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Effects of anatomical variation of muscle on composition and oxidation susceptibility of Atlantic mackerel (*Scomber scombrus*)

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ABSTRACT

Atlantic mackerel (*Scomber scombrus*) is a fatty fish with high ratio of dark muscle. The subcutaneous muscle on the dorsal and ventral side of the fillet is prone to yellow discolouration, attributed to lipid oxidation. The question is if this is solely due to the position of the muscle under the skin or if this muscle is more susceptible to oxidation than other dark muscle.

The aim of the study was to evaluate if anatomical variation in the muscle of Atlantic mackerel could affect the oxidation susceptibility and/or its processability. Water and lipid content, fatty acids composition, colour and lipid oxidation (assessed by heme-iron content) and hydrolysis (assessed by free fatty acid ratios) susceptibility were determined. Heme-iron content of dark muscle differed depending on anatomical position and was higher dorsal and ventral dark muscle than in other dark muscle, indicating higher oxidation susceptibility. Furthermore, the ventral and dorsal light muscle and the medial dark muscle had lower free fatty acid content (0.57–1.15 g FFA/100 g lipids) compared to lateral and ventral dark muscle (3.40–3.62 g FFA/100 g lipids). The results indicate that lateral, ventral and dorsal dark muscle are more susceptible to lipid oxidation and hydrolysis.

1. Introduction

Atlantic mackerel (*Scomber scombrus*) is a fatty fish species and its lipid content varies from 10 to 30% depending on catching season (Keay, 2011; Romotowska, Karlsdóttir, Gudjónsdóttir, Kristinsson, & Arason, 2016b). As a fatty species with high amounts of unsaturated fatty acids it is prone to lipid oxidation, and therefore most processing and preservation methods used to prolong shelf life of mackerel focus on hindering lipid oxidation and formation of rancid flavours and odours (Aubourg, Pérez-Alonso, & Gallardo, 2004; Aubourg, Rodríguez, & Gallardo, 2005; Losada, Barros-Velázquez, & Aubourg, 2007; Lugasi et al., 2007).

The muscle of fish, Atlantic mackerel included, is categorised as dark or light depending on its myoglobin content. The dark muscle lies underneath the skin and supports persistent swimming. The proportion of dark muscle corresponds to how much the species tend to swim (Belitz, Grosch & Schieberle, 2009). Atlantic mackerel, as a fatty migrant species, has a high ratio of dark muscle (Godø et al., 2004; Huss, 1995; Shahidi & Spurvey, 1996), which is spread beneath the skin and protrudes into the fillet on its transverse plane towards the spine of the fish (Huss, 1995).

Dark muscle has been reported to be richer in lipids and has been shown to be more sensitive towards lipid degradation than light muscle (Belitz et al., 2009; Dang et al., 2018; Karlsdottir et al., 2014; Shahidi & Spurvey, 1996; Undeland, Stading & Lingnert, 1998a; Undeland, I., Ekstrand, B., & Lingnert, H., 1998b). One of the compounds known to promote lipid oxidation is heme-iron. Richards and Hultin (2002) evaluated the effect of bleeding, and therefore presence of heme-iron, on lipid oxidation in light and dark muscle of Atlantic mackerel and trout. They observed in general that bleeding reduced oxidation of the muscle and that hemoglobin was the most abundant heme protein in the mackerel samples, bled and un-bled. The heme proteins are a possible binding site for oxygen and can therefore be a good indicator of oxidation susceptibility of the muscle.

Lipid oxidation products such as lipid hydroperoxides and thiobartbituric reactive substances are often measured to evaluate lipid oxidation during shelf life and the formation and breakdown of the

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compounds monitored through time. This can give information on the oxidation process and therefore relate to amount of pro-oxidant compounds in the samples. Lipid oxidation is however an immensely complex set of reactions and many different compounds are formed Due to the strong connection found in the literature linking heme-iron to susceptibility of lipid oxidation in fish muscle, heme-iron was chosen as an indicator of oxidation susceptibility (Carlsen, Møller, & Skibsted, 2005; Gomez-Basauri & Regenstein, 1992; Richards & Hultin, 2002; Undeland, Kristinsson, & Hultin, 2004).

Lipid degradation is not only caused by oxidation but also hydrolysis, and both reactions have been associated with quality deterioration. Hydrolysis of glycerol-fatty acids esters is catalysed by lipases and phospholipases and results in the release of free fatty acids (FFAs) (Chaijan, Benjakul, Visessanguan, & Faustman, 2006; Toyomizu, Hanaoka, & Yamaguchi, 1981). Furthermore, the free fatty acids can be more susceptible to oxidation resulting in the formation volatile compounds associated with rancid flavour and odour and other spoilage factors, limiting the shelf life of the product (Aubourg et al., 2004).

Processing fatty and sensitive raw material that is prone to lipid oxidation into high value products can therefore be challenging. A recent study by Sveinsdóttir et al. (2020) showed that the shelf life of individually quick frozen (IQF) fillets from Atlantic mackerel caught around Iceland in July–September was as low as 2.5 months in frozen storage at −25 ± 1.2 °C. Furthermore, industrial mackerel producers in Iceland have noted a yellow discolouration of the muscle underneath the skin and subcutaneous layer following 1–2 months of frozen storage. Evaluation of the product and the discolouration raised questions regarding the composition of the mackerel muscle, and whether lipid degradation and therefore oxidation susceptibility could depend not only on the traditional characterisation of muscle as light or dark but also by the anatomical position of the dark and light muscle on the fillet.

