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Non-volatile taste active compounds in the meat of Chinese mitten crab (*Eriocheir sinensis*)

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Abstract

The non-volatile taste active compounds, including soluble sugars, succinic acid, free amino acids and flavour 5′-nucleotides in the meat of Chinese mitten crab (*Eriocheir sinensis*) were analyzed, and their taste impacts were evaluated by taste active values (TAVs) and equivalent umami concentration (EUC) methods. The total free amino acid content of crab meat was 20.9 mg/g. Arginine, glycine and alanine were the major free amino acids, accounting for more than 70% of the total free amino acids. 5′-Adenosine monophosphate (AMP) was the main flavour 5′-nucleotide (75.3 mg/100 g), followed by 5′-inosine monophosphate (IMP) (34.4 mg/100 g) and 5′-guanosine monophosphate (GMP) (2.3 mg/100 g). Arginine, glycine, alanine, glutamic acid, IMP and AMP were of high TAV (greater than one), and they had strong taste impacts on the crab meat flavour. Glycine and alanine contributed to the major sweet taste, while glutamic acid, IMP and AMP contributed to the strong umami taste. As the TAVs of soluble sugar, succinic acid and bitter free amino acids were lower than one, thus those compounds are likely to have insignificant impact on the taste of the crab meat. The EUC was 4.2 g MSG/100 g crab meat, which meant that the umami taste of the crab meat was very intense.

Keywords: Chinese mitten crab; Taste active compounds; Free amino acids; Flavour 5′-nucleotides; Umami; Sweet

1. Introduction

Chinese mitten crab (*Eriocheir sinensis*) is a traditional savoury food in China. It has a unique pleasant aroma and a delicious taste (Chen & Zhang, 2006). The crab meat is famous for its very 'tián' and 'xiān' taste in Chinese cooking (Naiguang, 2004). The 'tián' taste means sweetness, while the 'xiān' taste could be described as delicious, palatable or savoury in Western cuisine, and "umami" in Japanese. Recently, umami taste has become widely accepted in Western countries, and umami is considered as the fifth basic taste sensation (Conn, 1992), along with sweet, salty, bitter and sour.

Umami was first defined as the characteristic taste elicited by glutamates, and has since also been associated with monosodium glutamate (MSG) (Yamaguchi, 1991). Umami is also provided by disodium salts of the 5′-nucleotides: IMP (disodium 5′-inosine monophosphate), GMP (disodium 5′-guanosine monophosphate) and AMP (disodium 5′-adenosine monophosphate). These compounds are naturally present in many protein-rich foods, such as meat, fish and fungi. There is a synergistic effect between MSG, IMP, GMP and AMP, which together in certain ratios produce a strong umami taste (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). The equivalent umami concentration (EUC) is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG-like amino acids and the flavour 5′-nucleotides. Therefore, EUC is very useful to evaluate the umami taste of food, such as mushroom (Chiang, Yen, & Mau, 2006; Tseng, Lee, Li,

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& Mau, 2005). Besides free glutamic acid and aspartic acid, free aromatic amino acids such as L-phenylalanine and L-tyrosine also play an important role in enhancing savoury or umami taste at their subthreshold concentrations in the presence of salt and free acidic amino acids (Lioe, Apriyantono, Takara, & Wada K, 2005; Lioe et al., 2004).

The taste active value (TAV) is calculated as the ratio of the concentration of an individual compound in the food matrix and its corresponding taste recognition threshold. Although, there is a limiting factor that the TAV method does not take into account the possibilities of masking, enhancing or additive effects of the simultaneously present compounds in the food matrix, TAV is widely used in evaluating the taste impact of those individual taste active compounds in the food matrix (Rotzoll, Dunkel, & Hofmann, 2006; Scharbert & Hofmann, 2005; Schlichtherle-Cerny & Grosch, 1998).

The contributions of extractive components to the taste of seafood have been reviewed (Fuke & Konosu, 1991; Komata, 1990; Ninomiya, 2002; Spurvey, Pan, & Shahidi, 1998). A series of taste panel assessments on synthetic extracts prepared by omitting or adding extractive component(s) was carried out by a triangle difference test, and changes in the taste profile were assessed. Twelve components, alanine, arginine, glutamic acid, glycine, betaine, AMP, CMP, GMP, Na^+, K^+, Cl^-, and PO_4^{3-}, were found to contribute more or less towards the characteristic taste of the snow crab (Hayashi, Yamaguchi, & Konosu, 1981).

