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Effect of *Zanthoxylum Bungeanum Maxim* on the lipid oxidation and fatty acid composition of dry-cured fish during processing

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Running title: Z. *Bungeanum* Maxim improves the quality of Layú

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Abstract The Chinese traditional dry-cured grass carp fish (Layú) was processed without (A) and with (B, 2%, W/W) Chinese prickly ash, *Zanthoxylum Bungeanum* Maxim, and the lipid oxidation and fatty acid composition of Layú were investigated. The addition of Chinese prickly ash showed no significant differences (P>0.05) on the nutritional contents of Layú, but significantly inhibited the increase of ratio of polar oil, the content of free fatty acid, peroxide value and TBA (P<0.05). ~~Besides, the increase of saturated fatty acid and the decrease of monounsaturated fatty acids and polyunsaturated fatty acids were depressed during the manufacturing process.~~ It also inhibited the increase of saturated fatty acids in oil, and the decrease of the content of polyunsaturated fatty acid and polyunsaturated fatty acids during processing. *Z. Bungeanum* Maxim could effectively delay lipid oxidative decomposition. The contents of DHA and EPA were significantly higher (P<0.05) in Layú B than Layú A. It can be concluded that *Z. Bungeanum* Maxim can display a protective effect against lipid oxidation during dry-cured fish processing~~improve the quality of Layú~~.

Keywords Layú; *Z. Bungeanum* Maxim; oxidation; fatty acids

Practical Application:

The addition of Chinese prickly ash can significantly inhibit the increase of ratio of polar oil, the content of free fatty acid, peroxide value and TBA (P<0.05). *Z. Bungeanum* Maxim can effectively delay lipid oxidative decomposition and improve the quality of Layú.

Introduction

Layú is a characteristic traditional fermented freshwater fish product in the south of China. It is popular with consumers due to its special flavor and desirable taste. There is a long history for residents living in the Yangtze River basin to make dry-cured Layú in winter. Different with other countries' traditional dry-cured fish, Layú is naturally fermented at low temperature and high salt concentration. Except salt, the dry-cured agents generally used also include spicery, especially Chinese prickly ash, which is used to improve flavor and extend shelf-life of Layú.

Chinese prickly ash processed as the spicery was actually the dry-aged pericarp of *Z. Bungeanum* Maxim, which belongs to *Rutaceaed and Zanthoxylum L.* The main chemical components of *Z. Bungeanum* Maxim include alkaloid, amide, lignans, essential oils and coumarin, etc (Gong et al., 2009). As natural functional ingredients, they are always the research focus in food processing. Since two thousand years ago, *Z. Bungeanum* Maxim has been applied as a kind of spicery in Chinese food.

Lipid, as an important component of the fish flesh, is a major factor which greatly influences both functionality and sensory properties of processed fish products. The quality of lipid in fish products depends on the content, composition of lipid and properties of fatty acids. The lipolysis and lipid oxidation in fish flesh during processing are closely associated with quality deterioration (Pacheco-Aguilar et al., 2000).Furthermore, fatty acids are generated due to lipolysis induced by lipases and phospholipases. Low-molecular weight compounds are produced by further oxidation of fatty acids, which lead to the rancid flavor and off-flavor of fish and fish products(Toyomizu et al., 1981). It has been reported that volatile compounds generated from lipid oxidation are primarily responsible for the characteristic flavor during the dry-curied process of anchovy (Triqui and Reineccius, 1995). There also has been much concentration on the effects of polyunsaturated fatty acids of fish products and oxidative degradation of lipid during storage on fish flavor. The hydrolysis of endogenous lipases, microbial catabolic enzymes and contact with air for a long time during the processing of dry-cured fish products lead to lipolysis and lipid oxidation and reduce the quality of lipid in dry-cured fish products. However, little information is known regarding the

changes in lipids that occurred in dry-dured fish products, and there is no report on the effects of spiceries on quality of lipid in dry-cured fish products.

This paper studied the effects of *Z. Bungeanum* Maxim on the lipid oxidation and fatty acid compositions of dry-cured grass carp during processing, in order to provide theoretical basis for the industrial production of dry-cured grass carp, Layú.

