0.8% of thyme UAM was still below 7 log CFU/g, while that of controls and 0.4% of thyme UAM attained a count of 8.45 and 7.44 for MVC and 8.20 and 6.47 for PVC. There was a significant difference between control, 0.4 and 0.8% of thyme UAM (P<0.05).

 Table 3- MVC and PVC changes in Hypophthalmichthys molitrix surimi samples including 0.4 and 0.8% ultrasound thyme extracted during storage

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$							
zero 3.48 ± 0.28^{aE} 3.47 ± 0.19^{aE} 3.28 ± 0.14^{aE} 3.25 ± 0.09^{aE} 3.34 ± 0.06^{aE} 3.24 ± 0.07^{aE} 4 5.44 ± 0.19^{aD} 4.59 ± 0.2^{bD} 4.26 ± 0.1^{bD} 5.31 ± 0.10^{aD} 4.35 ± 0.09^{bD} 4.19 ± 0.03^{bD} 8 6.72 ± 0.37^{aC} 6.07 ± 0.22^{bC} 5.49 ± 0.16^{cC} 6.37 ± 0.04^{aC} 5.51 ± 0.09^{bE} 5.23 ± 0.10^{cC} 12 8.45 ± 0.19^{aB} 7.44 ± 0.2^{bB} 6.36 ± 0.1^{cB} 8.20 ± 0.04^{aB} 6.47 ± 0.09^{bB} 6.24 ± 0.08^{cB}	Stanaga tima (dava)	MVC			PVC		
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Storage time (days)	Control	0.4%Extract	0.8% Extract	Control	0.4%Extract	0.8% Extract
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	zero	3.48±0.28 ^{aE}	3.47±0.19 ^{aE}	3.28±0.14 ^{aE}	3.25±0.09 ^{aE}	3.34±0.06 ^{aE}	3.24 ± 0.07^{aE}
$12 \qquad 8.45 \pm 0.19^{aB} 7.44 \pm 0.2^{bB} 6.36 \pm 0.1^{cB} \qquad 8.20 \pm 0.04^{aB} 6.47 \pm 0.09^{bB} 6.24 \pm 0.08^{cB}$	4	5.44±0.19 ^{aD}	4.59 ± 0.2^{bD}	4.26±0.1 ^{bD}	5.31±0.10 ^{aD}	4.35 ± 0.09^{bD}	4.19±0.03 ^{bD}
	8	6.72±0.37 ^{aC}	6.07±0.22 ^{bC}	5.49±0.16 ^{cC}	6.37±0.04 ^{aC}	5.51±0.09 ^{bC}	5.23±0.10 ^{cC}
$16 \qquad 9.36 \pm 0.26^{aA} 8.4 \pm 0.17^{bA} 7.4 \pm 0.12^{cA} \qquad 3.25 \pm 0.09^{aE} 3.34 \pm 0.06^{aE} 3.24 \pm 0.07^{aE}$	12	8.45±0.19 ^{aB}	7.44 ± 0.2^{bB}	6.36±0.1 ^{cB}	8.20 ± 0.04^{aB}	6.47 ± 0.09^{bB}	6.24±0.08 ^{cB}
	16	$9.36{\pm}0.26^{aA}$	8.4 ± 0.17^{bA}	7.4 ± 0.12^{cA}	3.25±0.09 ^{aE}	3.34 ± 0.06^{aE}	3.24 ± 0.07^{aE}

Means with the same small letters in a raw and capital letters in a column were not significantly different (P < 0.05).

In the present study, initial MVC and PVC counts indicate good surimi quality (Mexis *et al.*, 2009). Most of the available literature on fresh fish total viable count (TVC) reports bacterial counts of 2-4 (log CFU/g) (Gelman *et al.*, 2001). Fan *et al.*, (2008) have demonstrated the increase of TVC in fish meat during storage. By the day 12 of storage, TVC counts in controls and 0.4% treatments were higher than the maximal recommended limit of 7 log CFU/g for TVC in raw fish (Sallam, 2007).

Most of plant extracts have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal (Zaehner & Fiedler, 1995).

The gram-negative psychrotrophic bacterial count is major group of microorganisms responsible for spoilage of aerobically stored fish at chilled temperatures (Sallam, 2007). In the current study, the growth pattern of PVC was similar to MVC in different times. A significant increase in PVC with time has also been reported for salmon stored at 1°C for 15 days (Sallam, 2007). The active compound for the inhibition of *E. coli* and *Salmonella*

enteritidis was identified as Gallic acid. It has been reported that gram-negative bacteria have low susceptibility to plant extracts when compared to gram-positive bacteria. The resistance of gram-negative bacteria to antibacterial substances is related to presence of lipopolysaccharides in their outer membrane. Generally, the extent of the inhibitory effects of the extracts could be attributed to their phenolic composition (Hasani & Hasani, 2014; Tesaki *et al.*, 1999).