However, to our knowledge, the non-volatile taste active compounds in Chinese mitten crab meat have not been investigated previously. Chinese mitten crab is a promising fresh-water fishery industry in China, which has made significant advances during the last decade, from 200 000 tonnes in 2000 to 420,000 tonnes in 2004 (Yuan, 2005). Thus the Chinese mitten crab that was previously a luxury food has been turned into a common food in China today.

The objective of this paper was to evaluate the taste impacts of the non-volatile taste active compounds, including soluble sugars, succinic acid, free amino acids and flavour 5′-nucleotides present in the crab meat by TAVs and EUC methods.

2. Materials and methods

2.1. Sample preparation

Male Chinese mitten crabs, individually weighing about 150 g, were harvested and transported live to the laboratory in October 2005 (October is the best season for crab consumption) from Yangchenghu Lake, which is the most famous Chinese mitten crab locality in Suzhou City, Jiangsu Province, China. The crabs were steamed at 100 °C for 15 min before picking the claws, legs and abdomen meat by hand, and then the crab meat was minced and mixed. The crab meat was stored at −20 °C until further use.

2.2. Soluble sugar assay

Soluble sugars were extracted as described by Tseng (Tseng et al., 2005). Crab meat (2.00 g) was homogenized in 50 ml of 80% aqueous ethanol for 24 h at 4 °C and then filtered. The residue was washed three times with additional 25 ml portions of 80% ethanol. The combined filtrate was then rotary-evaporated at 40 °C and redissolved in deionised water to a final volume of 10 ml. The aqueous extract was filtered through 0.45 μm filters prior to HPLC analysis. Ten microliters of filtrate were injected into the high-performance liquid chromatograph (HPLC) (Agilent 1100). The column was a Sugarpak1, 6.5 mm i.d. ×300 mm. The temperature was controlled at 85 °C. The eluent used was pure water, the flow rate was set to 0.4 ml/min for 22 min. The refractive index detector was a waters 2410. All analyses were done in triplicate. The identity and quantity of the sugars were assessed by comparison with the retention times and peak areas of each standard.

2.3. Nucleotide analysis

The nucleotides were extracted and analysed by a method similar to that reported by Ryder (1985). Crab meat (5.00 g) was homogenized in 25 ml of 0.6 M cold perchloric acid (PCA) for 2 min. Then, the sample was filtered and neutralized with 1 M potassium hydroxide (KOH). All sample solutions were filtered through 0.45 μm filters prior to HPLC analysis. Ten microliters of filtrate were injected into the HPLC (Agilent 1100). The column was an Intersil ODS-3, 250 mm × 4.6 mm. The temperature was controlled at 30 °C. The eluents used were (A) methanol and (B) 0.05% of phosphoric acid. To achieve the nucleotide separations, the flow rate was set to 1.0 ml/min and the following gradient was performed: initial of 5% A for 10 min, linear change to 15% A for 5 min, linear change to 70% A for 6 min and finally linear change to 5% A for 4 min. The wavelength of the detector was set at UV 260 nm. All analyses were done in triplicate. The identity and quantity of the nucleotides were assessed by comparison with the retention times and peak areas of each nucleotide standard.

2.4. Succinic acid analysis

Crab meat (5.00 g) was homogenized in 25 ml of purified water for 2 min. Then, samples were centrifuged at 10,000*g* for 20 min. All sample solutions were filtered through 0.45 μm filters prior to HPLC analysis. The HPLC condition was almost the same as for the nucleotide analysis except that the detector wavelength was set at UV 215 nm. All analyses were done in triplicate. The identity and quantity of succinic acid was determined by comparison with the retention times and peak areas of the standard.

2.5. Free amino acid (FAA) analysis

Samples for FAA analysis were extracted in trichloroacetic acid (TCA) according to the method of Konosu, Watanabe, and Shimizu (1974). Then the free amino acids were separated on an Agilent 1100 HPLC using a 4.0 mm × 125 mm C18 column. All analyses were done in triplicate. The identity and quantity of the amino acids were determined by comparison with the retention times and peak areas of each amino acid standard.

