

Article

Taste Attributes of the “June Hairy Crab” Juveniles of Chinese Mitten Crab (*Eriocheir sinensis*) in Yangcheng Lake, China—A Pilot Study

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Abstract: This is the first report on the use of a taste sensing system to quantitatively evaluate the taste attributes of two groups of native “June hairy crab” juveniles (commonly referred to as “Liu-Yue-Huang”) of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853) from a net enclosure culture area in Yangcheng Lake (lake culture) and aquaculture ponds near the lake (pond culture). We showed that umami was the predominant basic taste of steamed June hairy crabs, followed by bitterness and astringency. The intensity value of saltiness was aberrant and could not be determined using this system. The average values of aftertaste-U reached 8.7 and 10.7 in the male June hairy crabs from the lake and pond cultures, respectively, which was significantly higher than their respective aftertaste-B and aftertaste-A values ($p < 0.01$). Female crabs did not have aftertaste-B, while their aftertaste-U was significantly higher than aftertaste-A ($p < 0.01$). Although principal component analysis and linear discriminant analysis were not able to completely distinguish among crabs from different cultures, they could robustly distinguish between male and female crabs.

Keywords: aquatic product; “Da-Zha-Xie”; “Rong-Ao-Xie”; Decapoda; Crustacea; consumer preference; food chemistry; taste sensing system; E-Tongue; flavor analysis



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1. Introduction

The Chinese mitten crab, also known as the Chinese hairy crab (*Eriocheir sinensis* H. Milne Edwards, 1853) is a traditional Chinese delicacy with high commercial values [1,2]. Consumers appreciate those from Yangcheng Lake as the tastiest hairy crabs in China, increasing their commercial value compared with crabs produced in other regions as a National Product of Geographical Indication for “Yangcheng Lake hairy crab” [3]. Generally, the crab is a typical famous autumn dish because the market-sized crabs with sexual maturity can only be obtained around October, when the crabs finish their growth through the 13–16th molts [2]. Therefore, crabs are mostly consumed in October–November; other months are usually off-season. With high demand for this delicacy by consumers and high profits by crab farmers also in off-season, a new crab product of *E. sinensis* juveniles, i.e., the June hairy crab (commonly referred to as “Liu-Yue-Huang”) has entered the consumer market. The June hairy crab is an immature, not fully grown crab, and it is usually collected and sold around the lunar June [4]. These June hairy crabs are highly favored owing to their particularly high proportion of hepatopancreas, an especially prized edible part because of its high lipid content [5,6]. To satisfy the consumer demand for hairy crabs in mid-summer and improve the economic benefits [7], the farmers in Yangcheng Lake have been selling June hairy crabs to consumer markets since 2016.

However, as a relatively new food product, the taste profiles of the June hairy crab have not been well studied to date. To fill the gap, using an INSENT Taste Sensing System (Intelligent Sensor Technology (INSENT), Inc., Atsugi, Kanagawa, Japan), the two primary aims of this study were (i) to explore the taste profiles of the hepatopancreas tissues of the June hairy crab from lake and pond culture areas of the Yangcheng Lake; and (ii) to compare the flavor attributes of the hepatopancreas tissues between male and female June hairy crabs.

In addition, although the aforementioned taste sensing system developed in Japan has been widely used for quantifying the taste of food or the bitterness of medicines [8–11], each sensor electrode with its lipid membrane must still address the perception of taste receptors when testing high-lipid foods. If the lipid part of a sample is completely removed, the important fat-soluble components, which convey mouthfeel, will not be detected by the electronic tongue system [12]. Thus, the purpose of this study also included testing the sensor response to the high-fat food of the crab hepatopancreas, given that the total lipid content in the hepatopancreas of *E. sinensis* has been reported to be as high as 31.77% [13].

2. Materials and Methods

2.1. Crab Materials

June hairy crabs were sampled in July 2021. “Lake crabs” had body weights ranging from 64 to 129 g (wet weight basis, similarly hereafter) and were collected from a large net enclosure culture area in Yangcheng Lake of Suzhou City in Jiangsu Province. “Pond crabs” were collected from an aquaculture pond near Yangcheng Lake and had body weights ranging from 61.2 to 139.3 g.

