Abstract

The aim of the present study was to evaluate the effects of various sous-vide time-temperature regimes and their interactions on quality parameters of Atlantic mackerel (*Scomber scombrus*) during chilled storage. The mackerel fillets were exposed to sous-vide treatment at 60°C, 75°C and 90°C for 10, 15 and 20 minutes and further stored for 1, 3 and 7 days at 4 ± 1°C before analysis. Changes in pH, water content and cook loss, amount of water- and salt-soluble proteins, texture and color parameters, as well as accumulation of lipid oxidation products in sous-vide cooked mackerel were assessed. Sous-vide cooking time and temperature had the lowest contribution to formation of primary and secondary products of lipid oxidation, as well as increase in yellowness of the fish flesh due to their accumulation; whereas duration of chilled storage led to a significant increase in oxidation and yellowness ($p<0.05$). Duration of chilled storage also affected structural and textural properties of the fish muscle, leading to a decreased cook loss. At the same time, sous-vide cooking decreased the firmness of the fish muscle.

Duration of chilled storage was found to have the highest significant effect ($p<0.001$) on all physicochemical characteristics of sous-vide cooked mackerel.

Keywords: sous-vide cooking, Atlantic mackerel, chilled storage, cook loss, lipid oxidation

1. Introduction

Nowadays, due to a continuously growing number of health-conscious consumers, a special interest is being paid to the use of minimally processed foods and mild heat treatments aiming to preserve nutritional value of the food products while ensuring their high quality and stability (Li et al., 2016; Iborra-Bernad et al., 2014). Since the discovery and control of fire, cooking has been used as the main heat treatment method applied to food with the aim of prolonging its storage period by destroying microorganisms and deactivating intrinsic enzymes. Heating also helps to improve digestibility and sensory characteristics of the end-product such as appearance, color, flavor and texture (Iborra-Bernad et al., 2014). Currently, the most commonly used cooking methods for meat and fish are boiling, microwave cooking, pan-frying, oven roasting, and grilling. Unfortunately, all these have a number of negative effects, such as degradation of thermo-labile vitamins and bioactive compounds, destruction of cell membranes and denaturation of proteins, as well as leakage of minerals and vitamins to the cooking water (Rondanelli et al., 2017; Sanchez Del Pulgar et al., 2012; Rodriguez-Estrada et al., 1997). Besides, due to use of relatively high temperatures and presence of oxygen in the cooking environment, most of these methods may significantly alter fatty acid composition and decrease the PUFA level of lipid-rich foods and lead to the formation of unwanted lipid oxidation products, especially during grilling or frying meat and fish (Oz & Seyyar, 2016; Gerber et al., 2009). Therefore, the choice of cooking method is very important for ensuring good quality and safety of food products.

In this regard, sous-vide cooking is gaining an increasing attention from both consumers and catering industries worldwide. The French term "*sous vide*" for "under vacuum" cooking is defined as thermal treatment of vacuum-packed food under controlled conditions of temperature and time (Baldwin, 2012). This method implies the use of lower heating temperatures (under 100°C) and

45 longer cooking times compared to traditional cooking procedures (Schellekens, 1996), followed
46 by a rapid cool-down to 0-4°C and subsequent chilled storage. Sous-vide cooking requires low-
47 cost operations to provide consumers with ready-to-eat food products. This emerging method is
48 able to ensure high quality of the product due to reduced concentration of oxygen inside the
49 vacuum-pack (Oz & Seyyar, 2016; Baldwin, 2012). The sensory and nutritional quality is
50 preserved by reducing the loss of water and both flavor and aroma volatile compounds in sous-
51 vide cooked food (Oz & Seyyar, 2016; Iborra-Bernad et al., 2014; Vaudagna et al., 2002). Sous-
52 vide cooking has also been found to enhance its textural characteristics by increasing tenderness
53 and juiciness, which is important for small children and elderly consumers (Botinestean et al.,
54 2016; Creed, 2001). Moreover, a joint application of low heat treatment and vacuum packaging in
55 sous vide technology may help to significantly prolong the shelf life of food products compared to
56 foods prepared by traditional cooking methods. This phenomenon is attributed to a delay in
57 oxidation of lipids and muscle pigments (Diaz et al., 2009; Wang et al., 2004), as well as reduction
58 of microbial spoilage of sous-vide cooked foods during chilled storage (Rodgers, 2004).
59 The quality deterioration of chilled-stored fish is the result of several complex biochemical and
60 microbiological processes, such as endogenous enzymatic activity, growth of anaerobic
61 microflora, and oxidative processes in protein and lipid components (Rodríguez et al., 2006;
62 Hultmann and Rustad, 2002). Fish products are highly prone to lipid oxidation during storage due
63 to high content of polyunsaturated fatty acids, which significantly reduces their quality (Nguyen
64 et al., 2013; Frankel, 2005). The sensory spoilage in chilled fish relates to detrimental changes in
65 flavor, taste, color and texture as a result of drip loss and emergence of off-odors due to rancidity
66 and formation of trimethylamine and total volatile base nitrogen (Richards and Hultin, 2002). By
67 combining low cooking temperatures with vacuum packaging, sous-vide treatment may delay the