2.6. Taste activity value (TAV)

TAV was calculated as the ratio between its concentration determined in the crab meat and its threshold value generally measured in water or in a simple matrix. The compounds whose TAV was greater than 1 were considered as active in food taste.

2.7. Equivalent umami concentration (EUC)

The equivalent umami concentration (EUC, g MSG/100 g) is the concentration of MSG equivalent to the umami intensity given by the mixture of MSG-like amino acids and the 5′-nucleotide and is represented by the following equation (Yamaguchi et al., 1971):

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right)$$

where Y is the EUC of the mixture in terms of g MSG/100 g; a_i is the concentration (g/100 g) of each umami amino acid (Asp or Glu); b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); a_j is the concentration (g/100 g) of each umami 5′-nucleotide (5′-IMP, 5′-GMP or 5′-AMP); b_j is the RUC for each umami 5′-nucleotide to 5′-IMP (5′-IMP, 1; 5′-GMP, 2.3 and 5′-AMP, 0.18) and 1218 is a synergistic constant based on the concentration of g/100 g used.

3. Results and discussion

Only one soluble sugar, glucose was found in Chinese mitten crab meat, and its content was 59 ± 26 mg/100 g (wet weight), much lower than the its taste threshold of 860 mg/100 g (Rotzoll et al., 2006), which TAV was 0.07. Glucose contributes a pleasant sweet taste to food flavour. But because of its relatively low concentration, glucose would be of insignificant impact to the sweet taste of the crab meat in our study.

Succinic acid was reported to be one of the main taste active components in some seafood, such as clam (Spurvey et al., 1998). It contributes a special sour and umami taste. In stewed beef juice, umami taste could be reduced by the omission of succinic acid (Schlichtherle-Cerny & Grosch, 1998). However, succinic acid was not detected in our study (less than 0.5 mg/100 g), in which TAV was lower than 0.05 (threshold: 10 mg/100 ml (Rotzoll et al., 2006)). Therefore, its taste impact must be negligible.

In our previous study(Chen, Zhang, & Sundar, in press), the main minerals were potassium (273 mg/100 g) and sodium (190 mg/100 g) (wet weight), while the taste recognition thresholds of potassium chloride and Sodium chloride were 130 and 180 mg/100 ml, respectively (Rotzoll et al., 2006). Their TAVs were greater than 1, so it could be concluded that these minerals could contribute salty taste to the crab meat flavour. As is usual in crab cooking, salt is added and so the contribution of these minerals to the taste would be minor.

Table 1 shows the contents of free amino acids in the crab meat. The total free amino acid content of crab meat was 20.9 mg/g (wet weight). Arginine, glycine and alanine were the major amino acids, accounting for more than 70% of the total free amino acids in Chinese mitten crab meat. There was no correlation between the contents of free amino acids and total amino acids. Although, glutamic acid and aspartic acid contributed high amounts to total amino acids (Chen et al., in press), they were minor contributors to free amino acids. One reason is that glutamine and asparagine have been deaminated to glutamic acid and aspartic acid during the acidic hydrolysis, so glutamic acid and aspartic acid contents in total amino acid analysis were over-measured. Aromatic amino acids, phenylalanine and tyrosine were present in very small amounts in the crab meat.

Amino acids usually contribute a sour, bitter or sweet taste. Those containing a sulphur atom have a sulphury note (Shallenberger, 1993). Aspartic acid and glutamic acid have a sour taste, but in the presence of sodium salt, their sodium salts were MSG-like components, which gave the umami taste that was the characteristic taste of MSG and 5′-nucleotides (Yamaguchi et al., 1971). Glycine and alanine have a pleasant sweet taste, and they are widely presented in large quantity in seafoods, such as snow crab, clam and scallop (Fuke & Konosu, 1991; Spurvey et al., 1998; Wu & Shiau, 2002). There is also a synergistic interaction of umami taste between sweet amino acids and IMP, and sweetness would increase in the present of IMP (Kawai, Okiyama, & Ueda, 2002). Arginine is widely present in large amounts in lots of seafood, it contributes a pleasant overall preference rather than a bitterness to those products (Spurvey et al., 1998). Those amino acids with the hydrophobic side chains usually have an unpleasant bitter taste.

