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The comparison and analysis of nutritional qualities of Chinese mitten crabs (*Eriocheir sinensis*) in rice field and pond culture modes

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ABSTRACT

The nutritional quality of three edible parts (gonads, hepatopancreas and muscles) of Chinese mitten crabs (CMCs) from rice field culture and pond culture was firstly compared in our current study. It was found that the contents of mineral elements and volatile compounds in rice CMCs were superior to those in pond CMCs, and the antioxidant enzyme activities of rice CMCs were markedly higher than those of pond CMCs. Besides, the total free amino acid levels in the edible parts of pond CMCs were higher than those of rice CMCs. Compared with other tissues, the nucleotide and equivalent umami concentrations of the gonads in female rice CMCs were the maximum. Overall, both types of crabs demonstrated good nutritional quality, which met human nutrition and dietary needs. In comparison, the quality of rice CMCs was better than that of pond CMCs.

1. Introduction

The Chinese mitten crab (CMC; *Eriocheir sinensis*) is a type of important aquatic animal, which is widely distributed in China. CMCs are famous for their delicious taste, pleasant aroma and rich nutrition, and thus have high economic benefits (Chen & Zhang, 2007; Chen, Zhang, et al., 2007; Guo, et al., 2014; Lu, Cheng, et al., 2023). Nowadays, the breeding of CMCs has become one of the pillar industries in Chinese aquatic areas. According to the report of China Academy of Commerce Industry, the market scale of CMCs in 2020 has reached 100 billion RMB (Bao, et al., 2022).

Common breeding modes for CMCs are lake stocking, lake fencing, pond farming and rice farming, of which pond farming and rice farming are the main patterns in the northern regions of China. The pond farming is a kind of intensive and high-density pattern, which is characterized by high yield (Bao, et al., 2022). The symbiotic relationship between rice and crabs can achieve the purpose of both rice growth and CMCs breeding. It has the advantage of providing a suitable habitat for crabs without reducing production, and thus, the double harvest of rice and crabs can be obtained. In addition, pests and weeds can be destroyed by crabs, which are harmful to rice growth, so as to reduce the demand for chemical fertilizers and pesticides, thus benefiting both crab and rice industries (Bao, et al., 2022). Studies have indicated that the nutrients of CMCs with various breeding modes are varied (Kong, et al., 2012). However, only a few studies have compared rice farmed crabs with pond farmed crabs, which is deserved to be explored.

CMCs mainly contain three edible tissues: hepatopancreas, gonads and muscles (Wu, et al., 2007). The nutritional quality of CMCs is usually analyzed by the compositions of conventional nutrients, such as protein, minerals, free amino acids (FAA) and nucleotides in these edible tissues. Besides, the antioxidant activity of crabs could also reflect the level of their body wellness (Wu, et al., 2007). This study aims to compare the nutritional quality of CMCs cultured in rice and pond modes, in order to better develop the resources of CMCs, provide useful nutritional information for crab consumers, and establish basic reference for the industry standards of CMCs.

2. Materials and methods

2.1. Specimen collection

This study was conducted in Panjin Photosynthetic Crab Industry Co., Ltd., Liaoning Province, from June 28, 2021 to September 28, 2021. The experiment was performed in a rice crab farm, which was divided into 6 fields: 3 for rice fields and 3 for pond fields. Healthy and similar-sized crabs were put into each field, and the density was 0.45 inds/m^2 in

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the rice field group and 0.75 inds/m^2 in the pond field group. The feed was bought from the Wellhope Foods Co., Ltd. The crabs were fed once a day, according to 3–5 % of their body weight.

2.2. Sample preparation

Healthy CMCs were randomly chosen from each field, then their wet weight was measured using an electronic scale and accurate to 0.01 g. The weighed CMCs were put on ice to render them unconscious. The gonads and hepatopancreas were removed with forceps. The muscles were chilled at −20 °C for two hours and then carefully dissected. Each sample was homogenised and stored at −80 °C for further analysis. The hepatopancreas index (HSI), gonadosomatic index (GSI), conditional factor (CF), muscle production (MY) and total edible amount (TEY) of CMCs were determined using the equations (1)-(5), respectively.

$$HSI\,(\%) = 100 \times \frac{\text{hepatopancreas wet weight}}{\text{body wet weight}} \quad (1)$$

$$GSI\,(\%) = 100 \times \frac{\text{gonads wet weight}}{\text{body wet weight}} \quad (2)$$

$$CF(\%) = 100 \times \frac{\text{body wet weight}}{\text{carapace length}^3} \quad (3)$$

$$MY\,(\%) = 100 \times \frac{\text{muscle wet weight}}{\text{body wet weight}} \quad (4)$$

$$TEY\,(\%) = MY + HSI + GSI \quad (5)$$

2.3. Proximate compositions analysis

Total ash, total lipid, crude protein and water content of each sample were measured in accordance with the Chinese national standards GB 5009.4-2016, GB/T 14772-2016, GB 5009.5-2016 and GB 5009.3-2016, respectively (Wu, et al., 2018).

2.4. Mineral analysis, heavy metal and health risk assessment

Mineral elements were measured according to Wu's report with slight modification (Wu, et al., 2020). Dried powder (2.00 ± 0.01 g) was obtained from the crab tissues, and the sediments were digested with 5 mL nitric acid (65 %) in microwave dissolver (MARS6, CEM, America) under a temperature gradient of 120–180 °C for 45 min. The digested specimens were cooled to room temperature and then diluted with deionized water. The concentrations of all elements were measured using an inductively coupled plasma mass spectrometer (Agilent 7500 series, USA).

The health risk assessment was determined according to the following parameters.

The estimated daily intake (EDI) of heavy metals was calculated by the following equation:

$$EDI = C_i \times \frac{IR}{BW} \quad (6)$$

where C_i is the heavy metal concentration of the tissue (mg kg^{-1}, dry weight). Depending on Maurya's report, the conversion factor 4.8 is used to convert wet weight to dry weight (Maurya et al., 2019); IR represents the ingestion rate, and the daily per capita intake of CMC is 0.0778 kg person^{-1}day^{-1}; BW is the body weight with adult 70 kg and children 20 kg (Xiong, et al., 2020).

The target hazard quotient (THQ) was a health risk assessment method established by the US Environmental Protection Agency (USEPA), which was used to determine the level of non-carcinogenic risk and calculated by the following formula:

$$THQ = \frac{ED \times EF \times EDI}{RfD \times AT} \times 10^{-3} \quad (7)$$

where ED,EF and EDI represent the exposure duration (70 years), exposure frequency (365 days/year) and estimated daily intake, respectively; RfD is the safe oral dose of the heavy metals (As, 0.3 μg kg^{-1} day^{-1}; Cr, 1500 μg kg^{-1} day^{-1}; Cd, 1 μg kg^{-1} day^{-1}; Pb, 4 μg kg^{-1} day^{-1}) and AT means the average exposure time, which is that 365 days per year multiply by the number of years of exposure (Xiong, et al., 2020).

The lifetime cancer health risk (CHR) for heavy metal exposure was calculated by the following formula:

$$CHR = \frac{EF \times ED \times IR \times CSF}{BW \times AT} \times 10^{-3} \quad (8)$$

where CSF represents the cancer slope factor (inorganic As, 1.5 mg kg^{-1} d^{-1}) (USEPA, 2016); The other parameters are the same as the formulas above.

2.5. Determination of antioxidant enzyme activities

The activities of total antioxidant capacity (*T*-AOC), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GSH-Px) were measured using appropriate detection kits (Nanjing Jiancheng Technology Co., Ltd.). The samples were diluted with normal saline (1:9), homogenized 10 times and centrifuged (3000 rpm, 15 min, 4 °C). The enzyme activities in the supernatant were measured according to the kit's instructions.

2.6. Determination of collagen content

The collagen content was estimated according to hydroxyproline (Hyp), accounting for 13.4 % of collagen proteins. The experiment was processed according to the instructions of the kit (Nanjing Jiancheng Technology Co., Ltd.). Briefly, the sample was added with 1 mL of 6 mol/L HCL and water bath at 95 °C for 5 h. After cooling, the indicator was added and the pH was adjusted to 6–6.8. Subsequently, an appropriate amount of activated carbon was added, mixed, and centrifuged at 3500 r/min for 10 min, and the supernatant was taken. After adding residual drugs, the Hyp concentration was detected at 550 nm by the microplate reader (Luo, et al., 2021).

