The Study of Analytical Identification on Main Monomer Compounds of Spoiled Grass Carp by High Performance Liquid Chromatography of Quadrupole Time of Flight Mass Spectrometry

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Abstract

**Background:** The change of monomer compounds of materials during preservation is becoming increasingly important in the background of food preservation. It can help to understand underlying causes of quality change of materials in preservation substantially. The objective of this study was to infer and analyze the change of main monomer compounds during spoiled Grass carp (*Ctenopharyngodon Idellus*), and in favor of understanding the quality change of Grass carp in preservation.

**Methods and materials:** The spoiled Grass carp was studied as materials by High Performance Liquid Chromatography of Quadrupole Time of Flight Mass Spectrometry (HPLC-Q-TOF-MS). HPLC-Q-TOF-MS has two scan modes, positive one and negative one. Special related substances and molecular components of this characteristic fragment ion were identified by quasi-molecular ion peaks and accurate molecular weight of fragment ion from high-resolution mass spectrometry.

**Results:** 46 kinds of monomer compounds were determined in spoiled Grass carp, which have 3 kinds of non-nitrogenous compounds and 43 nitrogenous compounds. 43 nitrogenous compounds including 6 kinds of amino acids (2 kinds of α-amino acids), 10 kinds of amines, 12 kinds of amide compounds, 2 kinds of nitro compounds, 12 kinds of heterocyclic nitrogenous compounds, and 1 kinds of nitriles compound.

**Conclusion and suggestion:** Structure of monomer compounds in fresh and perishable materials can be inferred and identified by HPLC-Q-TOF-MS. They can be used to increase efficiency in identification and analysis of chemical component. It will be benefit for identification, evolution, and deduction of active ingredients and new compounds of fresh material in preservation.

Keywords: High performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF MS); Analytical identification; Spoiled; Grass carp; Chemical compounds; Fragment ions

Introduction

Grass carp (*Ctenopharyngodon idellus*) which is one of the four major Chinese carps has been introduced into more than 100 countries [1]. It is an important economic species in freshwater and its global production is more than 4.5 million tons annually [2], it has become the most important freshwater fish in consumption worldwide [3]. Compared to other types of meats, Grass carp is beneficial to human’s body due to its low fat, low cholesterol and high unsaturated fatty acids. However, it is most at risk of damage and perishable as aquatic products because it is easy to cause the spoilage quickly and the freshness loss [4].

Similar to other types of fresh aquatic products, storage conditions play an important role in grass carp quality. Improper storage time and storage environment will bring about decrease in freshness and deterioration of fish, decrease in the freshness of the fish may influence meat quality and taste, or even deterioration that will bring about great loss to fisheries production. The fish spoilage is a multifaceted process; it involves physical, chemical and microorganism mechanisms and is related to color changes, texture collapse, protein denaturation, lipid oxidation, ATP degradation and microbial spoilage [5,6].

At present, There are many indicators in studying fish preservation, such as sensory assessment, cooking quality and structure, salt-soluble protein, thiol, disulfide bond, total volatile base nitrogen (TVB-N), electrical conductivity (EC), total viable counts (TVC), biogenic amines (BAs), freshness quality index (K value), SOD, MDA and ATP enzyme, changes in metal ion content, etc. [7-14], some of these indicators are formulated relevant standards in china, for example, the determination of total volatile base nitrogen (TVB-N) in aquatic products (SC/T3032-2007), the freshness index for fish muscle (K-value)-high performance liquid chromatography (SC/T3048-2014) and so on, they have laid the foundation for the research of preservation of fish and other aquaculture products.

However, during the process of fish preservation, most of these indicators reflected change of the apparent indexes not the internal material compositions, so there is insufficient in describing the preservation process or revealing the change rules.

In fact, the actual changes in apparent indexes are reflected by the existence and quantity of the monomeric compounds which is material foundation. Studying the monomeric compounds is necessary to reveal the changes in apparent indexes. During the period of storage, compounds decomposition and polymerization of Grass carp are always occurred by enzymolysis, microbial metabolism and
temperature and so on as well as other aquatic materials. Thus, low attainment of the high degree of purity of monomeric compounds from fish is main bottleneck in research and has been one of the hot points in the fish study in recent years.

