THE CONTRIBUTION OF FATTY ACIDS TO THE COMPOSITION OF THE TOTAL LIPIDS IN JUVENILE CAPE HAKE FILLETS.

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Abstract

Due to the decline in fish stocks more juvenile fish are being caught. The aim of this study was to investigate the contribution of short and medium chain length fatty acids to the composition of the total lipids in juvenile Cape hake fillets and the impact thereof the nutritional value. The fatty acids that most contributed to the total fatty acid composition were palmitic acid (C16:0) (46.52%), docosahexaenoic acid (C22:6) (25.68%) and stearic acid (C18:0) (9.4%). Saturated fatty acid contribution to the total lipid content was found to be higher than reported in literature in other hake species and no eicosapentaenoic acid was detected. Eicosanoic acid, was detected only in the medium sized hake fillets, indicating reduced nutritional value in juvenile hake fillets. The challenges of the fishing industry is thus not only to control the sustainability of fish resources, but also the size of the total allowable catch to ensure the best nutritional level.

Keywords: juvenile Cape hake, Fatty acids, nutrition

1. INTRODUCTION

South African Cape hake consists mainly of two morphologically similar species, with the distribution of each being depth dependent. The majority of the South African demersal total catch is harvested off the west coast and is dominated by the shallow-water hake Merluccius capensis Castelnau (A), and deep-water hake M. Paradoxus Franca (B) (Fairweather, Booth, Sauer and Leslie, 2006). These species are not distinguished commercially, and are sold for consumption together under the generic name of hake. Therefore, similarly as done in a previous study by Herrero, Huidobro and Careche (2003) the two species were not separated.

The Cape hake fishery is a considerable social and economic asset to South Africa (Siyema, 2010). Serious concerns have been voiced about the size and management of the catch in the past; therefore a management strategy for Cape hake is in place since 2006 (Butterworth and Rademeyer, 2005; Von der Heyden, Lipinski and Matthee, 2007; Siyema, 2010). The main concern identified, is one that is also a global concern - the need to contain fish catches at a sustainable level versus government policy to increase employment by broadening participation in the fishing industry and growing the economy

(Siyema, 2010; Field, Attwood, Jarre, Sink, Atkinson and Pieterson, 2013). In South Africa approximately one-third of the fresh and frozen catch is exported, and the same amount of the demersal catch reaches shore-based facilities in a fresh or processed state (Hutton, 2000; Siyema, 2010).

In fish, fatty acids predominantly arise from synthesis de novo via non-lipid carbon sources within the animal, or directly from dietary lipid (Henderson, 2003). Lipid digestion, absorption and transport in fish is generally similar to that in mammals as described by Tocher (2003) in a comprehensive review of the metabolism and functions of lipids and fatty acids. Marine lipids are characterised by a content of longer chain (up to C24) fatty acids (Huynh and Kitts, 2009). One group of lipids present in fish muscle, namely the polyunsaturated fatty acids (PUFA's) is considered “essential” (required but not synthesised) to humans and is acquired through the human diet (Batista, Vetter and Lucas, 2001; Kiessling, Pickova, Eales, Dosanjh, and Higgs, 2005; Huynh and Kitts, 2009). Most cultures in the world consume fish in some way or other and obtain ω3 PUFA's in this manner (Hsieh and Kinsella, 1986). These are known precursors of metabolic products that play an important role in blood clotting, immune response and vascular tone, and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Das, 2000; Maki, Davidson, Dicklin, Ingram, Cyrowski, Umporowicz, Bell and Elliot, 2003; Kiessling et al. 2005).

Numerous studies have been published describing fish lipids and fatty acids, with special emphasis on the ω3 PUFA in fish species, including species of hake (Wessels and Spark 1973; Méndez and González, 1997; Huynh and Kitts, 2009; Pacetti, Alberti, Boselli, and Frega, 2010; Medina, Castro and Pantoja, 2013). Despite these studies there is limited information on particularly the short and medium chain length fatty acids that form part of the total lipids of Cape hake muscle tissue, nor the implication on the nutritional value when juvenile fish are consumed. The aim of this study was to investigate the contribution of short and medium chain length fatty acids in Cape hake to the composition of the total lipids and whether fish size (maturity) influences the distribution thereof, and to compare the fatty acid composition to relevant literature on similar species in a more adult state, to highlight possible changes in the nutritional value of juvenile Cape hake.

