Effective discrimination of flavours and tastes of Chinese traditional fish soups made from different regions of the silver carp using an electronic nose and electronic tongue

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Effective discrimination of flavours and tastes of Chinese traditional fish soups made from different regions of the silver carp using an electronic nose and electronic tongue

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Abstract: Silver carp is a one of the most important freshwater fish species in China, and is popular when making soup in the Chinese dietary culture. In order to investigate the profile of fish soup tastes and flavours cooked using different regions of the same fish, the silver carp was cut into four different regions: head, back, abdomen, and tail. The differences in taste and flavour of the four kinds of homemade fish soup were investigated by an electronic nose and electronic tongue. The basic chemical components of the different fish regions and the SDS-PAGE profile of the fish soup samples were investigated. Two chemometrics methods (principal component analysis and discriminant factor analysis) were used to classify the odour and taste of the fish soup samples. The results showed that the electronic tongue and nose performed outstandingly in discriminating the four fish soups even though the samples were made from different regions of the same fish. The taste and flavour information of different regions of the silver carp fish could provide the theoretical basis for food intensive processing.

Keywords: fish soup; principal component analysis; discriminant factor analysis

Fish is a healthy food with excellent nutritional values, and provides high quality protein which is easily digestible and absorbable. As one of the most important protein sources in the world, it complements the dietary protein provided by cereals and legumes which are typically consumed in many developing countries. Silver carp (*Hypophthalmichthys molitrix*) has been intensively cultured and it is the second largest commercial fishery in China (Jia et al. 2013). It plays a very important role in providing protein sources due to its large annual production and relatively low cost (Maitena et al. 2004). Furthermore, silver carp is generally regarded as a popular food source for a healthy diet or processing surimi products (Fan et al. 2008; Fan et al. 2009; Zhou et al. 2016). Based on regular Chinese appetites, cooking fish soup is the most favoured style when preparing meals for most families, except when fried, steamed, boiled or grilled. Research shows that about 60% of Chinese households drink soup every day, an equivalent of about 500 million bowls of soup per day. Similar to China, many other countries generally have a dietary habit of drinking soup. Each country

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has its own special "soups", such as borscht in Russia, curry beef soup in United States, and cold soup in Spain, etc. (Sánchez-Moreno et al. 2005; Elez-Martínez et al. 2007; Rui et al. 2009; national standard of the People's Republic of China (GB 5009.5-2010). In China, consumers believe that fish soup has many essential nutrients which can improve or increase human body immune responses. Based on different dietary patterns and appetites, traditional families boil fish soup using different regions of the fish, such as the head, abdomen and tail. Both the taste and the flavour are very important factors that determine the quality of the fish soup. There are various approaches used to analyse the taste and flavour of fish soup, such as the spirit chromatography, gas chromatography (GC), liquid chromatography (LC) and solid phase micro-extraction (Fontanals et al. 2007; Iglesias et al. 2009; Selli et al. 2009) and GC or LC coupled with mass spectrometry (GC/MS or LC/MS).

In recent years, the electronic nose and tongue have mainly been used to analyse and identify the flavour and taste of different foodstuffs. The electronic nose can mimic the sense of smell in mammals, and acts as a very effective way for exploring the major characteristics of flavour profiles of foodstuff (Yang et al. 2016). The electronic tongue is considered as a promising approach that can simulate the human sense of taste for foodstuff analysis, and could obtain further comprehensive information about the analysed object (Yu et al. 2015). The electronic nose and tongue have been successfully applied to the recognition of features and properties of various foodstuffs based on the whole flavour or taste profile information, such as food freshness (Funazaki et al. 1995; Hammond et al. 2002), fruits (Radi et al. 2016; Sanaeifar et al. 2016), vegetables (Berna et al. 2005; Gómez et al. 2006; Yin et al. 2007), beverages (Hu et al. 2016; Nery et al. 2016), edible oil (Cosio et al. 2006; Ghasemi-Varnamkhasti, 2016; Xu et al. 2016), etc.

