Effect of different drying processes on the protein degradation and sensory quality of Layú: A Chinese dry-curing grass carp

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ABSTRACT

Five different drying methods, sun drying, intermittent drying, low-temperature drying at 5°C, low-temperature drying at 15°C and hot-air drying at 45°C, were comparatively evaluated based on physicochemical properties and sensory properties of dry-cured Layu. Sun drying and intermittent drying Layu showed superior sensory qualities compared with other dried samples. Based on the comprehensive comparison of sensory qualities and safety concerns, intermittent drying Layu was more acceptable compared to other dry-cured Layu and thus was recommended for dry-curing fish products as it could shortened the drying time with relative constant drying rate, increased flavor amino acid content and less safety concerns.

Keywords: Drying, dry-curing fish, protein, biogenic amine, sensory evaluation

Running Title: Effect of Drying Methods on Quality of Dry-curing Fish

INTRODUCTION

Up to now, Asia represents over 90% of world aquaculture production, among which, China was by far the biggest contributor to aquaculture with freshwater aquaculture accounts for about 56.6% of production. Curing and drying was the techniques available to preserve fish with a long history. [1,2] In Chinese tradition, fresh fish was usually salted and/or spiced, then dried for preserving and transportation, more importantly for the development of desirable flavor and unique texture. Layú is the traditional name for dry-cured grass carps (*Ctenopharyngodonidellus*) in Chinese. It was popular not only for its special chewiness and flavor but also long shelf life. [3] Layú is also a good source of high-quality proteins as well as vitamins and minerals. Fish proteins were well known for their excellent combination of amino acids that suit well for nutritional requirements. In South China, Layú is highly appreciated. [3]

In the curing processes, fish proteins were prone to degrade into relatively large peptides, then further into small peptides and free amino acids due to microbial and enzymatic activities. They could be further converted into even smaller compounds, such as ammonia, α-ketoacids, methylketones and amines. [4] High temperatures, high pH values and low salt concentrations can promote the accumulation of free amino acids and, therefore, stimulate the formation of biogenic amines. These biogenic amines were basic nitrogenous compounds usually formed by the decarboxylation of the precursor amino acids. [5,6] In cured products, large amount of specific biogenic amines indicate poor-quality raw materials, microbial contamination and adverse conditions during processing and storage. Therefore, curing processes must be carried out carefully without compromising product quality while extending shelf-life.

Drying was a complex curing process that depends on physicochemical (temperature, pH, ionic strength and water activity) and biochemical (lipid content, enzymes and bacterial species) parameters. It changed the properties of the fish tissue (including protein degradation, lipid oxidation, etc), thus the overall sensory properties of the fish. Sun drying and hot-air drying were two main traditional drying methods for fish products. Sun drying was an easy-to-perform and economic processing method, but very limited by the environmental conditions, and frequently associated with low quality and long processing time. Hot-air drying employed a convection system with high temperature, controlled air velocity and humidity, but involving high energy consumption, bacterial contamination and possible quality change.[7,8] To satisfy the demand for high-quality fish products and the availability to anywhere it needs, drying techniques had always been modified and developed to meet the requirements. In recent years, intermittent drying method combining multistage-drying conditions showed promising effects on improving dehydration ratio and dehydration quality, as well as maintaining good food quality.[9,10] However, due to the variations in species, shape and size of the raw materials, rarely a single drying method could fit in the needs for all fish products.

The objective of this study was to investigate the effects of drying process on the production of traditional Layu, which aimed at improving the product quality and process efficiency. Five different drying methods, sun drying (SD), low temperature drying at 5°C (LT5), low temperature drying at 15°C (LT15), hot air drying (HA) at 45 °C and intermittent drying (ID, 7 h in a vacuum with air velocity of 1 m/s at 5°C and relative humidity of 60%–65% and 5 h in sealed condition at 10°C as a cycle), were studied through evaluating drying curves and drying rates, sensory qualities, protein contents, biogenic amine and nitrate values. Comparisons were made with fresh and salted carps.

MATERIALS AND METHODS

Sample Preparation

Eighty fresh grass carps (*Ctenopharyngodon idellus*) were purchased from a local fish market (Hangzhou, Zhejiang province, China) and immediately brought to the laboratory on ice. Individual fish weight was 2.5 ± 0.5 kg. After washing, scaling, de-heading, and gutting, each fish was cut into half for salting. Salting with a fish to salt of 1:9 (w/w) was performed at 5°C for 6 days avoiding direct sunlight. After salting, fish was cut into fillets of 3 x 4 x 1.5 cm for dry-curing. Fish fillets were equally divided into five groups and dried to an end point of water content of 40% processed fish weight.