2. MATERIALS AND METHODS

2.1 Sample preparation

Cape hake samples were harvested by a leading South African fishing industry during the month of February from the South African shoreline close to the city of Cape Town (Méndez and González, 1997). The Cape Hake were mechanically scaled, headed, gutted and kept on ice (average muscle temperature 7±0.5 ºC) for 24 hours prior to laboratory analysis. From the c. 100 samples received, only ten were within the parameters of juvenile fish

(weighing less than 200 g). The selected fish, with an average weight of 166±25 g and length of 21±2 cm (headed), were then filleted. These fillets were divided into two groups namely Small (69.94±8.42 g) and Medium (81.32±0.24 g), using the average weight of each fillet. From each fillet, five 1 cm3 sections of tissue were removed aseptically from different areas of each fillet. These were then homogenised as a composite sample for further analysis.

2.2 Fatty acid extraction

Of the homogenate, 0.7 g was subjected to total lipid extraction, as described by Folsch, Lees, and Sloane Stanley (1957), using chloroform:methanol 2:1 (v/v). All reagents, solvents and standards were of analytical grade (Merck, RSA and Separations, RSA), and stored in dark bottles.

2.3 Fatty acid analysis

Trans-esterification of the fish lipids was done by addition of trimethylsulphonium hydroxide (TMSOH, Merck, Midrand RSA) according to the method of Butte (1983). Extracts were stored in glass vials and frozen at -18 ºC until chromatographic readings were performed. The fatty acid methyl esters (FAME) were analysed and separated on a Finnegan Focus GC equipped with a 30 m x 0.25 mm ZB-1 (Separations, RSA) glass capillary column. The column contained 100 % dimethyl polysiloxane (0.25 µm) with helium as carrier gas (constant flow – 3.0 ml. min-1) and functioned in a splitless mode of injection. The temperature programme is summarised as follows: 40 to 90 ºC at a rate of 8 ºC min-1, followed by a ramp from 90 to 280 ºC at 10 ºC min-1. The column was attached to a Finnegan Focus DSQ mass spectrometer for mass detection of fragments with m/z smaller than 1000. Mass analysis was performed at eV with an ion source temperature of 200 ºC. Integration of the peaks was performed on a Total Ion Chromatogram, using Xcalibur software version 1.4 SR1 (Finnegan). Fatty acid methyl esters were identified by comparison of their mass spectra and retention time using the internal library of GC–MS equipment and standard. From the 34 fatty acids originally detected, only 21, including cholesterol, were identified and are reported as weight percent of total fatty acids using mass response factors in Figure 1.