In the present study, the flavour and taste characteristics of four kinds of fish soups cooked with different regions of the same fish (head, back, abdomen, and tail) were investigated. Biomimetic sensor devices, an electronic nose and electronic tongue were used to explore the flavour and taste profiles of the fish soups, respectively. Two data dimensionality reduction algorithms, principal component analysis (PCA) (Branca et al. 2003; García et al. 2003; Lee et al. 2003) and discriminant factor analysis (DFA) (Lippolis et al. 2015; Kiani et al. 2016) were used to discriminate these four kinds of fish soup samples. Meanwhile, the proximate compositions of different regions of the fish were determined, and the differences among soluble proteins of the four samples were identified using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

MATERIAL AND METHODS

Materials and reagents

Live silver carp (*Hypophthalmichthys molitrix*, ~1 kg) were purchased from a local market in Huazhong Agricultural University and taken to the laboratory in a plastic bag within 15 min. The silver carp was supplied scaled, gutted and washed with tap water several times, then the whole fish body was divided into four portions (head, back, abdomen, and tail). This study was carried out with two batches of fish purchased at one-week intervals. Each batch included three fish, and a total of six silver carp were used for the whole study. All chemicals used were analytical grade.

Preparation of fish soup samples

With a ratio of 1 : 4 (fish **:** water), different regions of a fish as mentioned before were directly cooked and boiled for 1.5 h on weak fire. Then the fish soup was gradually cooled down to room temperature. Four different kinds of fish soup samples were collected into 50 mL polypropylene centrifugal tubes and three parallel samples from each region of the fish were separated. The total of 12 samples were centrifuged (3 200 g) for 15 min. The upper oil film was removed with a syringe, and the consomme fish soup was left. All the samples were stored in a refrigerator at –80 °C for further analysis.

Chemical components analysis

Moisture content. The determination of moisture content was calculated according to the national standard of the People's Republic of China (GB/T 5009.3-2010). Briefly, the fish soup sample was directly dried at 101–105 °C, and the dried sample weighted and the moisture content calculated.

Fat content. The fat content was determined according to the measurement of the People's Republic of China national standards (GB/T 5009.6-2003). Briefly, the fish soup sample (10.0 mL) was hydrolysed with hydrochloric acid (10.0 mL). Then, the sample was extracted with acetic ether, and the quantity after eliminating the solvent was the total free and combined fat.

Ash content. The determination of ash content complied with the measurement of the People's Republic of China national standards (GB 5009.4-2010). Briefly, the fish soup sample (5.0 mL) was initially

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dried in a crucible on a water bath. The crucible with the dried sample was placed on an electric hot plate with low heat until the substance carbonized without smoke. Then the crucible was transferred to a muffle furnace for heating (550 ± 25 °C, 4 h). When it cooled to 200 °C, it was transferred to a desiccator for 30 min. If there were any carbon particles in the residues, then a few drops of water were added and the above steps repeated until there were no carbon particles left. Then the ash content was calculated.

Protein content. The protein content was measured according to the Kjeldahl method following the national standard of the People's Republic of China (GB 5009.5-2010). Briefly, the fish sample was heated and catalytically digested, the protein was decomposed into ammonia, which reacts with sulfuric acid into ammonium sulfate. The ammonia was released by distilling the ammonium sulfate in an alkali solution, and absorbed in boric acid. Then it was titrated with standard sulfuric acid solution. The protein content was calculated by multiplying the volume of the titrant by the conversion factor.

SDS–PAGE

The SDS–PAGE method was determined according to the protocol (Fritz et al. 1989), and the parameters were set as following: separating gel concentration, 10%; concentration of the concentrated gum, 5%; and 10 μL samples. The voltage of electrophoretic conditions was set as following: Initial voltage 65 v, when the bromophenol blue migrated into the separating glue; increased to 90 v, when the dye tracer arrived at about 1 cm from separation with upper rubber soles, stopped the electrophoresis. The gel was stained with coomassie blue R-250 (w/v, 0.1%) in acetic acid (10%) and methanol (45%), destained overnight with gentle shaking in a solution of acetic acid (10%) and methanol (10%).

Electronic nose analysis

The volatile components in the headspace of the fish soup samples were detected by the commercial FOX4000 sensor array system (Alpha MOS., Toulouse, France) to distinguish the dissimilarity between the flavour profiles of the four samples. The electronic nose sensor array consists of an auto-sampling apparatus, a detector unit containing 18 metal oxide sensors (MOS), and a pattern recognition software for data recording and interpretation (Song et al. 2010). Three types of sensors (LY2-type, T-type and P-type) comprised the whole detector system which was located in three temperature-controlled chambers. Sensor chamber CL (High Performance Controlled in temperature) LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, LY2/gCT; Sensor chamber A (High Performance Controlled in temperature) T30/1, P10/1, P10/2, P40/1, T70/2, PA/2; Sensor chamber B (High Performance Controlled in temperature) P30/1, P40/2, P30/2, T40/2, T40/1, TA/2. The reference gas was filtered and dried air, with a purity quotient N99.999%. The pattern recognition software for data recording and interpretation was performed by the Alpha Soft 11.0 software (Alpha MOS, Toulouse, France).

