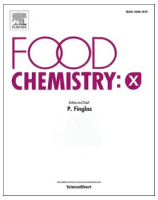
Food Chemistry: X 20 (2023) 100933

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Analysis of dipeptides in Chinese liquors based on dansylation combined with liquid chromatography–mass spectrometry

Yubo Yang [a], Xin Lu [b,c], Fan Yang [a], Zhen Jia [b,c], Xiaoyu Xie [b,c,d], Nian Cao [a], Di Yu [b,c], Fujian Zheng [b,c,d], Xinyu Liu [b,c], Li Wang [e,*], Guowang Xu [b,c,d,*]

[a] Guizhou Moutai Co., Ltd, Renhuai 564501, China
[b] CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China
[c] Liaoning Province Key Laboratory of Metabolomics, Dalian 116023, China
[d] University of Chinese Academy of Sciences, Beijing 100049, China
[e] China Guizhou Moutai (Group) Co., Ltd, Renhuai 564501, China

ARTICLE INFO

Keywords:
Chinese liquor
Dipeptides
LC-MS
Quantification analysis

ABSTRACT

Dipeptides have been shown to be an important taste substance in alcoholic beverages. However, the characterization of dipeptides in Chinese liquors was poor. Here, dansylation combined with liquid chromatography – mass spectrometry was employed to analyze dipeptides in eight liquors of two flavors. Consequently, 35 dipeptides were identified from liquors and 32 of them were quantified. Dipeptide quantification showed LODs smaller than 2.5 ng/mL. The calibration curves showed concentration spans from two to three orders of magnitude with satisfactory linearity. The matrix effects in low and high concentrations were from −25.71 % to 24.19 % and −14.82 % to 20.73 %, respectively. Intra- and inter-day precision is lower than 15 % for both low and high concentrations. The dipeptide contents in sauce flavor liquors were higher than those in strong flavor liquors. Ala- and -Phe dipeptides showed their unique trends between sauce and strong flavor liquors. This study provides new clues to evaluate taste of liquors.

1. Introduction

Chinese liquor has a long history as one of the embodiments of Chinese culture. With its unique flavors, the taste composition of Chinese liquor has always been of interest to food scientists and analysts. It has been reported that organic acids, aldehydes and esters play important roles in determining the taste of Chinese liquor (Liu & Sun, 2018; Niu et al., 2017). However, these reported compounds are still insufficient to reveal the formation of such complex tastes in Chinese liquors.

There have been many studies showing that several dipeptides possess various tastes such as bitter, umami, sour and salty, making them play important roles in adjusting the tastes of foods including ham, soy sauces and fermented foods (Gallego, Mora, & Toldrá, 2019; Yamamoto et al., 2014). Particularly, dipeptides were also identified in fermented alcoholic beverages such as sake and beer, and were demonstrated to influence tastes greatly (Takahashi et al., 2012). Despite these, dipeptides have not been reported and noticed in the studies of Chinese liquor, probably due to the analytical difficulty because of the lower contents of dipeptides.

Liquid chromatography-mass spectrometry (LC-MS) is the main approach used in the analysis of dipeptides. Meanwhile, the auxiliary use of chemical derivatization can significantly improve the MS sensitivity and chromatographic retention of dipeptides. LC-MS and 6-aminoquinolyl-*N*-hydroxy-succinimidel carbamate derivatization have been used to identify and quantity dipeptides in alcoholic beverages, presenting good sensitivity and chromatographic separation of dipeptides (Takahashi et al., 2012).

Therefore, considering the potential contribution of dipeptides to

* Corresponding authors at: Kweichow Moutai Distillery Co Ltd, Renhuai 564500, Guizhou, China. Tel.: +86 851 22386504 (L. Wang). CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China. Tel./fax: +86 411 84379530 (G. Xu).

E-mail addresses: yangyubo@jszx27.wecom.work (Y. Yang), luxin722@dicp.ac.cn (X. Lu), yangfan@jszx27.wecom.work (F. Yang), jiazhen@dicp.ac.cn (Z. Jia), xiexy@dicp.ac.cn (X. Xie), caonian@jszx27.wecom.work (N. Cao), yudi_1808@dicp.ac.cn (D. Yu), zhengfj@dicp.ac.cn (F. Zheng), liuxy2012@dicp.ac.cn (X. Liu), eileenjn@126.com (L. Wang), xugw@dicp.ac.cn (G. Xu).

https://doi.org/10.1016/j.fochx.2023.100933
Received 24 June 2023; Received in revised form 17 July 2023; Accepted 6 October 2023
Available online 12 October 2023

liquor taste, this study wants to investigate the differences in the content of dipeptides in eight commercialized Chinese liquors from the markets. To identify the dipeptides in the liquors with high sensitivity, dansylation combined with liquid chromatography-mass spectrometry was applied to perform the screening of dipeptides. On this basis, the selected dipeptides in the liquors were further confirmed and quantified by using corresponding standards. Finally, the differences in dipeptide contents of different liquors were compared and the potential contribution of dipeptides to the tastes of specific liquors were discussed. This study was designed to provide methods for the study of the taste substances of Chinese liquors.

2. Materials and methods

2.1. Samples

Eight Chinese liquors including Moutai (MT), Wu Liang Ye (WLY), Jian Nan Chun (JNC), 20 Nian Jiao Cang (20 yr), Si Cang Lao Jiu (SCLJ), Qing Hua Lang (QHL), Xi Jiu (XJ) and Guo Jiao (GJ) were purchased from the local markets. Among these liquors, GJ, WLY and JNC were classified as strong flavor (nongxiang (NX) based on Chinese typing) while the others were sauce flavor (jiangxiang (JX) based on Chinese typing). All of the liquors were stored airtight at room temperature until preparation.

