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Lipid Oxidation and Fatty Acid Composition in Salt-Dried Yellow Croaker (*Pseudosciaena polyactis*) During Processing

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Abstract Lipid oxidation in salt-dried yellow croaker (*Pseudosciaena polyactis*) was evaluated during processing with commonly used analytical indices, such as the peroxide value (POV), the thiobarbituric acid reactive substances (TBARS) value, and oxidative-relative lipoxygenase (LOX) activity. Additionally, fatty acids were analyzed using gas chromatography-mass spectrometry. Both POV and TBARS increased significantly ($P<0.05$) at the rinsing stage. POV reached its peak value of 3.63 meq O_2 per kg sample at the drying stage, whereas TBARS constantly increased from 0.05 to 0.20 mg MDA per kg sample. Processing of salt-dried yellow croaker had an extremely significant ($P<0.01$) effect on LOX activity. Twenty-six fatty acids were identified. Combined eicosapentaenoic acid (EPA; C20:5n3) and docosahexaenoic acid (DHA; C22:6n3) content varied between (19.20 ± 0.37) mg g^{-1} and (23.45 ± 1.05) mg g^{-1}. The polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio in yellow croaker was 0.73–1.10, and the n-6/n-3 PUFA ratio was approximately 0.13–0.20. The contents of most fatty acids varied significantly ($P<0.05$) during the different processing stages, and these differences were caused by lipid oxidation. C18:0, C16:1n7, C19:0, and C22:6n3 showed clear changes in principle component one of a principle components analysis. These fatty acids are potential markers for evaluating lipid oxidation in fish muscle because there was a significant correlation between these markers and TBARS and LOX activity ($P<0.05$) with Pearson's coefficients >0.931.

Key words salt-dried yellow croaker (*Pseudosciaena polyactis*); processing; lipid oxidation; fatty acids

1 Introduction

Small yellow croaker (*Pseudosciaena polyactis*) is a warm-temperate fish species widely distributed in the Yellow Sea and northern East China Sea. This fish is processed into various types of food products, such as salt-dried fish, stewed fish, and others. Salt-dried fish is a traditional seafood product in China and is popular because of its unique flavor. This fish is prepared by marinating and air dry ripening (Chung *et al*., 2007; Wu *et al*., 2013). Even today, production methods are based on workers' experience, and little is known about the biochemical changes that occur in the fish muscle. Researchers have primarily focused on detecting nitrite and nitrosamines in salt-dried fish, which may form carcinogenic nitrosamines during salting (Wu *et al*., 2012). However, other aspects, such as lipid oxidation, have been overlooked. Fish is a primary source of omega-3 (n-3) polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA; C22:6n3) and eicosapentaenoic acid (EPA; C20:5n3), which have many health benefits for humans (Arab-Tehrany *et al*., 2012). However, PUFAs are susceptible to physicochemical changes due to lipid oxidation. Such changes can cause sensorial and nutritional deterioration of the fish meat (Farvin *et al*., 2012), which can be a health concern. There is growing evidence of a correlation between lipid oxidation products and chronic diseases, such as asthma, atherosclerosis, Alzheimer's, and rheumatoid arthritis (Spiteller, 1998). Many studies on lipid oxidation have focused on the degree of primary oxidation by measuring the peroxide value (POV), which indicates the production of hydrogen peroxides (Capuano *et al*., 2010). Another method is to quantify secondary oxidation products by measuring thiobarbituric acid reactive substances (TBARS) (Rød *et al*., 2012). Lipoxygenase (LOX; EC 1.13.11.12), which catalyzes the oxygenation of polyunsaturated acids containing a cis,cis-1,4-pentadiene moiety to produce hydroperoxides, has been assumed to play an important role

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in lipid oxidation and in the occurrence of volatile compounds in foods (Jin *et al*., 2011). As the free fatty acids produced by lipolysis are the primary precursors of volatile compounds (Morita *et al*., 2003; Gianelli *et al*., 2012), the relationship between lipid oxidation and fatty acids is worth studying. Some studies about lipid oxidation with respect to the processing of salt-dried meat products, including dried cured goose (Wang *et al.*, 2016) and Cantonese sausage (Qiu *et al.*, 2013), have been conducted, but no similar studies have been conducted on salt-dried fish. Moreover, comprehensive analyses combining the study of lipid oxidation and fatty acids have rarely been carried out. Therefore, the objective of this study was to evaluate lipid oxidation using analytical indices (POV, TBARS, and LOX) and to profile the fatty acids formed in salt-dried yellow croaker during processing using gas chromatography-mass spectrometry (GC-MS).

