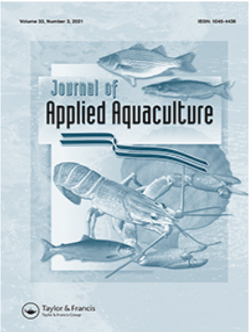

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ABSTRACT

Fishmeal and oils used for feed formulation contain polyunsaturated fatty acids (PUFAs) that are easily oxidized, and affect farmed fish. In this study, the effects of season (dry or wet), drying methods (i.e., artisanal: bare ground, rock surface, meshes laid on the ground or raised racks or adopted), as well as storage time on *Rastrineobola argentea* used for fishmeal in East Africa were examined. Lipid oxidation and proximate composition stability were determined at 30-day intervals over a period of 90 days. Lipid oxidation stability was monitored by determining free fatty acids (FFA), peroxide value (PV) and thiobarbituric acid-reactive substances (TBARs). Changes in proximate composition were based on moisture, ash, crude protein (CP), crude fat (EE), and gross energy (GE). The relationships among lipid oxidation and proximate composition parameters were also explored. Considerable oxidative rancidity reflected by significantly high levels of FFA, PV, and TBARs in dried *R. argentea* was recorded in the wet than in the dry season. Drying of *R. argentea* on bare ground and meshes laid on the ground led to higher lipid oxidation than drying on raised racks. Lipid oxidation was also significantly higher in salted and indirectly dried *R. argentea* than in the unsalted and directly dried samples. Dry matter tended to decrease with storage time, as ash was high in salted samples and those dried on bare ground. There was a general decrease in EE content with storage time that coincided with peroxidation. However, CP and GE were less affected by season, processing methods and storage time. Drying R. *argentea* on raised racks was the best among the processing method investigated.

KEYWORDS
Lipid oxidation; processing methods; proximate composition; *Rastrineobola argentea*; seasonality; storage time

Introduction

Dried silver cyprinid (*Rastrineobola argentea*, Common names: Mukene, Omena, or Dagaa), an indigenous small pelagic fish species of Lake Victoria, is used for

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fishmeal (FM) in East Africa. *R. argentea* is an important protein supplement in livestock feeds because of its rich amino acid and fatty acid profiles (Kubiriza et al., 2017b ; Mugo-Bundi et al. 2015; Mwanja et al. 2010). However, processed *R. argentea* is associated with lipid oxidation (Kubiriza 2017), which is known to negatively affect farmed fish (Kubiriza 2017; Maurente et al., 2000; Zhong, Lall, and Shahidi 2008). *R. argentea* fishmeal contains considerable proportions of highly unsaturated fatty acids (HUFA; Mwanja et al. 2010) that readily oxidize. Lipid oxidation generates off-flavors that affect feed quality and palatability. Products of lipid oxidation interact with proteins resulting in loss of protein functional properties and nutritional value of the feed. In extreme cases, the toxic products of lipid oxidation can cause death of the consuming organism (Frankel 1984; Ladikos and Lougovois 1990; Halver and Hardy (2002)). Indeed, feeding fish on oxidized diets can deter growth and reduce health and survival (Dong et al., 2011; Hamre et al., 2001; Kubiriza et al., 2017a; Maurente et al., 2000; Zhong, Lall, and Shahidi 2008). Lipid oxidation is exacerbated by exposing feeds and/or raw materials to high temperatures, oxygen, and catalytic metal ions such as copper, iron and/or zinc (Antolovich et al., 2002; Frankel 1984; Jensen 1990; Halver and Hardy (20022002)). Furthermore, inadequate processing, handling or storage of oil-laden raw materials and/or feeds can escalate lipid oxidation incidences (Jensen 1990). Complete drying of *R. argentea* takes about a day in the dry season and more than 2 days in the wet season. Half-dried *R. argentea* is associated with off-flavors (Kubiriza 2017) that are characteristic of secondary lipid oxidation (Antolovich et al., 2002; Frankel 1984) and tend to persist even after complete drying.

R. argentea is a short-lived species that breeds throughout the year, with two peaks in the dry seasons of July–August and December–February. Juvenile *R. argentea* is usually abundant in the months of November through December when food is adequate (Mwebaza-Ndawula 1998). The fish is harvested using light attraction by lamps during moonless nights and then piled up in canoes throughout the fishing period of about 7 h (overnight) until it is delivered ashore in the morning for sun drying. Artisan processors dry *R. argentea* on different surfaces, including bare ground, mesh laid on the ground, rocks, pebbles and, more recently, on raised racks (Masette 2010). *R. argentea* FM dried by the different methods exhibits varying magnitudes of lipid oxidation (Kubiriza 2017). The variations in the intensity of off-flavors may indicate varying levels of lipid oxidation products in *R. argentea* dried on different surfaces. Moreover, the dried *R. argentea* is packed in poly-sacks or woven polypropylene bags that are normally piled in poorly aerated stores and maintained at fluctuating ambient temperatures that may exacerbate lipid oxidation.

While the crude protein content, amino acid and fatty acid profile of *R. argentea* are adequate (Kubiriza et al., 2017b; Mugo-Bundi et al. 2015) for animal feed formulation, the processing and handling methods ostensibly affect its nutritional quality (Kubiriza 2017; Masette 2010). Studies have shown that during processing, *R. argentea* is contaminated with sand, dust, animal waste, and microbes (Masette 2010; Odoli et al. 2017), all of which reduce its quality. Unfortunately, very little (if anything) has been done to address the physical-chemical quality challenges associated with *R. argentea* fishmeal, and more so lipid oxidation whose effects on farmed fish are detrimental (Dong et al. 2012; Fontagné-Dicharry et al. 2014; Kubiriza, 2017; Kubiriza et al., 2017a). In E. Africa (mostly in Kenya, Uganda and Rwanda), cage fish farming is on the rise, and so is the demand for quality fish feeds. Accordingly, both feed manufacturers and fish farmers are concerned with raw material and feed quality deterioration. Dietary lipid oxidation is presently of particular interest because of its adverse effects on densely stocked fish, and more so in cages and tanks (Kubiriza et al., 2017a). While fish farmed in ponds access phytoplankton that can ameliorate the effects of lipid oxidation, those stocked at high densities in cages can easily succumb to dietary lipid oxidation (Kubiriza, 2017; ; Kubiriza et al., 2017). Indeed, oxidized lipids can impair growth of fish and in extreme cases lead to pathological side effects and mortalities (Dong et al. 2012; Fontagné-Dicharry et al. 2014; NRC 2011). Studies have shown that oxidative stress more intensively affects the larvae and juvenile fish stages as compared to adults (Dong et al. 2012; Fontagné et al. 2006; Fontagné-Dicharry et al. 2014; NRC 2011). However, this is a paradox because larvae and juvenile fish are reared in tanks and often fed on diets containing a considerable amount of oil-laden fishmeal to meet their high nutrient requirements (NRC 2011). In Siberian sturgeon, for example, dietary oxidative stress led to deformities, slow growth, and reduced survival of larvae (Fontagné et al. 2006). In Uganda, the survival of African catfish larvae fed on locally formulated diets containing up to 40–50% CP (mostly constituted by *R. argentea* fishmeal) ranges between 10% and 15%. Survival rates above 30% are seldom achieved in hatcheries when either artemia or zooplankton such as rotifer and cladocera are used (Pers. Observation) but tend to drastically reduce when artificial feeds are introduced. Even though other confounding factors (mostly related to water quality) may lead to mortalities in African catfish hatcheries, oxidative stress is of concern. Therefore, the processors of *R. argentea* used for fishmeal are being sensitized to and guided on how to control lipid oxidation. Drying of *R. argentea* on raised racks has been adopted to replace the poor artisan processing methods (e.g., drying on the ground, pebbles, and rock surfaces) in a bid to mitigate lipid oxidation, physical-chemical and microbial contamination. This study investigated the oxidative properties and proximate composition of *R. argentea* processed by artisan and adopted methods. The findings are to guide the processing of R. argentea used for fishmeal in livestock feed formulation and indeed humans in Eastern Africa.

