Activities of Endogenous Lipase and Lipolysis-Oxidation of Low-Salt Lactic Acid-Fermented Fish (Decapterus maruadsi)

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Abstract: There is increasing demand for low-salt meat products that retain traditional flavors. In this study, dry-salted fish (Decapterus maruadsi) were processed by 2 methods to obtain traditional salted fish (HS) and low-salt lactic acid-fermented fish (LAF). The relationship between lipolysis and lipid oxidation was investigated by evaluating changes in endogenous lipase (lipolytic enzymes; lipoxygenase, LOX), free fatty acid composition, thiobarbituric acid reactive substances (TBARS), and peroxide value (POV) during processing. Lipolytic enzyme activity showed a decreasing trend, in general. LOX activity initially increased and eventually decreased. Phospholipase, acid lipase, and neutral lipase activity was 0.33, 0.17, 0.57 (in HS) and 0.39, 0.25, 0.67 (in LAF) times in the final product than the activity levels observed in fresh fish. A principal component analysis indicated that phospholipase and neutral lipase play major roles in promoting lipid hydrolysis (in HS and LAF), the correlation between lipolytic activity and lipid oxidation in HS is greater than the correlation in LAF, and the contribution of LOX to lipid oxidation was minor in salted fish.

Key words: lipolysis oxidation, endogenous lipase, highly salted fish, low-salt lactic acid-fermented fish, Decapterus maruadsi

1 Introduction

The flavor of dry-cured meat products is largely generated from lipids. Lipolysis and lipid oxidation directly influence the final flavor of processed hams. Hydrolysis is the first step by which lipids are transformed to flavor compounds or aroma precursors, generating free fatty acids (FFAs), which are the main precursors of volatile compounds. FFAs are positively correlated with acid lipase, phospholipase, and neutral lipase activity in dry-cured ham. The oxidation of FFAs is the second step in the transition from lipids to flavor compounds or aroma precursors, and forms the characteristic aroma in final products. Lipid oxidation includes autoxidation and enzymatic oxidation, but the relative importance of these mechanisms is unclear. Some scholars believe that autoxidation is a more important role than the enzymatic oxidation of lipoxygenase (LOX) in Chinese traditional smoked-cured bacon and Jinhua ham. In contrast, LOX has been shown to contribute to lipid oxidation in dry-salted duck and unsmoked bacon. The relationship between lipolysis and lipid oxidation in dry-salted meat products is unclear; for example, according to Huang et al., phospholipid hydrolysis could promote lipid oxidation in smoke-cured bacon, whereas Jin et al. and Gandemer reported that lipolytic activity is essentially unrelated to lipid oxidation.

Traditional salted fish typically have a high salt content (20–30%). Based on health recommendations, consumer demand for less salty meat products, and particularly products with traditional unique flavors, but low salt contents, has gradually increased. Sodium chloride an important food ingredient, contributes to the flavor of meat products. Generally, less salty meat products couldn’t keep the traditional flavor of high salty products. There are studies found flavor compound formation is greater for dry-cured goose with a highly sodium chloride content than a low salt content. In addition, Corral et al. reported that slow-fermented sausages with different salt contents exhibit different volatile compounds. So, to produce...
a product with a low sodium chloride content and unique flavor, we previously invented a new method for salted-fish processing, i.e., low-salt lactic acid-fermented fish.

And endogenous lipase and lipolysis-oxidation in aquatic products are poorly understood. So, our study was used to explore the different impacts between our new method and traditional method on product quality. In this study, changes in lipase activity (acid lipase, neutral lipase, and phospholipase), LOX activity, the lipid oxidation parameters thiobarbituric acid reactive substances (TBARS) and peroxide value (POV), and fatty acid composition were examined during dry-salted fish processing using the traditional method with high salt (HS) and the new method with low-salt and lactic acid bacteria (LAF). Furthermore, differences in the development of endogenous lipase and lipolysis-oxidation in LAF and HS fish, the relationship between lipolysis and lipase, and the correlation between lipolysis and lipid oxidation were also assessed. These findings provide a basis for refining LAF methods to produce flavorful low-salt fish products.

