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The Effect of Different Preparation Methods on the Flavor of Farmed *Takifugu obscurus*

Zhuanxia Chen[1], Ningping Tao[1,*], Xiao Qin[1] and Dayong Liu[2]

[1]*College of Food Science and Technology, Shanghai Engineering Research Center of Aquatic-Product Processing and Preservation, Shanghai Ocean University 201306, China*

[2]*Jiang Su Zhongyang Ecological Fishes Company Ltd., 226600, China*

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ZC, NT, XQ and DL designed the study. ZC and XQ performed experimental work and analyzed the data. ZC and NT wrote the article.

Key words
***Takifugu obscurus*, Fried, Steamed, Boiling, Soup, Umami.**

ABSTRACT

The aim of this study was to investigate the tastes of the meat, liver and soup through different preparatory procedures which were used in traditional. The taste of *Takifugu obscurus* under different conditions were analyzed by sensory evaluation and electronic tongue technique. The characteristic of the taste substances were tested, such as free amino acids, nucleotides, trimethylamine oxide and betaine. Results showed that umami was the most prominent flavor. Compared with liver, IMP is the main flavor nucleotide in meat. However, the contents of the umami amino acids and betaine in liver are much higher than that of meat. Additionally, trimethylamine oxide is mainly found in the meat. After steaming, content of IMP in meat increased significantly. Compared with steamed meat, the flavor materials in boiled meat had a certain loss. Therefore, the addition of fried liver to the soup is beneficial to flavor.

INTRODUCTION

Wild puffer fish is widely known to be poisonous, but delicious, especially when it is made into a soup. Despite the lethal poison of puffer fish, people are willing to risk their life to try it. The puffer fish is a kind of anadromous fish (Cheng *et al*., 2016), widely distributed in the Sea of Japan, the East China Sea and the Yellow Sea (Ali *et al*., 2017). *Takifugu obscurus* is one of puffer fish, farmed *T. obscurus* is popular in China and Southeast Asia, because of its umami taste, high nutritional value and do not contain tetrodotoxin in the meat and liver. Additionally, the meat contains high protein, low fat and rich in essential amino acids, minerals and nucleotide substances (Noguchi *et al*., 2006). The liver of the puffer fish has a high content of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other n-3 series of polyunsaturated fatty acids, which plays a role in preventing atherosclerosis and thrombosis (Mnari *et al*., 2007). In addition, the concentrations of these nutrients are significantly higher than other well-known freshwater fishes (Jang *et al*., 2011).

The taste substances, including nitrogenous and non-nitrogenous compounds, are a kind of non-volatile matter which belongs to water-soluble compounds and have low molecular weight (Fuke and Konosu, 1991). Free amino acids (FAA), nucleotides, trimethylamine oxide (TMAO), and betaine are mainly included. Meanwhile, FAA and nucleotides are important nitrogenous taste substances in aquatic products; TMAO has a unique flavor and refreshing sweetness; betaine mainly shows sweetness. FAA, nucleotides and its associated product *et al*. are considered as active ingredients which can generate aroma substances (Yamaguchi *et al*., 1970). Konosu (1974) reported that in different species (red sea bream, stone flounder, flathead flounder, flounder, puffer, angler, common mackerel, and jack mackerel), the total content of the nitrogen in these constituents amount to nearly 90% or more of the total extractive nitrogen, which free amino acid-N is 7%-45%, and TMAO-N 2%-23%. However, the taste of fish is not just the simple accumulation of these substances, but the synergistic reaction to all, enhancing the overall flavor (Liu *et al*., 2009).

Abbreviations

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FAA, free amino acids; TMAO, trimethylamine oxide; RW, raw meat; RL, raw liver; SM, steamed meat; BM, boiled meat; SL, steamed liver; FL, fried liver; SLW, steamed liver water; FLW, fried liver water; SLS, the soup prepared by SL and meat; FLS, the soup prepared by FL and meat; PCA, perchoric acid; TMA, trimethylamine; TCA, trichloroacetic acid; ADP, adenosine diphosphate; AMP, adenosine; GMP, guanylic monophosphate; HxR, hypoxanthine ribonucleoside; IMP, inosinic monophosphate; Adr, adrenaline; Hx, hypoxanthine.

* Corresponding author: nptao@shou.edu.cn
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Currently, studies conducted on the *T. obscurus* were mainly focused on its toxicity (Ji *et al*., 2011), nutrition evaluation (Tao *et al*., 2012), and odour components (Tao *et al*., 2014), few studies had addressed the effects of different processing methods on the flavor. Li *et al*. (2016) reported that frying and cooking contributes greatly to the overall flavor of the soup.

The stew soup made out of farmed *T. obscurus'* meat is excellent, in order to increase the umami taste, the method of adding fried liver into soup is used traditionally. This research aimed to study the effects of different preparation methods on the flavor, and to provide theoretical basis for the delicious taste of *T. obscurus* soup, by using common processing methods such as steaming, boiling and frying. The soups were prepared by adding fried and steamed liver into the water and cooked together with meat, respectively.

MATERIALS AND METHODS

Materials and sample preparation

In this research, 20 two year old *Takifugu obscurus*, each (27±1.12) cm long, weighing (335±7.01) g and with a liver weight of (43±4.43) g were provided by Zhong Yang ecological fishes in Jiang Su Province. The fishes were killed immediately after conducting the standard biological measurement. The meat and liver were cleaned, and the eyes and skin were removed, and then stored at -80°C for further analysis.

