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Influence of high-pressure processing on quality attributes of haddock and mackerel minces during frozen storage, and fishcakes prepared thereof

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ABSTRACT

The study focused on assessing quality parameters of haddock and mackerel minces subjected to a high-pressure treatment (HP) at 200 and 300 MPa and frozen storage at −40 °C. Dry matter, water-holding capacity, protein solubility and oxidation, lipid oxidation, microbiological parameters, low molecular weight metabolites (LMW) and color parameters, were analyzed. The texture of fishcakes prepared on the basis of these fish minces was also studied, showing a decrease in firmness along with an increase in pressure. A marked inhibition of microbial growth was observed in fish minces when increasing the pressure level of HP-treatment. However, no significant effect ($p < 0.05$) on the content of primary and secondary lipid oxidation products was observed between untreated and 300 MPa-pressurized fish samples. The results suggested that HP-treatment could be successfully applied to both lean and fatty fish samples for reduction of microbial growth with minor changes in product quality.

Industrial relevance.

The application of high pressure (HP) treatment of 200 and 300 MPa could be successfully applied to both lean and fatty fish species before freezing for reduction of microbial growth. The degree of lipid oxidation is decreasing with an increase in pressure as a result of inactivation of prooxidative endogenous enzymes. Fish minces become slightly lighter and softer after HP-treatment conducted at 200 MPa due to denaturation of proteins, thus enhancing sensory properties of fishcakes prepared thereof.

1. Introduction

Consumer demand for fish and fish products has gradually increased over the last decades due to their high nutritive value and essential nutrients (Aubourg, 2018). Fish is a highly valuable source of bioactive compounds such as essential long-chain omega-3 fatty acids – docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), fat-soluble vitamins (E and D) and easily digestible proteins (Aubourg, 2018; Weichselbaum, Coe, Buttriss, & Stanner, 2013). These compounds are essential in the human diet and has shown a positive role in preventing a wide range of diseases (Aubourg, 2018). However, fish is highly perishable, demanding fast and efficient processing and storage operations after slaughter. Different factors are responsible for the quality loss of fish products, the major being microbial development, activity of endogenous enzymes, lipid and protein oxidation (Cropotova, Mozuraityte, Standal, & Rustad, 2019; Dalgaard, Madsen, Samieian, & Emborg, 2006; Hultmann & Rustad, 2004; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2005).

Freezing followed by frozen storage has been one of the best methods to ensure safety and retain quality attributes and nutritional value of fish products exposed to prolonged storage (Cropotova et al., 2019; Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013). However, the quality of frozen-stored fish is limited by freeze denaturation of fish muscle proteins and oxidation of lipids (Cropotova et al., 2019; Standal et al., 2018). This may lead to changes in functional properties (texture, water-holding capacity, etc.), reducing the value of raw material for further processing into mince used in production of prepared fish products such as fish balls, fishcakes, etc. Highly unsaturated fatty acids along with a large content of pro-oxidants can induce enzymatic and non-enzymatic rancidity thus impairing fish product quality after freezing and frozen storage (Suh, Kim, Shin, & Ko, 2017; Vázquez et al., 2013). Deterioration in flavor of fish products, including rancidity and

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off-flavor development may be due to the formation of low-molecular weight metabolites from lipid oxidation products during frozen storage (Secci & Parisi, 2016; Standal et al., 2018).

As a result of the continuously growing consumer demand for fish products that are safe and have superior sensory and nutritional characteristics, a number of advanced technologies such as high-pressure processing (HPP), pulsed electric field, oscillating magnetic fields (ohmic heating, dielectric heating, microwaves), ultraviolet radiation and plasma treatment have been developed (Aubourg, 2018). High-pressure processing is becoming increasingly popular among these non-thermal food processing technologies in regard to preservation of sensory and nutritional properties of fish through inactivation of endogenous enzymes and microbial populations (Ashie & Simpson, 1996; Aubourg, 2018; Chéret, Aránzazu, Delbarre-Ladrat, de Lambarrie, & Verrez-Bagnis, 2006; Heinz & Knorr, 2002; Ramírez-Suárez & Morrisey, 2006; Smelt, 1998). Besides its high efficiency in prevention of fish spoilage, HPP does not affect vitamins and flavor compounds, allowing preservation of nutritional value and sensory properties of the product (Aubourg, 2018).

High-pressure treatment applied to fresh fish products before chilled storage may significantly improve their quality and safety, leading to a substantial shelf-life extension (Ohshima, Ushio, & Koizumi, 1993; Amanatidou et al., 2000; Christensen, Hovda, & Rode, 2017; Aubourg, 2018). This beneficial effect can be achieved due to inactivation endogenous hydrolytic enzymes, such as lipases, phospholipases, peroxidases and lipoxygenases (Fidalgo, Saraiva, Aubourg, Vásquez, & Torres, 2014; Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2010; Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vázquez, 2014; Vázquez et al., 2013). Thus, HPP can be successfully applied as a pre-treatment before chilling or freezing for extension of the shelf life (Ortea et al., 2010; Torres et al., 2014; Vázquez et al., 2013).

