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Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchusmykiss*) fillets

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ABSTRACT

Carrageenan-based biocomposites have significant potential to develop packaging films and coatings for shelf-life extension of food products. In this study trout fresh fillets were separated into three groups: uncoated trout fillet (UTF), coated with carrageenan-based materials (coated trout fillet, CTF), and coated with carrageenan-based materials containing 1 g/100 mL essential lemon oil (active coated trout fillet, ACTF). The effect of carrageenan coating enriched with essential lemon oil on the quality of rainbow trout (*Oncorhynchusmykiss*) fillets during refrigerated storage (4 ± 1 °C) over a period of 15 days was evaluated. Trout fillets were analyzed for microbiological (total viable count, *Enterobacteriacea* counts, lactic acid bacteria, H_2S-producing bacteria), chemical (TVB-N, moisture, pH), and biochemical (fatty acids content) characteristics. This study demonstrates the effectiveness of an edible active carrageenan coating in preserving fresh trout fillets from lipid oxidation and microbial growth.

1. Introduction

The rainbow trout (*Oncorhynchusmykiss*) is one of the major aquaculture fish species worldwide. The production of rainbow trout has grown exponentially since the 1950s, due to increased inland and off-shore cages (FAO – Fisheries and Aquaculture Information and Statistics Service http://www.fao.org/fishery/en). Although, several aspects of rainbow trout farming industry are highly efficient, current research and development are continually attempting to increase production efficiency and sales, the latter accomplished by developing better marketing. Since fish fillets are highly perishable products, the development of preservation methods in order to extend their shelf-life would be desirable. Deterioration of fish mostly occurs in the fat-containing portions, especially in the unsaturated fatty acid component, which quickly oxidizes in presence of the environmental oxygen. In this respect trout is particularly sensitive to spoilage due to the high content of unsaturated fatty acids. Trout is an important source of high-quality proteins for humans, however, it is highly susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, relatively large quantities of free amino acids, high amount of polyunsaturated fatty acids, and presence of autolytic enzymes (Ghaly, Dave, Budge, & Brooks, 2010 for review).

Cold storage and freezing are the normally employed methods for fish preservation, however they cannot prevent oxidative spoilage. Thus, vacuum packaging, modified atmosphere (MAP), cold and hot smocking, salting, wrapping in aluminum foil or cling film and combinations of such methods, have been investigated for their effectiveness in prolonging the shelf-life of fish (Arashisar, Hisar, Kaya, & Yanik, 2004; Chytiri, Chouliara, Savvaidis, & Kontominas, 2004; Erkan, 2012; Erkan, Tosun, Ulusoy, & Üretener, 2011; Frangos, Pyrgotou, Giatracou, Ntzimani, & Savvaidis, 2010; Giménez, Roncalés, & Beltrán, 2002; Gómez-Estaca, 2007; Pyrgotou, Giatrakou, Ntzimani, & Savvaidis, 2010).Moreover, edible films and coating are possible opportunities to prolong the shelf-life of perishable food products such as fish fillets. Natural polysaccharides are good candidates as base ingredients of both edible films and coating to provide a shelf-life extender (Volpe, Malinconico, Varricchio, & Paolucci, 2010 for review). Edible coatings made of protein or polysaccharides (with the advantage of polysaccharides of being reported as no allergenic, tasteless and

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odorless), alone or in combination with biological or not biological materials (Polyvinylchloride-PVC, gelatin, proteins) seem to represent a valid alternative too and are actively investigated for fish shelf-life extension (Gómez-Estaca, 2007; Kilincceker, Dogan, & Kucukoner, 2009; Ojagh, Rezaei, Razavi, & Hosseini, 2010). Furthermore, ingredients additives, such as antioxidant substances and antimicrobial agents can be included in the formulations for edible films and coatings in order to increase the shelf-life of foods. Consumers are increasingly aware of the risk for health due to the presence of chemical substances added to food for their preservation and antimicrobial properties. In this respect, plant essential oils are gaining interest for their antimicrobial activity and their flavors that lead them to be widely used within the food industry (Ahn et al., 2007; Burt, 2004; Gutierrez, Barry-Ryan, & Bourke, 2009). Oregano, rosemary, thyme and garlic are amongst the most employed EO as antimicrobial agents against food-borne pathogens and spoilage micro flora in fish (Erkan, 2012; Erkan et al., 2011; Frangos et al., 2010; Gómez-Estaca, 2007; Mexis, Badeka, Riganakos, Karakostas, & Kontominas, 2009; Pereira de Abreu, Paseiro-Losada, Maroto, & Cruz, 2010; Pyrgotou et al., 2010). Citrus EO have also been successfully exploited for their antimicrobial activities on both Gram positive and Gram negative bacteria (Espina et al., 2011; Fisher & Phillips, 2006; Gutierrez et al., 2009; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). The lemon essential oil is antiseptic, cleansing and fungicide and can be useful for boosting the immune system and fight infection. Essential oils are a great alternative to the chemicals added to foods; in fact, their activities and their active constituents act on the membrane of bacterial cells limiting proliferation (Delaquis, Stanich, Girard, & Mazza, 2002).