Materials and methods

Experimental set up

Wooden raised racks with a surface area of 2 by 1.5 m and an elevation of 1.2 m above the ground were constructed. A one millimeter shed cloth was stretched to cover the surface area of each rack on which treated *R. argentea* (salted or unsalted) was spread and dried under direct or indirect sun-light energy. Under indirect sun drying, a wooden frame covered with transparent polythene paper and elevated at about 45° with a peak of 1 m high was constructed above the stretched shed cloth. The frame with transparent polythene bags contained slits/windows on either side to regulate temperature and moisture during drying.

R. argentea sample collection

Samples were collected from Kiyindi, one of the main landing sites where *R. argentea* is processed and traded on the Ugandan side of Lake Victoria. Sample handling history prior to landing was provided by fishermen. Briefly: *R. argentea* was harvested by light attraction to the lampara nets (mesh size: 10 mm stretched) during moonless nights between 22:00 and 06:00 h, and heaped in canoes until landing ashore. On landing, five-kilogram samples of freshly landed *R. argentea* were dried in triplicate groups by either artisanal or adopted processing methods. This protocol was repeated in the dry and wet seasons. After processing, samples were collected from the different drying methods (Figure 1), transported to the laboratory, and analyzed for proximate composition and lipid oxidation within 2–3 days. For subsequent analysis, batches of dried samples were packaged in poly-bags according to the processing method and stored at ambient temperature (as it is normally done at feed mills). Sub-samples were then drawn in triplicates for re-analysis at 30-day intervals over 90 days of storage.

Lipid extraction from *R. argentea* samples

To remove water molecules from the sample, 3 g (one teaspoon) of sodium chloride salt and 50 g of anhydrous sodium sulfate (Na_2SO_4 were added to 100 g of finely ground *R. argentea* in a 250 ml plastic extraction bottle). To extract the lipids, 100 ml of diethyl ether were added to the mixture and shaken using an electric shaker for one hour. The mixture was centrifuged for 15–20 min at 4000 rpm to obtain diethyl ether supernatant with extracted lipids on top. The diethyl ether supernatant was then filtered off through anhydrous sodium sulfate to further remove any remaining water. The mass of diethyl ether lipid supernatant was determined by pouring it into a pre-weighed evaporating flask. Using a rotor vapor set at 40–45°C, the solvent

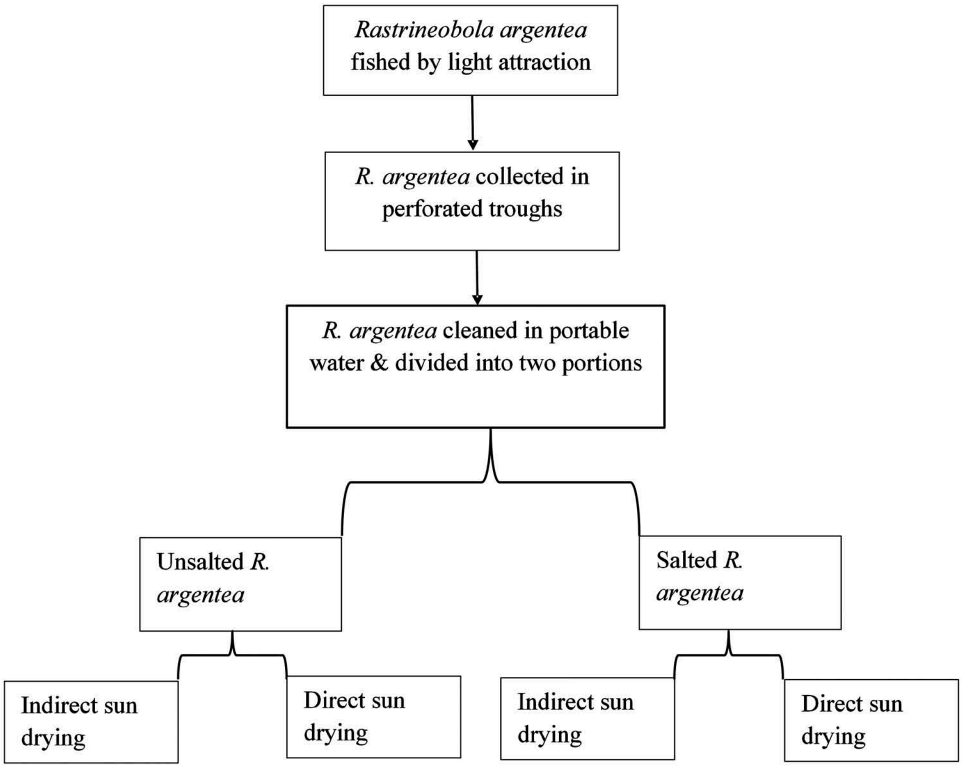

Figure 1. Adopted processing methods for *R. argentea*.

(diethyl ether) was evaporated to remain with oil/fat in the flask. This procedure was repeated for every category of dried *R. argentea*. The extracted oil was then used to carry out specific analyses as described below.

Analyses of oxidative properties

The oxidative characteristics of *R. argentea* dried by different methods were elucidated by monitoring changes in free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid-reactive substances (TBARs).

Free fatty acids (FFA) and acid value (AV) determination

One hundred milliliters of neutralized 2-propan (HPCL) were added to 5–10 g of oil and titrated against 0.1018 N sodium hydroxide (NaOH) with phenolphthalein indicator. The obtained titer value was then used to estimate the percentage of free fatty acids as oleic as follows: %FFA = (Volume of NaOH used (mls) *N*28.2)/mass of oil sample (g), where N is the normality of NaOH and 28.2 is a constant (AOCS 1998).

Lipid hydroperoxide (PV) determination

Lipid hydroperoxides were determined following standard methods by AOCS (1998). Fifty milliliters of Iso-octane (2,2,4-trimethyl pentane) were added to 5 g of the oil sample in a conical flask, followed by 3–5 drops (about 0.5 ml) of saturated potassium iodide (KI). The reaction was allowed to occur for 1 min after which it was stopped by adding about 30 ml of water. The liberated iodine was immediately titrated with 0.010 N sodium thiosulfate, and the titer value obtained was then used to calculate the PV (meq.O_2/kg of oil) as PV = (Vthio*N*1000)/m, where: Vthio-is volume of thiosulfate used, N-is the normality of sodium thiosulfate and m-is mass of the oil sample.

