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Edible coating enriched with rosemary extracts to enhance oxidative and microbial stability of smoked eel fillets

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ABSTRACT

The antioxidant and antimicrobial properties of rosemary essential oil (EO) and extracts, entrapped in carboxyl methyl cellulose (CMC) edible coating for smoked eel were investigated. Analysis of the EO indicated 1,8-cineole, L-camphor, a-pinene, and 1-borneol, as the main components. The solid residue generated by the EO distillation was extracted in an ultrasound bath with ethanol to give an extract rich in rosmarinic acid, diterpenes and flavonoids. The addition of the extract at 200–800 ppm (total phenol basis) in CMC coating provided antioxidant protection to smoked eel, which increased with concentration. Additionally, the combination of the extract (200 ppm) with EO (2000 ppm) retarded significantly the formation of both primary and secondary oxidation products, indicating possible synergistic effects. The antimicrobial activity of EO and extracts was moderate, with the extract at 800 ppm concentration showing the best results in decreasing the rate of total viable count, *Pseudomonas* spp., and lactic acid bacteria growth.

1. Introduction

European eel (Anguilla anguilla) production has steadily increased over the last 30 years, mainly due to the expansion of farming, which accounts for more than 80% of the world's consumption of the species (FAO, 2016; Nielsen & Prouzet, 2008). Greece shows important eel farming activity, following the Netherlands, Italy and Denmark. Eels are usually hot-smoked after brining or dry-salting, but the method differs considerably to suit local preferences. In Europe, eels are sold almost exclusively as skinned hot-smoked fillets. The market demand for smoked European eel has increased remarkably due to its flavour and high yield. Moreover, eels are rich in w-3 polyunsaturated fatty acids, and their consumption has been considered beneficial to human health. However, owing to this high polyunsaturated lipid content, fatty fish are susceptible to loss of nutritional quality and shortening of shelf life because of lipid oxidation. Nevertheless, microbial spoilage might be the main factor that defines the shelf life of the product (Baklori, Tsironi, & Taoukis, 2012).

Rosemary (*Rosmarinus officinalis,* L) is one of the most common aromatic herbs that grows wild and is also cultivated, while its antioxidant properties are well established and recognized by the fact that the only currently approved natural food antioxidant in the EU is an extract from this plant (Moreno, Scheyer, Romano, & Vojnov 2006; Dorman, Peltoketo, Hiltunen, & Tikkanen 2003). Rosemary extracts have been applied directly on fish flesh to help preserve refrigerated and frozen fish, and in most cases did exhibit antioxidant effects. Giménez, Roncalés, and Beltrán (2004, 2005) used a commercial liquid rosemary extract to preserve refrigerated gilt-head sea bream (*Sparus aurata*) and salmon (Salmo salar) fillets and found that lipid oxidation was slowed. Serdaroglu and Felekoglu (2005) and Vareltzis, Koufidis, Gavriililidou, Papavergou, and Vasiliadou (1997) also reported delayed lipid oxidation when a commercial rosemary extract was used as a preservative in the frozen storage of sardine and mackerel mince, respectively. The antimicrobial activity of rosemary extract was also examined. According to del Campo, Amiot, and Nguyen-The (2000), 0.5–1.0% of ethanolic solution of rosemary extract (100 mg/mL) in agar medium inhibited growth of gram-positive bacteria such as *Leuconostoc mesenteroides*, *Listeria monocytogenes* and *Staphylococcus aureus*. Lower concentrations (0.13 and 0.06%) were effective against *Streptococcus mutans* and *Bacillus cereus*, respectively. However, the appropriate extract concentration for microbial growth inhibition was significantly affected by the composition of the medium (pH, NaCl

Abbreviations: CD, conjugated dienes; CMC, carboxy-methyl-cellusose; EO, essential oil; GAE, gallic acid equivalents; PV, peroxide value; *p*-AV, p-anisidine value; TP, total phenols

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content) and storage temperature.

Essential oils (EOs) and extracts rich in antimicrobial or antioxidant components may be added to edible coatings instead of fish flesh. Pereira de Abreu, Paseiro Losada, Maroto, and Cruz (2010a, 2010b) used an ethyl acetate extract from barley skin to coat low density polyethylene and observed that the incorporation of the extract to the film protected Atlantic salmon and Atlantic halibut samples against oxidative deterioration during frozen storage. In the case of smoked fish, plant extracts added to edible coating may also impart certain slight flavour notes that can potentially positively impact sensory quality (Li et al., 2012).

To the best of our knowledge, the reported application of edible coatings enriched with plant extracts to smoked fish is limited to the study of Gómez-Estaca, Montero, Giménez, and Gómez-Guillén (2007), who used water extracts from oregano and rosemary in edible films and found that they were able to slow lipid oxidation, but failed to slow microbial growth of cold-smoked sardine muscle.

Therefore, the objective of the present study was to examine the potential of edible coating enriched with active substances from rosemary to extend the shelf life of smoked eel fillets during refrigerated storage. Carboxy-methyl-cellusose (CMC) was used to form the edible coating because it possesses certain desirable characteristics such as water-solubility, high viscosity, biocompatibility, biodegradability, hydrophilicity, moderate moisture and oxygen permeability and good film-forming ability. Also, CMC is odorless, tasteless, non-toxic, non-allergenic, flexible, and transparent (Tharanathan, 2003). Rosemary extracts were obtained from the herbal material remaining after the water-steam distillation process to recover EO. The commercial exploitation of rosemary for the production of EO generates high amounts of this solid residue that is not further processed and has a negative environmental impact. Consequently, its use to obtain high added value compounds is beneficial for the industry and the environment. The extract of rosemary was tested alone at several concentrations or in combination with EO to provide both antioxidant and antimicrobial activity.

