LWT - Food Science and Technology 134 (2020) 110011

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Impact of traditional and mild oven cooking treatments on antioxidant compounds levels and oxidative status of Atlantic salmon (*Salmo salar*) fillets

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ARTICLE INFO

Keywords:
Astaxanthin
Coenzyme Q_{10}
Steam oven
Sous-vide
Tocopherol

ABSTRACT

Atlantic salmon is widely consumed in the diet. When salmon fillets are submitted to cooking treatments, the high temperature could promote lipid peroxidation with a consequent reduction in product nutritional quality. For this purpose, the impact of traditional (convection) and mild (steam- and *sous-vide*) oven cooking treatments were assessed on the level of some selected antioxidant and oxidative status of salmon. Fatty acid profile, tocopherols (α and γ), astaxanthin and coenzyme Q_{10} (CoQ_{10}) levels, as well as markers for oxidative status [peroxide value (PV), thiobarbituric acid reactive substances (TBARS), percentage of oxidized CoQ_{10} on total CoQ_{10} amount] were determined in raw and cooked whole salmon fillets. As a result, the PV did not change as consequence of all treatments while TBARS level in raw sample (0.93 ± 0.16 μmol/g) was significantly higher than those found in all cooked ones, ranging from 0.43 ± 0.10 to 0.69 ± 0.17 μmol/g. Moreover, all oven treatments preserved ω3 PUFA and tocopherol fractions and led to a significant increase of CoQ_{10} availability, while solely the steam oven enhanced the availability of astaxanthin while reducing CoQ_{10} oxidative status in fillets.

1. Introduction

The potential health and nutritional benefits of fish consumption are mainly attributed to the lipid fraction, which is exceptionally rich in ω3 polyunsaturated fatty acids (ω3 PUFA) having lipid-lowering and anti-inflammatory effects (Pacetti, Mozzon, Lucci, & Frega, 2013). However, due to the high levels of polyunsaturated lipids, fish muscle is very susceptible to deterioration both by oxidation and hydrolysis leading to rancidity and development of off-flavours. Especially, when fish tissues are submitted to thermal treatments, which are the main way to increase safety and shelf life of products, to reduce antinutritional material as well as to increase protein digestibility, complex reactions involving many nutritional components are also activated. The high temperature generated during cooking promotes lipid peroxidation involving also the antioxidant compounds present in fish tissue (Leung, Galano, Durand, & Lee, 2018).

As concern the Atlantic salmon (*Salmo Salar*), lipid matter is characterized by high amounts of compounds prone to oxidation (i.e. ω3 PUFA, cholesterol) and copious levels of antioxidant molecules, such as astaxanthin (Ax), tocopherols, ubiquinones (Higuera-Ciapara, Félix-Valenzuela, & Goycoolea, 2006; Lerfall, Bendiksen, Olsen, Morrice, & Østerlie, 2016; Pravst, Žmitek, & Žmitek, 2010; Yu et al., 2020) which play an important role in protecting PUFAs from lipid peroxidation. Ax and α-tocopherol (α-T) operate via different mechanisms and at different stages of the oxidative deterioration of lipids in salmonid products. Hamre (2011) reported that high levels of α-T deposited in salmon flesh could be beneficial in the prevention of oxidative stress related to Ax depletion. Moreover, α-T exhibits a synergistic antioxidant action with coenzyme Q_{10} (CoQ_{10}), a fat-soluble quinone. CoQ_{10} is reversible converted from the oxidized form (ubiquinone) to the reduced one (ubiquinol). Ubiquinol represents a potent reducing agent able to interrupt the initiation of lipid peroxidation or to break the propagation by hydrogen donation to reduce peroxyl radicals. Furthermore, ubiquinol acts as a chain-breaking antioxidant in the membrane and it

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https://doi.org/10.1016/j.lwt.2020.110011
Received 16 March 2020; Received in revised form 30 July 2020; Accepted 1 August 2020
Available online 4 August 2020

regenerates tocopherol from tocopheryl radical, thus protecting the lipid environment from oxidation (Guescini et al., 2017). Depletion of ubiquinol and α-T in post-mortem fish muscle represents an important indicator of an ongoing process of oxidative stress. Passi, Cataudella, Tiano, and Littarru (2005) reported that a decrease in ubiquinone and α-T levels was accompanied by a significant increase of thiobarbituric acid reactive substances (TBARS) in salmon during storage. In view of this, the simultaneous monitoring of both antioxidants evolution and oxidative process, as consequence of different thermal treatments, would help to understand the engagement of antioxidant compounds on lipid oxidation and therefore the impact of different processing parameters (i.e. temperature, time, heat transfer mode) on the formation of oxidation by-products and loss of bioactive compounds in cooked fish.