spoilage of fish through inactivation of endogenous proteases and lipases, while preserving sensory and nutritional quality of the product (Baldwin, 2012).

Regardless of the fact that sous-vide technology has been widely applied to fish products since the early 90's (Baldwin, 2012), there is very little information in the scientific literature on its application to small pelagics such as Atlantic mackerel (*Scomber scombrus*). Nevertheless, these fish species have received greater attention due to its increasing capture production and economic importance in the latest years (FAO, 2015). According to European Market Observatory for Fisheries and Aquaculture Products, Atlantic mackerel is ranked among the top small pelagic commodity groups both in volume and value in Europe in 2017 (EUMOFA, 2018). Since Atlantic mackerel is a very perishable fish (Standal et al., 2018), 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.

Based on this, the aim of the present study was to investigate the effects of different sous-vide cooking regimes and chilling storage times on the physicochemical properties of Atlantic mackerel. The present study is very important both for food and catering industries dealing with processing of this fish.

2. Materials and Methods

2.1 Sample preparation and sous-vide cooking

The Atlantic mackerel fillets (*Scomber scombrus*) used as materials in the present study were supplied frozen from Pelagia A.S. (Selje, Norway) in January 2017. Average weight of the resulting skin-on fillets without bones was 85 ± 10 g with an average length of 27 ± 2 cm and

thickness of 0.7 ± 0.2 cm. Fish fillets were thawed at 0°C for 5h. Two fillets were placed in BST/SR type of bags and further heat-sealed using a vacuum sealing machine (Webomatic Vacuum packaging system, Super max, 3000 sensor), with extent of vacuum 99.6%. Temperature data loggers type SL52T (Signatrol, UK) with an embedded thermocouple probe were inserted inside the vacuum bags along with the fish fillets to monitor fluctuations of temperature over the sous-vide cooking and chilling experiment. The fish fillets were sous-vide cooked by applying different time-temperature treatments and subjected to further chilled storage according to a 3x3 factorial design. The samples were cooked in two water baths (Grant, UK) set at 60°C, 75°C and 90°C for 10, 15 and 20 min. Thus, the vacuum bags with fish fillets were dipped into the water baths when the temperature of water achieved 60 ± 1°C, 75 ± 1°C and 90 ± 1°C, and kept in water bath for 10, 15 and 20 min. The maximum core temperature reached for each of the temperature regimes was approximately 1.5 ± 0.5°C below the temperature in the water bath according to temperature loggers inserted in the vacuum packs. 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 and 7 days according to the planned experimental design displayed in Table 1. Thus, nine specific processing treatments of each of the temperature regimes were performed in the study. According to temperature loggers, the fillets achieved the temperature of 1.5 + 0.5°C in average 37 min. after cooking at 60°C, 45 min. after cooking at 70°C and 62 min. after cooking at 90°C. At each sampling day, the chilled mackerel samples were taken out of the vacuum packages and analyses performed. Changes in cooking weight losses, lipid oxidation, color and texture parameters as affected by different cooking regimes and storage duration were studied. Analyses were performed in two or three replicates for each vacuum package containing two mackerel fillets.

Multiple regression analysis was performed to explain the dynamics underlying the quality deterioration process in sous-vide cooked mackerel during chilled storage and to identify the contribution of each of the regime parameters to detrimental changes occurring in the product.

Table 1

2.2 Chemical and physicochemical assays

Measurement of pH

pH was measured at room temperature on homogenates of sous-vide mackerel samples in distilled water (1/10 w/w) (Vyncke, 1981) by using a MP-220 pH-meter (Mettler-Toledo, Hong Kong). Prior to pH measurements, the pH meter was calibrated with standard buffer solutions. The measurements were performed in triplicate.

