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Analysis of Nutrients in Fresh and Canned *Pseudobagrus*

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Abstract *Pseudobagrus* is a special economic fresh water fish in China. In order to evaluate the nutritional value, the contents of general nutritional compositions, amino acids, fatty acids and volatile flavor substances were compared between fresh and canned *Pseudobagrus*. The contents of moisture, ash, fat and crude protein in fresh *Pseudobagrus* were 71.36%, 1.46%, 10.4% and 10.4%, and those in its canned product were 50.97%, 5.82%, 17.8% and 16.7%, separately. The free essential amino acids (EAA) in fresh and canned *Pseudobagrus* were 41.52% and 44.53%, respectively. *Pseudobagrus* had more unsaturated fatty acids (86.45%) than its canned product (83.02%). Lower polyunsaturated fatty acids (PUFAs), docosahexaenoic acids (DHA, 22:6 n-3) contents and higher monounsaturated fatty acids (MUFAs), eicosapentaenoic acids (EPA, 20:5 n-3) levels were detected in fresh *Pseudobagrus*. After being processed, there were more kinds of volatile flavor substances in canned than fresh *Pseudobagrus*. Thus a new product of *Pseudobagrus* can be obtained in this processing method.

Keywords: *Pseudobagrus, amino acids, fatty acids, volatile flavor substances*

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1. Introduction

Pseudobagrus, commonly known as honk fish, yellow catfish, etc, belongs to siluriformes, bagridae [1]. It is a special economic fresh water fish in China and widely distributed in most lakes and reservoirs of natural water, especially the middle and lower reaches of the Yangtze river. Owing to its high protein, delicious meat, abundant nutrition and almost no fishiness, *Pseudobagrus* gains popularity among consumers and has enormous market potential at home and abroad [2].

However, research for *Pseudobagrus* mainly focuses on the biological characteristics, cultivation technique, dietary composition, disease prevention and control at present [3,4,5,6,7], there is still very rare products of *Pseudobagrus* on the market. By now, processing technology and analysis of nutritional ingredients on *Pseudobagrus* have not been studied systematically and reported. Therefore, it is the inevitable requirement of fishery industry to develop a widely accepted and recognized fish product. The aim of this research is to evaluate the nutritional value of fresh and canned *Pseudobagrus*, including amino acids, fatty acids and volatile flavor substances, which can provide theoretical basis and technical support for the development and mass production of *Pseudobagrus*.

2. Materials and Methods

2.1. Materials

Frozen *Pseudobagrus*, provided by Tianjin Jinbaodi Garden Food Company Limited. Edible salt, sugar, soy sauce, gringer powder, vegetable oil were purchased from Tesco supermarket in the third street of Tianjin Binhai New Area.

2.2. Instruments and Reagents

Electronic scales, oven, fryer, retorts, organization crushers, ultrasonic vibration analyzer, high-speed refrigerated centrifuge, automatic amino acid analyzer, GC-MS were used. The reagents used were of analytical grade, and deionized and distilled water was used.

2.3. Experimental Methods

2.3.1. Processing of Canned Pseudobagrus

(1) Thawing and cleaning: complete and disease-free *Pseudobagrus* with body length of 19-21cm was chosen and thawed 2-2.5 hours, then its tail, viscera and gills were removed and cleaned.

(2) Preservation: The thawed and clean *Pseudobagrus* was pickled in 8% saline for 20 minutes (fish: salt = 1: 1.5) and impurities attached to the fish surface was removed by running water, then it was cut into sections.

(3)Baking: the preserved *Pseudobagrus* was baked 30 minutes at 140°C and cooled to room temperature.

(4) Frying: after being baked, the fish was fried 5 minutes at 190-200°C, then the oil on fish surface was drained and immediately placed in a pre-cooled sauce for a dip 30s.

Seasoning Recipe: based on water, 20% soy sauce, 8% sugar, 1.5% salt, 0.1% ginger powder were mixed together.