Emerging oven cooking processes are based on mild thermal treatment of foods with minimal or rapid heating using steam as heat transfer medium. Modern commercial ovens allow to cook food by saturating oven chamber with hot steam or by submitting to saturated hot steam foods vacuum sealed in heat stable plastic (*sous-vide*). Such mild cooking techniques have found an adaptation from pot to oven. Steam has higher thermal conductivity than hot air used in convectional oven with the potential to effect rapid heating with minimal side effects. *Sous-vide* also reduces the oxygen load as factor promoting oxidation by limiting oxygen diffusivity (Aguilera, 2018; Kilibarda et al., 2018).

Although the impact of steaming and *sous-vide* cooking on fish quality characteristics (color, hardness, cooking loss, sensorial profile, fat retention, microbiological stability) were widely studied (Alexi, Kogiannou, Oikonomopoulou, Kalogeropoulos Byrne, Grigorakis, 2019; Bongiorno et al., 2018; García-Linares, Gonzalez-Fandos, García--Fernández & García-Arias, 2004; Głuchowski, Czarniecka-Skubina, Wasiak-Zys, & Nowak, 2019; Larsen, Quek, & Eyres, 2010; Picouet, Cofan-Carbo, Vilaseca, Carboné Ballbè & Castells, 2011; Singh et al., 2016), the information regarding the effects of such mild treatments on fish lipid oxidation is still scarce. Al-Saghir et al. (2004) noticed that moderate steaming of salmon did not enhance the primary and secondary oxidation products but significantly increased the content of cholesterol oxidation products. Cropotova, Mozuraityte, Standal, and Rustad (2019), who tested *sous-vide* cooking (performed in water bath) on Atlantic mackerel, revealed a positive correlation between higher temperature and cooking times and levels of secondary oxidation products such as conjugated trienes and tetraenes. Nieva-Echevarría, Manzanos, Goicoechea, and Guillén (2017) showed that steaming and *sous-vide* cooking of sea bass provoked a slight oxidation of unsaturated acyl groups, with the formation of compounds that modified the volatile profile of fish meat. However, no literature data has been found on the simultaneous analysis of both antioxidant levels and oxidative status of fish, including salmon, as a consequence of steaming and *sous-vide* processing.

Based on this, the aim of current study was to compare the effect of traditional (convection oven) and mild (steam- and *sous-vide*) oven cooking treatments on fatty acid profile, antioxidant levels (tocopherols, astaxanthin, coenzyme Q10) and oxidative status (peroxide value, TBARS, percentage of oxidized CoQ10 on total CoQ10 amount) of whole salmon fillet.

2. Materials and methods

2.1. Standards and reagents

Standards: α-tocopherol (α-T, >95%), γ-tocopherol (γ-T, >95%), δ-tocopherol (δ-T, >95%), astaxanthin (Ax ≥97%), coenzyme Q_{10} (CoQ_{10} ≥98%) were purchased from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO, USA) and solvents HPLC grade used for standards, sample preparation and for liquid chromatography were purchased from Merck (Merck, Darmstadt, Germany).

2.2. Sampling

Fresh farmed Atlantic salmon (*Salmon salar*) was purchased from a local distributor. Five samplings were performed. The fish specimens (size of 4–5 kg) were kept refrigerated with flake ice inside polystyrene boxes and transported to the laboratory within 1 h of purchase. Afterwards, fish specimens were cleaned and filleted. Considering that the distribution of fat, astaxanthin and tocopherols varied throughout the salmon body (Refsgaard, Brockhoff, & Jensen, 1998), fillets of 2.0 ± 0.2 cm of thickness and an average weight of 200.0 ± 20.0 g ($n = 5$) were obtained solely from the middle part of whole body salmon. After that, the fillets were submitted to different thermal treatments.

2.3. Cooking treatments

Three oven baking treatments were taken into consideration for cooking salmon: convection-oven (COT), steam-oven (SOT) and *sous-vide* (SVT) accomplished in oven. The steam oven (BOSCH Series 8, HSG636ES1) was purchased in the local distributor (MediaWorld Italy). COT was performed in a pre-heated oven at 180 °C for 20 min. SOT was achieved with the same oven by steam injection in the chamber (RH% = 100) and the salmon fillets were cooked at 65 °C for 20 min. For SVT, each fillet was vacuum-packed in a polypropylene heat-resistant (up to 120 °C) bag and submitted to steam oven cooking, with the same condition as SOT. In all procedures, the revealed core temperature of fillets was 60 °C, according to the internal cooked temperature recommended in the 2009 Food Code (FDA, 2009) for intact seafood products. Immediately after cooking, the core temperatures of the fillets were checked, and each fillet was minced in a grinder, cooled and subsequently used for analysis. Cooking times and temperatures of the three cooking methods were chosen in line with real household conditions. For this purpose, preliminary tests were performed in order to select the most suitable conditions for ensuring the satisfactory cooking of salmon fillets.

2.4. Extraction of total lipids

Total lipids were isolated as described by Bligh and Dyer (1959). Briefly, 20 g of minced fillet was homogenized in chloroform:methanol (60 mL, 1:2, v/v), the suspension was filtered through Whatman filter paper (Grade 4, 90 mm, Merck KGaA, Darmstadt, Germany) and the collected solution was washed three times, with 20 mL of KCl aqueous solution (0.88%, w/v). The organic solvent was dehydrated over sodium sulphate and evaporated with rotary evaporator (40 °C).