Water content

Water content was determined by drying samples of 2 g at 105 °C for 24 h to a constant weight, according to the official method (AOAC 2005). The analyses were run in triplicate.

Cook loss

For the determination of cook loss, sous-vide mackerel fillets were removed from vacuum bags and blotted dry with a tissue paper before weighing. The remaining liquid in the vacuum bag was weighed and the cook loss calculated as the percentage of fish weight loss after removing the liquid.

136

137 ***Soluble protein content***

138 Amount of water- and salt-soluble proteins (in % wet weight) was determined according to a
139 modification of the methods of Anderson and Ravesi (1968) and Licciardello, Ravesi, Lundstrom,
140 Wilhelm, Correia, and Allsup, (1982), as previously described by Hultmann & Rustad (2002).
141 Mackerel fillets were ground with a benchtop mixer to obtain a homogeneous mass. Then, 4 g
142 ground muscle was homogenized for 20 s in 80 ml phosphate buffer (0.05 M phosphate, 0.5%
143 triton X-405, pH 7.0) using an Ultra Turrax. After centrifugation at 8000 g for 20 minutes (samples
144 were kept all the time in ice 0+1°C), the volume was made up to 100 ml with phosphate buffer.
145 This represented the water-soluble fraction. The remaining precipitate was further homogenized
146 for 10 s in phosphate buffer with KCl (0.05M phosphate, 0.6M KCl, 0.5% tritonX-405, pH 7.0),
147 and centrifuged as described above. The supernatant was adjusted to 100 ml with KCl-phosphate
148 buffer. This was the salt-soluble fraction. The extraction procedure was carried out once on each
149 fillet. The extraction procedure was performed once on each fish fillet. The amount of proteins in
150 the extracts was determined with BioRad protein assay after centrifugation at 8000 g and 4°C for
151 20 minutes, using gamma globulin as a standard. The analyses were run in triplicate and the mean
152 value ±SD was calculated.

153

154 ***Texture parameters***

155 To investigate modification of sous-vide mackerel flesh firmness during chilled storage, breaking
156 strength of the fish muscle was measured with a TA.XT2 Texture Analyzer (SMS Stable Micro
157 Systems, Ltd., Surrey, UK) equipped with a 1 kg load cell according to the method described by
158 Hultmann & Rustad (2002). The resistance force was recorded in Newton (N) as the sample was

159 pressed by a flat-ended cylinder of 12 mm diameter at a constant speed of 1 mm s-1 until it had
160 reached 60 % of the fillet height. The holding time between the compressions was 5 s. From three
161 to five measurements were run on each fillet along its inner dorsal part, and the average was
162 calculated.
163
164 ***Primary and secondary products of lipid oxidation***
165 Primary lipid oxidation products were quantified by determination of peroxide value (PV) and
166 conjugated dienes (CD). PV was measured by using the iodometric titration method described in
167 AOCS official methods (Cd 8b-90) mentioned in the titration application issued by SI Analytics
168 (AOCS, 2003). The end point of titration was assessed potentiometrically with an automatic titrator
169 (TitroLine 7800, Xylem Analytics, Germany) coupled with a platinum electrode (Pt 62). The
170 analysis was performed in duplicate and the results were expressed in meqO_2/kg oil as a mean
171 value ± SD.
172 Spectrophotometric determination of conjugated dienes was performed in duplicate by UV
173 absorption at 233 nm according to AOCS standard method 2.501 (AOCS, 1998) by using a
174 GENESYS 10S UV-VIS spectrophotometer (Thermo Scientifc, USA). The results were expressed
175 in arbitrary units of absorbance (U.A.).
176 Secondary lipid oxidation products were assessed by determination of thiobarbituric acid reactive
177 substances (TBARS) and Schiff bases (SB).
178 TBARS were determined according to the method described by Ke and Woyewoda (1994), by
179 using a GENESYS 10S UV-VIS spectrophotometer (Thermo Scientifc, USA). The analysis was
180 carried out in three parallels for each vacuum-pack of fish and the results were expressed in mmol
181 TBARS/kg lipids as a mean value ± SD.

