Journal of Food Bioactives
International Society for Nutraceuticals and Functional Foods
Original Research
J. Food Bioact. 2023;23:46–57

Comprehensive profiling of amino and carbonyl compounds in the Hong Yao sour soup, a type of fermented sour soup, in Huangluo Yao Village of Guangxi, China

Zicai Wang[a], Xujun Shi[b] and Kenji Sato[a*]

[a]Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Kyoto 606 8502, Japan
[b]Guilin Chang Fa Xiao Zhai Biotechnology Co., Ltd, Guilin 541700, China
[*]**Corresponding author:** Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Kyoto 606 8502, Japan. E-mail: sato.kenji.7x@kyoto-u.ac.jp
DOI: 10.31665/JFB.2023.18353
Received: September 11, 2023; **Revised received & accepted:** September 30, 2023
Citation: Wang, Z., Shi, X., and Sato, K. (2023). Comprehensive profiling of amino and carbonyl compounds in the Hong Yao sour soup, a type of fermented sour soup, in Huangluo Yao Village of Guangxi, China. J. Food Bioact. 23: 46–57.

Abstract

Fermented sour soup is a traditional condiment in daily southwestern Chinese cuisine. Compounds found in Hong Yao sour soup were fractionated using size-exclusion chromatography. Amino compounds were identified through derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and liquid chromatography tandem mass-spectrometry (LC-MS/MS) with precursor scan, followed by identification using product ion scan. Other compounds lacking amino groups were isolated using solid-phase extraction with a strong cation exchanger and detected through LC-MS in full scan mode. Carbonyl compounds, including carboxylic acids, were detected and identified by derivatization with 2-nitrophenylhydrazine and LC-MS/MS. Besides amino acids and carboxylic acids including lactic and acetic acid, amino acid metabolites such as monoamines (tyramine, pyrrolidine, tryptamine), polyamines (cadaverine, putrescine), *N*-acetyl- and *N*-lacoyl-amino acids, and α-keto acids from Val, Ile, and Leu were also detected. However, unlike Japanese rice wine, only a few dipeptides were detected. This information aids in understanding the unique characteristics of Hong Yao sour soup.

Keywords: Hong Yao sour soup; Rice; Fermentation; Amino acid metabolites; Traditional Chinese food.

1. Introduction

Fermented sour soup, a traditional condiment in southwestern Chinese cuisine, is divided into two types based on ingredients and fermentation methods. The first type, red sour soup, is made from glutinous rice flour and vegetables such as tomatoes, red peppers, garlic, and ginger (Lin et al., 2020). An example of red sour soup is Kaili Hong Suan Tang. The second type, white sour soup, predates red sour soup and is primarily made from raw rice or glutinous rice flour without additional ingredient (Zhou et al., 2022b). Despite the differences, both types develop a sour taste after fermentation. A millennium ago, due to the humid climate and scarcity of salt, people in present-day Guizhou and Guangxi Province incorporated white sour soup into their diets as condiments (Wang et al., 2020). These fermented soups were believed to prevent illnesses like diarrhea and dysentery (Lin et al., 2022). Today, fermented sour soups are considered functional foods, reputed to aid digestion, boost appetite, and prevent obesity (Yuan et al., 2022; Zhou et al., 2022a). Moreover, fermented sour soup could be applied topically to prevent hair loss and maintain hair black.

Huangluo Yao Village, popularly known as "the long hair village," resides approximately 100 km from Guilin in the Longsheng Various Nationalities Autonomous County within northern Guangxi Zhuang Autonomous Region of China. This village is home to the Hong Yao ethnic group, among whom most women follow the tradition of maintaining long hair. The average hair length for adult Hong Yao women is 1.4 meters, with some reaching an impressive 2.3 meters. This earned them recognition from

Guinness World Records as the "longest hair group. " These women are renowned not only for their long hair but also for enduring black color even into their 80s. They attribute this hair care to their use of Hong Yao sour soup, a type of white sour soup, made from rice washing water at each home. A typical traditional preparation method in home is as follows. The rice washing water is boiled on a stove before fermentation. After cooling, it is fermented at room temperature for a few days without using a starter. Fermentation is facilitated by airborne bacteria. In Huangluo Yao Village, this soup doubles as a condiment and a hair treatment.

In recent years, metabolomics techniques involving gas chromatography-mass spectrometry (GC-MS) have gained traction for analyzing bioactive and flavor components. Several studies utilizing GC-MS have revealed the presence of organic acids, esters, alcohols, terpenoids, and amino acids in red sour soup (Wang et al., 2023). However, there is limited knowledge about the compounds within Hong Yao sour soup, which has been associated with maintaining black hair. Fermented products often contain an array of compounds, making their isolation and identification complex, even with GC-MS and LC-MS analyses. In our prior research, we employed LC-MS/MS in precursor ion scanning mode along with derivatization techniques to identify compounds with distinct chemical groups in intricate matrices, such as Japanese fermented soy paste, miso (Shirako et al., 2020). Our study aims to characterize amino and carboxyl compounds in Hong Yao sour soup using this approach to identify potential agents with hair-protecting properties.

2. Materials and methods

2.1. Sample

The Hong Yao sour soup was provided by Guilin Chang Fa Xiao Zhai Biotechnology Co., Ltd, (Guangxi, China). Large-scale preparation method is outlined in the Supporting information (Figure S1). Briefly, the Hong Yao soups were industry prepared essentially in the same way as traditional method except for the use of homemade Hang Yao soup as starter. For quality control of the fermentation process, pH and total acid number (TAN) are controlled to pH 2.5–3.0 and TAN>3.0, respectively. The sample was subjected to centrifugation at 17,000 *g* for 10 min at 4°C. The resulting supernatant was stored at −30 °C and utilized for subsequent experiments.

2.2. Reagents

Amino acids mixture standard solution (type H), triethylamine (TEA), phenyl isothiocyanate (PITC), n-butyric acid, isocaproic acid, propionic acid and sodium pyruvate were obtained from Wako Pure Chemical Industry (Osaka, Japan). 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ) was obtained from Toronto Research Chemicals (Toronto, Canada). Hydrochloric acid (6 mol/L), ammonium acetate, formic acid, 4-amino-n-butyric acid (GABA), ethanolamine, tyramine, phenyethylamine, cadaverine, histamine, putrescine, spermidine, spermine, acetic acid, lactic acid, malic acid, n-caproic acid, succinic acid, heptanoic acid, acetonitrile (HPLC grade), methanol, and ethanol were purchased from Nacalai Tesque. (Kyoto, Japan). Glyceric acid, lithium acetoacetate, pyrrolidine, tryptamine, 4-methyl-2-oxovaleric acid, and *N*-acetyl-L-glutamic acid (*N*-Ac-Glu) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Methionine sulfoxide, 2-ketobutyric acid, sodium 3-methyl-2-oxobutyrate, and 4-methyl-2-oxovaleric acid were purchased from Sigma Aldrich (St. Louis, MO, USA). The carboxylic acids assay kit, comprising 20 mM 2-nitrophenylhydrazine hydrochloride (2-NPH·HCl) in ethanol (reagent A) and 0.25 M 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) in ethanol/pyridine 97:3 (v/v) (reagent B), was purchased from YMC (Kyoto, Japan). Other chemicals used in this study were of analytical grade. Water purified through the Milli-Q system (Merck Millipore, Burlington, MA) was employed.

2.3. Amino acid analysis

Amino acid analysis was conducted using the percolumn PITC derivatization method with some modifications (Aito-Inoue et al., 2006). Glass tubes (50 mm × 6 mm i.d.) for hydrolysis were pretreated with 6 M HCl at 150°C for 1 h under vacuum. Following pretreatment, 20 µL of the sample were pipetted into the glass tubes and dried under vacuum. After drying, hydrolysis was performed using HCl vapor at 150 °C under vacuum for 1h, followed by removal of HCl by vacuum. To each tube containing the hydrolysate or non-hydrolysate, 10 µL of an alkaline solution consisting of methanol, TEA, and water in a ratio of 7:2:1 was added and then dried. The dried sample was reacted with a 20 µL derivatizing solution consisting of methanol, TEA, water, and PITC in a ratio of 7:1:1:1 for 20 min at 25 °C. Excess reagent was removed by vacuum, and the residue was dissolved in 200 µL of 5 mM sodium phosphate buffer containing 10% (v/v) acetonitrile (pH 7.4). The solution was passed through a 0.45 µm filter (Merck Millipore). PITC derivatives were separated using a Shimadzu LC-20A high performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with an L-column 3 C18 column (250 mm × 4.0 mm i.d., 5 µm; Chemicals Evaluation and Research Institute, Tokyo, Japan) maintained at 45°C. Eluent A comprised 0.15 M ammonium acetate buffer, pH 6.0, containing 5% (v/v) acetonitrile, while eluent B was 100% acetonitrile. The gradient elution conditions were as follows: 0–5 min, 0% B; 5.0–5.1 min, 0–10% B; 5.1–25 min, 10–47.5% B; 25–30 min, 47.5–100% B; 30–37 min, 100% B; 37.1–40 min, 0% B. The injection volume was 20 µL, and the flow rate was 0.6 mL/min. Absorbance at 254 nm was monitored.