2.2. Sample Pretreatment

Batch taste traits were conducted for the two types of crabs. Crabs were first washed with tap water to remove adherent impurities. After listing the samples, they were placed in a stainless-steel container and steamed for 30 min at 100 °C. Each crab carapace was opened, and the hepatopancreas were scooped out and weighed. Deionized water was added to a flask and heated in a 40 °C water bath. The warm deionized water was added to the cooked hepatopancreas at a hepatopancreas:water ratio of 1:10, and the organ was homogenized with a Nutribullet (JYL-Y912, Joyoung Company Limited, Qingdao, China) for 2 min. The homogenate was then transferred into 250 mL bottles and centrifuged at 3600 rpm for 15 min at room temperature around 25 °C. After the supernatant was filtered using Whatman qualitative filter paper (Merck KGaA, Darmstadt, Germany), 80 mL of each solution was divided into two aliquots and placed in two containers for parallel testing.

2.3. Taste Sensing System Testing

Taste evaluation measurements were conducted using an E-Tongue of the SA402B taste sensing system (Intelligent Sensor Technology (INSENT), Inc., Atsugi, Kanagawa, Japan) according to the procedure described previously [14]. In addition to the solutions described previously, an electrode maintenance solution (3.33 mol/L KCl in distilled water) and inner solution (3.33 mol/L KCl + 10 mg AgCl in water) were prepared. The reference solution was used to stabilize, preserve, and wash AAE (umami), CT0 (saltiness), C00 (anionic bitterness), CA0 (acidity), and AE1 (astringency) sensors. Preconditioning was performed by charging 0.2 mL of the inner solution into the taste sensors and reference electrodes, immersing the taste sensor surface in a reference solution, and placing the reference electrodes into the saturated KCl solution for at least 24 h. In addition, we performed the “Sensor Check” before the first measurement of crab roe or crab paste samples. The maintenance experiment was required to check whether the probe or sensor required cleaning or replacement before testing subsequent sample batches. Monosodium glutamate (MSG), quinine, and tannic acid were used as standard substances for umami, bitter, and astringent tastes, respectively [15]. To investigate how the taste sensor responds to the water-soluble components of crab fat and to detect sensors with abnormal output

values as early as possible, we also investigated different ways of testing crab meat; those included approaches such as increasing the frequency of the maintenance experiment and adding 40 °C deionized water instead of room-temperature water. Judging from the maintenance reports, we found that the function of the taste receptors could be effectively detected by the maintenance operation. The output value of the reference solution was set to the control value at the start of the “Taste analysis application”. We used the same tasteless definition described previously [14]. A radar chart was used to describe the taste traits of the June hairy crab.

2.4. Data Analysis

Mann–Whitney *U* test analysis was performed using SPSS 27.0 (IBM Inc., Armonk, NY, USA) to test the differences in taste intensity indicators between male and female crabs. Principal component analysis (PCA) and linear discriminant analysis (LDA) were performed using R 3.6.0 (R Foundation for Statistical Computing, <http://www.r-project.org>, accessed on 27 April 2022). Prior to PCA or LDA, all taste attributes were converted to Z-scores to standardize and normalize the taste scores.

3. Results

3.1. Taste-Active Values of June Hairy Crabs

First, we analyzed the output of each sensor probe. Sourness was regarded as tasteless because its value was far lower than “−13”. Similarly, the saltiness values of some samples were higher than 30, which exceeded the normal saltiness detection range of the instrument. The saltiness intensities of the samples were regarded as unreliable, as discussed in the Discussion Section. Therefore, the intensities of umami, bitterness, and astringency and their respective aftertastes were considered the six effective taste indicators for evaluating June hairy crabs (Table 1). Aftertaste of umami (aftertaste-U) had the highest intensity among the three aftertastes. Female crabs did not have an aftertaste of bitterness (aftertaste-B) and had only a low level of aftertaste of astringency (aftertaste-A); however, all male crabs exhibited some aftertaste-A and a low level of aftertaste-B (Table 1, Figure 1). From the three basic tastes, umami generally presented the highest intensity (14.7–16.6), followed by bitterness (9.9–12.7), and astringency (4.2–6.3) (Table 1, Figure 1). In addition, we were able to robustly distinguish between male and female crabs utilizing the taste-active values of all basic tastes and their aftertastes, while there was no significant difference in the tastes of the lake- and pond-cultured crabs (Table 2).