In this study, carrageenan was used to make an edible coating added with lemon EO (active coating) to extend the shelf-life of trout fillets. In particular, we have focused on the lemon EO due to the large production of lemons in the Campania region. To assess the effectiveness of the active coating, changes in pH, TBV-N, bacteria growth and changes in fatty acids composition were studied on rainbow trout fillets stored at 4 °C for 15 days. Moreover, the change of two indices of lipid quality, Index of atherogenicity (IA) and Index of thrombogenicity (IT), were assessed in function of shelf life.

2. Materials and methods

2.1. Preparation of trout fillets

Freshwater rainbow trout with an average weight of 700–800 g were purchased by a local fish farmer (Di Mella, Santacroce del Sannio, Benevento, Italy) and brought alive to the laboratory where they were decapitated, gutted and filleted by hands. To obtain uniform size of testing samples, skinless fillets were cut into 5 × 7 cm squares of 1 cm of thickness.

2.2. Chemical analysis of the essential lemon oil

The essential lemon oil (ELO) was kindly provided by SSEA – Experimental Station of Essential Oil and Citrus Products (Reggio Calabria, Calabria, Italy). The volatile fraction of ELO was analyzed by Gas chromatography at high resolution according to Siano and Cautela (2012). Briefly, a trace GC Thermo (Thermo, Rodano, Italy) equipped with a FID (flame ionization detector) and autosampler system model AS 3000 with 10 mL syringe set at 0.3 μL essential oil injecting in split modality 1:60, was employed. Analyses were conducted with a DB-5 (Restek Co.) column (30 m × 0.25 mm, film 0.25 μm) with the following temperature program: 70 °C for 10 min, programmed from 70 to 120 °C at 3 °C/min, from 120 to 220 °C at 4 °C/min for 5 min, from 220 at 280 °C at 15 °C/min for 10 min. Carrier gas was helium at constant flow of 1.5 mL/min, make-up gas was nitrogen at 30 mL/min flow rate. The injector and detector temperatures were both set at 250 °C. SGT filter was used as purifiers for carrier and make-up gas. Data acquisition was performed with a Intel *Core2* computer equipped with ChromQuest 5.0 integration software (Thermo, Rodano, Italy). Volatiles compounds contents was expressed as the percentage of GC area.

2.3. Preparation of the coating-forming solutions and treatments

Food-grade carrageenan (Sigma–Aldrich Co. LLC., USA) was used for the coating formulations. Preliminary tests with 0.5, 1 and 2 g/100 g of carrageenan were carried out for the optimal coating formulation. 1 g/100 g carrageenan gave the best results in terms of gel strength and was therefore used for the successive coating formulation. According to Erkan et al. (2011), 1 g/100 g of essential lemon oil was used in the carrageenan coating formulation. Carrageenan solution was prepared by mixing 1 g of carrageenan with 100 mL of distilled water and stirred at a temperature of 100 °C until the mixture became clear. Before gelation (about 30 °C) 1 g/100 g of ELO was mixed with the prepared carrageenan solution and stirred thoroughly. Trout fillets were divided into three groups to which the following treatments were randomly assigned: (UTF) uncoated trout fillets – fillets were stored in a container without any treatment; (CTF) coated trout fillet – fillets were individually submerged in 1 g/100 g carrageenan solution; (ACTF) active coated trout fillet – fillets were individually submerged in 1 g/100 g carrageenan solution with 1 g/100 g ELO. Fillets were then stored in a refrigerator at 0–4 °C. During storage, the samples were randomly taken every 3 days for analyses.

2.4. pH measurements

pH determinations have been carried out by a CRISON (Barcelona, Spain) mod. 507 pH-meter equipped with type 52-00 electrodes and a type 52-32 electrode for penetration analysis.

2.5. Determination of total volatile basic-nitrogen (TVB-N)

TVB-N was determined according to a modified micro-Kjeldahl distillation technique described by Cobb, Alaniz, and Thompson (1973). The TVB-N was expressed as milligrams of nitrogen per 100 g of sample, determined by micro-Kjeldahl, from the following equation:

(TVB-N) + $\{(V_1 - V_2)\ N \times 100 \times 14 \times 50\}/W \times 5$, where V_1 = volume (mL) of H_2SO_4 used for sample, V_2 = volume (mL) of H_2SO_4 used for blank, N = normality of H_2SO_4, and W = weight of sample (g).

2.6. Microbiological analysis

Samples (25 g) of UTF, CTF and ACTF were collected aseptically and transferred into a stomacher bag (Seward Medical, London, UK) containing 225 mL of Ringer solution. The mixture was homogenized for 1 min using a Stomacher (Lab Blender 400) at room temperature. Further serial dilutions were prepared from this homogenate. Appropriate dilutions were used for microbiological analyses. Analysis were carried out at 0, 3, 7, 12 and 15 days. The following media and incubation conditions were used: Plate Count Agar (PCA, Wade Road, Basingstoke, Hants RG24 8 PW England, Oxoid) for total viable counts (TVC), incubated at 28 °C for 72 h; Violet Red Bile Glucose Agar (VRBGA, Oxoid) for *Enterobacteriaceae* (including *Shewanellaputrefaciens*) (E) counts, incubated at 36 °C for 48 h; MRSagar (Wade Road, Basingstoke, Hants RG24 8 PW

England, Oxoid) for Lactic Acid Bacteria (LAB) counts, incubated anaerobically at 28 °C for 72 h; SPS agar (Wade Road Basingstoke Hants RG24 8 PW England, Oxoid) forH_2S-producing bacteria at 28 °C for 5 days. All microbiological counts were performed in duplicate and the results were expressed as the log of the number of colony-forming units per g ($\log_{10}$ cfu/g) of fillet.