Processing treatments

The samples were thawed under 4°C, the liver and the dorsal were then cut into 0.5 cm slices. In this paper, the cooking methods of the soup was mainly referred from the enterprise standard for white juice of puffer fish, formulated by Nakae Catering Management Co., Ltd., in Jiangsu province, China. Comparison between each material, raw meat (RW) and raw liver (RL) have also been made.

Steamed meat (SM): The meat was placed on a watch glass and steamed with boiling water for 30 min. The proportion of water and fish was 12:1.

Boiled meat (BM): The meat was cooked in boiling water for 30 min to ensure it was well-done. The proportion of water and fish was 10:1.

Steamed liver (SL): The liver was placed on a watch glass and steamed with boiling water for 30 min. The proportion of water and fish was 12:1.

Fried liver (FL): The oil was preheated to 120 °C in a wok. Then the liver was fried for 3 min until it appeared yellowish. The proportion of oil and liver was 3:7.

Steamed liver water (SLW): The SL was immersed in boiling water for 30 min. The proportion of water and liver was 10:1.

Fried liver water (FLW): The FL was immersed in boiling water for 30 min. The proportion of water and liver was 10:1.

The soup prepared by SL and meat (SLS): 35 g of SL and 150 g of RM were immersed in 2600 ml of pure water, and then boiled for 30 min.

The soup prepared by FL and meat (FLS): 35 g of FL and 150 g RM were immersed in 2600 ml of pure water, and then boiled for 30 min.

Sensory test

Experienced assessors (n=14; 6 males and 8 females aged 23–35 years) were selected from the 30 participants to take part in the experiments, according to the guidelines by Schiffman (2000) and Rhyu and Kim (2011). The scoring method (5-point scale intensity evaluation) was used, scale 1 represented no flavor, and 5 represented the strongest degree of flavor felt. In the sensory evaluation experiment, 10 ml of liquid samples and 10 g of solid samples were stored in 30 ml paper cup, each of which was marked with three random numbers for the evaluators to conduct the evaluation. Time interval of 5 min was set between two evaluation, and evaluators would rinse their mouth after completing an evaluation. During the evaluation, sensory officers put the sample into the mouth, use their tongue to taste it for 3 s and retain it in mouth for 5 s, then spit out and give a score according to the five basic intensity (sour, sweet, bitter, saline and umami) which was described by the trainer previously. The data of sensory evaluation was analyzed by MS Office Excel 2010 and Origin 8.0 statistical software.

Electronic tongue

Samples frozen under -80 °C were thawed. 5.00 g of solid samples were accurately measured, 50 ml ultrapure water was then added and homogenized for 2 min, left at room temperature for 30 min after ultrasonic for 5 min, then centrifuged at the speed of 12,000 g for 15 min at 4°C. The precipitate was dissolved in 30 ml water and then re-extracted once more as described above. The combined supernatant was collected and then measured at a volume of 100 ml. For liquid samples, the filtrate was detected using the E-Tongue after filtration.

The electronic tongue (Astree; Alpha MOS, Toulouse, France) system is made of seven different sensors: the UMS, GPS, SWS, SRS, STS, SPS and BRS. Among them, UMS, SRS and STS have a single response for umami, sour and saline, respectively. Sliver (Ag)/Silver chloride (AgCl) was used as the reference electrode. The chemical responses of the sensors were changed into electric signals by an electronic unit when the system is soaked in the flavor solution. In this experiment, the signal average ranging from 100 s to 120 s was collected for further analysis. Electronic tongue workstations Alpha Soft 14.0 was used to analyze the principal component.

Free amino acids

2.5 g (5 ml) samples were obtained and homogenized for 2 min after adding 15 ml of 5% hydrochloric acid then undergone ultrasonic extraction for 30 min and left to settle for 2 h. 10ml of supernatant was extracted, 5 ml of supernatant was taken after centrifuging at the speed of 10000 g for 15 min under 4°C. The pH was adjusted to 2.2, metering volume to 10 ml. After filtering through 0.22-µm filters, the extracted solution was analysed by an automatic amino acid analyser (L 8800; Hitachi Ltd., Japan).

Nucleotides

Samples were taken at 6 g (8 ml), respectively, 10 ml of 10% PCA was added, and homogenised for 2 min after through ultrasonic. Supernatant was extracted after centrifugation (10000 r/min, 10 min). Sediment was dissolved in 10 ml of 5% perchoric acid (PCA), homogenised, gone through ultrasound, and centrifuged. Two centrifugal supernatants were merged and then had the pH adjusted to 6.5 followed by the procedure of metering volume to 50 ml. After filtering through a 0.22-µm filter, the solution was collected and analyzed by an HPLC device (2695e; Waters Ltd., Milford, MA).

Analysis was performed by using a Waters E2695 Series HPLC system equipped with a UV-detector and Shimadzu C18 column (5 µm 250 mm×4.6 mm). The chromatogram was recorded at 285 nm and the peak areas were quantified. The mobile phase was methanol (A), 0.0125 mol/L monopotassium phosphate: dipotassium phosphate (1:1) (B), the pH was 5.75.