2.5. Primary and secondary products of lipid oxidation

Primary and secondary lipid oxidation products were quantified by determination of peroxide value (PV) and 2-thiobarbituric acid reactive substances (TBARS). PV was determined according to Crowe and White (2001) and the results were expressed in meq active O_2/kg lipids. TBARS were determined according to the method described by Pegg (2001) by using a Varian Cary 5000 UV–Vis–NIR spectrophotometer (Agilent, Santa Clara, CA, USA). The optical density of the pink-coloured water phase was determined at 532 nm. The results were expressed in μmol TBARS/g lipids.

2.6. Tocopherol determination

Salmon lipid fraction (200 mg) was diluted in 1 mL *n*-hexane and loaded on a UPLC Acquity H-Class system (Waters Corporation, Milford, MA, USA) equipped with a fluorimetric detector (FLD) and Ascentis Express HILIC (15 cm × 2.1 mm i.d., particle size 2.7 μm, Merck, Darmstadt, Germany) column set up at 30 °C. An isocratic elution (8 min) of *n*-hexane (95.5%), isopropanol (0.4%) and acetic acid (0.1%) at 0.3 mL/min was performed. FLD was set with an excitation and emission

wavelength of 290 and 330 nm, respectively. Tocopherols were identified by comparison of retention time with pure standards and quantified with external calibration. For the quantification, seven standard stock solutions of each tocopherol (α-T, γ-T, δ-T) in isopropanol were prepared in the range 3.5–100 μg/mL and analyzed to obtain the calibration curve (R^2 = 0.9836–0.9965) (Fig. S1, supplementary materials).

2.7. Fatty acid profile

Fatty acid methyl esters (FAME) were obtained from total lipids through alkaline transmethylation (Suter, Grob, & Pacciarelli, 1997) and were analyzed by capillary gas chromatography as reported by Balzano, Pacetti, Lucci, Fiorini, and Frega (2017). The fatty acid composition was expressed as percentage of fatty acid (% FA) of the total fatty acids and as mg FA/g salmon lipid. An example of fatty acids GC trace is reported in Fig. S2 (Supplementary materials).

2.8. Astaxanthin determination

Methanol (1 mL) was added to 50 mg of minced salmon tissue and vortexed. Then, 50 μL of solution was collected, diluted with 250 μL of methanol, centrifuged (20900 x g, 5 min. 4 °C) and injected into HPLC system (YL Instrument 9300, Amaze instrument, Uttar Pradesh, India) equipped with UV–Vis detector and a column Kinetex C18 100 A (250 mm × 4.6 mm i.d., 5 μm, Phenomenex, Torrance, California, USA). The mobile phase used was methanol:propanol (90:10, v/v) and the flow rate was 0.5 mL/min. Astaxanthin was quantified at 478 nm by using pure external standard obtaining the calibration curve as shown in Fig. S3 (Supplementary materials). The results were expressed as μg/mg.

2.9. CoQ_{10} determination

CoQ_{10} extraction method was similar to astaxanthin one but using 2-propanol (instead of methanol) for extraction and dilution of samples. After centrifugation 40 μl of extract was injected into HPLC system with electro-chemical detector (ECD) (Shiseido Co. Ltd.; Tokyo, Japan) characterized by a post-separation reducing column (Shiseido CQR) permitting to evaluate both oxidized and reduced CoQ_{10} forms, as described previously (Orlando et al., 2018). Total CoQ_{10} content in salmon and its oxidative status were expressed as μg/mg and as percentage of ubiquinone/total CoQ_{10}, respectively. The standard calibration curve of CoQ10 is reported in Fig. S4 (Supplementary materials).

2.10. Statistical analysis

Five fish sampling were performed. Three replicates were then performed for each sample and the results expressed as mean value ± standard deviation (SD) (n = 15). The significance of differences among the samples were evaluated using one-way ANOVA with Bartlett's test if significant. A p value < 0.05 was considered statistically significant and $p \leq 0.01$ highly significant. Pearson's correlation coefficient was used to evaluate the correlations among antioxidants quantified in the present study. Statistical analysis was performed using GraphPad Prism ® 6.0 Software.

3. Results

3.1. Lipid oxidation products formation

Primary and secondary oxidation products have been assessed in raw and cooked samples by determining PV and TBARS values, respectively (Table 1). No significant differences ($p < 0.05$) were revealed among PV of raw and cooked samples. Differently, TBARS values showed significant difference among the samples. TBARS in raw sample (0.93 ± 0.16 μmol/g) was significantly higher ($p < 0.05$) than that revealed in all cooked samples, ranging from 0.43 ± 0.10 to 0.69 ± 0.17 μmol/g.

Table 1
Peroxide value (PV) and TBARS value in extracted fat of raw salmon and cooked with convection (COT), *sous-vide* (SVT) and steam (SOT) oven treatments. Values are presented as the mean value ± standard deviation (n = 15, 5 fish sampling x 3 replicates of cooking process).