Conclusion

The shelf life of silver carp surimi is due to

the chemical and bacteriological activities in fish leading to loss of quality and succeeding spoilage. Current study showed that silver carp surimi treated with Thyme leaves extract had lower microbiological and chemical indices, during refrigerated storage, because of the antimicrobial and antioxidant properties of the extract. It can be concluded that natural extract from Thyme leaves can be used by the food industry to extent the shelf life because they exhibited promising antioxidant and antimicrobial effect.

References

Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J., & Mason, T. (2004). Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinus officinalis for the food and pharmaceutical industry. *Ultrasonics Sonochemistry*, 11(3), 261-265.

Amin, R. A. (2012). Effect of Bio Preservation as a Modern Technology on Quality Aspects and Microbial Safety of Minced Beef. *Global Journal of Biotechnology & Biochemistry*, 7(2), 38-49.

Asgharzadeh, A., Shabanpour, B., Aubourg, S. P., & Hosseini, H. (2010). Chemical changes in silver carp (Hypophthalmichthys molitrix) minced muscle during frozen storage: Effect of a previous washing process. *Grasas y aceites*, *61*(1), 95-101.

Asnaashari, E., Asnaashari, M., Ehtiati, A., & Farahmandfar, R. (2015). Comparison of adaptive neuro-fuzzy inference system and artificial neural networks (MLP and RBF) for estimation of oxidation parameters of soybean oil added with curcumin. *Journal of Food Measurement and Characterization*, 1-10.

Asnaashari, M., Farhoosh, R., & Farahmandfar, R. (2016). Prediction of oxidation parameters of purified Kilka fish oil including gallic acid and methyl gallate by adaptive neuro-fuzzy inference system (ANFIS) and artificial neural network. *Journal of the Science of Food and Agriculture*, *96*(13), 4594–4602.

Asnaashari, M., Farhoosh, R., & Sharif, A. (2014). Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. *Food Chemistry*, 159, 439-444.

Asnaashari, M., Hashemi, B., Mohammad, S., Mehr, H. M., & Asadi Yousefabad, S. H. (2015). Kolkhoung (Pistacia khinjuk) Hull oil and Kernel oil as antioxidative vegetable oils with high oxidative stability and nutritional value. *Food Technology and Biotechnology*, 53(1), 81-86.

Asnaashari, M., Tajik, R., & Khodaparast, M. H. H. (2015). Antioxidant activity of raspberry (Rubus fruticosus) leaves extract and its effect on oxidative stability of sunflower oil. *Journal of Food Science and Technology*, 52(8), 5180-5187.

Bagamboula, C., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri. *Food microbiology*, 21(1), 33-42.

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J. J., & Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agricultural and Food Chemistry*, 53(6), 1903-1906.

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2004). Characteristics and gel

properties of muscles from sardine (Sardinella gibbosa) and mackerel (Rastrelliger kanagurta) caught in Thailand. *Food Research International*, *37*(10), 1021-1030.

Chizzola, R., Michitsch, H., & Franz, C. (2008). Antioxidative properties of Thymus vulgaris leaves: comparison of different extracts and essential oil chemotypes. *Journal of Agricultural and Food Chemistry*, 56(16), 6897-6904.

Eshghi, N., Asnaashari, M., Haddad Khodaparast, M. H., & Hosseini, F. (2014). Evaluating the potential of natural curcumin for oxidative stability of soybean oil. *Natural product research*, 28(17), 1375-1378.

European-Union. (1995). Commission Decision 95/149/EC of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used (pp. 84-87).

Fahimdezhban, Y., Motallebi, A., Hosseini, E., Khanipour, A., & Soltani, M. (2014). Comparison of effect of Zataria multiflora and Rosemarinus officinalis extracts on quality of minced frozen silver carp. *Iranian Journal of Fisheries Sciences*, 13(1), 20-29.

Fan, W., Chi, Y., & Zhang, S. (2008). The use of a tea polyphenol dip to extend the shelf life of silver carp (Hypophthalmicthys molitrix) during storage in ice. *Food Chemistry*, *108*(1), 148-153.

Farahmandfar, R., Asnaashari, M., & Sayyad, R. (2015). Comparison antioxidant activity of Tarom Mahali rice bran extracted from different extraction methods and its effect on canola oil stabilization. *Journal of Food Science and Technology*, 1-10.

Farahmandfar, R., Safari, R., Ahmadi Vavsari, F., & Bakhshandeh, T. (2016). The Effect of Ajwain (Trachyspermum ammi) Extracted by Ultrasound-Assisted Solvent on Quality Properties of Silver Carp (Hypophthalmichthys molitrix) Surimi Stored at 4C. *Journal of Food Processing and Preservation*, 40(2), 291–297.