Several studies have been conducted comparing dark and light muscle of several fish species as mentioned, none categorize the muscle further based on its anatomical placement. However, a study focusing on the effect of position of muscle on lipid oxidation has been performed. Undeland, Stading, and Lingnert (1998) reported lipid oxidation of herring (*Clupea harengus*) fillets with and without skin during frozen storage, evaluating the oxidation of the fillets in three different horizontal layers. Lipid oxidation was reported highest in skinless fillets in the subcutaneous muscle layer followed by the medial muscle layer and the muscle layer closest to the vertebrae. Fillets with skin had slower rate of oxidation in the subcutaneous muscle layer although it was still the layer most affected by oxidation. However, information on potentially different composition of the dark muscle depending on its anatomical location on the fillet and evaluation of its degradation susceptibility is lacking. Reports from the Icelandic industry claiming yellow discolouration of muscle located under the skin of Atlantic mackerel fillets indicate that more information regarding the muscle stability is necessary to adjust processing practices towards full utilization of the raw materials. Therefore, the aim of this study was to evaluate whether the dark and light muscle of Atlantic mackerel might differ depending on its anatomical position on the fillet with regards to its water and lipid composition and oxidation (as assessed by heme-iron content) and hydrolysis (by free fatty acid formation) susceptibility.

2. Materials and methods

2.1. Raw material and handling

Atlantic mackerel (*Scomber* scombrus) caught on August 12th, 2017 of the coast of Iceland (65°6′ N 11°24′ W) was used in this study. The haul was 209 tons and the pull time was 3 h and 9 min. The catch contained mainly mackerel (92.3%) but other species included herring (*Clupea harengus*) (4.8%) and blue whiting (*Micromesistius poutassou*) (2.9%). The catch was rapidly cooled on board to −1.5 °C in cooling RSW-tanks in which they were stored for 2 days at −1.5 ± 0.5 °C prior to landing. Mackerel used in the study had an average weight of 450 ± 50 g and was graded a 0.8 on a scale of 0–5 for stomach filling (Eysteinsson, Jónasdóttir, Gislason, Arason & Gudjónsdóttir; 2020). Following landing and grading, the mackerel was mechanically filleted, IQF frozen (Skaginn, TCAB contact belt freezes, IQF freezer) and glazed. After freezing, the mackerel fillets were packed, 3 kg per box, in low-density polyethylene (LDPE) plastic bags (Kivo, Volendam, Netherlands) and corrugated cardboard boxes (Smurfit Kappa Narpapp AS, Dublin, Ireland). During transportation to the research facility samples were stored at −25 °C and were processed and analysed 5 weeks after catching and filleting. The fillets were processed while thawing one at a time in a cold room at 4 °C. The skin and subcutaneous layer were removed from the fillets, and the muscle was separated in to six parts depending on their muscle type and anatomical placement on the fillet. The muscle types included the dorsal (1) and ventral (2) light muscle, as well as the medial dark muscle (3) and subcutaneous lateral (4), dorsal (5) and ventral (6) dark muscle (Fig. 1). The yield of each muscle type was evaluated on 32 individual fillets. The muscle types from those 32 fillets were then divided into two samples, each containing the muscle collected from of the 16 fillets each, which were minced prior to further chemical and physical analysis. All chemicals used for analysis in this study were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), Sigma-Aldrich (Steinheim, Germany) and Fluka (Busch, Switzerland).

2.2. Water and lipid composition

The water content of the samples was measured by determining the difference in weight of the minced muscle samples following drying for 4 h at 102–104 °C (ISO, 1999). The total lipid content of the samples was measured according to the method of Bligh and Dyer (1959). Results of the content of water and lipids were presented as g per 100 g wet muscle. Each sample was analysed in duplicate.

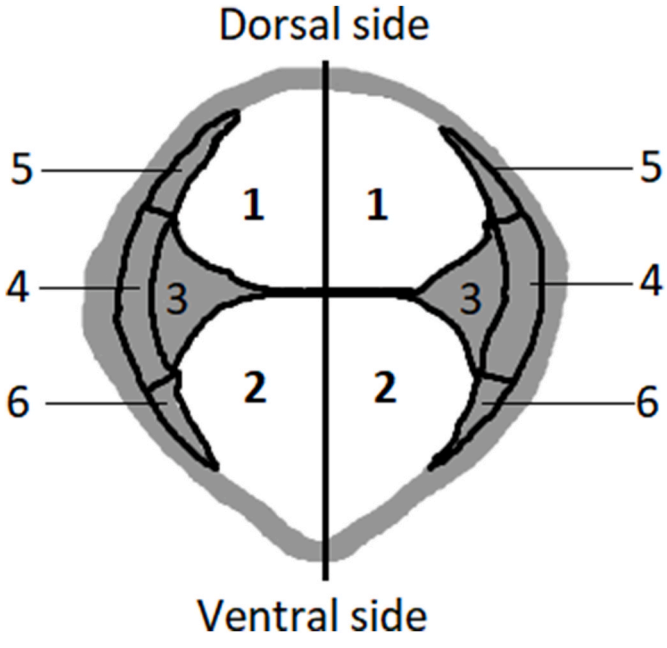

Fig. 1. Schematic image of how samples of Atlantic mackerel fillets were divided prior to analysis into six parts. Light muscle was divided into dorsal and ventral light muscle and dark muscle into lateral, medial, dorsal and ventral dark muscle.1: Dorsal light muscle, 2: Ventral light muscle, 3: Medial dark muscle, 4: Subcutaneous dorsal dark muscle, 5: Subcutaneous lateral dark muscle, 6: Subcutaneous ventral dark muscle.

2.3. Fatty acid profile

The fatty acid profile of the samples was determined from the total lipid extract by gas chromatography (GC) (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA). The methylation of the fatty acids was carried out according to the AOCS method (AOCS, 1998). Samples were analysed with an addition of methyl undecanoate (C23:0) as an internal standard and a program based on the AOAC-996.06 (2001) method as described by Romotowska et al. (2016b). Each sample was analysed in duplicate.

2.4. Heme-iron

The heme-iron content was determined according to the method described by Gomez-Basauri and Regenstein (1992) with slight modifications. Minced samples (2 g) were homogenized (Ultra-Turrax T-25 basic, IKA, Germany) with a 20 mM phosphate buffer at pH 6.8. Samples were then centrifuged at 3000 g for 30 min at 4 °C and filtered through Whatman No. 1 filter paper. Total heme-iron was determined by spectrophotometer at 525 nm. The heme-iron content was calculated based on the myoglobin content, which contains 0.35% iron, and expressed as mg heme-iron per 100 g muscle. Each sample was extracted in triplicate and extract measured in duplicate.