Materials and methods

Sample preparation

Fresh grass carp (*Ctenopharyngodon idellus*) was purchased from a local fish market (Ningbo, Zhejiang Province, China) and transported to the laboratory in ice within 30 min. Each fish (mean weight 3 ± 0.5 kg) was deheaded, gutted, and deboned immediately for curing. Dried Chinese prickly ash (*Z. Bungeanum* Maxim) produced in Sichuan Province, China, was triturated into the particle size (40-mesh) with a pulverizer, fastened in the PE bags, sealed in dry foam box and stored in the refrigeration at 4℃ for further use (Content of essential oil was 7.1 mL/100 g). The content of essential oil was determined according to the distillation stipulated by Forestry Standard of PRC-Quality Classify of Prickly Ash (LY/T 1652-2005).

Cured fish were processed according to the traditional processing standard of Zhejiang Province (Zhang et al., 2011). In Layú A, 50 kg of raw fish were salted with 5 kg NaCl and 1 kg Chinese prickly ash, forming piles alternating between fish and salt with spices. Based on the amount of Chinese prickly ash in the traditional Chinese salted fish and the flavor of dry-cured grass carp in previous study, 2% (w/w) Chinese prickly ash was used in this study. The temperature of the salting room was 2-5℃ and the relative humidity 80-90%. After curing for 15 days, the fish were taken out, brushed, hung on shelves and transferred to a room at 10℃ and relative humidity of 75%. There, the cured fishes undergo aging for 15 days. Layú B was treated similar to that of Layú A, except that no Chinese prickly ash was added.

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After 15 days of curing and another 15 days of aging as described above, the Layú became mature and can harvest. Two samples were taken during the curing and aging period, one sample right after curing 15days, the other after aging for 15 days. All the samples were minced in a high-capacity mincer (TQ-5, Henglian Food Machinery Co., Ltd, China). Fresh fish was minced to

obtain control sample. Minced samples obtained were stored at -80 ℃ for experimental analysis.

Determination of proximate composition and NaCl content

The dorsal white muscle from the Layú was used for analysis. The moisture contents were measured by the difference of the sample weight after drying at 105 ℃ for 20 h. Ash contents were estimated by heating the sample to a constant weight at 600℃. The protein contents were determined by the semi-micro Kjeldahl method.

Five grams of the sample was homogenized with 20 mL of distilled water and the NaCl content was measured as the conductivity of the homogenate using a digital salt meter with double electrodes (SS-30, Sekisui Chemical, Tokyo, Japan).

The crude fat was assayed following the method described by (Folch et al., 1957) Crude fat was extracted from 10 g sample with 100 mL×3 chloroform-methanol (2:1, v/v). Extracted fat was dried by rotating evaporation at vacuum conditions. Content of crude fat was obtained by weighing, and results were expressed as g/100g weight. The crude fat was dissolved with a little of chloroform, and stored at -20℃in darkness.

Lipid Fractionation (separated the neutral and polar lipid fractions) analysis

The lipid fractions were determined according to the method mentioned by (Zhang et al., 2013b). Lipids were separated into neutral and polar fractions on a 20 × 1.8 cm column of Silica Gel 60 (70-230; Merck). Lipid samples (0.1-0.2 g) were dissolved in 2 mL chloroform and applied to the column. Neutral lipids were eluted with 150 mL chloroform. Then the column was washed with 100 mL chloroform/methanol (49:1, v/v). Finally, polar lipids were eluted with 150 mL methanol. Yields of each fraction were determined gravimetrically.

FFA analysis

The FFA content was determined, according to the method of (Lowry and Tinsley, 1976), in the fat extracted by the (Bligh and Dyer, 1959). Toluene (2.5 mL) was used as the solvent, and 0.5 mL of 5% cupric acetatepyridine reagent was added to the tube and shaken for 2 min. The biphasic system was centrifuged for 10 min and the top layer was read at ~~725~~ 715 nm. A standard curve, using oleic acid solution, was used to calculate the content of FFA in the fat of the sample.

Peroxide value (POV) analysis

The POV was determined in the fat extracted by the (Bligh and Dyer, 1959) using a ferric thiocyanate method according to (Chapman and Mackay, 1949). No preliminary dilution with a benzene/methanol solution was necessary. A standard curve of ferric iron solution was used to calculate the content of peroxides in the fat of the sample.