2.7. Lipid analysis

2.7.1. Lipid extraction

The lipids of trout fillet were extracted with the Soxhlet method according to the Methods of analysis used for chemical control of food of the National Institute of Health, 1996 (Reports ISTISAN 96/34). A sample of 20 g was extracted using diethyl ether (Sigma–Aldrich CO. LLC., USA) as solvent, for 6 h. The solvent was removed with a rotary evaporator (Mod. Hei VAP Value, Heidolph, Germany). The residue was placed in a drier and weighed up to constant value.

2.7.2. Fatty acid methyl esters (FAMEs) preparation

The fatty acid profile was determined by gas chromatography, as fatty acid methyl esters (FAMEs) by gas chromatographic analysis, according to method AOAC 996.06 (AOAC, 1997). The oil was transferred to a pyrex test tube with screw cap, and 2 mL of methanolic-HCl solution 3 mol/L were added. Sample was placed in a water bath at 80 °C for 60 min. The fatty acid methyl esters were extracted with *n*-hexane, after addition of distilled water. The solution was filtered and directly injected into the gas chromatograph (GC) for analysis.

2.7.3. FAMEs gas chromatographic analysis

The fatty acid content of UTF, CTF and ACTF was determined using an SP-2560 (100 m × 0.25 mm × 0.20 μm, Supelco, Sigma–Aldrich CO. LLC., USA) capillary column. Sample was introduced by injection system split-splitless in split mode (ratio 1:100). The operating conditions utilized were in according to Bernardo-Gil, Oneto, Antunes, Rodrigues, and Empis (2001) and Kmiecik et al. (2011), with minor modifications. The oven temperature program started at 140 °C (held for 5 min), linearly increased to 260 °C (4 °C/min) and kept at this temperature for the remaining time of analysis. Fatty acids composition of trout fillets was obtained by comparison with retention times of the standard mixture FAMEs and was expressed as percentage. Data were recorded and processed by the ChromQuest 5.0 software (Thermo, Rodano, Italy).

2.7.4. Indices of lipid quality

From the data on the fatty-acid composition, the following were calculated:

$$\text{Index of atherogenicity (IA)} = 12:0 + (4 \times 14:0) + 16:0/n-6\ \text{PUFA} + n-3\ \text{PUFA} + \text{MUFA};$$

Index of thrombogenicity (IT) = 14:0 + 16:0 + 18:0/(0.5 MUFA) + (0.5 *n* − 6 PUFA) + (3 *n* − 3 PUFA) + (n − 3 PUFAI n − 6 PUFA). Such indices were proposed by Ulbricht & Southgate (1991) to better characterize the atherogenic and thrombogenic potential of the diet.

MUFA are monounsaturated fatty acids and PUFA polyunsaturated fatty acids.

2.8. Statistical analysis

All experiments were performed in triplicate. Data were analyzed by one-way analysis of variance (ANOVA) and any significant difference was determined at 0.05 level by Tukey's test. The results were expressed as mean ± standard deviation (SD). The analysis were carried out with the Statistica version 7.0 statistical package (Statsoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Chemical composition of the lemon essential oil

Qualitative and quantitative analyses of the ELO volatile profiles are shown in Table 1. The component present in the greatest amount was limonene, accounting for 62.12% of the total detected constituents, followed by β-pinene (14.34%), γ-terpinene (8.88%), sabinene (2.58%), α-pinene (1.58%), Myrcene (1.44%), geranial (1.12%) and β-bisabolene (1.0%). The concentrations of the remaining components were all below 1%. Limonene is the main volatile component of citrus fruits including, other than lemon, orange, bergamot and mandarin (Espina et al., 2011; Fisher & Phillips, 2006; Viuda-Martos et al., 2008). However, it has been demonstrated that the antimicrobial activity of citrus EO is not

Table 1
Chemical composition of lemon essential oil from Reggio Calabria (Italy).

N	Components	Percentage[a]
1	α-thujene	0.28
2	α-pinene	1.58
3	Camphene	0.06
4	Sabinene	2.18
5	β-pinene	14.34
6	Myrcene	1.44
7	Octanal	0.05
8	α-phellandrene	0.28
9	Δ_3-carene	0.01
10	α-terpinene	0.18
11	p-cymene	0.23
12	Limonene	62.12
13	(Z)-β-ocimene	0.35
14	(E)-β-ocimene	0.21
15	γ-terpinene	8.88
16	Cis-sabinene hydrate	0.07
17	Terpinolene	0.35
18	Trans-sabinene hydrate	0.01
19	Linalool	0.18
20	Nonanal	0.22
21	Cis-limonene oxide	0.01
22	Trans-limonene oxide	0.01
23	Camphor	0.01
24	Citronellal	0.13
25	Terpinen-4-ol	0.03
26	α-terpineol	0.33
27	Decanal	0.04
28	Octyl acetate	0.01
29	Nerol	0.35
30	Citronellol	0.01
31	Neral	0.60
32	Geraniol	0.06
33	Geranial	1.12
34	Bornyl acetate	0.04
35	Undecanal	0.04
36	Nonyl acetate	0.02
37	Citronellyl acetate	0.04
38	Neryl acetate	0.52
39	Geranyl acetate	0.54
40	Dodecanal	0.01
41	Decyl acetate	0.01
42	β-caryophyllene	0.33
43	α-bergamotene	0.77
44	Germacrene D	0.02
45	Valercene	0.06
46	β-bisabolene	1.00
47	Nootkatone	0.02

[a] Relative area percentage without using FID response correction factor.