Thiobarbituric acid-reactive substances (TBARs) determination

TBARs content of *R. argentea* FM was determined following methods described by Vyncke (1975). To about 5 g of sample, 30 ml of Trichloroacetic acid (TCA: 7.5%) were added and homogenized for about 1 min. The mixture was filtered to remove undissolved fat and any other residue. Five milliliters of 0.02M TBA reactant were added to 5 ml of the filtrate and the mixture boiled in a water bath at 100°C for about 40 min. The samples were cooled on ice before reading the absorbance at 350 and 600 nm (GENESYS 10 UV, Thermo Electron Corporation, Madison USA), respectively. The difference in the absorbance values obtained at the two wavelengths was used to estimate TBARs using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP). Results were expressed as μmol of malondialdehyde (MDA) per kg of oil.

Proximate composition analysis

Proximate composition of *R. argentea* sub-samples drawn at intervals of 30 days was analyzed using standard methods (AOAC 1995). Moisture content was determined by drying samples in an oven at 105°C for 24 h. Crude protein was estimated as N x 6.25, after determining Nitrogen (N) content of the sample using micro-Kjeldahl (AOAC 1995). Lipid extraction was done using soxhlet apparatus (Soxtec TM 2050 Avanti Extraction Unit), while ash was determined by the combustion of dry samples in a muffle furnace at 550°C for 8 h. Gross energy was determined by adiabatic bomb calorimetry (ISO 9831–1998: Gallenkamp auto-bomb, CABOO1.ABI.C (U.K) Ltd).

Statistical analysis

All statistical analyses were performed with PASW version 18 (SPSS, Chicago, IL, USA). Every 30 days, lipid oxidation and proximate data for artisan processed samples were compared among drying methods using one-way Analysis of Variance (ANOVA), while data from the adopted processing methods were analyzed by factorial ANOVA. In case of significant differences among groups, means were compared using Bonferroni technique. Normality was assessed by histogram, while equal variance was assessed by Bartlett's test. Significant differences were considered at p-values < .05. Ultimately, principal component analysis (PCA) bi-plots and loadings were constructed to explore the association of different parameters by season. .

Results

Oxidative characteristics of dried *R. argentea*

Free fatty acids (FFA)

The FFA concentration of dried *R. argentea* generally increased with storage time. Regardless of the processing methods, FFA content was significantly higher in the wet than in the dry season (Figures 2a-d). In *R. argentea* processed by the adopted methods in the wet season, FFA content was significantly higher ($p < .05$) in the unsalted and indirectly dried samples than in the salted and directly dried samples (Figure 2c). There were no distinct effects of processing methods on the FFA content of *R. argentea* in the dry season (Figures 2b and d).

Peroxide value (PV)

PV fluctuated close to 20 meq.O_2/kg of oil in *R. argentea* processed in the wet season by both artisan (Figure 3a) and adopted methods (Figure 3c) for the first 60 days of storage. Except in samples dried by artisan methods in the dry season (Figure 3a), PV peaked in all samples by day 90 (Figure 3 a, c and d). Among the artisan samples, PV was highest in samples dried on rock surface and lowest in those dried on raised racks (Figure 3a). In the dry season, PV did not significantly differ among artisan drying methods and storage time (Figure 3b). However, for the adopted methods, PV was low and comparable in the directly and indirectly dried *R. argentea* that was unsalted and slightly higher in the salted samples by day 90 (Figure 3d). In the wet season, PV was generally lower in the unsalted and directly dried samples than in the indirectly dried ones that were either salted or unsalted (Figure 3c).

TBARs

TBARs content of *R. argentea* dried by artisan methods in the wet season was about 22–28 μmMDA/kg immediately after processing, but increased with storage time to about 35–40 μmMDA/kg between day 30 and 60, and then dropped (Figure 4a). For the same drying methods in the dry season, the TBARs content of rock-surface dried *R. argentea* increased to above 140 μmMDA/kg by day 30 in storage and dropped drastically to a minimum of about 3–4 μmMDA/kg of oil (Figure 4b). TBARs content of *R. argentea* dried on raised racks lagged behind that of samples dried on mesh laid on the ground, though both increased with storage time (Figure 4b).

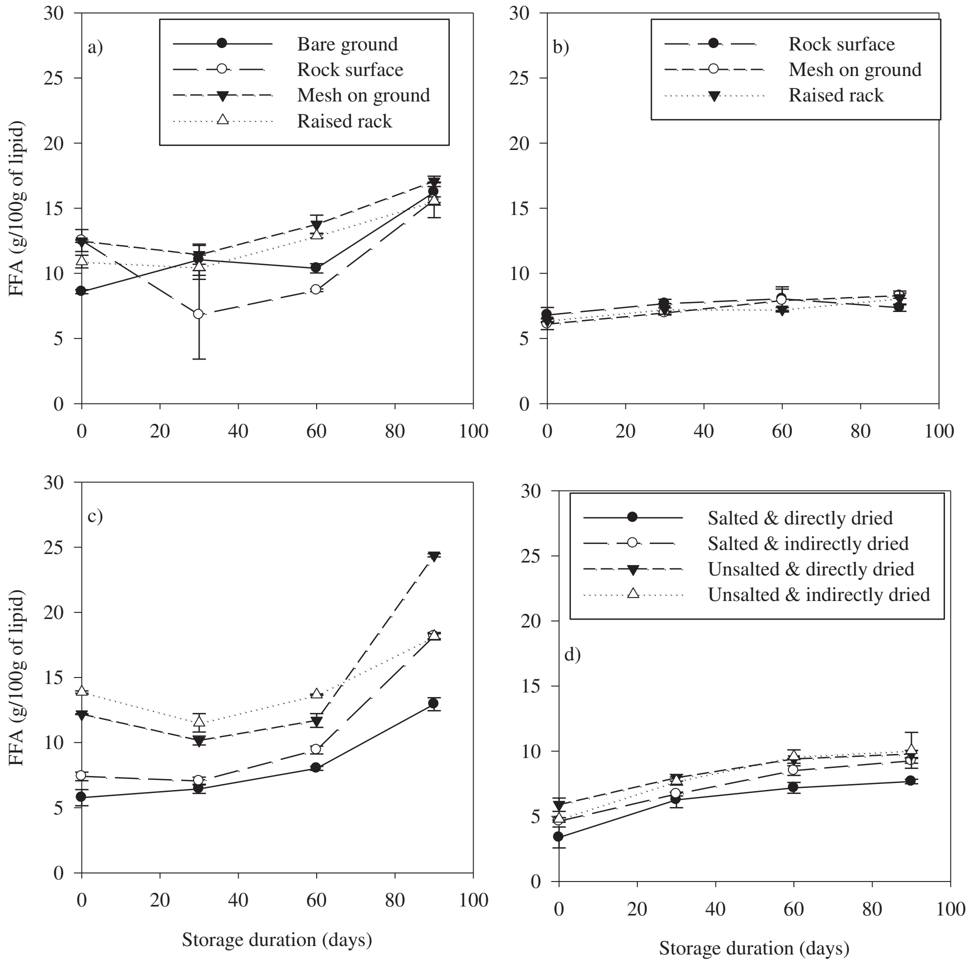

Figure 2. Effect of processing methods and storage duration on the FFA content (n = 3) of dried *R. argentea* (Artisan: (a) wet and (b) dry season: Adopted: (c) wet and (d) dry season).