3.2. Multivariate Statistical Analysis of the Taste Profiles

The factor load matrix in Table 3 determined that the first two principal components accounted for 84.81% and 9.37% (total 94.2%) of the cumulative variance contribution rate, respectively. Every taste coefficient (umami, bitterness, astringency, and their aftertastes) showed high component loading in PCA dimension 1. Umami and aftertaste-U had high component loadings in PCA dimension 2. Thus, the first component visibly separated female crabs from male crabs in the PCA score plot (Figure 2). Umami had the largest influence on PC2, which means this taste characteristic mainly depicted the outcomes for distinguishing the lake-cultured group from the pond-cultured group.

Table 1. Taste-active values of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crab of *Eriocheir sinensis* from the net enclosure culture area in Yangcheng Lake and aquaculture ponds by the lake.

Sampling Site	Sample Group Code	Sample Code	Umami	Aftertaste -U	Bitterness	Aftertaste -B	Astringency	Aftertaste-A
Yangcheng Lake	Lake—Female	Lake—Female 1	15.5	5.6	10.3	0.0	4.6	2.8
		Lake—Female 2	15.1	4.3	10.4	0.0	4.8	2.6
		Lake—Female 3	16.2	5.2	10.8	0.0	5.3	2.9
		Lake—Female 4	15.5	4.4	10.5	0.0	5.1	2.5
		(Mean ± SD)	15.6 ± 0.4	4.9 ± 0.6	10.5 ± 0.2	0.0 ± 0.0	4.9 ± 0.2	2.7 ± 0.2
	Lake—Male	Lake—Male 1	15.9	9.8	12.4	5.3	6.1	4.6
		Lake—Male 2	15.4	9.6	12.0	4.6	5.9	4.4
		Lake—Male 3	16.6	8.2	11.8	7.3	5.3	4.9
		Lake—Male 4	15.7	7.5	12.0	4.2	6.1	4.1
		Lake—Male 5	15.4	8.4	12.3	5.0	6.0	4.4
		(Mean ± SD)	15.9 ± 0.5	8.7 ± 0.9	12.1 ± 0.2	5.3 ± 1.1	5.9 ± 0.3	4.5 ± 0.3
Pond by Yangcheng Lake	Pond—Female	Pond—Female 1	15.1	6.4	10.3	0.0	4.6	2.7
		Pond—Female 2	15.7	5.9	10.3	0.0	4.5	2.8
		Pond—Female 3	14.7	5.9	10.0	0.0	4.5	2.4
		Pond—Female 4	14.7	5.6	9.9	0.0	4.5	2.3
		Pond—Female 5	14.7	4.7	9.9	0.0	4.2	2.1
		(Mean ± SD)	15.0 ± 0.4	5.7 ± 0.5	10.1 ± 0.2	0.0 ± 0.0	4.5 ± 0.1	2.4 ± 0.2
	Pond—Male	Pond—Male 1	15.8	8.3	12.0	3.9	6.0	4.1
		Pond—Male 2	16.2	12.2	12.5	4.9	6.1	4.7
		Pond—Male 3	15.8	10.3	12.6	4.6	6.1	4.4
		Pond—Male 4	16.2	10.6	12.7	4.4	6.3	4.5
		Pond—Male 5	15.5	12.2	12.5	4.0	6.3	4.2
		(Mean ± SD)	15.9 ± 0.3	10.7 ± 1.5	12.5 ± 0.2	4.4 ± 0.4	6.2 ± 0.1	4.4 ± 0.2

