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The Effect of Sous-Vide Cooking Parameters, Chilled Storage and Antioxidants on Quality Characteristics of Atlantic Mackerel (*Scomber scombrus*) in Relation to Structural Changes in Proteins[§]

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SUMMARY

The aim of the present study is to assess the influence of different sous-vide time-temperature regimes and use of two types of commercial antioxidants (rosemary extract and rosemary extract with ascorbyl palmitate) on quality parameters of Atlantic mackerel (*Scomber scombrus*) during chilled storage. The mackerel fillets were treated with the antioxidants, exposed to sous-vide cooking at 70 and 80 °C for 10 and 20 min, and further stored for 1, 3, 9 and 15 days at (0±1) °C. Changes in dry matter and ash, cook loss, protein oxidation and solubility, as well as texture parameters in sous-vide cooked mackerel during storage, were assessed by application of multiple regression analysis. It was revealed that duration of chilled storage had the highest contribution to the decrease in cook loss due to a possible reabsorption of water released during cooking by unfolded proteins. At the same time, this parameter increased protein carbonylation in mackerel samples, resulting in a decreased protein solubility due to aggregation of proteins and subsequent toughening of the fish muscle. However, the use of antioxidants has shown to be highly efficient in decreasing the protein carbonylation in the analysed fish samples.

Key words: Atlantic mackerel, sous-vide cooking, antioxidants, chilled storage, protein carbonylation

INTRODUCTION

Nowadays, the demand for fish products that are fresh, natural and safe to consume is continuously growing due to a number of documented health benefits of fish (*1*). According to several studies, a diet rich in fish can improve health and help to prevent some non-communicable diseases, such as obesity, diabetes, hypertension and cancer due to high content of bioactive compounds in their composition (*2,3*). The contribution of fish to health may relate to the presence of essential long-chain omega-3 fatty acids – docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), fat-soluble vitamins (E and D) and easily digestible proteins (*2,4*).

Fish has generally been consumed cooked or processed (except sushi and sashimi) for the sake of inactivation of pathogens and enhancement of palatability and digestibility of nutrients (*5*). Cooking is the main thermal treatment applied for fish products to prolong their shelf life by destroying microorganisms and deactivating proteolytic enzymes, and to improve digestibility and bioavailability of nutrients, as well as sensory attributes such as taste and flavour (*6*). However, thermal processing of fish can affect the content, functionality, activity and bioavailability of omega-3 fatty acids, fat-soluble vitamins and easily digestible proteins. At the same time, it promotes oxidation reactions, resulting in loss of sensory and nutritional quality (*6*). The extent of the quality loss depends on the temperature and duration of cooking, the presence of metal ions and pro-oxidants, as well as the use of antioxidants or chelating agents (*5,6*). Thermal treatment causes structural changes in the fish muscle, including the destruction of cell membranes and denaturation of proteins, as well as the texture loss due to solubilisation of collagen (*6,7*). Moreover, cooking at high temperatures can also lead to the formation of toxic compounds, such as the ones resulting from grilling and frying of fish (*8*). Therefore, the proper choice of cooking method is crucial for maximal preservation of healthy compounds naturally found in raw fish.

The most commonly used cooking methods applied to fish are boiling (otherwise called 'traditional cooking'), frying and stewing (*5*). All of them are conducted at high temperatures (higher than 95 °C) in the presence of oxygen, causing a decrease in nutritional value, destruction of valuable compounds and quality loss (*5,6*). Minimal cooking can help to maximally preserve bioactive compounds and essential nutrients naturally found in the fish (*1*). The increase in consumer demand for minimally processed fresh-like fish products with extended shelf life and enhanced safety and quality parameters has led to the development of sous-vide processing technology (*1*). Sous-vide cooking offers a number of benefits related to preservation of nutritional and sensory quality of fish products in comparison with traditional cooking methods (*1,9*). Therefore, this technology can become a possible strategy to reduce the loss of bioactive compounds, nutrients and vitamins during thermal processing of fish. The main technological advantage is that sous-vide treatment uses cooking temperatures below 90 °C with the absence of oxygen (*9*). Thus, a product is put inside a heat-stable vacuum pouch and slowly cooked under controlled conditions of temperature and time (*1,9*). Vacuum packaging prevents the direct contact between the product and oxygen, thereby reducing oxidation of lipids and proteins. Moreover, it improves safety and security of the end product, increasing its shelf life (*1,9*). Since sous-vide treatment is generally performed at much lower time-temperature cooking regimes than traditional cooking, it requires immediate chilling with ice and subsequent chilled storage at low temperatures (*9*).

Among small pelagic fish, Atlantic mackerel (*Scomber scombrus*) has received greater attention due to its increasing capture and economic importance according to FAO report (*10*). At the same time, European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) ranked this fish among the top small pelagic commodity groups both in the volume and value in Europe in 2017 (*11*). However, Atlantic mackerel is a very perishable fish due to high content of polyunsaturated fatty acids and haem-proteins found in dark muscle of the fish (*12*). Therefore, it is very important to apply a proper cooking technology to maximally preserve the nutritional and sensory quality of the fish, while reducing the rate of oxidation reactions and extending the shelf life.