Determination of 2-thiobarbituric acid (TBA)

2-Thiobarbituric acid (TBA) was detrmined by the method of (Cao et al., 2013), with slight modification. Approximately 10.0 g of minced samples was homogenized with 50 mL 7.5% (w/v) trichloroacetic acid (containing 0.1% EDTA), with a homogenisation at 15,000 rpm. The dispersion was filtered through Whatman No. 1 filter paper. The supernant (5 mL) was mixed with 5 mL 0.02 mol/L TBA first, and then it was heated in boiling water for 30 min to develop the rose-pink colour and cooled for 10 min in cold flowing water immediately. The absorbance was measured at 532 nm against the blank prepared with 5 mL distilled water and 5 mL TBA solution, using a UV spectrometer (2550, Shimadzu, Japan). Thiobarbituric acid reactive substances (TBARS) were calculated from a standard curve of malondialdehyde (MDA), which was freshly prepared by acidification of 1,1,3,3-tetraethoxypropane (TEP). TBA value was expressed as milligrams of malondialdehyde per kilogram of fish sample (mg MDA/kg sample).

Fatty acid analysis

The fatty acid composition of fish was determined by gas chromatography. Methyl esters were prepared using 2 M KOH in methanol and n-hexane according to the method described by (Ishihara et al., 1996) with minor modifications: 10 mg of extracted fat was dissolved in 2 mL hexane followed by 4 mL of 2 M methanolic KOH. The mixture was then vortexed for 2 min at room temperature and the hexane layer was used for GC analyses. The chromatographic separation was carried out using a Hewlett-Packard (HP 5890) chromatograph, a split/splitless injector and a flame-ionisation detector (FID) linked to an HP Chemstation integrator. A fused silica capillary column DB23 (60 m × 0.32 mm i.d. × 0.25 μm as film thickness) was used with nitrogen as the carrier gas, the flow rate of which was set at 0.44 mL min^{-1}; the temperature of the flame ionisation detector was maintained at 280°C and that of the injector at 270°C. The column temperature program was as follows: from 130 to 170°C at 6.5°C min^{-1}, from 170 to 215°C at 2.8°C min^{-1} 12 min isothermally, to 230°C at 40°C min^{-1} and 20 min isothermally. The standard

fatty acid methyl esters (FAMEs) were run under the same conditions. The FAME peaks were identified by comparison of their retention times with those of the standard mixtures and the areas under the peaks were automatically integrated using nonadecanoic acid methyl ester (C19:0) as the internal standard.

Statistical analysis

All the experiments were performed in triplicate. Analysis of variance (ANOVA) was used to evaluate the analysis data, and significant differences among means were determined by one-way ANOVA and Duncan's multiple range test (P = 0.05) (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL).

Results and Discussion

Nutritional constituent

As shown in Table 1, the dry matter content of fresh grass carp fish was 22.5 g/100g fresh flesh; and the contents of protein, fat and ash were 19.11, 1.85 and 1.15 g/100 g fresh fish, respectively. The elementary composition of grass carp fish was similar to the results reported by (Wu and Mao, 2008).

Both curing and aging had the effect of dehydrolysis, which led to the increase of dry matter content of Layú. The addition of Chinese prickly ash had no significant effects on the nutritional contents of salted Layú, and the contents of raw protein, fat, NaCl and ash showed no obvious differences between LayúA and Layú B.

The content of non-polar lipid (NPL) and polar lipid (PL)

The total fat content of grass carp changed to different degree after being curied and aged (Table 1), as well as the content of NPL and PL (Table 2).

Neutral lipid could be broken down into alcohols, fatty acids and other polarity material in controlled conditions. Neutral lipid was mainly depot lipid which included saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA), while polar lipid was membrane lipid which included more polyunsaturated fatty acid (PUFA)(Duckett et al., 1993).

Because of the impact of oxygen and microorganism in the air, the ratio of PL gradually elevated. Comparing the changes of NPL and PL content of Layú A and Layú B, it can be seen that Chinese prickly ash could restrain the increase of PL ratio to some extent.

Peroxide value (POV)

POV was used to measure the degree of oil oxidation in the initial stage of oxidation. As shown in Figure 1, POV of Layú A and Layú B increased gradually during the curing and aging process. However, the addition of Chinese prickly ash inhibited the increase of POV during the manufacturing process of Layú.

TBA

The thiobarbituric acid (TBA) value is the results of TBA reacted with malondialdehyde (MDA), which is the oxidative decomposition derivative from animal unsaturated fatty acids. TBA value indicates the extent of fat oxidation. MDA formed through hydroperoxides, which are the initial oxidative product of polyunsaturated fatty acids. In Figure 2, TBA values of Layú A and Layú B gradually increased during the curing and aging process and the increase of TBA value was inhibited because of the addition of Chinese prickly ash.

