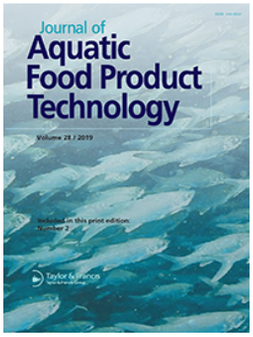

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Journal of Aquatic Food Product Technology

ISSN: 1049-8850 (Print) 1547-0636 (Online) Journal homepage: https://www.tandfonline.com/loi/wafp20

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To cite this article: Davis Chaula, Henry Laswai, Bernard Chove, Anders Dalsgaard, Robinson Mdegela & Grethe Hyldig (2019) Fatty Acid Profiles and Lipid Oxidation Status of Sun Dried, Deep Fried, and Smoked Sardine (*Rastrineobola argentea*) from Lake Victoria, Tanzania, Journal of Aquatic Food Product Technology, 28:2, 165-176, DOI: 10.1080/10498850.2019.1570992

To link to this article: https://doi.org/10.1080/10498850.2019.1570992

JOURNAL OF AQUATIC FOOD PRODUCT TECHNOLOGY
2019, VOL. 28, NO. 2, 165–176
https://doi.org/10.1080/10498850.2019.1570992

Fatty Acid Profiles and Lipid Oxidation Status of Sun Dried, Deep Fried, and Smoked Sardine (*Rastrineobola argentea*) from Lake Victoria, Tanzania

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ABSTRACT

Freshwater fishes contain long chain omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) of highest nutritional value. PUFAs in fish are susceptible to oxidative damage during processing and subsequent storage. Sardines (*Rastrineobola argentea*) are an important fish species of Lake Victoria, constituting 72.3% of the total landings by weight on the Tanzanian side of the lake. Fatty acid profiles and lipid oxidation status of sun-dried, deep-fried, and smoked sardines were investigated. Lipid oxidation was assessed by peroxide value, thiobarbituric acid reactive substances (TBARS), and free fatty acids. Fatty acids were analyzed by gas chromatography with flame ionization detector. The three omega-3 PUFAs: docosahexaenoic acid **(C22:6n-3)**, docosapentaenoic acid **(C22:5n-3)**, and eicosapentaenoic acid **(C20: 5n-3)** contributed 57–60, 63, and 38% of PUFAs in sun-dried, smoked, and deep-fried sardines, respectively. Lipid oxidation reactions were more pronounced in sardines dried on sand and rocks, with TBARS values 97.87 and 84.18 µmolMDA/kg, respectively. The polyene index was significantly lower ($p < 0.05$) in deep-fried sardines, indicating lower retention of PUFAs in the product. Lake Victoria sardines are a rich source of omega-3 PUFAs. PUFAs in sun-dried sardines are prone to oxidative damage. Smoking resulted in relatively higher retention of omega-3 fatty acids in products.

KEYWORDS

Dagaa; omega-3 fatty acids; freshwater fish; fish lipids; thiobarbituric acid reactive substances; lake Victoria; sardines; polyunsaturated fatty acids

Introduction

Sardine (*Rastrineobola argentea*), locally known as *dagaa* in Tanzania, is one of the important commercial fish species in the Lake Victoria region. Targeted by 54% of all engine propelled fishing crafts in the lake, *R. argentea* is ranked second most-important fish after Nile perch (*Lates niloticus*) (URT, 2014). The tiny, fatty, silvery freshwater fish are used for human consumption and as a major source of protein in animal feeds. They are considered a good source of high-quality protein, unsaturated fatty acids, vitamins A and D, and the vitamin B family, as well as minerals including calcium, iron, zinc, copper, and iodine (Kabahenda et al., 2011; Oduho et al., 2005; Owaga et al., 2010).The species constitute 72.3% of the total landings by weight on the Tanzanian side of the lake. The *dagaa* sub sector provides employment to 33,369 fishers and more others engaged in sardine fishery related activities, including processing, trading, transportation, and boat building (URT, 2014).

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Sardines contain saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and long-chain polyunsaturated fatty acids (LC-PUFAs). Elsewhere, it has been reported that the oil extracted from fresh *dagaa* contained 37.0% SFAs, 22.1% MUFAs, 12.5% omega-6, and 21.2% omega-3 PUFAs (Masa et al., 2011). If consumed in adequate amounts, PUFAs are known to confer health benefits to humans, including prevention of cardiovascular disease, psychiatric disorders, and some other illnesses, such as high blood pressure, atherosclerosis, thrombogenesis, cancer, and skin diseases (Gladyshev et al., 2012). Based on their chemical structure, PUFAs are categorized into two main groups, omega-3 and omega-6, depending on the position of the first double-bond from the methyl group of the fatty acid (Abedi and Sahari, 2014). The major omega-3 PUFAs deposits in fish that provide clinical benefits to humans include α-linolenic acid (ALA; 18:3), docosahexaenoic acid (DHA; 22:6), eicosapentaenoic acid (EPA; 20:5), and docosapentaenoic acid (DPA; 22:5). Omega-6 PUFAs include linoleic acid (LA; 18:2) and arachidonic acid (ARA; 20:4) (Abedi and Sahari, 2014). A daily intake of 250–500 mg of EPA+DHA decreases the risk of mortality from coronary heart disease and sudden cardiac death (EFSA, 2010). The cholesterol-lowering effect of LA, a major omega-6 PUFA is also well-recognized from human clinical trials (Mensink and Katan, 1992). Therefore, inclusion of PUFAs in human diets is inevitably a necessity given that they are not synthesized in the human body (Jabeen and Chaudhry, 2011). Hence, it is important to ensure that sustainable low-cost sources of PUFAs are available for proper maintenance of human health (Ozparlak, 2013).

