African Journal of Microbiology Research Vol. 6(9), pp. 2162-2168, 9 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1644
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Effect of eugenol oil and sauced treatments on fresh carp (*Cyprinus carpio* L.) fillets during storage at 4°C

Özlem Pelin Can

Department of Food Engineering, Faculty of Engineering, University of Cumhuriyet, Sivas, Turkey.
E-mail: peloni1@hotmail.com.

Accepted 13 January, 2012

In this study, the effect of different concentrations of eugenol added to a sauce containing different spices on sensory, microbiological (total mezophile aerobe bacteria, enterobacteriaceae, lactic acid bacteria, yeast and mould) and chemical (total volatile nitrogen and pH) changes during the storage of raw *Cyprinus carpio* L. fillets is investigated. 0.5% (A), 1.0% (B) and 1.5% (C) eugenol were added to the prepared sauce; then fillets were exposed to the sauce for 6 h at 4°C and were vacuum-packaged under aseptic conditions. Microbiological and chemical analyses were carried out on days 1, 7, 14, 28, 42, 56, 70, 84 and 98 of storage. The control groups samples were performed sensory analysis 28 day, while other groups up to 56 days. Spoilage was observed on 42nd day in control group and on 98th day of storage in eugenol added groups. The statistical analysis of the results showed that there was a significant difference between the control group and eugenol added group ($p < 0.05$), but there was no significant difference among the eugenol concentrations ($p > 0.05$).

Key words: *Cyprinus carpio*, eugenol, shelf life, sauce, storage.

INTRODUCTION

Fresh seafood has a short shelf life, which causes substantial practical problems for its distribution. Improvements in the shelf life of a product can have an important economic impact by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets. In addition, food borne illnesses are still a major threat, even in developed countries. Today, consumers demand additive-free, fresher and more natural flavored food products, which also maintain microbiological safety. While abundant data exists on preservation of fish using low-dose irradiation (Savvaidis et al., 2002), ice (Chytiri et al., 2004; Özoğul and Özoğul, 2004), ozonation (Neratzaki et al., 2005), packaging (Arashisar et al., 2004; Çaklı et al., 2006) including cold smoked using nitrite or potassium nitrate (Lyhs et al., 2001), antimicrobial combinations of lactic acid-nisin (Nychas and Tassou, 2000), sodium lactate (Öksüztepe et al., 2010), there is, in general, limited information in the literature on the effect of essential oils (EOs), such as oregano, thyme used singly or in combination with other preservative technologies, that is, salting, packaging on extension of shelf life of freshwater fish species, including carp (Erkan, 2010).

The use of natural antimicrobial compounds like organic acids and aromatic compounds, is thus of interest to the food industry (Arslan, 2002), People's need for animal protein sources is gradually increasing. Fish is the main protein suppliers among these demands. Fish can self-breed in natural sources, such as seas, lakes, dams and rivers and it is cheaper than other types of animal growing (Varlık et al., 1993). However, turning these natural sources in usable areas is not common globally. Fish can easily be spoilt owing to its composition. Thus, the shelf life of fish has great importance. Hence, fish must be either quickly consumed or technologically processed. Many researchers have attempted to improve the shelf life of fish using different methods such as salting, fumigation, food additive etc. (Gürel, 2003; Korkmaz, 2004). Traditional processing and preservation procedures can inhibit spoilage reactions but there is increasing interest in products with milder and more natural preservatives (Gould, 1996), EOs are natural antimicrobials with potential to extend the shelf life of seafood when used alone or in combination with other

Table 1. The chemical composition of sauce.