2.7. Electronic tongue and electronic nose

As for the detection of E-tongue (SA402B, INSENT, Japan), the chemical response of the sensor was converted into electrical signal through the following methods, when the system was immersed in flavor solution. Then, 5.00 ± 0.01 g sample was homogenized with 80 mL ultrapure water. The filtered transparent liquid was immediately detected by the E-tongue. Each sample was repeated for 4 times (Wang, et al., 2016).

The odor characteristics of crabs were analyzed by E-nose (PEN3, AIRSENSE, Germany). The sample (2.00 ± 0.01 g) was placed into a 10 mL brown headspace vial, and then transferred onto a tray at 4 °C. To ensure the uniform dispersion of the gas in the headspace bottle, the samples were heated in a water bath for 30 min and maintained for 10 min before injection. The injection volume was 2500 μL. The injection was completed within 1 s. The filtered dry air (purity > 99.999 %, flow rate = 150 mL/min) was employed as the carrier gas. The data acquisition cycle was 120 s, and the system rebalance needed another 600 s. E-nose testing was repeated for 8 times under the same conditions (Gu, et al., 2013).

2.8. Free amino acid (FAA) analysis

The measurement method was slightly modified according to Hua's study (Hua, et al., 2020). Each sample (2.00 ± 0.01 g) was added to 5 % trichloroacetic acid (W/V) solution in a centrifuge tube, homogenized for 2 min, fixed to 25 mL, sonicated for 20 min at room temperature, and left for 2 h. Subsequently, the filtrate obtained by filter paper filtration was subjected to centrifugation (10000 rpm, 30 min). Each sample was mixed with 20 μL of derivatization reagent AQC and heated at the constant temperature of 55 °C for 10 min. FAAs were analyzed by chromatography using the Agilent 1290 HPLC system (Agilent, USA). AccQ-TAG-C18 column (100 × 2.1 mm, 1.7 μm) was equipped and maintained at 55 °C.

2.9. Nucleotide analysis

The measurement of nucleotide was slightly modified according to Chen's study (Chen & Zhang, 2007). The sample (2.00 ± 0.01 g) was added to 10 % perchloric acid solution (20 mL) and vortexed for 1 min, followed by centrifugation (8000 r/min, 10 min). After removing the supernatant, 10 mL of 5 % perchloric acid solution was added and extracted again, and the supernatant was combined. The pH was adjusted to nearly 6.0 with 10 mol/L NaOH solution, and further adjusted to 6.0 with 1 mol/L NaOH solution. The adjusted pH solution was transferred to a pre-chilled volumetric flask, fixed to 50 mL with water at 4 °C, centrifuged (8000 r/min, 10 min), and filtered with membrane. The filtrate was then stored at 4 °C for measurement. Nucleotides in the edible parts of CMCs were identified by chromatography using the Agilent 1290 HPLC system (Agilent, USA). C18 column (5 μm, 4.6 mm × 250 mm) was equipped. The mobile phases consisted of A: 0.025 mol/L KH_2PO_4 and B: acetonitrile. The HPLC gradient program for nucleotides was as follows: 0–9.5 min, 100 % A; 9.5–10 min, 100 %-70 % A and then stopped for 5 min; 15–15.2 min, 70 %-100 % A and then stopped for 7 min. The temperature, flow rate and detector's wavelength were 30 °C, 0.8 mL/min and 260 nm, respectively.

2.10. Equivalent umami concentration (EUC) analysis

EUC (g MSG/100 g) is equivalent to the fresh taste intensity given by the mixture of monosodium glutamate, such as nucleotides and amino acids, and is expressed by the following formula (Yamaguchi, et al., 2010):

$$\text{Expressed by the equation: } EUC = \sum aibi + 1218\left(\sum aibi\right)\left(\sum ajbj\right) \quad (10)$$

where ai and aj are the concentrations (g/100 g) of umami amino acids (Glutamic acid (Glu), aspartic acid (Asp)) and nucleotides (Adenosine monophosphate (AMP), guanylate (GMP), inosine monophosphate (IMP)), respectively; bi and bj are the relative umami coefficients (RUC) of amino acids to MSG (Asp: 0.077, Glu: 1) and nucleotides relative to imp (Amp: 0.18, Gmp: 2.3, Imp: 1); 1218 is the synergy coefficient.

2.11. Volatile compounds analysis

Volatile compounds were identified using Wang's method with slight modification (Wang, et al., 2021). The levels of volatile compounds were assessed by gas chromatography-tandem mass spectrometry (GC–MS/MS; 7890B-5977B; Agilent, USA). Then, 2.00 ± 0.01 g of the sample in a 10-mL headspace flask was heated at 60 °C for 1 min. A single trap was used to absorb the volatile compounds, while a thermal desorption unit was used for desorption. The samples were injected into GC–MS through a cooled sampling system. Agilent DB-5MS (60 m × 0.32 mm × 1 μm) was equipped. The carrier gas, flow rate, injector temperature, ion source temperature and ionization energy were helium (99.999 %), 1.3 mL/min, 250 °C, 230 °C and 70 eV, respectively. The column temperature was programmed from 40 °C to 250 °C at 5 °C/min, held at 5 °C/min for 5 min.

2.12. Statistical analysis

The analyses of all data were conducted in triplicate, and the results were presented as mean ± standard deviation (SD) (n = 3). The differences between groups were compared with one-way ANOVA using SPSS v22.0. $P < 0.05$ and $P < 0.01$ were considered to be significant and highly significant, respectively.

3. Results and discussion

3.1. Tissue indices

The comparison of the biological indices of female and male CMCs with different breeding modes is presented in Fig. 1a. The body weights of both female and male crabs in rice fields were remarkably higher than those of pond crabs ($P < 0.01$), which indicated that in the same feeding pattern, rice field crabs gained more weight than pond crabs. The hepatopancreas index of male crabs in the rice fields was higher than that of male crabs in ponds ($P < 0.01$), while other biological indexes did not show significant differences. In addition, it was worth emphasizing that whether crabs were male or female, their hepatopancreas index was higher than the gonadal index, which was due to that hepatopancreas were the main sources of energy for gonadal development, resulting in the inevitable effect of hepatopancreas index level on the gonadal index (Wen, et al., 2001). Relative fatness and total edible rate of crabs are basic biological indicators to measure their commercial value and productive performance. There were no obvious differences in these two indicators between rice field crabs and pond crabs in our current study, indicating that both rice and pond breeding modes could produce high-quality commercial crabs.

3.2. Proximate compositions

The effects of the two breeding patterns on the proximate compositions of CMCs are shown in Fig. 1b. The content of crude protein in the gonads and muscles of CMCs with two breeding patterns showed significant difference ($P < 0.05$), in which the crabs in rice fields presented higher content, indicating that the gonads and muscles of rice field crabs possessed higher nutritional values than those of pond crabs. The total lipid content of gonads in female rice crabs was markedly higher than that of pond crabs ($P < 0.01$), while the total lipid content of hepatopancreas in female rice crabs was remarkably lower than that of pond crabs ($P < 0.01$). During the process of sexual maturity, the nutrition in hepatopancreas would gradually transfer to gonads. Our results showed that more lipid from hepatopancreas transferred to gonads in female rice crabs compared with ponds crabs. The water content of the gonads in male rice crabs was dramatically higher than that of pond crabs ($P < 0.01$), but there were no obvious differences in the crude protein, ash and moisture content of hepatopancreas in all treatment groups, indicating that the culture mode might not affect these compositions of the hepatopancreas.

The proximate compositions are a vital indicator for assessing the nutritional quality of the edible tissues of crabs. Worldwide scholars have studied the effects of different aquaculture environments on the proximate compositions of aquatic products. However, the obtained results were not consistent, and even the same aquatic product showed distinguished outcomes under various growth conditions, which might be due to the different regions and water environments where the crabs were cultured. The results obtained by Wu's report showed that culture conditions mostly influenced the crude protein of the hepatopancreas in CMCs, which was contrary to the results of this paper (Wu, et al., 2020). In our current study, the CMCs cultured in rice field had better sources of protein and fat.