Free fatty acids

The hydrolysis of glycerin fatty acid ester which releases free fatty acids is an important reaction during storage and processing of aquatic products. And it is usually catalyzed by lipase and phospholipase (Pacheco-Aguilar et al., 2000). The content of free fatty acid indicates the extent of oil hydrolysis. As shown in Figure 3, the content of free fatty acids gradually elevated in Layú A and Layú B during salting and aging. However, Chinese prickly ash prevented the increase of free fatty acids during the aging of Layú.

Fatty acids

As ~~shown~~ shown in Table 3, palmitic acid (C16:0) was the most abundant saturated fatty acids (SFA), oleic acid (C18:1n-9) was the most abundant monounsaturated fatty acids (MUFA), while linoleic acid (C18:2n-6) and DHA（C22:6n-3） ~~was~~ were the most abundant polyunsaturated fatty acids (PUFA). These results were consistent with those obtained by (Wu and Mao, 2008). Fatty acid composition changed to different degrees after curing and aging process (Bakar et al., 2008).

All SFA in grass carp fish significantly increased after curing, and contents of palmitic acid (C16:0) in Layú A and Layú B increased by 1.60% and 1.1%, stearic (C18:0) increased by 0.95% and 0.50%, total SFA increased by 4.50% and 2.21%, respectively. Similarly, dry-ripped process also significantly increased the contents of different SFA in grass carp fish. The contents of

palmitic acid (C16:0) in Layú A and Layú B increased by 2.40% and 1.12%, stearic (C18:0) increased by 1.70% and 1.40%, total SFA increased by 6.27% and 3.21%, respectively. And the results illustrated that Chinese prickly ash conspicuously inhibited the increase of SFA during the manufacturing process.

The content of each MUFA in grass carp fish changed variously after curing and dry-aging. The total MUFA contents in Layú A and Layú B increased by 1.59% and 2.99% respectively after curing, and decreased by 3.03% and 1.07% respectively after aging. The total MUFA contents were 2.73% higher in Layú B than those in Layú A. These results demonstrated that adding Chinese prickly ash could effectively prevent MUFA from hydrolysis and oxidation reactions. As far as lipid oxidation is concerned, salt has been treated as a pro-oxidant agent in several studies(Andrés et al., 2004; Kanner et al., 1991). Still now, the mechanism by which NaCl affects lipid peroxidation is not well understood. Literature reported that increasing the concentration of NaCl enhanced lipid peroxidation in raw minced muscle, especially after freezing-thawing process(Kanner et al., 1991). However, due to the implications of high salt content in the human diet on cardiovascular health (Brunner et al., 2001), the low content of salt was used in this study, could reduce the degree of lipid oxidation development in dry-cured fish.

Z. Bungeanum Maxim leaf (ZML) extract contains high amounts of phenolic compounds possessing high total antioxidant capacity and hydroxyl, 1, 1-dipheny-2-picrylhydrazyl (DPPH) free radical scavenging activity (Li et al., 2014). (Li *et al.*, (2015) investigated ZML extract as a natural antioxidant for the lipid stability of salted silver carp (Hypophthalmichthys molitrix) throughout salting and drying, found that it could be a source of natural antioxidants. Our results indicated that *Z. Bungeanum* Maxim had good antioxidant activity. The reason may be there are polyphenol compounds in *Z. Bungeanum* Maxim, which express high total antioxidant capacity and DPPH free radical scavenging activity. Fish protein hydrolysates during processing have been reported to exhibit antioxidant activity (Binsan et al., 2008; Zhang et al., 2013a), however the antioxidant activity of protein hydrolysates depends on the protease and hydrolysis conditions employed(Chia - Ling and Wen - Ching, 2002). Thus, the antioxidant activity of protein hydrolysates had been neglected in the present study.

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The curing and dry-aging decreased significantly PUFA in grass carp fish (P<0.05). After the

curing processing, the contents of EPA (C20:5n-3), DHA (C22:6n-3), and total PUFA were decreased by 0.30%, 0.47% and 3.30%, respectively in Layú A, and by 0.28%, 0.30% and 2.82% respectively in Layú B. While, for the dry-riping process, the contents of EPA (C20:5n-3), DHA (C22:6n-3), and total PUFA was decreased by 0.48%, 0.74% and 4.40% respectively in Layú A, and by 0.26%, 0.45% and 2.79% respectively in Layú B. Therefore, it indicated that the addition of Chinese prickly ash reduced the decrease of PUFA contents and ~~played a crucially important role in the inhibition of oxygenolysis of PUFA during the manufacturing process of Layú~~played a crucially important role in the inhibition of PUFA oxidation during the manufacturing process of Layú.