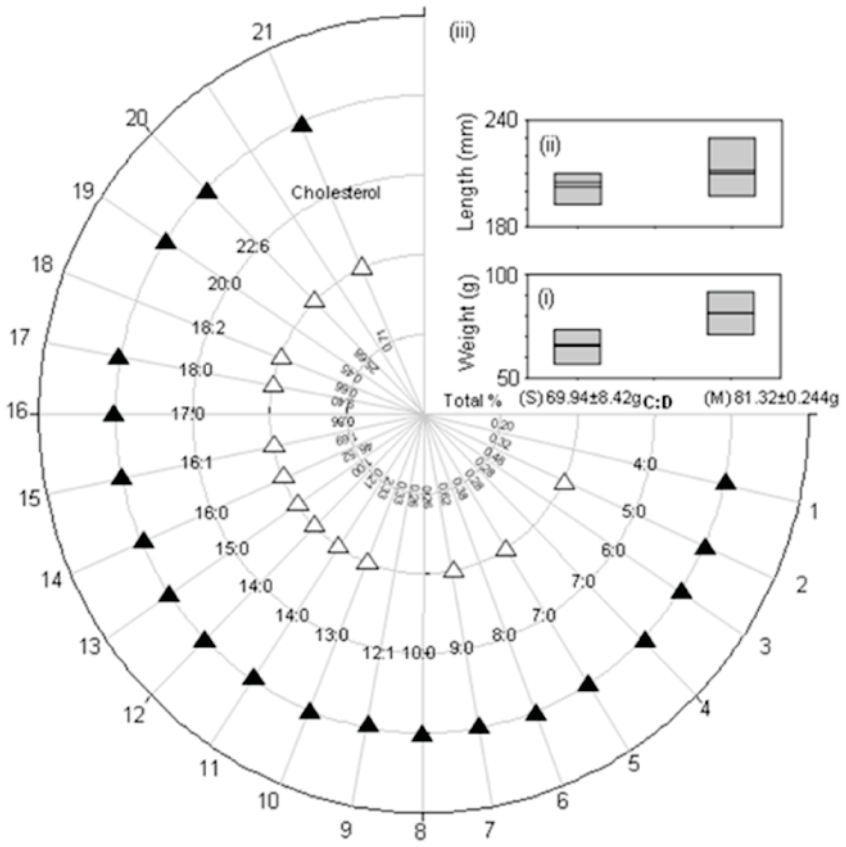

Figure 1. The distribution of the selected fatty acid methyl esthers (FAME's) and Cholestrol in the Small and Medium hake fillets. Section i represents the weight and ii indicates the length of the fillets while iii represents the absence/presence of the FAME's (1- 21) in the Small (S) and Medium (M) hake fillets. The FAME's are sorted according to Carbon length and dubble bond (C:D) as specified and the % of each FAME contributing to the composition of the total lipids are also indicated. The FAME's include 1.butanoic acid, 2-methyl-; 2. pentanoic acid, 3-methyl-, methyl ester; 3. hexanoic acid, methyl ester; 4. heptanoic acid, methyl ester; 5. 2-methylheptanoic acid; 6. octanoic acid, methyl ester; 7. nonanoic acid, methyl ester; 8. decanoic acid, methyl ester; 9. 9-dodecenoic acid, methyl ester, (E)-; 10. tridecanoic acid, 4,8,12-trimethyl-, methyl ester; 11. methyl tetradecanoate, 12. tetradecanoic acid, 10,13-dimethyl-, methyl ester; 13. pentadecanoic acid, methyl ester; 14. hexadecanoic acid, methyl ester; 15. 9-hexadecenoic acid, methyl ester, (Z)-; 16. heptadecanoic acid, methyl ester; 17. octadecanoic acid, methyl ester; 18. 9,15-octadecadienoic acid, methyl ester, (Z,Z)-; 19. eicosanoic acid, methyl ester; 20. 4,7,10,13,16,19-docosahexaenoic acid, methyl ester, (all-Z)- and 21. Cholesterol.

3. RESULTS AND DISCUSSION

There was a strong positive correlation (r=0.77) between the average length, and the weight of the fish fillets (Fig. 1 section i and ii). Section iii (Fig. 1) indicates the absence/presence of the selected FAME found in the Small and Medium groups of fillets. The fatty acids are sequentially presented in Fig. 1 from low to higher carbon containing fatty acids (refer to C:D indicating carbon and double bonds in the circle between the Small and Medium fillets, including cholesterol as number 21. Results of the fatty acids are reported individually, but grouped under saturated and unsaturated fatty acids and each component is numbered as per Fig. 1. Table 1 uses the same component numbers, but only the longer chain fatty acids are tabled and compared with previous studies on hake. However, since short chain fatty acids were not reported previously, these are not included in Table 1.

Table 1. Comparing the Fatty acid composition (weight % of total fatty acids) of the various hake species from this study and literature

