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Effect of processing steps on the physico-chemical properties of dried-seasoned squid

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Abstract

The effects of processing steps on the physico-chemical properties of dried-seasoned squid were studied using two kinds of squids. Browning was observed as indicated by an increase in yellowness (b^* value) during the semi-drying and roasting step. The temperature and time of heating and type of roasting, as well as the material itself were the key factors influencing the browning. A high level of trimethylamine oxide (TMAO), and low contents of trimethylamine (TMA) and dimethylamine (DMA) were detected in the raw material. TMAO gradually decreased during the processing, while TMA, DMA and formaldehyde (FA) increased significantly especially in boiled meat. The temperature of boiling was the most crucial factor impacting the production of FA. FA could be greatly decreased during the cooling process by washing with water or changing the roasting techniques. Therefore, to improve the quality of dried-seasoned squid, more attention should be paid to the temperature and roasting techniques during the processing.

Keywords: Dried-seasoned squid; Processing; Browning; FA; TMAO

1. Introduction

Dried-seasoned squid is very nutritional and popular in many countries of the world. Neon flying squid and jumbo squid are the main species available. Undervalued squid species are utilized in many countries of the world in the manufacture of a popular semi-moist seasoned product. The primary quality attributes of dried-seasoned squid are its colour and appearance. Brown discoloration caused by sugar–amino (Maillard browning) reaction in manufacturing is a critical problem in marine food industry, which can cause considerable loss to this industry. The susceptibility to browning formation, however, differs among different species of squid. The dried products of neon flying squid and Atlantic short finned squid undergo browning more readily than other squids (Sugiyama, Konosu, Hanabe, & Okuda, 1989; Haard & Arcilla, 1985). A number of studies have indicated that some amino acids such as Gly and Arg in neon flying squid, and Tau and Pro in Atlantic short-finned squid and Argentina squid might greatly contribute to Maillard browning reaction during the processing and storage (Tsai, Pan, & Kong, 1991b; Suyama & Kobayashi, 1980; Haard & Arcilla, 1985). Moreover, the breakdown products produced by O_2, light, and heat may be another important factor contributing to browning (Hayashi & Takagi, 1979; Morita, Kubota, & Aishima, 2002). In addition, temperature and water activity were also believed to significantly affect browning reaction (Tsai et al., 1991b). The degree of browning in cold air was lower than that in sun and hot air (Kim et al., 2000). In addition, the breakdown caused by enzymes from the squid can lead to undesirable fishy

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odours and flavours. Therefore an investigation into the effects of processing steps in manufacture of dried-seasoned squid is needed.

These days, individuals pay attention not only to the nutrition and savoury, but also to the safety of food. Trimethylamine oxide (TMAO) is a nitrogenous osmolyte distributed widely in marine organisms. High concentration of TMAO in squid is both a technological and toxicological problem because the breakdown of TMAO will lead to the production of harmful formaldehyde (FA). During frozen storage, TMAO degrades to FA and dimethylamine (DMA) or to trimehtylamine (TMA) through non-enzymic pathway (Spinelli & Koury, 1981; Kimura, Seki, & Kimura, 2002). Over 90% TMAO converts to TMA and DMA after heating for 1 h at 200 °C (Lin & Hurng, 1985). Moreover, TMAOase existing in the mantle of squid can induce TMAO degradation via enzymic pathway (Stanley & Hultin, 1984; Nitisewojo & Hultin, 1986; Fu et al., 2006). TMAOase is capable of catalyzing the conversion of TMAO to DMA and FA. So the production of harmful FA can not be avoided completely during processing of squid, which drew more and more attention as a crucial problem affecting food quality remarkably. However, till now, little information about chemical changes during the processing of dried-seasoned squid is available. The aims of the present study were to investigate the effect of processing steps on the physico-chemical properties of dried-seasoned squid, to find the key point affecting quality, to decrease the toxicity and improve the processing and the quality of products accordingly.

2. Materials and methods

2.1. Materials

Two different species *Ommastrephes bartrami* (neon flying squid) and *Dosidicus gigas* (jumbo squid) were used for analysis during the processing of dried-seasoned squid. The processing procedures and the samples taken for analysis were as follows.

2.1.1. Neon flying squid

(1) Neon flying squid (sample RM: raw material); (2) defrosting and removing viscera, head and fins (sample DM: defrosted meat); (3) skinning with protease (papain, 0.02–0.025%, 50–55 °C for 5–7 min; sample SM: skinned meat); (4) boiling (90–95 °C for 8–10 min; sample BM: boiled meat); (5) cooling (4–10 °C; sample CM: cooled meat); (6) primary seasoning (overnight; sample PS: primary seasoned); (7) drying (40–45 °C for 10 h; sample SD: Semi-dried meat); (8) storing in chilled room (about 15 days) and adjusting pH and water activity (sample AM: adjusted meat); (9) roasting (110–120 °C for 3–5 min) and stretching; (10) tearing (sample TM: torn meat); (11) second seasoning; and (12) drying (45–50 °C for 3–6 h); (13) final product (sample FP: final product).