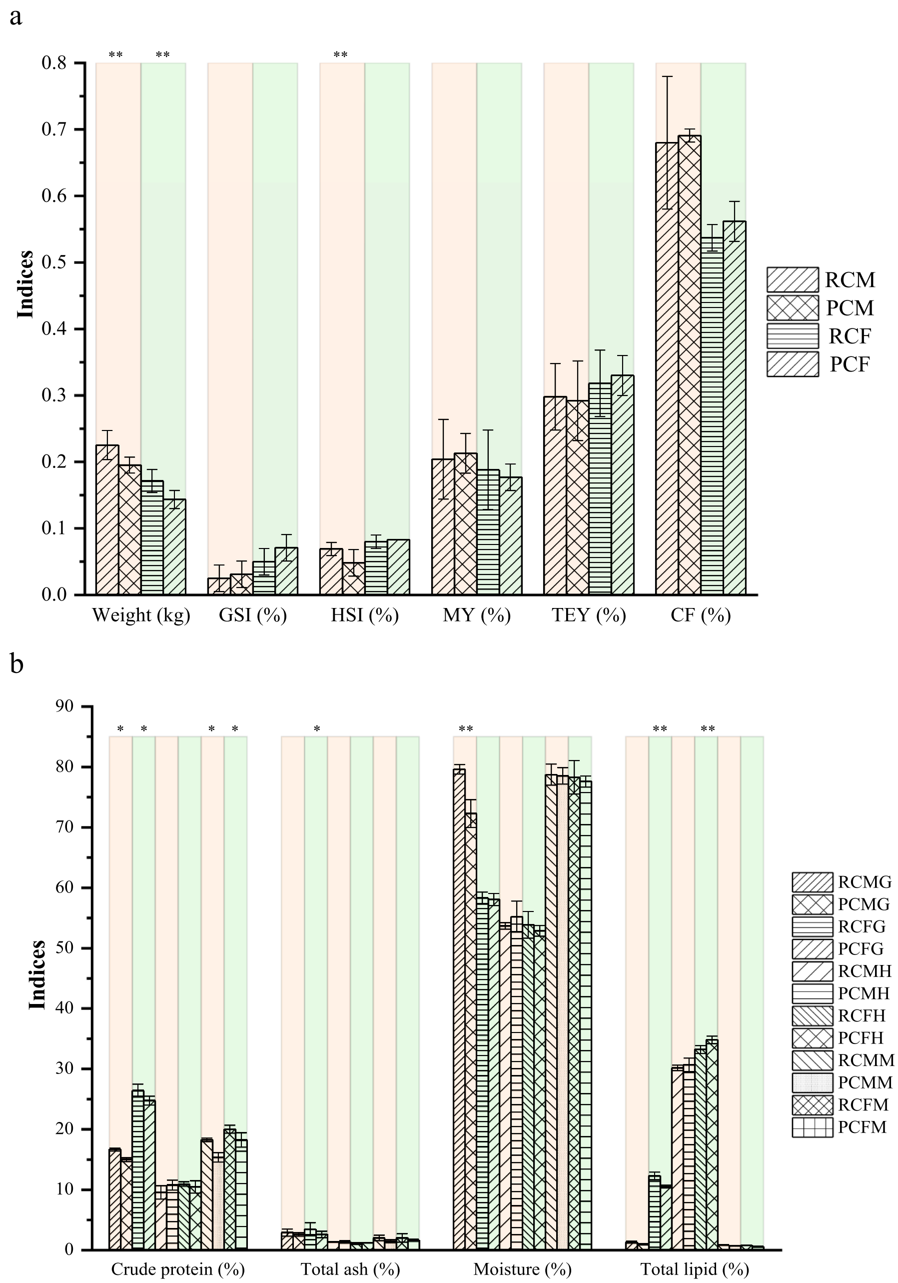


Fig. 1. Effects of different breeding patterns on the tissue indices and proximate compositions of Chinese mitten crabs (n = 3). (a) Tissue indices; (b) The content of proximate compositions. (The two sets of data under each background column were compared, asterisks indicated significant (*, $P < 0.05$) and highly significant effects (**, $P < 0.01$).) (RCM: Male rice crabs; PCM: Male pond crabs; RCF: Female rice crabs; PCF: Female pond crabs; RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

3.3. Mineral compositions

Fig. 2a shows the mineral content of three edible tissues of CMCs cultured in rice fields or ponds with a total of 5 macro elements (P, Ca, Mg, K, Na) and 3 trace elements (Zn, Cu, Mn). The Mg and K contents in the gonads of male rice crabs were remarkably increased compared to those of male pond crabs ($P < 0.01$), and the Mg and P contents in the gonads of female rice crabs were also markedly higher than those of female pond crabs ($P < 0.01$). As for the hepatopancreas, although the Na content of male rice crabs was dramatically higher than that of male pond crabs, the Zn, Cu, Mn, Ca and Mg contents of female rice crabs were obviously lower than those of female pond crabs ($P < 0.01$), which might be due to the fact that female rice crabs were in the molting period, resulting in a great loss of mineral elements in the hepatopancreas (Ahearn, et al., 2004). Overall, different breeding modes exerted the highest impact on the mineral elements of the hepatopancreas in CMCs, followed by the gonads and muscles.

The mineral is one of the seven essential nutrients for the human body, and indispensable for human health. Macro elements (P, Ca, Mg, K and Na) are important for human development and maintenance (Karnjanapratum, et al., 2013). Trace elements (Mn, Zn, Cu) are vital catalysts to various biochemical processes and metabolic activities in the human body (He, et al., 2017). Owing to that the organism body is not able to synthesize minerals, human must obtain them from food, drugs,

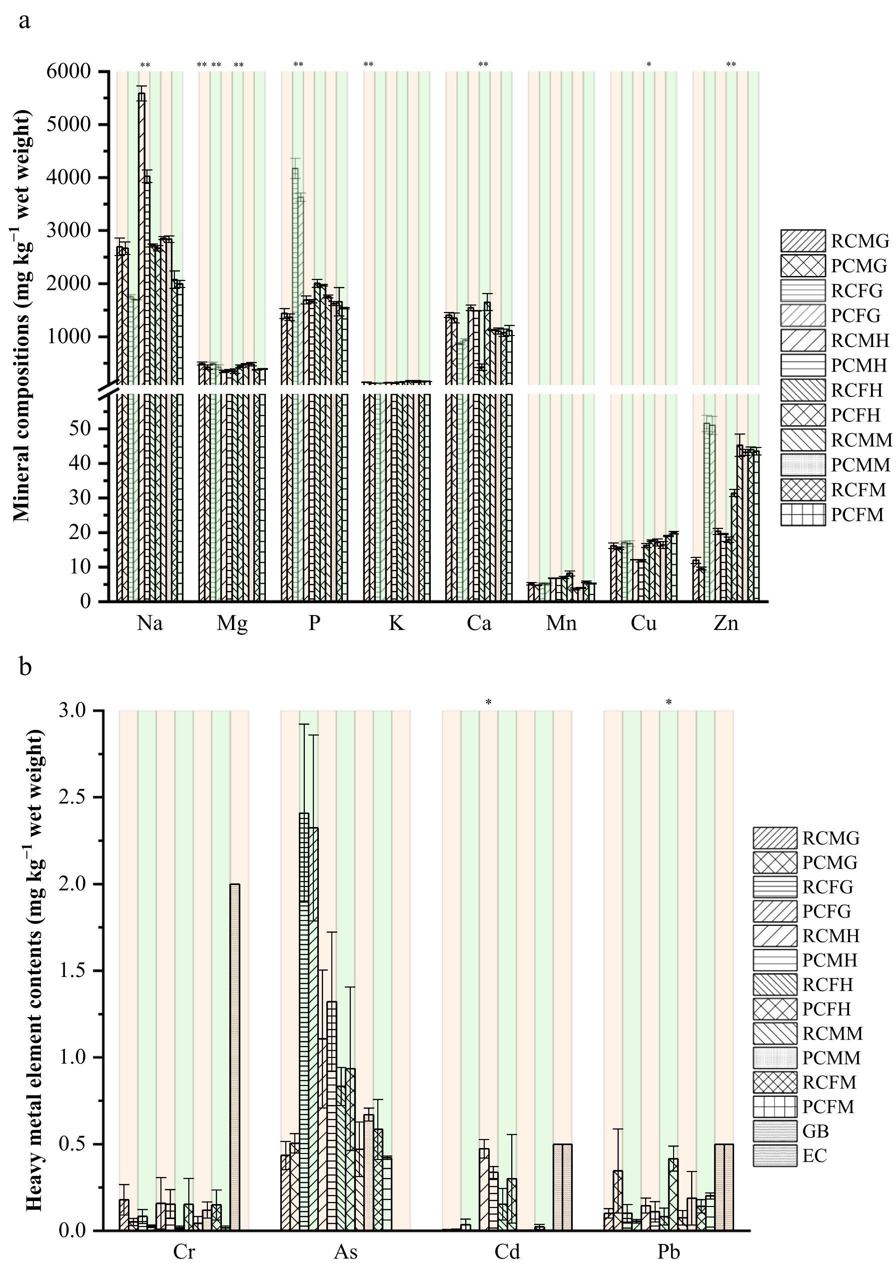


Fig. 2. Effects of different breeding patterns on the mineral compositions and heavy metals of Chinese mitten crabs (n = 3). (a) The content of mineral compositions (mg/kg wet weight); (b) The content of heavy metals (mg/kg wet weight). (The two sets of data under each background column were compared, asterisks indicated significant (*, $P < 0.05$) and highly significant effects (**, $P < 0.01$).) (RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