This paper aims to examine the effect of different sous-vide cooking parameters (temperature and time) and the use of antioxidants (rosemary extract and rosemary extract with ascorbyl palmitate) on quality parameters of Atlantic mackerel during chilled storage, and to provide insight into the oxidation-induced changes in the fish composition.

MATERIALS AND METHODS

Sample preparation and sous-vide cooking

Frozen fillets of Atlantic mackerel (*Scomber scombrus*) supplied from Pelagia A.S. (Selje, Norway) in January 2018, were used as materials in the study. The fish fillets were thawed at (0±1) °C overnight prior to experiment. Two fillets placed in each of the BST-090 type bags from Three Seal Bags series (Rolf Bayer Vacuumverpackung GmbH, Veitsbronn, Germany) with the following parameters: thickness 90 μm, gas permeability (O_2, N_2 and CO_2) up to 60 $cm^3/(m^2 \cdot day \cdot bar)$ and water vapour permeability up to 4 $g/(m^2 \cdot day)$. The vacuum bags with the fish were further heat-sealed using a vacuum sealing machine (SuperMax 3000 S; Webomatic, Bochum, Germany). Temperature data logger type SL52T (Signatrol, Tewkesbury, UK) was inserted into vacuum bags with the fish fillets to monitor fluctuations of temperature over the sous-vide cooking and chilling experiment. The antioxidant-treated and untreated fish fillets were sous-vide cooked by applying different time-temperature treatments and subjected to further chilled storage according to the planned experimental design displayed in **Table 1**. Two types of commercial antioxidants in liquid form were used: Fortium TR25 (rosemary extract) and Fortium RPT40 (a rosemary extract and ascorbyl palmitate) from Kemin Food Technologies, Herentals, Belgium. The antioxidants were manually distributed over the whole fillet surface by gentle rubbing, and left for 1 h to be absorbed by the fish flesh. The added amounts of antioxidants were the following: 0.001 mg/g of Fortium TR25 and 0.002 mg/g of Fortium RPT40. The fish samples were cooked in two water baths (Grant, Wilton, UK) set at 70 and 80 °C for 10 and 20 min. Immediately after the heat treatment, they were rapidly chilled with solid ice and put in chilled storage (in a cold room) at (4±1) °C for 1, 3, 9 and 15 days (**Table 1**). On each sampling day, the chilled mackerel samples were taken out of the vacuum packages and analysed. Control samples (raw mackerel fillets) were thawed at 0 °C overnight, vacuum-packed along with other experimental samples, and analysed after one day of chilled storage at (4±1) °C. Changes in cooking mass loss, protein solubility and oxidation, as well as texture parameters as affected by different cooking regimes and storage duration were studied. Analyses were performed in three and four replicates for each vacuum package containing two mackerel fillets.

Table 1. Experimental design for sous-vide cooking and chilled storage of mackerel samples

t(cooking)/°C	*τ*(cooking)/min	Use of antioxidant	*τ*(sampling)/day
70	10	0 A	1, 3, 9, 15
80	10	0 A	1, 3, 9, 15
70	20	0 A B	1, 3, 9, 15
80	20	0 A B	1, 3, 9, 15

0=no use of antioxidant, A=Fortium TR25 (rosemary extract), B=Fortium RPT40 (ascorbyl palmitate)

Chemical and physicochemical assays

Determination of dry matter and ash

Dry matter of the sous-vide cooked samples was determined by drying 2 g samples at 105 °C for 24 h to a constant mass, according to the AOAC Official Method 950.46 (*13*). Ash was determined by leaving the samples in the muffle furnace (Thermo Scientific Thermolyne, Thermo Scientific, Danville, IN, USA) overnight at 550 °C after determination of dry matter, as described in the same method (*13*). Both analyses were run in quadruplicate and the average values with standard deviation (S.D.) were calculated.

Determination of cook/drip loss

For the determination of cook and drip loss, sous-vide cooked and raw mackerel fillets (control samples) respectively, were removed from vacuum bags and blotted dry with a tissue paper before weighing. The remaining liquid in the vacuum bag was removed and the bag was also dried with a tissue paper before weighing. The cook loss was calculated as the percentage of fish mass loss after removing the liquid. The analysis was performed in triplicate and the mean value±S.D. was calculated.

Determination of protein solubility

Water- and salt-soluble proteins were extracted from mackerel muscle according to the method by Hultmann and Rustad (*14*) with a modification by Licciardello *et al.* (*15*). The extraction procedure was performed once on each fish fillet. Four fillets from each treatment were used for the extraction. Protein content in the extracts was determined by the method of Bradford (*16*), with bovine serum albumin (Sigma-Aldrich, Merck, Darmstadt, Germany) as a standard. The absorbance of the protein extracts was measured in a 10-mm QS quartz cuvette (Sigma-Aldrich, Merck) after the addition of BioRad protein dye reagent (Bio-Rad Laboratories AB, Oslo, Norway) at 595 nm with a UV-Vis spectrophotometer (Spectronic Genesys 10 Bio; Thermo Electron Corporation, Waltham, MA, USA). The analyses were run in four replicates and the mean value±S.D. was calculated.

