doi: 10.17221/188/2014-CJFS

Influence of Different Essential Oils on Refrigerated Fish Patties Produced from Bonito Fish (*Sarda sarda* Bloch, 1793)

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Abstract

Guran H.S., Oksuztepe G., Coban O.E., Incili G.K. (2015): **Influence of different essential oils on refrigerated fish patties produced from bonito fish** (*Sarda sarda* Bloch, 1793). Czech J. Food Sci., 33: 37–44.

The effects of different concentrations of thyme (group T), clove (group Cl), and rosemary (group R) extracts on the microbiological, chemical, and sensory attributes of fish patty made from the bonito fish (*Sarda sarda* Bloch, 1793) were investigated. The numbers of total mesophilic aerobic bacteria, coliform bacteria, *Staphylococcus–Micrococcus* spp. and yeasts and moulds in group R were lower than those of the other groups during storage ($P < 0.05$). During the storage period, total volatile basic nitrogen (TVB-N), thiobarbituric acid index (TBA-i), peroxide values, and free fatty acid content gradually increased in all groups, and significant differences were found between the groups ($P < 0.05$). The results of sensory evaluation showed that the shelf life of fish patties was 4 days for the control group, and 8, 10, and 14 days for the groups treated with thyme, clove and rosemary essential oil, respectively ($P < 0.05$). In conclusion, the addition of essential oils showed a positive effect on the product shelf-life; and in particular, rosemary essential oil produced a remarkable effect.

Keywords: bonito fish; thyme oil; clove oil; rosemary oil; shelf-life

Fish is one of the principal protein sources in the human diet. From an economic standpoint, people want to meet their need for animal proteins from cheaper food. As a result of the increasing number of working women and rapid urbanisation, the demand for ready-to-serve food products is rising. Ready-to-serve food products do not spoil for a period of time due to the processing techniques used. These products are consumed either directly or after being heated, and may be served either alone or with other products (Gökoğlu 1994). One of the products categorised as ready-to-serve food is fish patty. Fish patties are produced from cleaned and boiled fish, which is minced and subsequently mixed with spice additives. In hotels and restaurants, processed food products have gained increasing popularity as they offer a variety of aromas and alternatives.

Recently, several researches have been conducted to extend the shelf life of fish products which have a short shelf-life (Turan & Erkoyuncu 2004). In a relevant study, it was detected that fish balls containing raw or boiled anchovy had a shelf life of 9 days at 4 ± 1°C (Gökoğlu 1994). A research conducted by Öksüztepe *et al.* (2010) demonstrated that fresh rainbow trout fish balls containing 2% of sodium lactate had a shelf life of 16 days at 4 ± 1°C. A similar study by Çapkin (2008) pointed out that tench balls were consumable up to 10 days when preserved at 4 ± 1°C.

Mixtures of aromatic plants and essential oils have found common use to ensure the microbiological safety of food products and to extend their shelf life. The rosemary plant (*Rosmarinus officinalis* L.) has been used as an antioxidant or natural preservative in foods (Erkan *et al.* 2008). *T. vulgaris*, also known as common thyme, has long been used as a source of thyme essential oil and its constituents (such as thymol and carvacrol) have been derived from the different parts of the plant (Hudaib *et al.* 2002). These are phenolic compounds that give a unique odour and antioxidant property to thyme. What gives the specific odour and flavour to clove is the volatile oil "eugenol". Eugenol constitutes a significant part

doi: 10.17221/188/2014-CJFS

of carnation extract and has antioxidative properties. It is also known that these essential oils are classified as Generally Recognised as Safe (GRAS) (US FDA 2011).

The antimicrobial and antioxidant activities of essential oils (EOs) have been studied *in vitro*, using various model foods (Burt 2004), as well as commercial food products, such as seafood (Goulas & Kontominas 2007; Uçak *et al.* 2011; Emir Coban & Patir 2013; Ilhak & Guran 2014). Limited data, however, are available with regard to the application of EOs, including thyme oil, clove oil, and rosemary oil for extension of the shelf-life of fish patties. Thus, the aim of the present work was to evaluate the effects of rosemary, thyme, and clove essential oils on the microbiological, chemical, and sensory quality of bonito fish patties during storage at 4°C.