The small-pelagic fish *dagaa* are often caught and processed in large quantities, making it difficult to handle them well. Since fresh fish is highly perishable, drying, smoking, and salting have traditionally been used for preservation (Bellagha et al., 2007; FAO, 2008). Poor on-board handling of fresh *dagaa*, coupled with long fishing distances and long holding time before offloading from fishing vessels, often means that quality deterioration begins before landing. The traditional low-cost, low-technology, and weather-dependent open sun drying is the most common preservation method used by artisanal processors around Lake Victoria. The process is carried out by spreading fresh *dagaa* on a myriad of surfaces, varying from bare ground, rocks and grasses, to raised platforms. The preservation method is characterized by high postharvest losses and products with variable and low quality and limited shelf life (Bellagha et al., 2007). Other processing and preservation methods adopted to varying degrees around Lake Victoria include smoking, deep-frying, and freezing. Poor quality *dagaa* products due to inadequate technologies for value addition are among hindrances to promotion of its economic value in local, regional, and international markets (Odongkara et al., 2009). It is well-established that in the developing world, qualitative and quantitative food loss during the harvest, processing, and distribution stages remains a major concern (FAO, 2011).

Sun-drying, deep-frying, and smoking are reported to be the common methods of processing and preservation of fish in small-scale fisheries (FAO, 2010). Studies to understand the magnitude of quantitative post-harvest losses in *dagaa* subsector have been carried out (Akande and Diei-Ouadi, 2010; Diei-Ouadi and Mgawe, 2011; Ibengwe and Kristofersson, 2012). Proximate composition of fresh sardines has also been carried out (Ogonda et al., 2014). Other research has shown that fresh sardine has a high fat content comprised mostly of nutritionally significant long chain PUFAs (Robert et al., 2014a). Information about maintenance of the nutritionally important long-chain PUFAs during processing and subsequent storage of *dagaa* products is limited. Inappropriate handling, processing, storage, and transportation can lead to oxidation of nutritionally important but chemically unstable PUFAs. Furthermore, it has been proved that oxidation of PUFAs is linked to formation of off flavor compounds that affect sensory attributes of fish, rendering products unacceptable (Maqsood et al., 2012).

The present work characterizes *dagaa* products from artisanal fish processors around Lake Victoria, Tanzania. The study seeks to determine fatty acid profiles and status of lipid oxidation of sun-dried, deep-fried, and smoked *dagaa* products.

Materials and methods

Sample collection

Freshly sun-dried (on sand, rock, and grasses), deep-fried, and smoked *dagaa* samples were purchased from selected artisanal fish processors at landing sites in August, 2016. A total of 32 samples (16 sun-dried, 8 deep-fried, and 8 smoked) were collected and packed in 200 g air-tight polyethylene bags. Samples were airlifted to Denmark for laboratory analysis at the National Food Institute, The Technical University of Denmark. Prior to preparations for analysis, samples were stored at 2°C.

Sample preparation and storage

For each sample, 100 g portion of whole fish body was minced into fish meal using a mixer (Moulinex Moulinette S type 643 02 210, German).The fish meal was then stored at −40°C awaiting chemical analysis.

Moisture content

The moisture content was determined by weighing after drying a sample of approximately 2 g of homogeneous fish sample at 102°C for 18 h according to the **AOAC Method 930.15** (AOAC, 1996). The moisture content of fish samples was expressed as a percentage on dry weight basis.

Lipid content, fatty acid profiles, and polyene index

Lipids were extracted from 5 g of fish meal using chloroform, methanol, and water (1:1:0.8 v/v), following the method of Bligh and Dyer (1959) with modifications according to Knudsen et al. (1985). The lipid content was determined by gravimetry after evaporation of chloroform and expressed as percentage of dried fish.

Gas chromatograph (GC) condition

Lipid extracts were used for preparation of fatty acid methyl esters (FAMEs). FAMEs were analyzed using a gas chromatograph (Agilent Technology Model 7890A series GC, China) fitted with automatic sampler (Model 7693, Agilent Technology), fused silica capillary column (HP-88, 100 m × 0.25 mm × 0.20 µm film thickness; Agilent Technology), split injector, and flame ionization detector (FID). The carrier gas was helium with a flow rate of 0.38 mL/min and an inlet pressure of 51psi. The oven temperature program for separation was from 160 to 200°C, then from 200 to 220°C, and from 220 to 240°C at 10.6°C/min. All analyses were done in duplicate. The result of each fatty acid was expressed as g fatty acid/100 g lipid, whereas the polyene index (PI) was calculated as PI = (C20:5 + C22:6/C16:0) (Rodrıguez et al., 2007).