2.1.2. Jumbo squid

Jumbo squid (mantle; sample RM: raw material); defrosting and primary skinning; trimming; second skinning (65–70 °C for 10 min; sample SM: skinned meat) and the following processes were the same as neon flying squid.

2.2. Ingredients

The ingredients of the seasonings were the same for the two kinds of squids. The ingredients of the first seasoning included sucrose (8.5%), sorbitol (1%), salt (2%), monosodium glutamate (MSG; 0.6%), potassium sorbate (0.05%), nucleotide (0.05%), tartaric acid (0.2%), sodium tripolyphosphate (0.05%) and sodium succinate (0.2%). The ingredients of the second seasoning included sucrose (1%), lactose (8.5%), salt (1.35%), MSG (0.45%), potassium sorbate (0.1%), sorbitol (1.2%) and carboxyacetic acid (0.15%).

2.3. Analytical methods

2.3.1. Proximate composition and pH

The determination of moisture, crude protein, crude fat, and ash was carried out according to the methods of AOAC (1995). Moisture was quantified by oven-drying at 105 °C, total fat was evaluated by Soxhlet extraction, and crude ash by incineration in a muffle furnace at 550 °C. Crude protein level was estimated from the total nitrogen amount by multiplying by a factor of 6.25. Carbohydrate level (%) was calculated by the following equation: $100 - (\text{moisture} + \text{crude protein} + \text{crude fat} + \text{ash})$. Five grams of each sample were put into 10 volumes of deionized water, and homogenized. Then the pH was measured using a pH meter (Metrohm model 744, Switzerland).

2.3.2. Reducing sugar (RS)

Reducing sugar in the samples was extracted and then determined by a modification of the ferricyanide reduction method of Park and Johnson (1949).

2.3.3. Water activity

Samples were cut into small pieces, mixed well, and water activity was measured with a hygrometer (AWC 503C, Novasina, Pfaeffikon, Switzerland).

2.3.4. Colour measurement

The CIE L^* (lightness), a^* (redness) and b^* (yellowness) color values of the samples were measured with a colorimeter (WSC-S, Shanghai, China). A white standard board ($X = 1.1314$, $Y = 1.0220$, and $Z = 1.0042$) was used for calibration.

2.3.5. Determination of FAA

Samples were hydrolyzed under vacuum with 6 M HCl at 150 °C for 1 h. FAA level was analyzed with an Amino Acid Analyzer (Hitachi 835-50 Model, Japan).

2.3.6. TMAO, TMA, DMA and volatile basic nitrogen (VBN)

Each sample (50 g) was homogenized in 100 ml of 7.5% cold trichloroacetic acid (TCA) solution for 2 min, followed by centrifugation at 4000 × g for 15 min. The supernatant was filtered using a Whatmann #4, then the levels of TMAO, TMA, DMA and VBN were examined, respectively. TMAO was determined after reduction to TMA by the method of Yamagata, Horimoto, and Nagaoka (1969) with a slight modification. The extract (2 ml) was treated with 2 ml 1% $TiCl_3$ and incubated at 80 °C for 90 s, followed by cooling with running water. TMA was determined according to the method of Conway and Byrne (1936). TMAO was then calculated after subtracting indigenous TMA content of the samples. DMA was determined by the copper-dithiocarbamate method, as described by Dyer and Mounsey (1945). The concentration of VBN was measured by the method of Conway microdiffusion method (1950).

2.3.7. FA and free formaldehyde (F-FA)

FA includes free plus reversibly bound formaldehyde was determined by steam distillation of a mixture of 10 g mince and 10% phosphoric acid and 200 ml water with 30-min distillation. The amount of free plus reversibly bound FA in the distillate was determined by chromotropic acid reaction. F-FA was extracted at room temperature using 6% perchloric acid. The extraction was measured using acetylacetone reagent according to the method of Nash (1953).

2.3.8. Statistical analysis

Four parallel samples were prepared in each group and all the experiments repeated fore three times. Student's *t*-test, TrialRun (SPSS Inc., Chicago, IL, USA) and analysis of variance (ANOVA) were performed using Statview.

3. Results and discussion

3.1. Proximate composition, pH, and water activity

Tables 1 and 2 show the changes of proximate composition, pH and water activity during processing of two types of dried-seasoned squids. The jumbo squid had higher moisture than neon flying squid in the raw material. The crude protein decreased more than 20% in both species of squid during processing. On a dry weight basis, the crude fat and ash significantly changed during processing. The amount of ash decreased greatly during the boiling process, while the amount of carbohydrate was gradually elevated during all handing processes. The great increase of carbohydrate in the primary seasoned meats and the final products may be due to the seasoning treatment. In view of this, it is more suitable to express the results on the basis of carbohydrate-free dry meat in order to evaluate the actual changes influenced by the processing procedures as shown in Tables 1 and 2. Similar changes were observed in both types of squid (see Table 3).

A slight decrease in pH value was observed after processing. The changes of pH may be partially due to the treatment with seasoning, during which organic acid is added. It was reported that pH value influenced the flavour of squid products (Morita et al., 2002). Moreover, it was shown that water activity decreased from 0.96 to 0.65 and 0.64 in the final products of neon flying squid and jumbo squid, respectively. Dried-seasoned squids are intermediate moisture foods, so control of water activity is the most important key point affecting the shelf life of products. Similar changes were observed in both squid samples.