2.5. Lipid hydrolysis

The free fatty acid (FFA) content was determined using the method described by Lowry and Tinsley (1976) with modifications described by Bernárdez, Pastoriza, Sampedro, Herrera, and Cabo (2005). The amount was determined using an oleic acid standard curve ranging between 0 and 20 μmol. Each sample was analysed in duplicate.

2.6. Colour

The colour of the samples was determined with a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using the CIE Lab system. The L*-value, indicating lightness on the scale from black to white, 0 to 100 respectively, the a*-value, ranging from (+) red to (−) green, and the b*-value, ranging from (+) yellow to (−) blue were recorded by the instrument. The colour was measured on minced samples in three different places per sample.

2.7. Statistical analysis

Statistical analysis of data was performed using Microsoft Excel 2016 (Microsoft Inc. Redmond, Wash, USA) and IBM SPSS Statistics v. 26 (International Business Machines, Armonk, New York, USA). One-way analysis of variation (ANOVA) and Duncan's post hoc test was applied on all samples for each group and the significance level was set to $p \leq 0.05$ for all statistical analyses.

A principal component analysis (PCA) was performed using Unscrambler (Version 10.5.2, CAMO ASA, Trondheim, Norway). The data was centred, and all variables weighed with the inverse of the standard deviation to correct for different scales of variables. The model was fully cross validated.

3. Results and discussion

3.1. Yield

Fillets were separated depending on the type of muscle and their anatomical position on the fillet as described in Fig. 1. The fillets were mainly composed light muscle (73.7 ± 4.0%), divided by its position on the fillets into dorsal (45.8 ± 5.1%) and ventral (27.8 ± 5.5%) light muscle. The dorsal light muscle proportion was therefore significantly larger than the ventral light muscle part of the fillets. The fillets contained 21.6 ± 2.7% dark muscle in total, divided by position of the fillet into four parts. The medial and lateral dark muscle corresponded to 8.0 ± 1.7% and 13.0 ± 2.0% of the fillet weight, respectively, and the ventral and dorsal dark muscle types to 0.4 ± 0.3% and 0.2 ± 0.2% of the fillets total weight, respectively.

3.2. Water and lipid composition

The water content of the samples ranged between 47.4 and 63.0 g/100 g and differed as shown in Fig. 2. Overall, the dark muscle, apart from the medial dark muscle, had a lower water content than the light muscle ($p < 0.05$). Between the dark muscle types the ventral and dorsal muscles had the lowest water content, or 49.2 ± 1.6 and 47.4 ± 1.2 g water/100 g muscle, respectively. Furthermore, the light ventral muscle had a lower water content than the dorsal ($p < 0.05$). The water and lipid content of the different muscle types were negatively correlated ($r = -0.752$). The lipid content of the samples ranged between 17.7 and 26.2 g lipids/100 g muscle. The lowest lipid content, 17.7 ± 0.7 g lipids/100 g muscle was obtained in the medial dark muscle, followed by the light muscle on the dorsal side, which contained 20.8 ± 0.9 g lipid/100 g muscle. The other parts of the fillet, the lateral, dorsal and ventral dark muscle and the ventral light muscle all had higher lipid content in the range 24.3–26.2 g lipids/100 g muscle. However, no significant difference was observed in the lipid content of those muscle parts. The overall water and lipid composition of the muscle samples in this study is

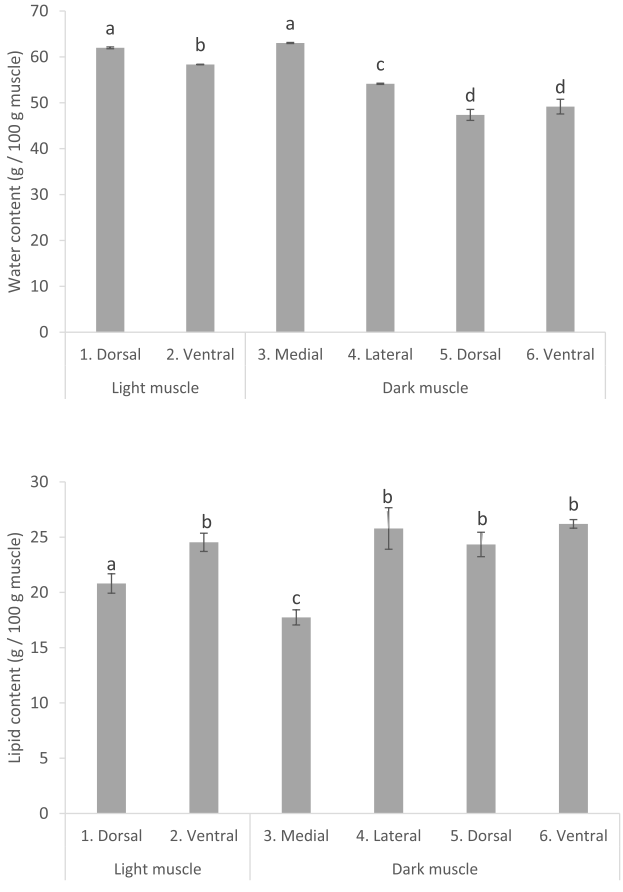

Fig. 2. Water (A) and lipid (B) content (g/100 g muscle) of different parts of Atlantic mackerel fillets (mean ± standard deviation, n = 2, each sample comprised of 16 individual fish). Different superscript letters between parts of the fillets indicate a significant difference ($p < 0.05$). Samples analysed were dorsal light muscle (1), ventral light muscle (2), medial dark muscle (3), subcutaneous lateral dark muscle (4), subcutaneous dorsal dark muscle (5), subcutaneous ventral dark muscle (6), separation detailed in Fig. 1.