Nevertheless, studies on potential benefits of HP-treatments prior to freezing and subsequent frozen storage of fish products are limited. Moreover, there is little information on potential destructive effects from applying HPP to fish products on their quality changes during frozen storage. According to several studies, High-pressure processing induces cell membrane damage, denatures proteins and causes changes in cell morphology (Arnaud, Lamballerie, & Pottier, 2018; Ohshima et al., 1993; Ramírez-Suárez & Morrisey, 2006). Weak energy bonds of proteins such as hydrogen and hydrophobic bonds can be irreversibly modified, leading to changes in their secondary, tertiary and quaternary structures (Ramírez-Suárez & Morrisey, 2006). A partial unfolding of proteins along with non-covalent (i.e., hydrophobic and hydrogen) interactions during HP-treatment trigger denaturation of protein molecules, thereby affecting water-holding capacity and texture parameters of the end product (Ramírez-Suárez & Morrisey, 2006). However, only one study was found on the influence of high pressure on generation of protein carbonyls as an indicator of protein oxidation in fish products subjected to HP-treatment prior to freezing and subsequent frozen storage (Arnaud et al., 2018).

Among lean and fatty fish species, haddock (*Melanogrammus aeglefinus*) and Atlantic mackerel (*Scomber scombrus*) have received great attention due to their increasing capture production and economic importance (FAO, 2015). Haddock is widely consumed in West-European countries in both fresh and frozen state, while its mince is largely used for different fish preparations (Andreetta-Gorelkina, Greiff, Rustad, & Aursand, 2016). According to European Market Observatory for Fisheries and Aquaculture Products (EUMOFA), Atlantic mackerel ranked among the top small pelagic commodity groups both in volume and value in 2017 in Europe (EUMOFA, 2018), while being an important food source due to high amount of essential nutrients and health benefits (Weichselbaum et al., 2013). Previous studies related to frozen storage of these species accounted for some spoilage pathways and quality loss (Aubourg & Medina, 1999; Standal et al., 2018). Therefore, it is very important to investigate the influence of high-pressure pre-treatment prior to freezing on quality parameters of these fish species in order to improve their quality during frozen storage.

In this study, moderate HP-treatment of 200 and 300 MPa was applied to raw haddock and mackerel minces prior to freezing. The fish minces were subjected to 1-month frozen storage and further used in preparation of fishcakes. This study is a part of preliminary research investigating the influence of HP-treatment on further changes in quality attributes of fish minces during short period of frozen storage. Physicochemical parameters of both the fish minces and fishcakes prepared thereof were determined to assess the efficiency and the quality changes in the products as induced by HP-treatment.

2. Sample preparation

Fillets of Haddock (*Melanogrammus aeglefinus*) and Atlantic mackerel (*Scomber scombrus*) caught in the Irish Sea in spring (March–April) were purchased from a seafood processor in Howth Co. Dublin (Ireland). Prior to mincing, fillets were kept on ice. Haddock and mackerel fillets were minced separately using a mincer (Mainca meat mincer, England) through a mesh plate (2 mm diameter holes). After mincing, portions of 500 g fish mince was vacuum packaged in flexible polyethylene bags (oxygen permeability 25 cm^3/m^2) (Sealed Air Ltd. Cryovac, Ireland). Samples were pressurized in a 200 L capacity high pressure machine (Hiperbaric, Burgos, Spain) in HPP Tolling facility, Dublin. Treatments at 200 and 300 MPa for 5 min were applied. Come up times were approximately 88 s and 129 s for 200 and 300 MPa treatment, respectively. The duration of treatment does not include the come-up time. After treatment, all the samples were stored at −30 °C for 3 weeks followed by shipping to Norwegian University of Science and Technology (NTNU) (Trondheim, Norway) via overnight post in frozen conditions.

2.1. Preparation of fishcakes

Mackerel and haddock minces (185 g; 1:1 *w*/w) were mixed together with 7.5 g of salt for 30 s in a food processor. Potato starch (12.5 g), pepper (0.5 g) were mixed in for 10 s followed by addition of 110 g of full cream milk and mixing for 35 s. From the mass, 60 g fish cakes were formed.

Fishcakes were fried in the rapeseed oil (3 tablespoons) for 2 × 3 min of each side. After frying, they were transferred to the kitchen steam oven to cook until inside temperature of the oven reached 75–80 °C.

2.2. Chemical and physicochemical assays

2.2.1. Dry matter and ash

Dry matter of the fish minces was determined by drying 2 g samples at 105 °C for 24 h to a constant weight, according to the official method (AOAC, 2005). Ash was determined by leaving the sample in the muffle furnace at 550 °C for 5 h after determination of dry matter, as described in AOAC method (2005). Both analyses were run in duplicate and the average was calculated.

2.2.2. Water holding capacity (WHC)

Water holding capacity (WHC) of the fish minces was determined according to the method previously described by Eide, Børresen, and Strøm (1982). This method allows determination of the ability of a fish mince to withhold water during centrifugation (Eide et al., 1982). Briefly, haddock and mackerel minces (~4 g) were centrifuged at 210 × *g* for 5 min. Dry matter content in the fish mince was determined before centrifugation as mentioned above and the water-holding capacity was calculated as the percentage of original water retained in the mince (Standal et al., 2018). The analyses were run in four replicates and the mean value with standard deviation (SD) was calculated.