3.2. Browning and flavour changes during the processing

L^*, a^* and b^* tristimulus colour values were measured to monitor the colour changes during processing of dried-seasoned squids. Tables 1 and 2 list colour changes during the processing of the two dried-seasoned squid. The L^*, a^* and b^* values in two kinds of squids differed somewhat. The a^* value was slightly enhanced in both neon flying squid and jumbo squid. After processing, L^* value showed a sudden rise in the semi-dried meat of neon flying squid and the torn meat of jumbo squid. The increase of L^* value seemed to be related to the decrease in moisture as well as the amount of seasoning coating the surface of the samples. The appearance colour of neon flying squid was more yellow than jumbo squid, that is, the b^* value of neon flying squid was higher than that of jumbo squid, implying that a much more significant browning happened in neon flying squid than that in jumbo squid during processing. The b^* value increased 50% in the semi-dried meat and 100% in the final product of neon flying squid, and increased 82% in the torn meat and 135% in the final product of jumbo squid compared with raw material. The dramatic increase of b^* value implied that brown discoloration occurred obviously in the semi-dried meat of neon flying squid and torn meat of jumbo squid.

Brown discoloration caused by sugar–amino reactions is a key quality problem in dried squid products during processing and the content of RS in products impacts the browning dramatically. As shown in Tables 1 and 2, the raw material of jumbo squid contained about 4.6 times RS as much as that in raw material of neon flying squid. RS decreased greatly during the processing especially during the cooling process, except that a slight increase appeared in the final products of both two kinds of squid because 8.5% lactose (a reducing sugar) was added during the second seasoning. The reduction of RS in jumbo squid was much more significant than that in neon flying squid during processing. The amount of RS in the cooled meat of jumbo squid lost about 92% compared with raw material. During the boiling process, muscle protein was denatured and the capability to absorb water decreased subsequently. After the cooling process, most RS was removed by washing with water. With the amount of RS

Table 1
Changes in levels of proximate composition, reducing sugar, pH, water activity, colour during the processing[A] of dried-seasoned neon flying squid

	RM	DM	SM	BM	CM	PS	SD	AM	TM	FP
Proximate composition										
Moisture(%)	71.0 ± 1.7^{e}	75.6 ± 0.9^{f}	76.7 ± 0.1^{g}	73.2 ± 0.6^{f}	74.6 ± 2.0^{fg}	65.0 ± 4.5^{d}	45.2 ± 0.3^{b}	48.2 ± 1.0^{bc}	49.2 ± 2.3^{c}	27.3 ± 0.7^{a}
Crude protein[B]	82.1 ± 1.2^{f}	82.2 ± 2.0^{f}	80.2 ± 0.9^{e}	81.3 ± 1.5^{e}	80.4 ± 1.6^{e}	76.6 ± 1.9^{d}	74.5 ± 2.1^{c}	75.3 ± 0.8^{c}	73.0 ± 0.4^{b}	64.6 ± 1.3^{a}
Crude fat[B]	1.9 ± 0.4^{ab}	2.3 ± 0.3^{b}	3.2 ± 0.4^{c}	3.2 ± 0.3^{c}	3.0 ± 0.1^{c}	3.0 ± 0.09^{c}	2.1 ± 0.07^{ab}	2.0 ± 0.1^{ab}	1.9 ± 0.2^{ab}	1.7 ± 0.2^{a}
Ash[B]	7.4 ± 0.6^{c}	7.0 ± 0.6^{c}	7.2 ± 0.6^{c}	5.1 ± 0.3^{a}	6.3 ± 0.3^{b}	8.3 ± 0.2^{d}	6.6 ± 0.6^{b}	8.2 ± 0.4^{d}	8.6 ± 0.3^{d}	9.2 ± 0.2^{e}
Carbohydrate[B]	8.6 ± 1.2^{a}	8.5 ± 0.9^{a}	9.4 ± 0.6^{ab}	10.4 ± 1.6^{b}	10.3 ± 2.0^{b}	12.4 ± 1.0^{c}	16.8 ± 2.5^{d}	14.5 ± 0.6^{cd}	16.5 ± 2.0^{d}	24.5 ± 1.4^{e}
Reducing sugar[C]	171.6 ± 33.5^{d}	165.2 ± 17.0^{d}	159.7 ± 30.9^{cd}	158.0 ± 15.4^{cd}	98.3 ± 6.7^{b}	110.2 ± 2.3^{c}	85.3 ± 5.3^{a}	84.9 ± 7.0^{a}	83.7 ± 5.6^{a}	141.3 ± 5.6^{cd}
pH	6.5 ± 0.1^{d}	6.5 ± 0.1^{d}	6.3 ± 0.2^{c}	6.5 ± 0.2^{d}	6.9 ± 0.1^{f}	6.4 ± 0.1^{d}	6.0 ± 0.1^{b}	6.3 ± 0.2^{c}	5.8 ± 0.2^{a}	5.9 ± 0.1^{b}
Water activity	0.96 ± 0.01^{e}	0.96 ± 0.01^{e}	0.96 ± 0.01^{e}	0.95 ± 0.01^{de}	0.95 ± 0.01^{de}	0.94 ± 0.01^{de}	0.92 ± 0.01^{cd}	0.90 ± 0.01^{c}	0.86 ± 0.01^{b}	0.65 ± 0.02^{a}
Colour										
L^*	2.6 ± 0.8^{a}	2.6 ± 0.4^{a}	2.7 ± 0.2^{a}	4.1 ± 0.3^{bc}	3.5 ± 0.4^{b}	4.5 ± 0.1^{c}	6.8 ± 0.1^{d}	6.1 ± 0.3^{d}	6.4 ± 0.4^{d}	7.6 ± 0.4^{e}
a^*	3.9 ± 0.2^{a}	4.0 ± 0.1^{b}	4.2 ± 0.1^{ab}	4.4 ± 0.3^{bc}	4.5 ± 0.05^{c}	4.2 ± 0.4^{bc}	4.3 ± 0.02^{c}	5.3 ± 0.07^{d}	5.5 ± 0.1^{de}	5.7 ± 0.2^{e}
b^*	2.2 ± 0.2^{a}	2.3 ± 0.3^{a}	2.4 ± 0.2^{a}	2.6 ± 0.09^{b}	2.8 ± 0.2^{bc}	2.5 ± 0.2^{ab}	3.3 ± 0.4^{c}	3.5 ± 0.1^{c}	4.4 ± 0.2^{d}	4.4 ± 0.1^{d}