Free fatty acids (FFAs)

FFA content was determined in the fish oil extract by acidometric titration using NaOH (0.1 M). The FFA content was calculated as oleic acid according to the **AOCS method Ca 5a-40** (AOCS, 1998). The results were reported as % oleic acid.

Peroxide value (PV)

Lipid hydroperoxides content was determined in fish oil extracts according to the method of Shantha and Decker (1994). All analyses were done in duplicate. Results were reported in milli-equivalents peroxide per kg oil (meq O_2/kg).

Thiobarbituric acid reactive substances (TBARS) assay

The TBARS in *dagaa* samples was determined by a previously reported assay using 1,1,3,3-tetra-ethoxypropane (TEP) as a standard (Salih et al., 1987). A 5 g sample of fish mince was homogenized in 30 mL of TCA solution (7.5% TCA, 0.1% EDTA, and 0.1% propylgallate) and filtered. Then, 5 mL of the aqueous extract were allowed to react with equal volume of 0.02M TBA-reagent in water bath at 90°C for 40 minutes. The absorbance of the pink-colored chromogen was measured at 530 nm. The assay was performed in duplicate and results reported in μmol malondialdehyde per kg fish (μmol MDA/kg fish).

Statistical analysis

Data were analyzed using IBM SPSS (SPSS for Windows Version 20.0, 2013, IBM, Bethesda, MD, USA). Data were reported as mean ± standard deviation. Differences between means were determined using one-way analysis of variance (one-way ANOVA) with Tukey's highest significant difference (HSD) post hoc test, according to the equal variance of different groups. The correlations among variables were determined using a two-tailed Pearson correlation coefficient. A p value <0.05 was considered statistically significant. Unscrambler v.10.5 (Camo Process AS, Oslo, Norway) was used for principal component analysis (PCA).

Results and discussion

Moisture and FFAs content

Moisture content of sun-dried sardines ranged from 8.0% ± 0.15 (on rock) to 13.9 % ± 3.94 (on raised rack). Deep-fried and smoked sardines had moisture content of 9.5 ± 0.65 and 10.03% ± 0.03, respectively (Table 1). These results indicate that sardines dried on grasses and on raised racks were not dry enough to stop enzyme activity, lipid oxidation, and hydrolytic reactions, which are favored by high water activity (Abbas et al., 2009). The East African Standard (2014) specifies that dried *R. argentea* shall comply with the requirement that its maximum moisture content is 12%. The moisture content of sardines dried on grasses and on raised platforms as analyzed in this study exceeded the 12% cut-off, and those dried on sand and rock had lower than 12%. Variations in moisture content among sun-dried sardine might be attributed to factors such as the modes of heat transfer on drying surfaces, surface area, air velocity, drying time, and frequency of turning the products during the drying period. While those dried on sand and rocks were expected to experience

Table 1. Moisture, fat (% dry weigh basis), and free fatty acids (% oleic acid) content of sun dried, deep fried, and smoked sardines.

Processing method		Moisture content	Fat	FFAs
Sun-drying	On sand	11.59 ± 2.96	14.87 ± 2.66	62.98 ± 6.42
	On raised rack	13.94 ± 3.94	17.39 ± 4.99	62.02 ± 5.29
	On rock	8.00 ± 0.15	16.18 ± 0.32	57.77 ± 1.74
	On grass	13.10 ± 1.56	12.79 ± 2.60	66.51 ± 2.64
Deep-frying		9.51 ± 0.65	34.22^{a} ± 2.17	5.37^{a} ± 1.38
Smoking		10.03 ± 0.03	13.60 ± 0.82	18.04^{b} ± 0.35

Values are expressed in mean ± standard deviation (n = 4).Means marked with different letters in a column are statistically significant (p < 0.05).

both conductive and convective modes of heat transfer, convective mode mainly dominates the process when products were dried on raised platforms and on grass.

