Matrix-assisted laser desorption/ionization mass spectrometry for the rapid and high throughput analysis of betaine and trigonelline in *Lycium chinense* Mill. and trigonelline in coffee

Peipei Zhou [a,1], Di Chen [b,1], Chang Liu [c], Liwei Liu [a], Tianyuan Zheng [a], Wenbo Cheng [d], Yunyu Duan [e], Yifei Wang [f], Lihua Zuo [a,*,1], Zhi Sun [a,*,1]

[a] *Pharmaceutical Department, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, 450052, China*

[b] *School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, Henan Province, 450001, China*

[c] *Department of Oral Emergency, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, 450052, China*

[d] *School of Biomedical Engineering, Division of Life Sciences and Medicine, University of Science and Technology of China; Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Science, Suzhou, Jiangsu Province, 215613, China*

[e] *School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan Province, 450001, China*

[f] *Department of Obstetrics and Gynecology, Shanghai Tenth People's Hospital, Tongji University, 301 Yanchang Road, Shanghai, 200000, China*

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ABSTRACT

A rapid, simple, effective, and green method for the determination of betaine and trigonelline from *Lycium chinense* Mill. (LCM) and the quantification of the trigonelline in coffee was proposed and validated by matrix-assisted laser desorption ionization time-of-flight mass spectrometric (MALDI-TOF MS) detection. Due to without chromatographic separation, the method greatly shortened the detection time. The detection of betaine and trigonelline concentration showed good linearity in the range of 1–100 μg/mL and 0.01–100 μg/mL, with correlation coefficients $r^2 = 0.9962$ and 0.9946, respectively. The good reproducibility and reliability of the method were demonstrated by excellent intraday and interday precisions with RSD <8.3%, and the recovery of betaine and trigonelline ranged from 92.2% to 116.0%. Analysis of LCM and coffee extracts (raw, light-roasted, and dark-roasted coffee beans) gave results in agreement with the literature. The method appeared as a fast and reliable alternative method for routine *Lycium chinense* and coffee analysis.

1. Introduction

Betaines are zwitterionic quaternary ammonium compounds produced by specific biosynthetic pathways, which perform physiological functions as an osmolyte and as a donor of methyl groups (Ares et al., 2022), including glycine betaine, proline betaine, trigonelline, valine betaine, γ-butyrobetaine and others. Betaine, namely glycine betaine, is a small zwitterionic compound that is produced by a wide variety of organisms. Chemically, compounds, glycine betaine analogues, related to betaine are collectively referred to as "betaines" (Lun Su, Leung, Huang, & Chen, 2003). In the work, the methodological research of rapid determination of betaine and trigonelline is mainly concerned with us. Since betaine is an important alkaloidal constituent of *Lycium chinense* Mill. (LCM), and according to China Pharmacopeia 2020, the content of betaine is not <0.50% of the dry weight of LCM, and it is one of the important indexes for quality control of LCM (Yu et al., 2023). Therefore, LCM was chosen as one of the sample matrices for this assay. Although LCM contains small amounts of trigonelline, not its main component, trigonelline can be found primarily in coffee beans and is closely related to the degree of roasting of the coffee beans and varieties. Consequently, two sample matrices were chosen to validate the methodological determinations of the present work.

Betaine is an alkaloid component that is generally recognized as a safe ingredient (Willingham, Ragland, & Ormsbee, 2020). It is a natural product in *Lycium* plant resources and many foods, including wheat, shellfish, spinach, beet, amaranth, quinoa, etc. (Sakamoto, Nishimura,

Ono, & Sakura, 2002). High levels of betaine are also accumulated in some common Chinese medicinal herbs, such as achyranthes and cistanche; what's more, betaine is widely found in mammals, one of the major functional components in LCM that regulate the organism's internal environment and enhance its activity (Arumugam et al., 2021). In addition to betaine, a small amount of trigonelline also exists in plants of *LyciumL.* (W. Liu et al., 2020). Betaines are important alkaloid components in LCM, with biological activities such as regulating lipid metabolism (Chen et al., 2022), adjusting blood sugar levels, anti-inflammation, neuroprotection, etc., which are the critical material bases for the medicinal plant resources of LCM and play a role in health care. Furthermore, betaine is considered an inexpensive feed additive that improves nutrient utilization in pigs and poultry. In addition to its potential health benefits for humans and animals, betaine is used as a stabilizer, emulsifier, and surfactant in a number of industrial applications (Dobrijević et al., 2023). On the whole, betaine is a non-essential amino acid with proven functional properties that play an essential role in food additives, moisturizing skin care, animal husbandry, and aquaculture, possessing a wide range of applications.