2 Materials and Methods

2.1 Materials and Reagents

Yellow croakers, with an average weight of 500 g (±20 g), were purchased from Vanguard Supermarket (Guangzhou, China). HPLC-grade boron trifluoride-methanol (14%, w/w), *n*-hexane, and the internal standard undecanoic acid (C11:0, purity > 99%) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Standard C_7–C_{40} straight-chain hydrocarbons (purity > 96%) were obtained from Anpel Laboratory Technologies Inc. (Shanghai, China). All other chemicals were of analytical grade and were acquired from the Guangzhou Chemical Reagent Factory (Guangzhou, China).

2.2 Sampling

The fish were stored at 0℃ within 48 h after death to maintain freshness. The fish were taken to the lab, scaled, and their internal organs were removed. They were prepared according to the method by Wu *et al.* (2013). Briefly, the fish muscle was cured with 20 g per 100 g coarse salt, marinated with saturated brine for 3 d at 20℃, and rinsed with fresh water for 6 h. Next, all fish samples were dried at 28℃ (±2℃) and 65% RH for 3 d. The five samples obtained during processing were: 1) minced raw material without salt and no processing (P); 2) minced material obtained after the salting stage and 2 days of processing (PY); 3) minced material obtained after the rinsing stage and 2.5 d of processing (PP); 4) minced material obtained at the drying stage and 4 days of processing (PG); and 5) minced material obtained at the end of processing (5 d) (PC). Minced material (150 g) from each stage was sampled for analyses. The minced raw materials contained 68.28% water, 0.21% salt, and 12.29% fat, and those obtained at the end of the processing contained 46.16% water, 5.62% salt, and 9.88% fat.

2.3 Lipid Oxidation Measurements

POV, which indicates the formation of primary lipid oxidation products, was measured by colorimetric determination using the ferric thiocyanate assay, according to the guidelines outlined in the National Standards of the People's Republic of China (GB/T 5009.37-2003). POV was expressed as milliequivalents of O_2 per kg of sample (meq O_2 per kg sample). TBARS was measured using the method described by Vyncke (1975) with minor modifications. 1,1,3,3-Tetraethoxypropane was used as the malondialdehyde (MDA) standard. The results are expressed as mg MDA per kg of sample (mg MDA per kg sample).

LOX was extracted according to the method described by Gata *et al.* (1996), and protein content was measured using the biuret method. LOX activity was determined using a UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan) at 20℃ by following the increase of absorbance at 234 nm for 1 min. Linoleic acid solution (2.8 mg mL^{-1}; Sigma, St. Louis, MO, USA) was used as the substrate for enzyme activity assay. One unit (U) of LOX activity was defined as the amount of enzyme required for an absorption increase of 0.001 min^{-1} (mg protein)$^{-1}$.

2.4 Fatty Acid Profiling

Lipids were extracted from the samples using a modified Folch's chloroform-methanol extraction method (Folch *et al.*, 1957). Briefly, each sample contained 50 mg of the internal standard undecanoic acid (C11:0) and was treated with a chloroform-methanol mixture (2:1), homogenized, and filtered. Sodium chloride (NaCl) solution (0.85%, 5 mL) was added to the filtrate, and the filtrate was centrifuged at 3000 r min^{-1} for 15 min. After centrifugation, the upper phase was discarded and the remaining organic phase was evaporated using the N-EVAP system (Organomation, Berlin, MA, USA) to obtain the total lipid (TL) extract. Fatty acids in the TL extract were transesterified to fatty acid methyl esters (FAMEs) using a boron-trifluoride (14% methanol, w/w)-catalyzed esterification protocol reported by Eymard *et al.* (2009).

FAMEs were measured using a GC-MS (QP-2010, Shimadzu). Samples (1 μL) were injected in split mode (20:1) onto a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm; Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas at a flow rate of 0.5 mL min^{-1}. Temperature was set to rise from 35℃ to 230℃ at a rate of 5℃ min^{-1}, and injector temperature was 250℃. The quantities of the fatty acids were calculated using the following equation:

$$\text{Content of fatty acids (mg g}^{-1}) = (\text{Peak area ratio (Fatty acid/C11:0) 50 mg}) (5\text{ g})^{-1}.$$

The fatty acids were calibrated using standard C_7–C_{40} straight-chain hydrocarbons (purity > 96%) (Anpel Laboratory Technologies Inc., Shanghai, China). Retention times were converted into Kováts index (KI) values and

2.5 Statistical Analysis

All experiments were performed in triplicates (n = 3).

identified by comparing their mass spectra with those in the NIST05 database (National Institute of Standards and Technology, Gaithersburg Library, Gaithersburg, MD, USA). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using JMP 15.0 software (SAS Institute, Cary, NC, USA). Significant differences among the five samples were analyzed using Tukey's HSD test. The relationships between the primary fatty acids and lipid oxidation indices (POV and TBARS) and LOX activity were analyzed using the Pearson's correlation coefficient analysis, and the fatty acid data were subjected to principal components analysis (PCA). The Pearson's correlation coefficient analysis and PCA were performed using software SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Figs.1–3 were drawn using OriginPro 8.5 software (OriginLab Inc., Northampton, MA, USA).