Determination of protein oxidation

Protein oxidation was measured as the formation of carbonyl groups. Protein carbonyl groups were determined by DNPH-based enzyme-linked immunosorbent assay (ELISA) performed in a 96-well polystyrene plate from the ELISA kit (STA-310 OxiSelectTM; Cell Biolabs, Inc., San Diego, CA, USA) as a measure of protein oxidation (*17*). This is a rapid and highly sensitive plate-based assay technique developed by Buss *et al*. (*18*). The method is based on derivatization of carbonyl groups with dinitrophenylhydrazine (DNPH; ELISA kit STA-310 OxiSelectTM; Cell Biolabs, Inc.) and probing of protein-bound dinitrophenyl (DNP) with an anti-DNP antibody (ELISA kit STA-310 OxiSelectTM, Cell Biolabs, Inc.). Carbonyl groups were determined in the four protein extracts (N=4) and the average value with S.D. was calculated. The results were expressed in nmol of carbonyl per mg of protein.

Determination of texture parameters

To investigate the modification of sous-vide mackerel flesh firmness during chilled storage, breaking strength of the fish muscle was measured with a TA.XT2 Texture Analyser (Stable Micro Systems, Ltd., Godalming, UK) equipped with a 1 kg load cell according to the method described by Hultmann and Rustad (*14*). The resistance force was recorded in Newton (N) as the sample was pressed by a flat-ended cylinder of 12 mm diameter at a constant speed of 1 mm/s until it had reached 60 % of the fillet height. The holding time between the compressions was 5 s. Four fillets were used for texture analysis and from three to five measurements were run on each fillet, and the average value was calculated.

Experimental design and statistical analysis

A factorial design with four independent variables was employed in the study (**Table 1**). The independent variables were: cooking temperature (t(cooking)/°C), cooking time (τ(cooking)/min), use of antioxidants and duration of chilled storage (τ(storage)/day) as shown in **Table 1**, while the response variables were the main physicochemical parameters of mackerel samples. Experimental runs were randomized for each sampling day to reduce the effects of unexpected variability on the observed response. The experimental plan was used to identify the influence of sous-vide cooking regimes, use of antioxidants and duration of chilled storage on quality parameters of mackerel fillets.

The quality changes in sous-vide cooked mackerel were assessed through multiple regression analysis. The experimental data was analysed with SigmaPlot software, v. 14 (*19*) to derive statistically significant regression models from all possible linear and quadratic interactions between variables. The adequacy of the derived models was assessed statistically by R-value.

Statistical significance of the experimental data was verified by using Student's t-test and analysis of variance (ANOVA). The coefficients of determination for all parameters were considered to have a good fit of the obtained regression models at the 95 % confidence level.

Principal component analysis (PCA) was carried out to establish the variations and relationships among quality parameters of sous-vide cooked mackerel on the basis of experimental results. The dry matter, cook loss, protein oxidation and solubility, as well as flesh hardness data were entered into the PCA procedure using SigmaPlot software, v. 14 (*19*).

RESULTS AND DISCUSSION

Dry matter and ash analysis

Mass fraction of dry matter in sous-vide cooked mackerel samples varied over a wide range of values (34.6 to 52.0 %)

during the storage (**Table 2**). On the first day of chilled storage the mass fraction of dry matter in all sous-vide cooked mackerel samples was significantly higher ($p<0.05$) than in the initial raw mackerel fillets used as control (38.6 %, data not shown) samples. During storage, in the majority of mackerel samples the mass fraction of dry matter decreased. One possible explanation for this phenomenon is that cooking at 70 and 80 °C caused the rupture of fish muscle cells in the analysed mackerel fillets, leading to higher water release in the form of cook loss right after the treatment, thus increasing the mass fraction of dry matter in the samples. However, the released liquid was reabsorbed after the 3rd day of chilled storage by the thermally denatured unfolded and gelled proteins (*20*), thus decreasing the dry matter content of sous-vide cooked mackerel samples during storage. No significant variation was found in ash mass fraction in differently treated (sous-vide cooking and use of antioxidants) mackerel samples.

Cook and drip loss analysis

According to **Table 2**, cook loss was significantly ($p<0.05$) reduced in most of the sous-vide cooked mackerel fillets by the end of chilled storage compared to raw mackerel (control samples, 4.53 %, data not shown). These data are in strong agreement with the results of a previous sous-vide cooking experiment performed on Atlantic mackerel (*7*). The tendency for reduction of cook loss in sous-vide treated mackerel samples during chilled storage may be explained by the reabsorption of liquid released during cooking by unfolded myofibrillar proteins and its distribution between the intra- and extracellular spaces (*20*). To identify the main factors influencing

Table 2. Physicochemical parameters of sous-vide cooked mackerel during chilled storage