2.2. Chemicals and reagents

Methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Dansyl chloride, sodium carbonate (Na_2CO_3), and sodium bicarbonate ($NaHCO_3$) were obtained from Sigma-Aldrich (Louis, MO). Hydrochloric acid (HCl, 12 M) was purchased from Kermel (Tianjin, China). Dipeptide standards including AQ, AI, AL, AF, NP, DV, GA, GV, IA, II, IL, LI, IM, LM, IF, IS, IT, IV, LA, LQ, LF, LP, LS, LT, LV, FI, PI, PL, SI, SL, TI, TL, VI and VL were synthesized by Nanjing Yuanpeptide Biotech Co. Ltd (Nanjing, Jiangsu, China). Isotope-labeled myclobutanil-d_9 was purchased from AccuStandard (New Haven, CT). Metronidazole-$^{13}C_2$, $^{15}N_2$ was obtained from J&K Scientific (Beijing, China).

2.3. Sample preparation

2.3.1. Pre-treatment of liquors and calibration solutions

All liquor samples were concentrated by 25 times with rotary evaporation at 42 °C and the whole concentration procedure was completed within 30 min. Dipeptide standards for generating calibration curves were dissolved with de-ionized water at concentrations of 1.25 pg/mL, 2.5 pg/mL, 5 pg/mL, 10 pg/mL, 25 pg/mL, 50 pg/mL, 125 pg/mL, 250 pg/mL, 500 pg/mL, 1 ng/mL, 2.5 ng/mL, 5 ng/mL, 12.5 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, 250 ng/mL, 500 ng/mL, 1.25 μg/mL, 2.5 μg/mL and 5 μg/mL. Dipeptide standards for optimizing MRM parameters were prepared with deionized water at 100 ng/mL. Quality control samples (QCs) were obtained by blending all liquors in equal proportions. Internal standards were prepared together in water at different concentrations. Myclobutanil-d_9 and metronidazole-$^{13}C_2$, $^{15}N_2$ were prepared at 10 and 100 ng/mL, respectively. Each 20 μL of liquor or calibration standard was mixed with 20 μL of internal standard stock and the mixture was further dried via rotation vacuum.

2.3.2. Dansylation

The process of dansylation was conducted and optimized based on the procedure reported previously (Lu et al., 2021). In brief, 60 μL of Na_2CO_3/$NaHCO_3$ buffer (pH = 9.14) was added to the sample residues followed by a 3-min vortex for re-dissolvation. Afterward, 20 μL of dansulfonyl chloride in acetonitrile (10 mg/mL) was added to the mixture and further vortexed for 2 min. The mixture was then incubated at 60 °C for 60 min followed by the centrifugation at 15,000 *g*. The supernatant was isolated for further preparation.

2.4. LC-MSMS analysis

The LC-MSMS analyses of dipeptides were conducted by using an ultra-performance LC-TQXS series mass spectrometer (Waters, Milford) in multiple reaction monitoring (MRM) mode. The chromatographic separation was performed with a BEH C8 column (2.1 mm × 100 mm, 1.7 μm (Waters, Milford, MA)). The chromatographic conditions were applied according to the method for dipeptide analysis used previously (Lu et al., 2023): column temperature: 50 °C, flow rate: 350 μL/min, aqueous phase (channel A): de-ionized water containing 0.1 % formic acid, organic phase (channel B): acetonitrile containing 0.1 % formic acid, chromatographic buffer gradient: 5 % B from 0 to 0.5 min, 5 % to 100 % B from 0.5 min to 24 min followed by a 4 min-maintenance, changed the gradient back to initial B by 28.1 min and further equilibrated 5 % B to 30 min.

For data acquisition, the collision energy (CE) and cone voltage (CV) for MRM analysis of each dipeptide were optimized based on the corresponding MS response. The ionization conditions were applied as below: ion mode: positive, spray voltage: 1500 V, source temperature: 150 °C, cone voltage: 30 V, desolvation gas flow: 600 L/h, desolvation temperature: 350 °C, nebulizer gas flow: 7 bar. Collision energy and cone voltage for dipeptide identification and quantification were optimized by using the dipeptide standards.

2.5. Data processing

The processes involving peak detection, smoothing, integration, and normalization were realized with TargetLynx (Waters). The setting conditions are given in Table S1. The peak area after integration was calibrated with internal standards. Calibration curves were obtained according to the peak areas of calibration standards of different concentrations.

2.6. Evaluation of analytical characteristics

To ensure the stability and reliability of quantitative results, analytical characteristics including intra- and inter-day precision, limit of detection (LOD), limit of quantification (LOQ), linearity range, accuracy, and matrix effect were evaluated. LOD was determined as the concentration of which MS signal-to-noise close to 3 while LOQ was determined based on the lower limit of the linearity range. Inter- and intra-day precisions were assessed with calibration solutions of 5 ng/mL and 50 ng/mL, which were assigned as low and high concentrations, respectively. Intra-day precision was obtained via the calculation of the relative standard derivations (RSDs) of dipeptides between six injections of the same solutions while inter-day precision was obtained based on the RSDs between calibration solutions prepared within three consecutive days. The linearity range was determined based on the MS responses of dipeptides in the liquors. To ensure the quantitative accuracy of samples of diverse concentrations, the responses of dipeptides in the calibration solutions were weighted according to the reciprocal of the concentration. The linearity range should cover the minimum and maximum concentrations of dipeptides in all liquors. Matrix effect was evaluated with the neat working solution, matrix solution and matrix-containing working solution. The neat working solutions were the calibration solutions of 5 ng/mL and 50 ng/mL. QC samples were applied to prepare the matrix solution. The matrix-containing working solution was obtained by resolving calibration standards with the matrix solution. The matrix effect was finally assessed based on Equation (1).

$$\text{Matrix effect} = (A_{mw}\text{-}A_m)/A_w \times 100\ \% \quad (1)$$

where A_{mw}, A_m, and A_w are the peak areas of a dipeptide obtained from the matrix-containing working solution, the matrix solution and the neat working solution, respectively.