MATERIAL AND METHODS

Raw material. The research material, the bonito fish *(Sarda sarda* Bloch, 1793), was obtained from a local market. For each repetition, approximately 7–8 kg of bonito fish was used, and each fish weighed approximately 300–450 grams. The skin of the fish was removed and their heads were cut off. Next their internal organs were cleaned and the fillets were boiled in hot water for 1–2 minutes. After boiling for 1–2 min the fishbone was removed, and the fillets were minced in a mincing machine equipped with a 3-mm screen.

Preparation of fish patty. The fish patties contained 82% of fish (boiled in water), 5.4% of mashed potatoes, 4.3% of chopped parsley, 2% of chopped onions, 2% of salt, 0.5% of cumin, 0.5% of black pepper powder, 0.5% of pimento, 1.8% of bread, and 1% of olive oil. The mixture was divided into 4 groups. The treatments used in the present study were as follows:

– Group 1: Control (no addition of any essential oil) (Cnt);
– Group 2: 880 µl thyme oil/kg fish patty (Group T);
– Group 3: 2.65 µl clove oil/kg fish patty (Group Cl);
– Group 4: 8.5 ml rosemary oil/kg fish patty (Group R).

Thyme, clove, and rosemary essential oils were purchased from Kalsec® Inc., Kalamazoo, USA. The amounts of undiluted 100% essential oils added to the fish patties were the maximum daily permissible intake (DPI) (Peter 2004). The mixtures were then shaped into fish patty weighing ca. 25 g each. The fish patties were placed on foam plates, and wrapped with plastic wrap and stored at 4°C in a refrigerated incubator (ES 120 model; Nuve, Ankara, Turkey). Microbiological, chemical, and sensory analysis was conducted following 0, 2, 4, 6, 8, 10, 12, 14, and 16 days of storage. The study was composed of three replicates.

Microbiological analysis. On each sampling day, two fish patties (25 g each) were taken from each group and transferred into the sterile stomacher bags. Each fish patty was homogenised in 225 ml of 0.1% sterile peptone water using a Stomacher 400 bagmixer (Interscience, St. Norm, France). Homogenised samples (10^{-1}) were serially diluted in 0.1% sterile peptone water. Total aerobic mesophilic bacteria (TAMB) were counted by the pour plate method using Plate Count Agar (LAB M, Lancashire, UK) incubated at 35°C for 48 hours. *Staphylococcus–Micrococcus* microorganisms were counted on Mannitol Salt Agar (LAB M, Lancashire, England) incubated at 30°C for 48 hours. Coliform bacteria were counted on Violet Red Bile Agar (LAB M, Lancashire, UK) incubated at 30°C for 24 hours and yeast and mould were counted on Potato Dextrose Agar (LAB M, Lancashire, UK) incubated at 21°C for 5 days. All microbiological tests were carried out in duplicate, and the results were expressed as the logarithm of colony forming units per gram (CFU/g).

Chemical analysis. After the end of microbiological analysis the pH value was recorded by immersing a pH electrode (EDT, GP 353 pH meter; Dover, Kent, UK) in the homogenate of the fish patties (Skandamis & Nychas 2001).

Total volatile basic nitrogen (TVB-N) was determined according to the method described by Varlik *et al.* (2004). For determining the total volatile basic nitrogen (TVB-N) a fish patty (10 g) was homogenised with 6% perchloric acid (90 ml) for 1 min in a homogeniser. Then homogenates were filtered through a filter paper (Whatman No. 1) and the filtrates were alkalized with NaOH (20%) before distillation. Duplicate filtrates were then distilled in a Kjeldahl distillation apparatus. After the filtrates were distilled, they were titrated with 0.01 N HCl. Next, the thiobarbituric acid index (TBA-i) was determined using the method described by Vyncke (1970). TBA-i is expressed as mg malondialdehyde/kg sample. A 5 g fish patty sample was homogenised with 25 ml of 7.5% trichloroacetic acid for 1 minute. The suspension was then filtered and 5 ml of the filtrate was added to 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in distilled water). The mixture was immersed in boiling water for 40 min, then cooled

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with water. The absorbance was measured at 538 nm. The peroxide value (PV), expressed as mmole active oxygen/kg lipids, was determined using the modified Wheeler method (Varlik *et al.* 1993). The free fatty acid (FFA) content of the lipid was determined volumetrically using aqueous sodium hydroxide (0.1 N) and phenolphthalein indicator (1% ethanol) according to Pearson's method (Pearson 1976).