3 Results and Discussion

3.1 Lipid Oxidation of Salt-Dried Yellow Croaker During Processing

Fish lipid oxidation is normally evaluated based on analytical indices such as POV and TBARS as indicators of primary and secondary oxidation products, respectively (Pourashouri *et al.*, 2009; Yerlikaya and Gokoglu, 2010). As shown in Fig.1, POV indicated a very low oxidation level (0.44 meq O_2 per kg sample) in P. Then, POV index significantly increased ($P<0.05$) at PP, and reached its peak value of 3.63 meq O_2 per kg sample at PG; however, it significantly decreased ($P<0.05$) at PC. POV measures the formation of peroxide or hydroperoxide groups, which are initial products of lipid oxidation. Hydroperoxides are unstable during the processing of meat products, and they decompose to form volatile compounds (Tian and Dasgupta, 1999; Qiu *et al*., 2013). However, POV is not always useful for marine lipids because it increases during the initial stage of oxidative deterioration, reaches a peak, and then decreases during processing (Saito and Udagawa, 1992). Therefore, another index (TBARS) is needed to evaluate lipid oxidation. The results for TBARS index (mg MDA per kg sample) are summarized in Fig.2. TBARS constantly increased until PC, but significantly increased ($P<0.05$) during PP, when the value increased from 0.05 to 0.20 mg MDA per kg sample, demonstrating that lipid oxidation products accumulated during processing. TBARS values for all samples were <1 mg MDA per kg sample; above this level, lipid oxidation may result in rancidity of the meat products (Alfaia *et al*., 2010).

The results of one-way ANOVA indicate that the processing of salt-dried yellow croaker had an extremely significant ($P<0.001$) effect on LOX activity. Fig.3 shows that LOX activities rapidly decreased from P to PP ($P<0.05$), and gradually decreased ($P>0.05$) between PP and PG stages, but then significantly decreased ($P<0.05$) at PC. Our results disagree with reports on other dry-cured meat products (Jin *et al.*, 2010; Wang *et al*., 2016), where LOX activities rapidly increased during curing and then decreased during drying ($P<0.01$) because the pro-oxidative effect of the salt can increase LOX activity (Devatkal and Naveena, 2010). However, only 4%–8% salt (w/w) was added in those cases, whereas 20% was added in our case; higher salt content may have been responsible for the decrease in LOX activity.

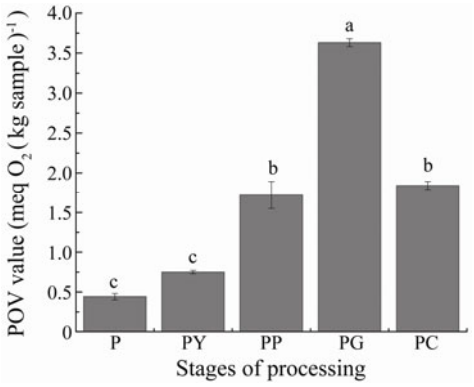

Fig.1 Changes in POV during salt-dried processing of yellow croaker (*Pseudosciaena polyactis*). Letters indicate significant differences between the different stages of processing ($P<0.05$).

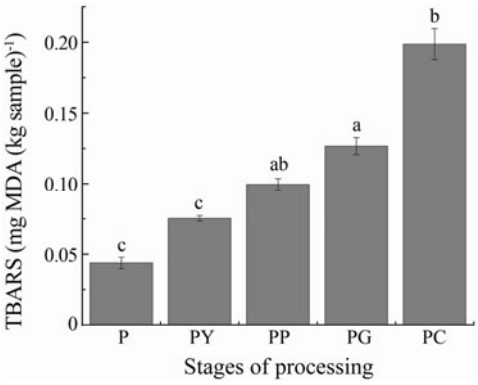

Fig.2 Changes in TBARS value during salt-dried processing of yellow croaker (*Pseudosciaena polyactis*). Letters a–c indicate significant differences between the different stages of processing ($P<0.05$).