[a–g] Means values in the same row of each processing bearing different superscripts differ significantly ($p < 0.05$).
[A] RM, raw material; DM, defrosted meat; SM, skinned meat; BM, boiled meat; CM, cooled meat; PS, primary seasoned meat; SD, Semi-dried meat; AM, adjusted meat; TM, torn meat; FP, final product.
[B] Expressed as % of carbohydrate-free dry meat.
[C] Expressed as μmol/g of carbohydrate-free dry meat.

Table 2
Changes in levels of proximate composition, reducing sugar, pH, water activity, colour during the processing[A] of dried-seasoned jumbo squid

	RM	DM	SM	BM	CM	PS	SD	AM	TM	FP
Proximate composition										
Moisture(%)	86.0 ± 1.8^{f}	84.1 ± 2.2^{f}	83.6 ± 0.1^{f}	76.7 ± 2.3^{e}	76.0 ± 2.3^{e}	68.0 ± 1.0^{d}	41.4 ± 3.4^{b}	48.5 ± 3.2^{c}	44.4 ± 0.9^{b}	21.3 ± 1.1^{a}
Crude protein[B]	87.7 ± 0.4^{i}	87.8 ± 0.8^{i}	83.3 ± 1.2^{g}	81.5 ± 0.8^{f}	81.4 ± 1.3^{f}	77.5 ± 1.4^{e}	73.7 ± 0.5^{c}	74.4 ± 0.6^{d}	72.6 ± 0.4^{b}	62.6 ± 0.2^{a}
Crude fat[B]	2.4 ± 0.2^{d}	2.2 ± 0.1^{d}	3.6 ± 0.09^{e}	2.2 ± 0.07^{d}	2.2 ± 0.1^{d}	2.0 ± 0.3^{c}	1.8 ± 0.2^{a}	1.8 ± 0.07^{a}	1.9 ± 0.1^{ab}	1.7 ± 0.3^{a}
Ash[B]	8.7 ± 0.4^{d}	7.8 ± 0.2^{c}	7.2 ± 0.4^{b}	4.9 ± 0.5^{a}	4.8 ± 0.1^{a}	4.8 ± 0.1^{a}	8.3 ± 0.3^{d}	8.0 ± 0.1^{c}	7.6 ± 0.4^{bc}	9.2 ± 0.3^{d}
Carbohydrate[B]	1.2 ± 0.06^{a}	2.2 ± 0.1^{a}	5.9 ± 0.6^{b}	11.4 ± 1.2^{c}	11.6 ± 2.8^{c}	15.7 ± 1.5^{d}	16.2 ± 2.0^{d}	15.8 ± 0.6^{d}	17.9 ± 0.5^{d}	26.5 ± 2.6^{e}
Reducing sugar[C]	787.0 ± 20.4^{d}	700.2 ± 30.1^{c}	657.2 ± 20.9^{c}	154.9 ± 39.1^{b}	62.7 ± 17.0^{a}	72.7 ± 16.6^{a}	69.2 ± 4.4^{a}	70.3 ± 5.0^{a}	73.5 ± 11.5^{a}	165.2 ± 6.7^{b}
pH	6.4 ± 0.06^{c}	6.3 ± 0.08^{c}	6.6 ± 0.08^{d}	6.4 ± 0.1^{c}	6.8 ± 0.1^{e}	6.4 ± 0.2^{c}	6.4 ± 0.2^{c}	6.1 ± 0.1^{b}	6.1 ± 0.08^{b}	5.9 ± 0.2^{a}
Water activity	0.96 ± 0.01^{d}	0.96 ± 0.01^{d}	0.96 ± 0.01^{d}	0.96 ± 0.01^{d}	0.97 ± 0.01^{d}	0.94 ± 0.01^{c}	0.86 ± 0.01^{b}	0.89 ± 0.02^{bc}	0.87 ± 0.01^{b}	0.64 ± 0.01^{a}
Colour										
L^*	2.4 ± 0.3^{a}	2.5 ± 0.2^{a}	3.3 ± 0.08^{b}	4.1 ± 0.4^{c}	4.2 ± 0.5^{c}	3.5 ± 0.3^{b}	3.0 ± 0.1^{b}	3.4 ± 0.3^{b}	7.4 ± 0.1^{e}	6.8 ± 0.4^{d}
a^*	3.5 ± 0.2^{a}	3.6 ± 0.3^{a}	3.9 ± 0.1^{bc}	4.2 ± 0.1^{d}	4.2 ± 0.3^{d}	4.1 ± 0.2^{d}	4.2 ± 0.1^{d}	4.2 ± 0.2^{d}	5.2 ± 0.08^{e}	5.5 ± 0.02^{e}
b^*	1.7 ± 0.1^{ab}	1.7 ± 0.2^{ab}	1.7 ± 0.3^{ab}	2.1 ± 0.2^{ab}	1.6 ± 0.06^{a}	2.4 ± 0.03^{b}	3.4 ± 0.2^{d}	2.5 ± 0.3^{bc}	3.1 ± 0.1^{cd}	4.0 ± 0.01^{e}