2.7. Statistics

Mann-Whitney *U* test was applied for evaluating the significance of differences. Hierarchical cluster analysis (HCA) was conducted to reveal the similarity between liquors and between dipeptides. Pearson correlation coefficients were calculated to characterize the correlation among liquors. GraphPad Prism 8 (GraphPad Software, USA) was used to perform Mann-Whitney test. HCA and Pearson correlation analyses were both conducted on MetaboAnalyst platform (https://www.metaboanalyst.ca/).

3. Results

3.1. Establishment of LC-MSMS methods

Due to the very low concentration of dipeptides in the Chinese liquor, dipeptides were defined by using LC-MSMS in MRM mode. The transitions and chromatographic conditions for MRM analysis of dipeptides were obtained according to the dipeptide library previously reported, which contains the retention times (t_R) and MSMS spectra for over 200 dipeptides (Lu et al., 2023). One transition is determined based on the derivatization-specific fragment with a good MRM response (Transition 1, t1) and the other is set based on the peptide bond-specific fragment

Table 1
Analytical characteristics of optimized LC-MS/MS method for liquor dipeptides.

Name	Intra-day precision (L, %)	Intra-day precision (H, %)	Inter-day precision (L, %)	Inter-day precision (H, %)	LOD	Linearity	R^2	Matrix effect (L,%)	Matrix effect (H,%)
Gly-Ala-t1	4.1	6.3	2.4	5.6	12.5 pg/mL	125 pg/mL-125 ng/mL	0.985	0.08	−0.35
Gly-Val-t2	14.0	10.2	6.8	4.2	50 pg/mL	250 pg/mL- 250 ng/mL	0.991	−6.93	−4.95
Ile-Ala-t1	7.9	3.6	6.8	5.6	1.25 pg/mL	250 pg/mL-125 ng /mL	0.994	−16.57	13.47
Leu-Ala-t2	9.2	3.9	10.9	3.6	5 pg/mL	125 pg/mL-50 ng/mL	0.9959	−13.63	−2.05
Ala-Ile-t1	4.6	3.6	3.6	1.8	1.25 pg/mL	125 pg/mL-125 ng/mL	0.994	−8.05	−1.64
Ala-Leu-t2	8.7	3.7	5.9	8.3	5 pg/mL	250 pg/mL-125 ng/mL	0.996	−0.94	4.52
Ala-Gln-t1	3.8	3.2	3.8	2.9	5 pg/mL	1.25 ng/mL-500 ng/mL	0.971	−25.71	6.78
Ile-Ser-t1	2.4	2.4	2.3	6.9	1.25 pg/mL	250 pg/mL-50 ng /mL	0.990	2.16	−0.99
Ser-Ile-t1	4.3	4.4	6.5	4.7	5 pg/mL	250 pg/mL- 50 ng /mL	0.985	24.19	20.73
Ser-Leu-t1	6.1	3.1	0.3	2.7	1.25 pg/mL	125 pg/mL-25 ng /mL	0.984	−14.27	2.09
Leu-Pro-t2	9.5	6.3	5.2	9.0	1.25 pg/mL	50 pg/mL-125 ng/mL	0.994	−18.84	−7.72
Pro-Ile-t2	5.7	5.4	2.2	1.3	5 pg/mL	1.25 ng/mL-500 ng/mL	0.994	−15.75	−5.05
Pro-Leu-t2	4.8	3.7	0.8	3.7	1.25 pg/mL	250 pg/mL-12.5 ng/mL	0.974	−19.04	−14.82
Val-Ile-t2	7.2	4.0	5.2	4.5	2.5 pg/mL	250 pg/mL-50 ng/mL	0.992	−8.89	−7.36
Val-Leu-t2	4.3	2.8	5.5	6.1	1.25 pg/mL	500 pg/mL-12.5 ng/mL	0.999	−18.32	−3.08
Ile-Val-t2	2.6	4.0	8.0	4.7	1.25 pg/mL	250 pg/mL-50 ng/mL	0.988	−3.19	7.21
Leu-Val-t2	8.1	4.0	5.2	5.0	12.5 pg/mL	50 pg/mL-12.5 ng/mL	0.982	−5.72	−1.12
Ile-Thr-t1	6.3	2.7	5.2	5.9	5 pg/mL	250 pg/mL-12.5 ng/mL	0.987	4.03	12.21
Leu-Thr-t1	10.3	4.6	11.7	1.1	12.5 pg/mL	250 pg/mL-50 ng/mL	0.991	−13.05	13.56
Thr-Ile-t1	5.2	6.0	3.1	5.0	5 pg/mL	250 pg/mL-50 ng/mL	0.992	−13.31	−1.80
Thr-Leu-t2	12.6	5.9	12.9	10.9	50 pg/mL	250 pg/mL-12.5 ng/mL	0.967	−3.10	−9.95
Ala-Phe-t2	5.7	5.0	8.8	3.8	500 pg/mL	500 pg/mL-25 ng/mL	0.984	−5.62	−9.79
Ile-Leu-t2/ Leu-Ile-t2	7.1	2.4	1.9	4.6	12.5 pg/mL	50 pg/mL-12.5 ng/mL	0.991	−4.71	5.89
Leu-Gln-t2	4.6	3.8	4.5	2.5	50 pg/mL	250 pg/mL-125 ng/mL	0.991	−10.97	6.42
Ile-Met-t1	8.0	2.8	6.2	4.9	2.5 pg/mL	2.5 ng/mL-125 ng/mL	0.999	−16.11	1.01
Leu-Met-t1	6.1	6.0	8.3	6.0	2.5 ng/mL	2.5 ng/mL-125 ng/mL	0.999	−10.22	−14.62
Ile-Phe-t2	6.9	5.0	10.8	1.5	500 pg/mL	1.25 ng/mL-50 ng/mL	0.999	−11.80	7.35
Leu-Phe-t2	10.2	3.7	7.0	8.2	1.25 ng/mL	1.25 ng/mL-50 ng/mL	0.965	−14.68	−9.82
Phe-Ile-t2	7.3	5.6	8.7	3.3	250 pg/mL	250 pg/mL-1.25 ng/mL	0.968	−11.91	5.04