	PV (meq O_2/kg lipid)	TBARS (mmol/g)
Raw	5.6 ± 1.2	0.93 ± 0.16 [c]
SOT	5.5 ± 2.7	0.43 ± 0.10 [a]
SVT	4.8 ± 0.7	0.64 ± 0.11 [b]
COT	5.3 ± 2.2	0.69 ± 0.17 [b]

Different letters in the same column indicate significant differences ($p \leq 0.05$) among the sample.

Among the cooked samples, the SOT presented the significantly lowest TBARS value ($p < 0.05$). No significant difference emerged between COT and SVT values.

3.2. Fatty acid composition

Fatty acid profiles of the raw and cooked fillets are shown in Table 2. The most abundant fatty acids were oleic, linoleic and palmitic acids. The ω3 PUFA was dominated by linolenic acid followed by docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. The changes in fatty acids content after oven cooking were marginal. No significant differences in the total fatty acid profile have been observed after oven treatments. In all the cooking treatments, the ω3 PUFA matter was well preserved.

3.3. Antioxidant compounds levels

The impact of different oven treatments on the levels of the main antioxidant molecules contained in salmon fillets (CoQ_{10}, Ax, α-T, γ-T) were assessed. Simultaneously, the oxidative status of CoQ_{10} was evaluated. An example of both forms of CoQ10 trace in raw and cooked salmon are reported in Fig. S5 (Supplementary materials).

The CoQ_{10} level in raw sample accounted for 0.72 ± 0.19 mg/100g fillets. This is consistent with data reported in the literature (Pravst et al., 2010). As reported in Fig. 1a, all performed oven treatments increased the availability of CoQ_{10} in salmon, a highly significant manner with respect to the raw product (COT = 1.17 ± 0.37, SOT = 1.15 ± 0.33, SVT = 1.04 ± 0.35 mg/100g). SOT and SVT treatments also promoted a highly significant reduction of CoQ_{10} oxidative status (Fig. 1b). The ratio (%) between the oxidized CoQ_{10} and the total level of CoQ_{10} revealed in SOT and SVT samples was significantly lower (SOT = 69 ± 13; SV = 62 ± 15) than that found in raw and COT samples (raw = 94 ± 8, COT = 85 ± 10, $p < 0.01$). As reported in Fig. 2 and Fig. S6 (Supplementary materials), SOT and SVT samples presented significantly higher Ax levels (SVT = 0.72 ± 0.18; SOT = 0.74 ± 0.16 mg/100g) in comparison with COT sample (COT = 0.60 ± 0.19 mg/100g). Only SOT presented higher ($p < 0.05$) Ax level than raw sample. Conversely, all cooking procedures did not significantly affect the α- and γ-tocopherols levels in fillets (Fig. 3; Fig. S7, supplementary materials). Considering the raw sample, the tocopherol levels markedly changed across the five fish sampled in the present study. α-T ranged from 1.7 to 4.8 mg/100g of flesh fillet with a mean value of 3.3 ± 1.0, whereas γ-T ranged from 1.3 to 3.3 mg/100g with a mean value of 1.99 ± 0.7. This high variability can be attributed to the fact that the tocopherol levels in salmon are strictly affected by the diet and dietary supplementations at different levels of α-T or γ-T, usually performed during salmon farming (Hamre, 2011; Menoyo et al., 2014). However, our findings were in line with the data reported in the literature where α-T was found to be the most abundant tocopherol isomer in salmon (Polat et al., 2013).

Evaluating the correlation between the antioxidant levels and the marker of lipid oxidation (Fig. 4), it is evident that in all samples, the

Table 2
Fatty acid compositions expressed as weight % of total fatty acid and as mg fatty acid/g lipid of raw salmon or cooked with convection (COT), steam (SOT) and *sous-vide* (SVT) oven treatments.