Fish soup samples (2 mL) from different fish regions were loaded into 5 mL glass vessels and immediately sealed with a metal screw lid. Each vessel was first equilibrated to 60 °C for 600 s before injection, and then 2 500 μL of the headspace gas was injected into the sensor chamber at 2 500 μL s^{-1}. Filtered and dried air (purity > 99.999%) was introduced as a carrier gas at a flow rate of 150 mL min^{-1} for electronic nose detection. The data acquisition period lasted for 120 s, and the delay time (about 300 s) was required for system recovery. For each sample, electronic nose detection was repeated five times under the same conditions.

Electronic tongue analysis

In order to identify the taste properties of different fish soups, a commercial electronic tongue α-ASTREE II Liquid Taste Analyser (Alpha MOS., Toulouse, France) was introduced in the current study. It is comprised of seven potentiometric sensors (ZZ, JE, BB, CA, GA, HA, JB by the Alpha MOS company), a reference electrode of Ag/AgCl, a mechanical stirrer to mix up samples, a 48-position auto-sampler (25 mL glass sample vials), an interface electronic module for signal amplification and an analog for digital conversion.

Every fish soup sample was centrifuged at 4 000 rpm for 15 min, and the upper oily film was removed. Clear fish soup (80 mL) was analysed five times by the electronic tongue and each analysis cycle lasted for 120 s. Prior to each analysis cycle, the sensors were firstly rinsed with deionized water, and then NaCl and sodium glutamate calibration solution (0.01 mol L^{-1}) were used as reference samples to monitor and correct the drift of sensors for 120 s, respectively.

Statistical analysis

All statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, USA). The differences among the flavours and taste profiles of the four fish soup samples were analysed using PCA and DFA. All graphs were drawn using the Origin 8.5 (OriginLab Corp., Hampton, USA).

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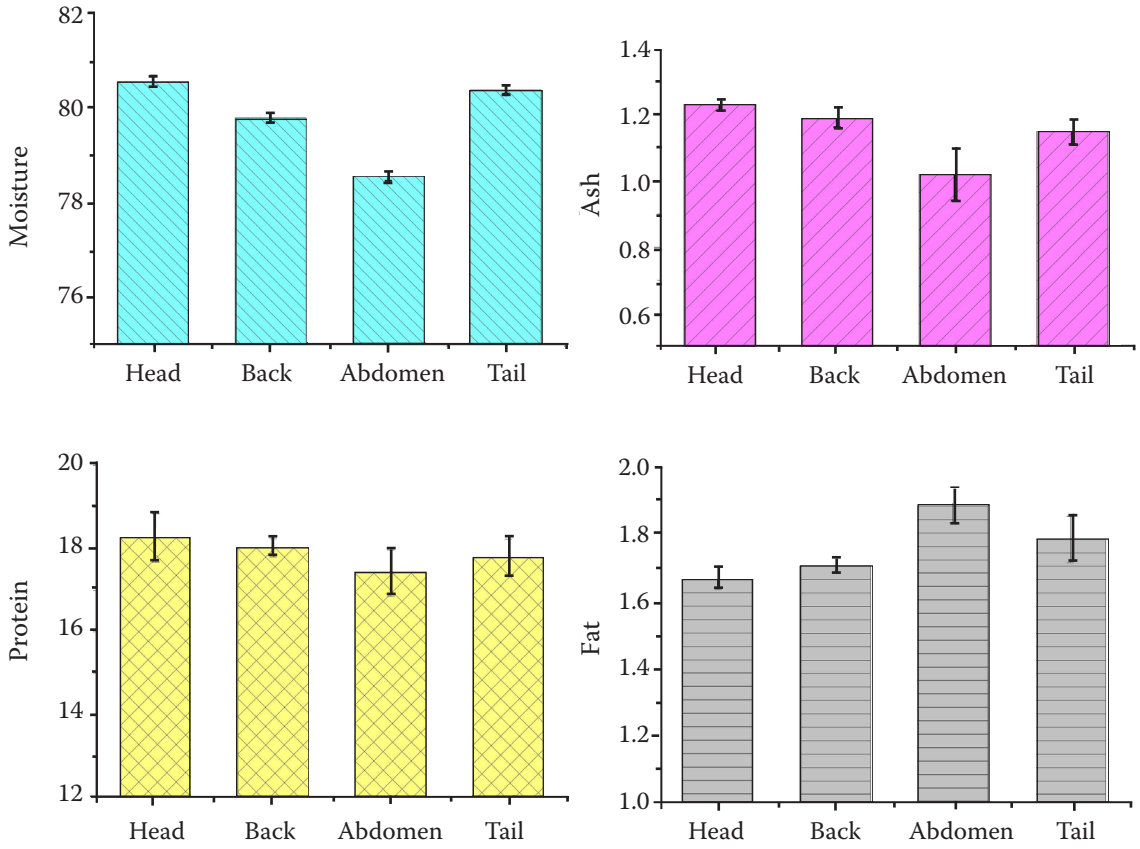