Sensory analysis. The sensory evaluation of fish patties was done by a five-member trained panel from the university. To conduct sensory analyses, fried fish patties were evaluated with respect to their colour, odour, flavour, appearance, texture, and general acceptability. The fish patties were fried separately in small amounts of vegetable oil until they turned brown before being presented to the panellists. After frying, they were cooled and samples were served to the panellists who were asked to evaluate on a 5-point hedonic scale ranging from very poor (1) to very good (5) where: 1 – very poor, 2 – poor, 3 – normal, 4 – good, and 5 – very good (Kurtcan & Gonul 1987).

Statistical analysis. The study has been composed of three independent repetitions. Microbiological data was formulated as $\log_{10}$ CFU/g and evaluated according to 3 × 4 × 1 × 9 (repetition number × application × preservation temperature × sampling day) factorial design. By using the SAS program, the data was processed by the analysis of variance with respect to main effects (application, preservation temperature and sampling days) and interactions between the triple variables: application, preservation temperature and sampling days. According to General Linear Model (GLM) procedures, averages of the least squares were designated through Fisher's Least Significant Difference (*LSD*) test and the statistical significance level was taken as 0.05 hereby (SAS 1999).

RESULTS AND DISCUSSION

Microbiological characteristics. Total aerobic mesophilic bacteria (TAMB) are used as an acceptability index for fish and fish products because of the effect of bacteria in spoilage (Emir Çoban & Patir 2013). TAMB of bonito fillets was determined to be 3.60 $\log_{10}$ CFU/g. However, the initial TAMB count of fish patties was 4.54–5.12 $\log_{10}$ CFU/g. These results indicate that the ingredients of fish patties such as parsley, onions, potatoes, and others could contribute high amounts of bacteria since these ingredients are not sterilised. TAMB count increased with an increase in storage time. Significant differences ($P < 0.05$) were determined between groups (Table 1). The control group contained high levels of TAMB compared to treatment groups. Thyme, clove, and rosemary oil can be considered effectively inhibitory on the growth of total aerobic

Table 1. Microbiological analyses of fish patties during storage at 4 ± 1°C ($\log_{10}$ CFU/g)

Microorganism	Fillet	Treatment	Storage time (days)							
			0	2	4	6	8	10	13	14
Total aerobic mesophilic bacteria	3.60	Cnt	5.12^{a}	5.56^{a}	6.48^{a}	NA				
		T	4.84^{b}	5.06^{b}	5.76^{b}	6.45^{a}	6.84^{a}	NA		
		Cl	4.77^{b}	4.95^{b}	5.16^{c}	5.55^{b}	6.14^{b}	6.75^{a}	NA	
		R	4.54^{c}	4.70^{c}	4.90^{d}	5.10^{c}	5.90^{c}	6.30^{b}	6.80^{a}	7.50^{a}
Coliform	2.10	Cnt	3.17^{a}	4.20^{a}	4.85^{a}	NA				
		T	3.10^{a}	4.00^{b}	4.25^{b}	4.80^{a}	5.35^{a}	NA		
		Cl	3.05^{a}	3.80^{b}	4.00^{b}	4.30^{b}	4.55^{b}	5.85^{a}	NA	
		R	2.60^{a}	2.90^{c}	3.15^{c}	3.55^{c}	3.90^{c}	4.40^{b}	5.00^{a}	5.40^{a}
Staphylococcus –Micrococus	1.05	Cnt	2.78^{a}	3.10^{a}	3.90^{a}	NA				
		T	2.50^{a}	2.90^{b}	3.55^{a}	3.90^{a}	4.20^{a}	NA		
		Cl	2.27^{a}	2.43^{b}	2.69^{b}	3.00^{b}	3.45^{b}	4.00^{a}	NA	
		R	1.40^{a}	1.60^{b}	1.75^{b}	2.00^{c}	2.34^{bc}	2.76^{b}	3.05^{a}	3.21^{a}
Yeast and mould	1.30	Cnt	2.48^{a}	2.89^{a}	3.20^{a}	NA				
		T	2.12^{a}	2.52^{a}	2.81^{b}	3.04^{a}	3.57^{a}	NA		
		Cl	2.00^{ab}	2.20^{ab}	2.53^{bc}	2.83^{ab}	3.10^{ab}	3.40^{a}	NA	
		R	1.46^{b}	1.68^{b}	1.80^{c}	1.97^{b}	2.28^{b}	2.79^{b}	2.95^{a}	3.17^{a}

$^{a–c}$means in the same column with different letters are significantly different from the others ($P < 0.05$); Cnt – control, T – thyme (880 µl thyme oil/kg fish patty); Cl – clove (2.65 µl clove oil/kg fish patty), R – rosemary (8.5 ml rosemary oil/kg fish patty); NA – not analysed

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flora. These findings are also parallel to the results of Gökoğlu (1994), Çetin and Bostan (2002), Çapkin (2008), and Öksüztepe *et al.* (2010).