Although, some amino acids were present in small amounts in the crab meat, their taste impacts were strong because of their low threshold values. To evaluate these compounds for their taste impact, TAV was a very useful index. As given in Table 1, arginine had both high concentration and high TAV of 10.3. The sweet taste amino acids, glycine and analine also had high TAV of 3.8 and 6.2, respectively, which could contribute significant sweet taste impacts to the crab meat. Glutamic acid with the umami taste in crab meat was over its threshold concentration

Table 1
The contents, taste attributes (+ = pleasant, − = unpleasant), taste thresholds and TAVs of free amino acids in the crab meat

FAA	Content (mg/g) Mean ± SD	Taste attribute	Taste threshold[a] (mg/ml)	TAV
Aspartic acid	0.30 ± 0.01	Umami (+)	1	0.3
Glutamic acid	0.62 ± 0.01	Umami (+)	0.3	2.1
Asparagine	0.77 ± 0.02	Flat/tasteless	–	–
Serine	0.15 ± 0.01	Sweet (+)	1.5	0.1
Glutamine	0.27 ± 0.02	Flat/tasteless	–	–
Histidine	0.05 ± 0.00	Bitter (−)	0.2	0.3
Glycine	4.97 ± 0.12	Sweet (+)	1.3	3.8
Threonine	0.05 ± 0.00	Sweet (+)	2.6	0.2
Alanine	3.69 ± 0.09	Sweet (+)	0.6	6.2
Arginine	6.64 ± 0.15	Bitter/sweet (+)	0.5	10.3
Tyrosine	0.10 ± 0.01	Bitter (−)	nd	
Cysteine	1.06 ± 0.11	Bitter/sweet/sulfurous (−)	nd	
Valine	0.15 ± 0.01	Sweet/bitter (−)	0.4	0.4
Methionine	0.10 ± 0.01	Bitter/sweet/sulfurous (−)	0.3	0.3
Tryptophan	0.21 ± 0.05	Bitter (−)	nd	
Phenylalanine	0.07 ± 0.01	Bitter (−)	0.9	0.1
Isoleucine	0.12 ± 0.01	Bitter (−)	0.9	0.1
Leucine	0.14 ± 0.01	Bitter (−)	1.9	0.1
Lysine	0.18 ± 0.00	Sweet/bitter (−)	0.5	0.4
Proline	1.21 ± 0.44	Sweet/bitter (+)	3	0.4
Total	20.9			

[a] Taste threshold value (mg/ml) and taste of free amino acids in water (Kato et al., 1989; Shallenberger, 1993).

by a TAV of 2.1, which could contribute significant umami taste impacts to the crab meat. As the concentration of all of the other amino acids were significantly below their corresponding taste detection thresholds, it was assumed that some of these amino acid might enhance the umami and sweet taste in their subthreshold concentrations. Phenylalanine and tyrosine are aromatic amino acids with a bitter taste, which have recently been discovered to be important components of the savoury fractions of soy sauce in addition to glutamate (Lioe et al., 2004). Phenylalanine was found to significantly enhance the umami tastes of the MSG/NaCl mixtures when it was added in a concentration range of 0.5–5.0 mM. However, neither the umami taste of MSG alone nor the salty taste of NaCl alone was intensified. In a further experiment, tyrosine at the subthreshold concentrations studied was shown to have the same activity as phenylalanine. Therefore, we would conclude that tyrosine and phenylalanine contribute insignificant direct taste impacts, but they might significantly enhance the umami taste of the crab meat.

The contents of the free amino acids in Chinese mitten crab were quite different as compared with those in the salt-water crabs, snow crab (Hayashi et al., 1981) and mud crab (Chio & Huang, 2003). Snow crab and mud crab had similar free amino acids contents. Snow crab contained high amounts of glycine, arginine, proline and alanine (6.23, 5.79, 3.27 and 1.87 mg/g, respectively), but small amounts of the umami taste amino acids, glutamic acid and aspartic acid (0.19 and 0.10 mg/g, respectively). The contents of the umami taste amino acids in Chinese mitten crab were three times higher than those in snow crab. Although, the individual content of glysine and alanine were different, the total content of the main sweet amino acids were almost equal (8.66 mg/g in Chinese mitten crab and 8.10 mg/g in snow crab). The contents of arginine in both crabs were similar. The bitter taste amino acids, such as phenylalanine and histidine, were present at lower than the threshold values, although their contents in snow crab were higher than in Chinese mitten crab.