In the adopted methods, TBARs stood at about 20 µmMDA/kg in *R. argentea* dried in the wet season, but, respectively, decreased in the unsalted and increased in the salted samples during storage (Figure 4c). The increase in TBARs for directly dried samples was significantly lower ($p < .05$) than in indirectly dried samples for the first 60 days of storage, but surpassed it by day 90 (Figure 4c). In the dry season, TBARs content was less affected by drying methods, though it was higher in the unsalted *R. argentea* than it was in the salted samples, immediately after drying. In unsalted *R. argentea*, TBARs content dropped to a minimum (almost zero) by day 60 and slightly increased to about 2–5 µmMDA/kg by day 90 (Figure 4d). On the contrary, TBARs content of salted *R. argentea* increased with storage time from about 18 µmMDA/kg at the end of processing to above 60 µmMDA/kg

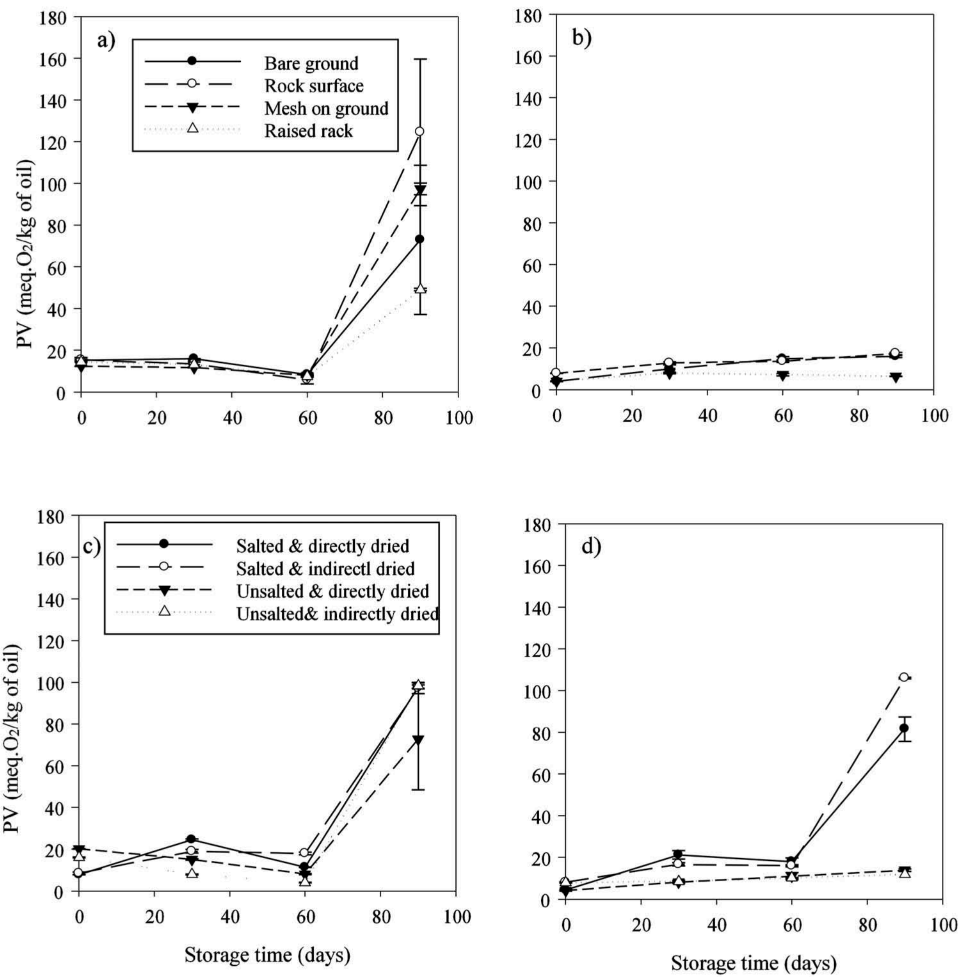

Figure 3. Effect of processing methods and storage duration on the PV (meq.O_2/kg of oil, n = 3) of dried *R. argentea* (Artisan: (a) wet and (b) dry season: Adopted: (c) wet and (d) dry season).

by day 60 and then leveled off (Figure 4d), with the directly dried samples lagging behind the indirectly dried ones (Figure 4d).

Changes in proximate composition of *R. argentea*

Effect of artisan methods

In the wet season, moisture content of *R. argentea* increased with storage time, being slightly higher in samples dried on the bare ground and on mesh laid on the ground, than in those dried on rock surfaces and raised racks (Table 1). In the dry season, moisture decreased from day 0 to day 60 in storage, but slightly increased by day 90, regardless of the drying methods (Table 1). Ash content of dried *R. argentea* was higher and more consistent during the dry season than in the wet season (probably due to reduced dust