In the present study, coliform bacteria counts of Cnt, T, Cl, and R groups were 4.85, 5.35, 5.85, and 5.40 $\log_{10}$ CFU/g at the end of the storage period, respectively. Based on the statistical analysis, there were no significant differences ($P > 0.05$) in coliform bacteria counts of any group on day 0 of storage while there were significant differences ($P < 0.05$) between the control and treated groups on the other days of storage (Table 1). The lowest coliform bacteria count was observed for Group R.

Staphylococcus–Micrococcus numbers detected as 1.05 $\log_{10}$ CFU/g in bonito fillets showed an increase in all groups starting from the first day of the preservation process. In the control group, it reached a maximum of 3.90 $\log_{10}$ CFU/g on the 4th day; in group T it reached a maximum of 4.20 $\log_{10}$ CFU/g on the 8th day; in group Cl it reached a maximum of 4.00 $\log_{10}$ CFU/g on the 10th day; and in group R it reached a maximum of 3.21 $\log_{10}$ CFU/g on the 14th day. The findings obtained from the control group had greater numbers of microorganisms than the other groups, which is a significant difference between the control and treatment groups. Additionally it was detected that the number of *Staphylococcus–Micrococcus* in group R throughout all preservation periods was 1 value of $\log_{10}$ CFU/g less than in the other groups ($P < 0.05$) (Table 1).

The initial yeast-mould counts of bonito fillets were determined as 1.30 $\log_{10}$ CFU/g. After 4 days, yeast-mould counts were 3.20, 2.81, 2.53, and 1.80 $\log_{10}$ CFU/g for Cnt, T, Cl, and R, respectively. Significant statistical differences were found between the samples ($P < 0.05$). Final populations of yeast-mould (3.57, 3.40, and 3.17 $\log_{10}$ CFU/g) were recorded for treatments T, Cl, and R, respectively (Table 1). These results may explain the effects of rosemary extract on yeast-mould. Emir Çoban *et al.* (2014) and Emir Çoban and Ozpolat (2013) reported that the rosemary extract treatment was effective in eliminating the growth of yeast-mould in smoked fish under refrigerated storage.

Chemical characteristics. The pH values of fish patties are shown in Table 2. At the beginning of storage, the pH value of bonito fillet was determined to be 5.65. Similar results were reported by Kaya *et al.* (2006) for bonito fillets. At the end of the storage period, pH values