SB measurements were performed according to Buege and Aust (1978), as follows. Mackerel samples were ground and homogenized with a high-speed IKA Ultra-Turrax homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). 70 µL aliquot of homogenate was mixed with 980 ml of chloroform-methanol mixture (2:1 V/V), adjusted to pH 2.3 with distilled water and then centrifuged at 1500 g for 10 min by using a Heraeus Multifuge X1 (Thermo Scientifc, USA). The SB were measured spectrofluorimetrically by using a luminescence spectrometer (LS 50B Perkin-Elmer, MA, USA) at 360 nm excitation and 430 nm emission wavelength. The assay was carried out in three parallels for each vacuum-pack of fish and the average result was expressed in arbitrary units of fluorescence (U.F.).

Color parameters

Color parameters of the fish fillets were measured instrumentally using a Minolta Chroma meter CR-400 (Konica-Minolta, Osaka, Japan). Before starting the analysis, the instrument was calibrated with a standard white plate. The measurements were performed on preselected locations at the inner surface of each mackerel fillet along its dorsal part at room temperature. The data were recorded in color coordinates of L* (lightness, black = 0, white =100), a* (redness >0, greenness <0), and b* (yellowness, b* >0, blue <0) according to the Commission Internationale de l'Éclairage (CIE) Lab scale. Color parameters were read three times on each sample and the average was calculated.

2.3 Experimental design and statistical analysis

A 2^3 two-level, full factorial design with added central and central axial points was employed in the study. The independent variables set in three levels were cooking temperature (t, °C), cooking

time (τ, min) and duration of chilled storage (d, days) as shown in Table 1, while the response variables were the main physicochemical parameters of studied mackerel samples. Experimental runs were randomized in each sampling day to reduce the effects of unexpected variability on the observed response. The experimental design was used to identify the influence of sous-vide cooking regimes and duration of chilled storage on quality characteristics of mackerel fillets.

The quality changes in sous-vide cooked mackerel were assessed through multiple regression analysis by the relative change (increase/decrease) in its main physicochemical and chemical parameters during chilled storage in comparison with initial raw mackerel (control) samples, as follows (Eq. 1):

- relative change in the studied quality parameter, %:

$$\Delta P = |P_{s\text{-}v} - P_c| / P_c \cdot 100\% \quad (1)$$

where ΔP is the relative change in a studied parameter of sous-vide mackerel samples in comparison to initial raw mackerel (control) samples (%); $P_{c/s\text{-}v}$ is the parameter value in raw (c) and sous-vide mackerel (s-v) samples. The untreated (raw) mackerel fillets from the same fish batch used as a control in the study, were put on ice and stored in the cold room at 4 ± 1°C side-by-side with the sous-vide processed fillets, and analyzed on day 1.

Statistical significance of the experimental data was verified by using Student's t-test and Analysis of Variance (ANOVA). The regression models describing the common effect of independent factors on a certain response variable were derived using Statgraphics Centurion XVI software, version 16.1.15. The coefficients of determinations for all parameters displayed a good fit of the

obtained regression models at 95% confidence level. Estimations of potential relationships between different parameters were conducted using linear correlation between mean values for each vacuum-packed samples.

The experimental data obtained from different sous-vide heat treatments and chilled storage periods was analyzed with Statgraphics Centurion XVI, using multiple regression analysis to derive a statistically significant regression model from all possible linear and quadratic interactions between variables. The ANOVA of the response was performed for finding the significance of variables and products of their interaction and all the insignificant terms ($p>0.05$) were rejected. The adequacy of the derived models was assessed statistically by R-value.

3. Results and discussion

pH

The initial pH value of raw mackerel samples was 6.31 ± 0.02 and for most samples there was a slight increase in pH after sous-vide cooking and storage (Table 2). However, the change in pH was different for the three groups of sous-vide cooking regimes. Thus, the highest value of pH (6.75) was observed for mackerel samples cooked at 60°C for 10 min on the 7th day of chilled storage, while the two remaining groups of sous-vide treatment regimes (cooking at 75°C and 90°C for 10 min) displayed lower pH values on the 7th day of chilled storage (6.61 and 6.42, respectively). Generally, an increase in pH values for sous-vide treated mackerel samples may be explained by the generation of trimethylamine and total volatile base nitrogen from either microbial or endogenous enzymatic degradation (Özyurt et al., 2017; Diaz et al., 2011; Fan, W et al., 2009; Richards & Hultin, 2002). However, none of the studied sous-vide samples indicated

spoilage during chilled storage, while maintaining a low, stable pH. These data are in accordance with several previous studies investigating sous-vide treatment of salmon (Diaz et al., 2011; González-Fandos et al., 2005; Garcia-Linares et al., 2004) and whiting (Mol et al., 2012).