(Transition 2, t2) with good specificity. The CE and CV values for both transitions were optimized by using dipeptide standards, As shown in Figure S1, both Transition 1 and Transition 2 of dipeptide standards showed the optimized CVs at 30 V. For CEs, Transitions 1 and 2 of dipeptide standards presented the optimized CEs at 35 V and 25 V, respectively. The identification procedure was conducted according to the workflow shown in Figure S2. The t_R of dipeptides in both transitions were matched with those in the library and further validated by using corresponding standards. As a sequence, a total of 35 dipeptides were defined and validated. The total ion chromatograms of the defined dipeptides in standards and Chinese liquor are presented in Figure S3. Information regarding MRM analyses of identified dipeptides including t_R, transition ion pairs, optimized CEs and CVs are given in Table S2.

3.2. Evaluation of analytical characteristics

LOD, inter- and intra-day precisions, linearity range, and matrix effect were evaluated to ensure the robustness of the analytical methods. As a result, the intra-day precisions in low and high concentrations ranged from 2.4 % to 14.0 % with a mean of 6.8 % and from 2.4 % to 10.2 % with a mean of 4.4 %, respectively (Table 1). The inter-day precisions in low and high concentrations ranged from 0.3 % to 12.9 % with a mean of 6.2 % and from 1.1 % to 10.9 % with a mean of 4.8 %. Among all 29 dipeptide standards, 17 dipeptides showed LODs no more than 5 pg/mL while LODs of 10 dipeptides ranged from 12.5 pg/mL to 500 pg/mL. Only two dipeptides, i.e. LF (1.25 ng/mL) and LM (2.50 ng/mL), respectively, had LODs greater than 1 ng/mL. Linearity was weighted according to the reciprocal of the concentration. The concentration spans of the calibration curves were from two to three orders of magnitude with satisfactory linearity. The matrix effects in low and high concentrations were from −25.71 % to 24.19 % and −14.82 % to 20.73 %, respectively. The above results showed satisfactory performance of the established methods.

3.3. Quantification of dipeptides in Chinese liquors

By using the LC-MSMS developed above, the concentrations of 32 dipeptides in 8 Chinese liquors of different flavors were quantified. As shown in Table 2, the contents of different dipeptides in different liquors varied widely, from a few μM to thousands of μM. It can be observed from Fig. 1A, the Chinese liquors with overall dipeptide content from low to high were GJ, MT, SCLJ, WLY, JNC, XJ, 20 yr and QHL, respectively. Among these liquors, GJ, WLY and JNC were classified as NX flavor while the others were JX flavor. It could be seen that the three liquors with the highest dipeptide content were all with JX flavor, suggesting the potential contribution of dipeptides to JX flavor. By comparing the concentration range of dipeptides in different liquors (Fig. 1B), it was observed that there were four liquors containing dipeptides in concentrations over 1000 μM, including three JX flavor liquors (QHL, 20 yr, XJ) and one NX flavor liquor (JNC). Pro-Ile was the only dipeptide with a concentration of over 1000 μM in all these liquors. It was also the only dipeptide with a concentration more than 10 mM (16.42 mM in QHL). In addition, these three JX flavor liquors also contained a large percentage of dipeptides (no less than 50 %) in the concentration range of 100 ~ 1000 μM. The other two JX flavor liquors (MT and SCLJ) and two NX flavor liquors (WLY and JNC) showed a large proportion of dipeptides in the concentration range of 10 ~ 100 μM. GJ was the only liquor that contained a large proportion of dipeptides in a concentration of smaller than 10 μM. Therefore, it could be summarized that the overall content of dipeptides in JX flavor liquors was higher

Table 2
Dipeptide concentration in eight Chinese liquors with different flavors.[a]