Figure 1. Basic chemical components (moisture, ash, protein, and fat) of different regions of the silver carp (head, back, abdomen, tail)

RESULTS AND DISCUSSION

Chemical compositions of the four different regions of fresh silver carp fish

The contents of four basic components (moisture, ash, fat, and protein) in different kinds of fish regions were determined (Figure 1). From Figure 1, it can be seen that the contents of moisture, ash and protein are highest in the fish head and lowest in the fish abdomen, while the content in the fish back is similar to that in the tail. Furthermore, the amount of fat is highest in the fish abdomen, whereas the other three regions of silver carp fish (tail, back, and head) were on the decrease.

SDS-PAGE analysis for soluble proteins

In general, the component fractions of fish protein can be divided into three categories, sarcoplasmic proteins, myofibrillar protein and connective tissue protein. Sarcoplasmic protein is soluble in water, dilutes in salt solution, and comprises about 20% to 30% of the total protein. The myofibrillar protein constitutes 50% to 55% of the total protein (Foegeding et al. 2002). Collagen is the main component of connective tissue protein making up about 25% of the total proteins (Sikorski et al. 1990). For fish, some of the flavour and taste substances come from solubleproteins which dissolve

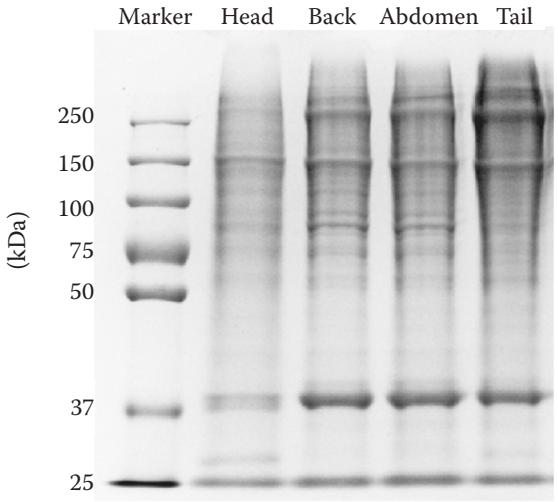

Figure 2. The SDS-PAGE profile of four kinds of fish soup samples made from different regions of the silver carp fish

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in water. Meanwhile, the characteristics of molecular distribution of proteins determine a different composition of flavours and tastes, especially for fish. The molecular distribution profile of soluble proteins dissolved in fish soup samples were investigated using the SDS-PAGE. The results of the SDS-PAGE analysis for four different kinds of fish soups are shown in Figure 2. The molecular weights of proteins dissolved in those four different fish soups are between 10–250 kDa.

As can be seen in Figure 2, the electrophoretic profile shows that, the characteristics of the soluble protein molecular size of the silver carp fish head soup are different from those of the other three kinds of soup. Low molecular weight bands at 38 kDa and 25 kDa are relatively intensive in the profile of protein from the fish head soup, two faint bands appeared at 150 kDa and 28 kDa. Four clearly observed bands were located at 250 kDa, 150 kDa, 38 kDa, and 25 kDa in the fish back, fish abdomen and fish tail soup, respectively.