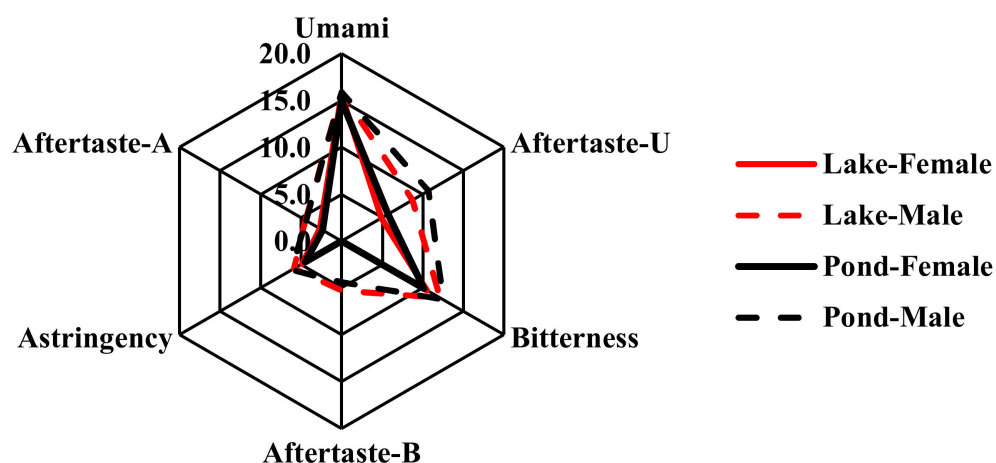


Figure 1. Radar chart of the mean taste values of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crabs (*Eriocheir sinensis*) from the net enclosure culture area in Yangcheng Lake and aquaculture ponds by the lake.

Table 2. Matrix for testing the significance of taste-active values among umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crabs (Mann–Whitney *U* test).

		Umami	Aftertaste-U	Bitterness	Aftertaste-B	Astringency	Aftertaste-A
Lake crab	Umami	-	**	**	**	**	**
	Aftertaste-U	*	-	**	**	**	**
	Bitterness	*	*	-	**	**	**
	Aftertaste-B	*	*	*	-	N.S.	N.S.
	Astringency	*	N.S.	*	*	-	**
	Aftertaste-A	*	*	*	*	*	-
Pond crab	Umami	-	**	**	**	**	**
	Aftertaste-U	**	-	*	**	**	**
	Bitterness	**	**	-	**	**	**
	Aftertaste-B	**	**	**	-	**	N.S.
	Astringency	**	**	**	**	-	**
	Aftertaste-A	**	**	**	**	**	-
Lake—Female vs. Pond—Male		N.S.	*	*	*	*	*
Lake—Female vs. Pond—Female		N.S.	N.S.	*	N.S.	*	N.S.
Lake—Female vs. Pond—Male		N.S.	*	*	*	*	*
Lake—Male vs. Pond—Female		N.S.	**	**	**	**	**
Lake—Male vs. Pond—Male		N.S.	*	*	N.S.	N.S.	N.S.
Pond—Female vs. Pond—Male		*	**	**	**	**	**
Total females vs. total males		*	**	**	**	**	**
Total lake crabs vs. Total pond crabs		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Note: The results of males or females from only the lake or pond crabs are marked in red and blue colors, respectively; * $p < 0.05$; ** $p < 0.01$; N.S., Not significant.

Table 3. Loading matrix, eigenvector, and cumulative contribution of the principal components of the taste values of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crabs.

	PC1		PC2	
	Loading	Eigenvector	Loading	Eigenvector
Umami	0.728	0.323	0.678	0.904
Aftertaste-U	0.907	0.402	−0.269	−0.359
Bitterness	0.986	0.437	−0.097	−0.129
Aftertaste-B	0.954	0.423	−0.064	0.085
Astringency	0.943	0.418	−0.127	−0.17
Aftertaste-A	0.983	0.436	0.027	0.036
Cumulative contribution (%)	84.81%		9.37%	

After LDA, three linear discriminant functions were extracted based on the taste intensity of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the June hairy crabs. The first (LD1) function explained 97% of the discriminative power, whereas the second (LD2) and the third (LD3) linear discriminants accounted for 1.9% and 1.1% of the variance, respectively. LD1 mainly separated the taste intensity of female June hairy crabs from those of the male crabs, while LD2 showed a slight separation between lake-cultured crabs and pond-cultured crabs. The accuracies of preliminary classification achieved 100% among female and male crabs (Figure 3). Cross-validation results presented accuracies between 80% and 100%.