FFAs content in deep-fried and smoked sardines was significantly lower ($p < 0.05$) than in sun-dried sardines. In sun-dried samples, FFAs ranged from 57.7 to 65.02% oleic acid, whereas deep-fried and smoked had 5.37 and 18.04% oleic acid, respectively (Table 1). Free fatty acids are produced by the hydrolysis of fish oils and fats. The level of FFAs is considered a crucial factor linked with the quality of fish products. High levels of FFAs in sun-dried sardines could be associated with high moisture and PUFAs content in the products, as the parameters were found to be correlated ($r = 0.54$ and $r = 0.59$) (Table 3). Such levels of FFAs were suggestive of activity of enzymes relevant to lipid structure, lipase, and phospholipase in sun-dried as opposed to deep-fried and smoked products. The enzymes are found in fish and could be produced by certain microorganisms, therein contributing to lipolytic breakdown of fish lipids (Kolakowska et al., 2002; Nayak et al., 2003). High temperatures during deep-frying and smoking processes might have killed microorganisms and inactivated the enzymes. Accumulation of FFAs in fish products causes unpleasant flavors and influences quality and shelf life. The flavor impairment caused by lipolysis is usually described as "soapiness" or rancid. Several studies have shown that lipid hydrolysis plays a key role in sensory deterioration (Baron et al., 2009; Green-Petersen et al., 2014). Thus, quantification of FFAs may serve as a support to the analysis of sensory acceptability of sardine products. Free fatty acids in products are known to have a pro-oxidant effect on oxidation of marine oils (Aubourg, 2001). Their pro-oxidant effect is due to the presence of carboxylic groups that catalyze the formation of free radicals (Aubourg, 2001) and the fact that free fatty acids reduce surface tension, which in turn increases oxygen transfer into the oil (Kittipongpittaya et al., 2014).

Lipid content

Lipid content in sun-dried and smoked products ranged from 12.8 to 17.4% (Table 1). Deep fried sardine had significantly high ($p < 0.05$) fat content (34.2% ± 2.17). The data agreed with the previous reports of 12.5–13.9% fat content (Kabahenda et al., 2011; Owaga et al., 2010) for sun-dried and 13.8% (Margaret et al., 2016) for oven-dried sardines. Slightly high values in this study could be due to the method used for fat analysis. Bligh and Dyer method is known for exhaustively extracting lipids from animal tissue. The extraction method is reported to be more efficient in extracting polar and nonpolar lipids (Ozogul et al., 2012). High-fat content in sardines obtained in this study also agreed with findings by Suseno et al. (2010), who reported that deep sea fish are low in fat (0.01–4.84%) compared to pelagic fish. Based on criterion used to classify fish according to fat content (Huss, 1998), sardines analyzed in this study are classified as fatty fish. The fat content of fish is known to affect the post-harvest quality and characteristics with respect to oxidative changes (Owaga et al., 2010). The lipids content of fish depends on factors such as water temperature, sex, age, season, food availability, and salinity of the different geographical locations (El-Tay and Abdeltif, 1998; Stansby, 1991). However, the high fat content in *R. argentea*, despite its small size, could be attributed to the fact that data were derived from analysis of the whole fish body with all its internal organs intact. Moreover, proximate composition of R. *argentea* is known to have seasonal variations with high content of protein, lipids, and trace metals during the wet season, indicating the impact of wash-off (Abdulkarim et al., 2016; Ongeri et al., 2012).

Fatty acid profiles and polyene index

A total of 33 fatty acids were identified and quantified in fish oil extracts (Table 2). The SFAs were relatively fewer (6) compared to unsaturated (27). Palmitic acid (C16:0) and stearic acid (C18:0) were found to constitute 13.92–28.72% and 0.87–7.2% of the total lipid content, respectively. Eighteen of the 27 unsaturated fatty acids were PUFAs, and 9 were MUFAs. Among the 18 PUFAs, the omega-3 fatty acids were relatively more abundant (9), followed by omega-6 (5). Deep-fried samples had the highest amount (28.72%) of C: 16:0; presumably some picked up from palm oil used to deep fry the

Table 2. Fatty acid profiles of sun-dried, deep fried, and smoked sardine (g fatty acid/100 g oil sample)[a].