In the fish back soup, fish abdomen soup and fish tail soup, the appearance of the high molecular weight band (250 kDa) could be responsible for the formation of protein which aggregated in the course of cooking the samples due to protein cross-linking. In the fish head soup, the low molecular weight band (28 kDa) might have been released by original collagen polypeptides from the fish head into the aqueous soup during the cooking process, as the connective tissue degrades readily during cooking. Along with bones, scales, skin, fins, etc., the fish head is usually discarded as inedible, however, in some Asian countries such as China, it is preferred and used to make fish soup as it provides better taste and nutritional value. Unique profiles of protein polypeptides in the fish head soup mean that protein hydrolysates (amino acids and peptides) might be different compared with those of others, which could result in different flavours.

Electronic nose analysis

Response signals of electronic nose sensors. The flavour characteristics of the four kinds of silver carp fish soups were determined by the eighteen metal oxide sensory panelists (LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, LY2/gCT, T30/1, P10/1, P10/2, P40/1, T70/2, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, TA/2). The corresponding intensity values of each sensor are shown in Figure 3. As it can be seen in Figure 3, each different sensor shows different responses when ex-

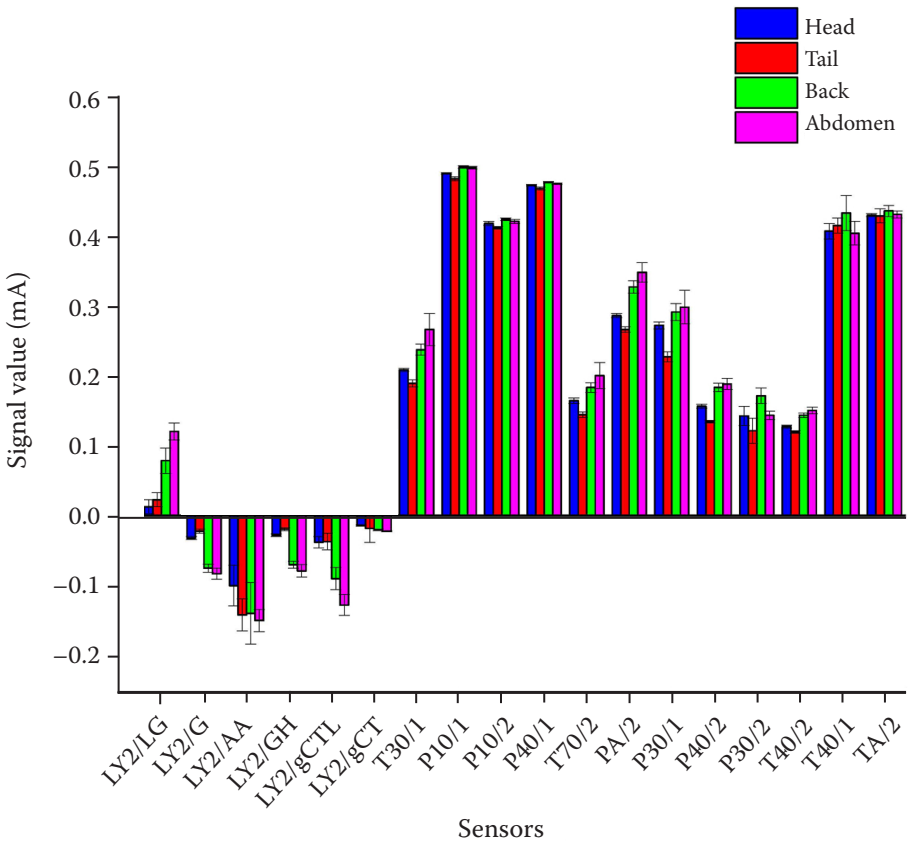

Figure 3. Responses of the flavour sensors of the electronic nose for the four different silver carp fish soup samples

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posed to headspace volatiles emitted from fish soups. Obviously, LY2 type sensors mainly displayed negative values except LY2/LG, which is sensitive to aldehydes. The max negative values were observed for LY2/AA, that is generally sensitive to alcohol, acetone and ammonia (Yu et al. 2012). The majority of positive values responded to the sensors including P-types and T-types. The two maximum positive values were generated by P10/1 and P40/1 sensors, volatile hydrocarbons and aromatic compounds were easily methane and ethane captured by P10/1 and P40/1, respectively. Among the 18 intensities of metal sensors, no significant difference among the four kinds of fish soup samples was observed in P10/1, P10/2, P40/1, TA/2 and LY2/gCT, but in the others. That means the flavours of different fish soup samples including hydrocarbons, methane, and some aromatic compounds were very similar, and were detected by the electronic nose.