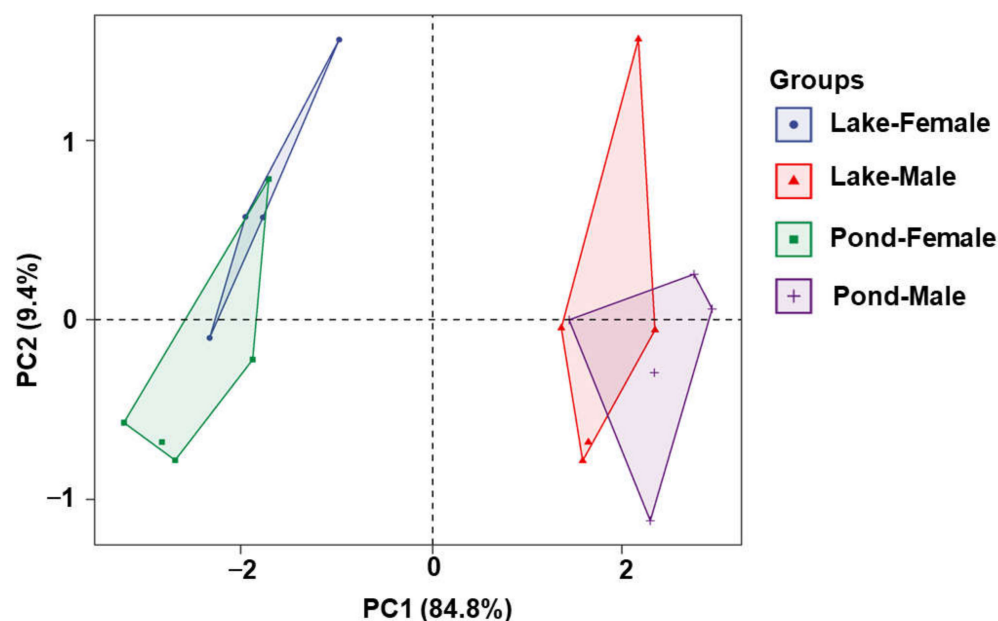


Figure 2. PCA of the taste values of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crabs.