2 Materials and Methods

2.1 Salted fish processing and sampling

Chilled round scad (Decapterus maruadsi) were purchased from a local supermarket with fresh weights of 100 ± 5 g. They were randomly divided into 2 equal groups, each group contained eight fish. One group (20% salt (w/w); HS fish) was prepared as follows. Salt was evenly spread on the fish, saturated salt water was used to cover the fish, and they were placed in an incubator at 4°C for 36 h. After the wet-cured procedure was complete, the fish were soaked in water for 1 h, and the desalination step was repeated 4 times. Then, fish were air dry-rinen at 28 ± 2°C with 10% relative humidity (flowing air speed, 6 m/s) until the salted fish water content was approximately 40%. The other group (LAF) was prepared by the procedures described above, but after finishing the desalination steps, fish were inoculated with mixture of Lactobacillus bacterial (10% (v/m)). The following bacterial species were established: the Lactobacillus plantarum (Lp), the Leuconostoc mesenteroides (Ln) and the Pediococcus pentosaceus (Pp) (separated from salted yellow croaker, Pseudosciaena crocea), the Lactobacillus acidophilus (La), and the Lactobacillus longer (Lb) (isolated from pickled fish three teeth, Otolithes ruber). All species were identified and lyophilized for preservation (composite ratio Lp: Pp: Ln: La: Lb = 4:4:2:3:4).

Specimens were sampled at 5 points: raw fish (sample A), end of salting (sample B), desalination (sample C), during low temperature heat pump drying (sample D), and the finished product (sample E). Approximately 10 fish have been sampled at each point. Samples have been vacuum-packed and stored at −80°C until analysis.

2.2 Lipase extraction and activity assays

2.2.1 Determination of lipase activity

A crude lipase solution was extracted according to the methods described Jin et al. Acid lipase activity was measured as described by Vestergaard et al., with minor modifications. A portion of the enzyme solution (0.1 mL) was diluted with 2.8 mL of buffer (acid lipase buffer: pH 5.0, 0.1 mmol/L disodium phosphate/0.05 mol/L citric acid, 0.05% (w/v) Triton X-100, and 0.8 mg/mL bovine serum albumin (BSA); phospholipase buffer: pH 5.0, 0.1 mmol/L disodium phosphate/0.05 mol/L citric acid, 0.05% (w/v) Triton X-100, 0.8 mg/mL BSA, 150 mmol/L sodium fluoride; neutral lipase buffer: pH 7.5, 0.22 mol/L Tris/His, 0.05% (w/v) Triton X-100). Finally, 0.1 mL of substrate buffer was added (1.0 mmol/L 4-methylumbelliferyl-oleate; Sigma, St. Louis, MO, USA). After incubation at 37°C for 30 min, the acid lipase and phospholipase reactions were stopped with 0.5 mL of 1 mol/L HCl and the incubated samples (neutral lipase) were immediately cooled in an ice-water mixture and measured within 1 min. Fluorescence was measured using a fluorescence spectrophotometer (Cary Eclipse; Varian, Palo Alto, CA, USA) (acid lipase and phospholipase: , neutral lipase: ). Lipase activity was calculated using a standard curve. One unit of activity (U) was defined as the amount of enzyme hydrolyzing 1 nmol 4-methylumbelliferyl-oleate per h at 37°C. Enzyme activity is expressed as units (U)/g protein.

2.3 Determination of LOX activity

Extraction of the crude LOX solution and the preparation of the substrate compound solution for the LOX activity assay were performed according to the method described by Jin et al.. LOX activity was assayed by measuring the increase in absorbance at 234 nm for 1 min using an ultraviolet spectrophotometer (Spectronic ® GENESYSTEM 5; Thermo Fisher Scientific, Waltham, MA, USA) at 20°C. The reaction medium contained 0.2 mL of substrate compound solution, 0.1 mL of enzymatic solution, and 2.9 mL of citric acid buffer (pH 5.5, 50 mmol/L). The blank sample contained 0.2 mL of substrate compound solution and 3.0 mL of citric acid buffer. Absorbance values at 234 nm were read before and after incubating with the ultraviolet spectrophotometer at 20°C (incubation time, 1 min). One unit of LOX activity (U) was defined as 0.001 unit of increased absorbance per min at 20°C at 234 nm. Enzyme activity is expressed as U/g protein.

2.4 Analyses of lipid oxidation and free fatty acids

The extent of lipid oxidation was evaluated based on TBARS and POV. TBARS values were determined accord-
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2.5 Statistical analysis

Using the NIST 0.5 database library, FFAs were retrieved and analyzed, and the fatty acid composition was determined based on comparisons with the standard spectrum of the MS gallery. Using the area normalization method, the relative contents of FFAs were calculated. Comparisons of endogenous lipase acid activity, POV, TBARS, and FFA composition at different stages of processing were performed by one-way analyses of variance using SPSS statistical software (Version 13; SPSS Inc., Chicago, IL, USA). The level of significance was set at $p<0.05$. The relationships among lipolysis (lipase activity and FFA) and lipid oxidation parameters (POV, TBARS, and LOX activity) during salted fish processing were analyzed by a principal component analysis (PCA) using SPSS statistical software.