or other external sources (Yan-Fang, et al., 2012). By the way, dietary intake is the most crucial way to meet basic body needs for minerals, which indicates that mineral-rich foods are very valuable. Our findings suggest that CMCs are a rich source for minerals. However, the mineral contents in various parts of CMCs, such as gonads, hepatopancreas and muscles, are not similar, which may be attributed to different physiological functions of the organs. Muscle is primarily involved in the mechanical movement of appendages and paws, and thus, it requires vital elements for neuromuscular function. Apart from serving as the center for nutrient absorption, synthesis and secretion of digestive enzymes, the hepatopancreas also act as a regulator of ions and heavy metals in order to reduce their load into the blood and other tissues (Chavez-Crooker, 2003). The gonads are the reproductive organs that require high nutritional supplements to produce the reproductive cells (Marques, et al., 2010). The results in our current study indicated that the edible tissues of CMCs were rich in mineral elements, especially P, Ca, K, Mg and Na, which were in agreement with Wu's report (Wu, et al., 2020). According to our comprehensive analysis, we reckoned that CMCs with rice breeding pattern provided a better source of minerals compared with pond breeding pattern.

3.4. Heavy metal elements and human health risk assessment

Fig. 2b shows the level of four toxic elements in three edible portions of CMCs from different breeding modes. The overall trend of heavy metal concentrations in the three edible parts of the gonads, hepatopancreas and muscles was As > Cr > Pb > Cd, As > Cd > Pb = Cr, and As > Pb > Cr > Cd, respectively. As was the most accumulated heavy metal in CMCs from different cultivated modes, while only small quantities of Cd accumulated in the gonads and muscles. In comparison, Pb content in the hepatopancreas of female rice crabs was markedly reduced compared to female pond crabs ($P < 0.05$). Moreover, the concentrations of these heavy elements were remarkably higher in the hepatopancreas than those in the gonads and muscles, which was also consistent with the earlier findings from Goretti's report (Goretti et al., 2016).

Heavy metals can pose health risk to humans due to the ingestion. For example, hexavalent Cr (VI) compounds could increase the risk of lung cancer (Ishikawa, et al., 1994); and Cd might cause renal failure and bone softening after chronic or high dose exposure to contamination (Gray, et al., 2005). Therefore, the content of heavy metals in food should be controlled in the safe threshold. Although the toxic elements Pb, Cr, Cd and As were identified in the edible portions of CMCs cultivated in rice fields and ponds, our results showed that they were all below the standard concentration in the Chinese national standard (GB2762-2017) and the EU standard (No. 629/2008; No. 1881/2006), indicating that it is safe to ingest CMCs from both rice and pond breeding pattern. In comparison, the concentrations of heavy metals in the edible parts of CMCs cultured in rice fields were lower than those in ponds.

The buildup of heavy metal elements in CMCs is detrimental to the consumer's health. Thus, health risk assessment is crucial for the consumption of CMCs. The estimated daily intakes (EDIs) of four heavy metals for adults (70 kg) and children (20 kg) were determined according to the concentrations of heavy metals in the gonads, hepatopancreas and muscles of the edible parts of CMCs (Supplementary material Fig. S1a). Cd had the lowest EDI value, and the average EDI values of Cd, Pb, As and Cr in adults and children were much lower than the provisional tolerable daily intakes (PTDIs) of heavy metals. Our findings indicated that the health risks posed by the intake of heavy metals due to the consumption of CMCs were very limited.

The gonads, hepatopancreas and muscles of CMCs are the three edible parts that can be employed to assess non-carcinogenic health risks according to the Target Hazard Quotient (THQ) (Anandkumar, et al., 2018). The THQ values of the four heavy metals in the three tissues are presented in supplementary material Fig. S1b. The THQ values of heavy metals in all three edible tissues were much lesser than 1, illustrating that CMCs originated from the two breeding patterns did not pose threat to human health.

Since only the cancer slope factor (CSF) of As was available, the cancer health risk (CHR) values of As were determined (Supplementary material Fig. S1c). The CHR values of gonadal As were higher than those of hepatopancreas and body flesh in CMCs. According to U.S. Environmental Protection Agency (USEPA), the lifetime cancer risk is negligible when the CHR value is below 10^{-6}, unacceptably when it is above 10^{-4}, and acceptable in the range of 10^{-6} to 10^{-4}. The CHR values of As in our experimental crabs were lower than 10^{-6}, indicating that there was no carcinogenic risk from the consumption of CMCs.

Heavy metal elements are known to be extremely harmful to humans and cannot be excreted from the body through metabolism. Therefore, high priority should be given to the content of heavy metal elements in CMCs. This toxicity profile was not detected by the assessment methods commonly used in some studies, which may be attributed to the fact that health risk indices (THQ and CHR) are determined on the basis of chronic contamination. However, we cannot exclude the acute contamination that may cause damage to human health. In this study, CMCs cultured in rice fields and ponds were harvested to evaluate the accumulation of four heavy metal elements and the risk to human health from the consumption of their edible parts. Interestingly, the results showed that the values of THQ and CHR were well below the acceptable thresholds (THQ < 1 and CHR < 10^{-6}), suggesting that the consumption of CMCs posed extremely low or no risk to human health. It is worth mentioning that the THQ and CHR values of rice crabs were lower compared to those of pond crabs, indicating that the consumption of rice crabs might be a healthier choice.

3.5. Antioxidative parameters

The antioxidative parameters of GSH-PX, GST, CAT and *T*-AOC in crabs were significantly influenced by the culture mode ($P < 0.01$) (Fig. 3a). The highest activities of GST and GSH-PX were observed in the gonads of pond female (6.1 ± 1.3 U/mL) and male (6.28 ± 0.27 U/mL) crabs, respectively. The highest activity of CAT was found in the hepatopancreas of rice male (1.17 ± 0.01 U/mg prot) crabs, and the maximum value of *T*-AOC was shown in the hepatopancreas of pond female crabs (1.306 ± 0.13 mmol/g prot). The activities of GSH-PX and GST in the three edible parts of pond crabs were remarkably increased than those of rice crabs ($P < 0.01$), while the activities of CAT in the three edible parts of rice field crabs were markedly higher than those of pond crabs ($P < 0.01$). The values of *T*-AOC in the gonads, hepatopancreas and muscles of male rice crabs were markedly increased compared to male pond crabs.

Unlike vertebrates, invertebrates only exhibited non-specific immunity, therefore the antioxidant defense system is vital to them (Johansson, et al., 1999). The antioxidative parameters (e.g., GSH-PX, GST, CAT and *T*-AOC) play important roles in preventing the attack of imbalances caused by reactive oxygen species (ROS) and also helping scavenge the accumulated ROS, which could protect the body from the damage of proteins, lipids, and DNA causing by oxidative stress (Li, et al., 2010). Thus, the level of antioxidant capacity can reflect the ability of disease resistance of CMCs. The outcomes in our current study showed that male crabs cultured in rice fields possessed higher antioxidant capacity compared to male crabs cultured in ponds, while female crabs cultured in ponds had higher antioxidant capacity compared to female crabs cultured in rice fields. On the basis of antioxidant results, rice cultured mode was adaptable for male crabs, while pond cultured mode was adaptable for female crabs.