Sample no.	Sous-vide cooking regime (t-τ(cooking)-τ(storage)-AO)	w(dry matter)/%	w(ash)/%	CL/%	w(water-soluble protein)/%	w(salt-soluble protein)/%	Hardness/N
1	70-10-1	47.6±3.3	1.0±0.1	6.2±1.7	0.6±0.1	0.31±0.11	5.6±1.3
2	70-10-3	43.6±3.0	1.1±0.0	4.8±1.1	1.1±0.6	0.52±0.32	5.9±1.4
3	70-10-9	34.8±1.0	1.1±0.0	4.2±1.2	0.8±0.2	0.53±0.14	6.6±1.5
4	70-10-15	40.3±5.3	1.1±0.1	4.2±0.8	0.9±0.1	0.5±0.2	6.7±1.2
5	70-20-1	41.4±1.9	1.1±0.0	6.8±0.7	1.28±1.20	0.6±0.4	5.2±0.9
6	70-20-3	45.8±2.9	1.1±0.0	4.8±1.4	0.4±0.1	0.38±0.14	6.5±1.1
7	70-20-9	50.7±4.6	0.9±0.1	4.6±0.6	0.42±0.10	0.3±0.1	7.2±1.7
8	70-20-15	39.3±4.6	1.1±0.1	4.3±1.1	0.7±0.1	0.55±0.22	7.4±1.6
9	80-10-1	46.4±1.5	1.0±0.0	6.9±1.3	0.4±0.1	0.3±0.1	7.0±0.9
10	80-10-3	51.3±0.4	1.8±1.3	5.2±0.4	0.5±0.1	0.28±0.03	6.3±1.4
11	80-10-9	44.9±5.7	1.0±0.1	4.8±0.7	0.35±0.03	0.3±0.1	6.4±0.9
12	80-10-15	52.0±3.2	0.8±0.1	4.5±0.5	0.50±0.02	0.27±0.03	6.5±0.8
13	80-20-1	40.0±1.5	1.1±0.0	6.3±1.2	0.42±0.10	0.37±0.13	7.6±1.8
14	80-20-3	43.5±5.0	1.0±0.0	4.3±0.6	0.51±0.12	0.34±0.12	7.7±1.3
15	80-20-9	41.0±3.5	1.1±0.1	5.9±2.8	0.5±0.2	0.3±0.1	7.7±1.4
16	80-20-15	46.8±4.2	1.0±0.1	4.9±0.8	0.4±0.1	0.4±0.1	7.8±1.7
17	70-10-1-A	44.8±2.4	1.1±0.1	5.6±0.3	0.6±0.3	0.52±0.03	5.6±0.7
18	70-10-3-A	48.1±7.3	1.0±0.1	4.1±0.3	0.82±0.23	0.5±0.1	5.6±1.1
19	70-10-9-A	34.6±5.8	1.2±0.1	4.9±0.6	0.7±0.1	0.5±0.1	6.3±1.1
20	70-10-15-A	43.8±0.6	1.0±0.0	3.5±0.4	0.7±0.1	0.57±0.02	5.8±1.8
21	70-20-1-A	43.4±4.1	1.1±0.1	7.9±0.3	0.64±0.34	0.4±0.3	5.5±1.3
22	70-20-3-A	46.5±5.0	1.0±0.1	5.4±1.2	0.55±0.10	0.45±0.04	7.6±1.1
23	70-20-9-A	49.6±21.0	0.9±0.1	4.6±0.2	0.58±0.10	0.4±0.1	7.8±1.5
24	70-20-15-A	41.2±11.1	1.1±0.2	3.6±0.3	0.6±0.2	0.7±0.4	7.9±1.3
25	80-10-1-A	42.6±2.5	1.1±0.1	5.8±1.0	0.6±0.1	0.35±0.04	6.6±0.6
26	80-10-3-A	42.8±6.0	1.0±0.1	5.9±0.9	0.87±0.42	0.38±0.10	6.0±0.6
27	80-10-9-A	49.7±1.7	0.9±0.0	4.9±2.4	0.41±0.04	0.3±0.1	6.1±1.1
28	80-10-15-A	48.7±0.1	0.9±0.0	3.5±0.6	0.48±0.03	0.38±0.04	6.3±0.8
29	80-20-1-A	48.2±3.4	1.0±0.1	7.3±1.0	0.84±0.22	0.3±0.1	6.6±1.0
30	80-20-3-A	46.2±0.0	0.9±0.0	7.0±1.0	0.5±0.1	0.3±0.1	6.6±1.5
31	80-20-9-A	43.0±3.6	1.0±0.0	6.8±2.0	0.4±0.1	0.19±0.02	6.7±1.1
32	80-20-15-A	43.9±2.7	1.0±0.1	4.5±1.1	0.6±0.1	0.35±0.02	6.9±1.8
33	70-20-1-B	51.5±2.0	0.9±0.0	7.8±1.5	0.43±0.10	0.16±0.03	7.1±1.2
34	70-20-15-B	48.0±6.8	1.0±0.1	4.2±0.4	0.58±0.03	0.49±0.04	7.4±2.1
35	80-20-1-B	50.7±4.5	0.9±0.1	8.3±2.1	0.39±0.04	0.2±0.1	6.9±1.4
36	80-20-15-B	44.2±6.3	1.0±0.1	5.1±0.8	0.4±0.1	0.3±0.1	7.1±0.5

t/°C=sous-vide cooking temperature, τ(cooking)/min=cooking time, τ(storage)/day=duration of chilled storage, AO=antioxidant: A=0.4 % Fortium TR25 (rosemary extract), B=0.2 % Fortium RPT40 (ascorbyl palmitate), CL/%=cook loss

the variations in cook loss in the fish samples (Fig. 1a) compared to raw mackerel fillets, the following regression equation was derived in terms of actual values, on the basis of multivariate regression analysis:

$$CL=0.865+0.057\cdot t(\text{cooking})+0.069\cdot\tau(\text{cooking})-0.159\cdot\tau(\text{storage})\ (R=0.821,\ R^2=0.674,\ p<0.001) \quad /1/$$

where CL is cook loss (%) in sous-vide cooked mackerel samples, t(cooking) is sous-vide cooking temperature (°C), τ(cooking) is the duration of sous-vide cooking (min), and τ(storage) is the duration of chilled storage (day). The regression model derived in the study has not revealed any effect of antioxidants on the fluctuations in cook loss during the storage.