Another type of betaines, trigonelline, also known as nicotinic acid *N*-methyl betaine, is an important component of coffee beans (Konstantinidis, Franke, Schwarz, & Lachenmeier, 2023). Among the various agricultural outputs, coffee is one of the most popularly consumed beverages all over the world. The quality of coffee beans and the degree of roasting determines the quality indicators of coffee drinks, such as taste, aroma, and color. The aroma and flavor components of coffee come from the changes in the composition of coffee beans during the roasting process, of which trigonelline is one of the ingredients that undergo significant changes during the roasting process (Kalaska et al., 2014). Trigonelline is also one of the sources of coffee bitterness and a vital precursor substance in the formation of the aroma of coffee beverages (Ludwig, Clifford, Lean, Ashihara, & Crozier, 2014). Coffee chemists believe that one of the reasons why the flavor of *Arabica* beans is superior to that of *Canephora* beans is the higher trigonelline content of *Arabica* beans compared to *Canephora* (Bicho, Leitão, Ramalho, De Alvarenga, & Lidon, 2011; Campa et al., 2004). Consequently, a rapid determination of the trigonelline in coffee is indispensable for evaluating its quality.

In general, the rapid determination of betaine and trigonelline is of great significance for the quality control of LCM and coffee. In previous detection methods, due to the special structural characteristics of betaines with quaternary ammonium and carboxylic acid group dipolar ions, betaine and their analogues have poor UV chromophores and are highly soluble in water, which brings great challenges to the extraction and quantitative studies of betaine and their analogues. At the present stage, the quantitative methods for the determination of betaine and trigonelline include thin-layer chromatography (Maria J. Martin, Pablos, Bello, & Gonzalez, 1997), HPLC-UV (Wang et al., 2016), LC-MS/MS (Caporaso, Whitworth, Grebby, & Fisk, 2018), ion chromatography (Arai et al., 2015), on-line solid phase extraction (Mohamadi, Sharififar, Pournamdari, & Ansari, 2021) and others (H. Liu et al., 2012). Although some of these methods have good accuracy and sensitivity, they are not suitable for the rapid determination of betaine and trigonelline in the daily food industry owing to the limitations of cumbersome operation, testing cost, and long time.

Compared with traditional detection methods such as liquid chromatography-mass spectrometry or other methods, matrix-assisted laser desorption ionization time-of-flight mass spectrometric (MALDI-TOF MS) has important advantages such as lower analysis costs, easier sample preparation, and more straightforward analysis procedures (Xu et al., 2019). The MALDI MS strategy was proposed in the present study, which is more suitable for the daily rapid analysis of betaines for quantitative determination. The method involved extraction and dilution, followed by mixing with matrix solution, then detected by MALDI-TOF MS without chromatographic separation, significantly shortening the detection time. In addition, betaine-d9 and trigonelline-d3 were
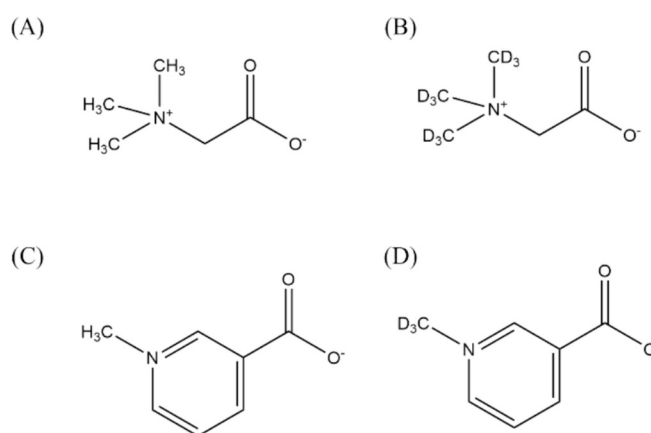