	Sun-dried					
Fatty acid	On sand	On raised rack	On Rock	On grass	Deep fried	Smoked
C14:0	3.35[a] ± 0.28	1.78 ± 0.79	1.29 ± 0.67	2.33 ± 0.63	0.54[b] ± 0.05	1.91 ± 0.21
C15:0	0.62 ± 0.07	0.67 ± 0.03	0.65 ± 0.02	0.64 ± 0.07	0.21[a] ± 0.03	0.49[b] ± 0.01
C16:0	21.72 ± 1.20	13.92[a] ± 1.3	23.25 ± 0.88	19.49 ± 3.2	28.72[b] ± 4.2	21.84 ± 0.2
C17:0	0.73[a] ± 0.21	0.34 ± 0.14	0.23 ± 0.09	0.47 ± 0.21	0.11 ± 0.08	0.24 ± 0.10
C18:0	7.2[a] ± 0.64	3.00 ± 0.38	2.15 ± 0.38	4.61[b] ± 0.40	0.87[c] ± 0.12	4.00[b] ± 0.54
C20:0	0.33 ± 0.10	0.33 ± 0.03	0.23 ± 0.01	0.40 ± 0.06	0.32 ± 0.01	0.15 ± 0.08
Total SFA	33.95 ± 8.29	20.04 ± 5.29	27.8 ± 9.15	27.94 ± 7.45	30.77 ± 11.56	28.63 ± 8.49
14:1	0.05 ± 0.03	0.08 ± 0.04	0.07 ± 0.01	0.06 ± 0.01	nd	0.05 ± 0.01
16:1 (n-7)	10.87[a] ± 1.05	6.43 ± 0.57	3.18[b] ± 0.61	5.5 ± 0.55	1.02[c] ± 0.18	5.92 ± 0.79
18:1 (n-9)	4.99[a] ± 0.31	3.79 ± 0.26	2.96 ± 0.33	3.74 ± 1.02	15.36[b] ± 1.43	2.67 ± 0.08
18:1 (n-7)	2.64[a] ± 0.16	0.91 ± 0.10	0.35 ± 0.03	1.21[b] ± 0.13	4.30[c]± 0.76	0.34 ± 0.01
20:1 (n-9)	0.99 ± 0.09	0.43 ± 0.05	0.19 ± 0.04	0.45 ± 0.06	0.11 ± 0.05	0.17 ± 0.01
20:1 (n-7)	0.28 ± 0.02	0.09 ± 0.01	0.04 ± 0.02	0.12 ± 0.02	0.01 ± 0.01	0.02 ± 0.001
22:1 (n-11)	0.08 ± 0.01	0.02 ± 0.001	0.02 ± 0.001	0.01 ± 0.001	nd	nd
22:1 (n-9)	0.06 ± 0.03	0.05 ± 0.03	0.05 ± 0.01	0.03 ± 0.01	nd	0.02 ± 0.01
24:1 (n-9)	0.86 ± 0.3	0.24 ± 0.03	0.36 ± 0.07	0.58 ± 0.06	0.2 ± 0.04	0.73 ± 0.22
Total MUFAs	20.82 ± 3.59	12.04 ± 2.26	7.22 ± 1.29	11.7 ± 1.97	21 ± 6.03	9.92 ± 2.09
16:2 (n-4)	1.44[a] ± 0.44	0.38[b] ± 0.04	0.39[b] ± 0.03	0.78[a] ± 0.40	0.21[b] ± 0.07	0.83[a] ± 0.34
16:3 (n-4)	1.61[a] ± 0.70	1.89[a] ± 0.06	0.94[b] ± 0.4	2.06[a] ± 0.35	0.13[b] ± 0.05	0.23[b] ± 0.04
18:2 (n-4)	0.43 ± 0.06	0.13 ± 0.08	0.13 ± 0.07	0.11 ± 0.07	0.02 ± 0.01	0.06 ± 0.001
18:3 (n-4)	1.97 ± 0.57	1.31 ± 1.20	0.52 ± 0.08	1.27 ± 1.01	0.47 ± 0.03	1.84 ± 0.05
Total (n-4)	5.45 ± 0.66	3.71 ± 0.82	1.98 ± 0.34	4.22 ± 0.82	0.83 ± 0.19	2.96 ± 0.80
16:4 (ω-3)	0.53 ± 0.2	0.26 ± 0.16	0.29 ± 0.15	0.40 ± 0.20	0.12[a] ± 0.03	0.29 ± 0.15
18:3 (ω-3)	0.32 ± 0.15	0.23 ± 0.16	0.16 ± 0.14	0.25 ± 0.16	0.10 ± 0.04	0.27 ± 0.08
18:4 (ω-3)	0.03 ± 0.03	0.03 ± 0.01	0.04 ± 0.04	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02
20:3 (ω-3)	nd	0.34 ± 0.20	0.42 ± 0.20	0.18 ± 0.02	0.12 ± 0.08	0.28 ± 0.03
20:4 (ω-3)	0.37[a] ± 0.13	3.98 ± 1.30	5.27[b] ± 1.30	2.54 ± 0.28	1.67 ± 1.01	3.59 ± 1.3
20:5 (ω-3), EPA	6.03[a] ± 0.87	2.42 ± 0.33	1.69 ± 0.34	3.53 ± 0.32	0.53[b] ± 0.11	3.25 ± 0.46
21:5 (ω-3)	0.34[a] ± 0.11	0.27 ± 0.15	0.29 ± 0.09	0.32 ± 0.08	0.09[b] ± 0.01	0.33 ± 0.01
22:5 (ω-3), DPA	1.84 ± 0.26	2.06 ± 0.25	2.06 ± 0.17	2.01 ± 0.26	0.88[a] ± 0.25	2.46 ± 0.01
22:6 (ω-3), DHA	9.04 ± 2.37	10.39 ± 1.71	10.19 ± 1.85	11.63 ± 1.75	4.94[a] ± 1.18	14.46[b] ± 0.44
Total (ω-3)	18.5 ± 3.37	19.98 ± 3.35	20.41 ± 3.40	20.88 ± 3.71	8.46 ± 1.59	24.95 ± 4.61
18:2 (ω-6)	2.13[a] ± 0.65	0.73 ± 0.08	0.47 ± 0.07	0.89 ± 0.71	6.04[b] ± 0.5	0.85 ± 0.1
18:3 (ω-6)	0.17[a] ± 0.04	0.19 ± 0.05	0.19 ± 0.02	0.25[b] ± 0.06	0.11[c] ± 0.01	0.17 ± 0.01
20:2 (ω-6)	0.26 ± 0.04	0.29 ± 0.03	0.29 ± 0.05	0.29 ± 0.04	0.09[a] ± 0.01	0.29 ± 0.06
20:3 (ω-6)	2.52[a] ± 0.25	0.89 ± 0.13	0.62[b] ± 0.11	1.66 ± 0.14	0.94 ± 0.23	2.58[a] ± 0.14
20:4 (ω-6)	0.26 ± 0.01	0.35 ± 0.06	0.25 ± 0.04	0.31 ± 0.04	0.09[a] ± 0.003	0.32 ± 0.02
Total (ω-6)	5.34 ± 1.16	2.45 ± 0.30	1.82 ± 0.18	3.40 ± 0.61	7.27 ± 2.59	4.21 ± 1.01
Total PUFAs	29.29 ± 2.39	26.14 ± 2.46	24.21 ± 2.53	28.50 ± 2.70	16.56 ± 1.73	32.12 ± 3.37
Total (ω-6 + ω-3)	23.84 ± 2.73	22.43 ± 2.77	22.23 ± 2.84	24.28 ± 3.04	15.73 ± 1.92	29.16 ± 3.78
PUFA/SFA	0.86	1.30	0.87	1.02	0.54	1.12
Polyene Index	1.35	1.88	1.04	1.46	0.58	1.47