The model clearly shows that the duration of chilled storage had the biggest contribution to the decrease in cook loss, thus leading to reabsorption of water by the unfolded denatured fish muscle proteins (*20*). Contrary to chilled storage time, temperature and duration of sous-vide cooking led to an increase in cook loss in mackerel samples, thus decreasing the product quality. However, according to regression coefficients of the model (Eq. 1), their influence was more than twice lower than of the chilled storage time. Therefore, we can conclude that the moisture lost in form of cook loss after sous-vide cooking of mackerel fillets was partially reabsorbed by the thermally denatured proteins during chilled storage.

Protein oxidation analysis

Oxidation (Fig. 1b and Fig. 1c) and solubility (Fig. 1d) of sarcoplasmic and myofibrillar proteins of Atlantic mackerel are affected by sous-vide cooking regimes and duration of chilled storage. During chilled storage, the carbonyl content significantly ($p<0.05$) increased both in the sarcoplasmic and myofibrillar proteins of sous-vide cooked mackerel samples (Fig. 2). This increase can be partially explained by the interaction of proteins with lipid oxidation products and various pro-oxidants (iron, myoglobin, *etc.*) liberated from the heat-disrupted cells during cooking (*21*). According to our hypothesis supported by earlier studies, sous-vide cooking broke down muscle structures, causing unsaturated fatty acids to react with haem pigments and other pro-oxidants, promoting autoxidation in the fish systems (*21*). The oxidized lipids came further in contact with proteins, leading to their carbonylation.

The initial carbonyl content in raw mackerel was 11.52 and 15.27 nmol/mg of sarcoplasmic and myofibrillar proteins, respectively, and it increased by 90 % in sous-vide cooked fillets at the end of chilled storage compared to control samples. To explain which process parameters influenced this drastic increase in experimental mackerel samples, the following regression models (Fig. 1b and 1c) were derived in terms of actual values:

$$\Delta w(C_{WSP})=27.505-5.221\cdot w(A)-44.225\cdot w(B)+2.138\cdot\tau(\text{storage})\ (R=0.879,\ R^2=0.689,\ p<0.001) \quad /2/$$

$$\Delta w(C_{SSP})=-58.946+1.485\cdot t(\text{cooking})-82.038\cdot w(B)+2.375\cdot\tau(\text{storage})\ (R=0.757,\ R^2=0.673,\ p<0.001) \quad /3/$$