Five drying methods were performed at the end of winter season (from late Dec. to early Jan.) with a constant daytime temperature of 5°C–15°C. Sun drying (SD) was used as the control under the condition of 8 h in direct sunlight at 5-15°C and 16 h in sealed condition at 10°C every day. Four other methods were: 5°C low-temperature drying (LT5) (in a vacuum of 77kPa with air velocity of 1 m/s at 5°C and relative humidity of 60%–65%), 15°C low-temperature drying (LT15) (in a vacuum of 77kPa with air velocity of 1 m/s at 15°C and relative humidity of 60%–65%), hot-air drying (HA) (in a convection oven with air velocity of 1 m/s at 45°C and relative humidity of 60%–65%), and intermittent drying (ID, 7 h in a vacuum of 77kPa with air velocity of 1 m/s at 5°C and relative humidity of 60%–65% and 5 h in sealed condition at 10°C as a cycle).

Ash Content

Ash content was determined by using a muffle furnace heated at 600°C for 2 h. The ash content was than calculated by the weight according to the AOAC standard method.[11]

Moisture Content and Drying Rate

Moisture content was determined by drying 5 g sample in a convection oven at 105°C until constant weight was obtained.[12] Drying rate was determined by dividing the water content with the drying time.

Protein Content

Proteins were extracted from samples according to the method described by Lefever et al.,[13] and protein concentration was determined by the Biuret method.[14] SDS-PAGE was performed for the extracted protein as described by Balange and Benjakul[15] with modifications. Ten milliliters of citric acid-phosphate buffer was added to a 200-mg sample. The mixture was centrifuged (10,000g) for 10 min. Then, the supernatant was analyzed by the SDS-PAGE using 4% stacking gel and 10.5% running gel with MW markers ranging from 6.5-200 kDa (Sigma-Aldrich., Shanghai, China).

Free Amino Acid Analysis

Free amino acid (FAA) contents from fish protein extracts were analyzed. A sample of 500 μL was first combined with 50 μL internal standard (0.325 mg/mL hydroxyproline), then deproteinized with acetonitrile and centrifuged. The supernatants were derivatized by using the method from Bidlingmeyer et al..[16] The derivatized amino acids were analyzed by reverse-phase high pressure liquid chromatography (HPLC) as previously described.[17]

Biogenic Amines Determination

Biogenic amines were extracted according to the method described by Saarinen.[18] Briefly, 5 g finely ground sample was homogenized with 20 mL of 5% TCA solution for 2 min and centrifuged. The residue was homogenized again with another 20 mL of 5% TCA solution and centrifuged. Supernatant from both centrifugations was combined and filtered, then analyzed by a Waters 2695 series HPLC system.[19]

The derivatization reagent was prepared by mixing 100 mg *o*-phthalaldehyde (OPA), 1 mL acetonitrile and 130 μL 2-mercaptoethanol, and diluting into 10 mL with 0.4 M borate buffer (pH 10.2). Pre-column derivatization with OPA was performed automatically. A reverse-phase Hypersil ODS C_{18} (125×4.60 mm, particle size 5 μm) column was used for separation. The column temperature and flow rate were set at 40°C and 1.0 mL/min, respectively. The mobile phase consisted of solvent A (pH 7.2), 7.35 mM sodium acetate solution:triethylamine:tetrahydrofuran (500:0.12:2.5, v/v), and solvent B (pH 7.2), 7.35 mM sodium acetate solution:methanol:acetonitrile (1:2:2, v/v). Fluorescence was monitored at an emission wavelength of 450 nm using an excitation wavelength of 340 nm.

All samples and standards were injected at least in duplicate. Repeatability tests were performed by injecting a standard and sample consecutively six times a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for three days under the same experimental conditions.

Nitrite Determination

Nitrite content of dried salted samples was determined using a colorimetric nitrite assay based on the Griess reaction.[20] Approximately 5 g finely ground fish sample was deproteinized and defatted by precipitation with 10 mL of 0.42 M ZnSO4 followed by filtration. 1mL of each color developing reagent, 0.2% sulfanilamide, 0.1% N-1-naphtyethylene diamine dihydrochloride, and 44.5% HCl, was added sequentially to the filtrate. The mixture was then kept at room temperature for 5 min. The OD value of the colored mixture was read at 538 nm on a Shimadzu UV2401 spectrophotometer (Shimadzu Inc., Tokyo, Japan) and determined by fitting into a standard curve of $NaNO_2$.