Table 2

Water content and cook loss

The water content in sous-vide cooked mackerel samples displayed relatively high variation (Table 2), ranging from 55.2% to 69.9% along the sampling days. The initial water content in raw mackerel fillets was 69.3 ± 0.4%. The water content did not show any trend neither according to sous-vide cooking regimes, nor to duration of chilled storage.

Contrary to water content, cook loss was significantly ($p < 0.05$) affected by cooking regimes and chilled storage in sous-vide treated mackerel samples. In order to assess the eventual effect of all independent factors influencing cook loss in sous-vide treated mackerel samples after thermal treatment and chilled storage in comparison to initial raw mackerel fillets, the following regression model (Eq. 2) was derived in terms of actual values, on the basis of experimental design ($p < 0.05$):

$$\Delta CL = 24.29 - 0.56 \cdot t + 4.11 \cdot d \qquad (R^2 = 0.517) \qquad (2)$$

where ΔCL is relative decrease in cook loss (%); t is sous-vide cooking temperature (°C); d is duration of chilled storage (days).

From the derived model, it is clearly seen that increased temperature of sous-vide cooking (t) leads to an increase in cook loss in mackerel samples, thus negatively influencing the product quality.

However, duration of chilled storage has a greater positive contribution on the retention of water in the fish. It decreases cook loss in mackerel samples, probably due to structural changes occurring in the fish muscle and connective tissue after cooking (Ofstad et al., 1993), involving reabsorption of water released by unfolded myofibrillar proteins and its distribution between the intra- and extra-cellular spaces (Belitz et al., 2009). Therefore, according to the derived model (Eq. 2), loss of moisture in sous-vide cooked mackerel fillets resulted from thermal denaturation and shrinkage of muscle proteins, which partially reabsorbed the released water during chilled storage.

Amount of water- and salt-soluble proteins

According to Table 2, total amount of extracted proteins was generally reduced for sous-vide cooked mackerel during chilled storage. The decrease in water-soluble (sarcoplasmic) and salt-soluble (myofibrillar) proteins indicated that they were heat denatured. The denaturation increased with increased duration of heat treatment and increasing temperature. From Table 2, it can be noticed that duration of sous-vide cooking has the main negative effect on solubility of sarcoplasmic and myofibrillar proteins in mackerel fillets. However, taking into account that the myofibrillar network is mainly responsible for retaining water in the fish flesh (Hultmann and Rustad, 2002), we assume that the reduction of solubility of salt-soluble proteins contributed to re-absorption of free water by swollen and solubilized myofibrillar proteins forming a gel after cooking. Therefore, cook loss was reduced due to structural changes in salt-soluble proteins: fibril swelling, gelling and re-absorption of fluid by denatured proteins (Cropotova et al., 2018; Schnepf, 1989). This assumption was confirmed by a positive significant correlation (R=0.403) between cook loss and amount of salt-soluble proteins in sous-vide cooked mackerel samples during chilled storage (Fig. 1A). Thus, sous-vide cooking significantly reduced the solubility of myofibrillar

297 proteins in mackerel fillets compared to water-soluble ones, thus increasing cook loss due to
298 detrimental changes in the myofibrillar network of the fish muscle.
299
300 **Figure 1**
301
302 ***Texture***
303 The sous-vide cooked mackerel samples displayed significantly lower ($p < 0.05$) values of
304 breaking strength on the 3rd and 7th day of chilled storage, compared to initial raw mackerel fillets.
305 At the same time, the fillets' firmness increased in proportion with increasing heating temperature,
306 and the maximum breaking strength was recorded for mackerel samples subjected to sous-vide
307 cooking at 90°C for 10 min (Table 2). This phenomenon may be explained by the heat-induced
308 toughening of the fish muscle, after denaturation of myofibrillar and sarcoplasmic proteins (Kong,
309 Oliveira, Tang, Rasco, & Crapo, 2008). Thus, solubilization of connective tissue in the temperature
310 range of 50-70°C leads to the flesh tenderization, while denaturation of myofibrillar proteins
311 occurring at higher temperatures, causes flesh toughening (Baldwin, 2012). This explanation helps
312 to interpret the difference in texture parameters between fish samples heated up to 60°C, 75°C and
313 90°C, since more proteins become denatured as the cooking temperature rises, thus leading to an
314 increase in cook loss and muscle toughening. In addition, more intensive water losses from the
315 muscle tissue upon severe heating (90°C) also contributed to mackerel flesh toughening.
316 Moreover, the texture modifications of sous-vide mackerel may also be associated with a thermal
317 transition of the fish muscle from a viscoelastic to an elastic state (Baldwin, 2012). Thus, mackerel
318 flesh increases in toughness up to a temperature of 80°C due to the viscous flow in the fluid-filled
319 channels between the muscle fibers and fiber bundles. Sous-vide heating up to 65-75°C increases