consistent with earlier reported proximate composition of Atlantic mackerel caught around Iceland, but data regarding composition of different portions of fillets from mackerel caught around Iceland are lacking (Romotowska et al., 2016; Romotowska et al., 2016b; Romotowska, Karlsdóttir, Gudjónsdóttir, Kristinsson, & Arason, 2016a). A study by Standal et al. (2018) reported lipid content of 13.8 ± 0.8 and 22.8 ± 1.2 g lipid/100 g muscle in light and dark Atlantic mackerel muscle respectively, which is lower than observed in the present project. This difference can be explained by seasonality. Standal et al. (2018) reported results from samples caught in February, when the mackerel is leaner than mackerel caught in the Icelandic fishing zone in July–September, following a heavy feeding period (Keay, 2011; Romotowska et al., 2016a, 2016b). The difference between light and dark muscle in the present project and the Standal study are however similar. Romotowska et al. (2016b) showed the seasonal variance of the composition of mackerel was also highly dependent on the season of catch within the summer months when it is present in Icelandic waters.

3.3. Fatty acid profiles

The fatty acid composition of the mackerel muscle samples (Table 1) was similar between the different muscle types and was in all cases dominated by monounsaturated fatty acids (MUFA, 39.3–42.3 g/100 g total lipids), followed by polyunsaturated fatty acids (PUFA, 30.0–34.2 g/100 g total lipids), but least of saturated fatty acids (SFA, 24.4–27.1 g/100 g total lipids). This is consistent with previously reported fatty acid composition of Atlantic mackerel (Romotowska et al., 2016a, 2016b, 2016; Standal et al., 2018). However, slight differences were observed in the proportion of polyunsaturated fatty acids (PUFA) between the muscle types. The most abundant fatty acid in the muscle was cetoleic acid (C22:1n11) ranging from 14.4 to 16.1 g/100 g total lipids in the different muscle types. Cetoleic acid was found in significantly but only slightly higher concentrations in all samples of the dark muscle, ranging from 15.7 to 16.1 g/100 g total lipids, than in the light muscle, ranging from 14.4 to 14.5 g/100 g total lipids ($p < 0.05$). The concentrations of eicosapentaenoic acid (EPA)) ranged between 7.6 and 8.2 and docosahexaenoic acid (DHA) 11.2–14.9 g/100 g of the total lipids, respectively. DHA was of lowest quantity in the dorsal and ventral dark muscle, 11.2 ± 0.2 and 11.6 ± 0.4 g/100 g lipids respectively, and highest in the lateral and medial dark muscle, 14.8 ± 0.6 and 14.9 ± 0.5 g/100 g total lipids respectively ($p < 0.05$). These fatty acids have shown to have many beneficial properties, for examples positively affecting heart health and early neurodevelopment (Calder, 2011; Mozaffarian & Wu, 2011). The dorsal and ventral dark muscle samples contained slightly lower PUFA concentrations than other parts of the fillets ($p < 0.05$), which could be attributed to lower DHA concentrations in the dorsal and ventral dark muscle and higher cetoleic acid in the dark muscle. Therefore, even though there are some variations in the fatty acid composition, some showing significant differences between the portions of the fillets, they are not thought to be a deciding factor contributing to differences in lipid oxidation susceptibility based on anatomical position.

3.4. Heme-iron

The heme-iron content of the muscle samples varied based on the position of the muscle on the mackerel fillet (Fig. 3). The dorsal and ventral light muscle, as well as the medial and lateral dark muscle, had a

Table 1
Fatty acid composition (g/100 g total fatty acids) of different parts of the Atlantic mackerel muscle (mean ± standard deviation, n = 2, each sample comprised of 16 individual fish). Different superscript letters between parts of the fillets indicate a significant difference ($p < 0.05$). Samples analysed were dorsal light muscle (1), ventral light muscle (2), medial dark muscle (3), subcutaneous lateral dark muscle (4), subcutaneous dorsal dark muscle (5), subcutaneous ventral dark muscle (6), separation detailed in Fig. 1.