Table 2. Chemical analyses of fish patties during storage at 4 ± 1°C

Analysis	Fillet	Treatment	Storage time (days)							
			0	2	4	6	8	10	12	14
pH	5.65	Cnt	5.58^{a}	5.52^{a}	5.59^{a}	NA				
		T	5.56^{a}	5.50^{a}	5.53^{a}	5.65^{a}	5.71^{a}	NA		
		Cl	5.43^{a}	5.38^{a}	5.43^{a}	5.57^{a}	5.65^{a}	5.73^{a}	NA	
		R	5.51^{a}	5.43^{a}	5.47^{a}	5.50^{a}	5.52^{a}	5.64^{a}	5.71^{a}	5.83^{a}
TVB-N (mg/100g)	11.24	Cnt	11.56^{a}	12.73^{a}	13.88^{a}	NA				
		T	11.45^{a}	12.11^{b}	13.12^{b}	13.76^{a}	13.91^{a}	NA		
		Cl	11.32^{a}	11.61^{bc}	12.10^{bc}	12.57^{b}	13.00^{b}	13.41^{a}	NA	
		R	11.29^{a}	11.40^{c}	11.74^{c}	11.90^{c}	12.05^{c}	12.70^{b}	12.93^{a}	13.24^{a}
TBA-i (mg MDA/kg)	0.27	Cnt	0.56^{a}	1.68^{a}	3.49^{a}	NA				
		T	0.43^{a}	0.78^{b}	1.91^{b}	2.64^{a}	2.94^{a}	NA		
		Cl	0.39^{a}	0.63^{bc}	1.57^{bc}	2.07^{b}	2.41^{b}	3.02^{a}	NA	
		R	0.33^{a}	0.51^{c}	1.04^{c}	1.61^{c}	2.01^{c}	2.23^{b}	3.64^{a}	4.04^{a}
Peroxide value (mmole active oxygen/kg lipids)	0.53	Cnt	0.78^{a}	1.30^{a}	2.28^{a}	NA				
		T	0.69^{a}	0.97^{b}	1.84^{b}	2.37^{a}	2.59^{a}	NA		
		Cl	0.60^{a}	0.82^{bc}	1.53^{bc}	1.83^{b}	2.17^{b}	2.53^{a}	NA	
		R	0.51^{a}	0.70^{c}	0.90^{c}	1.12^{c}	1.81^{c}	2.29^{b}	2.46^{a}	2.69^{a}
Free fatty acids (g oleic acid per 100 g lipids)	0.48	Cnt	0.81^{a}	1.18^{a}	1.36^{a}	NA				
		T	0.70^{a}	0.97^{b}	1.29^{b}	1.46^{a}	1.60^{a}	NA		
		Cl	0.60^{a}	0.82^{bc}	1.08^{bc}	1.22^{b}	1.39^{b}	1.57^{a}	NA	
		R	0.53^{a}	0.65^{c}	0.89^{c}	1.03^{c}	1.28^{c}	1.46^{b}	1.55^{a}	1.63^{a}

$^{a–c}$means in the same column with different letters are significantly different from the others ($P < 0.05$); Cnt – control, T – thyme (880 µl thyme oil/kg fish patty); Cl – clove (2.65 µl clove oil/kg fish patty), R – rosemary (8.5 ml rosemary oil/kg fish patty); NA – not analysed

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for Cnt, T, Cl, and R were found to be 5.59, 5.71, 5.73, and 5.83, respectively. The pH of all groups increased during storage, presumably due to the production of basic amines (trimethylamine and other volatile amines) by the spoilage bacteria (Ordonez *et al.* 2000).

The TBA-i is an index of lipid oxidation measuring malondialdehyde (MDA) content and other aldehydes. MDA is formed through hydroperoxides, which are the initial reaction products between polyunsaturated fatty acids and oxygen (Fernandez *et al.* 1997; Kostaki 2009). Changes in TBA-i in the different treatments during storage are shown in Table 2. The TBA-i indicating rancidity development in all fish balls remained low (< 5 mg MDA/kg) and below the limit level at which rancid flavours may become evident in fish (Nunes *et al.* 1992). Significant differences ($P < 0.05$) were found between T, Cl, and group R after 6 days of storage (Table 2). The TBA-i of fish patties reached 3.49, 2.94, 3.02, and 4.04 mg MDA/kg at the point of sensory rejection for samples Cnt, T, Cl, and group R, respectively. Similar results with essential oils were reported earlier (Kenar *et al.* 2010; Ucak *et al.* 2011; Emir Çoban *et al.* 2014). TBA-i values ranged from 0.33 mg MDA/kg to 4.04 mg MDA/kg for group R, and this may be attributed to the strong antioxidant properties of the rosemary essential oil. Kenar *et al.* (2010) found that the initial TBA-i value of 0.58 mg MDA/kg in vacuum packed sardines treated with rosemary extract (10 g/l) at 3 ± 1°C showed a little fluctuation and reached 0.84 mg MDA/kg at the end of the storage period (20 days). This stability may be related to the removal of oxygen from the pack (Alak *et al.* 2010).