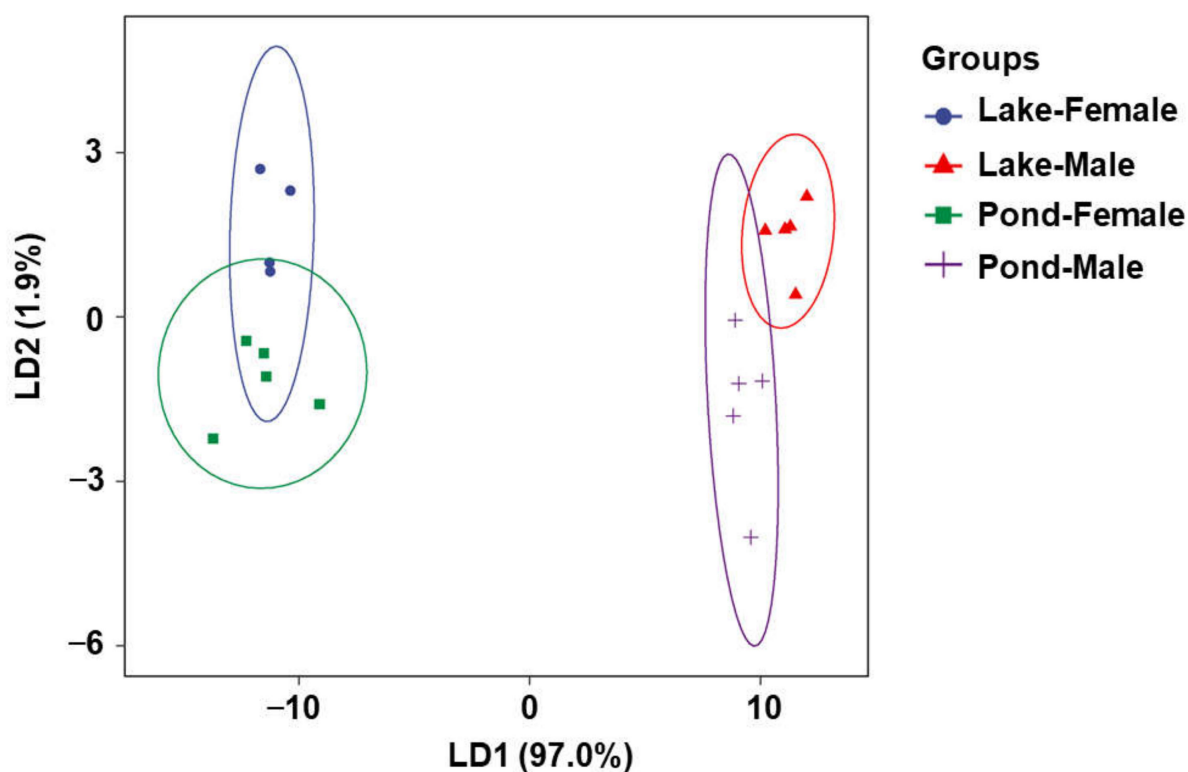


Figure 3. Discriminant function analysis of the taste values of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the hepatopancreas of June hairy crabs.

4. Discussion

4.1. Characteristics of Taste Profiles

This study sampled male and female June hairy crabs from a net enclosure culture in a lake or an aquaculture pond for the first time. Umami, one of the most basic tastes [16], was the most prominent taste of June hairy crabs (Figure 1). Generally, in the edible part of crabs, glutamic acid has the highest content among the flavored amino acids [7,17]. Wang et al. [4]

reported that the glutamic acid contents of the male and female June hairy crabs were 1.35 g/100 g dry weight and 1.25 g/100 g dry weight, respectively. Interestingly, free amino acid composition and flavored nucleotides, such as 5'-guanylate and 5'-adenylate, are similar between June hairy crabs and adult crabs [7]. Sasaki et al. [18] have reported that flavored nucleotides and free amino acids have a synergistic freshness effect. Therefore, it is understandable that the umami sensor shows the highest perceived intensity for the umami taste in the June hairy crabs of the present study.

The perceived intensity value of the salt taste in this experiment seemed to be unreliable. The measurement results of the sensing system SA402B presented two output types: a relative value (umami, bitterness, astringency, and saltiness) and changes in the membrane potential caused by adsorption (CPA) or aftertaste. The CPA value corresponded to the aftertaste, which represents a change in the membrane potential of the reference solution caused by adsorption before and after the sample measurement [8,19]. A sample solution with a pH greater than 8 will negatively impact the measurement output. However, even if the pH of a sample solution is less than 8, as long as it contains a preponderance of alkaline amino acids, the relative value will still be aberrant. The June hairy crab supernatant had a pH of 6.5, which was weakly acidic. However, previous literature indicated that it contained a high concentration of the alkaline amino acid arginine [4]. Therefore, the positive charge this amino acid carries might have a negative impact on the output of the saltiness sensor CT0. The response of the saltiness sensor CT0 to arginine made the relative value greater than the actual saltiness value.

June hairy crabs also exhibited some bitterness (9.8–12.7) and low astringency (4.2–6.5) intensity. The threshold of quinine compounds is 0.008 mM [20], and its taste value is approximately 10 in response to 0.01 mM quinine hydrochloride [21]. This means that, if the output of the bitter taste sensor is below 10, the sample cannot be perceived as bitter by humans [19]. In our experiment, the bitterness values obtained ranged between 9.8 and 12.7, which are considered low bitterness. Low bitterness is a critical component of building the intensity and complexity of flavor [12,22]. Ensuring that a small amount of bitterness and other tastes are present in food flavors is a major trend in the food industry [19,23]. Moreover, the most abundant amino acid in the edible part of the crab is L-arginine (L-ARG) [4,7,17], which can be perceived as both bitter and sweet [17]. Meanwhile, the taste sensor output of the 0.05% tannic acid reference was approximately 11 [21], but the maximum output indicated by the astringency sensor was 6.5 in our study. We speculate that the detection of astringency also indicated the richness of the hepatopancreas in June hairy crabs rather than its astringency. In addition, June hairy crabs are undergoing their last molting; the astringency taste may be related to their immature stage, as no astringency is measurable in commercial crab meat [14,24].