In recent years, with the development of the inspection instruments, the technology which combines both efficient separation of liquid chromatography with high sensitivity of mass spectrometry especially combined high resolution mass spectrometry with multi-stage mass spectrometry has been widely used in plant and animal components analysis and identification, it has opened up a new way on the study of natural products, heavy metals, pesticide residues and so on [15-20].

High Performance Liquid Chromatography of Quadrupole Time of Flight Mass Spectrometry (HPLC-Q-TOF-MS) is a kind of typical technology which has qualitative and quantitative analyses in effective ingredients by the combination of Liquid Chromatography and Mass Spectrometry, this technology can be utilized in the structure analysis of trace components without reference substance, it has highly efficient and sensitive advantages.

In this study, HPLC-Q-TOF-MS was utilized to analyze the main monomeric compounds of Grass carp (*Ctenopharyngodon idellus*) in the process of maintaining the freshness qualitatively; accurate information of molecular weight and fragment was got. At the same time, the main monomeric compounds were inferred according to structure databases of a variety of compound and the cracking rules of mass spectrometry. The aim was to provide the scientific basis for active ingredients identification and preservation research of Grass carp, and to provide a reference for chemical composition analysis and identification of fresh material.

In this experiment, chemical composition of the species and its change rule of Grass carp in different stages like fresh raw materials, preservation process and deterioration were studied. Study of chemical composition in deterioration of Grass carp is as follows.

### Materials and Methods

#### Materials and chemicals

In refrigerator, Grass carp was kept for 6 months during -10°C (fresh Grass carp was bred in Guangzhou Huadu district, weighted 1200 g ± 200 g, obtained from Guangzhou Grandview supermarket, Guangdong, China). It was removed from the refrigerator and placed at room temperature for three days until it smelled badly. At the same time, its content of TVB-N was 32.5 mg/100 g, and identified it as rotten fish according to the National Food Safety Standard of China for Fresh and Frozen Aquatic Animal Products (GB 2733-2015).

Methanol (CH\(_3\)OH) which was purchased from Sigma-Aldrich-(St. Louis, MO, USA) was used as a solvent in LC/MS/MS, it was used throughout the study. Ultra-pure water was produced by Milli-Q type 3 ultra-pure water machines (Millipore, USA).

#### Equipment

Ekspert“ ultraLC type 110-XL HPLC and AB SCIEX Triple TOF “ type 5600 Q-TOF MS, Duo Spray “ ion source, with AB SCIEX- Analyst “ TF Software, Multi Quant “ Software quantitative analysis Software were used. All equipment were supplied by the SCIEX companies in the United States.

Integln Milli-Q type 3 ultrapure water machine was supplied by the Millipore companies in the United States.

### Experiment methods

#### Preparation of sample solution: Firstly, 10 g of spoiled grass carp was obtained by a sterile knife respectively, they were extracted with 100 ml ultrapure water and 100 ml chromatographic pure methanol respectively, refluxing extraction (extraction temperature is 95°C in ultrapure water and 70°C for reflux in chromatographic pure methanol, respectively) for 2 hours. The extract was stand for 24 h during 0°C, then centrifuged it (12 000 r. min-1, 5 min). At the last, supernatant were filtered through 0.35μm filters and analyzed by HPLC–MS. Blank is frequently prepared as described above.

#### Analysis of test conditions

**HPLC analysis:** The chromatographic separation was carried on ZORBAX®RPHD Eclipse pluse C18 column (2.1 mm × 100 mm, 1.8 μm particle size). The mobile phase, which consisted of methanol (A) and 0.1% formic acid in water (B) was delivered at a flow rate of 0.5 mL/min under the following gradient program: 10% (A) from 0.1 to 5 min, 20% (A) from 5 to 10 min, 25% (A) from 10 to 15 min, 30% (A) from 15 to 20 min, and returning to the initial condition over 5 min. The sample injection volume was 10 μL. The column oven temperature was set at 40°C.

**MS analysis:** Each sample was analyzed in positive and negative ionization modes. Column effluent was directed to the ESI source. The curtain gas, nebulizer gas, and heater gas were set to 30, 50, and 55 psi. Source temperature was 550°C for both modes. In an ESI source, positive ion mode produced 4500V atomizing voltage (ISVF) and 100 V declustering potential; negative one produced -4500 V atomizing voltage (ISVF) and -100 V declustering potential.