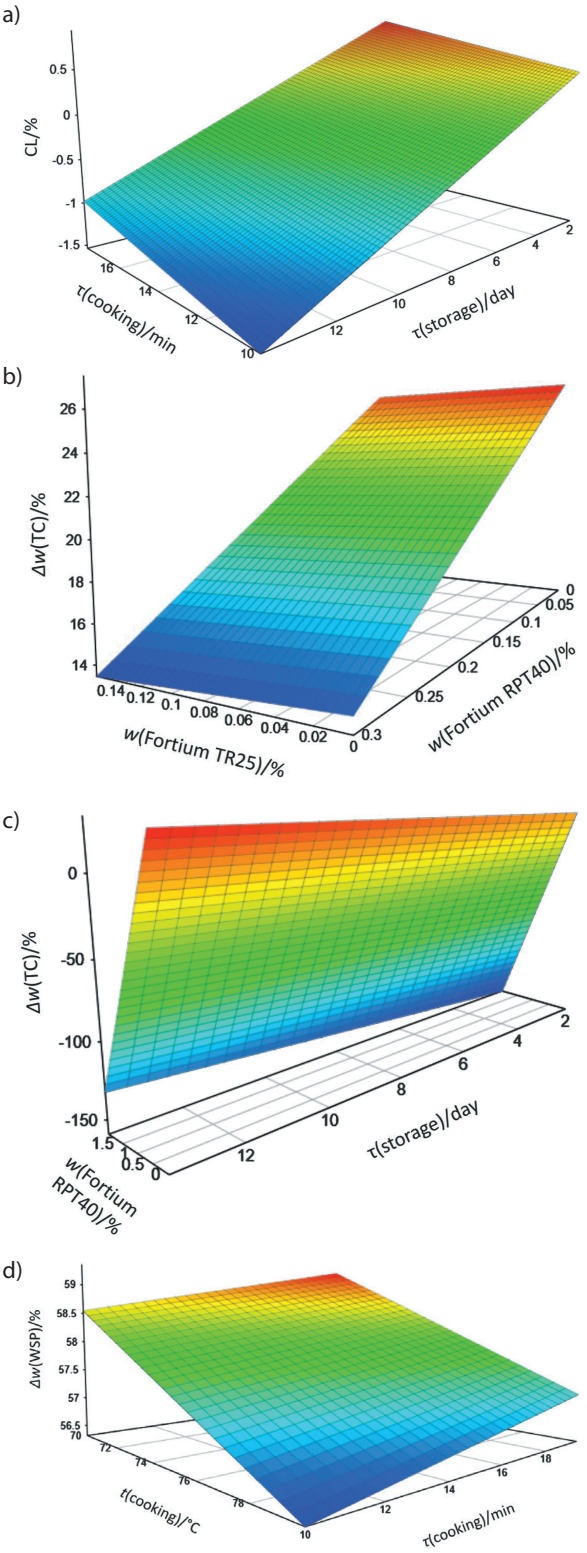

Fig. 1. 3D surface plots displaying the influence of sous-vide regime parameters on quality characteristics of Atlantic mackerel on: a) cook loss, b) relative increase in total carbonyls in water- and c) salt-soluble proteins, and d) relative decrease in water-soluble proteins, where CL/% is cook loss, τ(cooking)/min is duration of sous-vide cooking, τ(storage)/day is duration of chilled storage, w(Fortium TR25 or RPT40)/% is the mass of added antioxidant per product mass, Δw(TC)/% is relative increase in total carbonyl content and Δw(WSP)/% is relative decrease in water-soluble proteins

where $\Delta w(C_{WSP/SSP})$ is the relative increase in total carbonyls in water- and salt-soluble proteins of experimental samples of sous-vide cooked mackerel compared to control samples (%), w(A) is the mass fraction of antioxidant Fortium TR25 (rosemary extract) (%), w(B) is the mass fraction of antioxidant Fortium RPT40 (ascorbyl palmitate) (%), t(cooking) is sous-vide cooking temperature (°C), and τ(storage) is the duration of chilled storage (day).

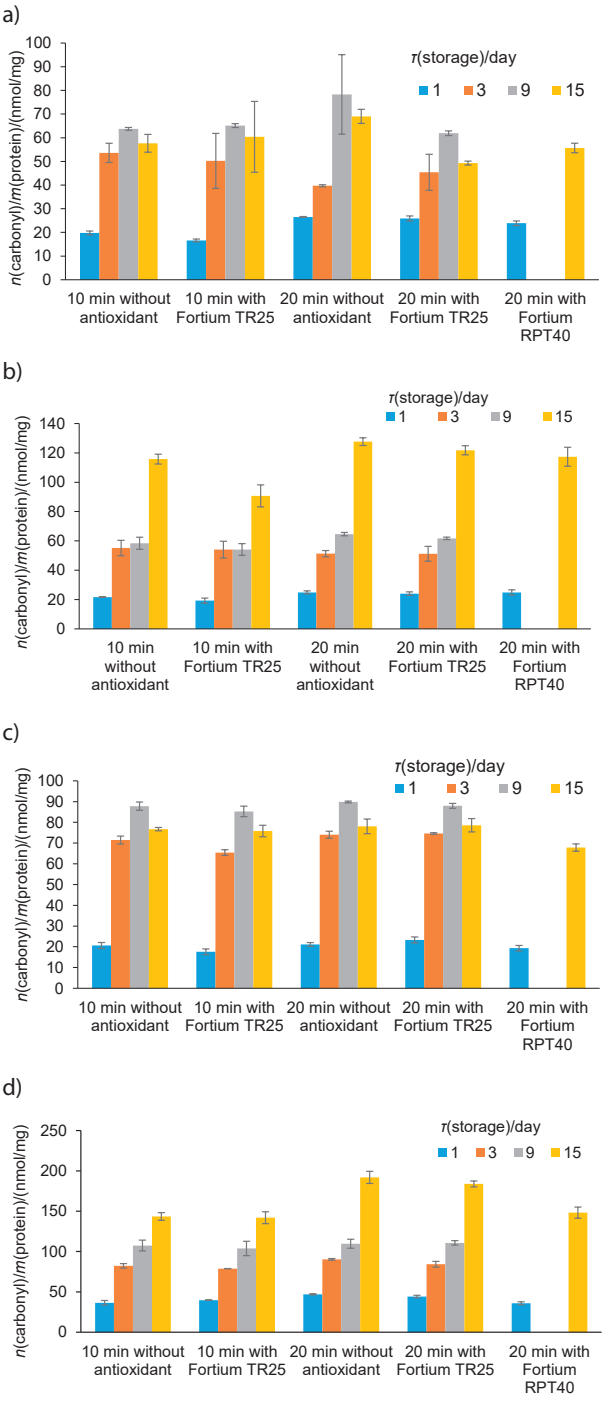

Fig. 2. Total carbonyl content in: a) and b) water-soluble (sarcoplasmic) and c) and d) salt-soluble (myofibrillar) proteins of Atlantic mackerel cooked at 70 °C (a and c) and 80 °C (b and d)