Sauce composition	Quantity	Rate (%)
Tomato sauce	200 g	20
Lemon juice	200 ml	20
Oil	200 ml	20
Garlic seed	100 g	10
Red pepper	10 g	1
Thyme	10 g	1
Black pepper	10 g	1
Salt	30 g	3
Water	240 ml	24

preservation techniques. However, some food components decrease the antimicrobial effect of EO and the use of EO for mild preservation of seafood remains little studied (Loapez-malo et al., 2000; Nychas, 2000; Pirie and Clayson, 1964; Sofos et al., 1998). Eugenol is a flavoring agent used in food products, which has no negative effect on human health (Blaszyk and Holley, 1998; Burt, 2004). Europe Union also advises it. It is well known that eugenol has antioxidant activity (Kalemba and Kunicka, 2003; Ouattara et al., 1997). Oxidative stress is produced when the balance between oxidative stimulation and various antioxidant systems is impaired (Arashisar et al., 2004). Because of these properties, it is more wanted to use the eugenol than other agents do.

The purpose of this study is to enhance both the shelf life of fish fillets using eugenol medium and to get more delicious fish by using species.

MATERIALS AND METHODS

Fish samples and sauce preparation

The *Cyprinus carpio* L. fish is used (approximately 10 kg of fish) were obtained from aquaculture farm located on Lake Keban in Turkey. The fresh fish samples were packed in polystyrene boxes with crushed ice and then transferred to the laboratory. The fish were harvested, beheaded, gutted manually, washed and then the fillets were sauced. The chemical composition of the sauce used is given in Table 1. The rate of used sauce was chosen as 20% of fish weight. This type of sauce was chosen preliminary studies, the most popular formula.

Treatments samples

Treatments included the following: conrol (control samples: sauced). A [treated samples: sauced/ eugenole 0.5% (v/w)], B [treated samples: sauced/ eugenole 1% (v/w)] and C [treated samples: sauced/ eugenole 1.5% (v/w)]. Eugenole was added in oil then mixed other ingredients. The samples were held in these sauce groups for 6 h at 4℃ and vacuum packed. Samp les were analyzed microbiologically, chemically and sensorially on days 0, 7, 14, 28, 42, 56, 70, 84 and 98. The process was been repeated for three times and their average was taken for a more accurate reading.

Chemical analysis

The pH value was measured as described by Lima Dos Santos et al. (1981), by using a digital pH meter (HANNA). Total volatile basic nitrogen (TVB-N, mg N/100 g) values were determined as described by Tarladgis et al. (1960).

Microbiological analysis

Approximately 10 g of the fish fillet were sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 90 ml of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a Lab Blender 400 Stomacher at room temperature. Microbiological analyses were conducted using standard microbiological methods. Total mezophile aerobe bacteria count was determined by the pour plate method, using Plate Count Agar (Oxoid CM 325) as the medium. Plates were incubated at 35±1℃ for 24–48 h (Harrigan and Mccance, 1976), Wort Agar (Merck 110130) was used for yeast counts. Plates were incubated at 30±1℃ for 5 days (Harrigan and Mccance, 1976). Sa bouraud Dextrose Agar was used for mould counts. Plates were incubated at 30±1℃ for 5 days (Harrigan, 1976). Lactic acid bac teria (LAB) were enumerated on Man Rogosa Sharpe Agar (MRS, pH 5.7, Merck 110661) incubated at 25℃ for 4–5 days. (Harrigan, 1976). Enterobactericeae were enumerated on Violed Red Bile Glucose Agar (VRBGA, Merck 110275) plates incubated at 30 ± 1℃ for 24-48 h (Harrigan and Mccance, 1976).

Sensory analysis

The sensory properties of fillets were estimated by a panel of eight trained panelists from the staff of the Department of Food Hygiene and Technology. Fırat University of Turkey. according to the freshness grade guide for fillets (Kurtcan and Gönül, 1981). The panelists were asked to evaluate all six parameters (color, odor, texture, flavor, view, and total assessment) on a scale from 1 (very bad), 2 (bad), 3 (normal), 4 (good) and 5 (very good). Samples were evaluated before being baked.

Statistical analysis

Analysis of the data was conducted using Statistical Analysis System (SAS) package programmed. Values between groups and within group-between days were compared. Data were subjected to variance analysis in accordance with 3 × 11 × 3 × 1 factorial design and in terms of fix effects and inter-variable interactions so that “repetition number × sampling time × test groups × number of samples examined at one instance from each test group”. According to General Linear Models (GLM) procedure, Fisher’s smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (Anonim, 1996). Alpha value was determined as 0.05.