(5) Packaging and sterilization: vacuum packaging and sterilization at 121°C, 20 minutes were used to get the final product.

2.3.2. General Nutritional Compositions

The determination of moisture, crude protein, fat and ash were according to GB5009.3-85, GB50095-2010, GB/T14772-2008, GB5009.4-2010, respectively.

2.3.3. Determination of Free Amino Acids

Prior to determination, blocky *Pseudobagrus* samples were ground into surimi (5 g) and placed in 25 mL centrifuge tube, added 10 mL 5% TCA solution, shaked up and down vigorously, and then extracted 30 minutes with ultrasonic. After extraction and standing for some time, the supernatant was pipetted in the centrifuge tube, centrifuged 10 minutes at 4°C, 10000 r/min, and then the supernatant in 5 mL EP tube was through 0.22 µm membrane water system, stored at 4°C to be determined on automatic amino acid analyzer.

2.3.4. Fatty Acid Composition

Extraction of fat oil: blocky *Pseudobagrus* samples were ground into surimi (10 g) and mixed with 50 ml petroleum ether (boiling point range 60-90°C) in 250 ml stoppered conical flask. After repeated oscillation and 1.5 hours ultrasonic extraction, the extract was allowed to stand for some time, poured in 50 ml centrifuge tube, centrifuged 10 minutes at 3000 r/min. The supernatant was removed completely to extract oil at 30°C, 100-110 r/min rotary evaporation. Then 5 ml n-hexane was added in and the extract was removed to be preserved at -20°C.

Fat oil methyl esterification: sample aboved (1 mL) and potassium hydroxide methanol solution (1 mL) were mixed in 5 mL EP tube and the mixture was shaked 30minutes on shaking table at 280 r/min. Analysis was performed using GC-MS after the supernatant was through 0.45 µm organic membrane.

2.3.5. Volatile Flavor Substances

Volatile flavor material acquisition:

Blocky *Pseudobagrus* samples were ground into surimi (2 g) and placed in 15 ml solid-phase extraction apparatus sampling bottle. Then anhydrous sodium sulfate (2 g) was added in to make samples into homogeneous granule with a glass rod stirring continuously. The sampling bottle was heated 80°C in water bath with a manual sampler containing 65 µm PDMS/DVB SPME (solid-phase microextraction) fiber head inserted in to acquire volatile flavor. One hour later, extraction head was pulled out and immediately inserted into the gas chromatograph sample mouth whose temperature was 250°C, then to inject sample after 30 minutes thermal desorption.

GC-MC (gas chromatography and mass spectrometry) detection conditions:

(1) Chromatographic column: HP-5MS (30 m × 0.25 mm × 0.25 um) elastic quartz capillary column was used, the column initial temperature was 40°C, kept 3 minutes, and 4°C/min up to 150°C, then 8°C/min up to 250°C, kept 6 minutes. Vaporizing chamber temperature was 250; The carrier gas was He with the purity of 99.999; The carrier gas flow rate was constant current 1 ml/min. The split ratio was 5:1.

(2) Mass spectrometry conditions: ion source was EI source; Ion source temperature was 220°C; electron energy was 70 eV and the quality range was 43-500 amu(atomic mass unit).

2.4. Statistical Evaluation

The results were analyzed at least in triplicates and data presented as mean ± standard deviation (n≥3). The means were subjected to simple ANOVA for significance test (p<0.05) using SPSS version 17.0.

3. Result and Discussion

3.1. General Nutritional Compositions

The contents of moisture, ash, crude protein, fat in fresh and canned *Pseudobagrus* were presented in Table 1. Compared with fresh *Pseudobagrus*, final product had lower moisture, higher ash and fat content.

Table 1. the contents of moisture, ash, fat and crude protein

name	moisture %	ash %	fat %	protein %
Fresh fish	71.36±0.01a	1.46±0.03a	10.4±1.12a	10.4±0.07a
Canned fish	50.97±0.04b	5.82±0.07b	17.8±0.91b	16.7±0.05b

Data were presented as mean ± SD (n=3). Mean values within each row followed by different letters differed significantly at p＜0.05.