Total Volatile Basic Nitrogen Profiles

Analysis of total volatile basic nitrogen (TVB-N) was carried out according to the method described by Sallam[21] with appropriate modification. Approximately 10 g sample was homogenized with 90 ml of 0.6 M perchloric acid and centrifuged. The supernatant was then filtered and analyzed by a Foss Kjeltec 2300 Analyzer to determine the hydrochloric acid consumption. The TVB-N was calculated using the following equation:

$$\text{TVB-N (mg N}\cdot 100\text{g}^{-1}) = \frac{(V-V0)\times 0.098134\times 14\times 1000}{m}$$ **Error! Bookmark not defined.**

Where V is the volume of hydrochloric acid consumption of the treated samples (ml); V0 is the volume of hydrochloric acid consumption of the control (mL); 0.098134 is the concentration of hydrochloric acid standard solution (M); 14 is the molecular weight of nitrogen ($g\cdot mol^{-1}$); m is the mass of the sample (g). The results were expressed in mg N per 100 g sample.

Sensory Evaluation

The sensory evaluation was performed by a trained sensory panel of 12 members. Samples were immersed in cold water for 10 min, and then steamed for 15 min. When samples cooled down to 40°C, sensory characteristics were evaluated in terms of texture, color, odor, taste and chewiness on a 1–10 scale with 10 corresponding to like extremely and 1 corresponding to dislike extremely. The percentage of each sensory character in the overall rating is: texture 15%, color 15%, smell 30%, taste 30% and chewiness 10%.[22]

Statistical Analysis

The data were analyzed using Microsoft excel 2003 and SPSS 16.0. All results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). Comparison of the mean values of different treatments was based on Duncan's multiple range test and statistical significance was considered at $p < 0.05$.

RESULTS AND DISSCUSIONS

Water Content Profiles for Layu

Salting and drying were the two most common methods in processing fish products. In this study, fresh carps were salted first, and then dry-cured. Figure 1 showed drying curves of the five drying methods, sun drying, low-temperature drying at 5°C, low-temperature drying at 15°C, hot-air drying at 45°C and intermittent drying at 15°C. During sun drying process, salted grass carps were dried at relatively low rates at 1.51%/h on day 1 and 0.75%/h on day 4. The time of SD samples reaching 40% of moisture content was 4 d. Low-temperature drying (LT5 and LT15) and hot-air drying was continuous processes. Specifically, LT5 sample drying rate was the lowest with small variations among LT5, LT15 and HA drying methods. HA sample drying rate was high at 3.61%/h during the first 5 h of drying and then decreased to 0.57%/h at the end. The drying time needed to reach 40% of moisture content was 48 h, 32h and 20 h for LT5, LT15 and HA samples, respectively. During these drying processes, the drying rates for all samples decreased with increasing drying time. This was partly due to the water movement from the center of the fish to the surface along with the salt migration. With the water evaporating, salt crust forms on the surface of the fish blocking further water evaporation, thus reducing the water loss rate (or drying rate) with time.[23] Meanwhile, salt ions attracted free water molecules in high salt concentrations. This also decreased the water loss rate with time. Thus no constant drying rates were shown during the drying period, and the rates decreased with time. This drying rate pattern also applied to intermittent drying method. Intermittent drying simulated sun drying with similar drying temperature and exposure time, but had controlled ventilation and humidity. After 4 cycles of drying and sealing, the moisture content of ID Layu reached 38.7%. The drying period in ID was 28 h, saving 20 h compared to LT5 and 4 h to LT15. The overall time needed to dry salted carps for ID was 36 h less than that for SD.

Sensory Evaluation for Layu

Sensory evaluation on color, texture, odor, etc., was used worldwide for the determination of food quality and thus consumer acceptance.[24,25,26]. In this study, dried salted samples were steamed for rehydration, and then evaluated by experienced panelists for sensory scores. Results showed that individual sensory score and the overall score for each dried sample group were higher than salted samples (Table 1). This indicates that dehydration led to major changes in tissue texture and flavor components, therefore resulting in sensory quality changes in dried fish. Among different dried sample groups, sensory scores for texture, color, odor, taste and chewiness varied. SD and ID Layu had higher scores in texture and taste than other samples. In addition, ID Layu had the highest scores in color and chewiness among all samples, whereas HA Layu had the lowest sensory scores in all five categories of sensory evaluation. From observation, LT15 and HA Layu had hard surface with dark brown color but soft center of the meat, which may have attributed to the high water loss rates and continuous drying during the drying process. Combining all sensory scores, ID Layu had the highest overall score among all samples followed by SD Layu, indicating high-quality products processed from intermittent drying as well as sun drying.