	Light muscle						Dark muscle											
	Dorsal (1)			Ventral (2)			Medial (3)			Lateral (4)			Dorsal (5)			Ventral (6)		
C14:0	8.1	±	0.2^{a}	8.3	±	0.1^{a}	7	±	0.2^{a}	7.2	±	0.3^{a}	8.1	±	0.1^{a}	7.8	±	0.1^{a}
C14:1	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}
C15:0	0.5	±	0.0^{a}	0.5	±	0.0^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}	0.5	±	0.0^{a}	0.4	±	0.0^{a}
C16:0	12.1	±	0.2^{a}	12.1	±	0.1^{a}	12.7	±	0.2^{a}	12.8	±	0.3^{a}	12.6	±	0.1^{a}	12.8	±	0.0^{a}
C16:1n7	3.7	±	0.1^{a}	3.8	±	0.1^{a}	3.7	±	0.1^{a}	3.9	±	0.1^{a}	4.3	±	0.1^{a}	4.2	±	0.1^{a}
C16:2n4	0.3	±	0.0^{a}	0.3	±	0.0^{a}	0.3	±	0.0^{a}	0.3	±	0.0^{a}	0.3	±	0.0^{a}	0.3	±	0.0^{a}
C17:0	0.4	±	0.1^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}
C16:3n4	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.1^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}
C17:1	0.6	±	0.0^{a}	0.6	±	0.0^{a}	0.6	±	0.0^{a}	0.6	±	0.0^{a}	0.7	±	0.0^{a}	0.7	±	0.0^{a}
C18:0	1.9	±	0.0^{a}	1.9	±	0.0^{a}	2.3	±	0.1^{b}	2.4	±	0.1^{b}	1.9	±	0.0^{a}	2.1	±	0.1^{b}
C18:1n9	7.5	±	0.2^{a}	7.5	±	0.1^{a}	7.1	±	0.1^{b}	7.1	±	0.2^{a}	7.4	±	0.1^{a}	7.4	±	0.0^{a}
C18:1n7	1.5	±	0.0^{a}	1.5	±	0.0^{a}	1.5	±	0.0^{a}	1.5	±	0.0^{a}	1.5	±	0.0^{a}	1.6	±	0.0^{a}
C18:2n6	1.9	±	0.0^{a}	1.9	±	0.0^{a}	1.7	±	0.0^{a}	1.8	±	0.1^{a}	1.8	±	0.0^{a}	1.8	±	0.0^{a}
C18:3n6	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}
C18:3n4	1.8	±	0.0^{a}	1.8	±	0.0^{a}	1.4	±	0.0^{c}	1.4	±	0.0^{c}	1.6	±	0.0^{b}	1.4	±	0.0^{c}
C18:3n3	6.3	±	0.1^{a}	6.3	±	0.1^{a}	4.5	±	0.1^{c}	4.6	±	0.1^{c}	5.3	±	0.1^{b}	4.8	±	0.1^{c}
C20:1	10.1	±	0.2^{a}	10.2	±	0.2^{a}	9.9	±	0.2^{a}	9.8	±	0.4^{a}	10.4	±	0.2^{a}	10.4	±	0.1^{a}
C20:2	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}
C20:3n6	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}
C21:0	0.3	±	0.0^{a}	0.3	±	0.0^{a}	0.4	±	0.0^{a}	0.4	±	0.0^{a}	0.3	±	0.0^{a}	0.4	±	0.0^{a}
C20:3n3	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}	0.1	±	0.0^{a}
C20:4n6	1.1	±	0.0^{a}	1.1	±	0.0^{a}	0.9	±	0.0^{b}	0.9	±	0.0^{b}	0.9	±	0.0^{b}	0.9	±	0.0^{b}
C20:5n3 (EPA)	8.2	±	0.2^{a}	8	±	0.1^{ab}	7.7	±	0.2^{c}	7.8	±	0.2^{bc}	7.9	±	0.1^{bc}	7.6	±	0.1^{c}
C22:1n11	14.4	±	0.3^{a}	14.5	±	0.4^{a}	15.6	±	0.4^{b}	15.7	±	0.9^{b}	15.7	±	0.4^{b}	16.1	±	0.3^{b}
C22:1n9	0.7	±	0.0^{a}	0.7	±	0.0^{a}	0.8	±	0.0^{a}	0.8	±	0.0^{a}	0.8	±	0.0^{a}	0.8	±	0.0^{a}
C22:2	0.5	±	0.0^{a}	0.5	±	0.0^{a}	0.4	±	0.0^{a}	0.5	±	0.0^{a}	0.5	±	0.0^{a}	0.5	±	0.0^{a}
C22:4n6	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}	0.2	±	0.0^{a}
C24:0	1.1	±	0.0^{a}	1.1	±	0.0^{a}	1.2	±	0.0^{a}	1.2	±	0.0^{a}	1.1	±	0.0^{a}	1.1	±	0.0^{a}
C22:6n3 (DHA)	13.6	±	0.4^{a}	12.7	±	0.2^{b}	14.9	±	0.5^{d}	14.8	±	0.6^{d}	11.2	±	0.2^{c}	11.6	±	0.4^{c}
C24:1n9	0.8	±	0.0^{a}	0.8	±	0.0^{a}	1	±	0.0^{a}	1	±	0.1^{a}	0.9	±	0.0^{a}	0.9	±	0.0^{a}
SFA	24.4	±	0.5^{a}	24.5	±	0.2^{a}	24.5	±	0.5^{a}	24.9	±	0.7^{a}	27.1	±	0.2^{b}	25.1	±	0.1^{a}
MUFA	39.3	±	0.5^{a}	39.8	±	0.7^{a}	40.3	±	0.7^{b}	40.6	±	1.4^{b}	39.9	±	0.7^{a}	42.3	±	0.4^{b}
PUFA	34.3	±	0.5^{a}	33.5	±	0.3^{a}	32.8	±	0.8^{a}	32.7	±	1.2^{a}	30.4	±	0.3^{b}	30	±	0.5^{b}

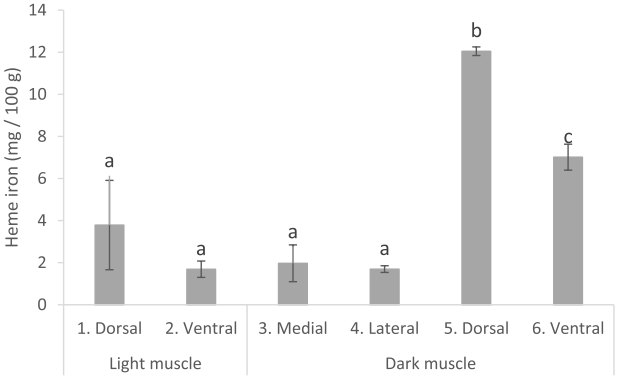

Fig. 3. Heme-iron content (mg/100 g muscle) of different parts of Atlantic mackerel fillets (mean ± standard deviation, n = 2, each sample comprised of 16 individual fish). Different superscript letters between parts of the fillets indicate a significant difference ($p < 0.05$). Samples analysed were dorsal light muscle (1), ventral light muscle (2), medial dark muscle (3), subcutaneous lateral dark muscle (4), subcutaneous dorsal dark muscle (5), subcutaneous ventral dark muscle (6), separation detailed in Fig. 1.