TVB-N is one of the most widely used indices of fish and fish product quality. It is a general term which includes the measurement of trimethylamine, dimethylamine, ammonia, and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss 1995). At the beginning of the storage period, TVB-N levels were found to be 11.56 mg/100 g for Cnt, 11.45 mg/100 g for T, 11.32 mg/100 g for Cl, and 11.29 mg/100 g for R. TVB-N levels increased from 11.56 to 13.88 mg/100 g in the control group at the end of storage. Significantly ($P < 0.05$) lower TVB-N levels were obtained from group R (rosemary treatment) during storage (Table 2). TVB-N levels increased in all groups during storage. An increase in TVB-N levels in all groups may be the result of deamination of free amino acids, oxidation of amines, and degradation of nucleotides by autolytic enzymes and microbial activity (Ocano-Higuera *et al.* 2011). Our results indicate that the rosemary essential oil was more effective at inhibiting enzymatic and microbial activity than the other treatments. These results are in agreement with those of Kenar *et al.* (2010), Ozogul *et al.* (2010), and Uçak *et al.* (2011), who reported that rosemary oil generally showed lower TVB-N values for fish and fish products. Lang (1983) suggested that the quality classification of fish and fish products regarding TVB-N values would be "high quality" up to 25 mg per 100 g and not exceeding the acceptability limit (35 mg/100 g), "good quality" up to 30 mg/100 g, "limit of acceptability" up to 35 mg/100 g, and "spoiled" above 35 mg/100 g. At the end of storage, TVB-N values did not exceed the acceptability limit (35 mg/100 g) for all groups. To our knowledge, very little information is available in the literature on the effect of EOs on TVB-N production in seafood.

The peroxide value is expected to be below 2 mmole active oxygen/kg lipids in very good material, and not to exceed 5 mmole active oxygen/kg lipids in good material. The initial PVs were 0.78 mmole active oxygen/kg lipids, 0.69 mmole active oxygen per kg lipids, 0.60 mmole active oxygen/kg lipids, and 0.51 mmole active oxygen/kg lipids for Cnt, T, Cl, and R, respectively. In all samples, it fluctuated between 0.51 and 2.69 mmole active oxygen/kg lipids during storage. Significant differences ($P < 0.05$) in PVs were found between Cnt and treated groups on the 2nd day (Table 2). A lower PV was observed for group R. This could be explained that rosemary essential oil prevents fish patties from undergoing oxidation or inhibits bacterial enzyme activity. The antioxidant attributes of rosemary extract stem from carnosol, carnosic acid, and rosmarinic acid found in its structure (Richheimer *et al.* 1996; Emir Çoban & Ozpolat 2013). It has been reported (Uçak *et al.* 2011) that the addition of rosemary extract into Atlantic mackerel fish burgers resulted in lower oxidation in treated compared to control groups in terms of peroxide values. These findings are similar to the results reported by Quitral *et al.* (2009) and Gao *et al.* (2014), who demonstrated that the peroxide levels in groups with added rosemary oil were lower compared to untreated groups.

A lower FFA content was observed for both Cl and R while the highest FFA content was determined for the control group (Table 2). Lipid hydrolysis developed at a slower rate in the samples treated with clove and rosemary extract compared to the control group. Similar results were reported by other researchers (Olley *et al.* 1969; Serdaroğlu & Felekoğlu 2005; Ozogul *et al.* 2010; Uçak *et al.* 2011; Emir Çoban

doi: 10.17221/188/2014-CJFS

et al. 2014). An increase in FFA results from the enzymatic hydrolysis of esterified lipids. The connection between lipolysis and lipid oxidation is that FFA oxidise more readily than esterified lipids (Hwang & Regenstein 1993; Ashton 2002; Uçak *et al.* 2011).

Sensory characteristics. The results of the sensory evaluation (colour, appearance, texture, odour, flavour, and general acceptability) of bonito fish patties are presented in Table 3. According to the statistical analysis, there were no significant differences ($P > 0.05$) in colour, appearance, odour, and texture between all groups during storage at 4 ± 1°C. However, significant differences ($P < 0.05$) were observed between the control and treated groups. Group R was mostly preferred by the panellists. The use of rosemary extract improved the sensory quality of fish patties. Similar results have been reported in the other fish products treated with rosemary extract (Corbo *et al.* 2009; Mahmoudzadeh *et al.* 2010; Uçak *et al.* 2011). Based on colour, appearance, texture, odour, taste, and general acceptability scores, the shelf life of fish patties was 4 days for the control, 8 days for group T, 10 days for group Cl, and 14 days for group R. In the present study, it was demonstrated that the addition of essential oils into bonito fish (*Sarda sarda* Bloch, 1793) patties did not affect certain sensory properties (colour, appearance, odour, flavour, and texture) of the product, but had an effect on the taste and general acceptability of the product. However, the fish patties used in the present study are a very complex food containing spices such as cumin, black pepper, pimento, onions etc. Presumably, the negative effect of essential oils on the sensory attributes of the fish patty may be masked by the ingredients and spices used in the production of fish patties. The use of higher concentrations than we used in the present study may result in a further increase of the shelf life of fish patties, but high EO concentrations would probably impart unpleasant sensory effects (strong odour and flavour, etc.) on the quality of fish patties.