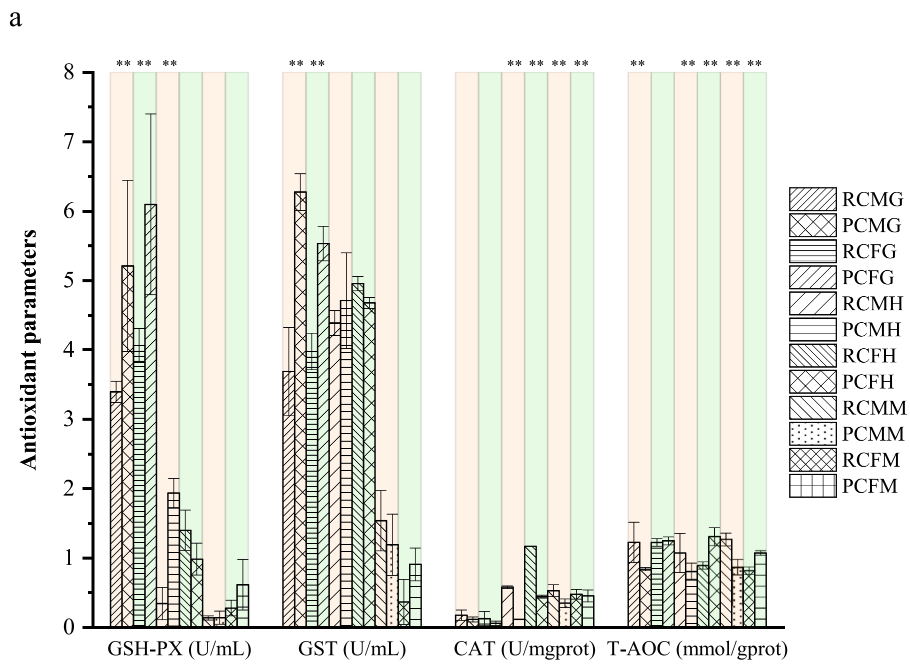
3.6. Determination of collagen content

The effects of different culture modes on the contents of Hyp and collagen in CMCs are shown in Fig. 3b and 3c. The highest contents of Hyp and collagen were found in the gonads of rice crabs with 0.15 ± 0.02 μg/mg wet weight and 1.09 ± 0.12 μg/mg wet weight, respectively. The contents of collagen in the gonads of rice crabs were remarkably increased compared to those of pond crabs ($P < 0.01$), while the contents of Hyp and collagen in the muscles of male pond crabs were markedly higher than those of male rice crabs ($P < 0.05$).

Hyp is a type of proline (Pro) metabolite, which is a unique amino acid specific in scavenging oxidants and regulating apoptosis (Phang, et al., 2010). Generally, the content of Hyp can better reflect the collagen content. CMCs suffer from several molts in their lifetime, and each molt period could increase their body size. However, during the process of molting, each time requires a large amount of collagen. Our results showed that the contents of Hyp and collagen of CMCs with rice culture mode were superior to those of crabs with pond culture mode, which indicated that rice cultivation was beneficial to the shelling and growth of CMCs. These outcomes were consistent with the previous consequences of body weight in our current study.

3.7. FAA

As shown in Fig. 4a, a total of 20 FAA were observed in three edible parts of CMCs from two culture modes. The FAA content of the three edible parts of CMCs from the two culture modes differed significantly, especially in the hepatopancreas and muscles of male crabs, in which pond crabs possessed significantly higher FAA content than rice crabs ($P < 0.05$). As for the gonads, alanine (Ala) (1.06–1.15 mg/g) dominated in the FAA of male crabs. The content of arginine (Arg; 1.44 mg/g) was the



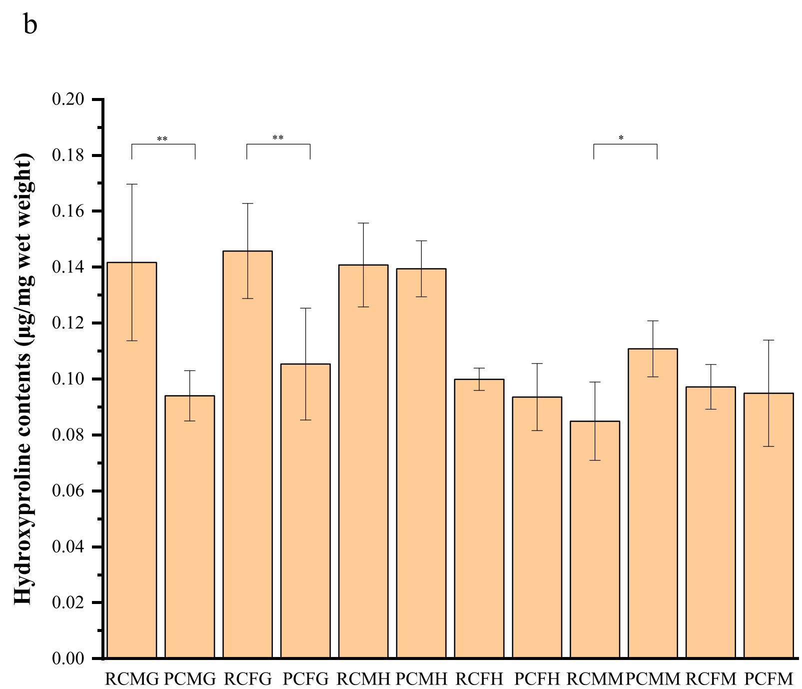


Fig. 3. Effects of different breeding patterns on antioxidant enzyme activities and collagen content in Chinese mitten crabs (n = 3). (a) Antioxidant parameters; (b) Total hydroxyproline contents (μg/mg wet weight); (c) The collagen contents (μg/mg wet weight). (The two sets of data under each background column were compared, asterisks indicated significant (*, $P < 0.05$) and highly significant effects (**, $P < 0.01$).) (RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

highest in female rice crabs, while the content of tryptophane (Trp; 1.33 mg/g) was the maximum in female pond crabs. Moreover, as for the hepatopancreas, the highest FAA content was Trp (1.33–3.13 mg/g) in female pond crabs, and for the muscle, the highest FAA content was Arg (2.29–4.25 mg/g) in male pond crabs.

The ratio of essential amino acid to total amino acid (EAA/TAA) is widely used to evaluate the nutrition level of fishery products. As shown in supplementary material Table S1, the EAA/TAA values of gonads and hepatopancreas in rice and pond crabs were matched with the standard value of 0.4 according to FAO/WHO (FAO/WHO, 1991), which demonstrated that both rice and pond crabs are adaptable sources of protein.

In this study, there were nine taste activity values (TAV) > 1 (Supplementary material Fig. S2a), which were Arg, Ala, lysine (Lys), Glu, histidine (His), valine (Val), methionine (Met), glycine (Gly), phenylalanine (Phe) in descending order. Among them, the taste FAAs included

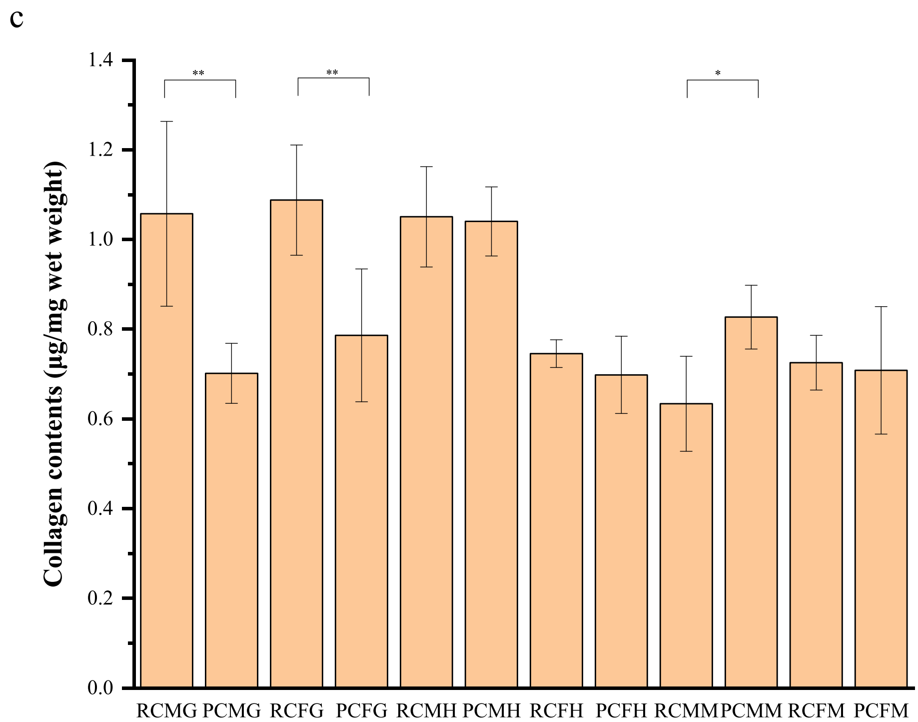


Fig. 3. (*continued*).