RESULTS AND DISCUSSION

The results of microbiological, chemical and sensory analysis were given in Tables 2, 3 and 4, respectively. It was shown in Table 2 that the number of mezophile aerobe bacteria (MAB) count increased from 4.84 to 5.3 in the first week. This increment continued until 42nd day of storage and reached to 8.58. As followed from Table 3 the same tendency was recorded for all groups.

Table 2. Result of microbiolgical analysis of raw fish filet sauced stored at 4℃. A: 0.5% eugenol, B: 1% euge nol and C: 1.5% eugenol. a. b. c : Means within a column lacking a common superscript letter are different (P<0.05). z. y : Means within a row lacking a common superscript letter are different (P<0.05). *: Not analyzed. Values are means (±SD) for three trials at each groups (n=4×3).

Microorganisms (log cfu/g)	Example	Storage (days)								
		0	7	14	28	42	56	70	84	98
Mezophile Aerob Bacteria	Control	4.81±0.1$^{c.z}$	5.67±0.3$^{bc.z}$	6.13±0.3$^{b.z}$	6.56±0.2$^{b.z}$	8.67±0.3$^{a.z}$	*	*	*	*
	A	4.51±0.3$^{b.z}$	4.72±0.3$^{b.z}$	5.33±0.2$^{b.z}$	5.59±0.1$^{b.z}$	5.67±0.4$^{b.y}$	5.64±0.3$^{b.z}$	5.63±0.3$^{b.z}$	6.47±0.2$^{a.z}$	7.33±0.2$^{a.z}$
	B	3.96±0.2$^{b.z}$	4.31±.10$^{b.z}$	4.89±0.1$^{ab.y}$	5.12±0.2$^{ab.z}$	5.67±0.1$^{ab.y}$	5.19±0.3$^{ab.z}$	5.79±0.3$^{ab.z}$	5.78±0.3$^{a.z}$	5.92±0.3$^{a.y}$
	C	3.84±0.1$^{b.z}$	4.10±0.1$^{b.z}$	4.63±0.1$^{ab.y}$	5.59±0.13$^{a.z}$	5.76±0.3$^{a.y}$	5.56±0.1$^{a.z}$	5.58±0.3$^{a.z}$	5.82±0.2$^{a.z}$	5.67±0.2$^{a.y}$
Lactic Acid Bacteria	Control	2.41±0.3$^{c.zy}$	4.87±0.2$^{b.z}$	6.84±0.2$^{ab.z}$	7.67±0.3$^{a.z}$	7.94±0.3$^{a.z}$	*	*	*	*
	A	2.93±0.3$^{c.z}$	3.65±0.2$^{bc.y}$	3.85±0.1$^{b.y}$	4.77±0.6$^{b.y}$	5.51±0.3$^{ab.y}$	5.79±0.3$^{ab.z}$	5.70±0.2$^{ab.z}$	6.13±0.2$^{a.z}$	6.89±0.2$^{a.z}$
	B	2.01±0.1$^{c.y}$	3.17±0.31$^{b.y}$	3.92±0.1$^{ab..y}$	4.92±0.3$^{a.y}$	5.33±0.2$^{a.y}$	5.74±0.3$^{a.z}$	5.58±0.3$^{a.z}$	5.52±0.3$^{a.z}$	5.77±0.2$^{a.z}$
	C	2.08±0.2$^{c.y}$	2.85±0.3$^{b.y}$	3.51±0.3$^{b.y}$	4.92±0.16$^{a.y}$	4.68±0.4$^{a.y}$	4.98±0.2$^{a.z}$	5.27±0.4$^{a.z}$	4.41±0.4$^{a.y}$	4.59±0.2$^{a.z}$