The results of fatty acid composition demonstrated that grass carp fish was rich with n-3 PUFA, which accounted for 17.66% of total fatty acid in grass carp fish (Table 3). Meanwhile, n-6 PUFA was also abundant in grass carp fish, which accounted for 17.21% of the total fatty acids in grass carp fish. Both contents of n-3 PUFA and n-6 PUFA decreased during the manufacturing process of Layú, especially in the drying process ($P<0.05$).

N-3 and n-6 PUFA in fish fatty acids are the integral essentials for human being's growth and development. They are the necessary ingredients for synthesizing structured lipids of human body and cell membrane. Besides, the main physiological function includes adjusting metabolism of cholesterol and triglyceride, synthesizing arachidonic acids and prostaglandin, protecting nervous tissue, strengthening the cardiovascular system, keeping the health of skin and hair, reducing swelling and inflammation and keeping the normal growth and function of brain and eye, etc. (Calder and Grimble, 2002; de Pablo and de Cienfuegos, 2000; Wong, 2005).

These two classes of PUFA competed for the same enzyme for conversion and derivatives in body and have many important opposing physiological functions. Therefore，the balance between them is very important for normal growth and development. The recommended ratio of n-6: n-3 in human diet should be more than 1:5, and the nutritionally ideal ratio of n-6:n-3 was 1:1 (Dawczynski et al., 2007; Simopoulos, 2002). The study indicated that the ratio of n-3: n-6 in each sample was in range of 1.02-1.07 during the manufacturing process of Layú. Therefore, ~~both the fresh grass carp fish and curried Layú were beneficial for human health~~ both fresh grass carp fish and curried Layú were beneficial for the balance of the two classes of PUFA.

The ratio of UFA: SFA in fish significantly decreased during curing processing (Table 3). However, the addition of Chinese prickly ash inhibited the decline of the ratio of UFA: SFA in Layú. It could be concluded that *Z. Bungeanum* Maxim could effectively slow down the oxidation of fatty acids in Layú.

Conclusion

The addition of *Z. Bungeanum* Maxim (2%, W/W) during curing process had no significant differences on the nutritional contents of salted Layú. Chinese prickly ash contained the natural chemical constitutes which had antioxidant and antimicrobial activity such as alkaloid, amide, lignin, essential oils and coumarins, etc. The addition of Chinese prickly ash significantly inhibited the increase of ratio of polar oil in aged Layú, POV, TBA, free fatty acids content in Layú and oil oxidation($P<0.05$), especially protected the ingredient of functional PUFA such as EPA and DHA, during the manufacturing of Layú. Therefore, *Z. Bungeanum* Maxim (2%, W/W) combined with salting during manufacturing of Layú can effectively improve the quality of Layú ~~and extend its shelf-life~~.

Acknowledgments

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Author Contributions

J. Zhang collected test data and drafted the manuscript. D. Xu and H. Mo did the experiments. Z. Fang prepared the samples. X. Zhao designed the study and interpreted the results.

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FIGURE LEGENDS

Figure.1 The peroxide value of grass carp lipid during the manufacturing process

Figure.2 Thiobarbituric acid (TBA) of grass carp lipid during the manufacturing process

Figure.3 The content of free fatty acid of grass carp lipid during the manufacturing process