2.4. Size-exclusion chromatography

Size-exclusion chromatography (SEC) analysis was performed using a Shimadzu LC-10A HPLC system. A Superdex Peptide 10/300 GL column (GE Healthcare, Piscataway, NJ) was utilized. Prior to separation, the sample was concentrated 5-fold under vacuum using a centrifuge concentrator. The injection volume was 200 µL. The eluent used was 0.1% formic acid, and the flow rate was set at 0.5 mL/min. Elution was monitored at 214 and 254 nm, and fractions were collected every 1 min.

2.5. Identification of compounds by LC-MS/MS

Amino compounds present in SEC fractions before the 45 minute mark were derivatized with AccQ and the resulting derivatives were subjected to analysis through LC-MS/MS in precursor ion scan mode, following the methods previously outlined by Boughton et al. (2011) and Ejima et al. (2018). Briefly, a 100 µL aliquot from each SEC fraction was dried using a vacuum centri-

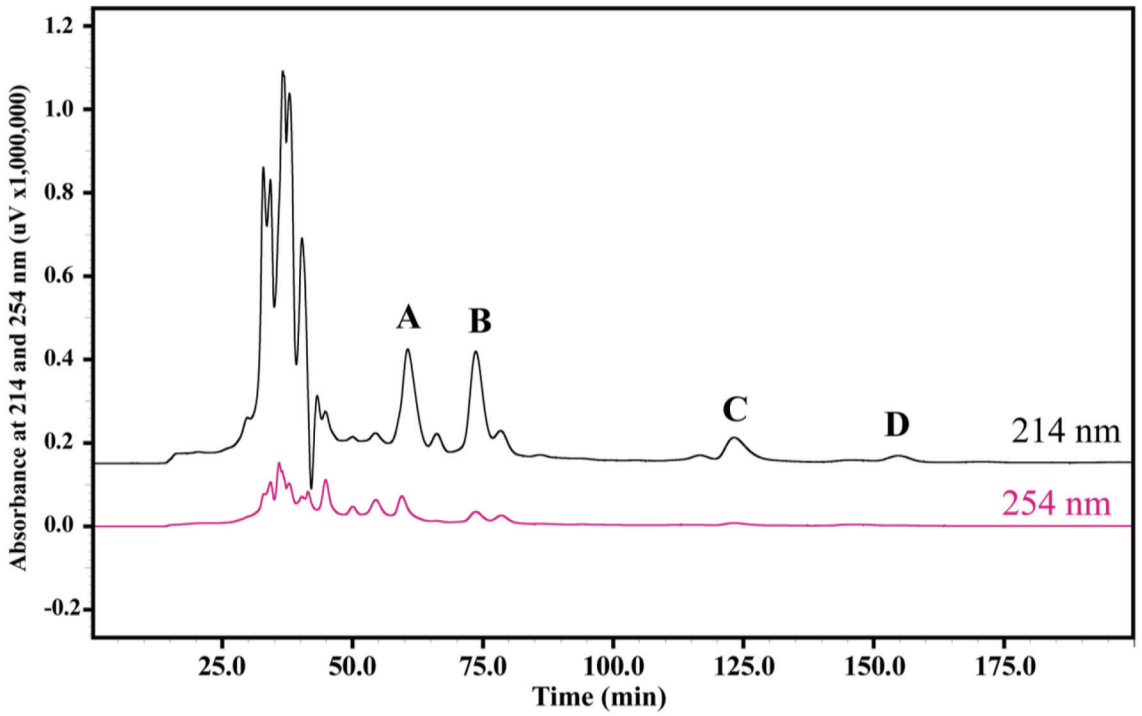

Figure 1. Size exclusion chromatogram of Hong Yao sour soup monitored at 214 and 254 nm. Fractions eluted from the Superdex Peptide 10/300 GL column were collected every 1 min from 27 min to 44 min. Four peaks eluted after 45 min were named A, B, C, and D, respectively.

fuge concentrator. The residue was dissolved in 10 µL of water and mixed with 20 µL of 0.3% AccQ acetonitrile solution and 70 µL of 200 mM sodium borate buffer (pH 8.8). This reaction mixture was then heated to 55°C for 10 min and subsequently filtered through a 0.45 µm filter. The filtrates were then analyzed using a Shimadzu LC-20A HPLC system coupled with a triple quadrupole mass spectrometer (LCMS-8040, Shimadzu) equipped with an electrospray ionization (ESI) source. Capillary voltage was set to 4.5 kV. Nitrogen drying gas and nebulizing gas flow rates were 15 L/min and 3 L/min, respectively. Collision-induced dissociation gas (Ar) pressure was 224 kPa. Heat block temperature and desolvation line (DL) temperatures were set to 400 °C and 250 °C, respectively. Chromatographic separation was achieved on an Inertsil OSD-3 column (250 mm × 2.1 mm i.d., 5µm, GL Science, Tokyo, Japan) at a column temperature of 40°C, and the injection volume was 10 µL. A gradient LC system was operated using 0.1% formic acid in water (Eluent A) and 0.1% formic acid in water : acetonitrile at 20:80 (v/v) (Eluent B) at a flow of 200 µL/min. The gradient elution conditions were as follows: 0–15 min; 0–30% B, 15–20 min; 30–50% B, 20–25 min; 50–100% B, 25–30 min; 100% B, 30.1–40 min; 0% B. Specific detection of AccQ-derivatives involved selecting precursor ion that generated product ion of the AccQ-moiety (b1 ion, *m/z* = 171.1) in positive mode (collision energy of −35 eV) within the scan range of *m/z* = 100–250, 250–300, 300–350, 350–400, and 400–500. The precursor ions *m/z* values were recorded and subsequently analyzed using product ion scan mode (collision energy of −15, −25, and −35 eV) to estimate the structure of the amino compounds. Data processing was conducted using LabSolutions LCMS Ver. 5.5. (Shimadzu).

SEC fractions eluted after 45 min were also collected, as represented by peaks A-D in Figure 1. Aliquots (100 µL) from fractions A-D were vacuum-dried and then reconstituted in 30 µL of water for direct LC-MS analysis. The gradient elution conditions were as follows: 0–15 min; 0–30% B, 15–20min; 30–50%, 20–25min; 50–100% B, 25–30 min; 100% B, 30.1–40 min; 0% B. MS measurements were conducted in full scan mode (*m/z* 50–300), followed by product ion scan mode. Other LC-MS conditions remained consistent with those previously detailed.

For isolation of compounds lacking positive charge in the Hong Yao sour soup, a strong cation exchange spin column technique was employed, following a previously described protocol (Sato et al., 1998). The strong cation exchanger (AG 50W-×8 resin, hydrogen form, 100–200 mesh, Bio-Rad, Richmond, CA, USA) was packed into a Centrifugal Filter Device with a 5.0 µm pore size, (Ultrafree-MC, Merck Millipore, Burlington, MA, USA) and washed twice with 200 µL of 50% methanol, followed by four washes with water. After washing, 200 µL of the Hong Yao sour soup was pipetted onto the resin and elution was performed via centrifugation at 2,000 *g* for 1 minute. The unabsorbed fraction was directly subjected to analysis using the same LC-MS system. The gradient elution conditions were as follows: 0–30 min; 0–30% B, 30–40 min; 30–100% B, 40.1–50 min; 0% B. MS measurements were conducted in full scan mode (*m/z* 120–500), followed by product ion scan mode.

For carbonyl compounds within the Hong Yao soup, conversion to their 2-nitrophenylhydrazides was achieved using the carboxylic acids assay kit with slight modifications following the procedure outlined by Chen et al. (2019). A 50 µL aliquot of the Hong Yao sour soup was combined with 50 µL of reagent A and 50 µL of reagent B in a 1.5 mL centrifugation tube, then incubated at 60°C for 20 min. After the reaction, the mixtures were cooled on ice for 1 minute before being filtered through a 0.45 µm filter. For precursor ion scan of the derivatives in negative mode, the product ion with *m/z* 137 derived from 2-NPH was selected. The scan encompassed the range of *m/z* 150 to 800, with a collision energy of 15 eV. A 10 µL aliquot was injected into the LC-MS/MS system and

chromatographic separation was carried out on the Inertsil OSD-3 column at a column temperature of 45°C. The gradient LC system was operated using 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B), with the following gradient elution conditions: 0–1 min; 5% B, 1–40 min; 5–95% B, 40–45.5 min; 95% B, 45.6–55 min; 5% B. Other LC-MS conditions remained unchanged.

Detected compounds were identified by matching precursor and product ion *m/z* values with commercial standards, data in existing literature, and databases such as MassBank (https://massbank.eu) and Human Metabolome Database (HMDB, https://hmdb.ca).

2.6. Synthesis of pyroglutamyl peptide and N-lactoyl-amino acids

Pyroglutamyl peptides and *N*-lactoyl-amino acids standards were synthesized chemically from pyrogutamate or lactate and Fmoc-amino acids using a Shimadzu PSSM-8 solid-phase peptide synthesizer. The synthetic standards were purified using RP-HPLC, and their purity was confirmed by LC-MS following the methods detailed by Ejima et al. (2018). The content of the purified standards was determined through amino acid release via HCl hydrolysis.