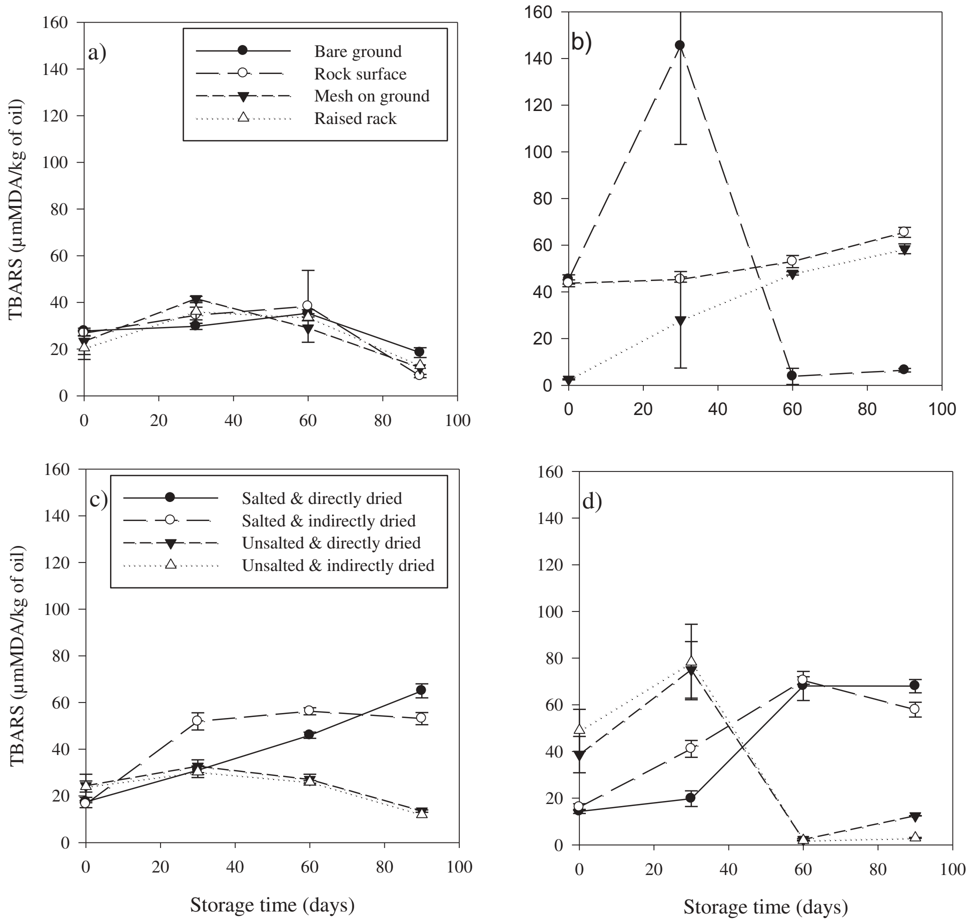

Figure 4. Effect of processing methods and storage duration on the TBAR content (n = 3) of dried *R. argentea* (Artisan: (a) wet and (b) dry season: Adopted: (c) wet and (d) dry season).

in the environment). Ash was higher in *R. argentea* dried on bare ground and rock surfaces than in that dried on mesh laid on the ground and raised racks (Table 1). Crude protein was not significantly affected by drying methods though it was generally higher in samples processed in the wet season than in those processed in dry season. Extractable crude lipids (EE) generally decreased with storage time, more so in the samples dried by artisan methods, in the dry season. In the wet season, EE significantly decreased ($p < .05$) in *R. argentea* dried on bare ground and on mesh laid on the ground than in samples dried on raised racks and rock surfaces. Gross energy was fairly constant throughout storage irrespective of season and drying methods.

Effect of adopted methods

Regardless of the season, moisture content increased with storage time, being slightly higher in the salted and indirectly dried *R. argentea* than in the

Table 1. Proximate composition of *R. argentea* (mean ±SE) processed in the wet and dry seasons by artisan methods and stored for 90 days.

		Days in storage							
		Wet season				Dry season			
Parameter (%)	Drying method	0	30	60	90	0	30	60	90
Moisture	Bare ground	6.55 ± 0.17^{a}	14.50 ± 0.23^{bC}	14.61 ± 0.18^{bB}	14.06 ± 0.18^{b}	-	-	-	-
	Bare rock	6.22 ± 0.25^{a}	7.06 ± 0.23^{aA}	10.64 ± 0.96^{bA}	13.78 ± 0.37^{b}	19.44 ± 0.82^{cB}	15.26 ± 0.17^{bB}	13.51 ± 0.37^{a}	16.24 ± 0.30^{c}
	Mesh on ground	6.15 ± 0.34^{a}	9.97 ± 0.11^{bB}	15.05 ± 0.14^{bB}	14.96 ± 0.02^{b}	15.26 ± 0.03^{bA}	13.80 ± 0.13^{aAB}	14.13 ± 2.22^{ab}	16.79 ± 0.75^{c}
	Raised rack	6.07 ± 0.14^{a}	6.63 ± 0.26^{aA}	11.29 ± 0.19^{bA}	12.89 ± 0.36^{b}	18.97 ± 0.15^{cB}	16.82 ± 3.36^{bBC}	13.02 ± 0.07^{a}	16.99 ± 0.36^{b}
Ash	Bare ground	22.42 ± 2.28^{cC}	14.78 ± 3.95^{bB}	20.73 ± 7.83^{cB}	17.15 ± 0.96^{bB}	-	-	-	-
	Bare rock	19.95 ± 5.41^{cC}	15.74 ± 2.92^{aB}	15.52 ± 0.76^{aA}	18.82 ± 9.74^{cB}	14.39 ± 0.36^{a}	14.44 ± 0.07^{a}	16.68 ± 0.62^{bB}	21.30 ± 0.35^{cC}
	Mesh on ground	15.98 ± 3.60^{bB}	13.09 ± 2.28^{aA}	14.75 ± 0.56^{abA}	12.28 ± 1.68^{aA}	14.63 ± 0.54^{a}	13.79 ± 1.63^{a}	14.71 ± 0.03^{aA}	17.66 ± 0.13^{bB}
	Raised rack	12.84 ± 5.27^{A}	12.06 ± 0.44^{A}	12.59 ± 6.99^{A}	10.70 ± 1.73^{A}	15.61 ± 2.12^{b}	13.07 ± 0.62^{a}	14.71 ± 0.02^{bA}	14.39 ± 0.09^{bA}
CP	Bare ground	56.76 ± 4.15^{a}	64.12 ± 0.80^{b}	62.59 ± 6.98^{ab}	62.70 ± 5.76^{ab}	-	-	-	-
	Bare rock	58.10 ± 2.30^{a}	58.99 ± 4.76^{ab}	63.92 ± 3.41^{b}	63.76 ± 5.64^{b}	75.36 ± 0.91^{cB}	61.70 ± 0.18^{b}	58.97 ± 0.75^{a}	58.87 ± 0.34^{a}
	Mesh on ground	57.67 ± 1.50^{a}	60.78 ± 0.54^{b}	66.72 ± 6.68^{ab}	62.41 ± 2.90^{b}	76.97 ± 2.47^{cB}	60.40 ± 9.31^{ab}	58.95 ± 1.24^{a}	60.27 ± 1.48^{b}
	Raised rack	55.67 ± 0.72^{a}	55.11 ± 0.93^{a}	58.95 ± 1.26^{b}	55.16 ± 2.59^{a}	68.92 ± 0.47^{cA}	62.85 ± 1.68^{b}	57.22 ± 0.19^{a}	59.09 ± 0.38^{b}
EE	Bare ground	12.43 ± 0.34^{bA}	10.90 ± 0.64^{bA}	8.48 ± 0.87^{aA}	7.19 ± 0.05^{aA}	-	-	-	-
	Bare rock	12.56 ± 0.14^{bA}	12.58 ± 0.09^{bB}	12.63 ± 0.38^{bB}	9.26 ± 0.03^{aB}	13.02 ± 0.26^{c}	10.92 ± 0.43^{bA}	8.23 ± 0.01^{aA}	8.14 ± 0.08^{aA}
	Mesh on ground	15.62 ± 1.24^{cB}	13.44 ± 0.13^{bB}	12.09 ± 0.30^{aB}	12.26 ± 0.00^{aC}	13.10 ± 0.05^{b}	11.61 ± 0.70^{abB}	9.87 ± 1.03^{aB}	9.81 ± 0.80^{aB}
	Raised rack	16.42 ± 0.69^{dC}	14.92 ± 0.33^{cC}	15.61 ± 0.20^{dC}	14.84 ± 0.07^{cD}	13.36 ± 0.15^{b}	12.54 ± 0.30^{bB}	10.01 ± 0.38^{aB}	9.36 ± 0.36^{aB}
GE (Kcal/g)	Bare ground	4.58 ± 0.10	4.68 ± 0.66	4.53 ± 0.99	4.62 ± 0.05	-	-	-	-
	Bare rock	4.45 ± 0.31	4.32 ± 0.31	4.64 ± 0.60	5.16 ± 0.22	5.82 ± 0.24	5.14 ± 0.15^{a}	5.01 ± 0.28	4.36 ± 0.01^{b}
	Mesh on ground	4.50 ± 0.53	4.26 ± 0.65	5.02 ± 0.20	5.04 ± 0.52	5.56 ± 0.09	4.95 ± 0.01^{a}	4.64 ± 0.02	4.69 ± 0.11^{c}
	Raised rack	4.17 ± 0.11	4.60 ± 0.38	4.50 ± 0.42	4.51 ± 0.15	5.39 ± 0.01	5.84 ± 0.14^{b}	4.73 ± 0.16	3.19 ± 0.01^{a}