	Raw	Oven-baked salmon			Raw	Oven-baked salmon		
		SOT	SVT	COT		SOT	SVT	COT
Fatty acid	weight % of total fatty acids				mg/g lipid			
C14:0	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.3	2.1 ± 0.2	11.5 ± 2.9	15.3 ± 4.0	14.6 ± 3.4	15.5 ± 3.2
C16:0	9.4 ± 0.6	9.1 ± 0.4	9.2 ± 0.3	9.4 ± 0.3	49.3 ± 7.1	62.3 ± 15.5	59.2 ± 11.7	67.6 ± 10.2
C18:0	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	13.0 ± 2.1	16.9 ± 4.0	16.0 ± 3.2	17.9 ± 3.1
∑ SFA	**14.1 ± 0.7**	**13.8 ± 0.6**	**13.9 ± 0.4**	**13.9 ± 0.4**	**73.8 ± 11.8**	**94.4 ± 23.0**	**89.8 ± 18.1**	**101.0 ± 16.1**
C16:1Δ9	2.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.2	12 ± 2.4	15.6 ± 4.9	14.8 ± 4.3	16.4 ± 2.8
C18:1 Δ9cis	42.8 ± 1.8	40.6 ± 2.7	40.7 ± 2.8	41.9 ± 2.7	225.8 ± 37.6	281.9 ± 58.4	265.2 ± 47.1	311.3 ± 62.5
C18:1Δ11	3.0 ± 0.3	3.1 ± 0.2	3.0 ± 0.3	2.9 ± 0.3	16.1 ± 3.2	21.3 ± 5.0	19.8 ± 4.6	21.3 ± 4.4
C 20:1	4.5 ± 1.2	5.4 ± 0.7	5.2 ± 0.8	4.9 ± 0.8	26.3 ± 8.6	37.2 ± 8.9	36.2 ± 6.3	34.5 ± 10.0
C22:1 Δ9	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	19.2 ± 8.2	25.1 ± 7.1	22.3 ± 6.5	20.7 ± 4.8
∑ MUFA	**55.9 ± 2.6**	**54.9 ± 3.9**	**54.7 ± 3.7**	**55.9 ± 2.9**	**299.4 ± 58.8**	**377.3 ± 79.6**	**337.0 ± 62.0**	**401.9 ± 78.9**
C18:2 Δ9,12ω6	14.7 ± 0.7	14.7 ± 0.9	14.7 ± 0.9	14.7 ± 0.8	76.1 ± 10.7	100.6 ± 21.8	93.7 ± 15.9	106.2 ± 17.0
C20:2 Δ14 ω6	1.2 ± 0.6	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	5.8 ± 1.7	8.2 ± 2.7	7.5 ± 2.2	8.7 ± 2.1
C18:3 Δ9,12,15ω3	6.3 ± 1.3	7.3 ± 1.8	7.3 ± 1.8	7.1 ± 1.6	30.7 ± 6.0	45.3 ± 15.4	40.9 ± 7.7	48.6 ± 10.0
C20:4Δ5,8,11,14ω6	0.8 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	3.8 ± 1.3	5.7 ± 2.5	5.2 ± 2.0	5.6 ± 1.3
C20:5Δ5,8,11,14,17ω3 (EPA)	2.3 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	12.4 ± 2.5	17.1 ± 4.9	16.0 ± 3.7	17.1 ± 2.7
C22:5Δ7,10,13,16,19ω3	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	5.1 ± 1.4	7.4 ± 2.6	6.8 ± 2.1	7.8 ± 1.6
C22:6Δ4,7,10,13,16,19ω3 (DHA)	3.7 ± 0.3	3.7 ± 0.6	3.9 ± 0.6	3.7 ± 0.2	19.4 ± 3.5	25.2 ± 7.0	24.1 ± 5.4	26.3 ± 4.9
∑PUFA	**30.1 ± 2.4**	**31.4 ± 3.7**	**31.5 ± 3.7**	**31.1 ± 2.8**	**153.3 ± 24.7**	**202.9 ± 48.7**	**188.3 ± 28.4**	**188.7 ± 89.1**
∑ PUFAω3	**13.4 ± 1.5**	**14.6 ± 2.5**	**14.7 ± 2.4**	**14.3 ± 1.8**	**67.6 ± 12.2**	**88.4 ± 28.6**	**81.9 ± 17.4**	**85.4 ± 40.2**
∑ PUFAω6	**15.5 ± 0.9**	**15.6 ± 1.1**	**15.5 ± 1.1**	**15.6 ± 0.9**	**80.0 ± 11.3**	**158.9 ± 39.0**	**147.7 ± 35.5**	**95.8 ± 45.3**

Results represent means values ± standard deviation (n = 15). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Cm:n Dx; m = number of carbon atoms, n = number of double bonds, x = position of double bond.

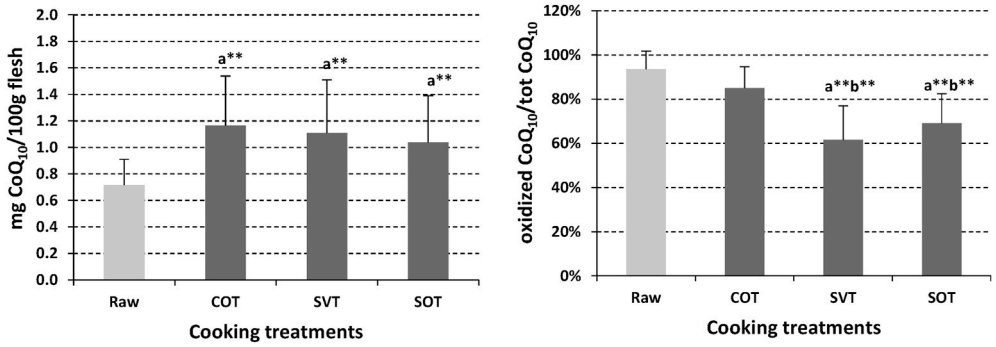

Fig. 1. Total CoQ_{10} levels (**a**) and its oxidative status of CoQ_{10} (**b**) in raw salmon or cooked with convection (COT), *sous-vide* (SVT) or steam (SOT) oven treatments. CoQ_{10} levels are expressed as mg of CoQ_{10}/100g of flesh salmon ± SD ($n = 15$), while its oxidative status as percentage of oxidized COQ_{10} respect to the total ± SD ($n = 15$). $^{**}p \leq 0.01$ vs raw (a), CO (b).