2.2.3. Protein solubility

Water and salt soluble proteins were extracted from mackerel and haddock minces by a modification of the methods of Anderson and Ravesi (1968) and Licciardello et al. (1982), as previously described by Hultmann and Rustad (2002). The extraction procedure was performed twice on each fish mince.

Protein content in the extracts was determined in duplicate using the method of Bradford (1976), with bovine serum albumin (BSA) as a standard. The analyses were run in triplicates and the mean value ± SD was calculated.

2.2.4. Protein oxidation

Protein carbonyl groups were detected by DNPH-based Enzyme-Linked Immunosorbent Assay (ELISA) performed in a 96-well polystyrene plate as a measure of protein oxidation (Dalle-Donne, Giustarini, Milzani, & Colombo, 2003). This is a rapid and highly sensitive plate-based assay technique developed by Buss, Chan, Sluis, Domigan, and Winterbourn (1997). The method is based on derivatization of carbonyl groups with dinitrophenylhydrazine (DNPH) and probing of protein-bound dinitrophenyl (DNP) with an anti-DNP antibody. The indirect ELISA kit, STA-310 OxiSelectTM, was purchased from Cell Biolabs, Inc. (San Diego, CA, USA). Carbonyl groups were determined in water-soluble (sarcoplasmic) and salt-soluble (myofibrillar) proteins in quadruplicate and the average value with standard deviation were calculated. The results were expressed in nmol carbonyls/mg protein.

2.2.5. Lipid oxidation products

The total amount of lipids was extracted from the mackerel minces by the Bligh & Dyer (B&D) method, which uses a binary mixture of chloroform and methanol diluted with distilled water as an extraction medium (Bligh & Dyer, 1959). The extraction was performed in duplicate. Extracted lipids in chloroform phase and oxidation products in water/methanol phase were stored at −80 prior to analysis of peroxide values and thiobarbituric reactive substances.

Thiobarbituric acid reactive substances (TBARS) in chloroform phase were determined as described by Ke and Wovewoda (1979) using 1.1.3.3-tetraethoxypropane (T 9889) as a standard. The results were expressed as μM of TBARS in 100 g muscle ± standard deviation of at least four parallels.

Determination of TBARS in methanol/water phase was performed as described by Schmedes and Holmer (1989) using 1.1.3.3-tetraethoxypropane (T 9889) as a standard. The results are expressed as mM of TBARS in 100 g muscle ± standard deviation of at least four parallels.

Peroxide value was measured in the total lipids by the use modified Shantana and Decker (1994) method as described by Baron et al. (2013). The 1 mL aliquot of total lipid extract was mixed with 10 mL of chloroform/methanol (7 + 3, v/v), then thiocyanate-solution (50 μL, 30%) was added and the reaction mixture was left to react for 5 min at room temperature. The absorbance was read at 500 nm using spectrophotometer (UV mini 1240, Shimadzu Corp., Japan). Peroxide value was determined using a standard curve with $FeCl_3$ as standard. Analysis were performed in triplicate. The results are expressed meq peroxide / 100 g muscle ± standard deviation.

2.2.6. NMR analysis of low molecular weight (LMW) metabolites in fish minces

Samples of the frozen fish mince ($n = 2$ from each treatment and raw material) were freeze dried and extracted by methanol, chloroform and water as previously described (two-step method by Wu, Southam, Hines, & Viant, 2008). Approximately 50 mg freeze dried samples were used in the extractions. The water/methanol phase was evaporated in a vacuum centrifuge (30 °C, 1 h), freeze dried, and dissolved in 550 μL phosphate buffered saline (PBS, pH 7.4) D_2O with 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS)-d6 at a final concentration of 0.5 mM and transferred to 5 mm tubes. Extracted LWM metabolites for the 16 samples were identified and quantified by the software Chenomx NMR suite 7.0 (Chenomx Inc., Canada).

The K-value was calculated as the molar ratio of Inosine (Ino) and Hypoxhantine (Hx) vs the sum of Inosine mono phosphate (IMP), Inos and Hx, while the Hx value was calculated from the molar ratio of Hx vs the sum of IMP, Inos, and Hx.

2.2.7. Color parameters

Color parameters of haddock and mackerel minces 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 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 (Cropotova et al., 2019). Color parameters were determined on each fish mince sample in three readings and the average was calculated.

2.2.8. Texture parameters of fishcakes

Hardness and cohesiveness of fishcakes prepared from HP-treated and untreated mackerel and haddock minces was measured on a TA.XT2 Texture Analyzer (SMS Stable Micro Systems, Ltd., Surrey, UK) equipped with a 1 kg load cell according to the method described by Hultmann and Rustad (2002). A flat-ended cylinder of 12 mm in diameter was pierced into the inner part of the fishcake (divided into two parts) at a constant speed of 1 mm s^{-1} until it had reached 60% of its height. The holding time between the compressions was 5 s. The samples' temperature was 20 ± 2 °C, and the analyzed surface area was 153.8 ± 2.4 cm^2. The maximum resistance force was recorded in Newton (N) and expressed as the average of 4 determinations per fishcake. Cohesiveness, which represents the force holding the integrity of fishcake structure, while preventing it from gaping, was calculated as the ratio of areas delimited by the curves of the second and the first compression. Exponent software version 6.1.16 of the Stable Micro Systems (SMS Stable Micro Systems, Ltd., Surrey, UK) was used for the data extraction.