From the regression equations displayed above, we can conclude that the addition of rosemary extract and ascorbyl palmitate strongly protected fish tissue from oxidation, while duration of chilled storage was the main factor promoting protein carbonylation in sous-vide cooked mackerel samples. The protective effect of rosemary extract and ascorbyl palmitate was much stronger than the negative influence of chilled storage. Thus, the values of the regression coefficients for antioxidants Fortium TR25 and RPT40 were 2.44 and 20.68–34.54 times higher (Eqs. 2 and 3) than the regression coefficients for duration of chilled storage, respectively. Also, commercial antioxidant Fortium RPT40 added in the mass fraction of 0.2 % retarded protein carbonylation in a more effective way than 0.4 % Fortium TR25. Moreover, mass fraction of Fortium TR25 is not included in Eq. 3, and thus did not have any positive influence on the decrease of myofibrillar protein carbonylation. In addition, temperature of the sous-vide cooking has the second largest contribution to the increase of total carbonyl content in salt-soluble proteins after chilled storage (Eq. 3). Generally, myofibrillar proteins were characterized by a significantly higher carbonyl content than sarcoplasmic proteins in all cooking regimes (**Fig. 1**). Similar results were obtained for raw Atlantic mackerel fillets subjected to chilled and frozen storage (*12*). This tendency can be explained by a higher susceptibility of salt-soluble proteins to denature both during processing and storage (*22*).

Protein solubility analysis

There were no significant ($p<0.05$) changes in the solubility of water-soluble (sarcoplasmic) and salt-soluble (myofibrillar) proteins of mackerel fillets subjected to cooking at 70 and 80 °C during storage (**Table 2**). However, solubility of sarcoplasmic proteins significantly ($p<0.05$) decreased in mackerel samples cooked sous-vide at 80 °C in comparison with cooking at 70 °C. The significant decrease in protein solubility can be explained by protein aggregation as a result of cross-linkages between polypeptides and proteins, occurring due to protein oxidation promoted by cooking at higher temperatures (*5*). At the same time, no significant difference was found in the solubility of myofibrillar proteins in the differently treated fish samples. However, to reveal all factors contributing to the decrease in solubility of sarcoplasmic proteins of sous-vide cooked mackerel during chilled storage, the following regression equation (**Fig. 1d**) was derived in terms of actual values:

$$\Delta w(\text{WSP})=73.874+0.231\cdot t(\text{cooking})+0.084\cdot\tau(\text{cooking}) \quad /4/$$
$$(R=0.712,\ R^2=0.608,\ p<0.001)$$

where Δw(WSP) is relative decrease in water-soluble proteins (%), t(cooking) is sous-vide cooking temperature (°C), and τ(cooking) is duration of sous-vide cooking (min).

According to the regression equation 4, the second important factor leading to the decrease in the solubility of sarcoplasmic proteins after cooking temperature is duration of sous-vide treatment. However, its effect is 2.75 times less

meaningful than the effect of cooking temperature (Eq. 4). Thus, according to the data displayed in **Table 2**, a 10 °C increase in cooking temperature reduced 1.33- to 1.72-fold the solubility of sarcoplasmic proteins in sous-vide cooked mackerel at the end of chilled storage. The regression model displayed above has not revealed any influence of antioxidants on the changes in protein solubility during the experiment.

Texture analysis

According to **Table 2**, there was a small but insignificant decrease in the hardness of mackerel fillets after sous-vide cooking compared to control samples ((8.0±1.0) N, data not shown). This tendency is in agreement with previous studies performed on sous-vide cooked Atlantic mackerel (*7*) and can be explained by heat-induced solubilisation of connective tissue in the temperature range of 50–70 °C leading to the flesh tenderization. However, the destruction of myofibrillar network is not severe before reaching a temperature of 70 °C resulting in the flesh toughening (*7,9*). Therefore, mackerel samples subjected to sous-vide cooking at 70 °C had significantly lower values of hardness than the mackerel fillets cooked at 80 °C on the first day of experiment (**Table 2**). Moreover, since more proteins are denatured as the cooking temperature rises, their water-holding capacity decreases leading to an increase in cook loss and subsequent muscle toughening. Thus, cooking at 70 °C leads to softening of the fish flesh due to aggregation of sarcoplasmic proteins forming a gel, whereas heat treatment at higher temperatures (80 °C and higher) makes the fish flesh tougher due to viscous flow in the fluid-filled channels between the muscle fibres and fibre bundles increasing the elastic modulus (*9*). However, hardness of most samples of the studied sous-vide mackerel fillets was gradually increasing during chilled storage.

Variations among quality parameters revealed by PCA

In order to identify the main variations among quality parameters of sous-vide cooked mackerel samples, as well as the distribution of mackerel samples depending on sous-vide cooking regimes, the experimental data (dry matter, cook loss, protein oxidation and solubility, as well as flesh hardness) were subjected to PCA analysis. Thus, the PCA of the fish samples was performed on mean values of seven quality parameters of each fish group (sous-vide cooking and storage regimes) aiming to identify similarities and differences. Projection of the variables (quality parameters) on the factor planes revealed that the 1st and 2nd axes of the PCAs display 37.16 and 32.59 % of the total variance (**Fig. 3**), respectively.