3.2. Determination of Free Amino Acids

Free amino acids in fresh and canned *Pseudobagrus* were determined and the results were showed in Table 2.

Table 2. the contents of free amino acids

name	Content(g/100g)	
	Fresh fish	Canned fish
Asp**	1.25±0.01	1.23±0.04
Thr*	0.43±0.00	0.73±0.03
Ser	4.18±0.04	8.23±0.28
Glu**	4.82±0.03	0.31±0.01
Pro	0.92±0.00	0.58±0.04
Gly**	4.23±0.04	0.03±0.00
Ala**	6.71±0.14	6.17±0.10
Cys*	8.98±0.11	8.63±0.28
Val*	4.61±0.01	3.58±0.14
Leu*	2.06±0.00	3.04± 0.03
His	6.60±0.71	6.15± 0.14
Lys*	4.32±0.06	---
Tyr*	---	---
Phe*	---	0.90±0.14
Ile*	---	1.35±0.04
Met*	---	---
TAA	49.11	40.92
EAA	20.39	18.22
DAA	17.02	7.74
EAA/TAA(%)	41.52	44.53
DAA/TAA(%)	34.66	18.92

*Essential amino acids, **Delicious amino acids, TAA represents total amino acids, EAA represents essential amino acids and DAA represents delicious amino acids.

There were 12 and 13 kinds of free amino acids in fresh and canned *Pseudobagrus*, respectively. After being processed, the content of free Thr, Ser, Leu, Phe, Ile in final products increased, especially Ser significantly. Besides, the content of other kinds of free amino acids decreased more or less. The change of amino acids mainly attributed to two aspects. On one hand, under the action of muscle proteolytic enzyme and aminopeptidase, fish muscle protein mainly muscle plasma protein, degrade and generate free amino acids in the process of curing. On the other hand, during baking and frying, amino acids and reducing sugar can generate aldehydes, ketones, alcohols and other volatile compounds of small molecule. The decrease of Cys was more likely due to the generation of sulfur compounds caused by Stretcher degradation [8]. This was in accordance with the detection of 2-hexyl thiophene in final products while not in the fresh *Pseudobagrus*.

There were mainly four kinds of amino acids, including Glu, Asp, Gly, Ala, that were related to the delicious component in the muscle of fish. Table 2 indicated that the content of delicious amino acids (DAA) in fresh and canned *Pseudobagrus* was 17.02 and 7.74 grams per one hundred grams, respectively. Besides, the content of essential amino acids (EAA) were accounted for 41.52% and 44.53% in total amino acids(TAA), respectively. According to the ideal mode of FAO/WHO, the composition of amino acids in protein which had better quality was that EAA/TAA was around 40% [9]. Thus, these data further confirmed *Pseudobagrus*, as a kind of freshwater fish, was delicious, rich in nutrition and popular with a large proportion of consumers, and its nutritional value had been improved to a certain extent after processing.

3.3. Fatty Acids Composition

Table 3 shows the variety and content of fatty acids in fresh and canned *Pseudobagrus*. It was concluded that raw fish had diverse fatty acids in which the kinds of unsaturated and saturated fatty acids were 16 and 11, respectively.The content of oleic and linoleic acid in fresh *Pseudobagrus* was 42.81% and 20.33%, respectively. Oleic and linoleic acids are beneficial to human health which can lower blood cholesterol levels and prevent atherosclerosis [10]. In addition, it also contained a small amount of high nutritional value of 5,8,11,14,17-eicosapentaenoicacid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA). After a series of process such as preservation, baking, frying, the relative contents of linoleic acid increased to 43.14% in final product. Besides, palmitate, nonadecanoate, eicosanoic acid and DHA also had a slight increase.