320 the fish tenderness because sarcoplasmic proteins aggregate forming a gel, whereas heat treatment
321 at higher temperatures (over 80°C) makes the fish flesh tougher due to an increase in the elastic
322 modulus (Baldwin, 2012).
323 The present study also revealed direct correlations between salt-soluble proteins and breaking
324 strength (R=0.541) in mackerel fillets (Fig. 1B), thus confirming the fact that heat denaturation of
325 myofibrillar proteins and their further degradation during chilled storage increase cook loss and
326 lead to flesh tenderization.
327
328 ***Lipid oxidation products***
329 One of the main causes for quality deterioration of fish products is lipid peroxidation (Frankel,
330 2005). In the present study, the PV and CD were used for determination of primary lipid oxidation
331 products during chilled storage of sous-vide mackerel samples. The initial PV and CD of raw
332 mackerel fillets of 3.15 ± 0.06 meq O_2/kg oil and 0.351 ± 0.03 U.A. respectively, displayed a
333 significant increase after sous-vide cooking and chilled storage. As shown in Figure 2 A-B, both
334 PV and CD gradually increased during the whole period of chilled storage for all sous-vide
335 mackerel fillets (from 5.73 to 24.20 meqO_2/kg oil for PV and from 0.715 to 0.958 U.A. for CD).
336 The PV values are considered to be relatively high, because in most of the cases they exceed the
337 acceptable level of 10 meqO_2/kg oil (European Pharmacopoeia, 2008), while denoting the
338 progressive oxidation in the product. The PV at day 1 for mackerel samples cooked at 60°C and
339 75°C for 10 and 15 min was lower compared to the PV of mackerel samples at day 1 submitted
340 to higher temperatures and for longer times (Fig. 2B), showing a gradual increase in primary
341 products of lipid oxidation with an increase in time and temperature of sous-vide cooking and
342 subsequent chilled storage. This phenomenon shows that the oxidation process in mackerel

samples subjected to moderate heat treatment and short chilled storage was in the propagation stage (Karoui and Hassoun, 2017), and was significantly accelerated with an increase of temperature and duration of thermal treatment. Similarly, the observation that PV increased faster for the most severely heat-treated mackerel samples (at 90°C for 15 and 20 min) during chilled storage implies that exposure of fish samples to higher temperatures and longer times of sous-vide cooking leads to accelerated lipid oxidation.

Figure 2

TBARs is a common measure of secondary oxidation products, which contributes to rancid flavor and off-odors in foods (Bensid et al., 2014; Connell, 1990; Igene et al., 1985). In our study, the initial TBARs value of raw mackerel fillets was 0.61 ± 0.22 mmol MDA/kg. However, it progressively increased during the chilled storage (Fig. 2C), reaching a maximum level of 5.23 mmol MDA/kg for mackerel samples cooked at a temperature of 90°C for 20 minutes and subjected to 7-day chilled storage. The highest oxidation levels is observed for mackerel samples subjected to longer times of sous-vide cooking (15 and 20 min) (Fig. 2C). Both the increase in sous-vide cooking temperature and time, as well as duration of chilled storage, significantly ($P < 0.05$) increased TBARS content in analyzed mackerel samples.

Formation of Schiff Bases (SB) resulting from a crosslinking reaction between aldehyde moiety from protein carbonyls and alkaline amino acids of proteins (Estévez, 2011), is also an important indicator of lipid oxidation rate. It is essential to avoid or reduce the formation of SB in sous-vide products, because these compounds involve progressive cross-linking, leading to protein denaturation, polymerization, during storage and impaired functionality, including loss of water-