2.7. Quantification of compounds in Hong Yao sour soup

To quantify pyroglutamyl peptides, *N*-acetyl-, and *N*-lactoyl-amino acids, we directly subjected 10 µL of samples or standards with varying concentrations (ranging from 0.01 µM to 5 µM) to LC-MS/MS in the multiple reaction monitoring (MRM) mode. Chromatographic separation was accomplished using the Inertsil OSD-3 column. We employed a gradient LC system using 0.1% formic acid (Eluent A) and 0.1% formic acid containing 80% acetonitrile (Eluent B). The gradient elution proceeded as follows: 0–10 min; 5–19% B, 10–27 min; 19% B, 27–40 min; 19–45% B, 40.01–46 min; 100% B, 46.01–55 min; 5% B.

For quantifying polyamines in the samples, we followed the method of Fu et al. (2016) with minor adjustments after derivatization with benzoyl chloride. Briefly, polyamine stock solutions (1 mg/mL) were prepared in purified water and stored at −30°C. Working standard solutions for calibration were prepared by diluting the stock solutions appropriately. To perform derivatization, 10 µL of standard or sample (diluted one hundred times) was mixed with 45 µL of 100 mM sodium borate buffer at pH 8.8 and 45 µL of benzoyl chloride (2% in acetonitrile, v/v). The mixture was vortexed for 5 min at room temperature and then centrifuged at 13,000 *g* for 10 min at 4°C. The supernatant was transferred to the sample vial for LC-MS analysis. Derivatives were analyzed by LC-MS/MS in MRM mode using a Cosmosil $5C_{18}$-MS-II column (150 mm × 2.0 mm i.d., 5µm, Nacalai Tesque). The column temperature was maintained at 40°C. A gradient LC system using 0.1% formic acid in water (Eluent A) and 0.1% formic acid containing 80% acetonitrile (Eluent B) at a flow rate of 200 µL/min was emplyed. The gradient elution proceeded as follows: 2% B (0–1 min), 2–100% B (1–18 min), 100% B (18–22.5 min), followed by 2% B for 7 min to equilibrate the column.

For the quantification of monoamines, we carried out sample derivatization with AccQ, as described above. The derivatives were then analyzed using LC-MS/MS in MRM mode. Chromatographic separation was accomplished on a Cosmosil $5C_{18}$-MS-II column. The gradient elution conditions were as follows: 0–15 min; 0–30% B, 15–20min; 30–50% B, 20–25min; 50–100% B, 25–30 min; 100% B, 30.1–40 min; 0% B.

To quantify carbonyl compounds, we converted the samples to 2-nitrophenylhydrazide, following the method mentioned above with minor modifications. In short, 100 µL of standard solutions or samples spiked with internal standard (IS) of heptanoic acid were mixed with 50 µL of 200 mM 2-NPH·HCl in 50% aqueous methanol and 50 µL of 120 mM EDC·HCl in pyridine/methanol (6:94, v/v). The mixtures were allowed to react at 40°C for 30 min. After the reaction, 800 µL of 50% aqueous acetonitrile was added. The derivatives were analyzed using LC-MS/MS in MRM mode with chromatographic separation was achieved on the Inertsil OSD-3 column. A gradient LC system using 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) at a flow rate of 200 µL/min was employed. The gradient elution proceeded as follows: 0–1 min; 10% B, 1–11 min; 10–40% B, 11–35 min; 40% B, 35–45 min; 40–80% B, 45.1–51 min; 95% B; 51.1–60 min 10% B.

For each compound, MRM transitions were optimized using LabSolutions LCMS Ver. 5.5 by directly injecting 1µL of the individual derivatized standard solutions. Both commercially available standards and chemically synthesized standards were used for optimization. Each compound was monitored by three transitions and the most specific one was selected for quantification. Quantifier transitions are provided in Table S1.

2.8. Statistical analyses

All statistical analyses were performed by using a GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± SD. Differences in amino acid contents before and after acid hydrolysis were analyzed using the unpaired *t*-test for two independent means. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Amino acid contents

The amino acid contents of non-hydrolyzed and acid-hydrolyzed Hong Yao sour soup are presented in Table 1. In the current chromatographic conditions, GABA coeluted with Thr. As a result, the combined sum of GABA and Thr is shown in Table 1. Subsequent LC-MS/MS analysis revealed that the GABA content exceeded that of Thr. Consequently, the principal free amino acids in Hong Yao sour soup were GABA, Ala, Gly, Val, and Leu. Only minor quantities of Glu, Arg, Tyr, and Ser were present. Following HCl hydrolysis, a significant increase was observed in most amino acids ($p < 0.05$), except for GABA/Thr and Leu, indicating the presence of considerable peptide amounts. The rise in hydrophobic amino acids, such as Leu, Ile, Val, Ala, and Phe, due to the hydrolysis was less pronounced than that of hydrophilic amino acids.

3.2. SEC fractionations

Superdex Peptide 10/300 GL is designed to operate within a mass range of 20,000 to 100 Da. In practice, the optimal separation occurs in the range of 7,000 to 100 Da (Tran et al., 2011). Figure 1 illustrates the chromatograms of the fermented sour soup on Superdex Peptide 10/300 GL. Some peaks emerged between 27 and 42 min, aligning with the elution time of oligopeptides with 1,000 Da and water, respectively (Sato et al., 2013). Fractions were col-

Table 1. Concentrations of amino acids in Hong Yao sour soup and its HCl hydrolysate (mM)

Amino acids	non-hydrolysate	hydrolysate
Asp	0.53 ± 0.02	1.26 ± 0.05*
Glu	0.114 ± 0.001	1.01 ± 0.04*
Ser	0.051 ± 0.003	0.59 ± 0.05*
Gly	0.94 ± 0.01	2.01 ± 0.09*
His	0.20 ± 0.01	0.36 ± 0.02*
Arg	0.03 ± 0.01	0.33 ± 0.02*
Thr/GABA	2.35 ± 0.02	2.47 ± 0.10
Ala	1.20 ± 0.01	1.82 ± 0.07*
Pro	0.52 ± 0.01	0.83 ± 0.03*
Tyr	0.06 ± 0.01	0.17 ± 0.02*
Val	0.72 ± 0.02	0.97 ± 0.04*
Met	0.25 ± 0.01	0.28 ± 0.02*
Ile	0.306 ± 0.001	0.45 ± 0.02*
Leu	0.84 ± 0.15	0.86 ± 0.02
Phe	0.369 ± 0.003	0.43 ± 0.02*
Lys	0.34 ± 0.01	1.08 ± 0.03*
Asn	0.317 ± 0.004	–
Gln	0.121 ± 0.004	–

The data from triplicate analyses are given as mean ± SD. *p< 0.05 represent significant difference between non-hydrolysate and hydrolysate group.

lected every minute from 27 to 44 min and labeled as SEC Fr. 28 to 45. Moreover, a series of broad and large peaks (A-D) appeared after the elution of water, persisting until 160 min. The compounds within peaks A-D were eluted subsequent to water, likely due to interactions with the column packing involving hydrophobic and/or electrostatic interactions. Peaks A-D were also collected.

3.3. Identification of compounds in SEC fractionations

Compounds in SEC Fr. 33 to 45 underwent derivatization with AccQ. This derivatization enhances the resolution and detection of hydrophilic compounds possessing primary and secondary amines through reversed-phase chromatography-mass spectrometry (Ejima et al., 2018). A precursor ion scan technique targeting the product ion of *m/z* 171 was employed for the specific detection of amino compounds. In SEC Fr. 33–35, only negligible peaks of amino compounds, aside from reagent peaks, were observed (data not shown). Figure 2a presents mass chromatograms of SEC Fr. 36–45, spanning scan ranges of *m/z* 100–250, 250–300, 300–350, 350–400, and 400–500. Peaks corresponding to amino acids were indicated using their one-letter abbreviations. Precursor ions within numbered peaks underwent product ion scan analysis. Peptide structure was deduced through immonium ions and sequence-specific ions—namely, a_n, b_n, x_n, and y_n ions, following the nomenclature in previous studies by Roepstorff and Fohlman (1984), and elaborated upon by Falick et al. (1993). This process resulted in the identification of 28 amino compounds, encompassing 6 dipeptides with Pro, Ala, and branched-chain amino acids, 3 monoamines (pyrrolidine, ethanolamine, and DOPA), 3 polyamines (cadaverine, putrescine, and *N*-acetyl spermidine), amino acids, and a modified amino acid (methionine sulfoxide). Table 2 provides a summary of retention time, precursor ion (*m/z*), product ions (*m/z*), and SEC fraction numbers for the estimated amino compounds. For compounds featuring a single amino/imino group, the AccQ derivative yielded a monovalent ion due to the presence of one AccQ moiety. Compounds with two amino/imino groups resulted in derivatives featuring two AccQ moieties, generating both monovalent and divalent ions. For instance, cadaverine and putrescine formed derivatives with 2 AccQ moieties, primarily generating divalent ions with *m/z* values of 222.2 and 215.2, respectively. However, a smaller portion of the putrescine derivative generated monovalent ions at *m/z* 429.3.