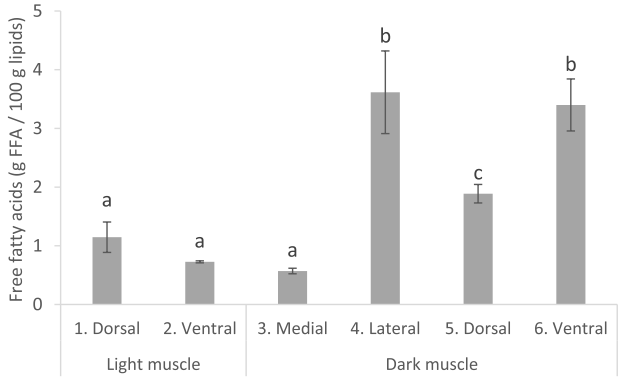

Fig. 4. Free fatty acid content (g/100 g lipids) of different parts of Atlantic mackerel fillets (mean ± standard deviation, n = 2, each sample comprised of 16 individual fish). Different superscript letters between parts of the fillets indicate a significant difference ($p < 0.05$). Samples analysed were dorsal light muscle (1), ventral light muscle (2), medial dark muscle (3), subcutaneous lateral dark muscle (4), subcutaneous dorsal dark muscle (5), subcutaneous ventral dark muscle (6), separation detailed in Fig. 1.

low average heme-iron concentration in the range of 1.7–3.8 mg heme-iron/100 g muscle. The dorsal and ventral dark muscle contained significantly higher ($p < 0.001$) heme-iron concentrations, of 12.0 ± 0.2 mg heme-iron/100 g muscle and 7.0 ± 0.6 mg heme-iron/100 g muscle, respectively compared to other portions of the fillet. This is in agreement with previous studies, which report that heme-iron content is generally higher in dark muscle than light muscle (Richards & Hultin, 2002). The pro-oxidant effects of heme-iron has been established in previous studies (Carlsen et al., 2005; Maestre, Pazos, & Medina, 2011; Richards & Hultin, 2002). Furthermore, Richards and Hultin (2002) showed that when hemoglobin extracted from Atlantic mackerel muscle and from trout were introduced to washed cod mince the extract collected from mackerel caused a significantly more lipid oxidation. Other forms of iron, e.g. non-heme iron and free iron, have also establish pro-oxidant effects. Therefore, in future studies the breakdown of heme-iron and amount of free iron in these different muscle groups could provide further insight into the oxidation reactions in the muscle (Gomez-Basauri & Regenstein, 1992).

The high concentration of heme-iron in the subcutaneous dark muscle both on the dorsal and ventral side of the fillets indicates that these muscle tissues are more susceptible to lipid oxidation than the lateral or medial dark muscle.

3.5. Free fatty acids

A large variance in the amount of free fatty acids (FFAs) in the different parts of the muscle (Fig. 4) was observed in the study. The light muscle contained low amounts of FFA, 0.73 ± 0.02 and 1.15 ± 0.26 g FFA/100 g lipids in the light ventral and dorsal muscles respectively. Some variation was observed amongst the dark muscle tissues. The medial dark muscle contained the lowest amount 0.57 ± 0.05 g FFA/100 g total lipids and the lateral and ventral dark muscle the highest, 3.62 ± 0.70 and 3.4 ± 0.44 g FFA/100 g lipids respectively. The dorsal dark muscle was lower in FFA than the two earlier mentioned dark muscle types (1.89 ± 0.16 g FFA/100 g lipids) ($p < 0.05$). Since FFA formation can be used to assess the degree of lipid hydrolysis, and therefore enzymatic activity in the muscle, these results indicated that more lipid hydrolysis had generally taken place in the subcutaneous dark muscle (lateral, dorsal and ventral dark muscle) compared to the light muscle and the medial dark muscle. The difference in the amount of FFA in the medial and lateral dark muscle when compared to the heme-iron content of those same muscle groups indicate not only that the anatomical position of the muscle is important when evaluating hydrolysis but also that the heme-iron content does not seem to be a deciding factor for amount of free fatty acids and therefore enzymatic activity in the muscle. The subcutaneous lateral dark muscle, despite having low heme-iron content compared to subcutaneous dark muscle with other positions, has high FFA content.

Previous studies have reported both slightly higher FFA in dark muscle of fish (Karlsdottir et al., 2014; Undeland, Ekstrand, & Lingnert, 1998) and lower (Dang et al., 2018). The results of the present project show that some information may be lost during this type of comparison due to the high variation of hydrolysis in dark muscles depending on their anatomical positions. When processing migrant fish with a high ratio of dark muscle this information is invaluable for producers when e.g. evaluating possibilities for producing skinless or deep skinned products.

3.6. Multivariate data analysis

A principal component analysis (PCA) was performed to analyse the similarities and differences between the muscle types based on the studied variables. A PCA bi-plot of the results is shown in Fig. 5, and PC 1 and 2 describe 88% of the variance within the data. PC 1 (55%) showed mainly the difference between the characteristics of the light and dark muscle, while PC 2 (33%) indicated the differences within the dark muscle depending on its anatomical position, separating the medial and lateral dark muscle from the ventral and dorsal dark muscle. The variables correlating strongly with the light muscle were high EPA and water content, as well as higher L* values, indicating that it was in fact lighter in colour than the dark muscle. The dark medial and lateral muscle types had higher lipid content, higher a* value indicating redder colour, and ventral and dorsal dark muscle higher b* value, indicating more yellow colour. Dark ventral and dorsal are also separated from the rest and each other through FFA and heme-iron content, the former of higher quantity in the dark ventral muscle and the latter higher in the dark dorsal muscle.