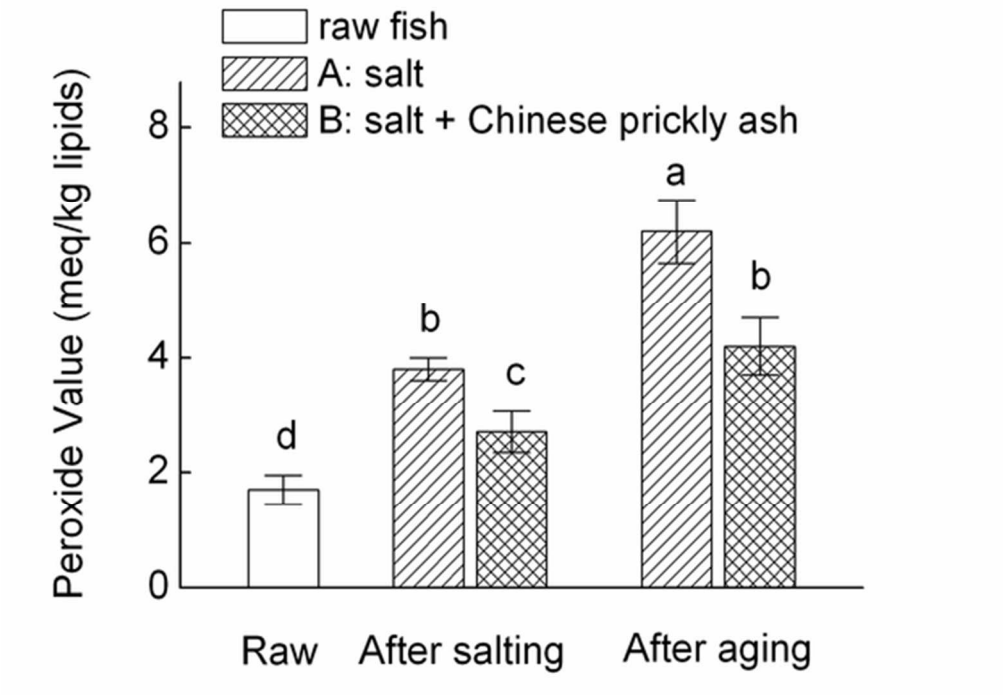

Figure1
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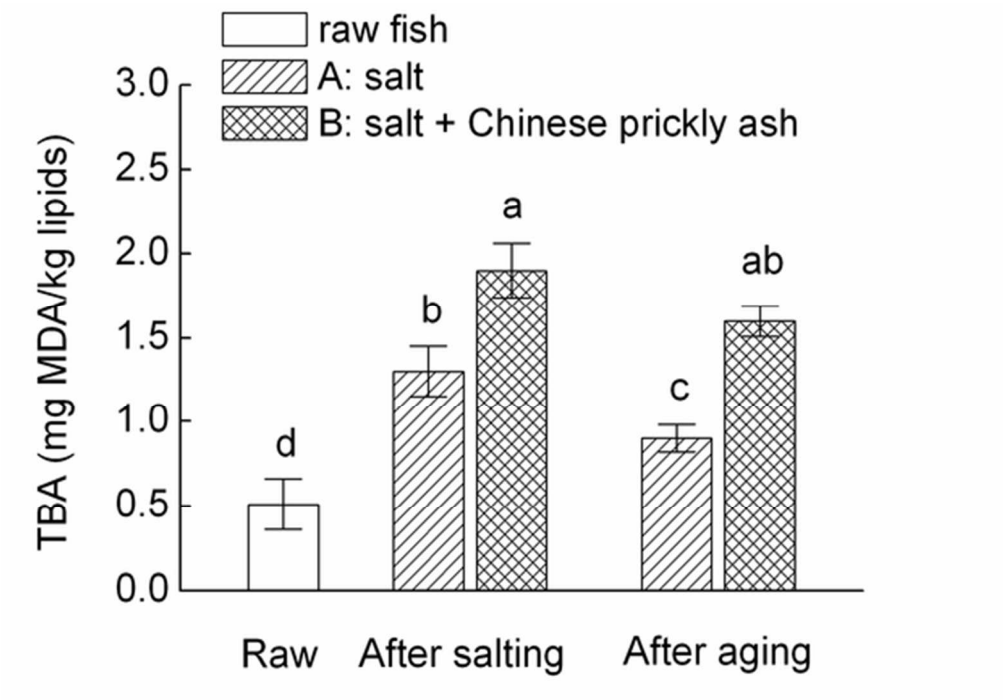

Figure2
52x36mm (300 x 300 DPI)