Enterobactericeae	Control	3.78±0.3$^{b.z}$	4.11±0.23$^{b.z}$	5.44±0.15$^{a.z}$	5.83±0.1$^{a.z}$	6.71±0.3$^{a.z}$	*	*	*	*
	A	3.52±0.2$^{a.z}$	3.66±0.1$^{a.z}$	3.69±0.2$^{a.y}$	3.57±0.2$^{a.y}$	3.47±0.3$^{a.y}$	3.85±0.2$^{a.z}$	3.81±0.2$^{a.z}$	3.85±0.3$^{a.z}$	3.96±0.3$^{a.z}$
	B	3.43±0.3$^{a.z}$	3.82±0.08$^{a.z}$	3.85±0.6$^{a.y}$	3.73±0.3$^{a.y}$	3.76±0.2$^{a.y}$	3.97±0.2$^{a.z}$	3.93±0.2$^{a.z}$	3.41±0.4$^{a.z}$	3.03±0.2$^{a.z}$
	C	2.63±0.3$^{b.z}$	2.78±0.1$^{b.z}$	3.36±0.1$^{a.y}$	3.74±0.1$^{a.y}$	3.53±0.1$^{a.y}$	3.59±0.3$^{a.z}$	3.71±0.42$^{a.z}$	2.22±0.13$^{b.z}$	2.15±0.2$^{b.z}$
Yeast	Control	4.84±0.3$^{a.z}$	4.96±0.1$^{a.z}$	5.01±0.3$^{a.z}$	5.19±0.3$^{a.z}$	5.36±0.3$^{a.z}$	*	*	*	*
	A	4.810.1±$^{a.z}$	4.84±0.3$^{a.z}$	4.97±0.3$^{a.z}$	4.39±.02$^{b.z}$	5.26±0.3$^{a.z}$	5.34±0.6$^{a.z}$	5.87±0.32$^{a.z}$	5.95±0.2$^{a.z}$	5.94±0.2$^{a.z}$
	B	4.12±0.3$^{b.z}$	4.23±.0.1$^{b.z}$	4.90±0.1$^{a.z}$	4.76±0.1$^{ab.z}$	4.89±0.1$^{b.z}$	4.82±0.1$^{b.z}$	5.16±0.1$^{a.z}$	5.67±0.1$^{a.z}$	5.93±0.1$^{a.z}$
	C	3.61±0.3$^{b.y}$	4.16±0.32$^{ab.z}$	4.38±0.13$^{ab.z}$	4.72±0.42$^{a.z}$	5.23±0.23$^{a.z}$	5.01±0.26$^{a.z}$	5.75±0.2$^{a.z}$	5.15±0.3$^{a.y}$	5.03±.0.1$^{a.z}$
Mould	Control	3.41±0.2$^{a.z}$	4.65±0.3$^{a.z}$	4.79±0.2$^{a.z}$	5.19±.10$^{a.z}$	5.43±0.3$^{a.z}$	*	*	*	*
	A	3.51±0.1$^{a.z}$	4.56±0.2$^{a.z}$	4.61±0.2$^{a.z}$	4.93±0.3$^{a.z}$	4.56±0.2$^{a.z}$	4.86±0.3$^{a.z}$	4.72±0.3$^{a.z}$	4.69±0.2$^{a.z}$	4.91±0.1$^{a.z}$
	B	3.71±0.1$^{a.z}$	4.91±0.2$^{a.z}$	4.77±0.2$^{a.z}$	4.83±0.1$^{a.z}$	4.87±0.3$^{a.z}$	4.81±0.2$^{a.z}$	4.89±0.2$^{a.z}$	4.93±0.1$^{a.z}$	4.99±0.2$^{a.z}$
	C	3.13±0.3$^{a.z}$	4.07±0.2$^{a.z}$	4.75±0.3$^{a.z}$	4.49±0.1$^{a.z}$	4.67±0.2$^{a.z}$	4.85±0.2$^{a.z}$	4.77±0.2$^{a.z}$	4.83±0.3$^{a.z}$	4.81±0.2$^{a.z}$

Statistical analysis results showed that there was an important difference between the control and A, B and C groups while eugenol containing groups showed no significant interaction between each other (Table 2). On the other hand, an increase was also recorded in the number of enterobactericeae and yeast and mould for control group based on the storage time. On the contrary, eugenol containing groups showed fluctuations.