366 holding capacity (Estévez, 2011). The initial fluorescence corresponding to amount of SB in raw
367 mackerel fillets was 45.6 ± 2.5 U.F. However, it increased significantly ($P < 0.05$) after sous-vide
368 cooking and chilled storage of the fish, reaching a maximum level of 902.9 ± 8.9 U.A. for mackerel
369 samples subjected to heat treatment at 90°C for 20 min followed by 7-day chilled storage.
370 According to Figure 2D, the formation of SB in sous-vide cooked mackerel was significantly
371 affected by both prolonged heating (15 and 20 min) and chilled storage (7 days). These data are in
372 agreement with Traore et al. (2012), who noticed a strong augmentation in SB fluorescence of pig
373 meat after increasing the time of cooking. The increase in SB fluorescence of sous-vide cooked
374 mackerel samples may be associated with impaired functionality of myofibrillar proteins (Estévez,
375 2011), reduced significantly during prolonged heat treatment and during chilled storage.
376 However, in order to estimate the influence of all independent factors on formation of primary and
377 secondary products of lipid oxidation (PV, CD, TBARS and SB) in sous-vide cooked mackerel
378 samples subjected to different time-temperature treatment regimes and subsequent chilled storage,
379 the following statistically significant ($p < 0.05$) regression models in terms of actual values (Eq.
380 3-6) were derived:
381
382 - for primary products of lipid oxidation:
383
384 $$\Delta PV = -491.66 + 6.18 \cdot t + 18.23 \cdot \tau + 26.44 \cdot d \qquad (R^2 = 0.939) \qquad (3)$$
385 and
386 $$\Delta CD = 31.877 + 0.609 \cdot t + 3.009 \cdot \tau + 3.639 \cdot d \qquad (R^2 = 0.717) \qquad (4)$$
387 - for secondary products of lipid oxidation:
388

$$\Delta TBARS = -936.31 + 8.67 \cdot t + 34.41 \cdot \tau + 38.75 \cdot d \qquad (R^2 = 0.858) \qquad (5)$$

and

$$\Delta SB = 49.56 + 0.26 \cdot t + 3.92 \cdot d \qquad (R^2 = 0.938) \qquad (6)$$

where ΔPV, ΔCD, $\Delta TBARS$ and ΔSB display the relative increase in PV-value, absorbance at 233 nm associated with accumulation of conjugated dienes, TBARS and SB content in sous-vide cooked mackerel compared to initial raw mackerel samples, respectively; t is sous-vide cooking temperature (°C); τ is duration of sous-vide cooking (min); d is duration of chilled storage (days). From the regression equations displayed above (Eq. 3-6) it can be seen that time and temperature of sous-vide cooking along with duration of chilled storage positively influence the accumulation of primary and secondary products of lipid oxidation in analyzed mackerel samples. However, according to the values of the regression coefficients of all independent variables from Eq. 3-6, the main factors affecting oxidation stability of the product are duration of sous-vide cooking and of chilled storage. The regression coefficients of duration of sous-vide cooking and of chilled storage are in average 3-6 times higher than for temperature of sous-vide cooking when it comes to formation of lipid peroxides, conjugated dienes and TBARS. Moreover, the strongest effect on generation of primary and secondary products of lipid oxidation in analyzed fish samples seem to be duration of chilled storage. Also, this parameter mostly influences the accumulation of Schiff bases in the product. Nevertheless, according to Eq. 6 the formation of Schiff bases as secondary oxidation products formed by the interaction between lipid-derived carbonyls with alkaline amino groups of proteins was promoted by both sous-vide cooking temperature and chilled storage time, as reported by Utrera et al. (2012).

412 ***Color parameters***

413 Both lightness (L*) and yellowness (b*) values displayed a significant ($P < 0.05$) increase
414 throughout storage (Figure 3A-C) in comparison to the color parameters of the raw mackerel fillets
415 (59.18 ± 3.89 and 8.48 ± 1.14). These results are in agreement with previous study of Karoui and
416 Hassoun (2017). Due to a high variation of a*-values for sous-vide mackerel fillets among
417 sampling days (Fig. 3B), it was impossible to conclude on the tendency. This was in a good
418 agreement with the results obtained by other researchers (Karoui and Hassoun, 2017). Thus, for
419 mackerel samples subjected to heat treatment at 60°C, redness values (a*) decreased throughout
420 the whole chilled storage time, while for mackerel samples exposed to severe heat treatment (90°C)
421 the tendency was opposite: a*-values gradually increased over the chilled storage. Surprisingly,
422 mackerel samples cooked at 75°C displayed a mixed tendency for the flesh redness. Thus, a*-
423 values increased for 15-minute heat treatment and decreased for 20-minute heating in comparison
424 with 10-minute cooking.