2. Materials and methods

2.1. Materials

Wild rosemary was collected in January 2016, in NTUA campus (Athens, Greece). The plant material was dried at room temperature, subsequently the leaves were separated from the stems and the dried rosemary leaves were stored at 4 °C until used. The extractions were carried out in ethanol 96°. Water, acetonitrile, methanol and hexane used for chromatography analyses were of HPLC and MS grade (Fisher Chemical, Leicestershire, UK). The standard compounds applied for the quantifications included quercetin dihydrate, rosmarinic acid (Sigma-Aldrich, Steimheim, Germany), caffeic acid, gallic acid (98% w/w, Acros Organics, Fair Lawn, New Jersey), as well as a standard C7-C30 alkanes mixture (Sigma–Aldrich, Steimheim, Germany) (for the determination of retention indices in GC–MS analyses). The reagents were DPPH radical, trolox (Sigma–Aldrich, Steimheim, Germany), Folin-Ciocalteu's Phenol Reagent (Merck, Darmstadt, Germany), sodium thiosulfate 0.1 N, potassium iodide, (for analysis, Carlo Erba Reagents, Val de Reuil Cedex, France), *p*-Anisidine and starch soluble (Panreac, Barcelona, Spain).

2.2. Extraction procedures

2.2.1. Essential oil distillation

The dried leaves of rosemary were subjected to water-steam distillation, in a pilot scale (17 L) distiller, to recover the EO, as described by Tsimogiannis et al. (2016). The resulting oil was dried over anhydrous sodium sulfate and stored at 4 °C in a dark glass vial. A yield of 4 mL oil/kg dry herb was obtained. The oil-free herb was dried in a forced air oven at 38 °C and ground in a laboratory mill (Moulinex, Multi Moulinette, type T71, EEC), equipped with a 0.5 mm sieve.

2.2.2. Ultrasound extraction

The dried ground material was extracted with ethanol in an ultrasonic bath (Elma Singen/Htw., type: S 30H, Hohentwiel Germany). Batches of 20 g were placed in Erlemayer flasks and 200 mL ethanol was added. The extraction was performed for 30 min. The obtained ethanol extracts were combined, dried in a rotary evaporator (Heidolph G1, Schwabach, Germany) under vacuum and kept in sealed glass vial, in the refrigerator until used.

2.3. Preparation of coating solution

Coating solutions were prepared according to Choulitoudi et al. (2016). Briefly, carboxy-methyl-cellulose (CMC) was dissolved in distilled water (15 g/L) under magnetic stirring at 80 °C. The CMC solution was mixed under magnetic stirring with tween 20, 2% (v/v), used as emulsifier. Emulsions with EO were obtained by adding rosemary EO, 2000 ppm, in CMC solutions, while the dried ethanol extract was added to reach final concentrations of 200, 500 and 800 mg total phenols/L. Homogenization of the emulsions was performed by a high-speed homogenizer (CAT Unidrive 1000, Paso Robles, California) at 8000 rpm, for 5 min, at room temperature. The emulsions remained for 15 min at room temperature to exhaust air bubbles formed during homogenization. The samples tested were: CMC (CMC), CMC with ethanol extract at different concentrations (CMC + 200, CMC + 500, CMC + 800), CMC with EO (CMC + EO), CMC with the combination of ethanol extract at 200 mg/L and EO (CMC + 200 + EO).

2.4. Raw material and sample preparation

Smoked eel fillets were provided by a Greek aquaculture unit. Smoking was conducted at 90–95 °C for 35 min. The average lipid content of the product was 23.9% and the content of unsaturated fatty acids was previously determined as 73.2% of total fatty acids, out of which 17.2% ω-3 polyunsaturated fatty acids (Baklori et al., 2012). Fillets were transported to the laboratory in polystyrene boxes covered with flaked ice (0 °C). They were cut into rectangular slices (weight: 50 ± 5 g) in a laminar flow hood. 12 fish slices were placed in each CMC solution for 5 min and then allowed to drain for 1 min. Untreated (Control) and treated samples were placed individually in Petri dishes and stored at controlled isothermal conditions of 4 °C in a high precision (± 0.2 °C) low temperature incubator (MIR – 254 – PE, Panasonic). Electronic, programmable, miniature dataloggers (COX TRACER®, Belmont, North Carolina) constantly monitored the temperature in the incubators. Duplicate samples were taken in appropriate time intervals during a 30-day period to allow for the study of lipid oxidation and microbial growth.

2.5. Chromatographic analysis of the EO and extracts

The GC–MS analysis of EO as well as the HPLC-DAD-ESI–MS/MS of ethanol extract were performed according to the previously documented protocols by Choulitoudi et al. (2016) and Tsimogiannis et al. (2016).

2.6. Total phenolic content (TP)

The Folin Ciocalteu method (Waterhouse, 2005) was used for the quantification of total phenols (TP) of the extracts. The results were expressed in gallic acid equivalents (GAE) through construction of a reference curve. All samples were analyzed in duplicate and the presented results are mean values of duplicate experiments.