2.3. Microbiological analysis

Microbiological analysis of samples was carried out as outlined in Stratakos et al. (2019) with slight modifications. Briefly, samples (10 g) were homogenized in 90 mL of Maximum Recovery Diluent (MRD, Oxoid Ltd., Basingstoke, England) using a Stomacher for 60 s. Decimal dilutions of the homogenate were prepared in MRD. Total viable aerobic bacterial counts were determined by the spread plate method, using plate count agar (Oxoid Ltd., Basingstoke, England). The inoculated plates were incubated at 30 °C for 48 h for total viable mesophilic counts, and at 6.5 °C for 10 days for psychrophilic counts.

2.4. Statistical analysis

Statistical analysis and data processing were conducted using Statgraphics Centurion XVI. Statistical significance of the experimental data was verified by using Student's *t*-test and Analysis of Variance (ANOVA). To establish a relationship between certain parameters, Pearson correlations were calculated. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Dry matter and ash

The physicochemical parameters of the analyzed fish minces are displayed in Table 1. A small but insignificant increase in dry weight of HP-treated haddock and mackerel minces compared to untreated

Table 1
Physicochemical parameters of mackerel and haddock minces subjected to HP-treatment and subsequent frozen storage.

Sample	Dry matter, %	Ash, %	WHC, %	Solubility of sarcoplasmic proteins, %wet weight	Solubility of myofibrillar proteins, %wet weight	Carbonyls in sarcoplasmic proteins nmol/mg protein	Carbonyls in myofibrillar proteins nmol/mg protein	Peroxide value, meq/kg oil	TBARS (M/W) mM/100 g muscle	TBARS(Cl) mM/100 g muscle	L*-value	a*-Value	b*-Value
Haddock mince													
200 MPa treated	18.9 ± 1.2[a*]	1.1 ± 0.0[a]	61.7 ± 1.8[a]	3.4 ± 0.1[a]	1.5 ± 0.1[a]	6.2 ± 0.0[a]	13.7 ± 0.2[a]	n.m.[**]	n.m.	n.m.	58.3 ± 1.3[a]	−1.1 ± 0.3[a]	−1.8 ± 0.4[a]
300 MPa treated	17.5 ± 0.6[b]	1.2 ± 0.2[a]	54.0 ± 2.7[b]	2.4 ± 0.1[b]	0.9 ± 0.1[b]	9.9 ± 0.2[b]	21.6 ± 1.1[b]	n.m.	n.m.	n.m.	68.5 ± 0.6[b]	−1.7 ± 0.6[b]	−1.1 ± 0.7[b]
Control (untreated)	16.9 ± 0.8[b]	1.2 ± 0.0[a]	57.1 ± 6.5[a]	3.5 ± 0.5[a]	1.4 ± 0.0[a]	3.0 ± 0.1[c]	8.1 ± 0.1[c]	n.m.	n.m.	n.m.	56.2 ± 1.0[a]	−1.1 ± 0.2[a]	−2.0 ± 0.3[a]
Mackerel mince													
200 MPa treated	29.9 ± 0.5[a]	1.2 ± 0.1[a]	49.6 ± 4.4[a]	4.0 ± 0.2[a]	1.5 ± 0.0[a]	12.1 ± 0.1[a]	19.4 ± 0.3[a]	12.7 ± 0.9[a]	8.1 ± 0.1[a]	1.6 ± 0.2[a]	53.0 ± 2.9[a]	5.3 ± 0.8[a]	9.3 ± 0.9[a]
300 MPa treated	31.6 ± 0.2[a]	1.2 ± 0.0[a]	53.6 ± 6.6[b]	3.3 ± 0.1[b]	0.8 ± 0.1[b]	23.5 ± 0.4[b]	25.6 ± 0.0[b]	5.6 ± 0.4[b]	4.1 ± 0.3[b]	0.5 ± 0.1[b]	60.9 ± 1.5[b]	3.5 ± 0.9[b]	16.3 ± 1.7[b]
Control (untreated)	29.3 ± 1.0[a]	1.1 ± 0.1[a]	66.0 ± 2.2[c]	5.4 ± 0.1[c]	1.9 ± 0.0[a]	5.3 ± 0.1[c]	12.8 ± 0.3[c]	7.4 ± 1.0[b]	5.4 ± 0.4[b]	1.0 ± 0.1[a]	49.2 ± 3.9[a]	3.9 ± 0.7[b]	8.0 ± 2.2[a]

[*] Values followed by the same letter in the same column are not significantly different at $p < 0.05$.
[**] Not meaningful.

(control) samples after 1-month frozen storage was observed (Table 1). This phenomenon could be due to the rupture of the fish muscle cells during high-pressure treatment at 200 and 300 MPa, leading to higher water release before freezing (Aubourg, 2018). Thus, compression expelled some of free fluid from the muscle during HP-treatment, resulting in increased dry matter. No significant variation between ash results for pressurized and untreated (control) fish samples was found.