425

426 **Figure 3**

427

428 To estimate possible effects from independent factors on lightness and yellowness of sous-vide
429 cooked mackerel samples after thermal treatment and chilled storage (in comparison to initial raw
430 mackerel fillets), the following regression models (Eq. 7 and 8) were derived in terms of actual
431 values, on the basis of experimental design:

432

433 $$\Delta L^* = -4.39 + 0.37 \cdot t + 0.20 \cdot \tau + 0.70 \cdot d \qquad (R^2 = 0.939) \qquad (7)$$

434 and

435 $$\Delta b^* = 26.86 + 0.92 \cdot \tau + 2.09 \cdot d \qquad (R^2 = 0.503) \qquad (8)$$

436

437 where ΔL^* and Δb^* display the relative increase in lightness and yellowness, respectively; t is sous-
438 vide cooking temperature (°C); τ is duration of sous-vide cooking (min); d is duration of chilled
439 storage (days).

440

441 According to the values of the regression coefficients, the main effect from all sous-vide regime
442 factors on lightness and yellowness of studied mackerel samples is attributed to duration of chilled
443 storage. Thus, these instrumental color parameters were significantly affected by chilled storage
444 time followed by sous-vide cooking of mackerel fillets (p=0.0000 for L*-value and P=0.0006 for
445 b*-value).
446 For L*-value, temperature of sous-vide cooking also had a significant effect (P=0.0012), by
447 increasing the fish lightness upon thermal treatment. A possible explanation for higher lightness
448 of mackerel flesh with increasing sous-vide cooking temperature could be higher denaturation and
449 aggregation of sarcoplasmic and myofibrillar proteins increasing light scattering (Christensen et
450 al., 2011). Probably, heating temperature and further chilled storage increased L*-value of
451 mackerel fillets due to the degradation of some thermo-labile protein compounds, as well as loss
452 of moisture which then affected the light refraction of the muscle (Oz and Seyyar, 2016; Nguyen
453 et al., 2013). There was a significant increase of b*-values as a consequence of both duration of
454 sous-vide cooking and storage time (P=0.0046 and P=0.0006, respectively). A similar trend has
455 also been discovered by Karoui and Hassoun, (2017) and Nguyen et al., (2013). The increase in
456 yellowness of sous-vide cooked mackerel throughout the storage time is most likely due to
457 accumulation of yellowish-colored compounds generated by decomposition of primary and

458 secondary products of lipid oxidation (Nguyen et al., 2013), giving significant ($p<0.05$) correlation
459 with R-values of 0.691, 0.844, and 0.705 with PV-value, conjugated dienes' absorbance and
460 TBARS, respectively (Fig. 4, A-C).
461
462 **4. Conclusion**
463 The present study demonstrated how different sous-vide regimes and chilled storage periods affect
464 chemical and physicochemical properties of Atlantic mackerel. Multiple regression models
465 derived in the study showed that duration of chilled storage exhibits the highest significant effect
466 ($p<0.001$) on changes in physicochemical parameters of sous-vide cooked mackerel, contributing
467 significantly to the formation of primary and secondary products of lipid oxidation and subsequent
468 increase in lightness and yellowness of the fish flesh. Therefore, the addition of some antioxidants
469 to control the oxidation development should be evaluated. It was also shown that chilled storage
470 time significantly affects the integrity of myofibrillar network responsible for retaining water in
471 the fish flesh, thus leading to an increase in cook loss in sous-vide heated mackerel samples during
472 chilled storage. Heating time and temperature were the parameters with the lowest contribution to
473 lipid oxidation and color modification, as well as structural and textural changes in the muscle of
474 sous-vide mackerel fillets during chilled storage. Also, values of PV, CD and TBARS were
475 positively correlated with b*-values of the fish flesh ($R = 0.691$, $R = 0.844$ and $R = 0.705$,
476 respectively), which suggests that experimental yellowish-colored mackerel samples were more
477 likely to have a higher lipid peroxidation level in comparison with raw mackerel.
478 Therefore, it was concluded that prolonged chilled storage of sous-vide cooked mackerel
479 negatively influences its physicochemical parameters, and thus in further studies, the use of
480 antioxidants should be evaluated to ensure the highest quality of the end product.

5. Acknowledgments

The study displayed in the paper is based upon work supported by the JPI project ProHealth "Innovative processing to preserve positive health effects in pelagic fish products", RCN 259582/E50.

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Tables

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

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