The number of lactic acid bacteria increased continuously for control group based on the storage time, while the number decreased for other groups. Holding time was determined to have no statistical influence except for 42nd day (Table 2). From the view of chemical analysis results, it was observed that there were no significant variations in the trend of pH values (Table 3). Table 3 showed us that the ratio of TVB – N was recorded as 44.8 mg/ 100 g. However, a sharp decrease was observed in TVB – N ratio for other groups. Note that the last column of Table 3

Table 3. Result of Chemical analysis of raw fish filet sauced stored at 4℃. A: 0.5% eugenol, B: 1% eugenol an d C: 1.5% eugenol. a. b. c : Means within a column lacking a common superscript letter are different (P<0.05). z. y : Means within a row lacking a common superscript letter are different (P<0.05). *: Not analyzed. Values are means (±SD) for three trials at each groups (n=4×3).

	Example	Storage (days)								
		0	7	14	28	42	56	70	84	98
pH	Control	6.13±0.12$^{a.z}$	6.15±0.14$^{a.z}$	6.12±0.17$^{a.z}$	6.51±0.23$^{a.z}$	6.45±0.15$^{a.z}$	*	*	*	*
	A	5.33±0.8$^{a.y}$	5.14±0.3$^{a.y}$	5.18±0.45$^{a.y}$	5.11±0.4$^{a.y}$	4.93±0.54$^{a.y}$	5.11±0.02$^{a.z}$	5.20±0.24$^{a.z}$	5.51±0.31$^{a.z}$	5.18±0.28$^{a.z}$
	B	5.31±0.1$^{a.y}$	5.26±0.3$^{a.y}$	5.52±0.2$^{a.zy}$	5.13±0.4$^{a.y}$	5.11±0.12$^{a.y}$	5.15±0.1$^{a.z}$	5.86±0.3$^{a.z}$	5.12±0.2$^{a.z}$	5.02±0.3$^{a.z}$
	C	5.28±0.3$^{a.y}$	5.33±0.7$^{a.y}$	5.84±0.3$^{a.zy}$	5.20±0.4$^{a.y}$	5.82±0.3$^{a.y}$	5.87±0.1$^{a.z}$	5.06±0.3$^{a.z}$	5.13±0.2$^{a.z}$	5.83±0.3$^{a.z}$
TVB-N	Control	12.6±5.17$^{b.z}$	19.6±5.31$^{a.z}$	28±5.01$^{ab.z}$	36±4.13$^{ab.z}$	42.2±4.22$^{a.z}$	*	*	*	*
	A	12.6±5.05$^{b.z}$	15.4±5.32$^{a.z}$	15.4±4.84$^{a.z}$	15.4±3.26$^{a.z}$	15.4±4.13$^{a.y}$	19.2±4.51$^{a.z}$	19.2±3.1$^{a.z}$	25±3.53$^{a.z}$	38.2±3.21$^{a.z}$
	B	12.6±4.13$^{a.z}$	16.1±4.52$^{a.z}$	16.1±5.01$^{a.z}$	18.2±2.56$^{a.z}$	18.2±3.21$^{a.y}$	16.1±4.13$^{a.z}$	24±4.1$^{a.z}$	28.2±3.01$^{a.z}$	28.2±3.3$^{a.y}$
	C	12.6±3.52$^{b.z}$	16±1.32$^{b.z}$	18±3.65$^{a.z}$	18±3.13$^{a.z}$	18±3.42$^{a.y}$	18±3.53$^{b.z}$	22.2±3.56$^{a.z}$	22.2±3.15$^{a.z}$	24.8±3.31$^{a.y}$

indicated an insignificant interaction between groups.

Sensory analysis results were given in Table 4. Sensory analysis was carried out until the 28th day of storage for control group samples and until the 56th day of storage for treatment group samples. Due to the microbiological quality of the control group samples, exceeded limit values were not sensorially evaluated. The control group samples were significant other groups samples in storage periods (in terms of odor and flavor).In this paper, the effect of different concentration on shelf life for *C. carpio* L fish was investigated experimentally. From the results obtained, which were given above, it was observed that all the microorganisms increased during holding time for control groups. However, this was not the case for eugenol containing groups.