[a–i] Means values in the same row of each processing bearing different superscripts differ significantly ($p < 0.05$).
[A] RM, raw material; DM, defrosted meat; SM, skinned meat; BM, boiled meat; CM, cooled meat; PS, primary seasoned meat; SD, Semi-dried meat; AM, adjusted meat; TM, torn meat; FP, final product.
[B] Expressed as % of carbohydrate-free dry meat.
[C] Expressed as μmol/g of carbohydrate-free dry meat.

Table 3
Changes in levels of free amino acids (mg/100 g carbohydrate-free dry meat) during processing of dried-seasoned squid

	Neon flying squid processing[A]				Jumbo squid processing[A]			
	RM	BM	SD	FP	RM	BM	SD	FP
Taurine	735 ± 23^{a}	750 ± 28^{a}	699 ± 35^{a}	660 ± 28^{a}	614 ± 68^{a}	591 ± 109^{a}	602 ± 88^{a}	616 ± 56^{a}
Aspartic acid	47 ± 5^{b}	36 ± 3^{ab}	27 ± 2^{a}	37 ± 6^{ab}	35 ± 4^{b}	27 ± 2^{a}	39 ± 3^{b}	33 ± 5^{b}
Threonine	181 ± 11^{b}	135 ± 19^{a}	152 ± 10^{ab}	131 ± 7^{a}	152 ± 13^{a}	160 ± 8^{a}	127 ± 5^{a}	140 ± 6^{a}
Serine	163 ± 7^{b}	125 ± 4^{a}	143 ± 10^{ab}	135 ± 14^{a}	145 ± 9^{ab}	145 ± 8^{ab}	118 ± 10^{a}	178 ± 18^{b}
Glutamic acid	1042 ± 90^{a}	1149 ± 80^{a}	1126 ± 32^{a}	1255 ± 54^{a}	889 ± 69^{a}	934 ± 76^{a}	1009 ± 103^{a}	1427 ± 47^{b}
Proline	1153 ± 37^{a}	878 ± 88^{ab}	840 ± 66^{b}	966 ± 50^{ab}	1092 ± 56^{a}	871 ± 41^{b}	822 ± 23^{b}	777 ± 54^{b}
Glycine	373 ± 45^{a}	380 ± 25^{a}	319 ± 11^{a}	352 ± 18^{a}	275 ± 11^{a}	247 ± 23^{a}	296 ± 16^{a}	254 ± 19^{a}
Alanine	444 ± 69^{a}	443 ± 27^{a}	399 ± 31^{a}	620 ± 33^{b}	430 ± 28^{a}	401 ± 25^{a}	412 ± 32^{a}	609 ± 33^{b}
Valine	73 ± 6^{c}	40 ± 2^{a}	67 ± 5^{bc}	55 ± 3^{ab}	52 ± 4^{ab}	43 ± 2^{a}	58 ± 2^{b}	49 ± 3^{ab}
Cystine	755 ± 30^{b}	708 ± 35^{ab}	689 ± 21^{ab}	644 ± 33^{a}	617 ± 76^{a}	587 ± 43^{a}	608 ± 89^{a}	570 ± 56^{a}
Methionine	277 ± 33^{a}	265 ± 29^{a}	259 ± 18^{a}	233 ± 30^{a}	231 ± 49^{a}	222 ± 23^{a}	217 ± 14^{a}	231 ± 28^{a}
Isoleucine	356 ± 44^{a}	354 ± 48^{a}	342 ± 27^{a}	321 ± 47^{a}	299 ± 33^{a}	292 ± 10^{a}	303 ± 17^{a}	314 ± 26^{a}
Leucine	418 ± 38^{a}	404 ± 25^{a}	382 ± 37^{a}	399 ± 25^{a}	353 ± 35^{a}	358 ± 29^{a}	318 ± 23^{a}	291 ± 25^{a}
Tyrosine	118 ± 10^{a}	180 ± 9^{b}	122 ± 11^{a}	99 ± 7^{a}	110 ± 13^{a}	117 ± 11^{a}	95 ± 8^{a}	161 ± 9^{b}
Phenylalanine	150 ± 14^{a}	213 ± 18^{b}	158 ± 9^{a}	146 ± 10^{a}	132 ± 5^{a}	145 ± 10^{ab}	117 ± 9^{a}	190 ± 20^{b}
Lysine	572 ± 33^{b}	583 ± 13^{b}	563 ± 18^{b}	405 ± 21^{a}	465 ± 41^{a}	452 ± 36^{a}	454 ± 37^{a}	390 ± 29^{a}
NH_3	145 ± 12^{b}	116 ± 10^{a}	125 ± 8^{b}	141 ± 13^{b}	178 ± 10^{b}	106 ± 15^{a}	117 ± 20^{ab}	126 ± 21^{ab}
Histidine	157 ± 11^{a}	167 ± 9^{a}	159 ± 12^{a}	186 ± 17^{a}	125 ± 12^{a}	127 ± 11^{a}	129 ± 4^{a}	132 ± 9^{a}
Arginine	748 ± 45^{b}	592 ± 32^{a}	567 ± 21^{a}	650 ± 36^{ab}	491 ± 38^{b}	308 ± 30^{a}	328 ± 25^{a}	311 ± 21^{a}
Total	7906 ± 334^{a}	7519 ± 299^{a}	7137 ± 301^{a}	7435 ± 175^{a}	6684 ± 346^{a}	6131 ± 210^{a}	6171 ± 286^{a}	6799 ± 166^{a}