Table 2
Fatty acid profile of rainbow trout (*Oncorhynchusmykiss*) fillets during 15 day cold storage.

% Area FA[a]	Day 0	Day 4			Day 7			Day 15		
	UTF	UTF	CTF	ACTF	UTF	CTF	ACTF	UTF	CTF	ACTF
4:0	3.11 ± 0.1	4.1 ± 0.3	3.79 ± 0.2	4.25 ± 0.5	2.34 ± 0.2	3.74 ± 0.3	3.51 ± 0.1	4.81 ± 0.5	3.94 ± 0.4	3.55 ± 0.4
16:0	12.19 ± 0.7	14.27 ± 0.5	13.66 ± 0.5	13.01 ± 0.2	16.66 ± 0.7	15.37 ± 0.2	13.94 ± 0.1	17.08 ± 0.8	16.21 ± 0.2	14.45 ± 0.2
16:1	3.45 ± 0.5	3.2 ± 0.1	3.33 ± 0.2	3.41 ± 0.2	2.7 ± 0.1	2.99 ± 0.1	3.23 ± 0.2	2.67 ± 0.3	3.01 ± 0.3	3.26 ± 0.5
18:0	3.03 ± 0.1	3.35 ± 0.2	3.22 ± 0.3	3.12 ± 0.1	4.92 ± 0.1	4.34 ± 0.3	3.55 ± 0.2	4.51 ± 0.5	4.28 ± 0.5	3.28 ± 0.1
18:1n9	27.77 ± 0.8	26.82 ± 1.5	27.27 ± 0.6	27.59 ± 0.5	21.09 ± 0.6	24.86 ± 0.8	26.91 ± 0.8	20.88 ± 1.9	21.45 ± 0.7	25.73 ± 0.9
18:2n6	26.67 ± 0.5	22.95 ± 1.2	24.53 ± 0.9	25.88 ± 0.8	17.04 ± 0.7	21.79 ± 0.7	25.81 ± 0.6	19.21 ± 0.5	19.27 ± 0.5	24.66 ± 0.9
18:3n3	4.44 ± 0.1	4.35 ± 0.1	4.22 ± 0.5	4.39 ± 0.2	3.56 ± 0.2	3.88 ± 0.1	4.39 ± 0.1	4.32 ± 0.2	3.39 ± 0.5	3.39 ± 0.2
20:1n9	0.5 ± 0.1	0.62 ± 0.1	0.64 ± 0.2	0.56 ± 0.3	0.65 ± 0.2	0.6 ± 0.1	0.54 ± 0.2	1.04 ± 0.5	0.78 ± 0.5	0.66 ± 0.2
20:2	1.03 ± 0.3	0.95 ± 0.3	1.01 ± 0.5	1.00 ± 0.2	0.75 ± 0.1	0.91 ± 0.2	0.97 ± 0.1	0.88 ± 0.4	0.94 ± 0.2	0.97 ± 0.5
20:3n6	0.41 ± 0.1	0.39 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	0.36 ± 0.1	0.39 ± 0.1	0.39 ± 0.2	0.35 ± 0.5	0.38 ± 0.1	0.39 ± 0.1
20:3n3	0.91 ± 0.2	0.87 ± 0.1	0.89 ± 0.2	0.90 ± 0.2		1.02 ± 0.1			1.01 ± 0.3	1.01 ± 0.4
20:5n3	2.9 ± 0.5	2.89 ± 0.5	2.9 ± 0.5	2.90 ± 0.5	2.76 ± 0.8	2.81 ± 0.4	2.85 ± 0.5	2.54 ± 0.2	2.8 ± 0.6	2.83 ± 0.1
22:2	0.46 ± 0.1	0.41 ± 0.2	0.4 ± 0.2	0.43 ± 0.1	0.39 ± 0.2	0.38 ± 0.1	0.38 ± 0.1	0.31 ± 0.2	0.35 ± 0.2	0.37 ± 0.1
22:6n3	7.05 ± 0.8	6.61 ± 0.5	6.79 ± 0.7	6.98 ± 0.7	6.01 ± 0.5	6.57 ± 0.1	6.87 ± 0.8	5.91 ± 0.5	6.33 ± 0.5	6.77 ± 0.9
SFA	18.84 ± 0.8	22.19 ± 1.2	20.67 ± 0.8	20.38 ± 0.5	23.92 ± 1.1	23.45 ± 0.7	21 ± 0.7	27.44 ± 1.5	24.43 ± 0.9	21.28 ± 0.8
MUFA	31.72 ± 1.1	30.64 ± 0.9	31.24 ± 1.2	31.56 ± 1.5	24.44 ± 1.0	28.45 ± 0.8	30.68 ± 1.4	24.59 ± 0.5	25.24 ± 0.5	29.65 ± 1.2
PUFA	44.26 ± 2.5	39.42 ± 2.4	41.14 ± 2.0	42.88 ± 1.8	30.87 ± 0.9	37.75 ± 1.5	41.66 ± 1.2	33.52 ± 1.0	34.47 ± 0.8	40.39 ± 2.1
PUFA/MUFA ratio	1.40 ± 0.5	1.29 ± 0.7	1.32 ± 0.5	1.36 ± 0.9	1.26 ± 0.5	1.33 ± 0.2	1.36 ± 0.5	1.36 ± 0.5	1.37 ± 0.2	1.36 ± 0.5
Total n6	27.47 ± 1.2	23.34 ± 1.1	24.93 ± 1.5	26.28 ± 0.9	17.40 ± 0.8	22.18 ± 0.9	26.20 ± 0.7	19.56 ± 0.8	19.65 ± 0.5	25.05 ± 0.6
Total n3	15.30 ± 0.5	14.72 ± 0.8	14.80 ± 0.8	15.17 ± 1.1	12.33 ± 0.8	14.28 ± 1.0	14.11 ± 0.9	12.77 ± 0.5	13.53 ± 0.5	14.00 ± 0.9
n3/n6 ratio	0.56 ± 0.1	0.63 ± 0.1	0.59 ± 0.1	0.58 ± 0.5	0.71 ± 0.2	0.64 ± 0.1	0.54 ± 0.2	0.65 ± 0.1	0.69 ± 0.1	0.56 ± 0.2
n6/n3 ratio	1.80 ± 0.7	1.56 ± 0.5	1.68 ± 0.5	1.73 ± 0.3	1.41 ± 0.7	1.55 ± 1.0	1.86 ± 0.8	1.53 ± 0.2	1.45 ± 0.7	1.79 ± 1.1
DHA/EPA ratio	2.43 ± 0.5	2.29 ± 0.2	2.34 ± 0.1	2.41 ± 0.5	2.18 ± 0.5	2.34 ± 0.2	2.41 ± 0.5	2.33 ± 0.2	2.26 ± 0.2	2.39 ± 0.7
DHA + EPA	9.95 ± 0.8	9.5 ± 0.5	9.69 ± 2.2	9.88 ± 0.8	8.77 ± 1.4	9.38 ± 1.1	9.72 ± 0.7	8.45 ± 0.9	9.13 ± 0.5	9.6 ± 0.5
IA[b]	0.16 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	0.18 ± 0.02	0.31 ± 0.01	0.24 ± 0.03	0.20 ± 0.01	0.3 0 ± 0.01	0.28 ± 0.04	0.21 ± 0.01
IT[c]	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.22 ± 0.02	0.20 ± 0.04	0.20 ± 0.01	0.22 ± 0.02	0.21 ± 0.01	0.20 ± 0.01