To detect the compounds within Peaks A-D, a total ion scan was executed using scanning ranges of *m/z* 50–120, 120–200, 200–250, and 250–300. Total ion chromatograms (TIC) are depicted in Figure 2b. Solely monovalent ions, discerned through *m/z* differences between isotopes, were observed. Product ion scans were conducted for all precursor ions within numbered peaks to infer their structures. Table 2 also provides a summary of observed retention time, precursor ions (*m/z*), product ions (*m/z*), and estimated structures for Peaks A-D. Precursor and product ion patterns were cross-referenced with databases (HMDB and MassBank). Based on this information, tyramine, tryptophan, and L-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid were identified, as shown in Table 2. Notably, Figure S2 showcases head-to-tail plots comparing the spectrum of *m/z* 217.1, obtained from product ion scan experiment at −15 V collision energy, with the corresponding in silico spectra predicted by HMDB. All identified compounds incorporate aromatic groups (phenyl and indole groups). Aside from the protonated molecular ion $[M+H]^+$ of tyramine at *m/z* 138.2, non-covalent homo-dimer ion, $[2M+H]^+$, was also detected at *m/z* 275.2.

3.4. Identification of compounds in cation exchanger non-absorbed fractionation

After separation through the cation exchange resin, compounds lacking positive charge, such as pyroglutamyl peptides, were eluted from the column. The eluates underwent analysis using LC-MS in the total scan mode. Figure 3 illustrates the TIC within scan ranges of *m/z* 120–200, 200–250, 250–300, and 300–500. The precursor ions within peaks marked with numbers were analyzed by LC-MS/MS in product ion scan mode. This analysis led to the identification of various compounds: organic acids, pantothenic acid, phosphorylated saccharides, pyroglutamyl peptides, and *N*-acetyl-amino acids. The summary of retention time, precursor and product ions, and estimated compounds is presented in Table 3. Conversely, precursor ions in peaks 9, 12, and 13 could not be definitively attributed to pyroglutamyl peptides, despite the presence of immonium ions corresponding to Gln (*m/z* = 84) or Ile or Leu (*m/z* = 86). The discrepancy between the full molecule masses and the estimated amino acid composition based on immonium ions implies the existence of *N*-lactoyl-amino acids. These compounds are formed through dehydration condensation between the carboxy group of lactic acid and the amino group of amino acids. The estimated *N*-lactoyl (Lac)-amino acids, namely *N*-Lac-Gln, *N*-Lac-Leu, and *N*-Lac-Ile, were successfully synthesized. Confirmation of the presence of these *N*-lactoyl-amino acids was achieved by comparing their retention time and spectra with synthetic peptide standards via LC-MS/MS. For quantifying *N*-lactoyl-amino acids, LC-MS/MS with MRM detection was employed. The *N*-lactoyl-amino acid standards exhibited good linearity ($R^2 > 0.99$), ensur-

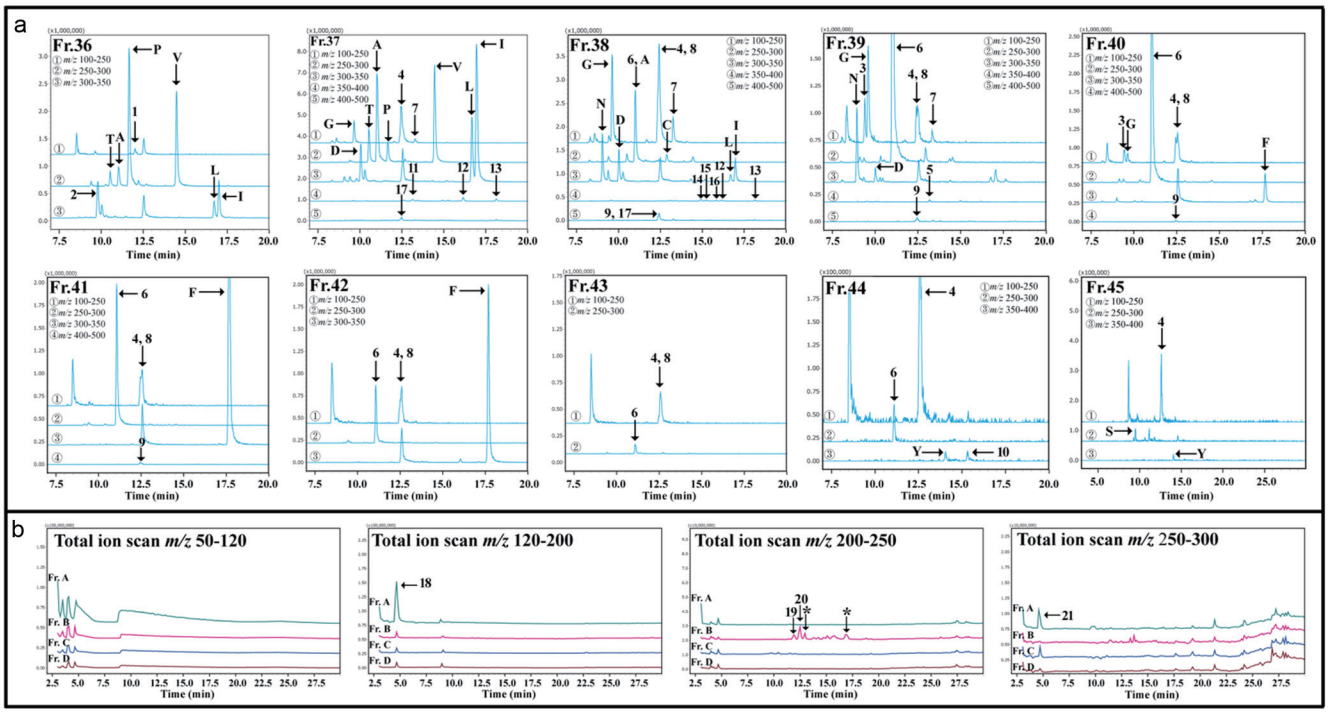

Figure 2. LC-MS chromatograms of SEC fractions of Hong Yao sour soup. (a): LC-MS precursor ion scan chromatograms targeting *m/z* 171 of AccQ-derivatives in positive ion mode in SEC fractions 36–45. (b): LC-MS full scan chromatograms of non-AccQ derivatives in SEC fractions A-D with scanning ranges *m/z* 50–300. Compounds in peaks with numbers were further analyzed by product ion scan for estimation of structure. Peaks of proteinogenic amino acids were directly indicated with one-letter abbreviations of amino acids on peaks. Peaks that could not be detected in the product ion scan analysis are marked with an asterisk.

ing dependable quantification. Notably, *N*-Lac-Gln registered at 1.43 μM, followed by *N*-Lac-Leu (0.51 μM), and *N*-Lac-Ile (0.34 μM) (Table 4).

3.5. Carbonyl compounds

Carbonyl compounds, encompassing carboxylic acids, aldehydes, and ketones, can react with 2-NPH to form hydrazones (Hofstetter et al., 2019). Although the reaction mechanism differs between carboxyl group and ketone/aldehyde groups with 2-NPH, both derivatives yield a strong and stable product ion with *m/z* 137 in negative mode. This ion serves for the precursor ion scan in negative mode to detect carbonyl compounds in biological samples (Peters et al., 2004). Through the precursor ion scan, numerous carbonyl compounds were detected (Figure 4). The most prominent peak with *m/z* 224 was identified as lactic acid derivative through comparison with the standard. Similarly, the second most significant peak was identified as acetic acid with *m/z* 193 (Figure S3a). Four minor peaks exhibited *m/z* values of 222, 236, 250, and 264 corresponding to molecular masses of 88, 102, 116, and 130, respectively, prior to derivatization. For identifying compounds with *m/z* 222 and 236, analogs of carbonyl compounds with these molecular masses were utilized as standards, including butyric acids (molecular weight of 88), pyruvic acid (88), 2-ketobutyric acid (102), and acetoacetic acid (102). By comparing the retention time and the product ions of the derivatives, compounds with molecular weights of 88 and 102 were identified as pyruvic acid and 2-ketobutyric acid, respectively (Figure S3b and c). Compounds with *m/z* 250 and 264 have molecular weights higher by 14 and 28 than 2-ketobutyric acid, indicating the presence of an additional methylene group. Based on comparisons with standards, peaks with *m/z* 250 and 264 were identified as 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, and/or 4-methyl-2-oxovaleric acid (Figure S3d). These α-keto acids have the potential to react with two 2-NPH molecules, although under the present conditions using 50 μL of 20 mM NPH, only derivatives with one 2-NPH molecule were observed.

To achieve complete derivatives with two 2-NPH for quantifying these α-keto acids, derivatization was conducted using 200 mM 2-NPH in 50% methanol. LC-MS/MS revealed the presence of derivatives with two 2-NPH molecules. All derivatives exhibited doublet peaks corresponding to *cis* and *trans* isomers (Han et al., 2013) (Figure S4). Given the limited separation of 3-methyl-2-oxovaleric acid and 4-methyl-2-oxovaleric acid under the current chromatographic conditions, the sum of these two compounds is reported in Table 4.