Ala, Glu and Phe. The taste FAA content was remarkably lower in the hepatopancreas of male rice field crabs than in pond crabs ($P < 0.05$), and these FAAs could directly affect the overall flavor of CMCs. The freshness of FAA is one of the main reasons for the unique flavor and high nutritional value of crabs, in which Asp and Glu are important FAA, since it could exert a synergistic effect with nucleotides, thereby enhancing the freshness of CMCs (Tu, et al., 2020). In our current study, the Asp content of edible parts in rice crabs was decreased compared to that in pond crabs, and the Glu content in the hepatopancreas of male rice crabs was markedly higher than that in male pond crabs ($P < 0.01$).

FAA is the basis of taste presentation in aquatic animals, and the variety of compositions and content can lead to the distinction of flavor characteristics, especially for their freshness and sweetness (Chen & Zhang, 2007). The present study showed that the breeding pattern exerted an effect on the FAA content of the edible parts of CMCs, which was consistent with Wu's findings (Wu, et al., 2020). The FAA contents of all edible parts of pond crabs were higher than those of rice field crabs, except for the gonads of female pond crabs, which were lower than those of female rice crabs. Overall, our results suggested that pond crabs possessed higher quality of FAA compared to rice crabs.

3.8. Nucleotides

Fig. 4b shows the effect of different breeding modes on the content of nucleotides in CMCs. The highest content of nucleotides in the edible tissues of crabs was IMP, followed by AMP and GMP. IMP was found in higher levels in gonads and muscles than AMP, while the opposite trend was found in the hepatopancreas. The nucleotide content of crab muscles was AMP > IMP > GMP, which was similar to the results obtained by Luo's study (Luo, et al., 2021). The thresholds of IMP, GMP and AMP were 25, 12.5 and 50 mg/100 mL, and their TAV values are shown in supplementary material Fig. S2b. Therefore, the contents of AMP and IMP might have a significant impact on the flavor of crabs, since their TAV values were greater than 1. In this study, the contents of AMP in the hepatopancreas and gonads in rice crabs were dramatically lower than those of pond crabs ($P < 0.05$). The GMP content of gonads in female rice crabs was significantly lower than that in female pond crabs ($P < 0.05$), while the IMP content of gonads showed the reverse outcome, which indicated that the alteration of culture mode could significantly affect the content of nucleotides.

The nucleotide is another important source of umami flavor in CMCs, among which AMP and IMP have a synergistic effect, while IMP and GMP can synergistically enhance freshness together with fresh FAA (Chen & Zhang, 2007). When the concentration of IMP is low, even a small amount of AMP can present umami and increase sweetness. Interestingly, the levels of IMP in female gonads were significantly higher than in other tissues, which might be the reason for the strong umami taste of female crabs.

EUC is the index to judge the umami intensity of CMCs, which can be derived from measuring the synergistic effect of taste FAA (Asp and Glu) and nucleotides (GMP, IMP and AMP). It has been widely used to assess the taste characteristics of food (Luo, Monroig, et al., 2021). The EUC values of CMCs in the two culture modes are shown in supplementary material Fig. S2c. Among the three edible parts, the EUC value of gonads was the highest, indicating that the strong flavor of female crabs mainly came from gonads, followed by the muscles and hepatopancreas. The EUC value of gonads in female rice crabs was 93.87 ± 21.03 g MSG/100 g, which was markedly higher than that in female pond crabs with 78.25 ± 9.82 g MSG/100 g, indicating that the umami intensity of gonads in female rice crabs was superior to that in female pond crabs ($P < 0.05$). Taken together, our findings demonstrated that the umami flavor of female crabs in rice fields was better than that of female crabs in ponds.

Based on the above results, it was found that the nucleotide played a dominant role in freshness compared to FAA. Among the nucleotides, the high content of IMP played an important role, which also suggested that the gonads of female rice crabs had the highest EUC content due to the abundant IMP content.

3.9. Volatile compounds

The analysis of volatile compounds revealed that 28 compounds were isolated and identified from the two breeding modes of CMCs (Fig. 5). Cluster analysis and heatmap visualization showed that volatile compounds from three edible parts of CMCs could be clearly

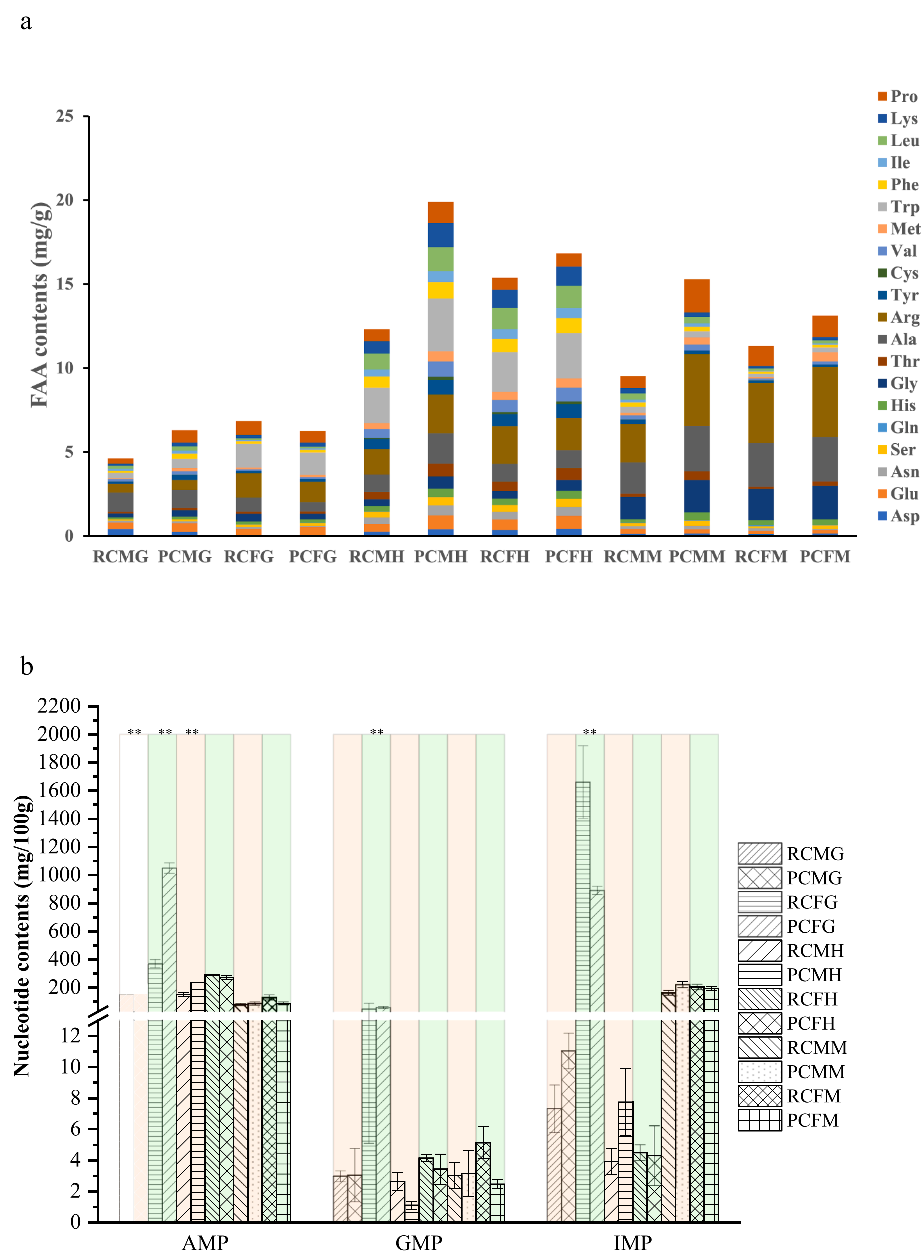


Fig. 4. Effects of different breeding patterns on FFAs and flavor nucleotides in Chinese mitten crabs (n = 3). (a) The content of FFAs (mg/g); (b) The content of nucleotide (mg/100 g). (The two sets of data under each background column were compared, asterisks indicated significant (*, $P < 0.05$) and highly significant effects (**, $P < 0.01$).) (RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

distinguished. Interestingly, 27 and 22 volatile compounds were identified from rice crabs and pond crabs, respectively, of which 21 components were identified as characteristic volatile compounds for CMCs since they were present in both species.