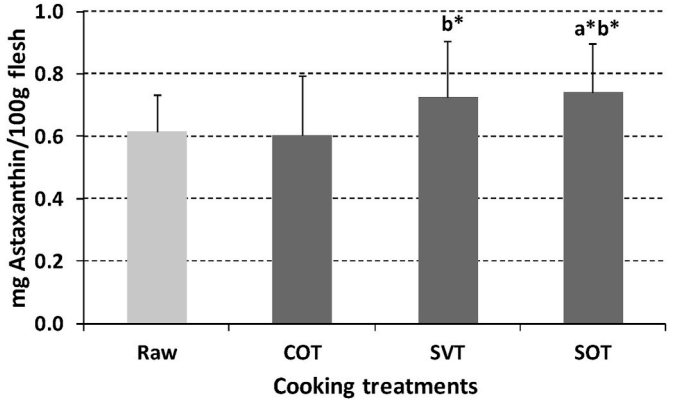

Fig. 2. Astaxanthin levels in raw salmon and cooked with convection (COT), *sous-vide* (SVT) or steam (SOT) oven treatments. Values are expressed as mg of astaxanthin/100g of flesh salmon ± SD ($n = 15$). $^{*}p \leq 0.05$ vs raw (a), CO (b).

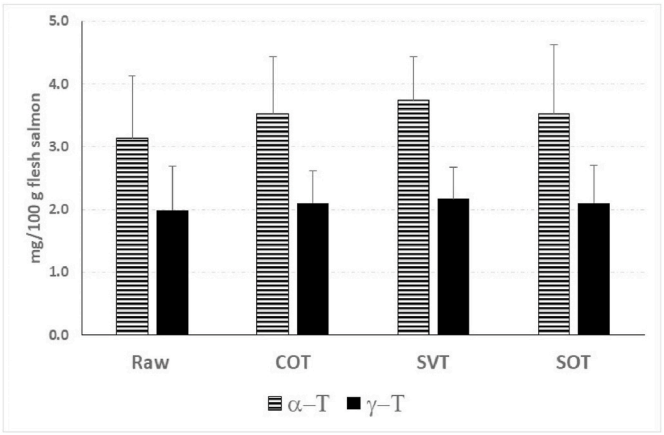

Fig. 3. α- and γ-tocopherol contents in raw salmon and cooked with convection (COT), *sous-vide* (SVT) or steam (SOT) oven treatments. Values are expressed as mg/100g flesh salmon ±SD ($n = 15$).

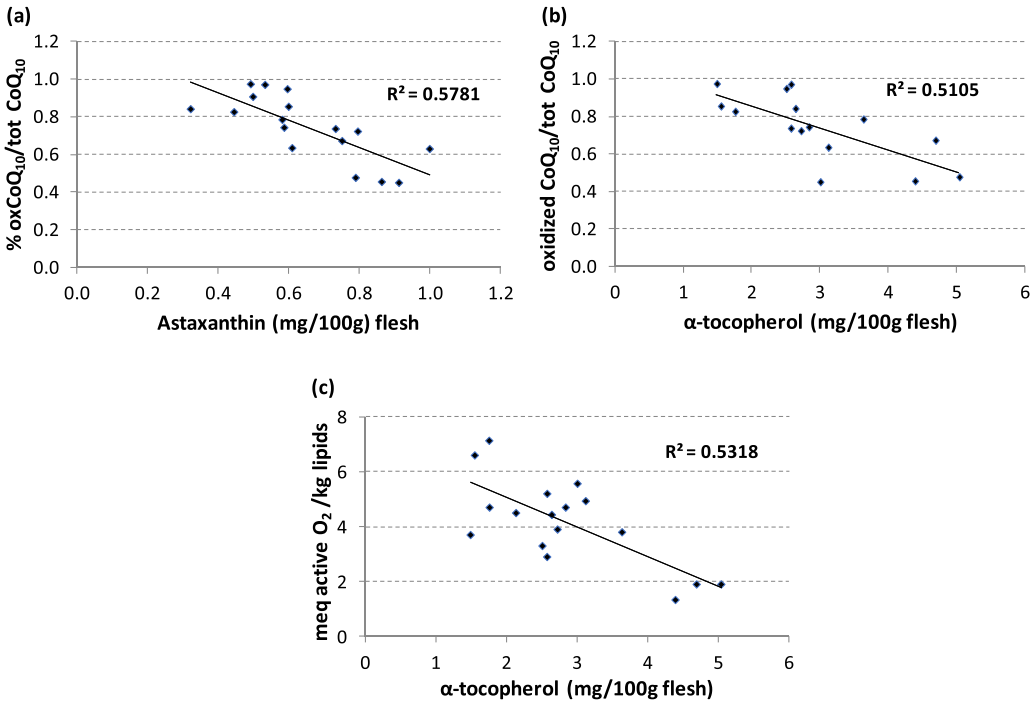

Fig. 4. Correlations between astaxanthin levels and percentage of oxidized COQ_{10} (**a**) and between α-tocopherol levels and both percentage of oxidized COQ_{10} (**b**) and PV (meq of active O_2/kg lipid) (**c**) in raw salmon and cooked with convection-oven (CO), *sous-vide* (SV) or steam-oven (SO).