Therefore, according to the concentrations of free amino acids, it can be concluded that Chinese mitten crab and snow crab have a similar sweet taste, while the umami taste of Chinese mitten crab was more intense than that of snow crab.

The concentrations and TAVs of flavour 5′-nucleotides, AMP, IMP and GMP were shown in Table 2. AMP was the main component (75.3 mg/100 g), follow by IMP (34.4 mg/100 g), and GMP was present in small amount, only 2.3 mg/100 g (wet weight). The TAVs of AMP, IMP and GMP were 1.5, 1.4 and 0.2, respectively. AMP and IMP may therefore contribute a significant impact on crab flavour as their TAVs were greater than one. While in the early reports about the flavour 5′-nucleotides in snow crab: AMP was the main component (32 mg/100 g), and IMP and GMP were present in small amounts (5 and 4 mg/100 g respectively). The concentrations of AMP and IMP in Chinese mitten crab were significantly higher than those in snow crab, while GMP was lower than that in snow crab.

IMP and GMP are intense flavour-enhancers of the umami taste, and are much stronger than MSG. The synergistic effect of flavour 5′-nucleotides with MSG-like components (Glu and Asp) might greatly increase the umami taste of soups (Yamaguchi et al., 1971). The taste profile contributed by AMP was dependent on the concentration in the samples. At low concentrations (50–100 mg/100 ml),

Table 2
The concentrations, taste thresholds and TAVs of nucleotides in the crab meat

Nucleotide	Concentration (mg/100 g) Mean ± SD	Taste threshold[a] (mg/100 ml)	TAV
AMP	75.3 ± 0.4	50	1.5
GMP	2.3 ± 0.2	12.5	0.2
IMP	34.4 ± 2.9	25	1.4

[a] Taste threshold value (mg/100 ml) of nucleotides in water (Fuke & Ueda, 1996; Yamaguchi et al., 1971).

Table 3
Values of a_i, b_i, a_j and b_j

FAA	a_i (g/100 g)	b_i	a_ib_i	Nucleotide	a_j (g/100 g)	b_j	a_jb_j
Glu	0.062	1	0.062	IMP	0.0343	1	0.034
Asp	0.030	0.077	0.002	GMP	0.0023	2.3	0.005
				AMP	0.0753	0.18	0.014

AMP contributed sweetness but no umami taste; however, there is a synergistic interaction between AMP and IMP in eliciting the umami taste. When a very low concentration of IMP was also present, umami and complexity tastes were elicited, and sweetness was increased (Fuke & Ueda, 1996).

According to the equation: $Y = \sum a_ib_i + 1218(\sum a_ib_i)(\sum a_jb_j)$, (data are shown in Table 3), the value of EUC was calculated as 4.2 g MSG/100 g (wet weight). In other words, the umami intensities of one gram of fresh Chinese mitten crab meat was equivalent to 0.042 g MSG. As the taste threshold of MSG was 30 mg/100 ml, the TAV of EUC was calculated as 140. Therefore, it could be concluded that the umami taste of the meat of Chinese mitten crab was very intense. Similarly, using the earlier reported data (Fuke & Konosu, 1991; Komata, 1990; Ninomiya, 2002; Spurvey et al., 1998), we had calculated the EUC value of the snow crab, and the value of EUC was 0.51 g MSG/100 g. The EUC value of Chinese mitten crab is about eight times higher than that of the snow crab, which indicated that the umami taste of Chinese mitten crab meat was also stronger than that of the snow crab.

In conclusion, arginine, glycine, alanine, glutamic acid, IMP and AMP were of high TAV greater than 1, and they had strong taste impacts on the crab meat flavour. Glycine and alanine contributed major sweet taste, while glutamic acid, IMP and AMP contributed a strong umami taste. As the TAVs of soluble sugar, succinic acid and bitter free amino acids were lower than 1, and those compounds might be of insignificantly direct impacts to the taste of the crab meat. The EUC of the crab meat was 4.2 g MSG/100 g, which meant that the umami taste of the crab meat was very intense.

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