[a]Fish lipid was extracted from whole fish body. Values are expressed in mean ± standard deviation (n = 4). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected. For each fatty acid, means marked with different letters are statistically significant ($p < 0.05$). Means without letter markings in a row did not have any statistically significant differences.

product. The total SFAs content varied from 20.04 to 33.95%. Proportionately, the total MUFAs and PUFAs ranged from 7.22 to 21% and 16.56 to 32.12%, respectively. The content of the two omega-3 PUFAs of highest nutritional interest, DHA and EPA, in all samples ranged from 4.94 to 14.46% and 0.53 to 6.03%, respectively. Other studies reported substantial amounts of EPA (5.2%) and DHA (6.4%) in oils extracted from fresh sardine (Masa et al., 2011; Robert et al., 2014a). Levels of DHA and EPA in *R. argentae* are comparatively higher than in Nile perch (*Lates niloticus*), which contains 10.45 and 3.63% of DHA and EPA, respectively (Ogwok et al., 2008). In each sample analyzed, the amount of DHA was higher than that of EPA. However, there were significant differences for DHA content ($p < 0.05$) among sun-dried, deep-fried, and smoked sardines. Significant differences for EPA content were observed between sardines dried on sand and deep-fried (Table 2).

Table 3. Pearson correlation matrix of the parameters.

	PV	TBARS	FFA	Fat	Moisture	SFAs	MUFAs	PUFAs	EPA	DHA
PV	1									
TBARS	.186	1								
FFA	.291	.550**	1							
Fat	−.484**	−.387*	−.800**	1						
Moisture	.375*	−.017	.543**	−.408*	1					
SFAs	−.418*	.171	−.110	.282	−.207	1				
MUFAs	−.408*	.176	−.106	.294	−.080	.707**	1			
PUFAs	.439**	.490**	.592**	−.726**	.290	.148	.051	1		
EPA	.062	.553**	.456**	−.376*	.174	.617**	.535**	.753**	1	
DHA	.606**	.041	.342*	−.647**	.234	−.393*	−.566**	.676**	.089	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

PV, peroxide value; TBARS, thiobarbituric acid reactive substances; FFA, free fatty acids; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

Results in this study showed that for all samples, the ratios of PUFAs to SFAs and omega-3 to omega-6 were in the range 0.54–1.30 and 1.16–11.21, respectively. The PI (0.58) of deep-fried sardines was significantly lower ($p < 0.05$) than that of sun-dried and smoked sardines, indicating lower retention of PUFAs in the product. The PI value refers to the ratio of PUFAs to the amount of the relatively stable palmitic acid (C16:0). In the current work, it was used to compare PUFAs damage among sun-dried, deep-fried, and smoked sardines. From Table 2, it can clearly be observed that deep-fried sardines had significantly lower amounts of EPA or DHA individually. Omega-3 LC-PUFAs, DHA, DPA, and EPA contributed 57–60, 63, and 38% of PUFAs in sun-dried, smoked, and deep-fried sardines, respectively. The dominance of EPA and DHA in freshwater fish species has been reported elsewhere (Görgün and Akpinar, 2012; Robert et al., 2014a; Zebene et al., 1998). The high amount of DHA and EPA in *R. argentae* is attributed to its feeding habit. The species are known to feed lower in the food chain, mainly on microalgae (diatoms and dinoflagellates), which are good sources of EPA, DPA, and DHA (Meziane et al., 2007; Mfilinge et al., 2005). EPA and DHA in freshwater fish species are also known to have seasonal variations (Antonio et al., 2008). Generally, the results show that smoking of sardines results in relatively higher retention of PUFAs than either sun-drying or deep-frying.