percentage of oxidized CoQ_{10} is inversely correlated with both Ax ($R^2 = 0.578, p = 0.001$) (Fig. 4a) and α-T ($R^2 = 0.511, p = 0.001$) (Fig. 4b). The PV was only correlated with α-T ($R^2 = 0.532$, $p = 0.006$) (Fig. 4c). TBARS was not correlated with either Ax or α-T (data not shown).

4. Discussion

Designing a thermal process for fish products is challenging since the heat load required for inactivating target microorganisms may cause undesirable quality changes in the lipid and protein fraction. New methods that focus on minimal heating or rapid heating of fish products are therefore of vital importance.

In the present work, the effect of traditional convection oven procedure (COT, 180 °C, 20 min) on antioxidant amounts and oxidative status of salmon fillets were compared with those of mild treatments, such as steam oven (SOT, 65 °C, 20 min) and *sous-vide* (SVT, performed in steam-oven at 65 °C, 20 min). To consider the complexity of the oxidation process, for the first time, changes in oxidative status of salmon as consequence of oven treatments were monitored by determining both traditional parameters (PV, TBARS) and an innovative index, such as the percentage of oxidized CoQ_{10}.

The PV levels were unaffected by COT, SOT and SVT treatments and in all samples (raw and cooked) a negative correlation with α-T was observed. This let us to suppose a possible involvement of α-T in the contrast of the primary lipid oxidation in both raw and cooked salmon. Al–Shaghir et al. (2004) also mentioned that the PV in salmon was unchanged after steaming. Unlike to PV, all cooking procedures led to a significant decrease of TBARS and the significantly lowest value was reached in SOT sample. However, the TBARS value did not show any correlation with investigated antioxidant compounds. The TBARS decrease could be caused by loss of the secondary oxidation products during thermal treatment. The interaction between lipid oxidation products and proteins is hypothesized even by Said Talab, 2014 for explaining the decreasing effect of the cooking method on carp lipid oxidation. Aubourg, Gallardo, and Medina (1997) also found a TBARS depletion in canned tuna after 60 min of heating at 115 °C.

The high oxidative stability of salmon lipid during the thermal treatments was also confirmed by the marginal changes of fatty acid profile occurring after the investigated oven procedures. Our results are in line with the literature data demonstrating that convection oven treatment accomplished at different time and temperature did not alter the EPA and DHA contents in various salmon species (Leung et al., 2018; Raatz et al., 2011). Since no other data concerning the effect of steam and *sous-vide* treatments, accomplished in oven, on salmon fatty acid damage already exists in literature, an extensive comparison of the presented data is difficult. Anyway, Şengör, Alakavuk, and Tosun (2013) reported that the salmon fatty acid profile was not distinctively altered by steam cooking performed with a steamer pod at 100 °C for 30 min, more invasive parameters than ours (SOT, 65 °C, 20 min). Similarly, Choo, Azlan, and Khoo (2018) did not find DHA and EPA variations in salmon after steaming (steamer pod, 100 °C, 10 min) and baking in foil (180 °C, 30 min), whereas they did after frying. Garcia–Linares et al. (2004) noticed as *sous-vide* accomplished in oven (90 °C for 10 min) maintained the ω3 PUFA level of salmon. In addition, different authors (Candela, Astiasarán, & Bello, 1998; Gladyshev, Sushchik, Gubanenko, Demirchieva, & Kalachova, 2007) indicated that the most common culinary treatments would not have the same effect on PUFA content in many fish species, including Salmoniformes. It was suggested that the higher amounts of Ax present in salmon compared to many seafood products could have a key role against the lipid oxidation. The higher antioxidant activity of Ax when compared to different carotenoids and tocopherols is confirmed in literature (Naguib, 2000).

Our findings reinforce the hypothesis suggesting the potential protective role of Ax toward lipid oxidative damage. We found an inverse correlation between Ax level and the percentage of oxidized CoQ_{10} in all samples (raw and cooked). The oxidation of CoQ_{10} is largely used as a sensitive marker of oxidative stress (Celano et al., 2016). Particularly, Passi et al. (2005) showed that ubiquinol, the reduced form of CoQ_{10}, is the most susceptible antioxidant to oxidative damage in different Mediterranean fish species because it is oxidized by oxidant agents. Furthermore, our results underlined the involvement of tocopherols on lipid oxidation protection. Besides the α-T participation in primary lipid oxidation (as above reported), in all samples the α-T resulted inversely correlated with oxidized CoQ_{10}. It is possible to presume that α-T was

able to counteract the CoQ_{10} oxidation together with the Ax. This finding is in agreement with Bell et al., 2009 who noticed the antioxidant synergism of vitamin E and Ax on the reduction of malondialdehyde formation in a vitro stimulation of microsomal lipid peroxidation of Atlantic salmon.