The PCA score plot was composed to determine whether sous-vide treated mackerel samples could be clustered pointing to similarities in quality parameters under the same cooking and storage regimes. The resulting PC1/PC2 score plot (**Fig. 3a**) discriminates the samples according to treatment conditions. The PCA revealed a temperature-related clustering of sous-vide cooked mackerel samples. Thus, according to

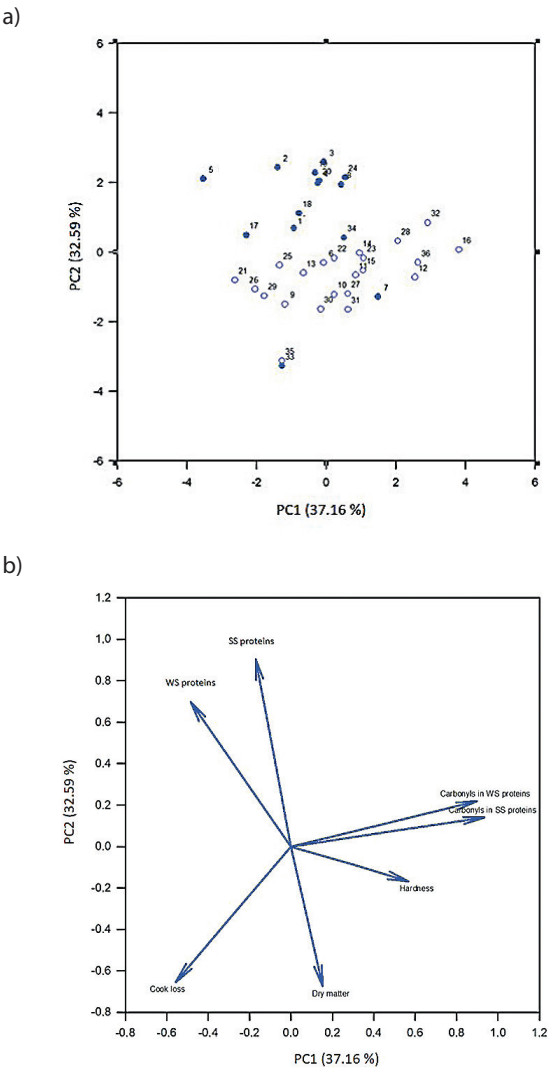

Fig. 3. PCA score plot (a) of mackerel samples cooked sous-vide at 70 °C (blue dots) and 80 °C (white dots); the numbers represent the sample numbers from the experimental plan, and (b) projection of quality parameters of the samples on the 1st and 2nd factor planes

the PCA score plot, data on mackerel quality parameters can be clustered on the basis of cooking temperature, and major changes in the quality characteristics are due to heating regime.

On the PCA factor plane, PC1 best explained the variation in carbonyls in water- and salt-soluble proteins (**Fig. 3b**), while PC2 the variability of protein solubility of water- and salt-soluble proteins. This tendency confirms the fact that an increase in protein carbonylation contributes to a decrease in solubility of sarcoplasmic and myofibrillar proteins in sous-vide cooked mackerel samples. Besides variation in protein solubility, PC2 best explains the variability of dry matter, cook loss and hardness: these variables appeared to be influenced by each other. However, it is clearly notable that hardness of the fish flesh is influenced by both the protein carbonylation and variations in dry matter and cook loss. Thus, we assume that heat denaturation and oxidation of the fish muscle proteins resulted in their reduced water-holding capacity, leading to an increase

in cook loss and subsequent muscle toughening. This assumption is in agreement with previous studies of quality changes in raw and sous-vide cooked Atlantic mackerel during chilled storage (*7,12*).

CONCLUSIONS

The present study revealed the influence of different sous-vide cooking regimes, chilled storage and use of antioxidants on quality parameters of Atlantic mackerel with regard to changes in protein characteristics. Multiple regression models derived in the study have shown that the duration of chilled storage led to a significant ($p<0.05$) increase in protein oxidation, resulting in toughening of the fish flesh due to a possible aggregation of proteins and conformational changes. However, mackerel samples cooked at 70 °C had less detrimental changes in quality characteristics during chilled storage than the fish samples cooked at 80 °C. Thus, we can conclude that sous-vide cooking at 70 °C is more beneficial in regard to quality loss of mackerel during prolonged storage. Nevertheless, chilled storage exhibited the most significant effect on the decrease in cook loss, probably due to the reabsorption of water by the heat-denatured fish muscle proteins. At the same time, both the temperature and time of sous-vide cooking affected the solubility of sarcoplasmic proteins as a result of protein carbonylation, leading to increased cook loss. The used antioxidants (rosemary extract and ascorbyl palmitate) had a much stronger positive influence on the decrease of protein oxidation in mackerel samples than the negative effect of sous-vide cooking parameters and chilled storage. The multiple regression analysis has revealed that ascorbyl palmitate retarded protein carbonylation in mackerel samples in a more effective way than it did rosemary extract. Therefore, it was concluded that prolonged cooking and subsequent chilled storage negatively influence the quality parameters of Atlantic mackerel with regard to changes in protein structure and properties. However, the used antioxidants (rosemary extract and ascorbyl palmitate) can effectively retard protein oxidation in the fish during both sous-vide cooking and storage, and thus are suggested for application.

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