3.2. Water holding capacity

High pressure significantly reduced ($p < 0.05$) water-holding capacity of mackerel minces compared to minced haddock (Table 1). According to Table 1, water-holding capacity (WHC) of the haddock mince subjected to pressure processing 300 MPa decreased only by 5.5%, whereas for the mackerel mince this decrease constituted 18.8%. The significant decrease in WHC of HP-treated fish minces may be explained by a pressure-induced denaturation of structural proteins like myosin and actin, impairing their ability to hold water (Christensen et al., 2017). These findings are in agreement with data obtained by Torres et al. (2014) and Christensen et al. (2017) studying the influence of high-pressure processing and subsequent storage on the water-holding capacity of mackerel, cod and salmon.

3.3. Protein solubility

The solubility of sarcoplasmic proteins in the haddock mince dropped by 0.4% and 42.0% after pressure treatment at 200 and 300 MPa in relation to control samples, respectively (Table 1). For the mackerel mince, the decrease was 34.1% and 63.1% for 200 and 300 MPa, correspondingly. The solubility of myofibrillar proteins also decreased in both the haddock and mackerel minces compared to untreated (control) samples. However, among the pressure-treated samples, haddock mince pressurized at 300 MPa showed a greater loss in protein solubility, than those treated at 200 MPa. Thus, solubility of myofibrillar proteins in the haddock mince was reduced by 52.1% from the initial value after pressure treatment at 300 MPa. These data suggest that pressurization of haddock and mackerel minces at 200–300 MPa for 5 min affects the functionality of their sarcoplasmic and myofibrillar proteins, leading to a decreased solubility due to formation of insoluble aggregates (Aubourg, 2018; Ramírez-Suárez & Morrisey, 2006). The possible explanation of the high pressure-induced molecular changes consists of two parts: 1) rupture of the weak non-covalent bonds leading to protein denaturation and 2) protein aggregation as a result of formation of new intra- and/or intermolecular noncovalent bonds (Aubourg, 2018). Thus, high pressure may affect both the protein conformation and integrity of hydrogen bonds, as well as hydrophobic molecular interactions, leading to protein denaturation, gelation or aggregation (Aubourg, 2018; Ramírez-Suárez & Morrisey, 2006).

3.4. Protein oxidation

According to data displayed in Table 1, there was a significant ($p < 0.05$) increase in carbonyls in both the sarcoplasmic and myofibrillar proteins of the pressurized fish minces in comparison with untreated minces after 1-month of frozen storage. After 1-month of frozen storage, there was a significant ($p < 0.05$) increase in carbonyls in both the sarcoplasmic and myofibrillar proteins of the pressurized fish minces compared to untreated minces (Table 1). The negative effect of high pressure on protein oxidation in the fish minces during frozen storage can be explained by a greater membrane damage compared to control samples (Arnaud et al., 2018). Arnaud et al. (2018) found that high-pressure treatment favors the rupture of the cell membranes of adipocytes, thereby liberating lipid oxidation products that further come in contact with proteins leading to protein oxidation. At the same time, HP-treated mackerel samples had significantly ($p < 0.05$) higher

carbonyl content in both the sarcoplasmic and myofibrillar proteins than HP-treated haddock samples after frozen storage. The protein oxidation could be promoted in the mackerel mince by a release of free iron during the HP-treatment. This is supported by earlier investigations of Tokur and Korkmaz (2007). Myoglobin is the predominant pigment in the dark muscle of Atlantic mackerel, and its degradation under high pressure may result in increased protein carbonylation due to released free iron (Tokur & Korkmaz, 2007). The higher sensitivity of mackerel to HP-induced protein oxidation compared to haddock could be also explained by the higher lipid content, which is in agreement with a previous comparative study of Arnaud et al. (2018) performed on pressurized cod and salmon subjected to frozen storage. Thus, the detrimental effect of HP-treatment on protein oxidation in the analyzed haddock and mackerel minces is probably the result of both the muscle cell damage promoting the release of free iron and lipid oxidation products emerged from the rupture of adipocytes (Arnaud et al., 2018; Ramírez-Suárez & Morrisey, 2006).

3.5. Lipid oxidation

Lipid oxidation was measured in HPP treated and non-treated mackerel minces as the raw material contained high amount of lipids (12.2–13.4% of wet weight) and therefore lipid oxidation is an important quality parameter.

Determination of PV is used as a quality parameter of oils and lipid-containing food products, despite that it is not directly related to the actual sensory quality of a product (Pegg, 2001). For some products like marine oils 10 meq/kg is the limit for an acceptable peroxide value (Pegg, 2001). Peroxide value measured in the mackerel minces varied between the samples (Table 1) with the highest amount obtained in 200 MPa treated samples. The measured thiobarbituric acid reactive substances (TBARS) values were also highest for 200 MPa treated samples. However, all the TBARS values measured in the chloroform phase were higher than the values obtained in our previous study by Standal et al. (2018). This indicates that mackerel minces were slightly oxidized. Lipid oxidation is a complex process involving the formation and breakdown of lipid oxidation compounds at the same time. Two opposite mechanisms could be considered to explain lipid changes observed: *prooxidative* - lipid oxidation increase by high pressure processing as a result of pressure-denaturing of iron containing protein increasing free metal iron and *antioxidative* high pressure processing inactivates the prooxidative endogenous enzymes (Pazos et al., 2015). Therefore, the lowest lipid oxidation obtained by 300 MPa could be explained by inactivation of prooxidative enzymes, while the highest amount of oxidation product at 200 MPa was the result of pressure-denaturation of iron containing protein increasing free metal iron and inactivated prooxidative endogenous enzymes that increased the oxidation (Tanaka et al., 1991).