Results of PCA and DFA analysis. In order to explore the inner properties which underlie the multiple dimensional data spaces, two effective dimensional reduction algorithms, principal component analysis and discriminant factor analysis were used to consider the response signals of the electronic nose (Guadarrama et al. 2001; Lippolis et al. 2015). The PCA plot is demonstrated in Figure 4 (containing points in total, 5 points per group, and different groups are marked with different colours). The total contribution variance of the first principal component (PC1) and the second principal component (PC2) is 86.153%, which means that the first two PCs already contained sufficient information to reflect the total variance of the whole dataset. As shown in Figure 4, the four kinds of fish soup samples are discriminable, and there is no overlap in the 2D space. The distance between each group is far, indicating that the odours of the different fish

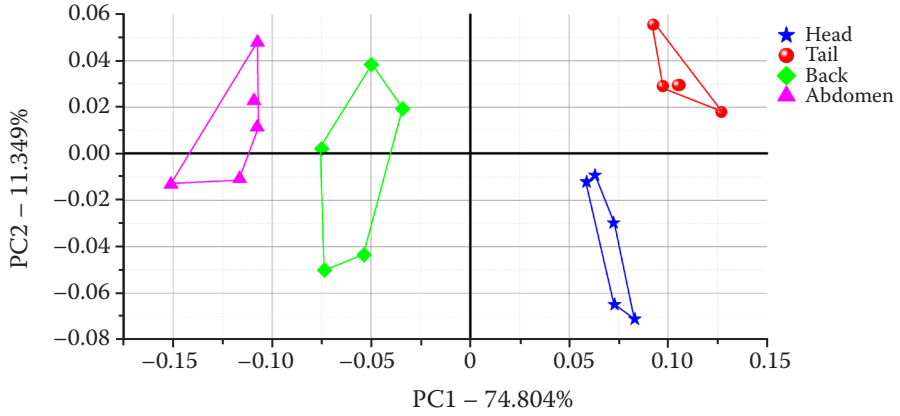

Figure 4. Two-dimensional PCA plot performed on electronic nose data about the different fish soup samples made from different regions of the silver fish

PCA – principal component analysis; PC1 – the first principal component; PC2 – the second principal component

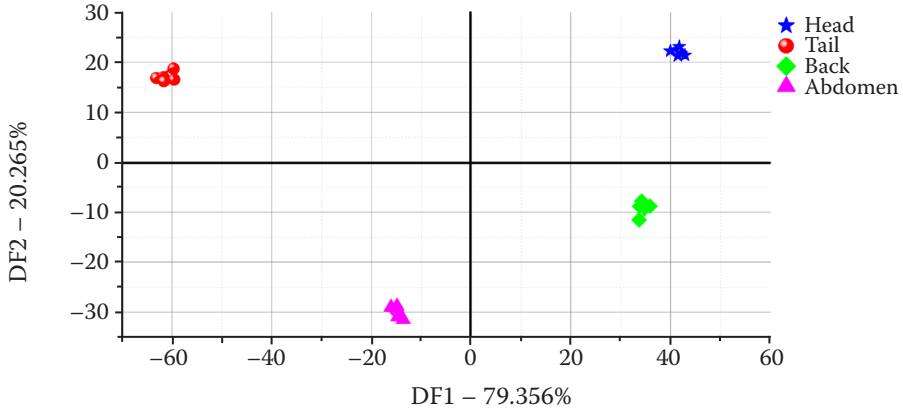

Figure 5. The DFA loading plot obtained from electronic nose data of the four different fish soup samples made from different regions of the silver fish

DFA – discriminant factor analysis; DF1 – the first discriminant factor; DF2 – the second discriminant factor

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soups are completely dissimilar. Therefore, the four fish soups, made from different regions of the silver carp fish, showed their own unique flavour properties, using the electronic nose equipment.

Furthermore, a supervised statistical method named discriminant factorial analysis (DFA) was used to make more reliable recognitions for different fish soup samples. DFA is a technology used to combine the sensor data by re-differentiation. A DFA score plot (Figure 5), with 99.6% of the total explained variance, was developed to evaluate the flavour differences between four kinds of fish soups. The DFA results showed that the samples were scattered in the four quadrants in the whole 2D space, head samples were mainly grouped in the first quadrant, tail samples in the second quadrant, back samples were distributed in the third quadrant and abdomen samples were clustered in the fourth quadrant of the score plot (Figure 5). More importantly, compared to results from the PCA and DFA, both showed that volatile profiles in the headspace emitted from the four fish soup samples were completely different, particularly as the DFA exhibited a more precise discrimination. These data demonstrate that the electronic nose could be used to discriminate the flavours of different regions of a silver carp fish.