Lipid oxidation

To determine the extent of lipid oxidation in sun-dried, deep-fried, and smoked sardines, PV and TBARS assays were respectively used to assess the primary and secondary lipid oxidation products. The peroxide values for sun-dried sardines ranged from 5.27 to 7.85 mEqO_2/kg. Smoked samples

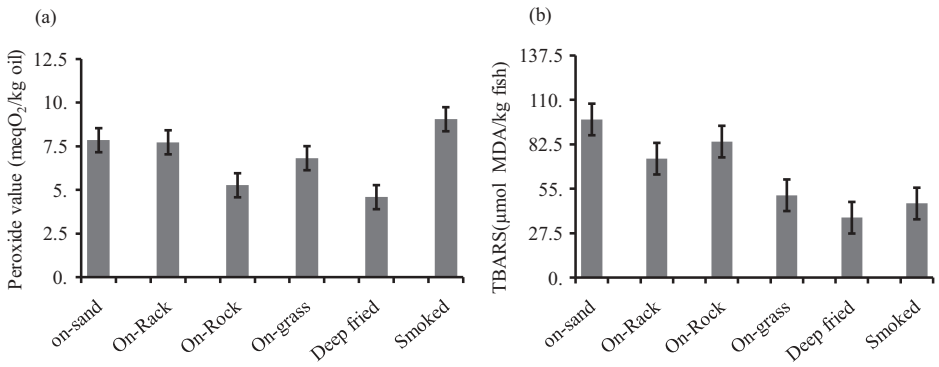

Figure 1. Lipid oxidation in sun-dried (on-sand, on-rack, on-grass), deep fried and smoked sardine: (a) peroxide value and (b) thiobarbituric acid reactive substances (TBARS).

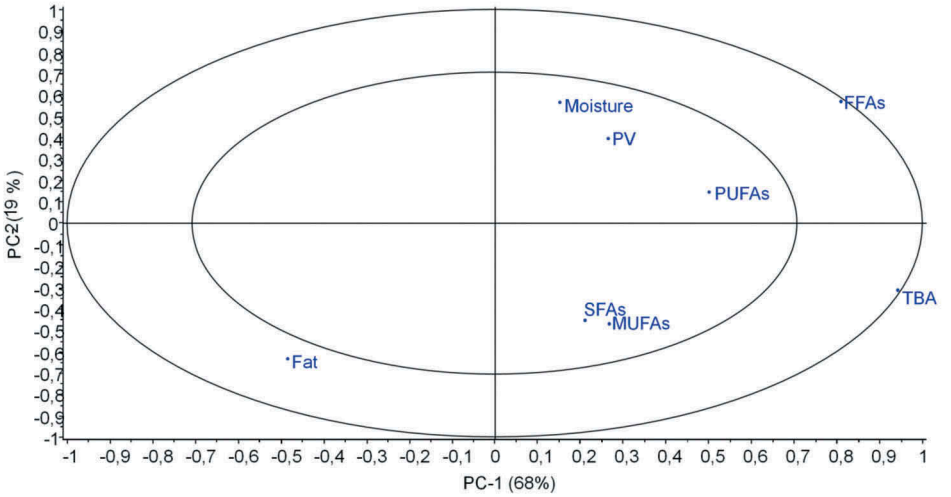

Figure 2. Correlation loadings of variables along principal components PC1 and PC2. PV: peroxide value, PUFAs: polyunsaturated fatty acids, MUFAs: monounsaturated fatty acids, SFAs: saturated fatty acids, FFAs: saturated fatty acids, TBA: thiobarbituric acid reactive substances.

had the highest peroxide value (9.05 mEq O_2/kg), whereas deep-fried samples had the lowest peroxide value (4.59 mEqO_2/kg) (Figure 1b). PV had positive correlation (r = 0.61) with DHA content in sardines. Differences in peroxide values among samples indicate that formation of hydroperoxides is not equally favored among samples. Based on palatability of fish oils, the Global Organization for EPA and DHA (GOED) in their voluntary monograph specifies that the PV of fish oil intended for human consumption should be below 5 mEqO_2/kg (Albert et al., 2013). The TBARS values ranged from 50.99 to 97.87 μmol MDA/kg fish (Figure 1a). The TBARS values for sardines from different sun drying options were such that on sand > on rock > on rack > on grass. Deep-fried and smoked sardines had significantly lower TBARS values than sun-dried samples ($p < 0.05$). Samples dried on sand had the highest TBARS value of 97.87 ± 25.47 μmol MDA/kg fish; whereas, deep-fried had the lowest TBARS value of 37.09 ± 11.33 μmol MDA/kg fish. Secondary products of lipid oxidation are known to be associated with changes in flavor, color, odor, mouth texture, and nutritional benefits in fish (Baron et al., 2009; Green-Petersen et al., 2014; Owaga et al., 2009).