3.6. NMR analysis of low molecular weight (LMW) metabolites

The profile of low molecular weight metabolites is relevant both for food quality and safety (Standal et al., 2018). By using NMR-based metabolomics – the effect of HPP processing on changes in compounds relevant for the quality of the minces (and measures on changes in enzymatic activities) were monitored. Haddock and mackerel have quite different composition of LMW metabolites – but the changes in e.g. adenosine triphosphate (ATP) related metabolites (due to endogenous enzymes), and formation of TMA from TMAO and biogenic amines (bacterial activity) and other enzymatic activities (e.g. formation of dimethylamine during frozen storage) can be monitored.

Adenosine triphosphate predominates in muscle of live animals under normal conditions, but after death a series of enzymatic reactions leads to decomposition of ATP to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate, inosine and hypoxanthine. ATP, ADP and AMP may be observed in fish muscle directly after killing the fish, by careful sample preparation and extraction (Standal, Gribbestad, Bathen, Aursand, & Martinez, 2007), while fresh fish for consumption usually shows IMP as the main ATP related compound. Inosine monophosphate contributes to the desirable taste of fresh fish, while Hx contributes to the bitter off-flavor of spoiled fish.

The degree of freshness of fish is often expressed as the K-value, defined as the ratio of the sum of Ino and Hx to the sum of all ATP related catabolites (Saito, Arai, & Matsuyoshu, 1959). The acceptable K-values differ among fish species, but a general limit for consumption of salmon and mackerel is approximately 80% (Erikson, Beyer, & Sigholt, 1997; Standal et al., 2018). Previous results on Atlantic mackerel have shown that the nucleotides are well preserved during frozen storage at −27 °C (Standal et al., 2018), so the 1 month frozen storage at −40 °C should not lead to changes in such metabolites. However, changes during freezing and thawing are expected, and the HPP treatment may impair endogenous enzyme activities (Fidalgo et al., 2014). The haddock mince control sample had a K-value close to 90% (Fig. 2A). The sample preparation/homogenization/freezing/thawing step may also have contributed to the relatively high K-value for these samples. The results show that the HPP treated haddock mince with 200 and 300 MPa have similar K values (60%), but lower K-values than the haddock control samples. A possible explanation is that the HPP treatment inactivates/reduces enzymatic activity involved in the degradation of IMP to Ino and Hx during the freezing/thawing step. Previous studies have reported that the effect of HPP on enzymatic activities (e.g. involved in nucleotide breakdown) varies among samples and pressure levels (Fidalgo et al., 2014; Ortea et al., 2010).

Another relevant freshness measure is the Hx value (the ratio of Hx to the sum of Hx), Ino and IMP (if any), and this has been suggested as a better measure on freshness of salmon rest raw materials due to the very fast degradation of nucleotides (IMP) in such tissues (Shumilina et al., 2016). The changes in Hx-values are analogous to the changes in the K-value for the haddock samples (Fig. 2A). The HPP treated samples have lower Hx-value than the control samples. This implies that the HPP treatment reduced the enzymatic degradation of Ino to Hx in the haddock minces.

For the mackerel mince samples, no IMP could be detected, i.e. all IMP had been degraded to Ino or Hx – resulting in a K-value of 100% (Fig. 2B). The Hx values of mackerel shows a different trend compared to the haddock samples (Fig. 2B). The Hx values are higher in the 200 MPa treated samples (24 ± 0.7%) compared to the control samples (17 ± 2.9%) and 300 MPa treated samples (16 ± 1.1%). A possible explanation of this observation is that the 200 MPa treated mince has higher enzymatic activity due to release of enzymes from the lysosomes as discussed in previous studies on acid phosphatase activities in HPP treated mackerel (Fidalgo et al., 2014).

The content of Trimethylamine (formed from TMAO by bacteria) and dimethylamine were not evaluated in this study since the sample preparation (i.e. evaporation of methanol from the water-methanol phase) showed that also such volatiles compounds are removed to different extents.

3.7. Color parameters

The color parameters for haddock and mackerel minces are shown in Table 1. The huge differences in redness (b*-value) between haddock and mackerel minces can be explained by the amount of myoglobin in their flesh (Tokur & Korkmaz, 2007). Myoglobin is the predominant pigment in dark muscle of Atlantic mackerel, which is found in very small amounts in haddock muscle. Color changes in haddock and mackerel minces subjected to different HP-treatments may mainly be explained by protein and lipid oxidation reactions (Arnaud et al., 2018). The b*-values increased towards the yellow for all HP-treated haddock and mackerel minces compared to control samples, which is in good agreement with similar study of Torres et al. (2014). These

Table 2
Texture parameters of fish cakes prepared from mackerel and haddock minces after HP-treatment and frozen storage.