3 Results and discussion

3.1 Changes in lipolytic enzyme activity during processing

Lipolytic enzyme activity levels decreased substantially at late stages (D and E) in the process compared to the initial activity levels in fresh fish (Figs. 1-3). These results were consistent with those obtained for other dry-pickled meats. Neutral lipase (in HS and LAF) demonstrated the highest activity throughout processing, followed by acid lipase, with phospholipase exhibiting the lowest activity. These results suggest that neutral lipase was the main hydrolytic enzyme for fat hydrolysis throughout the process, consistent with previous results in ham and dry-cured goose, but different from results obtained for smoked bacon, unsmoked bacon, and Parma ham, in which acid lipase activity was highest throughout the process.

Phospholipase activity was greater in HS samples than in LAF samples during processing (Fig. 1). The phospholipase activities were 33.33% (in HS) and 39.47% (in LAF) compared with the levels in fresh fish. Phospholipase activity was slightly inconsistent throughout processing (in HS and LAF), but ultimately decreased during the manufacturing process. Phospholipase activity declined ($p<0.05$) during HS processing, but decreased significantly (i.e., 53.5%) compared with fresh fish; $p<0.05$ at stage B and was then maintained at stable levels in stages C, D, and E during LAF processing. This pattern may be explained by the increase in compound lactic acid bacteria, but further experiments are necessary to determine the precise underlying factors. Acid lipase activity initially decreased ($p<0.05$) and then increased at the stage of C in HS and LAF (Fig. 2). The activity in HS samples reached a maximum at stage D, and decreased markedly thereafter ($p<0.05$). These results were consistent with those of Huang et al. for smoked bacon. Activity in LAF samples increased at stages D and E. The final acid lipase activity in LAF samples was significantly higher than that in HS samples ($p<0.05$). Neutral lipase activity decreased significantly ($p<0.05$ in...
lower than that of fresh fish. The activity in the final product was significantly lower than that of fresh fish (p < 0.05). The increase in the salt content and low water activity has an obvious inhibitory effect on lipolytic enzyme activity.

The activity of neutral lipase at stage E decreased significantly compared with stage D in HS (p < 0.05), but was stable in LAF (p > 0.05). The neutral lipase activity for HS was significantly higher than that of LAF, except at stage D (HS < LAF, p > 0.05).

In general, lipolytic enzyme activity showed a decreasing trend and the activity in the final product was significantly lower than that of fresh fish (p < 0.05 in HS and LAF). An increase in the salt content and low water activity has obvious inhibitory effects on lipolytic enzyme activity.

The lipolytic enzyme activity levels in HS fish were significantly higher than those in LAF, except at stage E (HS < LAF, p > 0.05); there was a slow increasing trend in LAF at the stage E. Certain concentrations of sodium chloride may promote lipolytic enzyme activity according to Wang and based on a study of dry-cured duck. The pattern observed in LAF can be attributed to the increase of compound.

3.2 Changes in LOX, POV, and TBARS during processing

LOX activity (in HS and LAF) increased during the first three stages, i.e., B, C, and D (Table 1), and may be explained by the activating effect of salt. At the final stages, activity decreased significantly in HS (p < 0.05), and decreased slightly in LAF (p > 0.05). The decrease in LOX activity may be related to processing conditions and the inactivation of hydroperoxides that accumulate during the process. The LOX activity in the final product increased by 24% and 32.8% compared with initial activity levels in HS and LAF, respectively, indicating that LAF is more suitable than HS with respect to LOX. The LOX activity in HS was significantly greater (p < 0.05) than that in LAF during the first three stages. It is possible that a higher salt content activates LOX activity to a greater extent and reduces antioxidant enzyme activity. In contrast, LOX activity in LAF was significantly greater (p < 0.05) than that in HS at the final stage, which reflected lactic acid bacteria that we used could release LOX.