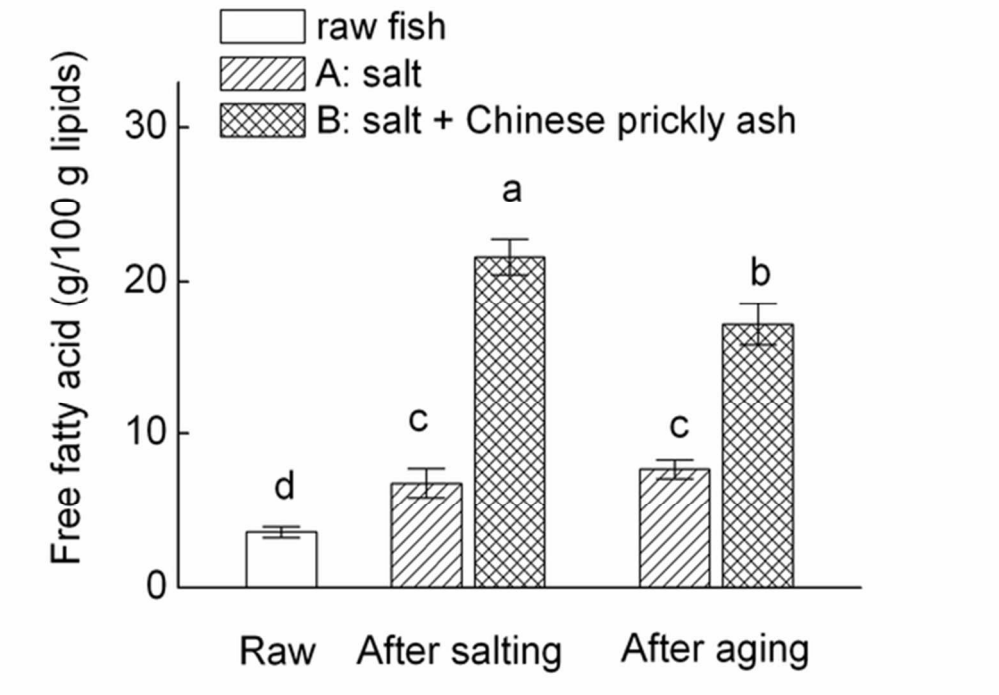

Figure3
52x36mm (300 x 300 DPI)

Table 1 Chemical composition of fresh, salted and aging grass carp (n =5)

Composition	Raw fish	A：Salt		B：Salt+ spicery	
		After salting	After aging	After salting	After aging
Dry matter (g/100g meat)	22.51±0.29c	29.35±0.57b	59.52±1.28a	29.52±0.72b	59.17±1.52a
Protein (g/100 g meat)	19.11±0.13c	22.50±0.76b	45.13±0.95a	22.63±0.61b	44.95±1.11a
Lipids (g/100 g meat)	1.85±0.05c	1.95±0.11b	4.01±0.17a	1.89±0.05bc	4.10±0.21a
Ash (g/100 g meat)	1.15±0.07c	5.17±0.32b	10.53±0.85a	5.09±0.42b	10.71±0.55a
Chloride (g/100 g meat)	0.12±0.02c	4.92±0.13b	9.81±0.46a	4.87±0.37b	9.79±0.57a
EPA (g/100 g meat)	0.039±0.007c	0.035±0.002c	0.053±0.007b	0.035±0.004c	0.064±0.005a
DHA (g/100 g meat)	0.176±0.001c	0.176±0.005c	0.333±0.013b	0.174±0.010c	0.360±0.008a

Values are expressed as means ± standard deviation (n=3). Different letters in two columns mean significant differences ($p < 0.05$).

Table 2 The content of non-polar lipid (NPL) and polar lipid (PL) fractions of fresh, salted and aging grass carp

Lipid characteristics	Raw fish	A：Salt		B：Salt+Spicery	
		After salting	After aging	After salting	After aging
NPL (%)	95.61 ± 0.72	93.51 ± 0.87	91.25 ± 1.02	93.67 ± 0.87	92.52±0.55
PL (%)	4.35 ± 0.21d	6.39 ± 0.12c	8.71 ± 0.35a	6.23 ± 0.27c	7.38 ±0.31b

Values are expressed as mean ± standard deviation(n=3). Different letters in two columns mean significant differences ($p<0.05$).

Table 3 Fatty acid content (% of total fatty acids) in raw, salted and aged grass carp