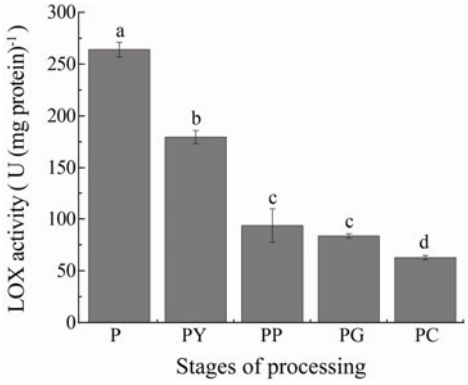

Fig.3 Changes in LOX activity during salt-dried processing of yellow croaker (*Pseudosciaena polyactis*). Letters a–d indicate significant differences between the different stages of processing ($P<0.05$).

Overall, POV observed in our study was higher than

that generally reported for salted herring (*Clupea harengus*) (0.4–1.1 meq O_2 per kg fish) during processing (Andersen *et al*., 2007). In this study, TBARS values were lower than those in horse mackerel (Giménez *et al*., 2011). Moreover, no undesirable odor was detected in our study.

3.2 Changes in Fatty Acid Composition During Processing

Standard C_7-C_{40} straight-chain hydrocarbons (100 mg L^{-1}) were used to ascertain the KI value of each fatty acid. Only $C_{15}-C_{30}$ straight-chain hydrocarbons gave the expected values, and the chromatogram of the standards is shown in Fig.4. $C_{15}-C_{30}$ hydrocarbons had retention times of 4.963–39.254 min, whereas the retention times of the fatty acids detected in this study were 10.413–39.194 min, which fell within those of the standard values. Therefore, the KI values of all fatty acids could be calculated and compared with those in the NIST05 database for accurate determination of identity.

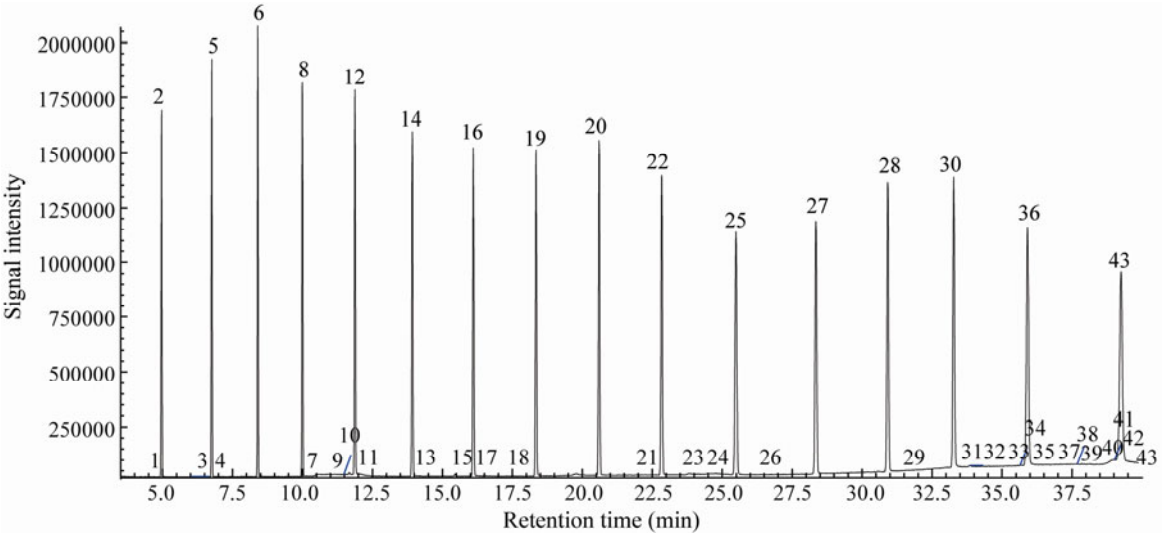

Fig.4 Chromatogram of $C_{15}-C_{30}$ straight-chain hydrocarbons standards (100 mg L^{-1}) using GC-MS. The peaks numbered 2, 5, 6, 8, 12, 14, 16, 19, 20, 22, 25, 27, 28, 30, 36, and 43 in the chromatogram correspond to $C_{15}-C_{30}$ from left to right.