4. Conclusion

This study reveals that there is a difference in the composition and susceptibility towards lipid degradation dependent upon the dark muscles anatomical position in Atlantic mackerel fillet. This information is relevant when evaluating the oxidative stability and shelf life of mackerel products. The heme-iron content of the dark ventral and dorsal muscles was 2–4 times higher than in other portions of the fillet. Lipid

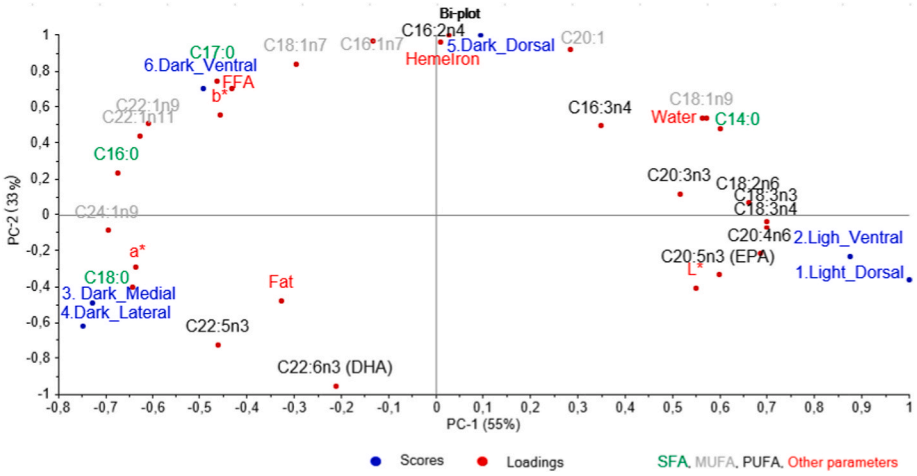

Fig. 5. Bi-plot showing scores and loadings from PC 1 and PC 2 from principal component analysis (PCA) of mackerel muscle. All samples and parameters were used. Scores are coloured in blue and loadings represented with red dot and lettering coloured based on parameter. Loadings with lettering coloured green are saturated fatty acids (SFA), grey are monounsaturated fatty acids (MUFA), black are polyunsaturated fatty acids (PUFA) and red are other measured parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hydrolysis appeared to be more rapid in the dark dorsal, ventral and lateral muscle, indacting higher enzymatic activity in these tissues. Although the dorsal and ventral dark muscle make up a very small portion of the fillet, they decrease the stability and shelf life of the whole fillet. Observations from industrial producers on the yellow discolouration of these tissues indicate that the dark muscle adjacent to the skin (ventral, dorsal and lateral dark muscle) should be removed from the fillet, leaving only the medial dark muscle, an apparently more stable dark muscle, on the fillet. Analysis of fatty acid composition showed that all portions of the fillets contain high concentrations of healthy long-chain PUFA so this could be done with minimal effect on the nutritional value of the product. Simultaneously the removal of the ventral, dorsal and lateral dark muscle with the skin and subcutaneous layers creates possibilities for further product development and value creation.

CRediT authorship contribution statement

Hildur Inga Sveinsdóttir: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Sandra Björk Sverrisdóttir:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Magnea G. Karlsdóttir:** Conceptualization, Project administration, Writing – review & editing. **Turid Rustad:** Writing – review & editing. **Sigurjón Arason:** Conceptualization, Writing – review & editing. **María Gudjónsdóttir:** Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

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

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References

AOAC-996.06. (2001). AOAC official method 996.06 fat (total, saturated, and unsaturated) in foods hydrolytic extraction gas chromatographic method. *American Oil Chemists' Society*.

AOCS. (1998). Official method Ce 1b-89. Fatty acid composition by GLC: Marine oils. *Official method and recommended practices of the AOC. D. Firestone. American Oil Chemists' Society*.

Aubourg, S. P., Pérez-Alonso, F., & Gallardo, J. M. (2004). Studies on rancidity inhibition in frozen horse mackerel (Trachurus trachurus) by citric and ascorbic acids. *European Journal of Lipid Science and Technology, 106*(4), 232–240. https://doi.org/10.1002/ejlt.200400937

Aubourg, S. P., Rodríguez, A., & Gallardo, J. M. (2005). Rancidity development during frozen storage of mackerel (Scomber scombrus): Effect of catching season and commercial presentation. *European Journal of Lipid Science and Technology, 107*(5), 316–323. https://doi.org/10.1002/ejlt.200401124

Fish, whales, Crustaceans, mollusks. In Belitz, H.-D., Grosch, W., & Schieberle, P. (Eds.), *Food chemistry*, (pp. 617–639). (2009) (pp. 617–639). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-69934-7_14.

Bernárdez, M., Pastoriza, L., Sampedro, G., Herrera, J. J. R., & Cabo, M. L. (2005). Modified method for the analysis of free fatty acids in fish. *Journal of Agricultural and Food Chemistry, 53*(6), 1903–1906. https://doi.org/10.1021/jf040282c

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Calder, P. C. (2011). Fatty acids and inflammation: The cutting edge between food and pharma. *European Journal of Pharmacology, 668*(Suppl 1), S50–S58. https://doi.org/10.1016/j.ejphar.2011.05.085

Carlsen, C. U., Møller, J. K. S., & Skibsted, L. H. (2005). Heme-iron in lipid oxidation. *Coordination Chemistry Reviews, 249*(3), 485–498. https://doi.org/10.1016/j.ccr.2004.08.028

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. *Food Chemistry, 99*(1), 83–91. https://doi.org/10.1016/j.foodchem.2005.07.022

Dang, H. T. T., Gudjonsdóttir, M., Karlsdóttir, M. G., Van Nguyen, M., Tómasson, T., & Arason, S. (2018). Stability of Golden redfish (*Sebastes marinus*) during frozen storage as affected by raw material freshness and season of capture. *Food Sciences and Nutrition, 6*(4), 1065–1076. https://doi.org/10.1002/fsn3.648

Eysteinsson, S. T., Jónasdóttir, S. H., Gislason, A., Arason, S., & Gudjónsdóttir, M. (2020). Biochemical characteristics of zooplankton entering Atlantic mackerel processing plants in Iceland as side- catch. *Submitted to Food Research International January 2020*.

Godø, O. R., Hjellvik, V., Iversen, S. A., Slotte, A., Tenningen, E., & Torkelsen, T. (2004). Behaviour of mackerel schools during summer feeding migration in the Norwegian Sea, as observed from fishing vessel sonars. *ICES Journal of Marine Science, 61*(7), 1093–1099. https://doi.org/10.1016/j.icesjms.2004.06.009

Gomez-Basauri, J. V., & Regenstein, J. M. (1992). Processing and frozen storage effects on the iron content of cod and mackerel. *Journal of Food Science, 57*(6), 1332–1336. https://doi.org/10.1111/j.1365-2621.1992.tb06850.x

Huss, H. H. (1995). Quality and quality changes in fresh fish. In *Food and agriculture organization of the united nations* (1995th ed.) http://www.fao.org/3/V7180E/V7180E00.htm.