In general, the higher content of unsaturated fatty acids (UFA) was in fat, the higher content of essential fatty acids was and the nutritional value was also relatively high [11]. The content of UFA in fresh and canned *Pseudobagrus* were 86.45% and 83.02%, and PUFA 27.14% and 46.20%, respectively. Because of baking and frying, the significant increase of content of PUFA made canned *Pseudobagrus* richer in nutrition. Under the action of lipoxygenase, PUFA would generate volatile carbonyl and alcohol compounds and they constitute the scent of fish such as plants sweet. Volatile carbonyl compounds produce connate and rich aroma, while volatile alcohol compounds more downy smell. So the flavor of canned *Pseudobagrus* would increase to an extent.

Table 3. the contents of fatty acids(%)

systematic name	shorthand name	Fresh fish	Canned fish
methyl myristate	C14:0	2.05±0.07	0.41± 0.00
6-methyl tetradecanoic acid methyl ester		0.11±0.00	0.02±0.00
methyl 12-methyltridecanoate		0.02±0.01	---
methyl pentadecanoate	C15:0	0.33±0.03	0.09± 0.03
cis-13,16,19-docosatrienoic acid methyl ester	C16:3	0.10±0.01	0.06± 0.01
methyl trans-2-hexadecenoate		0.37±0.01	0.07±0.00
methyl trans-9-hexadecenoate		8.10±1.41	1.72± 0.14
methyl palmitate	C16:0	4.67±0.03	8.04±1.44
7-methyl-6-hexadecenoic acid methyl ester		0.35±0.01	0.08±0.00
heptadecanoic acid-methyl ester	C17:0	0.14±0.00	0.04± 0.00
cis-7-hexadecenoic acid methyl ester		0.10±0.03	0.10±0.01
15-methyl hexadecanoic acid methyl ester		0.34±0.06	0.20±0.03
methyl linoleate	C18:2(n-6)	20.33±4.24	43.14±1.47
methyl oleate	C18:1	42.81±3.11	33.99±1.41
methyl 9-(Z)-octadecenoate		2.85±0.03	0.52±0.00
octadecanoic acid methyl ester	C18:0	5.21±0.14	1.14±0.06
methyl nonadecanoate	C19:0	0.11±0.00	5.29±0.28
5,8,11,14,17-eicosapentaenoicacid methyl ester	C20:5(n-3)	0.43±0.04	0.04±0.00
5,8,11,14-eicosatetraenoicacid methyl ester	C20:4(n-6)	2.58±0.28	0.18±0.03
7,10,13-icosatrienoic acid methyl ester	C20:3	0.66±0.04	0.81±0.01
11,14,17-eicosatrienoicacid methyl ester		1.92±0.03	0.28±0.04
11,14-eicosadienoicacid methyl ester		0.93±0.04	0.37±0.01
11-icosenoic acid methyl ester		3.72±0.14	0.28±0.04
eicosanoic acid methyl ester	C20:0	0.49±0.01	1.71±0.14
4,7,10,13,16,19-docosahexaenoic acid methyl ester	C22:6(n-3)	0.20±0.00	0.43±0.04
cis-docos-13-enoic acid methyl ester	C22:1	1.01±0.01	0.06±0.00
docosanoic acid methyl ester	C22:0	0.07±0.00	0.06±0.01
UFA		86.45	83.02
MUFA		59.31	36.82
PUFA		27.14	46.20

UFA represents unsaturated fatty acids, MUFA represents monounsaturated fatty acids and PUFA represents polyunsaturated fatty acid

3.4. Volatile Flavor Substances

The contents of volatile flavor substances in fresh and canned *Pseudobagrus* were presented in Table 4. There were 12 and 18 kinds of volatile flavor substances in raw fish and final product. Compared with fresh fish, pyrazine compounds had the most significant change in the canned *Pseudobagrus*.