As mentioned earlier, a substantial peak of the 2-NPH derivative of lactic acid, a glucose metabolite, was identified. Consequently, other carboxylic acids from glucose metabolites and short-chain fatty acids commonly observed in fermented rice soup were quantified through 2-NPH derivatization and LC-MS/MS using MRM mode. The typical separation chromatograms obtained from a mixture of standards are shown in Figure S5. Effective separations were achieved for ten 2-NPH derivatives. Calibration curves were constructed by plotting the peak area ratio of standards with varying concentrations to IS against the nominal concentration of each standard. Good linearity, indicated by high coefficients (R^2 > 0.99), and an extensive linear range were evident for all acids (Table 4). In Hong Yao sour soup, lactic acid exhibited the high-

Table 2. List of the identified compounds by LC-MS/MS analysis in the positive mode of AccQ-derivatives in SEC fractions 36–45 and non-AccQ derivatives in SEC fractions A and B from Hong Yao sour soup

Peak No.	Retention time (min)	precursor ion (*m/z*)	Main product ions[a] (*m/z*)	Estimated compound	SEC Fr.
1	12.1	$[242.1]^+$	116, 130, 171	Pyrrolidine	36
2	10.8	$[336.1]^+$	56, 74, 100, 145, 166, 272	Methionine sulfoxide	36
3	9.6	$[232.1]^+$	116, 128, 143, 145, 171	Ethanolamine	39–40
4	12.5	$[244.2]^{2+}$	84, 116, 128, 145, 171, 172	Lysine	37–45
5	13.2	$[368.1]^+$	144, 171, 186, 214	Dopa	39
6	11.0	$[274.1]^+$	116, 128, 145, 171	GABA	38–44
7	13.5	$[222.2]^{2+}$	86, 116, 128, 145, 171, 172	Cadaverine	37–39
8	12.5	$[215.2]^{2+}$	72, 89, 116, 128, 145, 171	Putrescine	39–42
9	12.5	$[429.3]^+$	72, 98, 115, 145, 171, 285	Putrescine	38–41
10	15.4	$[358.1]^+$	171, 188, 358	N-Acetylspermidine	44
11	13.2	$[357.2]^+$	116(y1), 171, 187(y2), 242(b2)	Ala-Pro	37
12	16.2	$[385.3]^+$	72(V*), 116(y1), 145, 171, 215(y2), 270(b2)	Val-Pro	37–38
13	18.2	$[399.2]^+$	86(L*), 116(y1), 171, 229(y2), 256(a2), 284(b2)	Leu-Pro	37–38
14	15.0	$[359.1]^+$	72(V*), 145, 171, 189(y2), 242(a2), 270(b2)	Val-Ala	38
15	15.3	$[373.2]^+$	86(L*), 132.6, 145, 171, 203(y2), 256(a2), 284(b2)	Leu-Ala	38
16	16.2	$[387.3]^+$	72(V*), 145, 171, 217(y2), 242(a2), 270(b2)	Val-Val	38
17	12.5	$[487.2]^+$	84(K*), 130, 145, 147(y1)	Lysine	37–38
18	4.7	$[138.2]^+$	51, 65, 77, 91, 103	Tyramine	A
19	12.0	$[205.1]^+$	29, 91, 115, 118.2, 132, 146, 159, 170, 188	Tryptophan	B
20	12.7	$[217.1]^+$	106, 130, 144, 145, 146, 154, 156, 158, 171, 173, 184, 199	L-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid	B
21	4.7	$[275.2]^+$	77, 91, 93, 103, 121	2×Tyramine	A

[a]Interpretation of a peptide CID spectrum relies mainly on the sequence-specific ions present (a, b, and c ion series and x, y, and z ion series and immonium ions (*).

est concentration (4,761.2 mg/L), followed by acetic acid (616.3 mg/L). The remaining acids registered concentrations below 100 mg/L, with isocaproic acid not being detected.

3.6. Biogenic amines

LC-MS analysis of the SEC fractions indicated the presence of biogenic amines. The contents of 10 biogenic amines are listed in Table 4. All calibration curves demonstrated good linear regression ($R^2 > 0.99$). GABA stood out as the most abundant at 166.4 mg/L, followed by tyramine (44.1 mg/L) and putrescine (18.5 mg/L).

4. Discussion

The primary objective of this study was to characterize Hong Yao sour soup, a type of Chinese white sour soup believed to have benefits in preventing hair graying and promoting hair growth in elderly women. The main components of the sour soup, including amino acids, peptides, and carboxylic acids, were initially analyzed. Previous research has indicated the involvement of certain bacteria—such as lactic acid bacteria, acetic acid bacteria, and *Clostridium* spp. in the fermentation process of a similar white sour soup from another province, Guizhou (Zhou et al., 2022b). These bacteria are responsible for producing lactic acid, acetic acid, propionic acid, and butyric acid. Our study also detected the presence of these carboxylic acids, confirming that the present preparation of Hong Yao sour soup exhibits the typical characteristics of Chinese white sour soup. The substantial amounts of lactic acid and acetic acid suggest that anaerobic fermentation by lactic and acetic acid bacteria occurs in Hong Yao sour soup. The presence of a smaller yet significant amount of butyric acid indicates the involvement of *Clostridium* spp. in the fermentation process. Due to the lack of pasteurization and the absence of starter use, variations in carboxylic acid levels may occur among producers, even though major carboxylic acids (lactic and acetic acids) exhibit similar levels. These carboxylic acids play a significant role as flavor compounds and preservatives in the sour soup. Additionally, aside from lactic acid and short-chain fatty acids, we identified five α-keto acids. Research has demonstrated that certain LAB species' aminotransferases can convert amino acids into corresponding α-keto acids (Gutsche et al., 2012). Our findings indicate the presence of α-keto acids derived from Thr, Ala, and branched-chain amino acids valine, leucine, and isoleucine in Hong Yao sour soup. These α-keto acids serve as precursors for other taste and flavor metabolites, such as aldehydes, alcohols, and carboxylic acids.

Free amino acids and peptides can contribute to taste of fer-

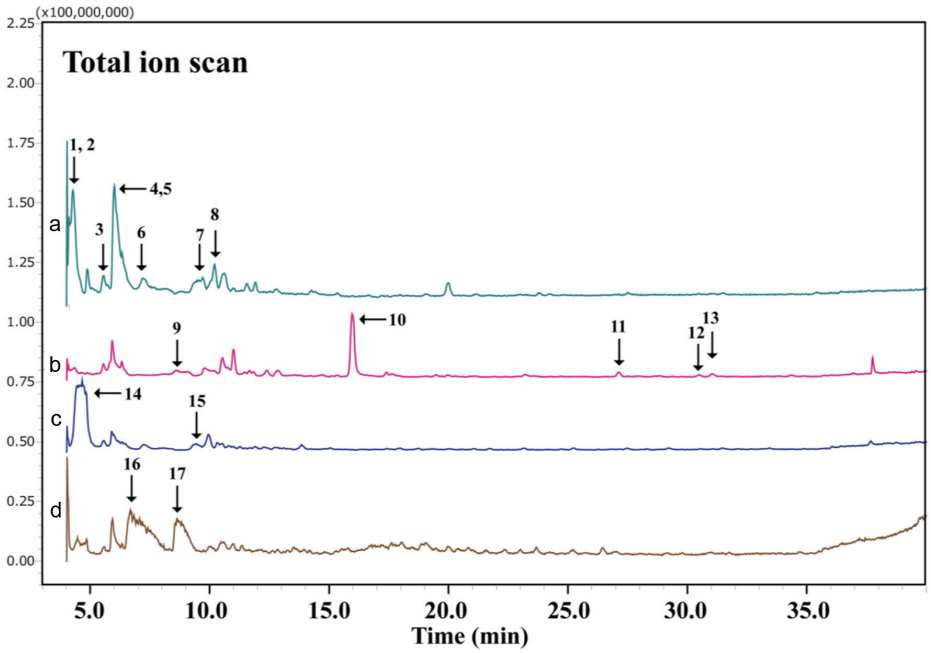

Figure 3. LC-MS full scan chromatograms obtained from the cation exchanger unabsorbed fraction at a scanning range of *m/z* 120–200 (a), 200–250 (b), 250–300 (c), and 300–500 (d) in positive ion mode. Compounds in peaks with numbers were further analyzed by product ion scan for estimation of structure.

mented condiments. Generally, peptides, particularly hydrophobic ones, tend to impart a bitter taste. Through HCl hydrolysis of soluble compounds in Hong Yao sour soup, hydrophilic amino acids, such as Asp, Glu, Gly, Ala, and Lys, were released predominantly, with only minimal amounts of hydrophobic amino acids such as Ile, Leu, and Phe being released. This observation indicates that the majority of peptides in the sour soup were composed of hydrophilic amino acids, while hydrophobic amino acids were present in their free form. This suggests the action of exopeptidases during fermentation, which preferentially re-