*Dash (-) indicates data were not available. Significant differences in proximate composition among days in storage within each processing methods (in a row) are shown by different lowercase superscripts, while significant differences among processing methods for similar days in storage are shown by different uppercase superscripts within a column. Values without superscripts are not significantly different at $P < .05$.

unsalted and directly dried ones (Table 2). In the wet season, ash content was higher in salted *R. argentea* ($p < .01$) than in unsalted samples (Table 2), regardless of drying methods and storage time. CP of *R. argentea* was less affected by season, drying methods and storage time. However, salted *R. argentea* had lower CP ($p < .0001$: particularly in the dry season) than in unsalted samples. The decrease in EE with storage time was significantly higher ($p < .001$) in salted *R. argentea* than in unsalted samples. There were no significant differences in EE of *R. argentea* dried by different methods within and between seasons. Gross energy was not affected by season, drying methods and/or storage time, though it remained slightly higher in salted than in unsalted samples (Table 2).

Discussion

Development of lipid oxidation during storage

The FFA, PV, and TBARs values were generally higher in the artisan-processed *R. argentea* than in the one processed by adopted methods. Among the artisan processing methods, lipid oxidation was lower in *R. argentea* dried on raised racks than in those dried on the ground, mesh laid on the ground and rock surfaces. Similarly, drying unsalted *R. argentea* on raised racks led to less lipid oxidation than salting and indirect drying, particularly in the wet season. These results indicate that lipid oxidation in dried *R. argentea* is affected by processing methods and seasonality, and to a lesser extent by storage time. The results further suggest that lipid oxidation in *R. argentea* is higher in the wet than in the dry season. The minimal oxidation in *R. argentea* dried on the raised racks as opposed to those directly dried on the ground and other surfaces can be attributed to minimal soil contamination (Masette 2010) and a fast drying rate resulting from effective air circulation across the racks. This facilitates water evaporation and drastically reduces moisture content of *R. argentea*.

The use of FFA, PV, and TBARs to assess lipid oxidation in fish products and fish oils is a widely accepted concept (Aidos 2002; Kirk and Sawyer 1998; Nguyen et al. 2012; Wu and Bechtel 2008). Usually, oils and meat products with FFA values in the range of 1–7% are regarded to be of food-grade quality (Bimbo 1998). In this study, the FFA values of *R. argentea* processed in the dry season were close to 7% throughout storage and across the processing methods, which indicated minimal hydrolysis and, hence, minimal quality deterioration. In the wet season, however, the FFA values of artisan-processed and unsalted *R. argentea* from adopted methods were above 10% at the end of processing and much higher during storage. This suggests that greater lipid oxidation occurred in *R. argentea* during the wet season than in the dry season, regardless of the processing method. Studies

Table 2. Proximate composition of *R. argentea* (mean ±SE) processed by adopted methods and stored for 90 days in the wet and dry seasons.

			Days in storage							
			Wet season				Dry season			
Parameter (%)	salt status	Drying method	0	30	60	90	0	30	60	90
Moisture	Salted	Direct	16.98 ± 0.36^{bC}	21.81 ± 0.34^{cC}	$23.80 \pm .36^{cB}$	22.19 ± 0.66^{cC}	14.18 ± 0.47^{aB}	18.60 ± 0.06^{bB}	21.97 ± 0.23^{cC}	22.64 ± 0.65^{cB}
		Indirect	19.28 ± 0.01^{cC}	$23.13 \pm .20^{cC}$	26.32 ± 0.70^{dC}	25.42 ± 1.97^{dD}	16.53 ± 0.32^{bC}	21.16 ± 0.07^{cC}	14.44 ± 0.30^{aA}	28.22 ± 0.49^{dC}
	Unsalted	Direct	14.47 ± 0.95^{aB}	13.04 ± 0.25^{aA}	17.16 ± 0.22^{bA}	12.57 ± 0.16^{aA}	14.95 ± 2.3^{aB}	13.44 ± 0.71^{aA}	14.65 ± 0.11^{aA}	16.67 ± 0.23^{bA}
		Indirect	8.91 ± 0.31^{aA}	16.55 ± 2.99^{cB}	18.38 ± 0.27^{cA}	17.19 ± 0.16^{cB}	8.61 ± 0.44^{aA}	13.69 ± 0.19^{bA}	15.02 ± 0.08^{bB}	16.32 ± 0.06^{cA}
Ash	Salted	Direct	18.77 ± 1.46^{bC}	18.64 ± 2.73^{bB}	18.62 ± 0.84^{bB}	19.48 ± 0.72^{bC}	22.73 ± 0.57^{cB}	22.53 ± 0.32^{bB}	22.71 ± 0.26^{C}	16.13 ± 0.29^{aA}
		Indirect	18.34 ± 3.64^{bC}	15.33 ± 2.95^{aA}	21.37 ± 0.78^{bC}	19.88 ± 1.96^{bC}	21.57 ± 0.32^{B}	21.14 ± 0.05^{bB}	19.28 ± 0.05^{bB}	17.36 ± 0.48^{aB}
	Unsalted	Direct	14.52 ± 3.76^{B}	14.23 ± 2.41^{A}	14.36 ± 3.09^{A}	12.24 ± 2.3^{A}	14.99 ± 0.16^{A}	14.26 ± 0.26^{A}	13.97 ± 0.05^{A}	15.63 ± 1.32^{A}
		Indirect	12.33 ± 2.57^{aA}	13.85 ± 1.94^{aA}	14.23 ± 3.04^{aA}	14.98 ± 3.73^{aB}	14.51 ± 0.09^{aA}	14.79 ± 0.53^{aA}	14.58 ± 0.16^{aA}	19.15 ± 0.73^{bC}
CP	Salted	Direct	65.09 ± 4.24^{C}	70.31 ± 1.06^{C}	66.64 ± 5.58	64.31 ± 1.36^{B}	60.03 ± 1.59^{A}	64.10 ± 2.55^{A}	61.79 ± 1.89^{A}	58.95 ± 0.16^{A}
		Indirect	56.44 ± 7.97^{A}	69.39 ± 3.37^{C}	72.43 ± 11.44	64.48 ± 1.23^{B}	68.35 ± 1.15^{B}	69.36 ± 0.92^{B}	57.70 ± 1.23^{A}	59.43 ± 0.62^{A}
	Unsalted	Direct	62.61 ± 9.69^{B}	66.32 ± 2.02^{B}	67.32 ± 9.78	60.21 ± 4.91^{AB}	73.20 ± 0.72^{B}	70.65 ± 2.71^{B}	65.98 ± 2.59^{B}	62.02 ± 0.31^{B}
		Indirect	61.69 ± 4.43^{B}	60.74 ± 6.04^{A}	62.54 ± 12.19	61.27 ± 0.68^{A}	63.23 ± 1.37^{A}	64.76 ± 0.38^{A}	64.20 ± 1.08^{B}	67.27 ± 0.96^{C}
EE	Salted	Direct	12.92 ± 0.17	11.64 ± 0.34	12.20 ± 0.33	10.86 ± 0.55	12.33 ± 0.20	11.73 ± 0.18	10.85 ± 0.07	11.50 ± 0.44
		Indirect	12.93 ± 0.93	11.20 ± 0.15	11.26 ± 0.62	10.13 ± 0.92	12.02 ± 0.15	13.02 ± 1.38	10.93 ± 0.27	8.85 ± 1.56
	Unsalted	Direct	13.18 ± 0.41	12.61 ± 0.10	11.69 ± 0.86	10.44 ± 0.39	13.53 ± 0.26	12.65 ± 1.35	11.84 ± 1.25	12.32 ± 0.01
		Indirect	12.12 ± 0.44	13.53 ± 0.24	11.85 ± 0.85	11.17 ± 1.56	12.99 ± 0.15	12.32 ± 0.16	12.63 ± 0.03	12.64 ± 0.09
GE (Kcal/g)	Salted	Direct	4.46 ± 0.18	5.53 ± 0.57	5.26 ± 0.31	4.94 ± 0.16	4.83 ± 0.10	4.630.13	4.94 ± 0.37	4.72 ± 0.04
		Indirect	5.12 ± 0.35	5.79 ± 0.22	5.98 ± 0.22	6.12 ± 0.14	4.64 ± 0.01	4.41 ± 0.09	4.15 ± 0.01	4.83 ± 0.04
	Unsalted	Direct	4.63 ± 0.61	4.29 ± 0.14	4.99 ± 0.12	4.60 ± 0.60	5.02 ± 0.17	4.46 ± 0.13	4.88 ± 0.01	5.19 ± 0.01
		Indirect	4.28 ± 0.48	4.60 ± 0.02	4.95 ± 0.45	5.61 ± 0.03	4.15 ± 0.05	5.17 ± 0.18	5.17 ± 0.00	5.65 ± 0.00