Trimethylamine oxides (TMAO)

According to the study of Wekell and Barnett (1991), reduction method was utilized to detect the content of TMAO. Fe-EDTA reagent was used as deoxidizer. Trimethylamine (TMA) and TMAO were analyzed in the TCA extracts via Dyer (1945) method, modified by Hashimoto and Okaichi (1957) and the method of Bystedt *et al*. (1959) modified by Yamagata *et al*. (1968), respectively. TMAO was calculated according to content difference of trimethylamine nitrogen before and after.

Betaine

The methods were mainly referenced from the study of Focht *et al*. (1956) and some modifications were made. Betaine was extracted from the processed samples, and then reinecke's salt was added into the extracting solution, the generated crystal was dissolved in 70% acetone and the solution was measured at 525 nm.

Statistical analysis

All analysis were performed at least in triplicate for each sample. Results were analysed by Analysis of Variance (ANOVA), which was conducted by Excel XP Software; and all figures were painted by Origin 8.0.

RESULTS

Sensory evaluation

Figures 1 and 2 shows that umami was the most prominent taste among the variety of samples, the second taste was saline. In terms of umami, SM had the highest score, followed by FL. Compared to steaming, the taste of BM had decreased. The FL had the higher scores than SL for its umami, saline, bitter and sweet scores, except sour. The umami score of FLS was higher than SLS.

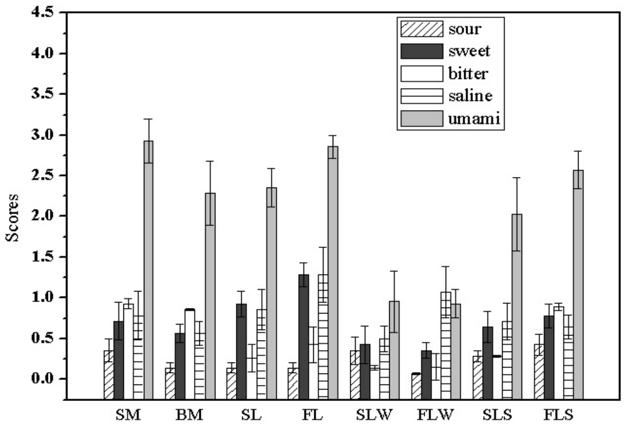


Fig. 1. *Takifugu obscurus* sensory evaluation results (n=14). RM, raw meat; SM, steamed meat; BM, boiled meat; RL, raw liver; SL, steamed liver; FL, fried liver; SLW, steamed liver water; FLW, fried liver water; SLS, steamed liver soup; FLS, fried liver soup.

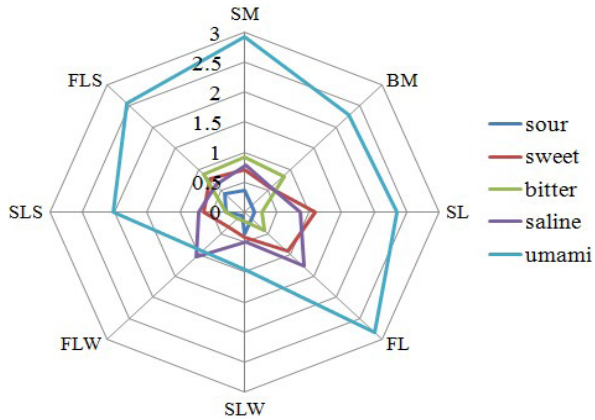


Fig. 2. Taste profile radar map of *Takifugu obscurus* sensory evaluation (n=14). For Abbreviations, see Figure 1.

Electronic tongue analysis

The contribution rate of PC1 + PC2 reached 97.371% (Fig. 3). The samples can clearly displayed on the picture, and there aren't superposition. Though SM and RM were close, they also can be differentiated clearly. BM was far from with RM and SM, while it was closed to FLS and SLS in terms of distance.

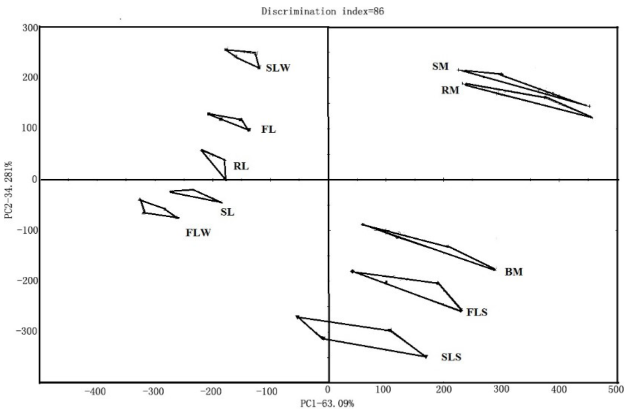


Fig. 3. Results of PCAs of E-tongue. For abbreviations, see Figure 1.

The analysis of the FAA

The FAA content of each sample, and the total amount of umami, sweet and bitter FAA in samples processed differently were shown in Table I and Figure 4, respectively. The contents of UAA, SAA and total FAA between SM and RM hadn't great difference, while BM decreased significantly in total. The contents of UAA in the liver were significantly higher than that of meat and soup, while BAA was lower. The FL had the more amino acids than SL. In contrast with the soup that was added with SL, the result of soup with FL showed that the contents of umami, sweet, bitter and the total FAA were significantly higher.