The fatty acid composition in salt-dried yellow croaker during processing is shown in Table 1. Twenty-six fatty acids were identified. The most abundant fatty acids were palmitic acid (C16:0), oleic acid (C18:1n9), and docosahexaenoic acid (DHA) (C22:6n3), which is in accordance with the results of Schneedorferová *et al.* (2015), who reported the aforementioned fatty acids as the major fatty acids in all studied fish species. Yellow croaker was rich in nutrients and contained both linoleic acid (C18:2n6) and linolenic acid (C18:3n3), which are essential for humans. Furthermore, the combined eicosapentaenoic acid (EPA) (C20:5n3) and C22:6n3 content varied between (19.20 ± 0.37) mg g^{-1} and (23.45 ± 1.05) mg g^{-1}. In addition, nervonic acid (NA; C24:1n9) has been proposed as an essential nutrient for neonatal development in humans. Both NA and DHA are incorporated in large amounts in structural lipids in the developing central nervous system (Bettger *et al.*, 2003). A higher PUFA/SFA ratio in meat has been recommended by nutritionists in view of its implications for human health (Liu *et al*., 2013). The PUFA/SFA ratio in yellow croaker was 0.73–1.10, which is higher than the recommended level of 0.4–0.5 (FAO/WHO, 1994). Although the PUFA/SFA ratio for yellow croaker was slightly higher than that for pork and lamb, the n-6/n-3 PUFA ratio was approximately 0.17, which is much lower than that for pork and lamb (Jiang *et al*., 2015; Botsoglou *et al*., 2014). Moreover, nutritional guidelines recommend that this ratio should be less than 4.0 (Simopoulos, 2003). The lower n-6/n-3 PUFA ratio is attributed to the fact that yellow croaker is rich in n-3 PUFAs, such as EPA and DHA. There is increasing strong evidence linking long-chain (LC) n-3 PUFA intake with cardiovascular health, and the beneficial effects of increased LC n-3 PUFA intake on the early development of the visual system have also been reported (Williams and Graham, 2006). PUFA damage, as measured by the polyene index (PI), had a higher range (1.12–1.48) in samples during processing than that reported for salmon (Aubourg *et al.*, 2005) and gilthead sea bream (Aubourg *et al.*, 2010). A decrease in the PI values from PP to PC indicates the decomposition of PUFAs. With such a decrease, POV and TBARS, which are the products of primary and secondary oxidation, usually increase.

Table 1 also shows the changes in the fatty acid composition during salt-dried processing of yellow croaker. One-way ANOVA showed that processing had an extremely significant ($P < 0.001$) effect on total SFA (∑SFA), total monounsaturated fatty acids (∑MUFA), total PUFA (∑PUFA), and on nonadecanoic (C19:0), tricosanoic (C23:0), and C22:6n3, as well as a highly significant ($P < 0.01$) effect on stearic (C18:0), palmitoleic (C16:1n7), C18:1n9, eicosadienoic (C20:2n7), C18:2n6, and arachidonic (C20:4n6) fatty acids. Additionally, the results of Tukey's HSD test showed significant differences in the amounts of the aforementioned nine fatty acids at different stages of processing ($P <$

0.05). ∑SFA significantly increased ($P<0.05$) during PP, but ∑MUFA and ∑PUFA significantly decreased ($P<0.05$) during PY and PG, respectively. This result shows that processing promotes the oxidation of unsaturated fatty acids (MUFAs and PUFAs), whose total content decreased, whereas that of SFAs increased. The decrease in unsaturated fatty acids as flavor precursors may result from the promotion of oxidation. This result is consistent with the significant increase in POV and TBARS during PP. Yang *et al.* (2005) suggested that the decrease in PUFAs during processing may result in the formation of volatile compounds in Xuanwei ham. Referring to the aforementioned nine fatty acids, the content of C18:0 and C19:0 significantly increased ($P<0.05$) at PC, whereas that of C23:0 significantly increased ($P<0.05$) during PY. The amount of C16:1n7, C18:1n9, and C22:6n3 significantly decreased ($P<0.05$) at PP, PC, and PY, respectively. The remaining three acids initially increased and then significantly decreased ($P<0.05$). C20:2n7 significantly increased at PP, and both C18:2n6 and C20:4n6 significantly increased ($P<0.05$) at PY. Subsequently, the amount of C20:2n7 and C20:4n6 significantly decreased ($P<0.05$) at PG, whereas that of C18:2n6 significantly decreased ($P<0.05$) at PP. Fu *et al.* (2015) reported that all drying processes largely decrease the relative contents of C20:5n3 and C22:6n3 ($P<0.05$) in silver carp slices. Anggo *et al.* (2015) investigated anchovy (*Stolephorus* sp.) and reported that almost all fatty acids decreased during salting fermentation except C18:0, C20:5n3, and C22:6n3.