The experiment was carried out to collect by using one TOF MS survey scan (250 ms) and 4 TOF MS/MS scans (100 ms each). The scan types of TOF MS was 100-1000 m/z, and the scan types of TOF MS/MS was 50-1000 m/z. Acquisition of MS/MS spectra was controlled by IDA function of the Analyst TF software (AB Sciex, Concord, Canada) with application of following parameters—dynamic background subtraction, charge monitoring to exclude multiply charged ions and isotopes, and dynamic exclusion of former target ions for 5 s. Rolling collision energy was set whereby the software calculated the CE value to be applied as a function of m/z. Data quality was corrected by CDS system (automated calibration delivery system, SCIEX, Concord, Canada) under Duospray source.

**Data processing:** MarkerView 2.0 (AB Sciex, Concord, Canada) was used to generate a peak table of m/z and RT for samples in the individual study using the following parameters. For peak detection: noise threshold of 50 counts, minimum chromatographic peak width of 3 scans, minimum spectra width of 10 mDa, background subtraction offset of 20 scans and subtraction multiplication factor of 1.2. For peak alignment: RT window of 0.7-10 min, RT tolerance of 0.3 min, mass tolerance of 12 ppm.

### Results and Discussion

**HPLC-Q-TOF-MS analysis of spoiled grass carp**

It is important to prevent monomeric compounds of spoiled Grass carp degrade and accumulate; it is the basis of the component identification. In this experiment, ultrapure water and methanol were used as solvent extractions at a relatively low temperature (reflux extraction temperature of ultrapure water is 95°C, and one of chromatographic pure methanol is 70°C). Then, scanning test of extract solution is processed by positive and negative ion modes, respectively.
The results showed that the characteristic information of the total ion current (TIC) under positive ion modes was stronger and with higher sensitivity than negative one in the methanol extract, and information of compounds in the methanol extract was more than one in aqueous extraction. Therefore, methanol extract under the scanning mode of positive ion (Fig. 1) were compared, analyzed and identified.

The analytical identification of main monomer compounds in spoiled grass carp

In experiment, possible elements (error is less than \( \pm 5 \times 10^{-5} \)) was calculated by the combination of the precise molecular mass from test of ESI positive ion mode with the high resolution data given by peakview 2.0 workstation. Then possible molecular formula of the main monomeric compounds was determined. The characteristic fragment ions and accurate chemical elements of the main monomeric one were obtained by the secondary mass spectrometry analysis.

46 kinds of monomeric compounds included 43 nitrogenous compounds and 3 non-nitrogenous compounds were identified by Combination of Chemspider database and fragment cracking rules of mass spectrometry. 43 nitrogenous compounds including 6 kinds of amino acids (2 kinds of α-amino acids), 10 kinds of amines, 12 kinds of amide compounds, 2 kinds of nitro compounds, 12 kinds of heterocyclic nitrogenous compounds and 1 kinds of nitriles compound. Cracking way of TOF MS/MS fragment ions for each compound were inferred the characteristics were shown in Tables 1 and 2.