Results in this study showed that lipid oxidation reactions were more pronounced in sun-dried sardines, particularly those dried on sand and rocks, which had high values of PV and TBARS. The TBARS levels in these samples were equivalent to 7.05 and 6.06 mg MDA/kg fish, respectively. A TBARS level below 6 mg MDA/kg fish is regarded as acceptable with regard to development of rancid flavor and odor in fish products (Freeman and Hearnsberger, 1994). Consequently, sardine dried on sand and rock, as analyzed in this study, surpassed the acceptable limit with regard to development of rancid odor. Smoking is a well-known fish preservation method that increases product stability and imparts characteristic flavor and color. Its antioxidation action results from the combination of dehydration and presence of smoke constituents (Goulas and Kontominas, 2005; Guillen and Errecalde, 2002). Lipid oxidation is indeed a very important chemical reaction leading to quality deterioration in sardines, as they contain high amounts of PUFAs. The reaction has proven to be responsible for production of undesirable flavor and odor and loss of nutritionally valued EPA and DHA (Azha and Nisa, 2006; Maqsood and Benjakul, 2011; Maqsood et al., 2012).The two PUFAs are reported to decrease the risk of mortality from coronary heart disease and sudden cardiac death (EFSA, 2010).

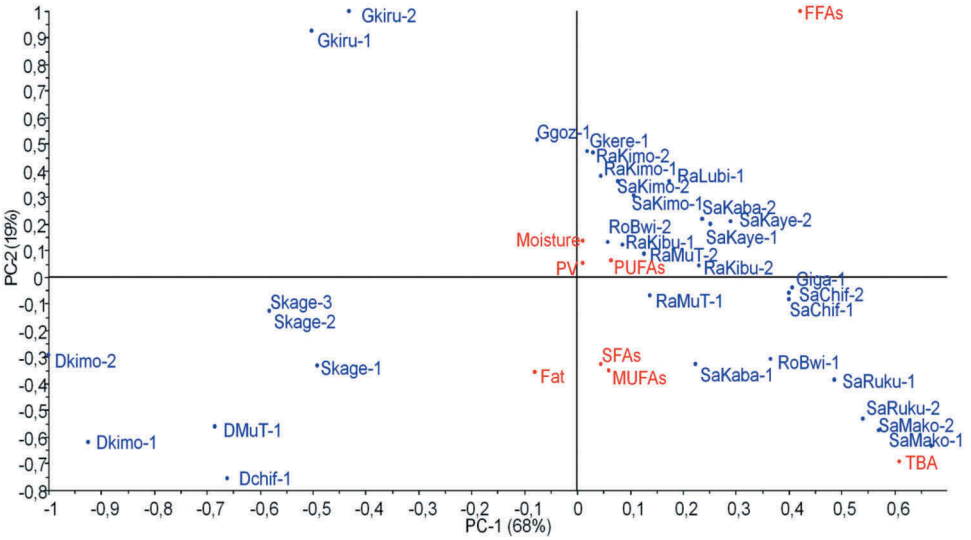

Figure 3. Principal component analysis bi-plot. S, smoked; D, deep fried; G, dried on grass; Ra, dried on raised rack; Ro, dried on rock; Sa, dried on sand; Kimo, MuT, Chif, Kage, kaba, Bwi, Mako, Kibu, Kaba, Kaye, Kere, goz, Kiru, Ruku and Lubi are landing sites at which samples were collected; FFAs, free fatty acids; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; PV, peroxide value; MUFAs, monounsaturated fatty acids; TBA, thiobarbituric acid reactive substances.

Furthermore, in order to visualize correlations between samples and parameters, PCA was performed. In Figures 2 and 3, the PCA on data from samples is shown. The correlation loadings plot revealed that two variables (TBARS and FFA) were significant for explaining the variations among samples (Figure 2). Five variables (moisture, MUFAs, PUFAs and SFAs) did not contain enough variation to discriminate the samples. The first two principal components in the PCA explained 68 and 19% of the total variance in the data set, respectively. Deep-fried and smoked samples are located on the left side of the graph (Figure 3), far away from the position of TBARS and FFAs, indicating that they are not associated with secondary oxidation products and free fatty acids, as opposed to sun-dried samples. The PCA revealed clustering of samples, which indicates the effect of processing method and sampling locations (Figure 3).

Conclusions

Fatty acid profiles and the extent of lipid oxidation in sun-dried, deep-fried, and smoked sardines were evaluated. Sardines from Lake Victoria are rich in long-chain PUFAs. Of particular interest is the high proportion of the nutritionally valuable ω-3 PUFAs in sun-dried and smoked products. Open sun-drying results in products prone to lipid oxidation accompanied by development of rancid odor and flavor, which impacts their sensory acceptability. Deep-frying resulted in low retention of PUFAs in the product due to oxidative damage at high temperature during processing. Further studies are needed to develop improved sardine handling and processing technologies to enhance products' oxidative stability.

Acknowledgments

The authors acknowledge for the financial support provided by the DANIDA supported project "Innovations and Markets for Lake Victoria Fisheries (IMLAF) DFC 14–P01–TAN)". National Food Institute, The Technical University of Denmark is acknowledged for granting permission and technical support during laboratory work.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Danida Fellowship Centre [DFC 14–P01–TAN].

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