Name	20 yr		GJ		JNC		MT		QHL		SCLJ		WLY		XJ	
	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)	Conc.	RSD (%)
Gly-Ala-2-t1	834.2	13.0	80.9	9.8	30.7	22.0	10.6	16.9	4976.4	9.8	26.4	12.6	216.5	14.6	293.0	14.9
Gly-Val-2-t2	291.8	25.7	11.9	38.6	13.1	18.4	17.0	28.6	1857.5	8.5	29.6	36.7	35.9	71.3	104.2	18.6
Ile-Ala-t1	1273.6	6.4	16.2	11.5	846.5	6.3	68.5	23.0	2983.9	4.1	102.6	3.6	236.1	6.9	288.2	3.7
Leu-Ala-t2	521.7	6.8	9.7	19.1	516.4	8.3	10.6	26.0	679.1	21.5	25.3	41.6	98.3	2.8	88.3	22.9
Ala-Ile-t1	782.3	5.0	7.6	36.5	95.2	6.0	30.4	15.9	3220.6	0.7	61.9	12.1	35.2	13.3	734.6	8.0
Ala-Leu-t2	1264.7	6.6	7.8	5.6	447.8	8.2	39.3	18.0	1742.3	3.9	68.6	11.9	144.4	10.4	460.5	6.9
Ala-Gln-t1	2907.5	8.5	46.5	6.6	65.5	6.4	73.8	2.8	6314.4	0.8	126.3	7.1	184.5	8.6	1106.4	3.4
Ile-Ser-t1	380.2	4.3	7.1	10.6	16.8	8.1	19.0	8.5	1092.0	5.0	18.9	16.4	51.2	19.1	167.1	5.8
Ser-Ile-t1	362.4	16.1	11.9	33.0	20.6	8.4	82.8	3.3	896.5	4.6	29.4	13.1	28.7	3.7	171.2	9.7
Ser-Leu-t1	604.0	4.3	4.5	34.2	10.3	19.9	12.5	13.2	571.8	6.0	18.3	14.5	35.3	14.4	62.3	4.1
Leu-Pro-t2	246.9	6.1	1.0	37.3	1718.6	11.8	5.5	21.6	185.6	7.9	7.0	23.4	73.0	5.8	30.7	8.9
Pro-Ile-t2	2266.1	7.8	260.3	19.6	4062.7	4.5	573.4	12.2	16424.4	0.8	589.2	12.1	750.1	17.0	2331.3	12.3
Pro-Leu-t2	74.2	8.8	8.9	8.4	310.1	4.2	15.6	7.3	340.2	4.1	17.1	7.7	25.1	2.3	68.5	8.5
Val-Ile-t2	389.1	11.3	5.2	13.7	41.2	9.8	13.8	14.3	1175.8	5.5	24.1	17.2	23.0	17.5	202.5	13.3
Val-Leu-t2	1385.9	8.4	13.7	9.8	2431.4	12.7	32.0	16.7	1425.6	5.4	48.9	9.7	152.0	0.9	270.7	11.6
Ile-Val-t2	289.2	3.8	8.7	3.7	46.2	4.9	12.1	12.1	835.6	2.3	22.6	14.9	22.6	8.1	581.6	10.8
Leu-Val-t2	204.6	9.0	2.4	24.9	221.2	0.3	5.0	28.3	298.5	3.9	9.8	8.3	16.6	11.2	53.0	17.2
Ile-Thr-t1	253.0	4.5	12.1	29.6	18.9	9.6	13.9	17.8	1710.5	3.8	22.8	14.5	33.1	5.3	155.5	9.3
Leu-Thr-t1	246.8	1.7	8.2	14.5	11.8	15.0	18.3	11.4	693.1	1.1	18.8	14.8	63.5	10.4	88.6	19.8
Thr-Ile-t1	359.6	13.2	14.1	12.1	16.4	24.3	19.7	27.7	1398.6	8.1	35.2	17.7	19.9	65.4	169.1	5.9
Thr-Leu-t2	67.9	17.0	8.6	37.4	7.6	19.6	7.6	36.4	228.2	19.6	4.6	63.1	12.4	48.1	18.4	10.1
Ala-Phe-t2	206.9	20.8	13.1	76.9	31.2	42.1	24.1	19.5	508.5	7.5	19.6	30.5	50.6	9.5	108.1	28.4
Ile-Ile-t2	67.1	4.7	0.7	15.9	7.0	21.8	2.9	17.4	335.9	7.9	3.6	7.4	3.3	3.5	44.1	10.2
Ile-Leu-t2/ Leu-Ile-t2	101.3	4.5	1.3	13.8	245.9	6.5	2.1	17.4	159.2	4.0	4.3	10.4	8.8	6.8	30.1	1.3
Leu-Leu-t2	34.5	6.9	0.4	21.3	102.0	4.8	0.4	33.1	54.2	8.9	1.5	9.9	4.1	25.0	7.8	5.6
Leu-Gln-t2	1098.5	7.6	9.4	14.1	14.5	26.7	13.2	22.4	1553.6	1.4	38.9	5.2	77.1	11.3	230.1	11.8
Ile-Met-t1	645.7	9.2	NQ	57.8	649.7	11.0	NQ	18.9	688.6	4.3	113.7	7.6	335.7	17.0	237.7	11.5
Leu-Met-t1	328.5	2.7	NQ	26.5	483.5	5.4	192.5	9.6	1008.6	2.9	159.0	7.3	189.5	14.0	443.6	6.6
Ile-Phe-t2	269.5	5.6	25.7	29.8	286.6	9.2	22.0	21.3	382.6	7.4	25.9	29.7	79.7	10.3	72.6	5.5
Leu-Phe-t2	117.8	2.6	7.1	8.4	105.4	15.9	7.6	81.4	137.2	13.1	11.8	20.0	25.4	26.1	22.7	13.6
Phe-Ile-t2	79.8	21.8	9.0	32.5	67.9	12.6	39.8	10.6	164.7	15.2	40.4	51.8	22.9	34.5	43.8	26.2
Leu-Ser-t2	73.1	0.9	0.3	27.7	6.4	42.8	2.1	33.4	149.2	2.9	4.1	21.7	15.8	5.4	22.1	29.7

[a] The concentration of dipeptides is in μM.