[a] Percentage area of total fatty acids.
[b] IA: Index of atherogenicity.
[c] IT: Index of thrombogenicity.

ascribable to Limonene, but instead it seems to be due to the presence of other constituents such as oxygenated monoterpenes (Chutia, DekaBhuyan, Pathak, Sarma, & Boruah, 2009). It has been proposed that oxygenated monoterpenes might be involved in the higher microbial activity of mandarin EO compared to lemon EO (Espina et al., 2011) although Viuda-Martos et al. (2008) showed that lemon EO possessed remarkable antibacterial activity against *Enterobacteriaceae* and Lactic Acid Bacteria.

3.2. General aspect

The effect of carrageenan coating and carrageenan coating with additional ELO on the general aspect of trout fillets is reported in Fig. 1. According to the visual inspection, after 7 days of cold storage the uncoated trout fillets (UTF) appeared grayish and dehydrated, while both the carrageenan coated (CTF) and carrageenan coated with additional ELO (ACTF) (not shown) were shiny and turgid and such characteristics maintain up to 15 days.

3.3. pH

pH trend of trout fillet without and with coating during storage at 4 °C for 15 days is shown in Fig. 2. The initial pH was 6.50, a value in agreement with that reported by Giménez et al., 2002 and Mexis et al. (2009) but slightly higher than that reported by Chytiri et al. (2004) and Arashisar et al. (2004). pH differences observed in fresh fish flesh are usually due to the dissociation of carbonic acid in general, which brings about the pH increase as the storage time progresses. The pH of fresh fish fillet is almost neutral but in the post-mortem period, decomposition of nitrogenous compounds leads to the increase in pH which affects the quality of the product during storage; especially, the sensorial characteristics such as odor, color, and texture are negatively affected (Abbas, Saleh, Mohamed, & Lasekan, 2009). In this study, the pH of the uncoated trout fillets (UTF) increased from 6.50 to 7.13 after 15 days. In the case of coated trout fillets (CTF) the pH reached a value of 6.82 after 15 days, while for the active coated trout fillets (ACTF) the pH value at 15 days was 6.75. Such results are likely due to a reduction of bacterial growth exerted by the carrageenan coating with ELO and, although at a lesser extent, by the carrageenan coating alone. Moreover, in presence of coating the fillet surface exposed to the atmosphere is protected from the spoiling action of the oxygen, which is known to increase the pH value likely a consequence of the basic amines production (Pastoriza, Sampedro, Herrera, & Cabo, 1996).