It is well known that the use of Eugenol essential oil does not allow or delay bacteria occurrence. On the other hand, the storage time has the most effect on bacteria occurrence. Mejlholm and Dalgaard (2002) showed that the essential oils cause cells to die by means of increasing the permeability of cytoplasmic membrane of microorganisms. Although several types of essential oils have been used for this purpose. Eugenol has proved to be the most important one with its more protective effect than others are, Farag et al. (1989) investigated the effect of thymol, menthol, eugenol and anathol on *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* and found that Eugenol had more significant effect than others.

Al-Bandak et al. (2009) showed *Majorana syriaca* extract delayed the microbial growth. Moroever; this extract decreased the oxidation (peroxide value and thiobarbituric acid substance) of minced tuna. This study showed that the potential of *M. syriaca* extract in extending the shelf life of tuna fish.

The changes in the microflora aacording to results of microbiolgical analysis of filleted carp samples were shown in Table 2. The initial (day 0) mezophile aerobe bacteria (MAB) of carp fillets was between 3.0 and 4.0 log cfu/g, which was a relatively low bacterial load, in agreement with the results of Chytiri et al. (2004). Similarly, low initial MAB (between 3 and 4 log cfu/g) were reported for rainbow trout (Özoğul. 2004). Carp fish samples (in groups of control and A) exceeded the value of 7 log cfu/g for MAB, considered as the upper acceptability limit for fresh marine species (ICMSF, 1986) on days 42 and 98 of storage, respectively, while samples of B and C groups did not reach this value throughout the 98-day storage period. Samples included A and B groups had significantly ($P < 0.05$) lower MAB count compared to C samples 98 of storage. The combination of vacuum packaged and eugenol resulted in a microbiological shelf-life extension of 98 days; especially the latter's phenolic components, carvacrol and thymol, known to exert antimicrobial activity (Burt, 2004). Mahmoud et al. (2004) found that dipping carp fillets in carvacrol/thymol solution (1%) both reduced the initial MAB and extended the shelf life from 4 days to at least 12 days at 5℃. according to microbiological results. Moreover, Giatrakou et al.

Table 4. Result of sensory analysis of raw fish filet sauced stored at 4℃. A: 0.5% eugenol, B: 1% eugenol and C : 1.5% eugenol. a. b. c : Means within a column lacking a common superscript letter are different (P<0.05). z. y : Means within a row lacking a common superscript letter are different (P<0.05). *: Not analyzed. Values are means (±SD) for three trials at each groups (n=4×3).