| No. | RT (min) | Molecular formula | Molecular weight (u) | Precursor ions \([M+H]^{+}\) (m/z) | The main fragment ions of TOF-MS/MS | Observed (u) | Theoretical (u) |
|-----|---------|-------------------|----------------------|-------------------------------|----------------------------------|--------------|----------------|
| 1   | 0.96    | C₁₇H₂₃NO       | 271.1781             | 100.07645                     | 120.0765, 86.0964                |              |                |
| 2   | 2.15    | C₂₀H₂₉NO       | 325.2381             | 103.12331                     | 103.1233, 86.0964                |              |                |
| 3   | 2.59    | C₁₇H₂₄N₂       | 284.1941             | 105.0704                      | 105.0708, 79.0556                |              |                |
| 4   | 0.56    | C₁₈H₂₅NO       | 302.2079             | 114.06642                     | 114.06618, 86.0729              |              |                |
| 5   | 0.78    | C₁₉H₂₆NO       | 310.2229             | 120.07097                     | 120.0806, 93.0716                |              |                |
| 6   | 1.89    | C₂₀H₃₂O        | 324.2381             | 121.06468                     | 121.0650, 93.0705, 77.0407      |              |                |
| 7   | 2.6     | C₂₁H₂₅N₂       | 315.2229             | 122.06964                     | 122.0695, 105.0710              |              |                |
| 8   | 0.57    | C₂₀H₂₅NO       | 302.2079             | 136.04588                     | 137.0460, 119.0354, 94.0407     |              |                |
| 9   | 1.79    | C₁₈H₂₃NO       | 291.1832             | 144.04776                     | 144.0478, 126.0371, 113.0300    |              |                |
| 10  | 2.71    | C₁₉H₂₆N₂       | 300.2229             | 144.08068                     | 144.0807, 128.0497, 103.0546    |              |                |
| 11  | 1.34    | C₂₀H₂₄N₂       | 310.2079             | 145.13337                     | 145.1334, 128.1080, 86.0978     |              |                |
| 12  | 2.58    | C₂₁H₂₇N₂       | 324.2381             | 145.16961                     | 145.1695, 86.979, 69.0719, 60.0827 |              |                |
| 13  | 0.52    | C₂₁H₂₅N₂       | 315.2229             | 146.11728                     | 146.1182, 87.0455, 60.0834      |              |                |
| 14  | 0.53    | C₂₁H₂₆NO       | 317.2081             | 159.14872                     | 159.1488, 142.1227, 86.0615     |              |                |
| 15  | 0.77    | C₂₂H₂₇N₂       | 326.2139             | 160.08598                     | 160.0860, 120.0813, 103.0547    |              |                |
| 16  | 0.92    | C₂₂H₂₉NO       | 328.2079             | 168.11287                     | 168.1127, 150.1028, 112.0872    |              |                |
| 17  | 2.27    | C₂₃H₂₇N₂       | 339.2139             | 173.16472                     | 173.1647, 156.1377, 100.751    |              |                |
| 18  | 1.5     | C₂₄H₂₉NO       | 348.2139             | 182.12861                     | 182.1285, 164.1193, 95.0607     |              |                |
| 19  | 8.14    | C₂₅H₃₁N₂       | 355.2229             | 183.07811                     | 183.07755, 97.9687              |              |                |
| 20  | 2.12    | C₂₅H₂₉NO       | 353.2081             | 188.07371                     | 188.0737, 131.1180, 117.1027    |              |                |
| 21  | 2.2     | C₂₆H₃₁NO       | 362.2139             | 188.17254                     | 188.1752, 152.0522, 117.1014    |              |                |
| 22  | 0.54    | C₂₆H₃₃NO       | 366.2079             | 189.15992                     | 189.1599, 144.1348, 130.0867    |              |                |
| 23  | 1.7     | C₂₇H₃₅N₂       | 370.2139             | 201.15954                     | 201.1595, 183.1488, 159.1495    |              |                |
| 24  | 0.53    | C₂₇H₃₃NO       | 368.2079             | 207.98815                     | 207.9882, 189.9757, 165.9665    |              |                |
| 25  | 2.76    | C₂₈H₃₅NO       | 374.2139             | 215.17458                     | 215.1749, 159.1490, 129.9939    |              |                |
| 26  | 10.13   | C₂₉H₃₇O₂       | 414.2381             | 217.10692                     | 217.10689, 137.0812, 156.0709, 111.0450, 83.0501 |              |                |
| 27  | 14.48   | C₂₉H₃₇NO       | 412.2381             | 228.23206                     | 228.2321, 88.0757, 70.659       |              |                |
| 28  | 0.56    | C₃₀H₃₉NO       | 420.2431             | 229.15465                     | 229.15465, 96.0823              |              |                |
| 29  | 5.71    | C₃₀H₄₁NO       | 422.2581             | 229.19083                     | 229.19093, 156.1383, 142.1231, 129.9960, 100.762 |              |                |
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Table 1: Dates and the main fragment ions from HPLC-Q-TOF-MS.