The analysis of the nucleotides

Figure 5 shows the nucleosides and their associated products graph on HPLC of mixed standard sample. The analysis of nucleotides and its associated products in each sample as shown in Figure 6. It can be seen that inosinic monophosphate (IMP) is the main flavor nucleotide in meat and FLS, but is low in liver. As shown in Figure 6A, after steaming, AMP, GMP, HxR, IMP and Adr had improved in the meat, especially IMP increased significantly, while ADP and Hx decreased. Compared to RM, with the exception of ADP and IMP decreased, the nucleotides contents of BM had not changed. In Figure 6B, HxR and Hx were the main nucleotides. From Figure 6C, results show that the IMP content in the soup added with FL was significantly higher than that added of SL.

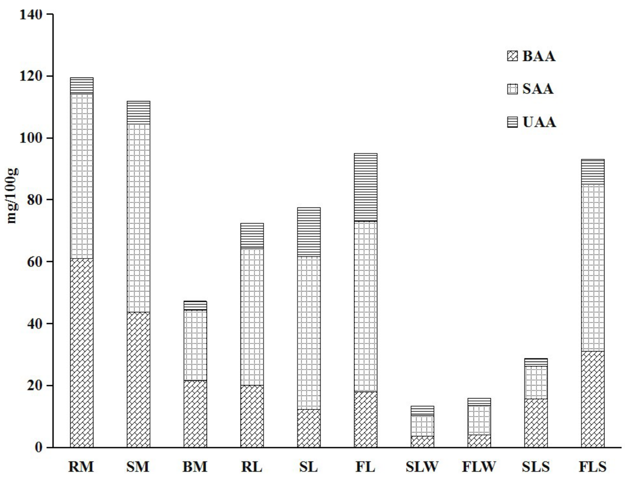


Fig. 4. Free amino acid contents in different treatments. UAA, umami amino acids; SAA, sweet amino acids; BAA, bitter amino acids. For abbreviations, see Figure 1.

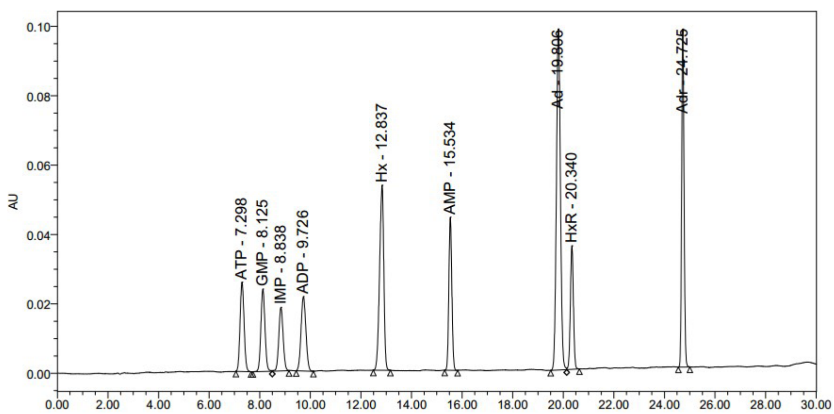


Fig. 5. Nucleosides graph on HPLC of mixed standard samples.

Table I.- Free amino acid contents in *Takifugu obscurus* at different preparation.

Names of amino acids	Flavor	Meat (mg/100g)			Liver (mg/100g)			Soup (mg/100ml)			
		RM	SM	BM	RL	SL	FL	SLW	FLW	SLS	FLS
Asp	Umami	1.86±0.02	4.28±0.28	1.61±0.10	1.46±0.21	1.09±0.01	1.59±0.01	0.26±0.01	0.52±0.09	0.73±0	2.69±0.23
Glu	Umami	3.43±0.07	3.11±0.22	1.14±0.05	6.57±0.06	14.70±0.21	20.19±0.33	2.60±0.20	1.80±0.34	1.71±0.07	5.35±0.41
Ser	Sweet	3.06±0.12	3.12±0.25	1.24±0.08	2.86±0.13	1.37±0.03	2.48±0.04	0.36±0.02	0.61±0.12	1.00±0.01	4.18±0.33
Gly	Sweet	17.54±0.37	24.84±1.86	8.85±0.57	3.22±0.03	3.65±0.05	3.92±0.04	0.63±0.05	0.71±0.13	2.15±0.04	13.74±1.04
Thr	Sweet	3.20±0.04	2.18±0.16	0.84±0.06	3.65±0.18	3.82±0.02	4.94±0.21	0.69±0.04	0.87±0.18	0.90±0.03	2.45±0.20
Pro	Sweet	8.99±1.03	6.14±0.67	2.85±0.06	8.88±0.35	-	-	-	1.93±0.04	1.89±0.02	6.43±0.54
Ala	Sweet	20.30±0.30	24.42±1.46	8.99±1.04	25.63±0.55	39.84±2.74	43.74±2.28	5.11±0.38	5.26±1.47	4.92±0.03	27.12±3.97
Cys	Sweet	-	-	-	-	0.76±0.06	-	-	-	-	0.06±0.01
Val	Bitter	1.77±0.10	0.95±0.31	0.44±0.18	2.85±0.17	0.66±0.02	2.15±0.10	0.33±0.01	0.57±0.12	0.67±0.01	0.49±0.02
Met	Bitter	5.55±0.01	3.12±0.26	1.41±0.13	2.14±0.06	0.93±0.02	1.32±0.05	0.30±0.02	0.43±0.12	1.21±0.07	1.72±0.12
Ile	Bitter	1.03±0.09	0.79±0.04	0.44±0.03	1.56±0	0.86±0.04	1.16±0.04	0.28±0.05	0.30±0.03	0.47±0.03	0.60±0.03
Leu	Bitter	2.06±0.03	1.51±0.08	0.79±0.03	3.45±0.06	1.85±0.03	2.44±0.08	0.58±0.04	0.72±0.08	0.93±0	1.14±0.07
Tyr	Bitter	2.03±0.07	1.28±0.07	0.74±0.22	-	1.97±0.12	1.28±0.09	0.41±0.05	0.20±0.06	0.64±0.11	0.87±0.13
Phe	Bitter	0.73±0.17	1.37±0.10	0.68±0.04	1.75±0.82	0.92±0.02	1.27±0.02	0.86±0.07	0.66±0.16	0.76±0.12	1.18±0.15
Lys	Bitter	31.75±0.15	17.10±1.47	7.99±0.36	7.90±0.26	3.94±0.08	5.21±0.21	0.71±0.07	1.25±0.23	8.32±0.14	13.49±1.08
His	Bitter	-	-	-	-	-	2.13±0.05	-	-	-	-
Arg	Bitter	16.17±0.06	17.66±1.40	9.17±0.31	0.45±0.06	1.07±0.05	1.04±0.06	0.17±0	-	2.70±0.16	11.62±0.84