Table 3. Identified compounds by LC-MS/MS analysis of the cation exchanger unabsorbed fraction in positive mode from Hong Yao sour soup

No.	Retention time (min)	precursor ion (*m/z*)	Main product ion (*m/z*)	Estimated compounds[a]
1	4.5	$[197.0]^+$	81,99	Ortophosphate
2	4.5	$[140.1]^+$	63,81,99	Ortophosphate+ACN
3	5.6	$[148.1]^+$	43, 60, 70, 88, 106, 130, 148	*N*-Ac-Ser
4	6	$[132.1]^+$	45, 91	Lactic acid+ACN
5	6	$[181.1]^+$	45, 91	2×Lactic acid
6	7.3	$[127.1]^+$	53, 63, 81, 99, 109	Ethylphosphate
7	9.5	$[130.1]^+$	28, 41, 56, 84	pyroGlu
8	10.2	$[190.1]^+$	43, 56, 84, 102, 130, 148, 172	*N*-Ac-Glu
9	8.8	$[219.1]^+$	56, 84, 90, 130, 184	*N*-Lac-Gln
10	16	$[220.1]^+$	30.5, 43, 72, 85, 90, 98, 124, 142, 184, 202	Pantothenic acid
11	27.0	$[243.1]^+$	43, 56, 84, 86(L*), 132(y1), 197, 226	pyrGlu-Leu
12	30.5	$[204.1]^+$	30, 41, 45, 69, 86(I*), 132(y1), 158	*N*-Lac-Ile
13	31.0	$[204.1]^+$	30, 44(L*), 45, 86(L*), 132(y1), 158	*N*-Lac-Leu
14	4.7	$[261.0]^+$	30, 43, 57, 81, 99, 109, 127, 145	Fructose 1-phosphate
15	9.3	$[259.1]^+$	84, 130	2×pyroGlu
16	7.0	$[341.0]^+$	43, 53, 81, 99, 109, 225, 243, 323	Fructose 1,6-bisphosphate
17	9.0	$[341.0]^+$	43, 53, 81, 99, 109, 225, 243, 323	Glucose 1,6-bisphosphate

[a]Abbreviations are: Lac-, Ac-, ACN: lactoyl-, acetyl-, acetonitrile, respectively.

Table 4. Contents of amino acid metabolites and carboxylic acids in Hong Yao sour soup

Compound[a]	Concentration[b]	Calibration range	Linearity (r^2)	Units
N-Lac-Gln	1.43 ± 0.01	0.2–5	0.9983	µM
N-Lac-Leu	0.51 ± 0.03	0.01–2	0.9997	µM
N-Lac-Ile	0.34 ± 0.03	0.01–2	0.9996	µM
N-Pyr-Leu	0.30 ± 0.03	0.1–10	0.9993	µM
N-Ac-Ser	3.63 ± 0.44	0.05–50	0.9980	µM
N-Ac-Glu	187.24 ± 10.03[c]	0.05–50	0.9998	µM
Pyruvic acid	0.52 ± 0.03	0.005–0.5	0.9999	mg/L
2-Ketobutyric acid	0.017 ± 0.002	0.0005–0.05	0.9998	mg/L
3-Methyl-2-oxobutyric acid	0.006 ± 0.001	0.0005–0.05	0.9994	mg/L
3-Methyl-2-oxovaleric acid and 4-Methyl-2-oxovaleric acid	0.10 ± 0.01	0.001–0.5	0.9999	mg/L
Succinic acid	87.91 ± 8.18[c]	0.001–5	0.9975	mg/L
Glyceric acid	1.68 ± 0.15	0.005–5	0.9918	mg/L
Malic acid	0.027 ± 0.003	0.001–2	0.9902	mg/L
Lactic acid	4,761.21 ± 115.28[c]	0.5–10	0.9976	mg/L
n-Caproic acid	0.56 ± 0.01	0.005–5	0.9982	mg/L
n-Butyric acid	0.05 ± 0.01	0.05–5	0.9986	mg/L
Propionic acid	14.16 ± 3.02[c]	0.005–2	0.9988	mg/L
Acetic acid	616.33 ± 63.20[c]	0.05–5	0.9980	mg/L
Cadaverine	5.43 ± 0.32[c]	0.001–0.1	0.9994	mg/L
Putrescine	18.52 ± 1.73[c]	0.001–0.1	0.9991	mg/L
Spermidine	0.47 ± 0.001[c]	0.001–0.1	0.9987	mg/L
Spermine	0.87 ± 0.03[c]	0.001–0.1	0.9972	mg/L
Ethanolamine	8.01 ± 0.14	0.05–10	0.9995	mg/L
Pyrrolidine	0.018 ± 0.001	0.001–0.5	0.9985	mg/L
Methionine sulfoxide	1.77 ± 0.08	0.05–5	0.9975	mg/L
GABA	166.39 ± 10.00[c]	0.05–5	0.9996	mg/L
Tyramine	44.09 ± 2.09[c]	0.005–0.5	0.9994	mg/L
Phenyethylamine	2.15 ± 0.03	0.05–5	0.9995	mg/L
Tryptamine	7.59 ± 0.58[c]	0.005–0.5	0.9998	mg/L
Histamine	1.14 ± 0.01	0.05–5	0.9999	mg/L

[a]Abbreviations are: Lac-, Pyr-, Ac-: lactoyl-, pyroglutamyl-, acetyl-, respectively. [b]The data from triplicate analysis are given as mean ± SD. [c]Sample was diluted for quantification.

lease hydrophobic amino acids from peptides. As a result, the sour soup contains only small amounts of hydrophobic peptides, which contribute to a mild bitter taste and, consequently, does not possess a strong bitter flavor.

In previous studies, we developed a solid phase extraction method using the strong cation exchanger (AG 50W-×8) packed into a mini spin column to isolate amino terminal blocked peptides. This method allowed the detection of pyroglutamyl peptides in various fermented foods. The present study also identifies the presence of pyroGlu-Leu. Pyroglutamyl peptides are shown to be formed non-enzymatically from peptides with glutaminyl residue at amino termina. Certain Japanese fermented foods such as Japanese rice wine (*sake*), soy paste (*miso*), and soy sauce (*shoyu*) contain relatively high levels of both hydrophilic and hydrophobic pyroglutamyl peptides, such as pyroGlu-Gln and pyroGlu-Leu, respectively (Shirako et al., 2019). In contrast, Hong Yao soup contained only a lower content (0.2 µM) of pyroGlu-Leu compared to Japanese fermented products (40–100 µM) (Kiyono et al., 2013). Furthermore, Hong Yao sour soup contained higher levels of *N*-acetyl- and *N*-lactoyl-amino acids compared to pyroglutamic peptides. Notably, *N*-acetylglutamate content exceeds that of free glutamate. In bacteria, *N*-acetyl-amino acids are synthesized from acetyl-CoA and amino acids through the enzyme *N*-acetylglutamate synthase (NAGS), which catalyzes the production of *N*-acetylglutamate from glutamate and acetyl-CoA. The high content of *N*-acetylglutamate and the low content of pyroglutamyl peptide distinguish Hong Yao sour soup from Japanese rice wine, although both are derived from rice.

N-lactoyl-amino acids were first identified in Parmesan cheese. It

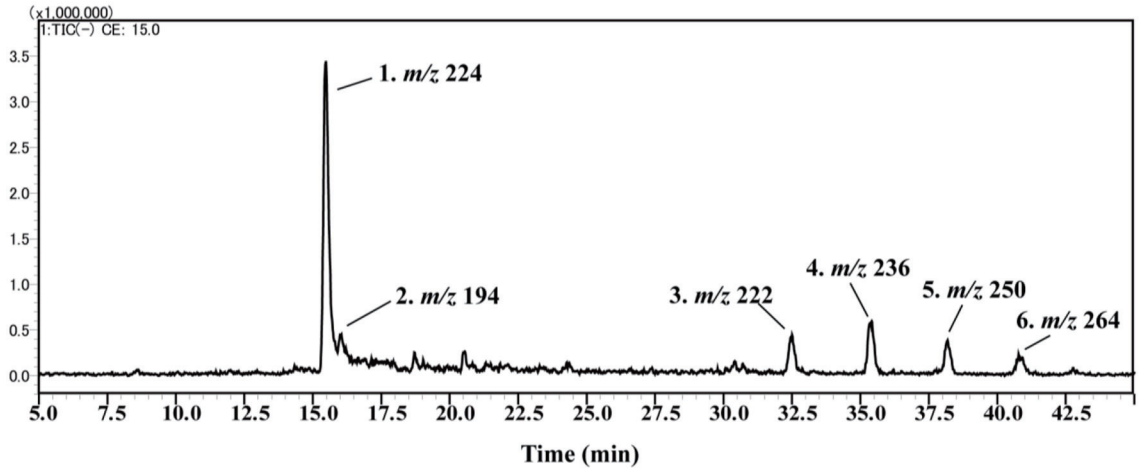

Figure 4. Precursor ion scan chromatograms targeting *m/z* 137 in negative ion mode of Hong Yao sour soup. Peaks identification: 1, lactic acid; 2, acetic acid; 3, pyruvic acid; 4, 2-ketobutyric acid; 5, 3-methyl-2-oxobutyric acid; 6, 3-methyl-2-oxovaleric acid and/or 4-methyl-2-oxovaleric acid.

