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Effects of CO_2 plant extracts on triacylglycerol oxidation in Atlantic salmon during cooking and storage

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ABSTRACT

Increasing concern of consumers on the safety of synthetic food additives has created high interest in natural preservatives in food industry. Plant extracts produced by supercritical CO_2 technology from rosemary (R), oregano (O) and an antimicrobial blend (AB) consisting of seven different plants were studied for their effects on lipid oxidation in Atlantic salmon (*Salmo salar*). Fish pieces were marinated with rapeseed oil containing 0, 1, 2 or 4 g of plant extracts/kg of fish. After cooking the pieces were stored in refrigerator for 26 days. Peroxide values (PVs) were determined and oxidised triacylglycerols (TAGs) measured by UHPLC–ESI/MS at 0, 7, 14 and 26 days of storage. During the first two weeks of storage, AB delayed oxidation by at least one week compared to control samples as shown by PVs (<10 meq. O_2) and by the oxidised TAGs. Oregano and rosemary showed also some antioxidative potential.

1. Introduction

Fish species with high fat content such as Atlantic salmon (*Salmo salar*) are rich in polyunsaturated n-3 fatty acids which are known to be especially healthy to humans. For example, in heart disease patients, several markers of human atherosclerosis were improved by a diet containing salmon with high ratio of n-3/n-6 fatty acids (Seierstad et al., 2005). Diets with higher n-3/n-6 ratio resulted in statistically significant lowering of serum triglycerides, vascular cell adhesion molecule-1, and interleukin-6 compared to the control groups. However, the highly unsaturated fatty acids are susceptible to oxidation during processing and cooking at high temperatures as well as during storage (Frankel, 2005). Deep-frying of fish, refrigerated storage, and reheating result in significant increase in peroxide value (PV) and in the level of thiobarbituric acid reactive substances (TBARS) (Nikoo, Ghomi, Rahimabadi, Benjakul, & Javadian, 2010). The lipid oxidation products found in e.g. fried foods are generally considered to be harmful for human health and may be linked to many diseases associated with western-type diet (Akbaraly et al., 2013). In addition, consumption of fish oil has been associated with increase in markers of lipid oxidation such as PV and TBARS in the tissues of experimental animals (Gonzalez, Gray, Schemmel, Dugan, & Welsch, 1992). This effect may be due to the increased intake of oxidised lipids along with the fish oil, which is easily oxidised during processing and storage.

Antioxidants are commonly used in food industry in order to prevent lipid oxidation. Safety and efficacy are important factors for consideration in application of food additives. Butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), or combination of TBHQ and α-tocopherol in the diet of experimental animals did not completely prevent the formation of lipid oxidation products in the tissues (Gonzalez et al., 1992). In fact, high BHT intake (2% of the amount of fish oil in the diet) resulted in accumulation of lipid oxidation products in certain tissues such as heart, skeletal muscle and mammary glands (Gonzalez et al., 1992).

Recently, increasing awareness and safety concerns of consumers towards synthetic food additives has created sufficient need in food industry for natural food preservatives (European Food Safety Authority, 2010). Essential oils of many herbs and other plants contain substances with antimicrobial and antioxidative properties (Baratta et al., 1998). Also, some of the polyphenol-rich ethanolic

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extracts of certain plants may be effective antioxidants in food. For example, it was previously reported that chromatographically fractionated phenolics from ethanolic extracts of apple peel inhibited the oxidation of fish oil as measured by TBARS assay (Sekhon-Loodu, Warnakulasuriya, Rupasinghe, & Shahidi, 2013). Flavonol-rich fractions inhibited lipid oxidation by 40–62% at a total phenolics concentration of 200 μg/mL, which surpassed the effects of α-tocopherol and BHT. In another study, clove bud and grape seed extracts showed protective effects on lipid and protein oxidation of silver carp fillets stored chilled (Shi, Cui, Yin, Luo, & Zhou, 2014). Plant essential oils are commonly prepared by steam distillation, which can cause degradation or oxidation of the highly sensitive components. Supercritical fluid extraction with CO_2 is a green technology yielding potentially valuable extracts for food industry without a risk of decomposition during processing (Fornari, Vicente, Vázquez, García-Risco, & Reglero, 2012).

In this study, we examined the effects of supercritical CO_2 extracts of rosemary, oregano, and an antimicrobial blend of 6 different plants on triacylglycerol (TAG) oxidation in Atlantic salmon (*S. salar*) during cooking and cold storage. Determination of lipid oxidation is a complex issue and generic methods such as PV measurements should be accompanied with more specific methods (Barriuso, Astiasarán, & Ansorena, 2013). Fish lipids contain many different TAG species. TAGs containing up to 16 double bonds were identified from several fish species by silver ion high-performance liquid chromatography (HPLC) (McGill & Moffat, 1992). As oxidation of lipids progresses from the primary oxidation products, lipid hydroperoxides, to secondary oxidation products, the complexity of the lipid oxidation products is not resolvable by traditional methods for oxidation analysis. Here, we present an ultra-HPLC–mass spectrometric (MS) method that enables the measurement of the TAG oxidation products along with the intact TAGs.

2. Materials and methods

2.1. Materials

Acetonitrile, acetone, chloroform and methanol were of HPLC grade and supplied by VWR International (Radnor, PA, USA). Formic acid (98%) was ACS quality and supplied by Merck Co. (Merck KGaA, Darmstadt, Germany). Water was purified with Elga Purelab Ultra water purification system (Elga LabWater, Woodrifge, Il, USA) equipped with 0.2 μM particle filter. Lithium formate monohydrate (98%) was supplied by Sigma Aldrich (St. Louis, MO, USA). 3,3′-bis(N,N-di[carboxymethyl]aminomethyl)-*O*-cresolsulfonephtalein (xylenol orange), $BaCl_2{\cdot}2H_2O$, $FeCl_3$, $FeSO_4{\cdot}7H_2O$, HCl 38%, H_2O_2 (30%) were of ACS grade and supplied by Sigma–Aldrich.