| No. | Derivation pathways of the main fragment ions | Classification |
|-----|---------------------------------------------|----------------|
| 1   | [M+H-HO\_2]^+82.0670, [M+H-HO\_2-CN]^+56.0517 | Heterocyclic nitrogenous |
| 2   | [M+H]^+103.1233, [M+H+NH\_3]^+86.0964 | Amines |
| 3   | [M+H]^+105.0708, [M+H-C\_2\_H\_4\_N\_O]^+79.0556 | Non-nitrogenous compounds |
| 4   | [M+H-HCOOH]^+117.1054 | Heterocyclic nitrogenous |
| 5   | [M+H]^+120.0815, [M+H+CN]^+93.0716 | Heterocyclic nitrogenous |
| 6   | [M+H]^+121.0650, [M+H-C\_2\_H\_4\_N\_O]^+77.0407 | Non-nitrogenous compounds |
| 7   | [M+H]^+122.0695, [M+H+NH\_3]^+105.0710 | Amines |
| 8   | [M+H]^+137.0460, [M+H-HO\_2]^+119.0354, [M+H+H\_2\_O-N\_H\_3]^+94.0407 | Heterocyclic nitrogenous |
| 9   | [M+H-HCOOH]^+144.0478, [M+H+O\_2]^+105.0374, [M+H+O\_2+H\_2\_O]^+87.0382 | Heterocyclic nitrogenous |
| 10  | [M+H]^+144.0807, [M+H+H\_2\_O]^+128.0497, [M+H+H\_2\_O]^+110.0546 | Non-nitrogenous compounds |
| 11  | [M+H]^+145.1334, [M+H-HCOOH]^+128.1080, [M+H+H\_2\_O]^+86.0978 | Heterocyclic nitrogenous |
| 12  | [M+H]^+145.1695, [M+H-C\_2\_H\_4\_N\_O]^+86.0979, [M+H+H\_2\_O]^+60.0827 | Amines |
| 13  | [M+H]^+146.1182, [M+H+H\_2\_O]^+87.0455, [M+H+H\_2\_O]^+60.0834 | Amines |
| 14  | [M+H]^+159.1488, [M+H+H\_2\_O]^+142.1227, [M+H+H\_2\_O]^+86.0615 | Amines |
| 15  | [M+H]^+166.0860, [M+H+HCOOH]^+120.0813, [M+H+HCOOH]^+110.0547 | Amino acids |
| 16  | [M+H]^+168.1127, [M+H+H\_2\_O]^+150.1028, [M+H+H\_2\_O]^+112.0872 | Nitriles compound |
| 17  | [M+H]^+173.1647, [M+H+H\_2\_O]^+156.1377, [M+H+H\_2\_O]^+100.0751 | Amines |
| 18  | [M+H]^+182.1286, [M+H+H\_2\_O]^+164.1193, [M+H+H\_2\_O]^+95.0607 | Amines |
| 19  | [M+H-HCOOH]^+155.0465, [M+H-HCOOH]^+127.0414, [M+H-HCOOH]^+97.9687 | Nitro compounds |
| 20  | [M+H]^+188.0737, [M+H+H\_2\_O]^+131.1180, [M+H+H\_2\_O]^+117.1027 | Amines |
| 21  | [M+H]^+188.1752, [M+H+H\_2\_O]^+152.0522, [M+H+H\_2\_O]^+117.1014 | Amines |
| 22  | [M+H]^+189.1599, [M+H+H\_2\_O]^+144.1384, [M+H+H\_2\_O]^+130.0867 | Amid compounds |
Table 2: The N compounds mainly fractured fragment ions derivation and classification.