Electronic tongue analysis

Response signals of electronic tongue sensors. The taste substance profiles of the four kinds of silver carp fish soups were investigated using the electronic tongue. The average values [means ± standard deviation (SD)] of intensity measured with 7 liquid cross-sensitive sensors (ZZ, JE, BB, CA, GA, HA, and JB) are presented in Figure 6. From Figure 6, it can be observed that each sensor gave a different intensity based on the sensitivity of the sensors to the taste properties in fish soup samples. On the whole, most of sensors did not show any significant difference except for sensors CA and BB. That means sensors CA and BB have the capability to identify the taste of four kinds of fish soup samples made from different regions of a silver carp fish body. According to previous research (Maitena et al. 2004; Fan et al. 2008; Jia et al. 2013), both sensors BB and CA are very sensitive to sweet and acid substances, but the other sensors including CA, HA, JB and BA are only sensitive to acid. That means the different kinds of fish soup samples showed significant differences on sweet characteristics.

Results of PCA and DFA analysis. In fact, a wide array of redundant information of electronic tongue outcomes occur due to taste sensors that are partially cross-sensitive. Most of the redundant information would confuse the discrimination of models. PCA was used to reduce the dimensions of the intensities of seven taste sensors by dropping unnecessary data information, in order to simplify the data analysis process of analysing the problem. The 2D PCA plot is shown in Figure 7 (containing points in total, 5 points per group, and different groups are marked by different colours). The total contribution variance of the first principal components (PC1) and the second principal components (PC2) is 94.471%, indicating that the first two PCs already contained sufficient information that reflect the total variance of the whole dataset. As shown

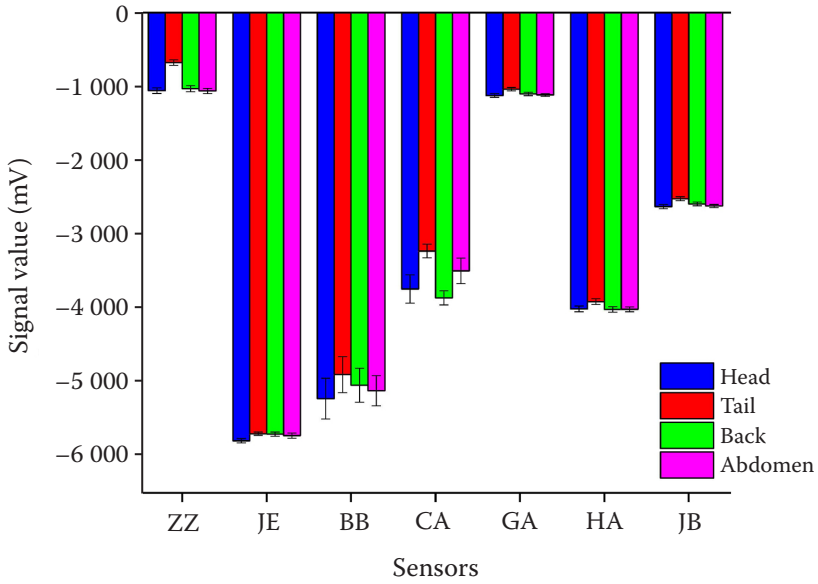

Figure 6. Responses of the taste sensors of the electronic tongue for the four different silver carp fish soup samples

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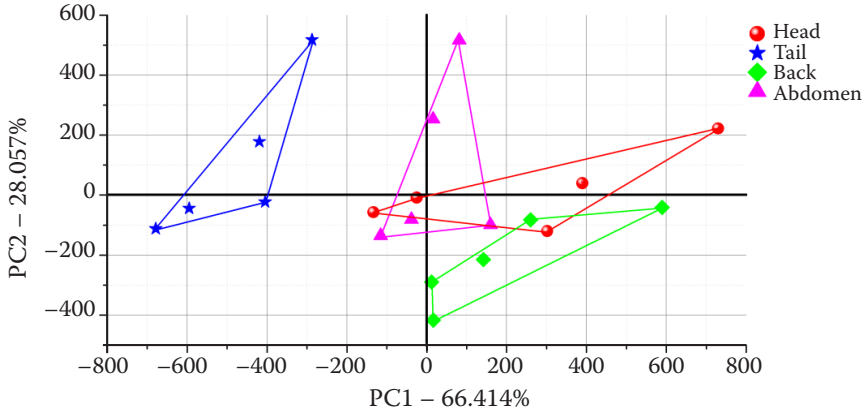