Pyrazine, a kind of heterocyclic compounds containing two nitrogens, can be used as an effective flavor enhancer [12] and its optimal formation temperature is 120°C to 150°C. It was considered to be the product of maillard reaction which had baked sweet and nuts, popcorn, coffee flavor characteristics [13] and low threshold value. Jian-Feng Wu [14] considered pyrazine compounds could be used to identify wine as flavor compounds. Pyrazine compounds in *Pseudobagrus* were only produced in the process of frying, which had prominent effect on the increase of final product flavor. Therefore, pyrazine compounds were peculiar to canned *Pseudobagrus*. They were flavor substances accepted and approved pervasively by the consumers.

Hydrocarbon compounds were one of the main volatile flavor substances in aquatic products. Because of high threshold, small contribution to the flavor, they had no odor or smell. They mainly came from homolysis of oxygen free radicals in the fatty acids and some hydrocarbon compounds were important intermediates during the formation of heterocyclic compounds. So they were important basal that could not be ignored to improve the overall flavor of samples [15]. The table below showed that there were little variation in the kinds and contents of hydrocarbon compounds, thus they had small contribution to improvement of flavor during processing of *Pseudobagrus*.

Besides, alcohol, ketone, aldehyde and other compounds also had some changes in kinds and contents because of thermal oxidation or degradation of unsaturated fatty acids [12]. Owing to their low threshold, they could make big contribution to flavor even at low concentration. The overall trend showed that most of them had increased in kinds and contents, which made canned *Pseudobagrus* more delicious.

Table 4. the contents of volative flavor substances(%)

kind	retention time	name	relative content %	
			Fresh fish	Canned fish
pyrazine	13.681	2-ethyl-5-methylpyrazine	---	---
	13.842	2-ethyl-3-methylpyrazine	---	2.36±0.01
	16.678	3-ethyl-2,5-dimethylpyrazine	---	1.63±0.03
	16.922	2-ethyl-3,5-dimethylpyrazine	---	0.44±0.01
	17.032	2,6-dimethylpyrazine	---	---
	19.759	2-butyl-3,5-dimethylpyrazine	---	0.21±0.03
alcohol	6.389	2-methyl-(1R,2S)-rel-cyclopentanol,	1.37±0.00	0.41±0.00
	15.209	4-ethyl cyclohexanol	---	---
	17.758	(Z)- non-2-en-1-ol	---	5.59±0.04
	17.914	benzaldehyde dimethylacetal	25.98±1.44	30.54±2.86
hydrocarbon	21.528	1,9-nonanediol	1.75±0.01	---
	21.614	2-butyl-1-octanol	---	3.94±0.01
	29.614	(E,E)-3,7,11-trimethyl-2,6-dodecadien-1-ol	0.78±0.03	1.85±0.00
	14.779	(S)-Limonene	2.04±0.00	0.71±0.01
	17.458	3-methyl decane	15.48±1.43	12.82±1.46
	18.305	p-cymene	1.6±0.00	0.95±0.00
	28.779	caryophyllene	38.78±2.90	4.25±0.00
	30.666	1-methyl-4-(6-methylhept-5-en-2-yl)benzene	7.74±0.06	10.83±1.37
ketone	9.447	2-heptanone	---	---
	17.217	methyl n-octyl ketone	0.16±0.00	---
	20.979	dodecan-2-one	---	9.54±0.00
aldehyde	12.414	benzaldehyde	---	8.71±0.01
	14.942	4-methylcyclohex-3-enecarbaldehyde	---	---
	25.528	(Z)-3-methoxy-2-methyl-2-vinylhexa-3,5-dienal	---	---
others	13.261	2-pentylfuran	4.47±0.03	3.27±0.07
	25.308	2-n-hexylthiophene	---	1.95±0.00

4. Conclusion

Based on the nutritional analysis including general nutritional compositions, free amino acids, fatty acids, and volatile flavor substances, it was concluded that *Pseudobagrus* had relatively high nutritional value and worth further research and comprehensive development. The result of the present study provided important baseline information on *Pseudobagrus* as valuable source, as well as potential nutrient supplement. Besides, after a series of processing, canned *Pseudobagrus* increased in nutrition and flavor to some extent, which provided an effective method in terms of processing of *Pseudobagrus.*

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