Components as per Fig.1.	Selected fatty acids C:D(x)[a]	Cape hake current study	Pacific hake[b] *Merluccius productus*	European hake[c] *Merluccius merluccius*	Cape Hake[d] *Merluccius paradoxus*	Cape Hake[d] *Merluccius capensis*	Atlantic hake[e] *Merluccius hubbsi*
10	C13:0	0.33					
11	C14:0	2.33	1.06	2	1.1	1.74	2.8
12	C14:0 (dimethyl)	0.21	-	-	-	-	-
13	C15:0	1.00	-	0.6	0.11	0.17	-
14	C16:0	46.52	21.7	18.3	23.28	23.57	18
15	C16:1 (9)	1.69	0.62	2.9	4.67	4.95	5.2
16	C17:0	0.86	1.08	0.8	0.43	0.68	-
17	C18:0	9.40	7.18	6.1	3.78	4.36	3.2
	C18:2 (9,12)	-	1.18	1.3	0.57	0.66	-
18	C18:2 (9,15)	0.66	-	-	-	-	-
19	C20:0	0.45	-	0.5	0.1	0.3	-
20	C22:6 (4,7,10,13, 16,19)	25.68	22.08	35	27.81	24.01	25.7
21	Cholesterol	0.71	-	-	-	-	-

[a]C=number of carbon atoms, D=number of double bonds, x=position of double bond(s). Huynh and Kitts, 2009; [c] Pacetti et al., 2010; Wessels and Spark 1973; Méndez and González, 1997

3.1 Saturated fatty acids

Based on the structures of the short-chain fatty acids (component 1, butanoic acid, 2-methyl-; component 2, pentanoic acid, 3-methyl-, methyl ester-; component 3, hexanoic acid, methyl ester, in Fig. 1), and the medium chain length fatty acids (component 4, heptanoic acid, methyl ester-; component 5, 2-methylheptanoic acid-; component 6, octanoic acid, methyl ester-; component 7, nonanoic acid, methyl ester; component 8, decanoic acid, methyl ester in Fig. 1), each could be classified as saturated fatty acids.

Although short-chain fatty acids are an important nutritive source to fish, limited information is available on the lipid transport mechanisms in fish intestines (Titus and Ahearn; 1988). Short-chain fatty acids are known products of anaerobic microbial fermentation in the hindgut of terrestrial vertebrates and are used as a blood fuel, either for energy purposes, or for lipid synthesis Clements, Gleeson and Slaytor, 1994). In Fig. 1 the short- and medium chain compounds (C4-C10) were found in all the Medium fillets and in three instances in the Small fillets. The exceptions were components 2 (pentanoic acid, 3-methyl-, methyl ester), 5 (2-methylheptanoic acid) and 7 (nonanoic acid, methyl ester) that also occurred in the smaller fillets. The short-chain fatty acid that contributed most to the total fatty acid composition is component 3 (hexanoic acid), at 0.48%. The medium-chain fatty acid that contributed most to the total fatty acid composition was component 7 – (nonanoic acid) at 0.62%.

Component 10 (4,8,12-trimethyltridecanoic acid), also known as an isoprenoid fatty acid, occurred in both fillet sizes. The total contribution of this component was 0.33% of the total fatty acid composition. This component derived from phytol, which in turn, is derived from chlorophyll thus indicating the possibility of this component to be from a dietary source has chemotaxonomical significance for both marine and freshwater sponges (Dembitsky, Rezanka and Srebnik, 2003). Another fatty acid known to originate from the diet of fish, is myristic acid (component 11). Previous studies on fatty acids found in hake species, as seen in Table 1, reported exclusively on fatty acids from medium chain length of 14 carbons and higher. Referring to section iii (Fig. 1), component 11 (myristic acid/methyl tetradecanoate C14:0) occurred in both sizes of fish fillets. Occurrence of this component was slightly higher (2.33%) than previously reported (Table 1). Myristic acid can originate, not only from the diet of fish, but can also be biosynthesised de novo by the fatty acid synthase pathway that produces predominantly palmitic acid (component 14) and minor amounts of myristic acid (Tocher, 2009).

Component 12, tetradecanoic acid (C14) also occurred in both sizes of fillets. The total contribution of this component was 0.21% of the total fatty acid composition. Although limited information is available regarding this dimethyl branched fatty acid-, it appears to have similar characteristics to component 10 (4,8,12-trimethyltridecanoic acid) and could possibly even be a precursor of component 10. However, none of the previously mentioned studies on hake has referred to this component.

Branched fatty acid chains such as tetradecanoic acid could implicate bacterial activity on the fillets (Carballeira, Maldonado, Rivera and Porras, 1989; Dembitsky, Rezanka and Srebnik, 2003). Pentadecanoic acid (component 13) is an uneven carbon chain component, making it unusual. This component also occurred in both groups of fillets (Tocher, 2009).