[a–c] Means values in the same row of each processing bearing different superscripts differ significantly ($p < 0.05$).
[A] RM, raw material; BM, boiled meat; SD, semi-dried meat; PF, final product.

sharply decreased, the browning would be greatly weakened during the following processes. Notably, the content of RS in jumbo squid was much lower than that in neon flying squid, which may be one of the reasons that the browning in jumbo squid is fainter than that in neon flying squid.

The total amounts of FAA in the raw material were 7906 ± 334 and 6684 ± 346 mg/100 g carbohydrate-free dry meat for neon flying squid and jumbo squid, respectively (Table 3). Glu and Pro were present in particularly high amounts, followed by Cys, Arg and Tau. These five amino acids together accounted for 56% and 43% of the total amount of FAA in neon flying squid and jumbo squid, respectively. In addition, Lys, Ala, Leu, Iso, Gly and Met were also found in relatively large amounts in both raw materials, ranging from 231 to 572 mg/100 g of carbohydrate-free dry meat. In the neon flying squid, the content of most of the FAA decreased during the processing and the major constituents, such as Tau, Cys, Pro, Arg and Lys in the final product reduced by 10–30% of their initial levels. However, Lys, Pro and Arg in the final product of jumbo squid decreased 16–37% compared with raw material. These findings revealed that the FAA in neon flying squid might be involved in and destroyed remarkably by browning reaction, which was accordant with the observation that b^* value exhibited a sudden rise at the same stage. It has been reported that Lys is one of the amino acids with the highest browning rate in the stimulated model system of dried squid (Tsai, Kong, & Pan, 1991a). Tau, Met, and Lys have also been reported as very active precursors of Maillard browning in the mantle muscle of Atlantic short finned squid (Haard & Arcilla, 1985). It was also shown that the non-enzymatic browning of seasoned-dried squid foods could be caused by abundance of Arg and Gly.

The drying process for the two kinds of squid at 45–50 °C might increase the browning rate and subsequently cause loss of amino acids. Temperature had a more pronounced effect on browning rate than water activity and it seemed desirable to dry squid at a centre temperature below 23 °C to avoid excessive browning (Tsai et al., 1991b). By examining the combination of three stages of seasoning, Hayashi and Takagi (1979) indicated that the secondary seasoning greatly contributed to the brown discoloration reaction of saki ika at 30 °C.

It is well known that squid is rich in proteins and free amino acids, especially has high amounts of taurine (Suyama & Kobayashi, 1980). FAA was the substrate to react with RS to form "roast" and "sweet" flavour in dried-seasoned squid due to browning. The taste amino acid of Pro and Ala in neon flying squid and Ala and Glu in jumbo squid exhibited a significant increase during the roasting processing to form special flavour of squid caused by high temperature heating. The increase of Glu might be due to the treatment of secondary seasoning with MSG as the ingredient.