The trends in POV and TBARS during HS and LAF processing were similar, both of them increased in the first three stages and decreased in the final stage (Table 1). POV (in HS and LAF) was highest at the mid-low temperature heat pump drying stage and decreased significantly (p < 0.05 in HS and LAF) at the end of processing. POV did not differ significantly between the finished products (in HS and LAF, p > 0.05). Hydroperoxides (the primary products of lipid oxidation, easily decomposed) determined the POV and indicated that lipid oxidation mainly occurs at stage D (in HS and LAF). TBARS (in HS and LAF) was highest at stage D, but remained stable at the final stage, and the differences in TBARS among stages were non-significant (p > 0.05). TBARS depends on the MDA content, which are secondary products of lipid oxidation. At the final stage, automatic oxidation and enzymatic oxidation...

![Image](https://via.placeholder.com/150)

Fig. 3 Changing of Neutral lipase activities of Decapterus maruadsi during different method of dry-salted processing. a-d Means with the same letter are not significantly different in different processing (p > 0.05); A-B Means with the same letter are not significantly different in different groups (p > 0.05).

Table 1 The changes of LOX, POV values, TBARS during different method of dry salted processing.

| Index     | Method | A       | B       | C       | D       | E       |
|-----------|--------|---------|---------|---------|---------|---------|
| LOX       | HS     | 92.44 ± 1.02<sup>ad</sup> | 120.36 ± 0.55<sup>ab</sup> | 122.48 ± 1.53<sup>ab</sup> | 136.42 ± 1.2<sup>ab</sup> | 115.48 ± 1.43<sup>bc</sup> |
| (U)       | LAF    | 92.44 ± 1.02<sup>ac</sup> | 107.72 ± 1.03<sup>bc</sup> | 109.48 ± 1.07<sup>ab</sup> | 122.13 ± 1.12<sup>ab</sup> | 121.56 ± 1.21<sup>ab</sup> |
| POV       | HS     | 0.35 ± 0.06<sup>bc</sup> | 0.85 ± 0.05<sup>ab</sup> | 0.95 ± 0.07<sup>ab</sup> | 2.52 ± 0.04<sup>ab</sup> | 0.72 ± 0.08<sup>ab</sup> |
| (mg/kg)   | LAF    | 0.35 ± 0.06<sup>bd</sup> | 0.70 ± 0.05<sup>bc</sup> | 0.84 ± 0.05<sup>bc</sup> | 2.37 ± 0.05<sup>ab</sup> | 0.89 ± 0.01<sup>ab</sup> |
| TBARS     | HS     | 0.10 ± 0.00<sup>bc</sup> | 0.23 ± 0.01<sup>ab</sup> | 0.27 ± 0.03<sup>ab</sup> | 0.73 ± 0.01<sup>ab</sup> | 0.70 ± 0.01<sup>ab</sup> |
| (ug MDA/g muscle) | LAF    | 0.10 ± 0.00<sup>bd</sup> | 0.20 ± 0.02<sup>bc</sup> | 0.25 ± 0.02<sup>ab</sup> | 0.69 ± 0.03<sup>ab</sup> | 0.68 ± 0.01<sup>ab</sup> |

A-E Means with the same letter are not significantly different in different processing (p > 0.05). a-b Means with the same letter are not significantly different in different groups (p > 0.05).
could cause primary products (hydroperoxides) of lipid oxidation to be further oxidized to secondary oxidation products, explaining the non-significant decreases in TBARS values.

As shown in Table 1, the trend in TBARS was more similar to that of LOX activity than was POV. It follows that TBARS more accurately reflects LOX trends. LOX activity and TBARS exhibit a significant positive correlation. Lipid oxidation occurs mainly at stages D and E. At stage D, the lipid oxidation indexes in LAF samples were lower than those in HS samples. However, at stage E, the numerical difference in the lipid oxidation indexes (POV and TBARS) was non-significant in HS and LAF, confirming that compound lactic acid bacteria could accelerate the process of lipid oxidation.

### 3.3 Changes in free fatty acids during processing

Changes in the main FFA contents during the entire procedure for the two dry-salted processes (HS and LAF) are summarized in Table 2 and Fig. 4. PUFA was the dominant component throughout the entire process, followed by SFA, with MUFA exhibiting the lowest percentage. The MUFA content increased (HS, $p > 0.05$; LAF, $p < 0.05$) at stage B, was reduced (HS and LAF, $p > 0.05$) at stage C, and decreased again (HS and LAF, $p < 0.05$) at stage E.