Fatty acids	Raw fish	A：Salt		B：Salt+Spicery	
		After salting	After aging	After salting	After aging
C12:0	2.32 ± 0.07a	2.29 ± 0.06a	2.11 ± 0.02c	2.18 ± 0.06b	2.21 ± 0.05b
C14:0	0.64 ± 0.02c	0.92 ± 0.07b	1.32 ± 0.01a	0.83 ± 0.05bc	1.05 ± 0.03b
C15:0	0.86 ± 0.03a	0.75 ± 0.01c	0.81 ± 0.01b	0.86 ± 0.01a	0.81 ± 0.03b
C16:0	13.10 ± 0.15d	14.70 ± 0.13c	17.10 ± 0.17a	14.2 ± 0.21c	15.32 ± 0.72b
C17:0	1.64 ± 0.05bc	1.83 ± 0.04a	1.69 ± 0.01b	1.72 ± 0.06ab	1.61 ± 0.15c
C18:0	7.71 ± 0.21d	8.75 ± 0.09c	10.51 ± 0.11a	8.21 ± 0.68c	9.61 ± 0.05b
C20:0	0.21 ± 0.04e	0.37 ± 0.11d	1.59 ± 0.03a	0.52 ± 0.68c	1.32 ± 0.06b
C22:0	0.85 ± 0.02d	1.52 ± 0.03b	2.87 ± 0.06a	1.35 ± 0.01c	1.25 ± 0.12c
ΣSFA	27.31 ± 0.47d	31.81 ± 0.25bc	38.18 ± 0.55a	29.52 ± 0.69c	32.83 ± 0.75b
C14:1*n*-5	0.69 ± 0.01a	0.35 ± 0.02c	0.32 ± 0.05d	0.37 ± 0.01c	0.42 ± 0.01b
C16:1*n*-7	8.21 ± 0.37a	7.53 ± 0.06b	6.32 ± 0.08c	7.25 ± 0.15b	6.57 ± 0.65c
C17:1	0.84 ± 0.02a	0.61 ± 0.04c	0.71 ± 0.02b	0.77 ± 0.05ab	0.62 ± 0.01c
C18:1*n*-9	20.2 ± 1.84c	21.5 ± 1.07b	20.3 ± 1.41c	22.1 ± 1.47a	21.5 ± 1.18b
C20:1*n*-9	3.22 ± 0.03c	3.82 ± 0.05b	3.48 ± 0.03bc	4.17 ± 0.17a	3.92 ± 0.15b
C22:1*n*-9	3.21 ± 0.17d	4.97 ± 0.08a	3.95 ± 0.24c	4.52 ± 0.32ab	4.38 ± 0.21b
ΣMUFA	36.62 ± 1.21c	38.21 ± 0.12ab	35.18 ± 0.15d	39.61 ± 0.41a	37.91 ± 0.72b
C18:2*n*-6	7.01 ± 0.42a	6.57 ± 0.05b	5.53 ± 0.61d	6.63 ± 0.03b	5.82 ±0.52c
C18:3*n*-6	0.21 ± 0.01a	0.17 ± 0.01b	0.20 ± 0.02a	0.21 ± 0.01a	0.19 ± 0.02a
C18:3*n*-3	3.80 ± 0.32a	3.05 ± 0.21c	2.72 ± 0.32d	3.28 ± 0.02b	2.92 ±0.21c
C20:2*n*-6	1.81 ± 0.04a	1.35 ± 0.17b	1.05 ±0.12c	1.41 ± 0.29b	1.23 ± 0.11bc
C20:3*n*-6	3.05 ± 0.17a	2.61 ± 0.06b	2.17 ± 0.25c	2.57 ± 0.13b	2.41 ± 0.52bc
C20:4*n*-6	5.12 ± 0.52a	5.04 ± 0.57b	4.66 ± 0.15c	5.06 ± 0.52b	4.76 ± 0.21c
C20:5*n*-3	2.11 ± 0.11a	1.81 ± 0.04b	1.33 ± 0.11d	1.83 ± 0.02b	1.57 ± 0.37c
C22:5*n*-3	2.23 ± 0.09a	2.08 ± 0.06ab	2.05 ± 0.01b	2.06 ± 0.03b	1.92 ± 0.09c
C22:6*n*-3	9.52 ± 0.72a	9.05 ± 0.37b	8.31 ± 0.65c	9.22 ± 0.05ab	8.77 ± 0.05bc
ΣPUFA	34.91 ± 1.01a	31.61 ± 0.77c	27.21 ± 0.65e	32.09 ± 0.97b	29.30 ± 1.89d
Σ*n*-3	17.66 ± 1.93a	15.99 ± 0.86bc	14.41 ± 0.57d	16.39 ± 0.21b	15.18 ± 1.18c
Σ*n*-6	17.21 ± 1.07a	15.74 ± 0.43b	13.51 ± 0.83d	15.88 ± 1.03b	14.41 ± 0.62c
n-3/*n*-6	1.03 ± 0.01b	1.02 ± 0.01b	1.07 ± 0.02a	1.03 ± 0.01b	1.05 ± 0.01ab
UFA/SFA	2.62 ± 0.08a	2.19 ± 0.05c	1.63 ± 0.03e	2.43 ± 0.10b	2.05 ± 0.03d

Values are expressed as mean ± standard deviation(n=3). Different letters in two columns mean significant differences($p<0.05$).