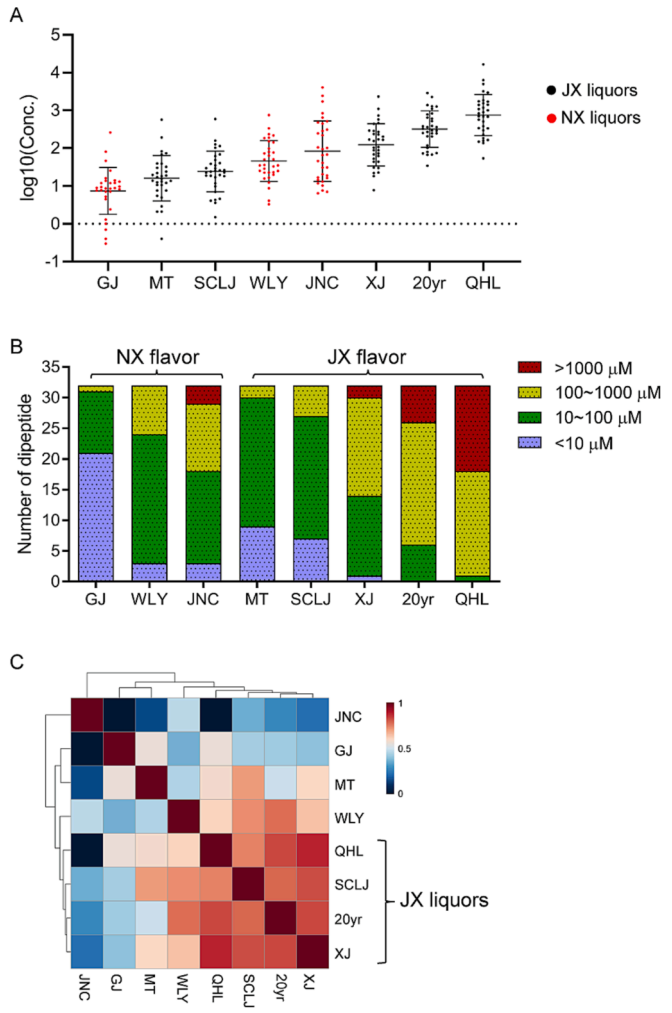


Fig. 1. Difference and correlation of dipeptides between different liquors A. Relative amounts of dipeptides among different liquors, B. Percentages of different concentrations of dipeptides in liquors, C. Correlation of dipeptide profiles among different liquors. JX: sauce flavor, NX: strong flavor.

than that in NX flavor liquors. On this basis, the correlation among different liquors was also investigated, JX flavor liquors including QHL, SCLJ, 20 yr, and XJ revealed high correlations with each other (Fig. 1C), suggesting that they had similar ratios among dipeptides, and this high correlation was not seen among NX flavor liquors.

The differences in the content of dipeptides with specific residues were further characterized. According to the pattern generated by HCA (Fig. 2A), the dipeptides could be generally grouped into three clusters, namely Clusters I, Cluster II and Cluster III which contained 10, 10, and 12 dipeptides, respectively. Overall, the dipeptides in Clusters I, Clusters II and Clusters III showed a high to low tendency in the normalized content of all liquors. Relatively, this manner was more pronounced in JX flavor liquors that the content of dipeptides in Cluster II was significantly higher than the content in Cluster III whereas no such significant variations were observed in NX flavor liquors (Fig. 2B). Pro-Ile and Leu-Leu were the dipeptides showing the highest and lowest overall content in liquors, respectively. The concentration range of Pro-Ile in all liquors was from 260.3 to 16024.4 μM while that of Leu-Leu was from 0.4 to 102.0 μM. Notably, the dipeptides containing Ala were mainly gathered in Cluster I while those containing Phe were mainly gathered in Cluster III. Among these dipeptides, the ones with Ala at *N*-terminal (Fig. 2C) and those with Phe at *C*-terminal (Fig. 2D) showed overall higher and lower levels in JX flavor liquors than in NX flavor liquors, respectively. Therefore, it could be concluded that the dipeptide concentration varied greatly between different liquors and between different species of dipeptides. Also, the two flavors of liquors showed differences in the profiles of the dipeptides with specific residues.

4. Discussion

This study is the first targeted identification and quantification of dipeptides in Chinese liquors. Dansylation was employed to enhance the chromatographic performance and mass spectrometry response of dipeptides in Chinese liquors. Because of the presence of large amounts of carboxylic acid compounds in Chinese liquors (Xie et al., 2022), the very low pH of concentrated liquors can easily lead to the failure of the buffer system during dansylation (Meseguer Lloret, Molins Legua, & Campins Falco, 2002). Therefore, the volume of derivatization reagents was evaluated in this study (not shown) to ensure the derivatization efficiency and to control the dilution concentration. In general, high-resolution mass spectrometry is undoubtedly the preferred approach for identifying unknown compounds. However, due to the low content of dipeptides in Chinese liquors, LC-MRM-MS with higher sensitivity was used for dipeptide identification and confirmation based on the established dipeptide library (Lu et al., 2023). A non-negligible issue is that the dipeptide library we built does not include all theoretically existing dipeptides, limiting the scope of identification.