3.4. TVB-N changes during storage

TVB-N is one of the most widely used indices of fish quality. High values of TVB-N are not desirable since they indicate the existence of nitrogenous materials deriving from the degradation, operated by proteolytic bacteria, of nitrogenous containing molecules, such as proteins and nucleic acids. According to the European regulation (ECC 95/149) the highest TVB-N limit is set at 35 mg N/100 g of fish flesh. Since no limit of acceptability has been specifically established for rainbow trout, there is a general consensus that 25 mg N/100 g of trout flesh is the highest acceptable level as proposed by Giménez et al., 2002. However, TVB-N value as fish freshness indicator has been recently disputed by the evidence that TVB-N value is still within the acceptability range even when the fish is rejected on the basis of the sensory evaluation (Chytiri et al., 2004; Erkan, 2012; Erkan et al., 2011; Mexis et al., 2009).

In this study, trout fillets TVB-N increased during the 15-day storage in all groups (Fig. 3). In both UTF and CTF, the TVB-N value was beyond the acceptable limit after 15 days, while in ACTF the final TVB-N was less than 25 mg N/100 g. These results indicate that, although the carrageenan coating is capable of slowing down the TVB-N production, the presence of ELO is crucial

Fig. 1. Trout fillets after 7 days of storage at 4 °C. Left: uncoated trout fillets. Right: carrageenan coated trout fillets.

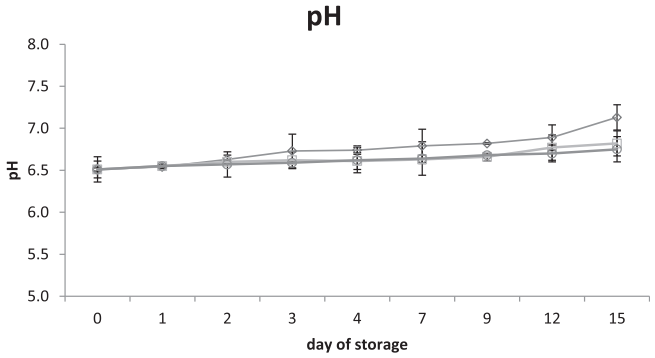

Fig. 2. pH changes in uncoated trout fillets (◊), carrageenan coated trout fillets (□) and trout fillets coated with carrageenan added with essential lemon oil (○). Points represent mean values of three determinations ± standard error.

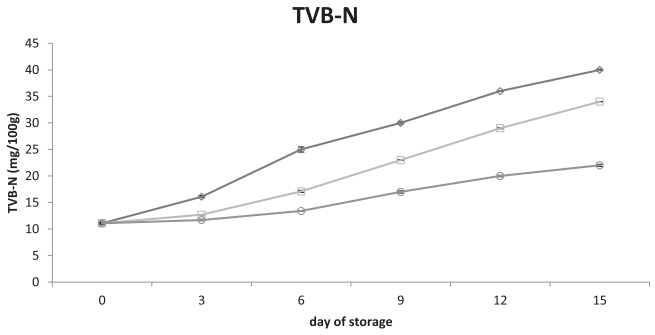

Fig. 3. Total volatile basic nitrogen (TVB-N) in uncoated trout fillets (◊), carrageenan coated trout fillets (□) and trout fillets coated with carrageenan added with essential lemon oil (○). Results have been expressed as mg of TVB-N per 100 g of muscle.

for inhibiting TVB-N production. To our knowledge, few studies have dealt so far with the effect of essential oils in combination with coating in trout. Ojagh et al. (2010) reported TVB-N values lower than 25 mg/100 g of trout fillets after 16 days of storage at 4 °C in presence of chitosan coating and chitosan coating plus cinnamon essential oil. Erkan et al. (2001) reported that trout fillets stored at 2 °C under a combination of VP and ELO showed TVB-N values slightly above the acceptability level after 7 weeks of storage.

3.5. Microbiological analysis

Fishes live in water and as a consequence contain large numbers of microorganisms on their body surface, depending on water conditions and temperature. Bacterial counts of freshwater fish species have been reported in literature to be widely variable, being comprised between 2 $\log_{10}$ cfu/g and 6 $\log_{10}$ cfu/g (Austin, 2006). In this study, the initial value of total viable counts (TVC) was around 4 $\log_{10}$ cfu/g. However, such value in the fresh trout fillets (UTF) reached 12 $\log_{10}$ cfu/g at 15 day after refrigerated storage. After12 days of storage, TVC value was about 6 $\log_{10}$ cfu/g in both coated (CTF) and active coated (ACTF) trout fillet, a value below the maximal recommended limit of 7 log10 cfu/g for TVC in raw fish. However, TVC increased up to about 9 $\log_{10}$ cfu/g at 15 day after refrigerated storage in CTF, while it was still about 6 $\log_{10}$ cfu/g in ACTF (Table 3).

Initial populations of Lactic acid bacteria (LAB) were approximately 2 $\log_{10}$ cfu/g and did not exceed 3 $\log_{10}$ cfu/g in UTF after 15 days of storage. In both CTF and ACTF final populations of LAB were below 3 $\log_{10}$ cfu/g throughout the storage period (Table 3).