has been reported that Korean fermented vegetable referred to *kimchi* and dry cured ham also contain *N*-lactoyl-amino acids (Christa et al., 2022; Paolella et al., 2018). In *kimchi,* lactoyl-hydrophilic amino acids like Gly, Ala, Glu, Gln, and Asn were present. In contrast, Parmigiano-Reggiano cheese and ham contained lactoyl-hydrophobic amino acids such as Tyr, Met, Ile, Leu, and Phe. In Hong Yao sour soup, *N*-lactoyl-Gln, -Ile, and -Leu were found in amounts of 0.3–1.4 μM, while free Gln was present at a lower level than other amino acids. These *N*-lactoyl-amino acids are reported to be enzymatically synthesized from lactic acid and amino acids during fermentation involving *L. helveticus* or *L. rhamnosus*, although the enzyme responsible for this synthesis in these bacteria has not been identified (Sgarbi et al., 2013). Jansen et al. (2015) identified an enzyme, cytosolic nonspecific dipeptidase 2 (CNDP2), in HEK 293 cells, that catalyzes the formation of *N*-lactoyl-amino acids. These findings suggest that *N*-lactoyl-amino acids are synthesized by exotype peptidases with certain substrate specificity, rather than by non-enzymatic reactions in Hong Yao sour soup. Studies have demonstrated that *N*-acetyl- and *N*-lactoyl-amino acids contribute to taste and taste modulation (kokumi perception) (Li et al., 2022; Zhao et al., 2016). However, the concentrations of these compounds in Hong Yao sour soup were considerably lower than the threshold concentrations for taste and kokumi perception.

Hong Yao sour soup contained monoamines and polyamines. Research has demonstrated that non-controlled autochthonous LAB or non-starter LAB are primarily responsible for the production of biogenic amine (BA) in fermented foods (Barbieri et al., 2019). The expression and/or activation of amino acid decarboxylation systems in LAB are considered adaptive responses to an acidic environment (Pereira et al., 2009). GABA, tyramine, and putrescine were the main BAs found in Hong Yao sour soup, with other amines also present. The content of GABA and tyramine exceeded that of their precursor amino acids Glu and Tyr, respectively, suggesting significant decarboxylation, which may respond to accumulation of lactic acid and acetic acid. Extensive amino acid decarboxylation is a distinctive feature of Hong Yao sour soup.

Fermented sour soup serves not only as a culinary condiment but also as a traditional remedy for nurturing hair among Hong Yao women, who maintain long and black hair even in old age. It has been hypothesized that certain compounds in Hong Yao sour soup contribute to hair darkening and growth. However, there is currently no research data available regarding the active compounds responsible for promoting hair health in Hong Yao sour soup.

Hair graying is a common sign of aging in both animals and humans, regardless of gender. Numerous theories have been proposed to explain this phenomenon. Previous studies have suggested that the incomplete maintenance of melanocyte stem cells (MSCs) is a fundamental cause of hair graying (Nishimura et al., 2005). Another theory attributes hair graying to melanocyte apoptosis induced by oxidative stress (Arck et al., 2006). Hair loss is a more complex hair disorder influenced by aging, genetic predisposition, dietary habits, drugs, and hormonal and metabolic abnormalities (Hordinsky et al., 2002; Morinaga et al., 2021).

Our present study highlights that Hong Yao sour soup contains common compounds such as amino acids and lactic acid. Additionally, it contains small quantities of specific compounds including short-chain fatty acids, peptides, BAs, *N*-lactoyl-amino acids, and *N*-acetyl-amino acids. Some of these compounds in Hong Yao sour soup may contribute to its hair-benefiting properties through antioxidant activity, protection, or activation of MSCs. Biogenic amines play essential roles in various cellular functions, including cellular proliferation and differentiation, nucleic acid regulation, protein synthesis, brain development, and nerve growth and regeneration (Shukla et al., 2010). In Hong Yao sour soup, the dominant BAs are GABA, tyramine, and putrescine. Recently, their potential role in hair follicle growth and pigmentation has garnered attention. Kovacevic et al. (2019) formulated a novel shampoo containing tyramine, which reduced hair shedding in women by contracting the *arrector pili muscle* through the trace amine-associated receptor. Liu et al. (2002) reported that injecting additional spermidine into Merino lamb skin stimulated cell proliferation in the wool bulb and led to fiber growth. Ramot et al. (2010) reviewed evidence indicating that the polyamine spermidine stimulates melanin production in human anagen hair follicles cultured in vitro. Hong Yao sour soup contains all the aforementioned BAs, making them potential candidates as active compounds contributing to hair health among Hong Yao women. Additionally, other compounds like *N*-acetyl-amino acids, *N*-lactoyl-amino acids and short-chain fatty acids might also play a role in hair protection.

5. Conclusion

In this study, we used an LC-MS/MS to analyze compounds in

Hong Yao our soup. Consequently, 63 compounds including amino acids, carboxylic acids, BAs, *N*-acetyl- and *N*-lacoyl-amino acids, and pyroglutamyl peptides were detected in the sour soup. This study provides insights into the chemical constituents of Hong Yao sour soup. Based on existing literature, we hypothesize that BAs like tyramine and spermidine could contribute to hair protection. Ongoing research using animal models aims to explore the hair-protective activity of Hong Yao sour soup and its bioactive compounds. Therefore, our findings about the bioactive compounds in Hong Yao sour soup hold promise for both culinary and hair health perspectives.

Conflict of interest

The authors declared that there are no conflict of interests with respect to the research, authorship, and/or publication of this article.

Supplementary material

Figure S1. Preparation of Hong Yao sour soup in home and industry scale.

Figure S2. Head-to-tail plot of experimental and predicted LC-MS/MS spectra of L-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid.

Figure S3. LC-MS/MS chromatograms of the 2-NPH derivatized standards.

Figure S4. The representative MRM LC-MS/MS chromatograms of the 2-NPH derivatives of a mixed standards of keto acid.

Figure S5. The representative MRM LC-MS/MS chromatogram of the 2-NPH derivatives of a mixed standards.

Table S1. Optimized mass spectrometric parameters for MRM analysis.

References

Aito-Inoue, M., Ohtsuki, K., Nakamura, Y., Park, E.Y., Iwai, K., Morimatsu, F., and Sato, K. (2006). Improvement in isolation and identification of food-derived peptides in human plasma based on precolumn derivatization of peptides with phenyl isothiocyanate. J. Agric. Food Chem. 54(15): 5261–5266.

Arck, P.C., Overall, R., Spatz, K., Liezman, C., Handjiski, B., Klapp, B.F., Birch-Machin, M.A., and Peters, E.M. (2006). Towards a "free radical theory of graying": melanocyte apoptosis in the aging human hair follicle is an indicator of oxidative stress induced tissue damage. FASEB J. 20(9): 1567–1569.

Barbieri, F., Montanari, C., Gardini, F., and Tabanelli, G. (2019). Biogenic amine production by lactic acid bacteria: A review. Foods 8(1): 17.

Bidlingmeyer, B.A., Cohen, S.A., and Tarvin, T.L. (1984). Rapid analysis of amino acids using pre-column derivatization. J. Chromatogr. 336(1): 93–104.

Boughton, B.A., Callahan, D.L., Silva, C., Bowne, J., Nahid, A., Rupasinghe, T., Tull, D.L., McConville, M.J., Bacic, A., and Roessner, U. (2011). Comprehensive profiling and quantitation of amine group containing metabolites. Anal. Chem. 83(19): 7523–7530.

Chen, Z., Gao, Z., Wu, Y., Shrestha, R., Imai, H., Uemura, N., Hirano, K.I., Chiba, H., and Hui, S.P. (2019). Development of a simultaneous quantitation for short-, medium-, long-, and very long-chain fatty acids in human plasma by 2-nitrophenylhydrazine-derivatization and liquid chromatography-tandem mass spectrometry. J. Chromatogr B Analyt. Technol. Biomed. Life Sci. 1126-1127: 121771.

Christa, P., Dunkel, A., Krauss, A., Stark, T.D., Dawid, C., and Hofmann, T. (2022). Discovery and identification of tastants and taste-modulating *N*-acyl amino acid derivatives in traditional Korean fermented dish Kimchi using a sensomics approach. J. Agric. Food Chem. 70(24): 7500–7514.

Ejima, A., Nakamura, M., Suzuki, Y.A., and Sato, K. (2018). Identification of food-derived peptides in human blood after ingestion of corn and wheat gluten hydrolysates. J. Food Bioat. 2: 104–111.

Falick, A.M., Hines, W.M., Medzihradszky, K.F., Baldwin, M.A., and Gibson, B.W. (1993). Low-mass ions produced from peptides by high-energy collision-induced dissociation in tandem mass spectrometry. J. Am. Soc. Mass Spectrom. 4(11): 882–893.