Feature	Example	Storage (days)					
		0	7	14	28	42	56
Color	Control	4.62±0.1$^{a.z}$	4.81±0.1$^{a.z}$	4.81±0.1$^{a.z}$	4.62±0.1$^{a.z}$	*	*
	A	4.62±0.1$^{a.z}$	4.68±0.1$^{a.z}$	4.5±0.2$^{a.z}$	4.81±0.1$^{a.z}$	4.56±0.1$^{a.z}$	4.62±0.2$^{a.z}$
	B	4.37±0.2$^{a.z}$	4.43±0.3$^{a.z}$	4.24±0.1$^{a.z}$	4.43±0.3$^{a.z}$	4.37±0.1$^{a.z}$	4.24±0.1$^{a.z}$
	C	4.62±0.1$^{a.z}$	4.56±0.1$^{a.z}$	4.68±0.3$^{a.z}$	4.62±0.1$^{a.z}$	4.56±0.1$^{a.z}$	4.5±0.1$^{a.z}$
Odor	Control	4.68±0.1$^{a.z}$	4.68±0.2$^{a.z}$	4.56±0.2$^{a.z}$	4.18±0.1$^{a.z}$	*	*
	A	4.18±0.3$^{a.z}$	4.21±0.3$^{a.z}$	4.36±0.1$^{a.z}$	4.20±0.2$^{a.z}$	4.33±0.2$^{a.z}$	4.21±0.1$^{a.z}$
	B	2.81±0.9$^{a.y}$	2.74±0.6$^{a.y}$	2.5±0.6$^{a.y}$	2.61±0.8$^{a.y}$	2.63±0.9$^{ab.z}$	2.49±0.9$^{ab.zy}$
	C	2.51±0.6$^{a.y}$	2.43±0.8$^{a.y}$	1.99±0.8$^{ab.y}$	1.95±0.8$^{ab.y}$	1±0.8$^{b.y}$	1±0.01$^{b.y}$
Texture	Control	4.81±0.08$^{a.z}$	4.81±0.1$^{a.z}$	4.49±0.3$^{a.z}$	4.75±0.2$^{a.z}$	*	*
	A	4.81±0.08$^{a.z}$	4.6±0.1$^{a.z}$	4.5±.02$^{a.z}$	4.56±0.3$^{a.z}$	4.31±0.1$^{a.z}$	4.5±0.1$^{a.z}$
	B	4.75±0.3$^{a.z}$	4.56±0.3$^{a.z}$	4.93±0.6$^{a.z}$	4.92±0.5$^{a.z}$	4.56±0.2$^{a.z}$	4.4±0.3$^{a.z}$
	C	4.6±0.3$^{a.z}$	4.5±0.1$^{a.z}$	4.81±0.1$^{a.z}$	4.76±0.6$^{a.z}$	4.63±0.2$^{a.z}$	4.59±0.1$^{a.z}$
Flavor	Control	4.6±0.3$^{a.z}$	4.6±0.1$^{a.z}$	4.56±0.1$^{a.z}$	4.31±0.3$^{a.z}$	*	*
	A	4.43±0.2$^{a.z}$	4.31±0.3$^{a.z}$	4.39±0.1$^{a.z}$	4.43±0.4$^{a.z}$	4.56±.30$^{a.z}$	4.31±0.3$^{a.z}$
	B	2.56±0.7$^{a.y}$	2.75±0.7$^{ab.y}$	1.93±0.3$^{ab.y}$	1.18±0.6$^{b.y}$	1±0.42$^{b.y}$	1±0.06$^{b.y}$
	C	2.12±0.7$^{a.y}$	1.93±0.9$^{b.y}$	1.81±0.9$^{ab.y}$	1±0.09$^{b.y}$	1±0.5$^{b.y}$	1±0.03$^{b.y}$
View	Control	4.75±0.09$^{a.z}$	4.6±0.1$^{a.z}$	4.61±0.3$^{a.z}$	4.6±0.3$^{a.z}$	*	*
	A	4.42±0.09$^{a.z}$	4.56±0.3$^{a.z}$	4.59±.20$^{a.z}$	4.63±0.1$^{a.z}$	4.67±0.2$^{a.z}$	4.65±0.3$^{a.z}$
	B	4.49±0.08$^{a.z}$	4.31±0.2$^{a.z}$	4.31±0.2$^{a.z}$	4.44±0.3$^{a.z}$	4.56±0.1$^{a.z}$	4.39±0.2$^{a.z}$
	C	4.33±0.03$^{a.z}$	4.39±0.3$^{a.z}$	4.42±0.3$^{a.z}$	4.51±0.3$^{a.z}$	4.59±0.1$^{a.z}$	4.45±0.3$^{a.z}$
Total assessment	Control	4.67±0.01$^{a.z}$	4.67±0.01$^{a.z}$	4.55±0.3$^{a.z}$	4.21±0.2$^{a.z}$	*	*
	A	4.53±0.01$^{a.z}$	4.69±0.09$^{a.z}$	4.61±0.3$^{a.z}$	4.4±0.2$^{a.z}$	4.39±0.4$^{a.z}$	4.45±0.1$^{a.z}$
	B	3.72±0.51$^{a.zy}$	3.99±0.08$^{a.zy}$	3.98±0.9$^{a.zy}$	3.56±0.1$^{a.zy}$	3.85±0.3$^{a.z}$	3.87±0.3$^{a.z}$
	C	2.9±0.19$^{a.y}$	2.9±.001$^{a.y}$	2.7±0.03$^{a.y}$	2.65±0.3$^{a.y}$	2.5±0.3$^{a.y}$	2.32±0.3$^{a.y}$

(2008) reported that the combined use of modify atmosphere packaging (MAP) and oregano oil 0.1% v/wt extended the shelf-life of swordfish fillets by 8 days, as determined by sensory and microbiological analysis.