RM, raw meat; SM, steamed meat; BM, boiled meat; RL, raw liver; SL, steamed liver; FL, fried liver; SLW, steamed liver water; FLW, fried liver water; SLS, steamed liver soup; FLS, fried liversoup. **-**, not detected.

Table II.- The contents of TMAO and betaine in *Takifugu obscurus*.

Names	Meat (mg/100g)			Liver (mg/100g)			Soup (mg/100mL)			
	RM	SM	BM	RL	SL	FL	SLW	FLW	SLS	FLS
TMAO	14.09±0..41	7.66±0.22	3.41±0.21	0.17±0.02	0.32±0.13	0.36±0.28	0.36±0.08	0.34±0.03	1.28±0.01	1.08±0.28
Betaime	200.70±0.50	158.59±0.35	133.85±0.50	568.14±0.32	518.60±0.09	619.17±2.87	119.77±0.11	100.49±0.06	146.68±0.54	306.64±0.61

RM, raw meat; SM, steamed meat; BM, boiled meat; RL, raw liver; SL, steamed liver; FL, fried liver; SLW, steamed liver water; FLW, fried liver water; SLS, steamed liver soup; FLS, fried liver soup.

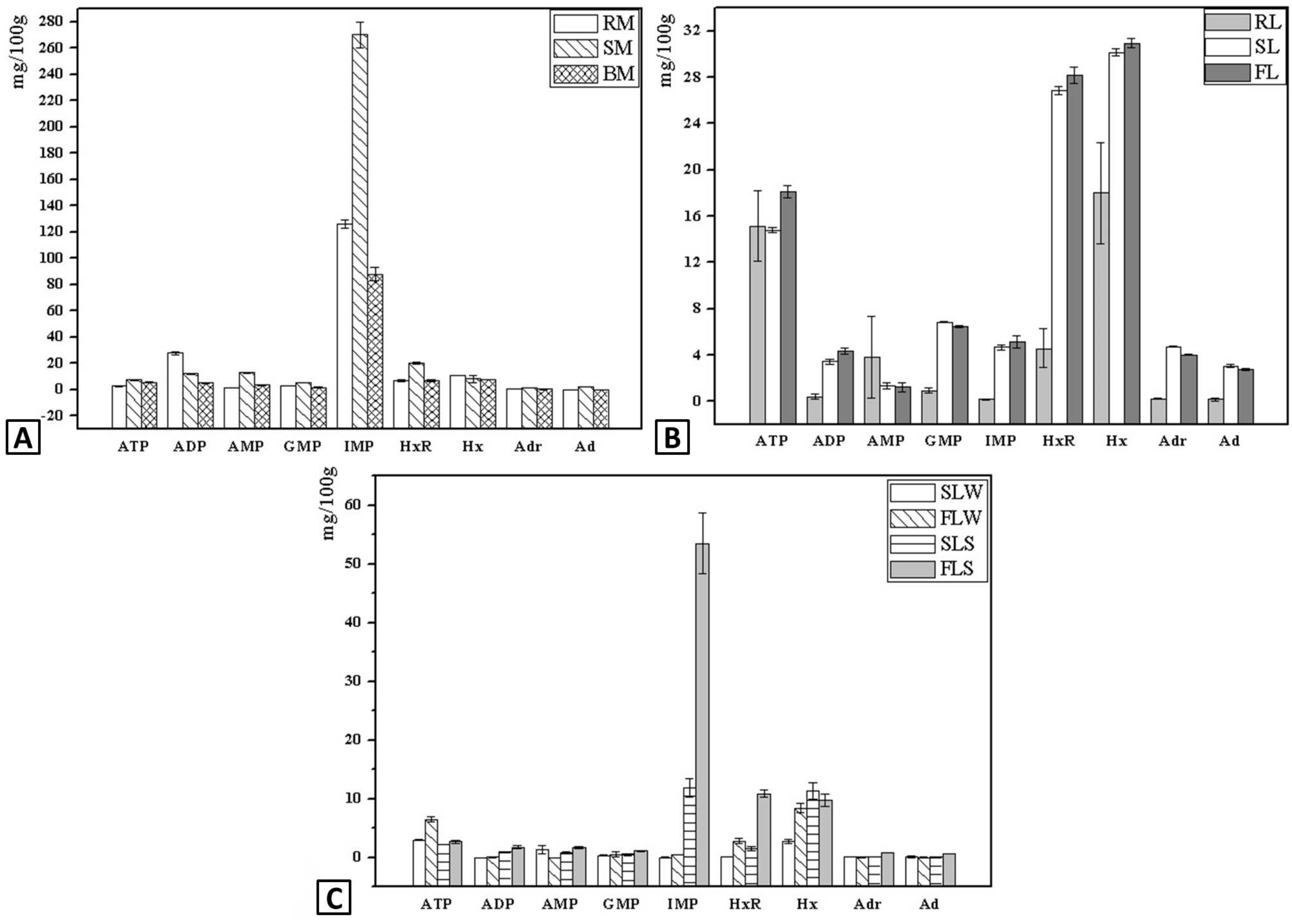


Fig. 6. Nucleotide contents in *Takifugu obscurus* at different preparation: A, meat; B, liver; C, soup. For abbreviations, see Figure 1.

The analysis of the TMAO and betaine

The contents of TMAO and betaine in each sample were shown in Table II. TMAO mainly existed in meat, but very low in liver. After cooking, the content of TMAO in meat was reduced. The content of betaine in liver was much higher than that in meat, and the contents of betaine in SM and BM were less than RM, besides, BM was lower than SM. At the same time, FL and FLS were much higher than that of SL and SLS, respectively.

DISCUSSION

Sensory evaluation

No seasonings were added during processing. As shown in Figures 1 and 2, the mainly flavor of the *T. obscurus* were umami, saline and sweet, umami was the most prominent taste. SM had the highest level of umami, the second was FL. The BM had the fewer flavors than SM; this may be caused by the loss of taste materials dissolved in water. Compared with SL, the short-term high temperature of FL had some differences for its umami, saline and sweet tastes. Sensory evaluation results showed that adding FL into the soup can significantly improve the umami taste, which indicated that thermal oxidation and decomposition of fatty acids during the frying process played an important role in the formation of flavor, especially polyunsaturated fatty acids and Maillard reaction (Liu *et al*., 2009; Mottram, 2008). The absorption of volatile substances in oil might also contribute to the taste.

Electronic tongue analysis

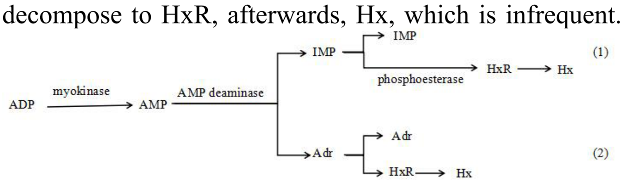