Due to the contribution of dipeptides in regulating food taste, identification and quantification of dipeptides in alcoholic beverages such as beers and Japanese sake have been conducted using LC-MS. Similar to the present study, 6-aminoquinolyl-*N*-hydroxy-succinimidel carbamate derivatization coupled to LC-MSMS was applied to quantify 32 dipeptides in Japanese sake (Takahashi et al., 2012). The LODs exhibited by the proposed method in this study ranged from approximately hundreds to tens of thousands of pg/mL, which was much higher than the LODs obtained in the presented study, in which the derivatization and analytical conditions were systematically optimized. The concentrations of dipeptides in sake ranged from 1.1 μM to 97.2 μM, with a mean of 16.1 μM. These values were significantly smaller than those of the liquor dipeptides quantified in the present study. It could be seen from Table S3, the tastes of 12 dipeptides studied in this study were uncovered previously, 10 of them, mainly Ile/Leu containing dipeptides, were confirmed to have a bitter taste. Of the dipeptides we studied, 25 out of 32 were with Ile or Leu, a proportion much higher than that of dipeptides (6 out of 32) in the sake study. The higher content and the proportion of bitter dipeptides may have contributed to the bitterness of liquors.

Chinese liquors can have different flavors and tastes depending on the raw materials, malt and brewing process (He, Gaca, & Jeleń, 2020). Sauce flavor and strong flavor are two of the major types of flavors that account for roughly 80 % of the market for all Chinese liquors. Particularly, sauce flavor liquors are well known for their unique “soy sauce”-like flavor, making them the most collectible liquors. It has been reported that the contents of carboxylic acids in sauce flavor liquors are significantly higher than those in other flavors of liquors (Xie et al., 2022). However, these findings are still not enough to interpret the specificity of the taste. In the present study, the contents of some dipeptides of sauce flavor liquors were significantly different from those in strong flavor liquors. More importantly, compared to the three strong flavor liquors, sauce flavor liquors have more similar profiles to specific residues. These dipeptides have proved to play important roles in regulating tastes of foods such as soy sauce and ham (Apriyantono, Setyaningsih, Hariyadi, & Nuraida, 2004). These differences may contribute to the distinction between the taste and flavor of the two types of liquors.

5. Conclusions

In this study, dipeptides in two flavors of eight Chinese liquors were identified and quantified by using dansylation combined with LC-MSMS.

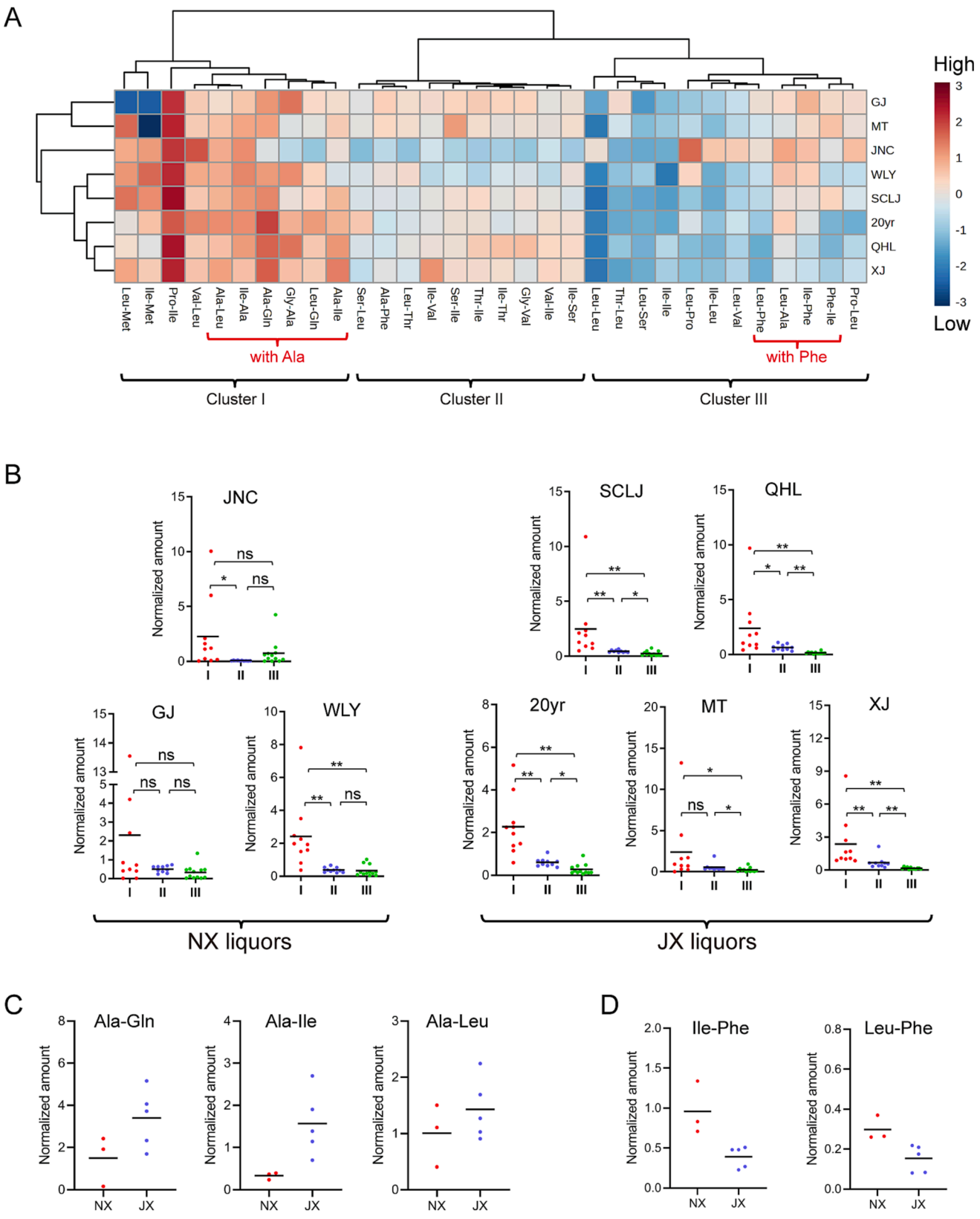


Fig. 2. Differences of dipeptides in two flavors of liquors A. HCA diagram showing difference and similarity of dipeptide profiles, B. Relative content of dipeptides of different grouping clusters among liquors, C. Relative content of Ala- and -Phe dipeptides in sauce flavor (JX) and strong flavor (NX) liquors.