| Compound Type          | Formula                        | Fragment Ions                        |
|------------------------|--------------------------------|--------------------------------------|
| Amino acids            | [M+H]+                         | 171.1409, [M+H-H2O]+100.0764         |
|                        | [M+H-C4H8]+159.1490           | [M+H-C4H8-C4H8]+129.9960            |
|                        | [M+H-C2H5O]+142.0861          | [M+H-C2H5O-C2H5]+114.0939           |
|                        | [M+H-H2O]+142.0861            | [M+H-C2H5O-CH2]+100.0762            |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |
|                        | [M+H]+                        | 173.1662, [M+H-C4H8]+173.0812       |
|                        | [M+H-C4H8]+173.0812           | [M+H-C4H8-C4H8]+140.0336            |
|                        | [M+H-C2H5O]+173.0812          | [M+H-C2H5O-C2H5]+140.0336            |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |
| Amide compounds        | [M+H]+                        | 177.0596, [M+H-C4H8]+175.0475       |
|                        | [M+H-C4H8]+175.0475           | [M+H-C4H8-C4H8]+142.0861            |
|                        | [M+H-C2H5O]+175.0475          | [M+H-C2H5O-C2H5]+142.0861            |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |
| Amino acids            | [M+H]+                        | 171.1409, [M+H-H2O]+100.0764         |
|                        | [M+H-C4H8]+159.1490           | [M+H-C4H8-C4H8]+129.9960            |
|                        | [M+H-C2H5O]+142.0861          | [M+H-C2H5O-C2H5]+114.0939           |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |
| Amide compounds        | [M+H]+                        | 177.0596, [M+H-C4H8]+175.0475       |
|                        | [M+H-C4H8]+175.0475           | [M+H-C4H8-C4H8]+142.0861            |
|                        | [M+H-C2H5O]+175.0475          | [M+H-C2H5O-C2H5]+142.0861            |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |
| Amino acids            | [M+H]+                        | 171.1409, [M+H-H2O]+100.0764         |
|                        | [M+H-C4H8]+159.1490           | [M+H-C4H8-C4H8]+129.9960            |
|                        | [M+H-C2H5O]+142.0861          | [M+H-C2H5O-C2H5]+114.0939           |
|                        | [M+H-C10H20]+88.0757          | [M+H-C10H20-H2O]+68.0757            |
|                        | [M+H-C10H20-H2O]+70.659       | Amide compounds                      |

Notes: The name of the compound is the same as Table 1.

Figure 1: TIC in methanol extract from spoiled Grass carp under the positive ion mode.
took off NH$_2$ and COOH, redundant H reset to the other atoms), and to produce fragment ions at m/z 298.1756; the second one was to take off the peptide chain and lost C$_6$H$_{13}$NO (115 u), then to create fragment ions at m/z 142.0863, 124.0757 and 96.0814 m/z. Its possible fragment pathways were shown in Figure 3.

Amines: Amines are formally derivatives of ammonia, wherein one or more hydrogen atoms have been replaced by a substituent such as an alkyl or aryl group. Molecular ion of Amines is formed by loss of the electrons of N atom. β-fragment is the most fragment pathway in collision induced dissociation. R group (substituent group) of fat amine was lost easily, CH$_2$ = CH$_2$ (28u) group of ring amine was lost easily, too, and HCN (27u) of aromatic amine was lost easily [25]. 10 amine compounds were analyzed and identified in this experiment. Using 1,8-Diaminoctane as an example, it was produced the proton molecular ion peak [1,8-Diaminoctane +H]$^+$ (m/z145.1695) by electrospray ionization. The MS$^2$ spectrum shown in Figure 4. In MS/MS spectrum, under collision induced dissociation, the [1,8-Diaminoctane +H]$^+$ lost C$_3$H$_9$N (59 u) firstly to produce ions at m/z 86.09, then the fragment ions fractured to lose NH$_3$ (17u) so as produced ions at m/z 69.07. Or C$_5$H$_{11}$N (85 u) was lost to produce fragment ions at m/z 60.08 ions which is stable and highly abundant. Its possible fragment pathways were shown in Figures 4 and 5.
Amide: Amide is a special form of amines; the simplest amides are derivatives of ammonia wherein one hydrogen atom has been replaced by an acyl group. Amide can also be considered as compounds that hydroxyl group of carboxylic acid molecules are replaced by amino or phenyl group. Its fragment pathway was similar to carboxylic acid, McLafferty rearrangement is the important pathway among them [26]. 12 amide compounds were analyzed and indentified in this experiment. For example, Propamocarb produced the proton molecular ion peak [Propamocarb+H]^+ (m/z 189.1599) by electrospray ionization. The MS^2 spectrum is shown in Figure 6.

In MS/MS spectrum, under collision induced dissociation, the [Propamocarb+H]^+ ions lost C_2H_6NH (45u) firstly to produce ions at m/z144.1384, then the fragment ions continue to fractured to lose CH_3 (14u) and CH_2O (56u)and formed ions at m/z130.0867 and m/z84.0819. Its possible fragment pathways were shown in Figure 7.