Iron(II) solution was prepared by dissolving separately $FeSO_4{\cdot}7H_2O$ (0.5 g) and $BaCl_2{\cdot}2H_2O$ (0.4 g) in water (50 mL), and by slowly mixing the solutions, followed by addition of HCl (2 mL, 38%). The solution was filtered with Whatman no. 1 filter paper. Iron(III) solution was prepared by dissolving $FeCl_3$ (0.5 g) in HCl (50 mL, 38%) and adding H_2O_2 (1 mL, 30%). The solution was boiled for 5 min and diluted to 500 mL with water after cooling.

GLC HPLC # G-1 triacylglycerol mixture that contained saturated TAGs from tricaprin to tristearin and also tripalmitolein, triolein, trilinolein, and trilinolenin, was purchased from Nu-Check Prep, Inc. (Elysian, MN, USA). Several synthesized standards of oxidised lipids were used as references: 22:0–18:1(keto)–22:0, 22:0–18:1(hydroxy)–22:0, 22:0–18:1(hydroperoxy)–22:0, 22:0–9:0(aldehyde)–22:0, 22:0–18:1(epoxy)–22:0, 18:0–18:1(hydroxy)–18:0, 18:0–18:1(epoxy)–18:0, 18:1(hydroperoxy)–18:1–18:0, 18:0–18:2(diepoxy)–18:0, 18:0–18:2(hydroperoxy)–18:0, 18:1(epoxy)–18:1(epoxy)–18:0, 18:0–18:1(hydroperoxy)–18:0, 18:1(hydroperoxy)–18:1(hydroperoxy)–18:0 (underlined double bonds are replaced by the epoxy groups, and other double bonds may have moved during the oxidation). 1,2,3-tripentadecanoyl-*sn*-glycerol was purchased from Larodan Fine Chemicals AB (Malmö, Sweden). Freshly extracted fish oil was oxidised in a convection oven at 60 °C for 48 h to serve as a highly oxidised reference.

Norwegian sea cage-grown Atlantic salmon (*S. salar*) was purchased as fresh (two filets from two fishes, 2 kg each) from a local grocery store and packed in ice. The filets were cut in approximately equal weight (120–140 g) pieces and the ends of the filets and the belly flaps were discarded.

Supercritical CO_2 extracts of rosemary (*Rosmarinus officinalis*) (R), oregano (*Origanum vulgare*) (O), and an antimicrobial blend (AB) were provided by Flavex Naturextrakte GmbH (Rehlingen, Germany). The rosemary extract was composed of carnosic acid (218 mg/g) + carnosol (12 mg/g, calculated as carnosic acid), ursolic/oleanolic acids (13 mg/g), and essential oil (24 mg/g). The oregano extract contained carvacrol (501 mg/g), thymol (93 mg/g), p-cymene (82 mg/g), and thymoquinone (51 mg/g). The anti-microbial blend was composed of a mix of seven supercritical extracts: 30% sage leaf (*Salvia fruticosa*), 20% hop (*Humulus lupulus*), 15% licorice root (*Glycyrrhiza uralensis*), 15% curcuma (*Curcuma xanthorrhiza*), 10% clove bud (*Syzygium aromaticum*), 5% oregano leaf, and 5% ajwain seed (*Trachyspermum ammi*). The principal components in the antimicrobial blend were eugenol (138 mg/g), ar-curcumene (96 mg/g), xanthorrhizol (108 mg/g), β-curcumene (126 mg/g), isoflavans (17 mg/g), humulones (69 mg/g), lupulones (31 mg/g), carvacrol (25 mg/g), thymol (28 mg/g), and carnosic acid (46 mg/g). The composition of the extracts were based on certificates of analysis provided by the manufacturer and they were determined by gas chromatography (GC)–MS and HPLC.

2.2. Marination and cooking of fish

Marination of the fish was done by placing randomized pieces of fish in plastic bags, followed by addition of 1, 2 or 4 g of marinade (approximately 5 mL) per kg of fish and by removal of excess air from the bags under water. The marinades, which were made in rapeseed oil, contained 30, 60, or 120 mg/mL of plant extract or mixture of plant extracts. One control piece was marinated with just the rapeseed oil. The marination was then continued in a refrigerator at 6 °C for 16 h. The fish were then baked in a convection oven for 17 min at 175 °C. The inner temperature was between 77–82 °C at the end of the baking. One additional control fish piece was cooked without any marinade, and one piece without any marinade was stored uncooked as raw control. After cooking, half of each fish piece was homogenized with a food processor for immediate extraction of lipids and the other half refrigerated at 6 °C for 26 days. Samples were taken from the fish pieces in the refrigerator, after 7, 14 and 26 days of storage. Three subsamples taken from a single piece of fish at each time point were pooled into one sample. The samples were homogenized before lipid extraction.

2.3. Extraction of lipids

Lipids were extracted by a modified Folch method (Folch, Lees, & Sloane Stanley, 1957). Approximately 350 mg of homogenized fish was weighed accurately into a disposable glass test tube. Chloroform (2 mL) and methanol (1 mL) were added, the test tube was capped and vortexed for 10 s, and ultrasonicated for 20 min. KCl solution (400 μL, 0.88%, w/v) was added into the test tube and the content was mixed thoroughly by vortexing. Proportions of chloroform/methanol/water in the mixture were 8:4:3 (by volume), when the water in tissue was taken into account. The test tube was then centrifuged (1000×*g*), and the lower phase was collected into a new test tube. New, pure lower phase (1.7 mL