3.3. Changes of the TMAO, TMA, DMA, FA and VBN

TMAO is an endemic substance in marine animals. Its decomposition can be catalyzed nonenzymatically by iron and various reductants, or enzymatically by trimethylamine-*N*-oxide demethylase (TMAOase) (Fu et al., 2006). The nonenzymatic reaction was reported to be the crucial

one during heating (Spinelli & Koury, 1981; Nitisewojo & Hultin, 1986). Fig. 1 shows the changes of TMAO, DMA and TMA in neon flying squid and jumbo squid during the processing. The amounts of TMAO, as well as its breakdown products DMA and TMA in jumbo squid were much higher than those in neon flying squid. High levels of TMAO and low contents of TMA and DMA were detected in the raw material. The TMAO levels exhibited a gradual decline during the whole processing in both of two kinds of squid. During skinning process, DMA level in jumbo squid manifested a significant increase, while DMA content sharply increased during boiling process in neon flying squid. The amounts of TMA remarkably increased in the torn meat, as well as in the final product of neon flying squid, while the torn meat and final product of jumbo squid contained relatively high level of DMA. The remarkable enhancement of DMA and TMA levels in the boiled meat might be attributed to the high temperature during the processing. Judging from the initial levels and the alteration of these amines, it could be concluded that the most important and principal precursor of DMA and TMA in dried squid was TMAO.

The degradation of TMAO was initially observed in raw material during freezing storage. It was reported that a high level of DMA is responsible for the appearance of unpleasant odours and flavours, so the DMA content in frozen squid mantle were generally inversely related to the quality of the cooked mantle. The FA accumulation during storage would result in cross-linking of proteins and textural toughening (Stanley & Hultin, 1984). In the two squids, neon flying squid has less DMA and FA than jumbo squid in the raw materials, manifesting a better quality for neon flying squid. The production of a great deal of DMA in jumbo squid during skinning process might closely associate with the TMAOase in it with optimal temperature of 70 °C (Fu et al., 2006). Many studies have shown that heating of squid meat could induce a significant production of TMA and DMA from TMAO, but the conversion varies depending on the heating temperature (Lin & Hurng, 1985; Synowiecki & Sikorski, 1988). On the basis that drum-dried and freeze-dried hake produced maximal amounts of DMA, and that reducing constituents might induce degradation of TMAO to DMA, it was suggested that the process could be non-enzymatic. Spinelli and Koury (1981) have further elaborated this theory with findings that Fe^{2+} and catabolites of cysteine (cysteine sulfinic acid, hypotaurine, and taurine) could degrade TMAO to DMA.

The amounts of FA and F-FA were analyzed next. As shown in Fig. 2, the contents of FA and F-FA altered similarly except that the F-FA changed more significantly than FA both in neon flying squid and jumbo squid. The origi-

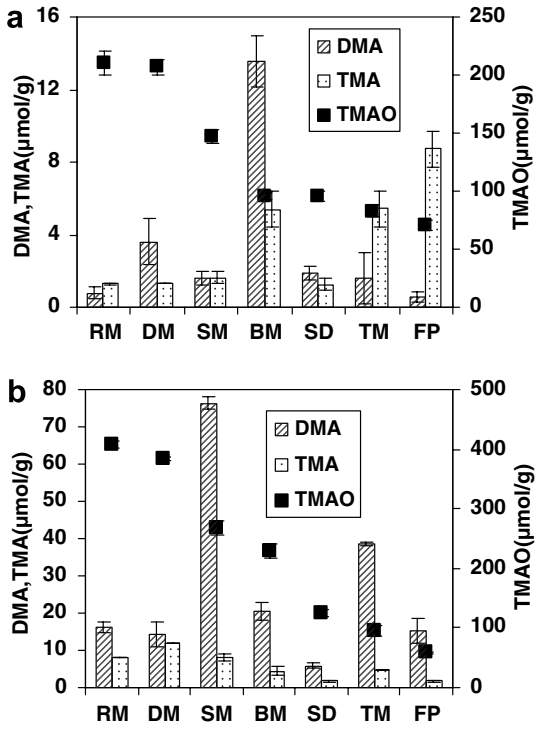

Fig. 1. Changes in levels of TMAO, TMA and DMA (μmol/g of carbohydrate-free dry meat) during processing of dried-seasoned squids. (a) Neon flying squid; (b) jumbo squid. RM, raw material; DM, defrosted meat; SM, skinned meat; BM, boiled meat; SD, Semi-dried meat; TM, torn meat; FP, final product.