Fu, Y.Q., Zhou, Z.H., Li, Y.L., Lu, X., Zhao, C.X., and Xu, G.W. (2016). High-sensitivity detection of biogenic amines with multiple reaction monitoring in fish based on benzoyl chloride derivatization. J. Chromatogr. A 1465: 30–37.

Gutsche, K.A., Tran, T.B., and Vogel, R.F. (2012). Production of volatile compounds by Lactobacillus sakei from branched chain alpha-keto acids. Food Microbiol. 29(2): 224–8.

Han, J., Gagnon, S., Eckle, T., and Borchers, C.H. (2013). Metabolomic analysis of key central carbon metabolism carboxylic acids as their 3-nitrophenylhydrazones by UPLC/ESI-MS. Electrophoresis 34(19): 2891–2900.

Hordinsky, M., Sawaya, M., and Roberts, J.L. (2002). Hair loss and hirsutism in the elderly. Clin. Geriatr. Med. 18(1): 121–133.

Hofstetter, CK, Dunkel, A, and Hofmann, T (2019). Unified flavor quantitation: Toward high-throughput analysis of key food odorants and tastants by means of ultra-high-performance liquid chromatography tandem mass spectrometry. J. Agric. Food Chem. 67(31): 8599–8608.

Jansen, R.S., Addie, R., Merkx, R., Fish, A., Mahakena, S., Bleijerveld, O.B., Altelaar, M., IJlst, L., Wanders, R.J., Borst, P., and van de Wetering, K. (2015). *N*-lactoyl-amino acids are ubiquitous metabolites that originate from CNDP2-mediated reverse proteolysis of lactate and amino acids. Proc. Natl. Acad. Sci. U S A 112(21): 6601–6606.

Kiyono, T., Hirooka, K., Yamamoto, Y., Kuniishi, S., Ohtsuka, M., Kimura, S., Park, E.Y., Nakamura, Y., and Sato, K. (2013). Identification of pyroglutamyl peptides in Japanese rice wine (sake): presence of hepatoprotective pyroGlu-Leu. J. Agric. Food Chem. 61(47): 11660–11667.

Kovacevic, M., McCoy, J., Goren, A., Situm, M., Stanimirovic, A., Liu, W., Tan, Y., Vano-Galvan, S., Shapiro, J., and Sinclair, R. (2019). Novel shampoo reduces hair shedding by contracting the arrector pili muscle via the trace amine-associated receptor. J. Cosmet. Dermatol. 18(6): 2037–2039.

Li, Q., Zhang, L., and Lametsch, R. (2022). Current progress in kokumi-active peptides, evaluation and preparation methods: a review. Crit. Rev. Food Sci. Nutr. 62(5): 1230–1241.

Lin, L., Wu, J., Chen, X., Huang, L., Zhang, X., and Gao, X. (2020). The role of the bacterial community in producing a peculiar smell in Chinese fermented sour soup. Microorganisms 8(9): 1270.

Lin, L.J., Zeng, J., Tian, Q.M., Ding, X.Q., Zhang, X.Y., and Gao, X.Y. (2022). Effect of the bacterial community on the volatile flavour profile of a Chinese fermented condiment - Red sour soup - During fermentation. Food Res. Int. 155: 111059.

Liu, S.M., Murray, A., Schlink, A.C., Mata, G., and Masters, D.G. (2002). The effects of intradermal injections of spermidine on the growth rate of fibres and mitosis of wool follicles in Merino lambs. Animal Science 75: 33–40.

Morinaga, H., Mohri, Y., Grachtchouk, M., Asakawa, K., Matsumura, H., Oshima, M., Takayama, N., Kato, T., Nishimori, Y., Sorimachi, Y., Takubo, K., Suganami, T., Iwama, A., Iwakura, Y., Dlugosz, A.A., and Nishimura, E.K. (2021). Obesity accelerates hair thinning by stem cell-centric converging mechanisms. Nature 595(7866): 266–271.

Nishimura, E.K., Granter, S.R., and Fisher, D.E. (2005). Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science 307(5710): 720–724.

Paolella, S., Prandi, B., Falavigna, C., Buhler, S., Dossena, A., Sforza, S., and Galaverna, G. (2018). Occurrence of non-proteolytic amino acyl derivatives in dry-cured ham. Food Res. Int. 114: 38–46.

Pereira, C.I., Matos, D., San Romao, M.V., and Crespo, M.T. (2009). Dual

role for the tyrosine decarboxylation pathway in *Enterococcus faecium* E17: response to an acid challenge and generation of a proton motive force. Appl. Environ. Microbiol. 75(2): 345–352.

Peters, R., Hellenbrand, J., Mengerink, Y., and Van der Wal, S. (2004). On-line determination of carboxylic acids, aldehydes and ketones by high-performance liquid chromatography-diode array detection-atmospheric pressure chemical ionisation mass spectrometry after derivatization with 2-nitrophenylhydrazine. J. Chromatogr. A 1031(1-2): 35–50.

Ramot, Y., Pietila, M., Giuliani, G., Rinaldi, F., Alhonen, L., and Paus, R. (2010). Polyamines and hair: a couple in search of perfection. Exp. Dermatol. 19(9): 784–790.

Roepstorff, P., and Fohlman, J. (1984). Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed. Mass Spectrom. 11(11): 601.

Sato, K., Egashira, Y., Ono, S., Mochizuki, S., Shimmura, Y., Suzuki, Y., Nagata, M., Hashimoto, K., Kiyono, T., Park, E.Y., Nakamura, Y., Itabashi, M., Sakata, Y., Furuta, S., and Sanada, H. (2013). Identification of a hepatoprotective peptide in wheat gluten hydrolysate against D-galactosamine-induced acute hepatitis in rats. J. Agric. Food Chem. 61(26): 6304–6310.

Sato, K., Nisimura, R., Suzuki, Y., Motoi, H., Nakamura, Y., Ohtsuki, K., and Kawabata, M. (1998). Occurrence of indigestible pyroglutamyl peptides in an enzymatic hydrolysate of wheat gluten prepared on an industrial scale. J. Agric. Food Chem. 46(9): 3403–3405.

Sgarbi, E., Lazzi, C., Iacopino, L., Bottesini, C., Lambertini, F., Sforza, S., and Gatti, M. (2013). Microbial origin of non proteolytic aminoacyl derivatives in long ripened cheeses. Food Microbiol. 35(2): 116–120.

Shirako, S., Kojima, Y., Hasegawa, T., Yoshikawa, T., Matsumura, Y., Ikeda, K., Inagaki, N., and Sato, K. (2020). Identification of short-chain pyroglutamyl peptides in Japanese salted fermented soy paste (miso) and their anti-obesity effect. J. Food Bioat. 12: 129–139.

Shirako, S., Kojima, Y., Tomari, N., Nakamura, Y., Matsumura, Y., Ikeda, K., Inagaki, N., and Sato, K. (2019). Pyroglutamyl leucine, a peptide in fermented foods, attenuates dysbiosis by increasing host antimicrobial peptide. NPJ Sci. Food 3: 18.

Shukla, S., Park, H.K., Kim, J.K., and Kim, M. (2010). Determination of biogenic amines in Korean traditional fermented soybean paste (Doenjang). Food Chem. Toxicol. 48(5): 1191–1195.

Tran, B.Q., Hernandez, C., Waridel, P., Potts, A., Barblan, J., Lisacek, F., and Quadroni, M. (2011). Addressing trypsin bias in large scale(phospho) proteome analysis by size exclusion chromatography and secondary digestion of large post-trypsin peptides. J. Proteome Res. 10(2): 800–811.

Wang, C., Song, X., Li, C., He, L., Wang, X., and Zeng, X. (2023). Mixed fermentation with *Lactobacillus plantarum, Bifidobacteriμm animalis* subsp. *lactis* and *Candida utilis* improves the fermentation quality of Hong Suan Tang. Food Chem. 402: 134488.

Wang, C., Zhang, Q., He, L., and Li, C. (2020). Determination of the microbial communities of Guizhou Suantang, a traditional Chinese fermented sour soup, and correlation between the identified microorganisms and volatile compounds. Food Res. Int. 138(Pt B): 109820.

Yuan, Q., Zhou, Q., Wang, N., Zheng, Y., Hu, H., Hu, S., and Wang, H. (2022). Integrative proteomics and metabolomics of Guizhou Miao Sour Soup affecting simple obese rats. Front Nutr. 9: 1019205.

Zhao, C.J., Schieber, A., and Ganzle, M.G. (2016). Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations - A review. Food Res. Int. 89(Pt 1): 39–47.

Zhou, X., Liu, Z., Xie, L., Li, L., Zhou, W., and Zhao, L. (2022a). The correlation mechanism between dominant bacteria and primary metabolites during fermentation of red sour soup. Foods 11(3): 341.

Zhou, X., Zhou, W., He, X., Deng, Y., Li, L., Li, M., Feng, X., Zhang, L., and Zhao, L. (2022b). Effects of post-fermentation on the flavor compounds formation in red sour soup. Front Nutr. 9: 1007164.