Muscle Proteins and Free Amino Acids Profiles for Layu

Fish muscle proteins could be degraded by proteases when the fish is alive, and even after the fish dies.[13,27] Peptides and free amino acids were then released to form the unique flavor of the processed seafood which was highly appreciated by consumers.[2] In Figure 2, the SDS-PAGE profile clearly showed the disappearance of the band for myosin heavy chain (200 kDa) in the samples from SD and ID Layu meat, suggesting the major component of fish muscle proteins was hydrolyzed during the drying process.

The values of total FAA and total essential amino acid (EAA) in all dried samples were significantly higher than those in fresh and salted samples, indicating the effects of drying process on protein hydrolysis and elevation of FAA levels (Table 2). Specifically, FAA and EAA in dried samples followed a descending trend of SD ≈ ID > LT15 > LT5 > HA. Glutamic acid and asparagic acid were the two major flavor amino acids. The combined values for these two amino acids were: SD (4.23 mg/kg DW) > ID (3.8 mg/kg DW) > LT15 (2.23 mg/kg DW) > LT5 (2.10 mg/kg DW) > HA (1.36

mg/kg DW). The values for sweet flavor amino acids, such as Gly, Ala, Ser, Thr, Pro, Lys, Cys and Met, decreased in the sequence of ID > SD > LT15 > LT5 > HA. From the above analysis, SD and ID Layu apparently had overall high values in total FAA and EAA, as well as individual flavor amino acids, which may be in correspondence with long drying time. Compared to SD and ID, HA had advantages in shortening drying time and improving drying efficiency, but not in the aspects of promoting flavor and nutrition. The results from FAA analysis were included with those from the sensory evaluation.

Total Volatile Basic Nitrogen Profiles for Layu

Total volatile basic nitrogen (TVB-N) including primary, secondary and tertiary amines, which contributed to the unpleasant odor in fish, was used as an indicator for fish deterioration.[23] Previous study showed that during the preservation and processing of fish and fish products, the endogenous proteases and microbial contamination are the main sources causing the increase in TVB-N value [24]. TVB-N contents for fresh fish, salted fish, and each dried sample group were compared in Figure 3. All dried samples had significantly higher TVB-N values than fresh and salted samples, indicating the effect of curing process on the increase of TVB-N levels. Based on the current legal limit of 30 mg/100 g,[28] all dried samples had TVB-N values lower than the legal limit of China. Among the dried samples, SD Layu showed the highest TVB-N value, whereas HA Layu had the lowest value. The length of the drying time played an important role in the levels of TVB-N. When compared to SD Layu, ID Layu had significantly lower TVB-N value, suggesting ID Layu was less deteriorated.

Biogenic Amine Profiles for Layu

Biogenic amines are organic basic nitrogenous compounds formed mainly by decarboxylation of amino acids, or by amination and transamination of aldehydes and ketones, which were ubiquitous constituents of food.[29] The level of biogenic amines could be used as an indicator of food spoilage or freshness.[30,31] Recently, six major biogenic amines, including putrescine, cadaverine, spermine, spermidine, histamine and tyramine, received considerable attention due to their detrimental effects on human health, such as migraine, hypertension, hypotension, rash and digestive problems.[6] They were considered to be potential precursors of carcinogenic N-nitroso compounds, and their presence may lead to death in very severe cases.[31]

Table 3 listed biogenic amine values for fresh and salted fish, and dry-cured Layu. Four biogenic amines, cadaverine, spermine, spermidine and tyramine, were detected from fresh samples. After salting and drying, two additional biogenic amines, putrescine and histamine, were detected in salted and dried samples. The biogenic amine values increased from 38.40 mg/kg DW in fresh samples to 59.36 mg/kg DW in salted samples due to the additional two biogenic amines. The biogenic amine values in dried samples varied among different drying methods, with a descending sequence in SD > ID > LT15 ≈ LT5 > HA. SD Layu had the highest biogenic amine level among all dried samples, which was consistent with the analysis for TVB-N. Among the biogenic amines, histamine and tyramine are considered to be the most hazardous to human health, and usually used as the indices for biogenic amine levels. FDA index for histamine is less than 500 mg/kg food. EU index of mackerel for histamine is less than 100 mg/kg and for tyramine is less than 100-800 mg/kg.[30] Histamine and tyramine levels in all dried samples were lower than the legal limits.