As shown in Figure 3, the differences of each flavor profile between samples can be fully displayed on the principal component surface, besides, the farther the interval was, the wider the taste differ. Along PC1 and PC2 axis, there was superior discrepancy among the samples. Although the taste of SM and RM were similar, there also hadn't overlapping. And a great difference was existed in the BM content, this may suggest that there was some loss of taste materials. The distance of BM was close to FLS and SLS, it indicates that the meat mainly contributes to the flavor of the soup, and when combined with liver, the taste of the soup can be enhanced.

The analysis of the FAA

As shown in Table I and Figure 4, there wasn't a great difference between SM and RM for the contents of umami amino acids (UAA), sweet amino acids (SAA) and total FAA, while BM decreased significantly, which may be related to the loss of FAA in the soup (Chi *et al*., 2012). The liver had higher contents of umami and bitter amino acids than that of meat. Compared to SL, FL generated more flavor substances after short-term high-temperature frying, this may be related to the reactions (Trevisan *et al*., 2016), the higher the temperature, the more severe the thermal oxidation and decomposition of fatty acids as well as the Maillard reaction. In contrast with the soup that was added with SL, the result of soup with FL showed that the contents of umami, sweet, bitter and the total FAA were significantly higher.

The analysis of the nucleotides

In Figure 6, inosinic monophosphate (IMP) is the main flavor nucleotide in meat, but is low in liver. The large quantity of IMP was also seen in the yellowtail muscle (Kubota *et al*., 2002). After steaming, the improvement of AMP, GMP, HxR, IMP and Adr in the meat, especially IMP increased significantly, while ADP and Hx decreased (Fig. 6A), it might have followed the degradation pathway of nucleotides: ADP degrades into AMP under myokinase, then under the action of AMP deaminase, AMP has two degradation pathway: (1) Reduced to IMP, which is predominant in the meat degradation, and a part of IMP will further decompose to HxR under the effect of phosphoesterase. (2) Reduced to AdR, and a part of Adr will further decompose to HxR, afterwards, Hx, which is infrequent.



Compared to RM, the decreased of ADP and IMP of BM had not changed, it may because that nucleotides were soluble and dissolved in liquid. The contents of HxR and Hx in liver significantly increased after short-term high temperature frying, other nucleotides also had some changes. This meant that the degradation process of AMP mainly go through the second pathway generating more HxR and Hx, while generating less IMP.