Table 1 Fatty acid composition of salt-dried yellow croaker (*Pseudosciaena polyactis*) during processing

Fatty acid	Retention time (min)	KI	SIG	Fatty acid content (mg g^{-1})				
				P	PY	PP	PG	PC
C12:0	10.413	1493	n.s.	0.08±0.01^{a}	0.06±0.01^{a}	0.06±0.00^{a}	0.05±0.00^{a}	0.07±0.01^{a}
C13:0	12.344	1591	n.s.	0.03±0.01^{a}	0.04±0.01^{a}	0.85±0.32^{a}	2.23±1.15^{a}	1.78±1.03^{a}
C14:0	14.442	1689	n.s.	2.79±0.28^{a}	2.63±0.16^{a}	3.40±0.35^{a}	3.63±0.31^{a}	4.23±0.55^{a}
C15:0	16.656	1786	n.s.	0.58±0.06^{a}	0.56±0.02^{a}	0.72±0.03^{a}	0.55±0.05^{a}	0.67±0.04^{a}
C16:0	18.950	1884	n.s.	19.83±0.82^{a}	19.06±0.37^{a}	19.85±0.11^{a}	21.71±0.25^{a}	20.12±0.07^{a}
C17:0	21.178	1981	n.s.	1.43±0.71^{a}	1.70±0.35^{a}	1.63±0.14^{a}	1.57±0.33^{a}	1.57±0.17^{a}
C18:0	23.482	2079	**	7.41±0.64^{c}	7.84±0.30^{c}	9.07±0.01^{bc}	10.29±0.57^{ab}	11.03±0.46^{a}
C19:0	26.298	2177	***	0.17±0.03^{b}	0.22±0.01^{b}	0.39±0.10^{b}	0.23±0.01^{b}	0.89±0.11^{a}
C23:0	29.087	2573	***	0.32±0.02^{c}	0.50±0.00^{a}	0.58±0.01^{a}	0.42±0.01^{b}	0.58±0.04^{a}
C24:0	31.483	2671	ND	ND	0.03±0.00	0.03±0.00	ND	0.03±0.00
C26:0	33.859	2868	ND	ND	ND	0.20±0.01	0.15±0.02	0.15±0.01
C14:1n3	14.159	1687	n.s.	0.08±0.01^{a}	0.06±0.00^{a}	0.05±0.00^{a}	0.05±0.01^{a}	0.05±0.01^{a}
C16:1n10	20.088	1890	n.s.	0.71±0.02^{a}	0.92±0.18^{a}	1.01±0.01^{a}	0.83±0.31^{a}	0.91±0.08^{a}
C16:1n7	18.428	1882	**	11.58±0.38^{a}	10.54±0.28^{ab}	9.53±0.64^{b}	9.58±0.36^{b}	9.22±0.19^{b}
C18:1n9	22.940	2085	**	18.49±0.42^{a}	17.97±0.49^{a}	17.36±0.27^{ab}	18.22±0.04^{a}	16.29±0.22^{b}
C19:1n9	25.704	2175	ND	ND	ND	0.13±0.03	ND	0.14±0.04
C20:1n9	28.333	2478	n.s.	2.05±0.38^{a}	1.47±0.47^{a}	1.62±0.13^{a}	1.02±0.02^{a}	1.19±0.12^{a}
C24:1n9	39.194	2676	n.s.	0.24±0.04^{a}	0.26±0.04^{a}	0.32±0.01^{a}	0.19±0.02^{a}	0.32±0.00^{a}
C20:2n7	28.155	2478	**	0.15±0.02^{b}	0.14±0.01^{b}	0.22±0.01^{a}	0.15±0.01^{b}	0.18±0.01^{ab}
C20:3n7	27.576	2476	ND	ND	0.13±0.00	0.18±0.03	0.11±0.03	0.12±0.02
C18:2n6	22.642	1988	**	1.51±0.03^{bc}	2.64±0.27^{a}	1.53±0.04^{b}	1.43±0.05^{bc}	1.00±0.03^{c}
C20:4n6	26.837	2474	**	2.37±0.09^{b}	3.45±0.10^{a}	3.13±0.05^{a}	2.43±0.10^{b}	3.07±0.27^{a}
C18:3n3	18.097	1795	n.s.	0.33±0.13^{a}	0.41±0.06^{a}	0.37±0.03^{a}	0.42±0.01^{a}	0.42±0.07^{a}
C20:4n3	27.733	2476	n.s.	0.70±0.02^{a}	0.78±0.08^{a}	0.84±0.04^{a}	0.74±0.06^{a}	0.81±0.09^{a}
C20:5n3	27.171	2475	*	10.38±0.56^{ab}	10.93±0.28^{a}	10.23±0.42^{ab}	8.98±0.27^{b}	9.85±0.17^{ab}
C22:6n3	29.784	2523	***	18.85±0.21^{a}	17.50±0.29^{b}	16.74±0.07^{c}	15.52±0.11^{d}	15.37±0.00^{d}
∑SFA			***	32.61±0.23^{c}	32.62±0.10^{c}	36.76±0.06^{b}	40.78±0.45^{a}	41.11±0.25^{a}
∑MUFA			***	33.14±0.37^{a}	31.21±0.16^{b}	30.02±0.47^{bc}	29.87±0.13^{c}	28.11±0.21^{d}
∑PUFA			***	34.26±0.15^{b}	35.97±0.54^{a}	33.23±0.53^{b}	29.76±0.01^{c}	30.79±0.04^{c}
PUFA/SFA				1.05±0.00	1.10±0.02	0.90±0.02	0.73±0.01	0.75±0.01
n-6/n-3 PUFA ratio				0.13±0.01	0.20±0.01	0.17±0.00	0.15±0.00	0.15±0.01
PI				1.48±0.06	1.49±0.07	1.35±0.07	1.12±0.08	1.25±0.19