Nitrite Profiles for Layu

Experimental mammalian studies had shown that high levels of nitrite adversely affected reproductivity including of fetus loss, reduced number of litters and live births, and neonatal mortality.[32,33] Nitrite was reported to form during the traditional curing of fish products.[34] After consumption, nitrite can be converted into nitrosamine, a strong carcinogen, in the stomach by microbial reaction.[35] However, deoxidizing microbes, such as *Escherichia coli*, *Staphylococcus aureus* or mold, existing in fish meat can help reduce nitrate into nitrite. In addition, trimethylamine produced by fish protein hydrolysis can catalyze nitrite formation from nitrate. Nitrite contents for fresh and salted fish, and dry-cured Layu were compared in Figure 4. All salted and dried samples had higher nitrite values than fresh samples, indicating salting and drying processes promoting nitrate formation. Nitrite value in SD samples was higher than in all other dried samples, which was consistent with TVB-N and biogenic amine analysis. There were no significant differences in nitrite values among LT5, LT15, ID and HA samples. According to national standard (GB2760-2011) of China, nitrite content in cured products should be kept equal or less than 30 mg/kg. Based on the national standard, all dried samples had acceptable nitrite contents.

CONCLUSIONS

Layu, as a traditional fish product, is highly appreciated by Chinese consumers. Drying was one of the most important methods for processing and preservation of Layu. The quality of Layu determined by physicochemical and biochemical properties eventually depends on dry-curing methods used in the process. Proper drying methods could improve sensory properties and inhibit hazardous components, producing high-quality and safe cured products. From the present study, sun drying for 8 h in direct sunlight at 5-15°C, as a traditional drying method, was found to have great advantages in drying fish products with superior sensory properties and overall flavor compared with low-temperature at 5°C and hot-air drying at 45°C. However, sun drying had its limitations on producing high levels of TVB-N, biogenic amines and nitrate, which may have safety concerns. The extended drying time and environmental dependence of sun drying method would also affect the drying efficiency. Intermittent drying (7 h at 5°C and 5 h in sealed condition at 10°C) produced comparable quality fish products as sun drying with relatively low hazardous material formation. In addition, intermittent drying improved drying efficiency with less overall drying time and less dependence on the environment. Studies on different combinations of drying time and temperature should be explored to further improve the quality and efficiency of drying process for commercial applications.

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Captions of Figures

FIG. 1. Effect of drying methods on the drying curves of Layú

FIG. 2. Electrophoresis patterns of fresh meat, salted meat and Layú meat by 5 different drying methods

FIG. 3. TVB-N content during manufacturing process of dry-cured Layú

FIG. 4. Nitrite content during manufacturing process of dry-cured Layú

Captions of Tables

TABLE 1 Sensory evaluation of texture, color, odor, taste and chewiness for dry-cured Layu

TABLE 2 Main FAA values (expressed as mg/kg DW) for fresh fish, salted fish and dry-cured Layu

TABLE 3 Biogenic amine content (expressed as mg/kg DW) for fresh fish, salted fish and dry-cured Layu

FIG. 1.