Figure 7. Two-dimensional PCA plot performed on the electronic tongue data about the different fish soup samples made from different regions of the silver fish

PCA – principal component analysis; PC1 –the first principal component; PC2 – the second principal component

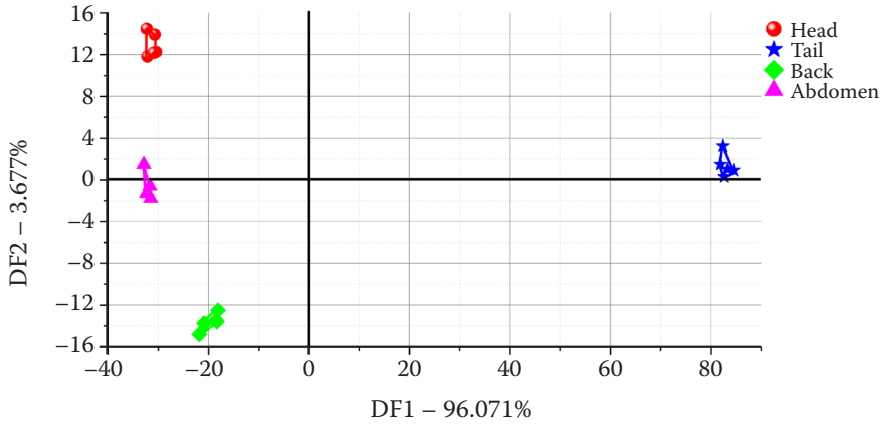

Figure 8. The DFA loading plot obtained from the electronic tongue data of the four different fish soups samples made from different regions of the silver fish

DFA – discriminant factor analysis; DF1 – the first discriminant factor; DF2 – the second discriminant factor

in Figure 7, the four kinds of fish soup samples are clustered together except for the fish head region, and there is so much overlap in the 2D space of the first two PCs. The distance between each group is very close, suggesting that the taste of different fish soups sourced from the silver carp fish regions (back, abdomen and tail) are very similar as detected by the electronic tongue.

Furthermore, a more accurate method, the discriminant factorial analysis (DFA) was used to distinguish the differences in taste of four kinds of fish soup samples. DFA is a technology that combines the sensor data by re-differentiation. A DFA score plot (Figure 8), with 99.75% of the total variance, was developed to evaluate the differences in taste between four fish soup samples. DFA results showed that the four kinds of fish soup were scattered in four quadrants in the whole 2D space. The head samples were grouped in the centre of the first and fourth quadrants, clearly showing the location on the positive x-axis; tail samples were mainly grouped in the second quadrant; back samples were clustered in the third quadrant; abdomen samples were distributed on the negative x-axis of the score plot (Figure 8). It is clear to see that the distance between different groups was very significant, there was no overlap in the DFA 2D space. Compared to the results of the PCA approach, the supervised statistical method DFA had better recognition capability on the tastes of the different kinds of fish soups.

CONCLUSION

In this study, an electronic nose and electronic tongue were used to explore the flavour and taste profiles in four kinds of silver carp fish soups. The exper-

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imental results showed that the electronic nose and tongue have a high capability for discriminating different fish soups, even fish soups made from different regions of the same fish body. Two-dimension reduction methods PCA and DFA gave a well-defined separation or cluster of the four groups in the 2D space. After data analysis, the performance of the PCA was very good in identifying the flavours of the four different fish soup samples, however the results for tastes was not so good. The supervised method DFA was more superior in distinguishing both the favour and taste of the fish soup, the recognition accuracy of DFA was better than that of PCA. Through the determination of four chemical components (moisture, ash, protein, and fat) from different regions of the silver carp fish, there were few differences shown based on the results of the approximate analysis. The molecular weight distribution of protein which dissolved in the fish soup was investigated using SDS-PAGE, the different molecular size profiles of the four regions showed that protein hydrolysis was the main contributor to the flavours and tastes of fish soups. According to these results, the electronic tongue and electronic nose combined with the chemometric methods (PCA and DFA) were good at rapid discrimination of the fish soups sourced from different regions of the silver carp fish. In addition, the taste and flavour information of different regions of the silver carp fish could provide the theoretical basis for food intensive processing.

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