Table 2: Changes in the main kinds of fatty acid composition and relative contents of *Decaptorus maruadsi* during different method of dry salted processing.

| FFA (mg/g) | Group | A     | B     | C     | D     | E     |
|-----------|-------|-------|-------|-------|-------|-------|
| C12:0     | HS    | ND    | ND    | ND    | ND    | 0.15 ± 0.02<sup>a</sup> |
|           | LAF   | ND    | ND    | ND    | ND    | 0.12 ± 0.01<sup>a</sup>  |
| C14:0     | HS    | 1.83 ± 0.10<sup>b</sup> | 1.56 ± 0.40<sup>b</sup> | 1.80 ± 0.13<sup>b</sup> | 1.85 ± 0.16<sup>b</sup> | 3.30 ± 0.65<sup>b</sup> |
|           | LAF   | 1.83 ± 0.10<sup>a</sup> | 2.25 ± 0.42<sup>a</sup> | 2.09 ± 0.74<sup>a</sup> | 2.54 ± 0.29<sup>a</sup> | 1.99 ± 0.20<sup>b</sup>  |
| C15:0     | HS    | ND    | 0.35 ± 0.06<sup>c</sup> | 0.47 ± 0.03<sup>c</sup> | 0.60 ± 0.12<sup>b</sup> | 0.78 ± 0.91<sup>c</sup> |
|           | LAF   | ND    | 0.62 ± 0.14<sup>a</sup> | 0.57 ± 0.17<sup>a</sup> | 0.60 ± 0.06<sup>a</sup> | 0.66 ± 0.25<sup>a</sup>  |
| C15:5     | HS    | 1.08 ± 0.02<sup>b</sup> | 1.95 ± 0.63<sup>a</sup> | 1.65 ± 0.13<sup>a</sup> | 1.87 ± 0.36<sup>a</sup> | 1.31 ± 0.21<sup>b</sup>  |
|           | LAF   | 1.08 ± 0.02<sup>a</sup> | 1.32 ± 0.36<sup>a</sup> | 1.48 ± 0.34<sup>a</sup> | 1.59 ± 0.34<sup>b</sup> | 1.16 ± 0.22<sup>b</sup>  |
| C16:0     | HS    | 15.62 ± 0.45<sup>Ab</sup> | 15.76 ± 0.02<sup>a</sup> | 15.92 ± 0.28<sup>a</sup> | 14.96 ± 0.29<sup>b</sup> | 13.63 ± 0.40<sup>b</sup> |
|           | LAF   | 15.62 ± 0.45<sup>a</sup> | 14.49 ± 0.34<sup>b</sup> | 14.84 ± 0.16<sup>b</sup> | 14.23 ± 0.13<sup>b</sup> | 14.74 ± 1.05<sup>b</sup> |
| C16:1     | HS    | 3.15 ± 0.19<sup>b</sup> | 3.87 ± 0.46<sup>a</sup> | 4.05 ± 0.21<sup>a</sup> | 4.30 ± 0.15<sup>b</sup> | 6.40 ± 0.57<sup>a</sup>  |
|           | LAF   | 3.15 ± 0.19<sup>b</sup> | 5.43 ± 0.91<sup>a</sup> | 4.53 ± 0.38<sup>b</sup> | 5.18 ± 0.49<sup>b</sup> | 4.83 ± 0.44<sup>a</sup>  |
| C17:0     | HS    | 1.00 ± 0.10<sup>bc</sup> | 0.71 ± 0.32<sup>c</sup> | 0.97 ± 0.04<sup>c</sup> | 1.09 ± 0.12<sup>b</sup> | 2.31 ± 0.25<sup>c</sup>  |
|           | LAF   | 1.00 ± 0.10<sup>a</sup> | 1.53 ± 0.30<sup>a</sup> | 1.21 ± 0.33<sup>a</sup> | 1.35 ± 0.12<sup>a</sup> | 1.68 ± 0.59<sup>a</sup>  |
| C18:1     | HS    | 0.28 ± 0.07<sup>b</sup> | 0.48 ± 0.20<sup>Ab</sup> | 0.55 ± 0.08<sup>Ab</sup> | 1.04 ± 0.35<sup>b</sup> | 1.41 ± 0.35<sup>b</sup>  |
|           | LAF   | 0.61 ± 0.14<sup>a</sup> | 0.49 ± 0.21<sup>a</sup> | 0.62 ± 0.11<sup>a</sup> | 0.40 ± 0.34<sup>b</sup> | 0.72 ± 0.34<sup>a</sup>  |
| C18:0     | HS    | 12.11 ± 0.23<sup>a</sup> | 12.18 ± 0.18<sup>a</sup> | 12.58 ± 0.24<sup>a</sup> | 12.25 ± 0.31<sup>a</sup> | 11.27 ± 0.18<sup>b</sup> |
|           | LAF   | 12.11 ± 0.23<sup>a</sup> | 11.90 ± 0.53<sup>a</sup> | 11.93 ± 0.67<sup>a</sup> | 11.90 ± 0.11<sup>b</sup> | 12.73 ± 0.35<sup>b</sup> |
| C18:1     | HS    | 14.84 ± 0.05<sup>bc</sup> | 15.50 ± 0.31<sup>Ab</sup> | 14.32 ± 0.25<sup>Ac</sup> | 14.31 ± 0.53<sup>c</sup> | 16.33 ± 0.73<sup>c</sup> |
|           | LAF   | 14.84 ± 0.05<sup>a</sup> | 15.01 ± 0.62<sup>Ab</sup> | 16.00 ± 0.83<sup>b</sup> | 14.68 ± 0.23<sup>b</sup> | 16.42 ± 0.59<sup>c</sup> |
| C18:2     | HS    | 1.44 ± 0.33<sup>a</sup> | 0.90 ± 0.20<sup>b</sup> | 1.41 ± 0.20<sup>Ab</sup> | 1.77 ± 0.18<sup>a</sup> | 1.71 ± 0.07<sup>b</sup>  |
|           | LAF   | 1.44 ± 0.33<sup>a</sup> | 1.75 ± 0.33<sup>a</sup> | 1.70 ± 0.14<sup>a</sup> | 1.72 ± 0.21<sup>a</sup> | 1.26 ± 0.10<sup>a</sup>  |
| C19:0     | HS    | ND    | ND    | 0.29 ± 0.01<sup>b</sup> | 0.40 ± 0.06<sup>a</sup> | 0.50 ± 0.03<sup>a</sup> |
|           | LAF   | ND    | 0.39 ± 0.07<sup>a</sup> | 0.34 ± 0.01<sup>a</sup> | 0.37 ± 0.06<sup>a</sup> | 0.35 ± 0.05<sup>a</sup>  |
| C20:0     | HS    | ND    | ND    | 0.26 ± 0.00<sup>b</sup> | 0.27 ± 0.04<sup>b</sup> | 0.49 ± 0.02<sup>b</sup>  |
|           | LAF   | ND    | 0.38 ± 0.1<sup>a</sup> | 0.29 ± 0.02<sup>a</sup> | 0.32 ± 0.06<sup>a</sup> | 0.33 ± 0.02<sup>a</sup>  |
| C20:2     | HS    | ND    | ND    | ND    | ND    | 0.42 ± 0.04<sup>a</sup>  |
|           | LAF   | ND    | ND    | ND    | ND    | 0.31 ± 0.05<sup>ab</sup> |
| C20:3     | HS    | ND    | ND    | ND    | ND    | 0.49 ± 0.02<sup>a</sup>  |
Table 2 Continued.