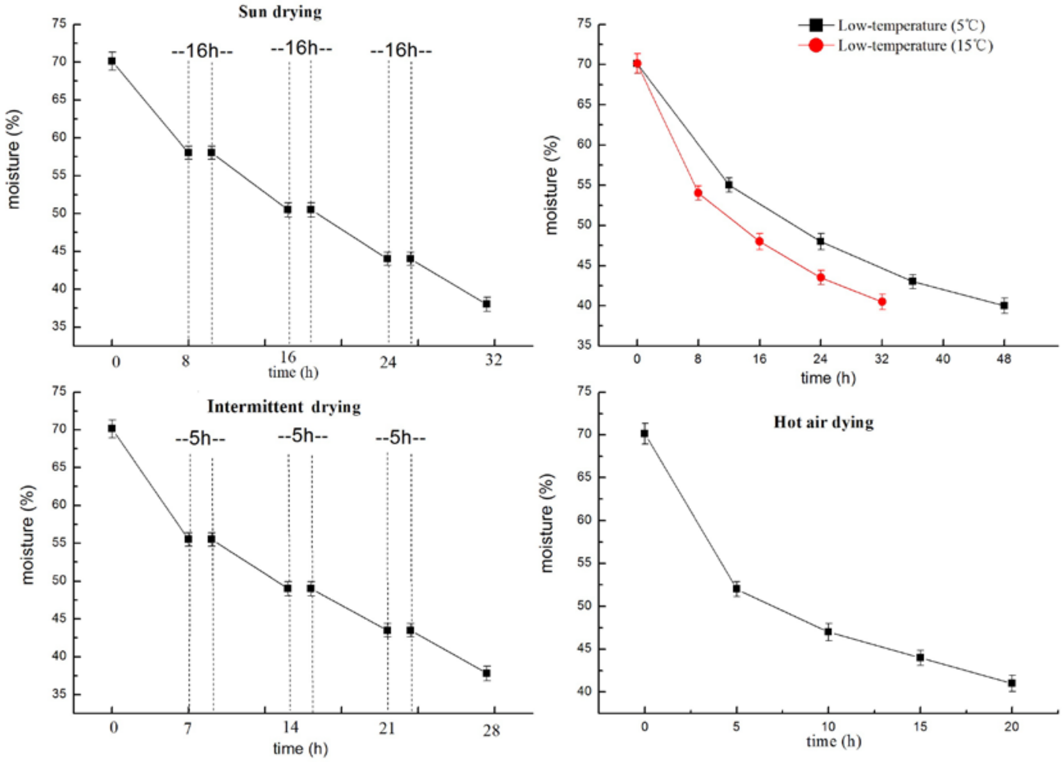

FIG. 2.

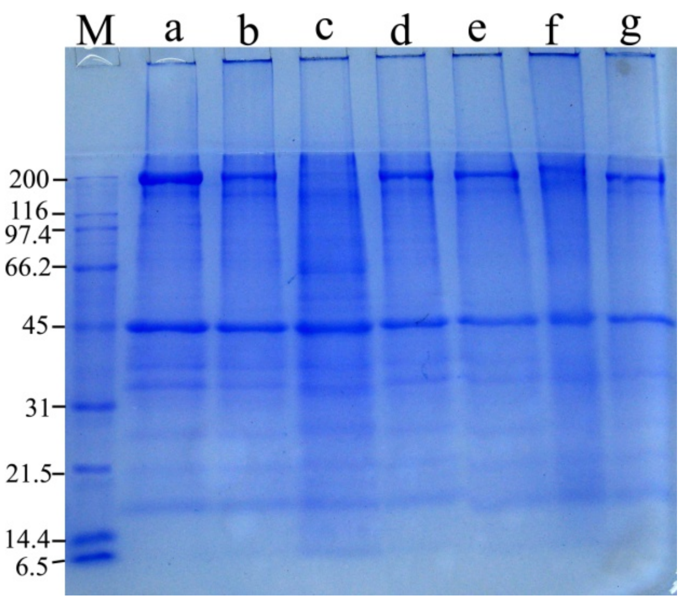

FIG.2. Note M: Marker; a: fresh fish; b: salted fish; c：Sun drying；d：Low temperature drying at 5℃；e：Low temperature drying at 15℃；f：Intermittent drying；g：Hot air drying

FIG. 3.

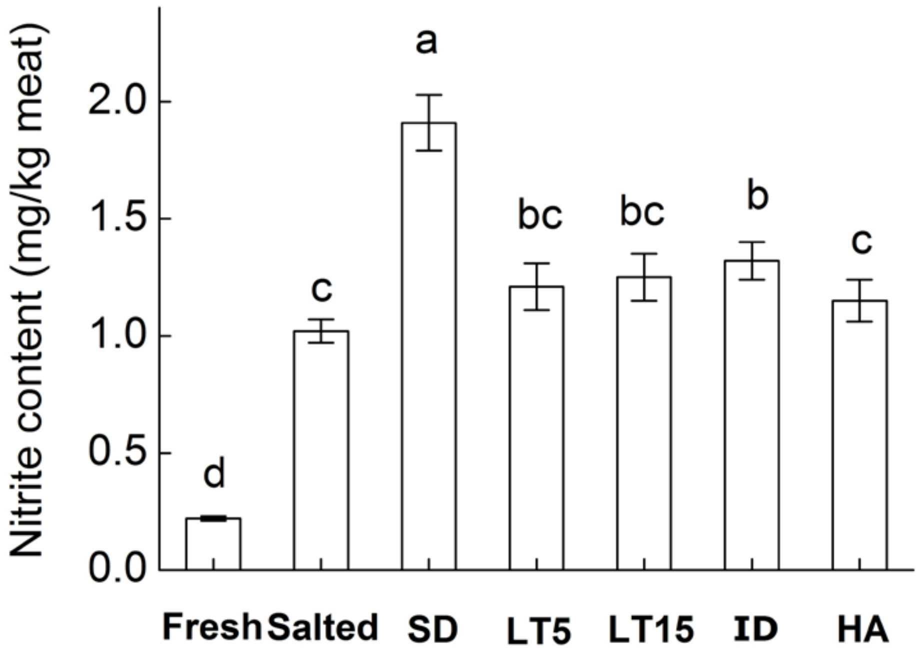
Nitrite content (mg/kg meat)
0.0
0.5
1.0
1.5
2.0
d
c
a
bc
bc
b
c
Fresh
Salted
SD
LT5
LT15
ID
HA

FIG. 4.