Farhoosh, R., Johnny, S., Asnaashari, M., Molaahmadibahraseman, N., & Sharif, A. (2016). Structure–antioxidant activity relationships of o-hydroxyl, o-methoxy, and alkyl ester derivatives of p-hydroxybenzoic acid. *Food Chemistry*, 194, 128-134.

Farhoosh, R., Sharif, A., Asnaashari, M., Johnny, S., & Molaahmadibahraseman, N. (2016). Temperature-Dependent Mechanism of Antioxidant Activity of o-Hydroxyl, o-Methoxy, and Alkyl Ester Derivatives of p-Hydroxybenzoic Acid in Fish Oil. *Journal of the American Oil Chemists' Society*, *93*(4), 555-567.

Gandomi, H., Misaghi, A., Basti, A. A., Bokaei, S., Khosravi, A., Abbasifar, A., & Javan, A. J. (2009). Effect of Zataria multiflora Boiss. essential oil on growth and aflatoxin formation by Aspergillus flavus in culture media and cheese. *Food and chemical toxicology*, 47(10), 2397-2400.

Gelman, A., Glatman, L., Drabkin, V., & Harpaz, S. (2001). Effects of storage temperature and preservative treatment on shelf life of the pond-raised freshwater fish, silver perch (Bidyanus bidyanus). *Journal of Food Protection*, 64(10), 1584-1591.

Goulas, A. E., & Kontominas, M. G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (Sparus aurata): Biochemical and sensory attributes. *Food Chemistry*, 100(1), 287-296.

Hasani, S., & Hasani, M. (2014). Antimicrobial Properties of Grape Extract on Common Carp (Cyprinus carpio) Fillet During Storage in 4 C. *Journal of Food Technology*, *12*(2), 48-53.

Jin, S.-K., Kim, I.-S., Kim, S.-J., Jeong, K.-J., Choi, Y.-J., & Hur, S.-J. (2007). Effect of muscle type and washing times on physico-chemical characteristics and qualities of surimi. *Journal of food engineering*, *81*(3), 618-623.

Kowalski, R., & Wawrzykowski, J. (2009). Effect of ultrasound-assisted maceration on the quality of oil from the leaves of thyme Thymus vulgaris L. *Flavour and fragrance journal*, 24(2), 69-74.

Lakshmanan, P. (2000). Fish spoilage and quality assessment.

Lanier, T. C., Yongsawatdigul, J., & Carvajal-Rondanelli, P. (2013). Surimi Gelation Chemistry

4 Surimi and surimi seafood (pp. 101).

Manju, S., Jose, L., Gopal, T. S., Ravishankar, C., & Lalitha, K. (2007). Effects of sodium acetate dip treatment and vacuum-packaging on chemical, microbiological, textural and sensory changes of Pearlspot (Etroplus suratensis) during chill storage. *Food Chemistry*, *102*(1), 27-35.

Mexis, S., Chouliara, E., & Kontominas, M. (2009). Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 C. *Food microbiology*, 26(6), 598-605.

Misharina, T., & Polshkov, A. (2005). Antioxidant properties of essential oils: autoxidation of essential oils from laurel and fennel and of their mixtures with essential oil from coriander. *Applied Biochemistry and Microbiology*, 41(6), 610-618.

Moosavi-Nasab, M., Alli, I., Ismail, A. A., & Ngadi, M. O. (2005). Protein structural changes during preparation and storage of surimi. *Journal of food science*, 70(7), c448-c453.

Namulema, A., Muyonga, J., & Kaaya, A. (1999). Quality deterioration in frozen Nile perch (*Lates niloticus*) stored at–13 and–27° C. *Food Research International*, *32*(2), 151-156.

Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry*, *120*(1), 193-198.

Özogul, F., Polat, A., & Özogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (Sardina pilchardus). *Food Chemistry*, 85(1), 49-57.

Pezeshk, S., Rezaei, M., & Hosseini, H. (2011). Effects of Turmeric, Shallot Extracts, and Their Combination on Quality Characteristics of Vacuum-Packaged Rainbow Trout Stored at $4\pm1^{\circ}$ C. *Journal of food science*, 76(6), M387-M391.

Razavi Shirazi, H. (2007). Seafood technology: principles of handling and processing: Pars Negar Press, 325p.(In Persian).

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat science*, 70(3), 531-541.

Sallam, K. I. (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *Food control*, 18(5), 566-575.

Sayyari, Z., & Farahmandfar, R. (2016). Stabilization of sunflower oil with pussy willow (Salix aegyptiaca) extract and essential oil. *Food Science & Nutrition*.

Shafiee, A., & Javidnia, K. (1997). Composition of essential oil of Zataria multiflora. *Planta medica*, 63(4), 371-372.