| FFA (mg/g) | Group | A     | B     | C     | D     | E     |
|-----------|-------|-------|-------|-------|-------|-------|
| C20:5     | HS    | 7.95 ± 0.08<sup>a,b</sup> | 6.91 ± 0.58<sup>c</sup> | 4.59 ± 0.49<sup>a,b</sup> | 9.04 ± 0.08<sup>a</sup> | 10.49 ± 0.02<sup>a</sup> |
|           | LAF   | 7.95 ± 0.08<sup>a</sup> | 7.32 ± 0.43<sup>b</sup> | 4.74 ± 0.67<sup>BCa</sup> | 8.78 ± 0.31<sup>BC</sup> | 10.92 ± 0.70<sup>a</sup> |
| C22:4     | HS    | 4.15 ± 0.23<sup>a</sup> | 3.42 ± 0.03<sup>a</sup> | 3.60 ± 0.35<sup>ab</sup> | 4.01 ± 0.44<sup>a</sup> | 4.17 ± 0.36<sup>a</sup> |
|           | LAF   | 4.15 ± 0.23<sup>a</sup> | 4.65 ± 0.78<sup>a</sup> | 4.36 ± 0.10<sup>a</sup> | 3.87 ± 0.53<sup>a</sup> | 3.80 ± 0.39<sup>ab</sup> |
| C22:6     | HS    | 26.14 ± 0.32<sup>a</sup> | 25.26 ± 0.33<sup>BCa</sup> | 30.00 ± 0.32<sup>a</sup> | 24.31 ± 0.23<sup>ab</sup> | 17.78 ± 0.46<sup>a</sup> |
|           | LAF   | 26.14 ± 0.32<sup>a</sup> | 23.33 ± 0.39<sup>ab</sup> | 25.98 ± 0.65<sup>ab</sup> | 24.14 ± 0.89<sup>BC</sup> | 19.34 ± 0.40<sup>a</sup> |
| C24:1     | HS    | ND    | ND    | ND    | ND    | 0.31 ± 0.02<sup>a</sup> |
|           | LAF   | ND    | ND    | ND    | ND    | 0.19 ± 0.00<sup>a</sup> |
| C18:4     | HS    | ND    | ND    | ND    | ND    | ND    |
|           | LAF   | ND    | ND    | ND    | ND    | ND    |
| C19:1     | HS    | ND    | ND    | ND    | ND    | ND    |
|           | LAF   | ND    | ND    | ND    | ND    | ND    |
| C22:5     | HS    | ND    | ND    | ND    | ND    | ND    |
|           | LAF   | ND    | ND    | ND    | ND    | ND    |