Initial *Enterobacteriaceae* counts were about 2 $\log_{10}$ cfu/g. After 15 days of storage *Enterobacteriaceae* counts reached a value of about 2 $\log_{10}$ cfu/g, 3, 5 $\log_{10}$ cfu/g and 5 $\log_{10}$ cfu/g in ACTF, CTF and UTF respectively (Table 3). Such a slow growth of *Enterobacteriaceae* is also reported by Chytiri et al. (2004) for whole gutted and filleted trout stored in ice, likely a consequence of a slow growth rate presented by these bacteria with respect to other Gram-negative psychrotrophic spoilers.

H_2S producing bacteria initial counts were about 2 $\log_{10}$ cfu/g and remained at low levels throughout the storage period in ACTF. The final value after 15 days was 6 $\log_{10}$ cfu/g in UTF and 5 $\log_{10}$ cfu/g in CTF (Table 3).

Taken together the microbiological results presented here indicate that a preservation measure such as a carrageenan coating coupled with the presence of ELO can slow down bacterial growth at least up to 15 days. This is in agreement with studies on plant essential oils as effective in reducing microbial population including pathogenic and spoilage bacteria in meats and fish. It is known that the ELO contains a well known antimicrobial compound as geranial, neral and linalool, and other compounds with antimicrobial activity (Chutia et al., 2009). The observed reduction in microbial populations presented in this study is in agreement with numerous previous observations carried out on trout fillets stored under diverse refrigerated conditions (vacuum or MAP, smoked, chitosan coated) with the addition of essential oils such as oregano, rosemary, black cumin and lemon oil (Arashisar et al., 2004; Chytiri et al., 2004; Erkan, 2012; Erkan et al., 2011; Frangos et al.; 2010; Giménez et al., 2002; Mexis et al., 2009; Ojagh et al., 2010; Pyrgotou et al., 2010).

Table 3
Microbiological analysis of rainbow trout (*Oncorhynchusmykiss*) fillets during 15 day cold storage. Results have been expressed as $\log_{10}$ CFU/g of muscle. Points represent mean values of three determinations ± standard error.

Storage (days)	Total viable counts ($\log_{10}$ cfu/g)			Lactic acid bacteria ($\log_{10}$ cfu/g)			Enterobacteriaceae ($\log_{10}$ cfu/g)			Sulphide-reducing microorganisms ($\log_{10}$ cfu/g)		
	UTF	CTF	ACTF	UTF	CTF	ACTF	UTF	CTF	ACTF	UTF	CTF	ACTF
0	4.02 ± 0.05	4.02 ± 0.10	4.02 ± 0.05	2.00 ± 0.02	2.00 ± 0.02	2.00 ± 0.03	1.00 ± 0.05	1.00 ± 0.11	1.00 ± 0.07	1.85 ± 0.01	1.85 ± 0.15	1.85 ± 0.10
3	4.49 ± 0.10	4.49 ± 0.15	4.39 ± 0.13	2.18 ± 0.01	2.03 ± 0.01	2.04 ± 0.01	1.00 ± 0.03	1.00 ± 0.05	1.00 ± 0.05	2.83 ± 0.03	1.94 ± 0.22	1.88 ± 0.05
6	7.53 ± 0.15	4.83 ± 0.07	4.53 ± 0.08	2.05 ± 0.05	2.10 ± 0.07	2.10 ± 0.03	1.00 ± 0.05	1.00 ± 0.15	1.00 ± 0.15	3.43 ± 0.05	2.33 ± 0.06	1.92 ± 0.07
9	8.88 ± 0.10	5.02 ± 0.10	4.82 ± 0.10	2.20 ± 0.07	2.14 ± 0.10	2.26 ± 0.09	1.78 ± 0.14	1.34 ± 0.10	1.14 ± 0.08	3.87 ± 0.10	2.45 ± 0.10	2.15 ± 0.07
12	9.51 ± 0.07	5.41 ± 0.08	5.31 ± 0.08	2.78 ± 0.15	2.37 ± 0.05	2.32 ± 0.08	1.97 ± 0.01	1.55 ± 0.08	1.33 ± 0.02	4.33 ± 0.01	2.77 ± 0.12	2.21 ± 0.05
15	12.09 ± 0.07	8.70 ± 0.09	5.70 ± 0.05	2.93 ± 0.10	2.58 ± 0.10	2.38 ± 0.01	2.36 ± 0.07	1.71 ± 0.10	1.46 ± 0.10	4.79 ± 0.05	2.99 ± 0.05	2.29 ± 0.05

3.6. Lipid profile

The fatty acids (FAs) composition of trout fillets is shown in Table 2. At the beginning of the storage period the percentage content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the muscle of fresh trout fillets was 18.84 ± 0.8, 31.72 ± 1.1 and 44.26 ± 2.5, respectively. Among SFA palmitic acid (16:0) was the most abundant, followed by butyric acid (4:0) and stearic acid (18:0). The most abundant MUFA was oleic acid (18:1 n − 9) and the predominant polyunsaturated fatty acids (PUFA) were eicosapentaenoic acid (EPA) (20:5 n − 3) and docosahexaenoic acid (DHA) (22:6 n − 3).