Table 3. Results of sensory analyses of fish patties during storage at 4 ± 1°C

Sensory parameter	Treatment	Storage time (days)						
		2	4	6	8	10	12	14
Colour	Cnt	4.75^{a}	4.00^{a}	NA				
	T	4.79^{a}	4.46^{a}	4.24^{a}	4.00^{a}	NA		
	Cl	4.84^{a}	4.79^{a}	4.58^{a}	4.40^{a}	4.15^{a}	NA	
	R	4.96^{a}	4.88^{a}	4.75^{a}	4.69^{a}	4.54^{a}	4.49^{a}	4.32^{a}
Appearance	Cnt	4.75^{a}	4.63^{a}	NA				
	T	4.80^{a}	4.78^{a}	4.51^{a}	4.46^{a}	NA		
	Cl	4.88^{a}	4.82^{a}	4.68^{a}	4.52^{a}	4.37^{a}	NA	
	R	4.94^{a}	4.89^{a}	4.85^{a}	4.61^{a}	4.40^{a}	4.28^{a}	4.09^{a}
Odour	Cnt	4.75^{a}	4.70^{a}	NA				
	T	4.85^{a}	4.85^{a}	4.76^{a}	4.52^{a}	NA		
	Cl	5.00^{a}	4.76^{a}	4.71^{a}	4.58^{a}	4.00^{a}	NA	
	R	5.00^{a}	4.85^{a}	4.70^{a}	4.00^{a}	3.90^{a}	3.40^{a}	2.70^{a}
Texture	Cnt	4.75^{a}	4.50^{a}	NA				
	T	4.80^{a}	4.75^{a}	4.10^{a}	3.60^{a}	NA		
	Cl	4.85^{a}	4.60^{a}	4.10^{a}	3.90^{a}	3.45^{a}	NA	
	R	5.00^{a}	4.75^{a}	4.30^{a}	4.00^{a}	3.80^{a}	3.30^{a}	2.55^{a}
Flavour	Cnt	4.70^{a}	4.50^{a}	NA				
	T	4.75^{a}	4.20^{a}	3.50^{a}	3.40^{a}	NA		
	Cl	4.88^{bc}	4.40^{b}	3.95^{b}	3.80^{b}	3.35^{a}	NA	
	R	5.00^{c}	4.80^{c}	4.10^{c}	3.88^{c}	3.55^{b}	3.30^{a}	2.45^{a}
General acceptability	Cnt	4.25^{a}	4.00^{a}	NA				
	T	4.50^{a}	4.35^{a}	4.10^{a}	3.98^{a}	NA		
	Cl	4.55^{bc}	4.45^{bc}	4.35^{b}	4.00^{b}	3.80^{a}	NA	
	R	5.00^{c}	4.88^{c}	4.75^{c}	4.60^{c}	4.20^{b}	3.85^{a}	3.55^{a}

$^{a–c}$means in the same column with different letters are significantly different from the others ($P < 0.05$); Cnt – control, T – thyme (880 µl thyme oil/kg fish patty); Cl – clove (2.65 µl clove oil/kg fish patty), R – rosemary (8.5 ml rosemary oil/kg fish patty); NA – not analysed

doi: 10.17221/188/2014-CJFS

Natural preservatives such as essential oils can be used as a safe method for storage of fish patties. It must be noted that our study is the first to report on the use of thyme, clove, and rosemary essential oils in the shelf life evaluation of bonito fish patties during storage at 4°C. The present study showed that a treatment with 8.5 ml rosemary oil/kg fish patty could effectively retard microbial growth, delay chemical deterioration, maintain or improve sensory attributes, and extend the shelf life of fish patty samples for 14 days during refrigerated storage. However, further studies are needed with regard to the preservation of fish patties using natural preservatives, including essential oils, in view of increasing the consumer demand for preservative-free seafood.

Acknowledgements. The authors thank Dr. Osman İrfan İlhak for the contributions to the manuscript and thank Eric McCoy for improvement in the language of the manuscript.

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Received: 2014–04–04
Accepted after corrections: 2014–06–27

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