Sharafati Chaleshtori, R., Rafieian Kopaei, M., Rokni, N., Mortezaei, S., & Sharafati Chaleshtori, A. (2013). Antioxidant Activity of Zataria Multiflora Hydroalcoholic Extract and Its Antibacterial Effect on Staphylococcus Aureus. *Journal of Mazandaran University of Medical Sciences (JMUMS)*.

Sharififar, F., Moshafi, M., Mansouri, S., Khodashenas, M., & Khoshnoodi, M. (2007). In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. *Food control*, *18*(7), 800-805.

Shaviklo, G. R., & Johannsson, S. R. (2006). Quality assessment of fish protein isolates using surimi standard methods. *Fisheries Training Programme, Final Project*.

Sparkman, O. D. (2005). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. *Journal of the American Society for Mass Spectrometry*, 11(16), 1902-1903.

Tesaki, S., Tanabe, S., Moriyama, M., Fukushi, E., Kawabata, J., & Watanabe, M. (1999). Isolation and identification of an antibacterial compound from grape and its application to foods. *Journal of the Agricultural Chemical Society of Japan (Japan)*.

Willian, H. (2000). Association of Official Analytical Chemists (AOAC). Official Methods of

Analysis International. Washington, DC.

Xia, T., Shi, S., & Wan, X. (2006). Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. *Journal of food engineering*, 74(4), 557-560.

Zaehner, H., & Fiedler, H.-P. (1995). *The need for new antibiotics: possible ways forward*. Paper presented at the Symposia-Society For General Microbiology.

Zolfaghari, M., shabanpour, B., & falahzadeh, S. (2011). Paper: Study of trend of chemical and microbial changes of rainbow trout (oncorhynchus mykiss) to determine the its optimum shelf-life during storage in refrigerator temperature (4 c). *Iranian Journal of Natural Resource*, 64(2), 107-119.



خصوصیات شیمیایی و میکروبی سوریمی ماهی کپور نقرهای غنی شده با عصاره برگ آویشن

رضا فرهمندفر^{1*}، رضا صفری²، فهیمه احمدی واوسری²، تهمینه بخشنده² تاریخ دریافت: 1396/12/19 تاریخ پذیرش: 1397/08/02

چکیدہ

در این تحقیق، تاثیر عصاره فراصوت آویشن بر خصوصیات ماهی کپور نقرهای مورد ارزیابی قرار گرفت. به این منظور، برگهای آویشن خشک، آسیاب و در متانول (10:1 وزنی/ حجمی) به مدت 30 دقیقه در دمای 2°45 خیسانده و در فرکانس kHz 30 به مدت 15 دقیقه در 2°40 فراصوت دهی شد. عصاره در دی متیل سولفوکسید حل و با سوریمی (0/4 و 0/8 درصد وزنی/ وزنی) مخلوط شد. سپس آنالیز شیمیایی (اسید چرب آزاد، عدد پراکسید، اسید تیوباربیتوریک و ترکیبات فرار بر پایه نیتروژن) و میکروبی (میزان باکتریهای مزوفیل و سرمادوست) نمونهها در فاصلههای زمانی معین صفر، 4، 8. 12 و 16 روز در دمای 2° 2 انجام شد. نتایج آنالیز شیمیایی و میکروبی نشان داد که غلظت 8/0% آویشن میتواند عمر ماندگاری سوریمی کپور 8. 12 و 16 روز در دمای 2° 2 انجام شد. نتایج آنالیز شیمیایی و میکروبی نشان داد که غلظت 8/0% آویشن میتواند عمر ماندگاری سوریمی کپور نقرهای را افزایش دهد و تفاوت معنیداری بین نمونه شاهد و تیمارها وجود دارد. علاوه بر این، نتایج نشان دادند که به دلیـل مقادیر زیاد ترکیبات نقرهای را افزایش دهد و تفاوت معنیداری بین نمونه شاهد و تیمارها وجود دارد. علاوه بر این، نتایج نشان دادند که به دلیـل مقادیر زیاد ترکیبات آنتیاکسیدانی و ضد میکروبی همچون تیمول (7/1/5%)، 9 – سیمن (20/14%)، کارواکرول (1/19%) و γ-ترپینن (4/4%) تاثیر معنیداری بر جلوگیری از اکسیدانی و ضد میکروبی همچون تیمول دارد. نتایج نشان داد که فراصوت روش مفید برای استخراج ترکیبات مؤره گیاهی می دارد. با هر این

واژههای کلیدی: آنتیاکسیدان، ماهی، مدت ماندگاری، سوریمی، آویشن

¹⁻ استادیار، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ایران.
2- دانشجوی دکتری، گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ایران.

⁽r.farahmandfar@sanru.ac.ir :نویسنده مسئول)