Pentadecanoic acid has previously been reported in hake, with a total contribution of 1% of the total fatty acid composition (Wessels and Spark 1973; Pacetti et al., 2010).

The biosynthetic reactions for the formation of new endogenous lipid, namely lipogenesis has previously been characterised in fish and is catalysed by the cytosolic fatty acid synthetase multienzyme complex. The main products of fatty acid synthetase are palmitic acid (hexadecanoic acid; component 14; C16:0) and stearic acid (octadecanoic acid component 17; C18:0) (Tocher, 2009). Palmitic acid is possibly the most common fatty acid found in nature. This acid contributes to about half (46.52%) of the total fatty acids found in this study, and was found in Medium and Small fillets. Acetyl-CoA is the carbon source for the biosynthesis of new lipids (either from the decarboxylation of pyruvate or the oxidative degradation of various amino acids). In other studies on hake (Table 1), palmitic acid has also been found to be a high contributor to the total fatty acids (Wessels and Spark 1973; Méndez and González, 1997; Huynh and Kitts, 2009; Pacetti *et al.,* 2010; Medina, Castro and Pantoja, 2013).

The prevalence of octadecanoic acid (component 17)-, was higher (9.4%) than previously reported (Table 1) and also occurred in both fillet groups. Octadecanoic acid has been identified to occur in high concentrations in lean fish muscle, such as hake, and is a known product of fatty acid synthetase (Huynh and Kitts, 2009; Tocher, 2009). Low amounts of arachidic acid have previously been detected, together with significant amounts of palmitic acid and stearic acid in phosphoglycerides, which constitute animal cell membranes (Tocher, 2009). Arachidic acid (C20:0), also known as eicosanoic acid (component 19), was reportedly found in European hake and Pacetti *et al.* (2010) reported similar results of approximately 0.45% of the total fatty acids. However none were detected in Pacific hake or Atlantic hake species (Table 1) and only 0.1% and 0.3% were recorded in Cape hake in previous studies (Wessels and Spark 1973; Méndez and González, 1997; Huynh and Kitts, 2009; Pacetti *et al.,* 2010; Medina, Castro and Pantoja, 2013).

3.2 Unsaturated fatty acids

It is important to note that, similar to humans, a marine teleost fish's dietary intake determines the composition of unsaturated fatty acid in muscle and other tissue, since the essential polyunsaturated fatty acids are not synthesised de novo in teleost fish (Henderson, 1996; Tocher, 2009). Monounsaturated fatty acids (MUFA's) are the primary energy source for embryonic development, while PUFA's such as EPA and DHA are required by marine fish for optimal growth and development (Tocher and Ghioni, 1999; Medina, Castro and Pantoja, 2013).

9-Dodecenoic acid (C12:1) (component 9) is a mono-unsaturated fatty acid that only occurred in the medium sized fillets. This component constituted 0.26% of the total fatty acid composition. Although previous investigations implicated cytochrome P450 mono-oxegenases pathway (predominant Phase I oxidation enzymes in vertebrates), relatively little is known regarding its function and regulation within aquatic organisms, such as fish. Cytochrome P450 mono-oxegenases enzymes are important in the bioactivation of certain toxins, as well as the oxidation of various fatty acids, such as arachidonate and lauric acid (Mosadeghi, Furnes, Matsuo and Schlenk, 2007). The occurrence of component 9 (9-dodecenoic acid) possibly indicates the exposure of hake to environmental chemical contaminants, for example, specific chlorinated herbicides, chlorinated solvents, perfluorinated acids and phtahalate esters (also collectively referred to as peroxisome proliferating agents). Environmental chemical contaminants are known to affect lipid metabolism in mammals by perturbation of fatty acid metabolism pathways, to adversely affect reproduction and development, and also to cause hepatocellular carcinogenesis after long-term exposures (Haasch, Henderson and Buhler, 1998).