The volatile compounds found in the edible parts of CMCs mainly included alkanes, esters, alcohols and aldehydes. Alcohols, alkanes and aldehydes were the most abundant in the hepatopancreas, and alkanes were the richest in hepatopancreas. The high threshold of esters, alcohols and alkanes demonstrated that these volatile compounds contributed slightly to the aroma of CMCs (Wang, et al., 2016). In comparison, aldehydes were classified as the main flavor sources, since their content was higher and threshold was lower than other volatile compounds (Wu, et al., 2014).

Numerous studies have suggested the contribution of aldehydes to the flavor of CMCs (Wu, 2017), which are considered to be the main influential volatile compounds (Chung & Cadwallader, 2010). Furthermore, the odor of 2,4-Decadienal, (E,E) presents strong buttery fragrance with pleasant smell, which may be another important factor for the good taste of CMCs (Yu & Chen, 2010). In our current study, the contents of phenylacetaldehyde, 2,4-Decadienal, (E, E), and hexadecanal in rice crabs were increased compared to those in pond crabs. However, unpleasant odor compounds in rice crabs, such as benzaldehyde, were higher than those in pond crabs. Furthermore, hexanal, derived from the oxidation of n-6 PUFAs with a grassy odor (Frankel, et al., 1989), was detected only in the rice fields crabs. Therefore, in comparison, the flavor of rice crabs is superior to that of pond crabs.

3.10. E-nose and E-tongue

E-nose and E-tongue are often used to differentiate the aroma and

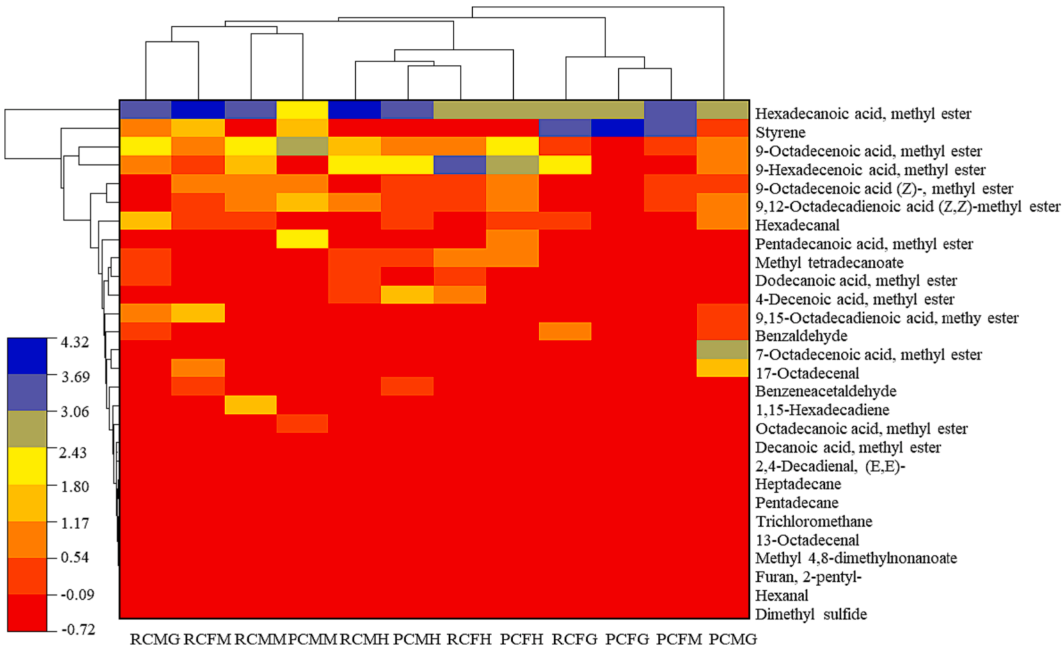


Fig. 5. Effects of different breeding patterns on volatile compounds in Chinese mitten crabs (n = 3). (RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

taste of food, especially muscle product, beverage and alcohol (Qiu, et al., 2014). The taste signal response values of edible parts in CMCs with different breeding modes were obtained by E-tongue via the detection of bitter, astringent, sour, salty, fresh and sweet sensors. The radar plots are drawn in Fig. 6a, 6b and 6c. The richness response values of the edible parts of female pond crabs were remarkably increased compared to female rice crabs, while the aftertaste-astringency (aftertaste-A) response values of the hepatopancreas of male rice crabs were higher than those of male pond crabs. Additionally, the sourness and bitterness response values of the muscles of male rice crabs were higher than those of male pond crabs.

The E-nose consists of a signal processing system, an odor-sensitive sensor array and some auxiliary devices. The sensor array identifies volatile odors in the sample based on the response curve, and then analyzes the detected odors. Fig. 6d, 6e and 6f show the radar plots of the three edible parts in CMCs with different culture methods. Notably, the W5S sensor response values of the edible parts in pond crabs were remarkably increased compared to those in rice crabs, indicating higher content of ammonia and nitrogen compounds in pond crabs. Besides, the W1W sensor response values of the edible parts in rice crabs were markedly higher than those in pond crabs, indicating a higher content of sulfide in rice crabs, which may be due to the lesser water condition in rice crab breeding. The W1C and W2S sensor response values of muscle in male rice crabs were significantly higher than those of male pond crabs, indicating that the aromatic components, alcohols, aldehydes and ketones were higher in the muscles of male crabs cultured in rice field were higher, which was consistent with the above findings of volatile compounds.

Previous studies have shown that different edible fractions of CMC can be clearly and easily separated by their principal component analysis (PCA) results (Gu, et al., 2013). In this study, the PCA results of E-nose and E-tongue are shown in Fig. 6g, 6h, 6i, 6j, 6k and 6l, it could be seen that the samples of CMCs from rice and pond culture were clustered together, respectively, indicating good reproducibility of the E-nose and E-tongue results. The PCA results of E-nose and E-tongue showed that the three edible parts of CMCs with rice and pond culture could be completely distinguished, indicating that the taste and aroma of CMCs varied across rice and pond culture methods. The results of E-nose and E-tongue were consistent with the results of EUC and volatile compounds, better explaining that rice field crabs and pond crabs had significant taste, especially the rice field crabs.

4. Conclusion

The quality of CMCs in rice fields and ponds was firstly compared based on several indexes in our current study. The crabs cultured in rice fields presented superior proximate compositions, mineral content, antioxidant activities, collagen content, nucleotide content, EUC value and volatile compound content, while those cultured in pond fields showed superior FAA content. All crabs from these two breeding patterns contained very little harmful heavy metal, and were safe to human health. In comparison, we reckoned that the nutritional value and flavor of rice crabs were better than those of pond crabs. The results of this experiment can assist farmers to produce commercial crabs with higher nutritional value so as to meet consumer's demand, and also provide reference basis for establishing the industry standard of CMCs.

Ethical Guidelines

All procedures were performed in compliance with the Care and Use of Laboratory Animals of the National Institutes of Health and the recommendations of the Guidelines for Care and Use of Laboratory Animals, ethics approval was not required for this research.

CRediT authorship contribution statement

Jie Mei: Investigation, Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **Xiaochen Liang:** Investigation, Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **Yilin Yu:** Conceptualization, Resources, Project administration. **Yuxi Lang:** Supervision, Writing – review & editing. **Xiaodong Li:** Conceptualization, Resources, Project administration.