Fishcake samples from haddock and mackerel mince (1:1, *w*/w)	Hardness, N	Cohesiveness
200 MPa treated	6.9 ± 0.5[a]	0.4 ± 0.0[a]
300 MPa treated	2.8 ± 0.4[b]	0.2 ± 0.1[b]
Control (untreated)	7.5 ± 1.0[a]	0.4 ± 0.0[a]

Values followed by the same letter in the same column are not significantly different at $p < 0.05$.

changes were more pronounced with increasing pressure. The gradual increase in yellow pigmentation for all fish minces with increased pressure could probably be due to accumulation of secondary lipid oxidation products, including the ones formed from the interaction between aldehyde groups generated during oxidation of lipids with free amino groups of phospholipids and proteins (Thanonkaew, Benjakul, Visessanguan, & Decker, 2006; Tokur & Korkmaz, 2007). This hypothesis is supported by a significant correlation between b*-value and amount of protein carbonyls formed in both the mackerel ($r = 0.975$, $p < 0.05$) and haddock mince ($r = 0.962$, $p < 0.05$).

The L*-value of both the HP-treated haddock and mackerel samples increased with increasing hydrostatic pressure compared to control samples. These results are in full agreement with results from other studies (Torres et al., 2014), while suggesting that the color of the fish mince becomes lighter with increasing hydrostatic pressure due to conformational changes in proteins (Arnaud et al., 2018).

3.8. Texture parameters of fishcakes

Fishcakes prepared from HP-treated haddock and mackerel minces had lower hardness and cohesiveness compared to fishcakes prepared from untreated fish minces (Table 2). The reduced hardness and cohesiveness of the fishcakes may be explained by a pressure-induced degradation of structural proteins such as myosin and actin, which could partially be assisted by proteolytic enzymes liberated from HP-damaged cells (Christensen et al., 2017). It was previously shown that moderate HP-treatment (below 400 MPa) may activate some proteolytic enzymes such as cathepsins B and D in fish tissue, thereby promoting protein degradation (Chéret et al., 2006; Teixeira et al., 2014). Thus, we suggest that tenderization of the fish tissue initiated during HP-pressure treatment is due to protein degradation assisted both by high pressure and activity of proteolytic enzymes (Chéret et al., 2006; Christensen et al., 2017). Therefore, fishcakes prepared from pressurized fish minces were characterized by the lower hardness values. Our hypothesis is that high pressure initiated, while subsequent freezing and cooking further promoted, rupturing of myofibrillar proteins, followed by gelation and solubilization of collagen (Chouhan, Kaur, & Rao, 2015; Cropotova, Mozuraityte, Standal, & Rustad, 2018).

3.9. Microbiological parameters

Total aerobic mesophilic and total aerobic psychrophilic bacteria counts are shown in Fig. 1. Initial mesophilic counts in non-treated mackerel and haddock mince were 5.77 log CFU/g and 5.16 log CFU/g respectively. High pressure treatment significantly reduced the mesophilic and psychrophilic counts. In case of mackerel mince pressurized at 300 MPa for 5 min, a reduction of 1.69 log CFU/g and 2.23 log CFU/g were observed in mesophilic and psychrophilic bacteria counts respectively. More than 1 log CFU/g reduction was also obtained in pressurized haddock mince samples.

Similar reductions in microbial load on fish samples has been observed in a range of fish samples including sardines (Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007). Treatment conditions and microbial reductions observed in this study corroborates with the previous studies. For example, Erkan et al. (2011) investigated cold smoked salmon in a pressure range of 220 to 330 MPa for 5 and 10 min and observed significant reductions both in total plate and viable count. Similar reductions were also observed by Karim et al. (2011) in the case of herring and haddock treated at 200 MPa for 3 min. The microbial inactivation due to pressure is mainly associated with the denaturation of proteins, alterations of cell membrane and cell morphology leading to membrane permeability and leakage of cellular content (Zhao, de Alba, Sun, & Tiwari, 2018).

4. Conclusion

Different high-pressure treatments (200 and 300 MPa for 5 min) were tested to identify how the processing conditions may affect the quality of haddock and mackerel minces subjected to 1-month frozen storage. HP-treatment significantly ($p < 0.05$) affected protein solubility and promoted oxidation in both sarcoplasmic and myofibrillar proteins of pressurized haddock and mackerel minces compared to untreated samples. The effect of HPP on the degradation of IMP to hypoxanthine and inosine varied among species and pressure levels. For haddock both K-value and Hx-value was lower in the HPP treated minces, while for mackerel an increase in Hx level was observed for the