Considering the engagement of the antioxidant compounds on lipid oxidation process, the levels of Ax, CoQ_{10}, and tocopherol were evaluated in raw and cooked samples. The data show that CoQ_{10} level was significantly enhanced as effect of all treatments whereas the Ax levels increased only as consequence of SOT. α-T and γ-T amounts remained stable in all treatments. As reported by Ovissipour, Rasco, Tang, and Sablani (2017), the thermal treatments lead to physicochemical changes in muscle foods, such as protein denaturation, probably making the antioxidant compounds more available to protect lipids from oxidation. Anyway, the effect of heat changes on antioxidant availability varied according the kind of antioxidant compound and the thermal operative conditions. The CoQ_{10} levels in salmon tissue were enhanced independently on the temperature (180 °C or 65 °C) and humidity reached in the oven treatments. Differently, the stronger favourable effect of SOT and SVT with respect to COT on Ax availability could be related to the synergistic impact of operative conditions with lower temperature and higher humidity adopted in SVT and SOT than in COT. As hypothesized for canthaxanthin in steamed and dry cooked fillets of rainbow trout (Choubert & Baccaunaud, 2010), the heat treatment of the salmon could lead to a degradation of the Ax-actomyosin complex or Ax-F-actin complex with consequently release of Ax in muscle. Simultaneously, when dry air cooking at high temperature is performed, as we done in COT (180 °C), the released Ax can be decomposed. Different authors reported that rainbow trout Ax is susceptible to temperature in dry air cooking more than moist cooking techniques such as steaming (Henmi & Hata, 1989; Young, Pritchard, Lowe, Crampton, & Buttle, 2017). It is important to notice that the heating rate during air convection is lower than in hot steam which has a higher thermal conductivity; thus, the superficial layers are longer exposed to high temperature than core and a non-uniformed degradation of Ax may occur. In addition, plastic bags of *sous-vide* provide a protection against the diffusivity of exogenous oxygen and a controlled heating rate.

The sensitivity of Ax to high temperature was also reviewed by Rao et al., (2007) who found that the edible oil added of Ax (0.1%) were effective in retaining 90% of Ax when heated at 90 °C whereas at 120 °C and 150 °C the Ax loss was significant. Moreover, extracts containing 20 mg/kg of Ax were found to have antioxidant activity in the studied oils at at 90 °C and 120 °C. This finding let us to suppose that a level of 6–7.5 mg/kg of Ax revealed in our salmon samples can be sufficient to inhibit lipid oxidation during SOT and SVT treatments.

Finally, the low vulnerability of tocopherols toward all the operative conditions revealed in the present study are in accordance with previous findings. Al-Saghir et al. (2004) demonstrated that in steamed salmon fillets, tocopherol levels remained almost stable and were not affected by the oxidation. The good resistance of both tocopherol isomers to microwave cooking (300 MHz, 2 min) was found for different fish species, including Atlantic salmon (Polat et al., 2013). No further literature concerning the investigation of tocopherol profile as effect of oven steam cooking, including *sous-vide* was found. However, the inverse correlation between α-T level and both the oxidized CoQ_{10} and the PV, emerged in this study, highlights the antioxidant protection of this molecule against the oxidation of salmon lipid.

5. Conclusions

Based on the results, all investigated traditional and mild oven treatments were able to preserve the salmon ω3 PUFA fraction from the oxidation. They also influenced the antioxidant availability in salmon tissue. However, such influence varied according to the antioxidant compounds, as well as to the thermal operative conditions.

All treatments did not alter the α-T and γ-T amounts and significantly enhanced the CoQ_{10} availability while, solely mild procedures (SOT and SVT) reduced the CoQ_{10} oxidative status in fillets. Moreover, the Ax level increased only as consequence of SOT. Overall, these results prove that SOT is a very mild process in terms of its effect on lipid oxidation and a very effective treatment in enhancing the availability of antioxidant compounds in salmon.

CRediT authorship contribution statement

Patrick Orlando: Investigation, Data curation, Validation, Writing - original draft. **Alessandra Giardinieri:** Investigation, Writing - review & editing. **Paolo Lucci:** Data curation, Writing - review & editing. **Ancuta Nartea:** Data curation, Writing - original draft. **Michele Balzano:** Investigation. **Deborah Pacetti:** Conceptualization, Methodology, Supervision, Writing - original draft, Project administration. **Natale G. Frega:** Conceptualization, Funding acquisition. **Sonia Silvestri:** Investigation, Writing - review & editing. **Luca Tiano:** Supervision, Methodology, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Polytechnic University of Marche [Grant, Frega, Ricerca di Ateneo 2018].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110011.

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