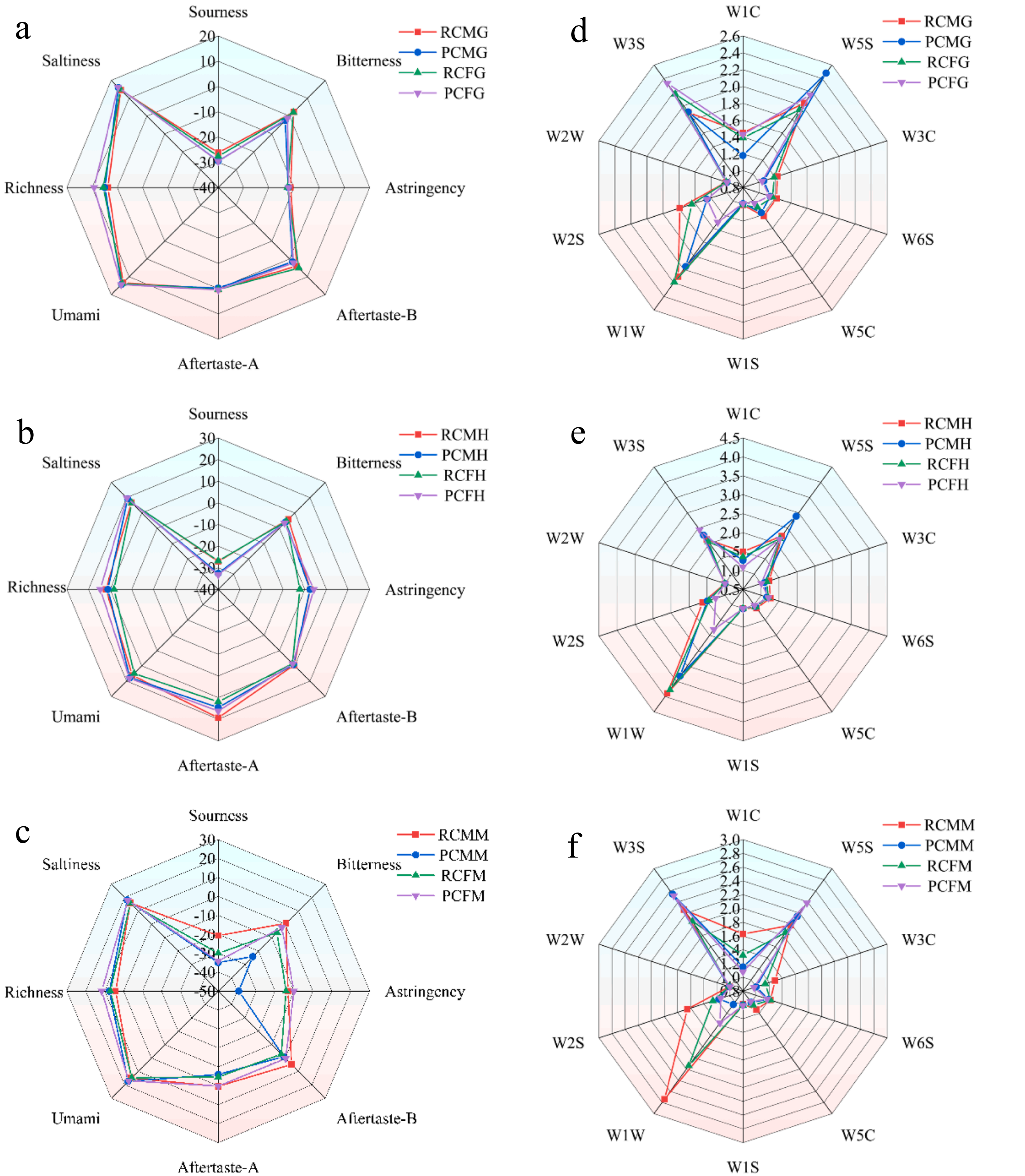


Fig. 6. Effects of different breeding patterns on the electronic tongue and electronic nose of Chinese mitten crabs (n = 3). (a), (b) and (c): Electronic tongue radar chart; (d), (e) and (f): Electronic nose radar chart; (g), (h) and (i): PCAs of electronic tongue; (j), (k) and (l): PCAs of electronic nose. (RCMG: Gonads of male rice crabs; PCMG: Gonads of male pond crabs; RCFG: Gonads of female rice crabs; PCFG: Gonads of female pond crabs; RCMH: Hepatopancreas of male rice crabs; PCMH: Hepatopancreas of male pond crabs; RCFH: Hepatopancreas of female rice crabs; PCFH: Hepatopancreas of female pond crabs; RCMM: Muscles of male rice crabs; PCMM: Muscles of male pond crabs; RCFM: Muscles of female rice crabs; PCFM: Muscles of female pond crabs).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

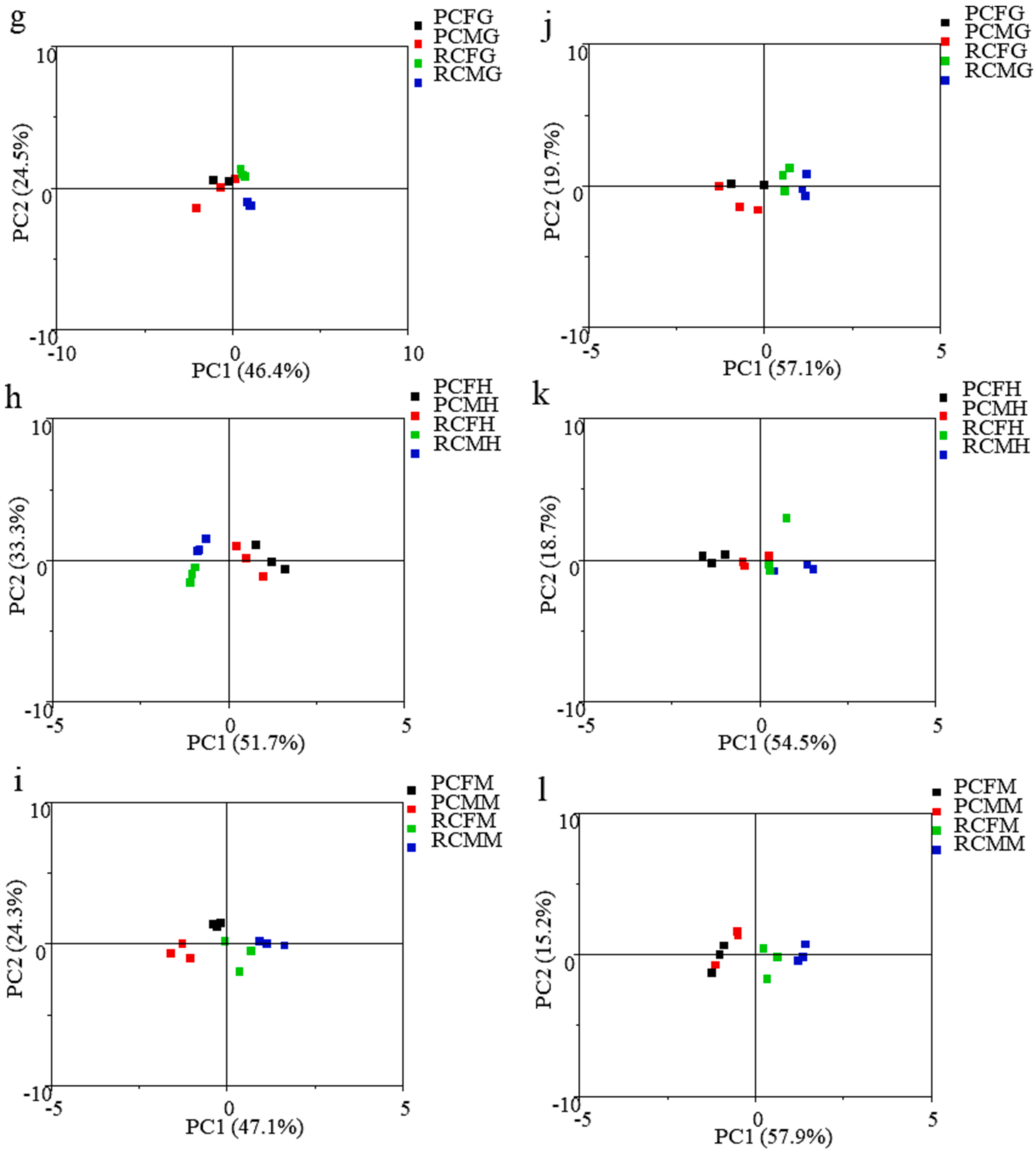


Fig. 6. (*continued*).

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100937.

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