The multichannel lipid membrane device can also sense three kinds of aftertastes, aftertaste-U, aftertaste-B, and aftertaste-A. An aftertaste is a taste persisting in the mouth after the substance that caused it is no longer present. The average values of aftertaste-U in male June hairy crabs were significantly higher than the values of aftertaste-B and aftertaste-A (Table 2). Female crabs did not have aftertaste-B, and their aftertaste-U was significantly higher than that of aftertaste-A (Table 2). Having a high aftertaste-U is widely acclaimed in the food industry. It is considered even more desirable than umami [12] because it is an important sign of taste richness and complexity. For example, glutathione is barely detected as an umami taste, but its aftertaste-U is high, and glutathione has been identified as a general taste enhancer that enhances umami [25]. Therefore, the outstanding aftertaste-U might be the key taste attribute to the deliciousness of the June hairy crabs.

4.2. Comparison of the Differences in the Taste of Male and Female June Hairy Crabs

PCA and LDA have been successfully applied to assess and visualize food flavor qualities and taste properties [26–29]. The main taste intensity indicators, such as bitterness, aftertaste-U, aftertaste-B, and aftertaste-A, had highly significant differences in male and female samples (Table 2). However, the effect of different production methods (i.e., lake

culture and pond culture) could not be robustly determined using our approach, i.e., no significant differences were observed between the males or females from lake and pond cultures. Male and female samples were well distinguished regarding PC1, covering 84.8% of variance. However, there was some overlap in male and female crabs on PC2, accounting only a minor portion (9.37%) of variance (Figure 2). Additionally, considering LDA, we verified that LD1 explained 97.0%, while LD2 and LD3 discriminant functions explained the remaining 3%. The analyses of umami, aftertaste-U, bitterness, aftertaste-B, astringency, and aftertaste-A in the steam-cooked crab hepatopancreas revealed comprehensive discriminant accuracies of 100% between male and female *E. sinensis* crabs. From the cross-validated rates, data discriminant accuracy was as high as 94.74% (Figure 3). Although almost no previous studies are available for comparison, Wang et al. [4] showed that the fatty acid content and the total content of free amino acids in male June hairy crabs were significantly higher than those in female crabs, with the umami amino acid contents of male crabs being twice as high as those of female crabs. The authors also revealed that methionine varied significantly by sex. The high content of free glutamic acid and aspartic acid in the hepatopancreas of male crabs made their MSG content significantly higher than that of female crabs [4]. Therefore, consumers would expect the hepatopancreas of male June hairy crabs to be more delicious than those of female crabs.

5. Conclusions

An SA402B Taste Sensing System equipped with five liquid membrane sensors and two corresponding reference electrodes was used, for the first time, to quantitatively evaluate the taste characteristics of off-season-marketed June hairy crabs. The taste properties of the steam-cooked hepatopancreas of the crabs were accurately depicted, with high values for umami and aftertaste-U. Female June hairy crabs had no aftertaste-B, whereas male June hairy crabs had prominent aftertaste-U and miscellaneous bitterness values. PCA and LDA were able to robustly distinguish between male and female crabs.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Animal Care and Use Committee of the Freshwater Fisheries Research Center at the Chinese Academy of Fishery Sciences. The analysis was carried out following the Guidelines for the Care and Use of Laboratory Animals set by the Animal Care and Use Committee of the Freshwater Fisheries Research Center (2003WXEP61). All operations were carried out under field permit no. 20181AC1128.

Data Availability Statement: Data that support the findings of this study are available from the corresponding author upon reasonable request (Y.J.: jiany@ffrc.cn).

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Conflicts of Interest: The authors declare no conflict of interest.

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