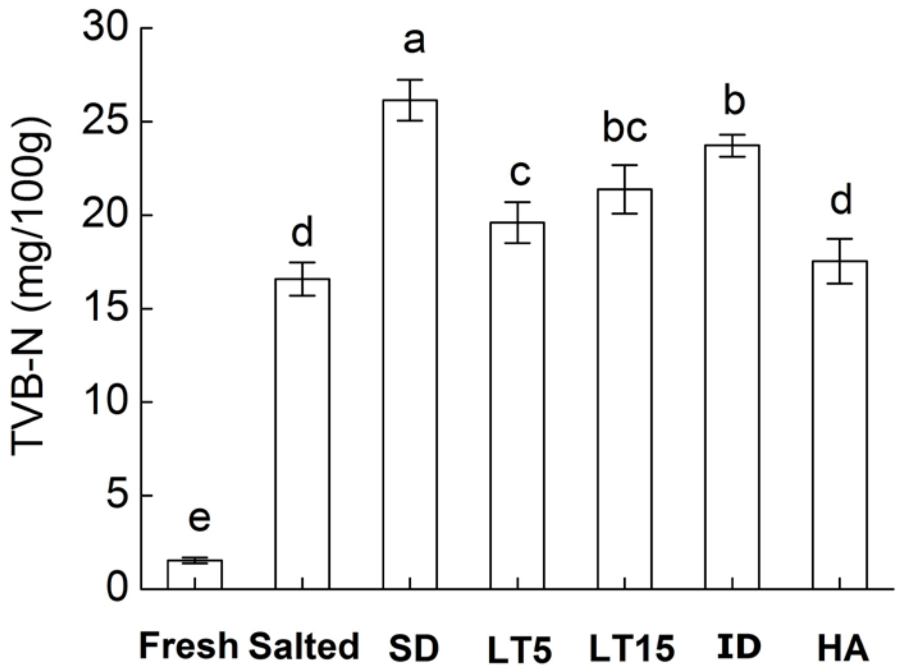
TVB-N (mg/100g)
0
5
10
15
20
25
30
e
d
a
c
bc
b
d
Fresh
Salted
SD
LT5
LT15
ID
HA

TABLE 1

Sensory evaluation of texture, color, odor, taste and chewiness for dry-cured Layu

Sensory Score	Salted	SD	LT5	LT15	ID	HA
Texture	6.1	8.6	8.4	8.1	8.8	7.5
Color	7.7	8.1	7.8	8	8.4	7.5
Odor	6.5	8.5	8.2	8.3	8.4	8.2
Umami	7.6	9.1	8.1	8.5	8.9	8.2
Chewiness	5.5	7.6	7.4	7.2	8.2	7.5
Total	33.4	41.9	39.9	40.1	42.7	38.9

TABLE 2

Main FAA values (expressed as mg/kg DW) for fresh fish, salted fish and dry-cured Layu

Amino acid	Fresh	Salted	SD	LT5	LT15	ID	HA
Asp	0.10 ± 0.03e	0.67 ± 0.02d	2.45 ± 0.35a	1.03 ± 0.07c	1.28 ± 0.07bc	2.25 ± 0.16a	1.05 ± 0.06c
Ser	0.04 ± 0.01d	0.90 ± 0.03c	2.02 ± 0.21a	1.07 ± 0.09bc	1.11 ± 0.05b	2.13 ± 0.23a	1.07 ± 0.08bc
Glu	0.12 ± 0.05e	0.27 ± 0.06d	1.78 ± 0.18a	1.07 ± 0.05c	0.95 ± 0.05c	1.55 ± 0.08b	0.31 ± 0.02d
Pro	ND	ND	0.37 ± 0.05b	0.17 ± 0.02c	0.39 ± 0.02b	0.68 ± 0.05a	0.11 ± 0.03c
Gly	0.51 ± 0.09d	1.16 ± 0.17c	3.87 ± 0.31a	1.95 ± 0.10b	1.83 ± 0.11b	4.05 ± 0.42a	1.46 ± 0.05bc
Ala	0.58 ± 0.06c	1.39 ± 0.13b	3.57 ± 0.27a	1.37 ± 0.07b	1.55 ± 0.07b	3.24 ± 0.25a	1.52 ± 0.07b
Cys	ND	0.12 ± 0.02d	1.31 ± 0.17b	0.66 ± 0.05c	0.73 ± 0.05c	1.53 ± 0.13a	0.33 ± 0.03d
Tyr	ND	0.22 ± 0.05a	0.22 ± 0.05a	0.17 ± 0.02b	0.27 ± 0.02a	0.15 ± 0.02b	0.12 ± 0.03b
His	0.04 ± 0.01e	1.16 ± 0.03d	3.83 ± 0.15a	1.42 ± 0.05c	1.73 ± 0.03b	3.78 ± 0.26a	1.43 ± 0.10c
Arg	ND	ND	0.72 ± 0.03a	0.43 ± 0.03c	0.68 ± 0.02a	0.61 ± 0.05b	0.37 ± 0.05c
EAA							
Thr	0.19 ± 0.04e	1.26 ± 0.05d	3.58 ± 0.17a	2.59 ± 0.21bc	2.85 ± 0.15b	3.27 ± 0.26ab	2.03 ± 0.09c
Val	0.30 ± 0.03d	1.55 ± 0.20c	3.41 ± 0.21a	1.75 ± 0.13bc	2.02 ± 0.12b	3.16 ± 0.21a	1.77 ± 0.15bc
Mst	0.26 ± 0.05e	0.41 ± 0.09d	1.27 ± 0.15a	0.81 ± 0.07bc	0.86 ± 0.05b	1.16 ± 0.06a	0.63 ± 0.07c