The established LC-MRM-MS strategy proved to be effective in identifying low-abundance dipeptides in complex liquor samples. The dipeptides in sauce flavor liquors were overall more abundant than those in strong flavor liquors, while the dipeptide profiles in sauce flavor liquors had a strong correlation, which was not significant in strong flavor liquors. The dipeptides with specific residues differed consistently among the two flavors of liquors. This study can provide new clues and tools for taste research and quality evaluation of Chinese liquors.

CRediT authorship contribution statement

Yubo Yang: Methodology, Resources, Validation, Project administration, Writing – review & editing. **Xin Lu:** Methodology, Software, Formal analysis, Investigation, Writing – original draft. **Fan Yang:** Conceptualization, Resources, Project administration, Funding acquisition. **Zhen Jia:** Software, Formal analysis, Writing – review & editing. **Xiaoyu Xie:** Investigation, Data curation. **Nian Cao:** Investigation, Validation, Resources. **Di Yu:** Investigation, Writing – review & editing. **Fujian Zheng:** Software. **Xinyu Liu:** Writing – review & editing. **Li Wang:** Resources, Supervision, Writing – review & editing, Funding acquisition. **Guowang Xu:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the foundation (21904123, 21934006) from the National Natural Science Foundation of China and the Youth Innovation Promotion Association CAS (2021186).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100933.

References

Apriyantono, A., Setyaningsih, D., Hariyadi, P., & Nuraida, L. (2004). Sensory and peptides characteristics of soy sauce fractions obtained by ultrafiltration. *Advances in Experimental Medicine and Biology, 542*, 213–226. https://doi.org/10.1007/978-1-4419-9090-7_15

Gallego, M., Mora, L., & Toldrá, F. (2019). The relevance of dipeptides and tripeptides in the bioactivity and taste of dry-cured ham. *Food Production, Processing and Nutrition, 1*(1). https://doi.org/10.1186/s43014-019-0002-7

He, X., Gaca, A., & Jeleń, H. (2020). Determination of volatile compounds in Baijiu using simultaneous chromatographic analysis on two columns. *Journal of the Institute of Brewing*.

Liu, H., & Sun, B. (2018). Effect of Fermentation Processing on the Flavor of Baijiu. *Journal of Agricultural and Food Chemistry, 66*(22), 5425–5432. https://doi.org/10.1021/acs.jafc.8b00692

Lu, X., Dou, P., Li, C., Zheng, F., Zhou, L., Xie, X., … Xu, G. (2023). Annotation of Dipeptides and Tripeptides Derivatized via Dansylation Based on Liquid Chromatography-Mass Spectrometry and Iterative Quantitative Structure Retention Relationship. *Journal of Proteome Research*.

Lu, X., Dou, P., Xie, X., Wang, X., Liu, X., Hua, R., & Xu, G. (2021). Development of a novel analytical method for inflammation and immunity-related metabolites in serum based on liquid chromatography tandem mass spectrometry. *Talanta, 234*, Article 122631. https://doi.org/10.1016/j.talanta.2021.122631

Meseguer Lloret, S., Molins Legua, C., & Campins Falco, P. (2002). Preconcentration and dansylation of aliphatic amines using C18 solid-phase packings: Application to the screening analysis in environmental water samples. *Journal of Chromatography A, 978*(1), 59–69. https://doi.org/10.1016/S0021-9673(02)01431-0

Niu, Y., Yao, Z., Xiao, Q., Xiao, Z., Ma, N., & Zhu, J. (2017). Characterization of the key aroma compounds in different light aroma type Chinese liquors by GC-olfactometry, GC-FPD, quantitative measurements, and aroma recombination. *Food Chemistry, 233*, 204–215. https://doi.org/10.1016/j.foodchem.2017.04.103

Takahashi, K., Tokuoka, M., Kohno, H., Sawamura, N., Myoken, Y., & Mizuno, A. (2012). Comprehensive analysis of dipeptides in alcoholic beverages by tag-based separation and determination using liquid chromatography/electrospray ionization tandem mass spectrometry and quadrupole-time-of-flight mass spectrometry. *Journal of Chromatography A, 1242*, 17–25. https://doi.org/10.1016/j.chroma.2012.03.076

Xie, X., Lu, X., Zhang, X., Zheng, F., Yu, D., Li, C., … Xu, G. (2022). In-depth profiling of carboxyl compounds in Chinese Baijiu based on chemical derivatization and ultrahigh-performance liquid chromatography coupled to high-resolution mass spectrometry. *Food Chem X, 15*, Article 100440. https://doi.org/10.1016/j.fochx.2022.100440

Yamamoto, S., Shiga, K., Kodama, Y., Imamura, M., Uchida, R., Obata, A., … Fukusaki, E. (2014). Analysis of the correlation between dipeptides and taste differences among soy sauces by using metabolomics-based component profiling. *Journal of Bioscience and Bioengineering, 118*(1), 56–63. https://doi.org/10.1016/j.jbiosc.2013.12.019