Significant differences in proximate composition among days in storage within each processing methods (in a row) are shown by different lowercase superscripts, while significant differences among processing methods for similar days in storage are shown by different uppercase superscripts within a column. Values without superscripts are not significantly different at $P < .05$.

by Aidos et al. (2001) and Chitra Som and Radhakrishnan (2013) alluded that fatty acid composition varies widely in fish species depending on the geographical location and season. Indeed, the present findings are in conformity with earlier studies because lipid oxidation was higher in the wet than in the dry season. This further affirms season-related fatty acid degradation in fish. Indeed, the abundance of the principal food item in Lake Victoria, the phytoplankton on which *R. argentea* feed, varies with season and is more abundant in the wet season than in the dry season (Mwebaza-Ndawula 1998). Naturally, fish derive much of their polyunsaturated fatty acids (PUFAs), particularly the eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA), from unicellular phytoplankton (Chitra Som and Radhakrishnan 2013). Therefore, the high lipid oxidation in *R. argentea* harvested in the wet season can be attributed to buildup of the highly unsaturated EPA and DHA derived from abundant phytoplankton. Furthermore, the initiation and propagation of the reactive oxygen radicals that lead to oxidative spoilage is facilitated by moisture content of the substrate (Nguyen et al. 2012), which is usually high in the wet season. Indeed, in this study, moisture positively correlated with FFA, PV, and TBARs regardless of the season (Figures 5 and 6). In agreement with the present findings, lipid oxidation and moisture content were positively correlated in salted cod (Nguyen et al. 2012), suggesting that lipid spoilage is influenced by water activity, regardless of fish species. In this study, *R. argentea* took about 2 days longer to completely dry in the wet season than in the dry season. Hence, the high FFA content in the wet season was a reflection of moisture-facilitated lipid oxidation. Earlier findings by Labuza and Dugan (1971) revealed a strong positive correlation between FFA and lipid oxidation development. Similarly, in this study, the FFA were positively correlated with the observed PV and TBARs (Figures 5 and 6). Regardless of the season, FFA content was significantly associated with primary and much less with secondary oxidation (Figures 5 and 6). Aidos (2002) and Labuza and Dugan (1971) pointed out that FFA are more susceptible to oxidation than esterified acids and reflect commencement of lipid spoilage. Similarly, the high FFA value in the unsalted, freshly processed *R. argentea* may reflect fast lipid spoilage and susceptibility to oxidation as compared to the salted samples. Salting seems to have had an immediate effect of dehydrating freshly processed *R. argentea* and reducing water activity. These findings mirror those reported by Nambudiry (1980), where an increase in salt led to a decrease in FFA of sardine muscle tissue. In contrast, salting was found to increase lipid oxidation in the Indonesian salted-dried marine catfish (Smith, Hole, and Hanson 1990), anchovies (Hernández-Herrero et al. 1999), cod (Nguyen et al. 2012) and bacon (Jin et al. 2010). Irrespective of the season and drying method, PV content was fairly stable for the first 60 days of R. argentea storage, and in harmony with TBARs content. This can be

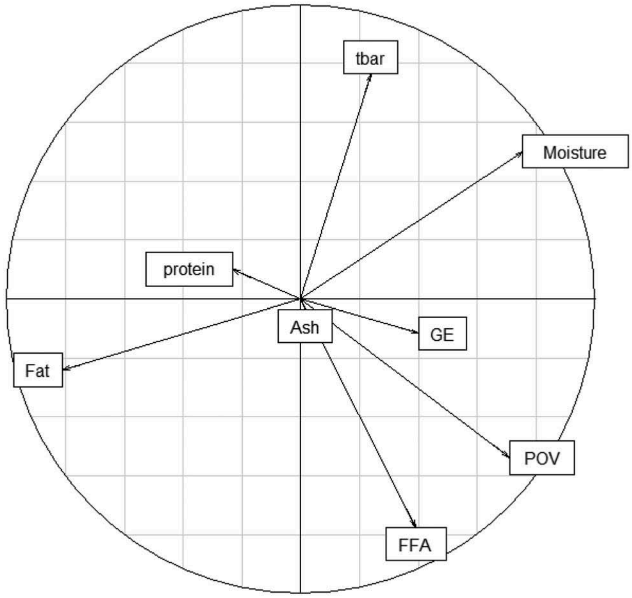

Figure 5. Overall principle component analysis (PCA) bi-plot of scores and loadings for different parameters of *R. argentea*. (n = 3) dried by artisan and adopted method in the wet season.