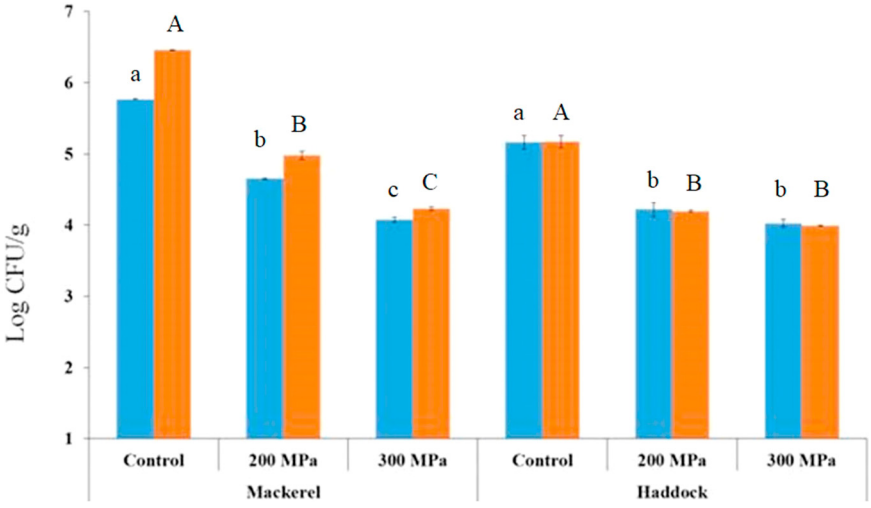

Fig. 1. Microbiological parameters of mackerel and haddock minces: blue color represents mesophilic and orange psycrophilic bacteria. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

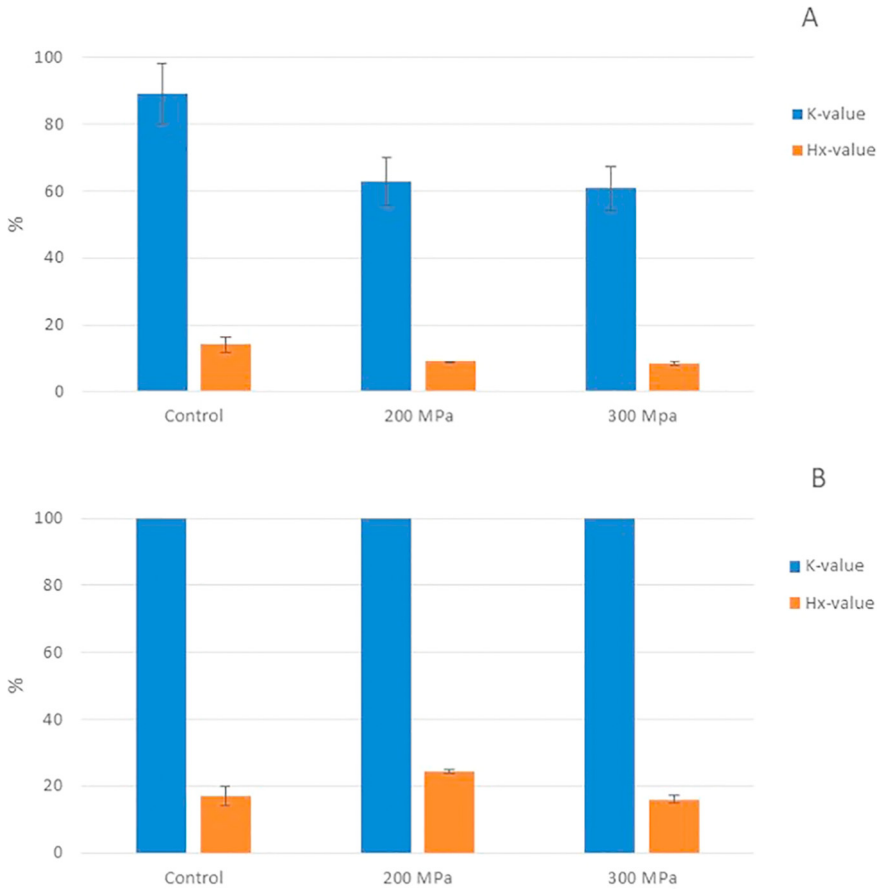

Fig. 2. K-value (ratio of IMP and Hx to the sum of all ATP related catabolites) and Hx-value (ratio of Hx to the sum of Hx, Ino and IMP) for haddock (A) and mackerel (B) minces submitted to different high pressure treatments (200 and 300 MPa for 5 min).

200 MPa treated fish mince compared to the control and 300 MPa treated fish. The breakdown of fish proteins under high-pressure treatment promoted changes in water-holding capacity and color characteristics of haddock and mackerel minces, affecting texture properties of fishcakes prepared thereof. The fish minces became lighter with increasing pressure from 200 MPa to 300 MPa. Hardness and cohesiveness of fishcakes prepared from HP-treated minces decreased significantly ($p < 0.05$) compared to untreated samples. The lowest degree of lipid oxidation was measured at 300 MPa and could be the result of inactivation of prooxidative endogenous enzymes, while the highest amount of lipid oxidation products at 200 MPa could be the result of prooxidative denaturation and release of prooxidative iron from proteins and not inactivated prooxidative endogenous enzymes. A significant ($p < 0.05$) reduction of microbial growth was observed in both haddock and mackerel with an increase in pressure level from 200 to 300 MPa compared to control (untreated) samples. This suggests that HP-treatment of 200 and 300 MPa may be successfully applied to both lean and fatty fish for reduction of microbial growth with minor changes in the product quality.

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