Another mono-unsaturated fatty acid, 9-hexadecenoic acid (C16:1) (component 15) is known to occur in fish. Compared to other studies done on hake, less palmitoleic acid was detected than previously documented (1.69% vs. 4.67%) as seen in Table 1. Fish are capable, like most organisms, of desaturating certain saturated fatty acids, for example, C16:0 (palmitic acid) to C16:1 (palmitoleic acid) via an aerobic process, utilising CoA-linked substrates and requiring Nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen (Tocher, 2009).

It is noteworthy that no compounds occurred only in the small-sized fish fillets. When a component was present in Small fillets-, it was also detected in the medium-sized fillets in all cases except for C18:2 (component 18). 9,15-Octadecadienoic acid a C18:2 polyunsaturated fatty acid is also known as mangiferic acid (first isolated from mango). In this study, aproximately 0.66% of the total fatty acids were from 9,15-octadecadienoic acid. This fatty acid was previously not recognised as a typical C18:2 fatty acid present in fish lipids, such as linoleic acid (9,12-octadecadienoic acid). However, 9,15-octadecadienoic acid has also been detected in a microalgae (Scenedesmus obliquus). Most of the PUFAs present in fish tissue are the result of the consumption of marine microalgae, which are considered to be the primary producers (Rasoul–Amini, Ghasemi, Morowvat and Mohagheghzadeh, 2009). 9,15-Octadecadienoic acid is an ω3 PUFA that may form part of the ω6 PUFAs pathways, which include linoleic and linolenic acid. 9,15-Octadecadienoic acid is listed in this study to indicate that, although present in small quantities, interconversion and uncommon alteration of unsaturation of C18:2 ω6 do occur.

Component 20 (4,7,10,13,16,19-docosahexaenoic acid, (C22:6ω3) is also known as DHA. On its own it contributes to almost one third of the total fatty acids (25.68%) isolated from the fish tissue, and occurred in both fillet groups. According to previous studies DHA in lean fish muscle, such as hake, are proportionally much higher than EPA (Huynh and Kitts, 2009). It is important to note that neither DHA nor EPA can be synthesised in fish. It is hypothesised that fish lipid deposits mainly originate from the phytoplankton and zooplankton at the base of the marine food web (Rasoul-Amini *et al.,* 2009; Tocher, 2009).

Cholesterol (component 21) is a well-known 27 carbon simple lipid (not containing fatty acids), that originates from a two-carbon precursor, namely acetate, and is present in all animals and humans. Cholesterol was detected in both fillets groups, and the contribution of this component constituted to 0.71 % of the total fatty acids. Although cholesterol and other steroids cannot be degraded to smaller molecules, it is degraded primarily by conversion to bile salts, which facilitates the emulsification and absorption of dietary fat. Small amounts of cholesterol are used to synthesise powerful steroid hormones. Similarly, cholesterol in fish is obtained by dietary intake and is released from intracellular stores, or by de novo synthesis, and acts as a precursor to all steroid hormones (Sharpe, Drolet, MacLatchy, 2006). However, it is believed that the triglyceride content is elevated in fish at the expense of cholesterol esters in teleost lipoproteins, and that fish has a higher concentration of circulating cholesterol. In contrast, the traditional detrimental effects associated with the high plasma cholesterol in humans, are absent in fish (Tocher, 2009).

4. CONCLUSIONS

In marine teleost fish, including hake, the de novo synthesising and the catabolism pathways of dietary polyunsaturated fatty acids have previously been established. Most of the fatty acid compounds reported in this study were detected in the Medium fish fillets. The three fatty acids that most contributed to the total fatty acid composition were palmitic acid (C16:0) (46.52%), DHA (C22:6) (25.68%) and stearic acid (C18:0) (9.4%). The contribution of saturated fatty acids to the total lipid content was higher in comparison to studies on other hake species, and no EPA was detected. Furthermore, one of the well-known fatty acids, namely eicosanoic acid, methyl ester (component nr 19), only occurred in the Medium hake fillets. This indicates a reduced nutritional value of juvenile hake fillets when consumed by humans, when compared to adult hake fillets (>500 g as per previous studies) harvested for retail stores. This study confirms that, concerning the fishing industry, the size of the catch matters in more than one way. The size and age can influence the total nutritional value of hake and most probably, in other fish species as well.

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