Ile	0.05 ± 0.01e	0.22 ± 0.04d	2.11 ± 0.17a	1.92 ± 0.11b	1.87 ± 0.11b	2.01 ± 0.15ab	1.66 ± 0.11c
Leu	0.37 ± 0.05e	0.57 ± 0.07d	2.61 ± 0.09a	1.61 ± 0.07bc	1.72 ± 0.31b	2.73 ± 0.13a	1.39 ± 0.09c
Phe	ND	0.02 ± 0.01e	1.52 ± 0.07a	0.68 ± 0.05c	0.75 ± 0.07c	1.31 ± 0.09b	0.27 ± 0.05d
Lys	0.11 ± 0.03d	0.18 ± 0.11d	2.03 ±0.08a	1.34 ± 0.09c	1.75 ± 0.11b	2.12 ± 0.12a	1.32 ± 0.06c
Total FAA	2.68 ± 0.76f	10.13 ± 0.58e	36.67 ± 1.27a	20.04 ± 1.22c	22.34 ± 1.37b	35.73 ± 1.81a	16.84 ± 1.05d
Total EAA	1.27 ± 0.58e	4.22 ± 0.18d	16.53 ± 0.62a	10.7 ± 0.75bc	11.82 ± 0.58b	15.76 ± 0.86a	9.07 ± 0.86c

Note ND, not detected. Data shown in Mean ± Standard deviation (n=3). Letters in same column represent significant difference ($p<0.05$)

TABLE 3

Biogenic amine content (expressed as mg/kg DW) for fresh fish, salted fish and dry-cured Layu

Biogenic amine	Fresh	Salted	SD	LT5	LT15	ID	HA
putrescine	ND	2.74 ± 0.16d	22.05 ± 0.95a	7.57 ± 0.16c	9.53 ± 0.19c	15.25 ± 0.71b	2.94 ± 0.17d
cadaverine	25.28 ± 0.75f	31.97 ± 1.28e	77.09 ± 1.88a	41.97 ± 1.28c	43.07 ± 1.20c	51.54 ± 1.45b	35.97 ± 1.22d
spermidine	1.44 ± 0.46d	1.69 ± 0.17c	2.13 ± 0.08a	1.92 ± 0.11b	1.52 ± 0.17cd	2.07 ± 0.07ab	1.93 ± 0.12b
spermine	2.04 ± 0.31b	2.25 ± 0.07ab	1.53 ± 0.09c	2.15 ± 0.12ab	2.05 ± 0.32b	1.72 ± 0.18c	2.52 ± 0.08a
histamine	ND	5.32 ± 0.29e	25.58 ± 0.67a	7.82 ± 0.29d	8.32 ± 0.31c	12.11 ± 1.38b	5.52 ± 0.25e
tyramine	9.64 ± 0.78c	15.39 ± 0.69b	17.27 ± 0.68a	15.91 ± 0.69ab	15.02 ± 0.67b	16.21 ± 0.77a	15.25 ± 0.61b
Total	38.40 ± 1.65f	59.36 ± 1.44e	145.65 ± 4.37a	77.34 ± 2.53cd	79.51 ± 2.37c	98.90 ± 4.72b	64.07 ± 2.85d

Note ND, not detected. Data shown in Mean ± Standard deviation (n=3). Letters in same column represent significant difference ($p<0.05$)