Such FAs composition is in line with fresh rainbow trout fillets reported by Ozden (2005), although the FAs composition of different individual fish of the same species can vary because of several environmental factors such as the diet and the temperature. Indeed, FAs composition of fish fillet inevitably reflects the FAs composition of the diet (Turchini, Torstensen, & Wing-Keong, 2009). In general, the FA content reported here for rainbow trout is similar to that one of freshwater fish, characterized by containing higher levels of C18 PUFA and lower levels of EPA and DHA with respect to marine fish (Ozogul & Ozogul, 2007).

Due to the high amounts of PUFA, EPA and DHA, fish may contribute to the prevention of diseases related to geriatric and cardiovascular disorders and certain forms of cancer (Swanson, Block, & Mousa, 2012). Oxidation of fat affects product quality and shortens shelf life. Thus, finding methods of preventing fish deterioration while maintaining the organoleptic characteristics of the fresh product would be highly sought. The active coating utilized in this study was able to preserve the fresh trout fillets from lipid oxidation, up to 15 days of storage. A constant increase in the value of SFA was observed in the trout fillets. The UTF showed the highest levels of SFAs throughout the entire period of storage. The presence of carrageenan coating and carrageenan coating with ELO helped to keep the SFA levels in CTF and ACTF lower than in UTF and ACTF showed the lowest SFA levels at 15 days.

This behavior is likely due to the protective action of the carrageenan that shows low water vapor permeability and great tensile strength. This is even more evident with the addition of ELO. There are several reports regarding the antioxidant activity of the essential oils measured through diverse methods. Miguel (2010), reviewed the antioxidant ability of several essential oils extracted from aromatic plants, as well as some factors that can influence the chemical composition of the oils and consequently the biological activity, although the correlation between the biological activity (i.e., the antioxidant activity) of essential oils and their chemical composition is often very complicated.

Both MUFA and PUFA decreased throughout the storage period. Also in this case the presence of carrageenan coating and carrageenan coating with additional essential lemon oil slowed down such decrease and ACTF showed the highest MUFA and PUFA levels at 15 days. This result may be ascribable to the protective barrier effect against oxygen shown by the carrageenan coating, with consequent reduction of lipid oxidation (Tang, Kumar, Alavi, & Sandeep, 2012).

Moreover, these results confirm the efficacy of natural substances in slowing down oxidative lipid damage of fish flesh. The use of natural plant extracts to prevent lipid oxidation in fish has been reported in *Sparusaurata* (Giménez, Roncalés, & Beltrán, 2004) and *Salmosalar* (Giménez, Roncalés, & Beltrán, 2005). Similarly, salmon lipid damage was slowed down by natural antioxidants derived from barley husks (Pereira de Abreu et al., 2010). Coating with edible films enriched with an oregano or rosemary extracts lowered the oxidation rate in cold smoked sardine (Gómez-Estaca, 2007).

The n6/n3 ratio is considered to be a good health indicator. The maximum recommended value by UK Department of Health (HMSO, 1994) is 4.0. Higher values are harmful to health and may promote cardiovascular diseases. It is important to note that in this study the initial ratio was 1.80 and the final ratio was 1.53 for UTF, 1.45 for CTF and 1.79 for ACTF. All n6/n3 values were quite low, even after 15 days of storage, indicating a high degree of lipid oxidation resistance.

In this study, DHA/EPA ratio was quite stable in trout fillets. The ratio was initially 2.43, while decreased until to 2.33 for UTF, 2.26 for CTF and 2.39 for ACTF sample, the last value quite near to those of fresh trout fillet. These values are in agreement with those reported in some freshwater fish species (Ozogul & Ozogul, 2007) and rainbow trout (Saglik Aslan, Guven, Gezgin, Alpaslan, & Tekinay, 2007).

3.7. Indices of lipid quality

The following indices were calculated during the 15-day storage period of trout fillets. Index of atherogenicity (IA), indicating the relationship between the sum of the SFAs and UFAs, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory systems), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases) (Ulbritcth & Southgate, 1991).

Index of thrombogenicity (IT), indicating the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic FAs (MUFA, PUFA-n6 y PUFA-n3), shows the tendency to form clots in the blood vessels (Ulbritcth & Southgate, 1991).

Both indices increase throughout the storage period, however, such increase was lower in CTF and ACTF with respect to UTF (Table 2).

4. Conclusions

This study shows that both carrageenan coating and carrageenan coating incorporating ELO had good antimicrobial activity and limited lipid oxidation of fresh trout fillets stored at 4 °C for 15 days. However, carrageenan coating incorporating ELO was more efficacious with respect to the carrageenan coating alone in increasing rainbow trout fillets shelf-life. These results demonstrate the efficacy of the ELO in slowing down microbial growth and lipid oxidation while the carrageenan polymeric matrix proved to be a good method to keep the shiny and fresh aspect of trout fillets well beyond 7 days of cold storage. We are confident that the results reported here, on the effect of carrageenan coatings on the keeping quality of fresh trout, with and without added ELO, will be of interest to stakeholders in the production and processing industries, due to the simple manufacturing methodology and the straightforward effects of ELO to increase the shelf life of trout fillets.

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