Nitro compounds: Nitro compounds are organic compounds that contain one or more nitro functional groups (−NO₂), mainly including aliphatic and aromatic nitro compounds. It has a little or no proton molecular ion peak. The ion peaks of M-OH, M-CO and M-NO were easy produced in MS/MS spectrum by rearrangement of r-hydrogen atoms [27]. 2 nitro compounds were identified in this experiment. For example, the obvious proton molecular ion peak was not produced by 4-Methoxy-N-methyl-2-nitroaniline of electrospray ionization. The MS^2 spectrum is shown in Figure 8.

In MS/MS spectrum, under collision induced dissociation the [4-Methoxy-N-methyl-2-nitroaniline +H]^+ ions lost C_6H_5 (45u) firstly to produce ions at m/z78.0924, then the unstable ions at m/z127.0157 was formed by CO (28u) lost and ions at m/z 98.9851 was formed by C_H_3 (28u) lost. Its possible fragment pathways were shown in Figure 9.

Other compounds containing N: 13 other nitrogen compounds which include 12 N-heterocycles and 1 nitriles were identified in this experiment. Although the fragment pathways of other nitrogen compounds are complicated, there is a general rule in the secondary mass spectrometry, that [M+H]^+ was broken into different daughter ions, by the loss of neutral molecules such as H_2O, 2H_2O, 3H_2O, CH_3OH, 2CH_3OH, 3CH_3OH, CO, CO_2, CHNH_2, CHCH_2, NO, NH_3 would be lost [28]. The details are shown in Table 2.

Non-nitrogenous compounds: 3 Non-nitrogenous compounds were identified in this experiment. Non-nitrogenous compounds were not identified in fresh fish and in the prophase of fish preservation processing, non-nitrogenous compounds which were not identified were produced of loss of N compounds in the process of preservation (Specific data published article). Thus, the quantity of non-nitrogenous compounds (type and quantity) can be used as an index of fresh fish. For example, 4-Acetyl-4-methylheptanedioic acid proton molecular ion peak [4-Acetyl-4-methylheptanedioic acid +H]^+ (m/z 217.1071) was produced by electrospray ionization. The MS^2 spectrum is shown in Figure 10.
Figure 8: MS² spectrum of 4-Methoxy-N-methyl-2-nitroaniline under positive ion mode.

Figure 9: Fragmentation pathways of 4-Methoxy-N-methyl-2-nitroaniline under positive ion mode.

Figure 10: MS² spectrum of 4-Acetyl-4-methylheptanedioic acid under positive ion mode.

Figure 11: Fragmentation pathways of 4-Acetyl-4-methylheptanedioic acid under positive ion mode.
In MS/MS spectrum, the [4-Acetyl-4-methylheptanedioic acid +H]+ ions first lost CH3COOH (60u) to produce ions at m/z 256.9781 by collision induced dissociation, then the fragment ions lost COOH (45u) to produce ions at m/z 211.0450 and CH3 (28 u) continued to fractured to produce ions at m/z 83.0514. Or it may lose CH2O, (44u) to produce ions at m/z 173.0791. Its possible fragment pathways were shown in Figure 11.

Conclusion

46 kinds of monomeric compounds has which 3 kinds of non-nitrogenous compounds, 43 nitrogenous compounds in bad grass carp were determined by HPLC-Q-TOF-MS. 43 nitrogenous compounds includes 6 kinds of amino acids (2 kinds of α-amino acids), 10 kinds of amines, 12 kinds of amide compounds, 2 kinds of nitro compounds, 12 kinds of heterocyclic nitrogenous compounds and 1 kinds of nitriles compound.

Compared with the low-resolution MS methods such as quadrupole, triple quadrupole and ion trap mass spectrometry, HPLC-Q-TOF-MS has relative high resolution and the extraction functions of ion characteristics, it can measure mass of both parent ion and fragment ions accurately. And it can provide selectivity because it has ability to discriminate peak which has same nominal masses but different exact masses between interference and mass peaks having similar [29,30]. Consequently, the role of Q-TOF MS/MS instruments has high efficiency on identifying non-target compounds in complex matrices when the reference compounds were unavailable [29,30].

HPLC-Q-TOF-MS has less complex pretreatment such as excessive chromatographic separation and simplifies the research process, increases the efficiency of analysis [30]. For fresh materials like Grass carp, it is difficult to obtain monomer compounds of high purity by traditional extraction, separation and purification and is easy to decompose and polymerize the compounds in traditional one. Therefore, HPLC-Q-TOF-MS used have important significance for studying monomeric compounds.

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