A-D Means with the same letter are not significantly different in different processing (p > 0.05). a-b Means with the same letter are not significantly different in different groups (p > 0.05); ND Means unchecked out; acid lipase activity (ALA), neutral lipase activity (NLA), phospholipase activity (PLA), LOX activity (LOXA), single unsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), C20:5 (EPA), C22:6 (DHA). C18:1 (a) means trans fatty acids (Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester)

tcreasing slightly (HS and LAF, p > 0.05) at stage D, and was maximal at stage E. The MUFA content during the entire process (HS and LAF) exhibited a generally increasing trend. SFA exhibited a similar trend to that of MUFA during all stages, and showed a gradual increase during the process. However, SFA in HS, p > 0.05) exhibited a gradual decrease during stage D. The MUFA and SFA contents in the final product in HS and LAF did not differ significantly. Different trends in PUFA content were observed for HS and LAF. In HS, the PUFA content decreased markedly (p < 0.05) at stage C, was stable (p > 0.05) at stage C, and decreased significantly (p < 0.05) during stages D and E. In LAF, the PUFA content exhibited a decreasing trend (non-
in LAF is more suitable to avoid oxidation than in HS.

C20:5 (EPA) and C22:6 (DHA) are PUFAs derived from the hydrolysis of phospholipids. The EPA content (in HS and LAF) initially decreased at stages B and C, and increased at stages D and E. However, the DHA content (in HS and LAF) initially increased (at stages B and C) and decreased at stages D and E. Thus, compared with EPA, DHA exhibited the opposite trend during the entire HS and LAF processes. The observed trend illustrated that fat degradation mainly produced DHA before the desalting stage, but the generation of EPA was greater than the consumption after this stage. The final EPA content increased compared with the initial content to different extents in HS and LAF.

And, the increased content in the final product for LAF was higher (37.5% of the original content in fish) than that of HS (31.9% of the original content). However, the final DHA content was lower than the initial content in fresh fish. This may be because DHA is more used for oxidation than EPA. The decrease in DHA at the last two stages may reflect a negative correlation with the increased oxidation. The amounts that were retained (in LAF and HS) relative to the initial content were 74% and 68%, respectively.

3.4 Relationships among endogenous lipase, lipolysis, and lipid oxidation during dry-salted fish processing

The relationship between endogenous lipase and lipolysis-oxidation during dry-salted fish processing was evaluated by a PCA. Three principal components in HS (Principal Component 1: 61.51%; Principal Component 2: 25.3%; Principal Component 3: 9.77%) and two principal components (Principal Component 1: 74.23%; Principal Component 2: 16.89%) in LAF were extracted, explaining 96.58% and 91.09% of the total variance, respectively. Figure 4 (a, HS; b, LAF) shows the loadings of different variables on scatter plots of principal component 1 and principal component 2, which could be defined as lipolysis factors and lipid oxidation factors, respectively. The relationship between the two variables could be explained by their location in the loading plots.