ISO. (1999). *Animal Feeding Stuffs. Determination of moisture and other volatile matter content*. International Organization for Standardization. ISO 6496:1999.

Karlsdottir, M. G., Sveinsdottir, K., Kristinsson, H. G., Villot, D., Craft, B. D., & Arason, S. (2014). Effect of thermal treatment and frozen storage on lipid decomposition of light and dark muscles of saithe (Pollachius virens). *Food Chemistry, 164*, 476–484. https://doi.org/10.1016/j.foodchem.2014.05.068

Keay, J. N. (2011). *Handling and processing mackerel*. Retrieved October 29th 2019 from: http://www.fao.org/3/x5938e/x5938e01.htm#Composition%20of%20mackerel.

Losada, V., Barros-Velázquez, J., & Aubourg, S. P. (2007). Rancidity development in frozen pelagic fish: Influence of slurry ice as preliminary chilling treatment. *LWT - Food Science and Technology, 40*(6), 991–999. https://doi.org/10.1016/j.lwt.2006.05.011

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists Society, 53*(7), 470–472. https://doi.org/10.1007/BF02636814

Lugasi, A., Losada, V., Hóvári, J., Lebovics, V., Jakóczi, I., & Aubourg, S. (2007). Effect of pre-soaking whole pelagic fish in a plant extract on sensory and biochemical changes during subsequent frozen storage. *LWT - Food Science and Technology, 40*(5), 930–936. https://doi.org/10.1016/j.lwt.2005.09.021

Maestre, R., Pazos, M., & Medina, I. (2011). Role of the raw composition of pelagic fish muscle on the development of lipid oxidation and rancidity during storage. *Journal of Agricultural and Food Chemistry, 59*(11), 6284–6291. https://doi.org/10.1021/jf200686z

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology, 58*(20), 2047–2067. https://doi.org/10.1016/j.jacc.2011.06.063

Richards, M. P., & Hultin, H. O. (2002). Contributions of blood and blood components to lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry, 50*(3), 555–564. https://doi.org/10.1021/jf010562h

Romotowska, P. E., Gudjónsdóttir, M., Kristinsdóttir, T. B., Karlsdóttir, M. G., Arason, S., Jónsson, Á., et al. (2016). Effect of brining and frozen storage on physicochemical properties of well-fed Atlantic mackerel (Scomber scombrus) intended for hot smoking and canning. *LWT - Food Science and Technology, 72*, 199–205. https://doi.org/10.1016/j.lwt.2016.04.055

Romotowska, P. E., Karlsdóttir, M. G., Gudjónsdóttir, M., Kristinsson, H. G., & Arason, S. (2016a). Influence of feeding state and frozen storage temperature on the lipid stability of Atlantic mackerel (Scomber scombrus). *International Journal of Food Science and Technology, 51*(7), 1711–1720. https://doi.org/10.1111/ijfs.13146

Romotowska, P. E., Karlsdóttir, M. G., Gudjónsdóttir, M., Kristinsson, H. G., & Arason, S. (2016b). Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (Scomber scombrus) caught in Icelandic waters. *Journal of Food Composition and Analysis, 49*, 9–18. https://doi.org/10.1016/j.jfca.2016.03.005

Shahidi, F., & Spurvey, S. A. (1996). Oxidative stability of fresh and heat-processed dark and light muscles of mackerel (Scomber scombrus). *Journal of Food Lipids, 3*(1), 13–25. https://doi.org/10.1111/j.1745-4522.1996.tb00051.x

Standal, I. B., Mozurairyte, R., Rustad, T., Alinasabhematabadi, L., Carlsson, N.-G., & Undeland, I. (2018). Quality of filleted atlantic mackerel (Scomber scombrus) during chilled and frozen storage: Changes in lipids, vitamin D, proteins, and small metabolites, including biogenic amines. *Journal of Aquatic Food Product Technology, 27*(3), 338–357. https://doi.org/10.1080/10498850.2018.1436107

Sveinsdóttir, H. I., Karlsdóttir, M. G., Arason, S., Stefánsson, G., Sone, I., Skåra, T., et al. (2020). Effect of antioxidants on the sensory quality and physicochemical stability of Atlantic mackerel (Scomber scombrus) fillets during frozen storage. *Food Chemistry. Accepted manuscript.* https://doi.org/10.1016/j.foodchem.2020.126744

Toyomizu, M., Hanaoka, K., & Yamaguchi, K. (1981). Effect of release of free fatty acids by enzymatic hydrolysis of phospholipids on lipid oxidation during storage of fish muscle at minus 5 degrees centigrade. *Bulletin of the Japanese Society of Scientific Flsheries*. ISSN: 0021-5392.

Undeland, I., Ekstrand, B., & Lingnert, H. (1998). Lipid oxidation in herring (Clupea harengus) light muscle, dark muscle and skin, stored separately or as intact fillets. *Journal of the American Oil Chemists' Society, 75*(5), 581–590. https://doi.org/10.1007/s11746-998-0069-9

Undeland, I., Kristinsson, H. G., & Hultin, H. O. (2004). Hemoglobin-mediated oxidation of washed minced cod muscle phospholipids: Effect of pH and hemoglobin source. *Journal of Agricultural and Food Chemistry, 52*(14), 4444–4451. https://doi.org/10.1021/jf030560s

Undeland, I., Stading, M., & Lingnert, H. (1998b). Influence of skinning on lipid oxidation in different horizontal layers of herring (Clupea harengus) during frozen storage. *Journal of the Science of Food and Agriculture, 78*(3), 441–450. https://doi.org/10.1002/(SICI)1097-0010(199811)78:3<441:AID-JSFA156>3.0.CO;2-Y