Notes: Different superscript letters indicate significant differences among the different stages of processing within a row ($P<0.05$), $n=3$; ND=not detected; SIG: Significance levels: * $P<0.05$, ** $P<0.01$, *** $P<0.001$; n.s.=not significant; KI: Kováts index. The polyene index (PI):PI=(C20:5+C22:6)/C16:0 was used as a measure of PUFA damage (Šimat *et al.*, 2015).

3.3 Correlations Between the Primary Fatty Acids and Lipid Oxidation Indices During Processing

The correlation analyses of the primary aforementioned nine fatty acids with the lipid oxidation indices (POV and TBARS) and LOX activity are shown in Table 2. C18:0, C16:1n7, C19:0, and C22:6n3 showed the highest correlations. The Pearson's correlation coefficients obtained between the formation of C18:0 and TBARS index and LOX activity were 0.961 ($P<0.01$) and −0.909 ($P<0.05$), respectively; those between the formation of C16:1n7 and C22:6n3 and LOX activity

Table 2 Pearson's correlation coefficients between the primary fatty acids and lipid oxidation indices

	P	T	L	f1	f2	f3	f4	f5	f6	f7	f8	f9
P	1											
T	0.561	1										
L	−0.740	−0.844	1									
f1	0.763	0.961**	−0.909*	1								
f2	−0.708	−0.836	0.999**	−0.903*	1							
f3	−0.104	−0.833	0.702	−0.694	0.722	1						
f4	0.106	0.312	−0.528	0.325	−0.551	−0.574	1					
f5	−0.419	−0.645	0.432	−0.664	0.410	0.463	−0.441	1				
f6	−0.313	0.121	−0.273	−0.012	−0.318	−0.506	0.270	0.513	1			
f7	0.134	0.906*	−0.648	0.741	−0.68-59	−0.971**	0.453	−0.590	0.327	1		
f8	0.077	0.566	−0.713	0.481	−0.748	−0.845	0.679	−0.058	0.805	0.695	1	
f9	−0.817	−0.883	0.977**	−0.956	0.967**	0.640	−0.356	0.459	−0.154	−0.627	−0.584	1

Notes: P, POV; T, TBARS value; L, LOX activity; f1–f9 C18:0, C16:1n7, C18:1n9, C20:2n7, C18:2n6, C20:4n6, C19:0, C23:0, and C22:6n3; ** significant correlation at the 0.01 level (double-sided); * significant correlation at the 0.05 level (double-sided).

were 0.999 ($P<0.01$) and 0.977 ($P<0.01$), respectively; and those between the formation of C19:0 and TBARS index was 0.906 ($P<0.05$). No correlation was observed between POV and composition of any fatty acid, which indicates that POV may not be a good index for revealing lipid oxidation in salt-dried fish during processing. According to these results, C18:0, C16:1n7, C19:0, and C22:6n3 were chosen as the best markers of lipid oxidation. Because of the high influence ($P<0.01$ and $P<0.001$) of processing and the high levels of C18:0, C16:1n7, C19:0, and C22:6n3 formed during salt-dried processing, along with high correlations with the chemical indices (TBARS and LOX activity) for assessing the extent of oxidation, these compounds are potential markers for the evaluation of lipid oxidation in salt-dried fish muscle. The basis of this evaluation was similar to that reported by Iglesias and Medina (2008).