The content of IMP in FLS was significantly higher than that of SLS (Fig. 6C), which might be one of the important reasons why the soup with FLS was more delicious. When oil droplets dissolved into the water, the soup becomes milky white from clear. In addition, more dissolved substances further enhanced the taste. However, further studies should be made on whether the routine of nucleotides degradation changes

The analysis of the TMAO and betaine

TMAO is one of the main flavor ingredients, as well as the main fishy flavor component in aquatic products, its taste threshold is 1000 mg/g. Betaine as one of the main flavoring substances in aquatic products, mainly tasted sweet and also contributes to umami (Kani *et al*., 2008), its taste threshold is 2.5 mg/g.

After cooking, the content of TMAO (Table II) was reduced and it might have converted to trimethylamine. TMAO mainly existed in meat, while betaine mainly in liver, which the result was consistent with Kani *et al*. (2007) study on Loliginidae squids.

The content of betaine after cooking had decreased; it might be connected to the long-time heating treatment process (Chi *et al*., 2012). The content of betaine in FL was higher than that of SL, which was consistent with the sensory evaluation results that the sweet score of FL was higher than that of SL. Therefore, the use of FL as a soup ingredient can significantly increase the sweetness of the soup.

CONCLUSION

Through the sensory evaluation, it can be concluded that the taste of *Takifugu obscurus* mainly present umami, sweet and saline. The taste differences were distinguished on the PC graph of the electronic tongue between meats, liver and soup under the different preparations. IMP is the main flavor nucleotide of meat, but its content in liver was very low. The umami amino acids content in the liver was higher than that in meat. TMAO mainly exists in the meat. However, betaine mainly existed in the liver. After steaming, the content of IMP in the meat increased significantly, at that time, nucleotides mainly go through the first way to accumulate the IMP. Compared with SM, BM have more loss in taste materials. Therefore, steaming is a better cooking method for meat. The HxR and Hx content in liver significantly increased after short-time high-temperature frying, due to the degradation of nucleotides to HxR and Hx. When FL is added into soup and cooked together, the contents of umami, sweet amino acids, IMP and betaine significantly increases. Therefore, the addition of FL is more effective than SL in further enhancing the flavor of the soup.

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Statement of conflict of interest

There is no conflict of interest regarding publication of this manuscript.

REFERENCES

Ali, S., Neubauer, H., Melzer, F., Khan, I., Akhter, S., Jamil, T. and Umar, S., 2017. Molecular identification of bovine brucellosis causing organisms at selected private farms in Pothohar Plateau, Pakistan. *Pakistan J. Zool.,* **49**: 1111-1114. https://dx.doi.org/10.17582/journal.pjz/2017.49.3.sc2

Bystedt, J., Swenne, L. and Aas, H., 1959. Determination of trimethylamine oxide in fish muscle. *J. Sci. Fd. Agric.*, **10**: 301-304. https://doi.org/10.1002/jsfa.2740100601

Cheng, C.H., Luo, S.W., Ye, C.X., Wang, A.L. and Guo, Z.X., 2016. Identification, characterization and expression analysis of tumor suppressor protein p53 from pufferfish (*Takifugu obscurus*) after the Vibrio alginolyticus challenge. *J. Fish. Shellf. Immunol.*, **59**: 312-322. https://doi.org/10.1016/j.fsi.2016.10.040

Chi, A.Y., Ji, H.W., Gao, J.L., Lu, H.Y., Lan, W.B. and Meng, L.Y., 2012. Effects of heating methods on the taste components of *Litopenaeus vannamei*. *J. Mod. Fd. Sci. Technol.,* **28**: 776-779.

Dyer, W., 1945. Amines in fish muscle: I. Colorimetric determination of trimethylamine as the picrate salt. *J. Fish. Res. Bd. Can.*, **6**: 351-358. https://doi.org/10.1139/f42-042

Focht, R.L., Schmidt, F.H. and Dowling, B., 1956. Sugar beet processing, colorimetric determination of betaine in glutamate process end liquor. *J. Agric. Fd. Chem.*, **4**: 546-548. https://doi.org/10.1021/jf60064a007

Fuke, S. and Konosu, S., 1991. Taste-active components in some foods: A review of Japanese research. *Physiol. Behav.,* **49**: 863-868. https://doi.org/10.1016/0031-9384(91)90195-T

Hashimoto, Y. and Okaichi, T., 1957. On the determination of trimethylamine and trimethylamine oxide: A modification of the Dyers' method. *Nippon Suisan Gakkaishi*, **23**: 269-272. https://doi.org/10.2331/suisan.23.269

Jang, H.J., Son, H.H. and Lee, D.S., 2011. Optimization of disk sorptive extraction based on monolithic material for the determination of aroma compounds from *Lantana camara* L. by gas chromatography-mass spectrometry. *Bull. Korean Chem. Soc.*, **32**: 4275-4280. https://doi.org/10.5012/bkcs.2011.32.12.4275

Ji, Y., Liu, Y., Gong, Q.L., Zhou, L. and Wang, Z.P., 2011. Toxicity of cultured puffer fish and seasonal variations in China. *Aquacult. Res.*, **42**: 1186-1195. https://doi.org/10.1111/j.1365-2109.2010.02707.x