3.4.1 The relationship between endogenous lipase and lipolysis

As shown in Fig. 6 (a and b), acid lipase activity (ALA), neutral lipase activity (NLA), phospholipase activity (PLA), and PUFA formed a cluster (I for HS; i for LAF), and SFA and MUFA were located in a separate area (III for HS; iii for LAF), indicating that lipolytic enzyme activity was closely related to the PUFA content phospholipase and neutral lipase. As shown in Fig. 6 (a), we observed a positive loading of PUFA (0.75) and high positive loadings of PLA, NLA, and ALA (0.97, 0.96, and 0.85, respectively) in principal component 1, indicating that PLA, NLA, and ALA had accelerating effects on the hydrolysis of salted fish during the HS process. As shown in Fig. 6 (b), PLA, NLA, and ALA (loadings of 0.98, 0.98, and 0.80) were more closely related with PUFA (loading, 0.995) in principal component 1. PUFA is mainly derived from the hydrolysis of phospholipids. PLA and NLA played more important roles than ALA in promoting the hydrolysis of phospholipids during the processing of dry-salted fish (in HS and LAF). The high positive loadings of PLA, NLA, and ALA (HS: 0.97, 0.96, and 0.85; LAF: 0.98, 0.98, and 0.80, respectively) and the high negative loadings of SFA and MUFA (HS: −0.82 and −0.90; LAF: −0.83 and −0.84) illustrated that the relationship between endogenous lipase and lipolysis-oxidation during dry-salted fish processing was evaluated by a PCA. Three principal components in HS (Principal Component 1: 61.51%; Principal Component 2: 25.3%; Principal Component 3: 9.77%) and two principal components (Principal Component 1: 74.23%; Principal Component 2: 16.89%) in LAF were extracted, explaining 96.58% and 91.09% of the total variance, respectively. Figure 4 (a, HS; b, LAF) shows the loadings of different variables on scatter plots of principal component 1 and principal component 2, which could be defined as lipolysis factors and lipid oxidation factors, respectively. The relationship between the two variables could be explained by their location in the loading plots.
LAF: \(-0.82\) and \(-0.96\), respectively) in principal component 1 (Fig. 6) as well as their low loadings in principal component 2 indicated that the SFA and MUFA contents were not closely related to lipolytic enzyme activity or that SFA and MUFA had inhibitory effects on lipolytic enzyme activity in salted fish. These findings differed from those of other studies\(^2, 6, 8\), in which acid lipase was the main enzyme for the decomposition of grease during the processing of dry-cured meat products. This difference among studies may be explained by differences in physical and chemical indicators during the manufacturing process as well as differences in material characteristics.

3.4.2 The relationship between LOX activity and lipid oxidation

LOX activity and POV (in HS; Fig. 6(a)) showed high loadings \((0.80-0.92)\) in principal component 2 and were clustered in the same group (II). However, LOX activity did not cluster with TBARS in group (II), suggesting that LOX had a significant accelerating effect at the early processing stages of salted fish, but had little effect at the end of processing. In contrast, as shown in Figure 6b, LOXA, POV, and TBARS (in LAF) clustered within the same group (ii), but LOXA had a lower loading in principal component 2 than that shown in Fig. 6(a). Thus, the contribution of LOX to lipid oxidation was minor in salted fish. Huang et al.\(^6\) and Zhou & Zhao\(^7\) also reported that auto-oxidation was the main cause of muscle lipid oxidation in smoke-cured bacon and Jinhua ham, respectively.

3.4.3 The relationship between lipolysis and lipid oxidation

The lipolysis-related variables (PUFA, SFA, MUFA, DHA, EPA, NLA, PLA, and ALA) were not strongly related to the lipid oxidation indices (POV and TBARS) and were independent, located in different areas in Fig. 6(b). However, SFA, MUFA and TBA were located in the same area in Fig. 6(a). The above conclusion indicating that the correlation between lipolytic activity and lipid oxidation in HS is greater than the correlation in LAF. Thus, Jin et al.\(^8\) and Gandemer\(^9\) obtained similar results in studies of other meat products. In contrast, Coutron-Gambotti & Gandemer\(^11\) found that intense lipolysis could promote lipid oxidation in dry-cured hams, and Huang et al.\(^6\) found that phospholipid hydrolysis could promote lipid oxidation in smoke-cured bacon.

4 Conclusion

Lipolytic enzyme activity was significantly lower in final fish products than in fresh fish. Neutral lipase demonstrated the highest activity in HS and LAF, followed by acid lipase, with phospholipase exhibiting the lowest activity. Phospholipase and neutral lipase mainly promoted lipid hydrolysis in HS and LAF. LOX (in HS and LAF) increased during the first three stages and decreased in the final stage, to different extents. Endogenous lipase activity was higher in HS than in LAF, except at the final stages of processing. The trends in lipid oxidation indexes illustrated that lipid oxidation occurs mainly at stages D and E. PCA indicated that lipolytic enzyme activity is closely related to PUFA, but not to SFA and MUFA contents, in HS and LAF. The contribution of LOX to lipid oxidation was minor in salted fish, and lipolytic activity was not strongly related to lipid oxidation in salted fish (in HS and LAF).

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