3.4 PCA of Fatty Acids

PCA is an effective mathematical method that reduces the dimensionality of multivariate data, while preserving most of the variance (Jin *et al.*, 2015). In this study, two principal components (PCA1 and PCA2) were extracted, and accounted for 91.25% of the total variance in the data. Fig.5 represents the loading plots for variations in the nine aforementioned fatty acids detected in salt-dried fish samples at four different stages of processing (PY, PP, PG, and PC). The first component, PCA1, represented 52.99% of the total variance, and was positively associated with the amount of the following fatty acids: C18:0, C19:0, C23:0, and C20:2n7 and negatively associated with C16:1n7, C18:1n9, C20:4n6, C18:2n6, and C22:6n3. The second component, PCA2, explained an additional 38.26% of the total variance, and was positively linked to C19:0, C23:0, C20:2n7, C20:4n6, C18:2n6, and C22:6n3, and negatively associated with the remaining three fatty acids.

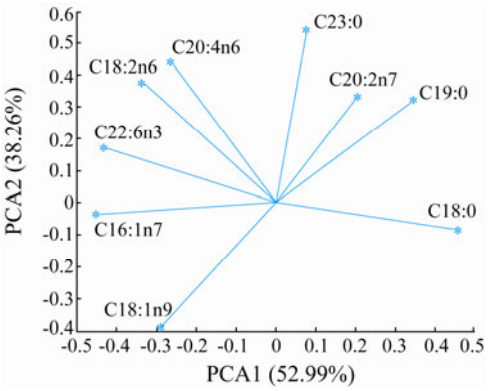

Fig.5 Principal components analysis (PCA1 and PCA2) of salt-dried fish samples at different stages of processing (PY, PP, PG, and PC).

The eigenvalues, proportion of variation (nine fatty acids), and scores of the samples at the four stages of processing obtained using PCA are shown in Tables 3 and 4. On PCA1, the different salt-dried fish samples were clearly separated during the processing steps. Furthermore, the scores of the samples increased from −2.93 to 2.26 and were arranged from low to high as: PY<PP<PG<PC, which is the same order as the processing order. Therefore, clear differences were observed between the samples as processing progressed. The variations with positive eigenvectors were C18:0, C19:0, C23:0, and C20:2n7, which showed that these fatty acids increased as processing progressed; moreover, these were mostly SFAs. The variations with negative eigenvectors were C16:1n7, C18:1n9, C20:4n6, C18:2n6, and C22:6n3, which revealed that these fatty acids decreased as processing progressed; moreover, all of them were unsaturated fatty acids, primarily PUFAs. These results indicated that SFAs clearly increased, while MUFAs and PUFAs decreased during processing, which is highly consistent with the results of one-way ANOVA and Tukey's HSD test analyses. In addition, the fatty acids that showed marked changes were C18:0, C19:0, C16:1n7, and C22:6n3; these had the highest correlations with TBARS lipid oxidation index and oxidation-related enzyme LOX according to the Pearson's correlation coefficient analysis. Thus, the significant changes in these four fatty acids were caused by lipid oxidation, and they could become potential markers for evaluating the lipid oxidation of salt-dried yellow croaker during processing.

Table 3 Eigenvectors of variation with each principal component

Variation characters	Eigenvectors of PCA1	Eigenvectors of PCA2
Stearic (C18:0)	0.448	−0.079
Nonadecanoic (C19:0)	0.337	0.316
Tricosanoic (C23:0)	0.082	0.530
Palmitoleic (C16:1n7)	−0.445	−0.045
Octadecenoic (C18:1n9)	−0.291	−0.399
Eicosadienoic (C20:2n7)	0.179	0.318
Arachidonic (C20:4n6)	−0.262	0.433
Linoleic (C18:2n6)	−0.332	0.369
DHA (C22:6n3)	−0.430	0.168

Table 4 Scores of samples with each principal component

Sample characters	Scores of PCA1	Scores of PCA2
PY	−2.93	0.26
PP	−0.08	1.16
PG	0.74	−2.68
PC	2.26	1.26

4 Conclusions

Lipid oxidation during the processing of salt-dried fish products has rarely been considered, and there have been few comprehensive analyses that combined the study of lipid oxidation and composition of fatty acids. The most abundant fatty acids were identified during each processing stage and nutritive values of the lipids, including the PUFA/SFA and n-6/n-3 PUFA ratios and PI values, were investigated. The effects of salt-dried processing on the lipid oxidation indices and fatty acids, as well as significant differences among the stages of processing, were identified. The correlations between the nine primary fatty acids and lipid oxidative indices and LOX were investigated and shown as the Pearson's correlation coefficients. The combined analyses revealed that C18:0, C16:1n7, C19:0, and C22:6n3, which showed marked changes in PCA1, were potential markers for evaluating lipid oxidation in salt-dried fish muscle. In the future, changes in other lipidomics data during the processing of salt-dried fish should be examined to obtain a deeper understanding of lipid oxidation. A comprehensive mechanism of lipid oxidation could provide theoretical support for the production of salt-dried fish, which is a traditional aquatic processing product in China.

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