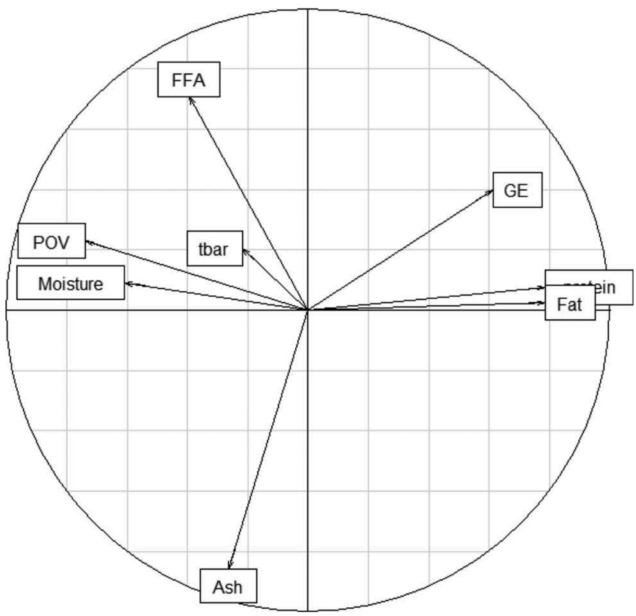

Figure 6. Overall principle component analysis (PCA) bi-plot of scores and loadings for different parameters of *R. argentea*. (n = 3) dried by artisan and adopted method in the dry season.

attributed to simultaneous and fairly equal rates of hydrogen peroxide formation and breakdown into secondary products (Nguyen et al. 2012). Indeed, freshly dried *R. argentea* (particularly the half-dried) exhibits off-flavors that are characteristic of secondary lipid peroxidation (Antolovich et al., 2002; NRC 2011), suggesting that peroxides are simultaneously converted into volatile ketones and aldehydes (Antolovich et al., 2002; Frankel 1984). A considerable increase in PV content of dried *R. argentea* by day 90 in storage may reflect high peroxide formation and simultaneously low conversion to secondary products, which is in agreement with the findings by Nguyen et al. (2012).

Unexpectedly, TBARs were much higher than PV in freshly dried *R. argentea*, whose concentration increased later and dropped during storage. It is not clear whether this surge in PV and TBARs is attributable to variability in processing methods, storage duration, contamination or sampling, and measurement errors. However, this phenomenon may suggest that substantial primary oxidation occurs in *R. argentea* during sun drying to generate a considerable amount of peroxides that are subsequently converted to secondary products that build up as the product dries and in storage. Indeed, the unexpectedly high TBARs levels and conversely low PV in freshly dried *R. argentea* are evidence of secondary lipid oxidation. The second upward surge in PV vs. low TBARs could reflect a reproducible peak in oxidation (Aidos 2002; Aidos et al. 2001) and/or the occurrence of lipid oxidation through separate pathways (Antolovich et al., 2002). It has been suggested that lipid oxidation occurs through different pathways: as a (1) random non-enzymatic free radical-mediated chain reaction, (2) non-enzymatic, non-radical photo-oxidation, or (3) specific enzymatic reaction (Antolovich et al., 2002; Niki et al. 2005). Along these three pathways, lipid oxidation occurs at different speeds (Antolovich et al., 2002); hence, we suspect that different pathways generated the observed trends in the PV and TBARs of *R. argentea* during storage. Overall, salted *R. argentea* was more oxidized than the unsalted regardless of drying method and storage duration, probably due to the presence of inorganic and organic contaminants that facilitate lipid oxidation (Nguyen et al. 2012; Niki et al. 2005). Indeed, Hansen, Skibsted, and Andersen (1996) and Nguyen et al. (2012) indicated that the anticaking compounds used in table salt, e.g., potassium ferrocyanide, facilitate lipid oxidation in fish muscles.

Changes in proximate composition

The dry matter content of *R. argentea* generally decreased with storage time, regardless of drying method. Ash content was influenced by drying method and less by season and storage time; being higher in *R. argentea* dried on bare ground or rock surfaces than in that dried on meshes laid on the ground or raised racks. As expected, salted *R. argentea* had higher ash content than unsalted. Crude protein and EE of dried *R. argentea* were affected by processing methods, storage time and season, whereas GE was least affected.

These results suggest a strong interactive effect of drying methods, storage time and season on the lipid and crude protein content of *R. argentea*. An earlier study by Ladikos and Lougovois (1990) revealed that processing methods and storage conditions interact to affect the rate of lipid oxidation in processed fish. In agreement, Castell (1971) reported that processing methods and storage conditions lead to the formation of lipid-protein complexes, thereby reducing their extractable proportions and digestibility when included in diets. The oxidized fatty acids can also react with proteins to form lipid-protein polymers (Ladikos and Lougovois 1990), resulting in a decrease in both EE and CP. Hence, the observed decrease in lipid and protein content of processed *R. argentea* could be attributed to complex formation. Ladikos and Lougovois (1990) further asserted that lipid hydroperoxides and their secondary products interact with protein or amino acids to impact flavor stability during processing, cooking, and storage. Moreover, a blend of suitable temperature, pH and moisture influences the interaction between protein, the reactive oxygen species (ROS, i.e., the radicals) and secondary oxidation products of lipid oxidation (Hall 1987). Through non-covalent bonds, some volatile compounds react with proteins to form complexes that increase their stability and subsequently reduce the extractable protein. The radical-induced cross-linkages or scission of proteins lead to nutritional losses (Ladikos and Lougovois 1990). The primary products of lipid oxidation (the hydroperoxide radicals) particularly react with sulfur and amine functional groups of the amino acids, while the secondary products (the aldehydes and epoxides) react with the thiols from cysteine (Gardner 1979; Wu, Zhang, and Hua 2009). Nutrient loss and organoleptic changes can also occur through non-enzymatic browning (maillard) (Ladikos and Lougovois 1990). In this study, salted and indirectly dried *R. argentea* developed brown coloration during processing and storage, probably due to non-enzymatic nutrient loss. In salted cod, color changes were attributed to lipid oxidation-related changes in the chemical composition of fish muscle (Nguyen et al. 2012). In heavily salted cod fillets, yellowness/browning was attributed to reactions between lipid oxidation products and protein components aided by reduced copper (Lauritzsen, Martinsen, and Olsen 1999).

Conclusion

The present findings show that oxidative stability and proximate composition of *R. argentea* are influenced by season, processing methods and storage time. Substantial lipid oxidation occured in the wet season, more so when *R. argentea* was dried on bare ground and rock surfaces. Drying on raised racks was associated with minimal lipid oxidation and consistent proximate composition. Therefore, we recommend the adoption of raised racks by artisan processors for effective drying of *R. argentea*. Additional treatment of *R. argentea* with antioxidants to stabilize lipids before drying on raised racks should be considered.

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Disclosure statement

None of the authors had conflict of interest in the study and data presented in this manuscript.

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