Kani, Y., Yoshikawa, N., Okada, S. and Abe, H., 2008. Taste-active components in the mantle muscle of the oval squid *Sepioteuthis lessoniana* and their effects on squid taste. *Fd. Res. Int.*, **41**: 371-379. https://doi.org/10.1016/j.foodres.2008.01.001

Kani, Y., Yoshikawa, N., Okada, S. and Abe, H., 2007. Comparison of extractive components in muscle and liver of three Loliginidae squids with those of one *Ommastrephidae* species. *Fish. Sci.*, **73**: 940-949. https://doi.org/10.1111/j.1444-2906.2007.01417.x

Konosu, S., 1974. Distribution of nitrogenous constituents in the muscle extracts of eight species of fish. *Nippon Suisan Gakkaishi,* **40**: 909-914. https://doi.org/10.2331/suisan.40.909

Kubota, S., Itoh, K., Niizeki, N., Song, X.A., Okimoto, K., Ando, M., Murata, M. and Sakaguchi, M., 2002. Organic taste-active components in the hot-water extract of yellowtail muscle. *Fd. Sci. Technol. Res.,* **8**: 45-49. https://doi.org/10.3136/fstr.8.45

Li, J.L., Tu, Z.C., Zhang, L., Lin, D.R., Sha, X.M., Zeng, K., Wang, H., Pang, J.J. and Tang, P.P., 2016. Characterization of volatile compounds in grass carp (*Ctenopharyngodon idellus*) soup cooked using a traditional Chinese method by GC-MS. *J. Fd. Process. Preserv.,* **41**: e12995. https://doi.org/10.1111/jfpp.12995

Liu, J.K., Zhao, S.M., Xiong, S.B. and Zhang, S.H., 2009. Influence of recooking on volatile and non-volatile compounds found in silver carp Hypophthalmichthys molitrix. *Fish. Sci.*, **75**: 1067-1075. https://doi.org/10.1007/s12562-009-0116-y

Mnari, A., Bouhlel, I., Chraief, I., Hammami, M., Romdhane, M., El Cafsi, M. and Chaouch, A., 2007. Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. *Fd. Chem.*, **100**: 1393-1397. https://doi.org/10.1016/j.foodchem.2005.11.030

Mottram, D.S., 2008. Flavour formation in meat products: A review. *Fd. Chem.*, **62**: 415-424. https://

doi.org/10.1016/S0308-8146(98)00076-4

Noguchi, T., Arakawa, O. and Takatani, T., 2006. Toxicity of pufferfish *Takifugu rubripes* cultured in netcages at sea or aquaria on land. *Comp. Biochem. Physiol. Part D: Genom. Proteom.*, **1**: 153-157. https://doi.org/10.1016/j.cbd.2005.11.003

Rhyu, M.R. and Kim, E.Y., 2011. Umami taste characteristics of water extract of Doenjang, a Korean soybean paste: Low-molecular acidic peptides may be a possible clue to the taste. *Fd. Chem.*, **127**: 1210-1215. https://doi.org/10.1016/j.foodchem.2011.01.128

Schiffman, S.S., Sattely–Miller, E.A., Graham, B.G., Bennett, J.L., Booth, B.J., Desai, N. and Bishay, I., 2000. Effect of temperature, pH, and ions on sweet taste. *Physiol. Behav.*, **68**: 469-481. https://doi.org/10.1016/S0031-9384(99)00205-X

Tao, N.P., Wang, L.Y., Gong, X. and Liu, Y., 2012. Comparison of nutritional composition of farmed pufferfish muscles among *Fugu obscurus*, *Fugu flavidus* and *Fugu rubripes*. *J. Fd. Comp. Anal.*, **28**: 40-45. https://doi.org/10.1016/j.jfca.2012.06.004

Tao, N.P., Wu, R., Zhou, P.G., Gu, S.Q. and Wu, W., 2014. Characterization of odor-active compounds in cooked meat of farmed obscure puffer (*Takifugu obscurus*) using gas chromatography–mass spectrometry–olfactometry. *J. Fd. Drug Anal.*, **22**: 431-438. https://doi.org/10.1016/j.jfda.2014.02.005

Trevisan, A.J., De, A.L.D., Sampaio, G.R., Soares, R.A. and Markowicz-Bastos, D.H., 2016. Influence of home cooking conditions on Maillard reaction products in beef. *Fd. Chem.*, **196**: 161-169. https://doi.org/10.1016/j.foodchem.2015.09.008

Wekell, J.C. and Barnett, H., 1991. New method for analysis of trimethylamine oxide using ferrous sulfate and EDTA. *J. Fd. Sci.*, **56**: 132-135. https://doi.org/10.1111/j.1365-2621.1991.tb07993.x

Yamagata, M., Horimoto, K. and Nagaoka, C., 1968. On the distribution of trimethylamine oxide in the muscles of yellow fin tuna. *Nippon Suisan Gakkaishi*, **34**: 344-350. https://doi.org/10.2331/suisan.34.344

Yamaguchi, S., Yoshikawa, T., Ikeda, S. and Ninomiya, T., 1970. Studies on the taste of some sweet substances: Part I. Measurement of the relative sweetness, Part II. Inter-relationships among them. *Agric. Biol. Chem.*, **34**: 181-197. https://doi.org/10.1271/bbb1961.34.181