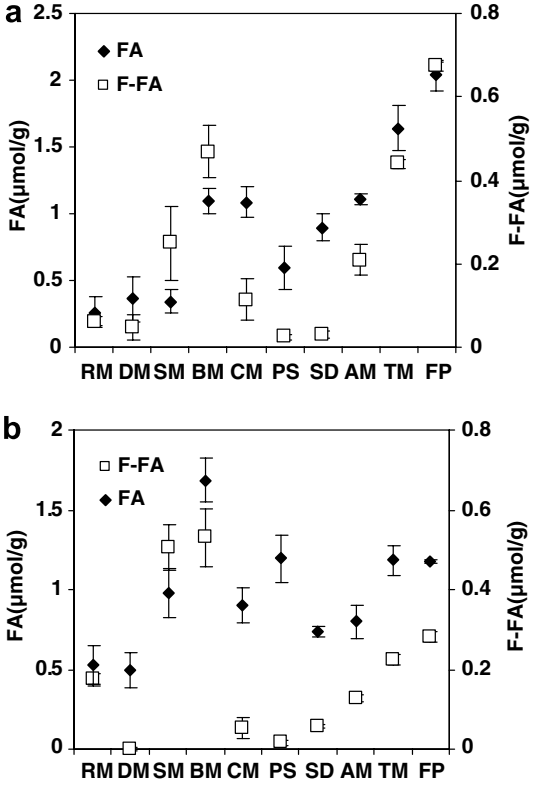

Fig. 2. Changes in levels of FA and F-FA (μmol/g of carbohydrate-free dry meat) during processing of dried-seasoned squids. (a) Neon flying squid; (b) jumbo squid. RM, raw material; DM, defrosted meat; SM, skinned meat; BM, boiled meat; CM, cooled meat; PS, primary seasoned meat; SD, Semi-dried meat; AM, adjusted meat; TM, torn meat; FP, final product.

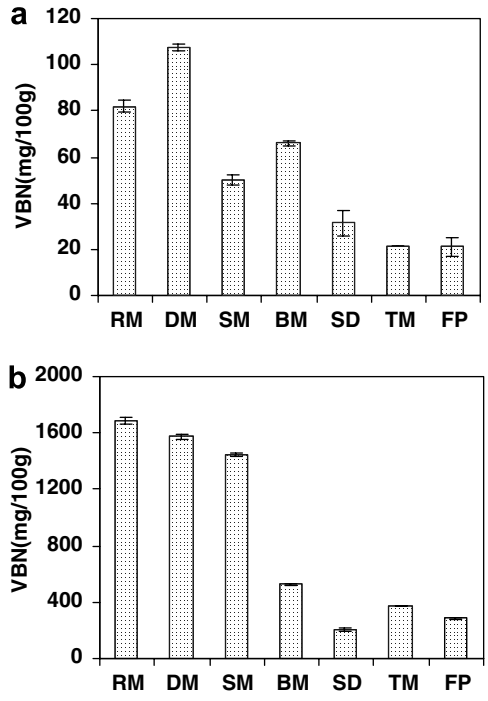

Fig. 3. Changes in levels of VBN (mg/100 g of carbohydrate-free dry meat) during processing of dried-seasoned squids. (a) Neon flying squid; (b) jumbo squid. RM, raw material; DM, defrosted meat; SM, skinned meat; BM, boiled meat; SD, Semi-dried meat; TM, torn meat; FP, final products.

nal amounts of FA and F-FA in raw material and those in defrosted meat were quite low, followed by a gradual enhancement and a peak value in boiled meat. After that, the contents of FA and F-FA decreased to a valley level in primary seasoned sample, followed by another increase up to peak value in the final product. Recently more attention was paid to the content of FA in squid to address safety issues. Low content of FA in seafood products is generally considered to be nontoxic, but in some cases, FA content exceed the acceptable level dramatically and this impacts the safety of the products and causes a great economic loss. Various alternatives inhibiting the production of FA have been proposed, such as storage under oxidizing condition (Lundstrom, Correia, & Wilhelm, 1982), heat treatment, removal of substrate and cofactors by washing (Landolt & Hultin, 1981), or the addition of enzyme inhibitors (Parkin & Hultin, 1982; Careche & Tejada, 1990). In the present study, the amount of reversibly bound FA exhibited no remarkable alteration during processing. So the level of FFA became a control factor, which can be greatly reduced by improving the handling process, such as by a washing with water. High temperature and instantaneous roasting were also well used to decrease the amount of FA.

The alteration of the volatile basic nitrogen (VBN) content during processing is shown in Fig. 3. The jumbo squid contained about 20-fold of VBN as that in neon flying squid. The amount of VBN was gradually reduced during the processing of jumbo squid, while VBN in neon flying squid manifested a rise both in thawed and boiled meat, followed by a gradual decrease. The VBN amounted to 21.1 and 285.7 mg/100 g carbohydrate-free dry meat in the final products of neon flying squid and jumbo squid, which decreased 74% and 83%, respectively, compared to the raw material. It was reported that the acceptable limit of VBN was dependent on the species and storage conditions and thus highly variable (Paarup et al., 2002; Lapa-Guimarães, Eduardo de Felício, & Contreras Guzmán, 2005). The high contents of VBN in jumbo flying squid may be due to the environment and physiological conditions.

4. Conclusions

Browning problem, TMAO and its breakdown products especially the production of formaldehyde is the centre of attention during processing of dried-seasoned squid. Herein, the effects of processing steps on the physicochemical properties of dried-seasoned squid were studied in detail using two kinds of squid. Browning occurred mainly during semi-dried and roasting process. The temperature and time of heating and type of roasting, as well as the material itself were key factors influencing the browning and the quality of products. Skinning, boiling and roasting were key processes to produce FA, and the temperature of boiling was the most crucial factor impacting the production of FA. FA could be greatly decreased during the cooling process by washing with water or changing the roasting techniques. Moreover, the physicochemical properties differed among different squid species. Therefore, to decrease the hazard of toxicity and improve the quality of the products, more attention should be paid to the temperature and roasting techniques according to the character of the material.

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