LAB and enterobacteriaceae (to a lesser extent), being facultative anaerobic bacterial species, were also found to be a significant part of the microbial flora of carp fillets, irrespective of packaging and antimicrobial treatment (Table 2). The LAB count growth in the control groups samples storage periods. The dominance of LAB has also been confirmed in vacuum packaged trout (Lima, 1986). In other studies, Tassou et al. (1995) observed that the addition of olive oil/lemon juice/oregano oil on cold fresh fish fillets, under MAP, reduced the final LAB counts, by only 0.5 log cfu/g, as compared to the control, whereas Stamatis and Arkoudelos (2007) found high final counts of LAB in the microbial flora of refrigerated fish species, stored under various MAP conditions. Recently, Kykkidou et al. (2009) reported that the use of thyme EO in combination MAP did not have a significant effect (P > 0.05) on the reduction of LAB population in swordfish fillets. The limited action of EOs is attributed to the high tolerance of LAB against the action of EOs, due to their ability to generate ATP and to deal with conditions of osmotic stress (Burt, 2004). It is also possible that the greater resistance of the LAB is related to their better ability to deal with conditions of osmotic stress and respond more effectively to K^+ efflux caused by many of these antimicrobials.

Enterobacteriaceae produced lower final (day 98) counts (ca. 3.96, 3.03 and 2.15 log cfu/g for A, B and C carp samples, respectively) than the other species examined in this study, probably due to the action of vacuum packaged and the antimicrobial effect of the eugenol (Table 2). Tassou (1995) reported that treatment of fresh sea bream fillets with a mixture of olive oil, lemon

and essential oil (oregano) reduced the final Enterobacteriaceae counts by approximately 2.5 log cfu/g, compared to the groups C.

TVBN may be considered as a quality index for fish. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Özoğul, 2004). The initial (day 0) control groups sample TVBN value of 12.6 mg N/100 g is in quite good agreement with Chytiri et al. (2004) and Neratzaki et al. (2005) for fish samples (Table 3). Among the treatments of the present study, A, B and C produced significantly lower ($P < 0.05$) TVBN values as compared to the control samples after 42 days. The inhibition of Neratzaki et al. (2005) reported lower TVBN values (ca. 40 mg N/100 g) for ozonated trout as compared to control, non-ozonated trout (ca. 62 mg N/100 g) on final day (15). Mahmoud et al. (2004) reported a TVBN value of 30 mg N/100 g after 12 days of storage at 5℃, a fter dipping carp fillets in a solution of 0.5% carvacrol and thymol (v/v), whereas the control reached this value after only 4 days. Furthermore, Goulas and Kontominas (2007) reported that the combination of light salting, MAP and oregano essential oil (0.4-0.8% v/wt) extended the shelf life of fresh sea bream by ca. 11–18 days.

Conclusions

From the review of literature, it is observed that although eugenol is an important essential oil, it is rather rarely used. In the present contribution, the usage of eugenol is introduced in different concentrations. The usage of eugenol increased the shelf life of fish for approximately 56 days. However, there was no significant difference between eugenol containing groups in the mean of shelf life. Thus, it is possible to say the 0.5 eugenol containing sauces are sufficient for protection.

Eugenol concentrations used in the study was extended storage period. However, sensory analysis results were evaluated, scored higher than group A samples. Concentration between the chemical and microbiological results when there is no difference between the lowest concentration group was the best sensory aspects. According to the results of this study, suggested eugenol concentration 0.5% (A group). It can be recommended that a work on the effect of sauce containing less than 0.5% eugenol.

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