Fig. 1. The chemical structures of (A) betaine, (B) betaine-d9, (C) trigonelline, (D) trigonelline-d3.

applied to normalize matrix effects in the MALDI MS spectra efficiently during analysis (Fig. 1). This work aimed to develop a fast analytical method for the quantitative determination of betaine and trigonelline in LCM and trigonelline in coffee beans with different degrees of roasting to meet daily testing in the field of beverages, food additives, and animal husbandry and aquaculture. Based on the MALDI MS analytical method, we evaluated the analytical performance of betaine analogues in the determination of LCM and coffee extracts, including linearity, intra-day precision, inter-day precision, and recovery. This method can be routinely applied to determine different types of LCM and coffee beverages in the market.

2. Materials and methods

2.1. Standards and chemicals

Betaine and trigonelline were obtained from MUST Biotechnology Co., Ltd. (Chengdu, China), Betaine-(trimethyl-d9) hydrochloride was purchased from RHAWN Chemical(RH486977, ≥98 atom %D, Shanghai, China), Trigonelline-d3 (chloride) was purchased from Cayman Chemical (0639350–3, Michigan, USA), Trifluoroacetic acid (TFA; LC-MS grade) was obtained from J&K Scientific Technology Co., Ltd. (Beijing, China). 2,5-Dihydroxy benzoic acid (DHB), α-Cyano-4-hydroxycinnamic acid (CHCA), and 1,5-diaminonaphthalene (DAN) were acquired from TCI Development Co., Ltd. (Tokyo, Japan). 9-Aminoacridine (9-AA) was purchased from Sigma-Aldrich (ST. Louis, USA). Acetonitrile and methanol (LC-MS grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). LC-grade water was purchased by Wahaha Group Co.,Ltd. (Hangzhou, China) throughout the experiments.

2.2. Samples

Lycium fruits were purchased from Beijing TongRenTang Traditional Chinese Medicine Decoction Piece Co. LTD. Raw coffee beans, light-roasted coffee beans, and dark-roasted coffee beans of typicati were obtained from Baoshan, Yunnan, China. About 50 g sample of coffee beans was finely powdered using an electrical blender (FW100 high-speed universal disintegrator) and stored in an airtight plastic bag.

2.3. Sample preparation

1 g of LCM was weighed into a 100 mL conical flask, 30 mL of 80% methanol was added, and the extract was ultrasonicated at 50 °C for one hour. At the same time, 1 g of coffee beans of different roast levels (light and dark roasted) and 1 g of green raw beans were weighed, 10 mL of

50% methanol were added and extracted by ultrasonication at 60 °C for one hour, then the extract was taken out and cooled down to room temperature, and weighed again to make up for the loss of weight with 80% methanol and 50% methanol, respectively. For the determination of betaine in LCM extract, the LCM extract was diluted 10-fold for analysis; for the trigonelline in light-roasted coffee bean extract, the extract was diluted 50-fold for analysis; and for the determination of trigonelline in LCM extract, the LCM extract was not diluted.

2.4. MALDI-TOF MS analysis

For betaine analysis of LCM preparation, the LCM extracts diluted 10 times by 80% methanol was emerged in a mixture of an internal standard (IS) solution consisting of trimethyl-d9-betaine (25 µg/mL), which was deposited on the stainless steel. The MALDI target probe was crystallized using a dried droplet method followed by 2 µL of CHCA (20 mg/mL) with 70% ACN/0.1% TFA as substrate precipitation on the sample. For trigonelline analysis of LCM and coffee preparation, the LCM extract and the coffee extract diluted 50 times by 50% methanol were mixed with the IS solution, trigonelline-d3 (5 µg/mL), were deposited on MALDI stainless steel target; similarly, CHCA (20 mg/mL, in 70% ACN/0.1%TFA as a matrix for crystallization. After the samples were dried, All MALDI-TOF mass spectra were acquired with a QuanIMAGE MALDI TOF mass spectrometer (Intelligene Biosystems Co. Ltd) with an Nd: YAG laser (5000 Hz , 349 nm) in the positive-ion mode. Ions with m/z values in the range of 50–600 were acquired. The m/z values were calibrated using the exact peaks of CHCA. Other parameters were set as follows: Pulse Frequency 1000 Hz; Detector Voltage −0.57 kV;
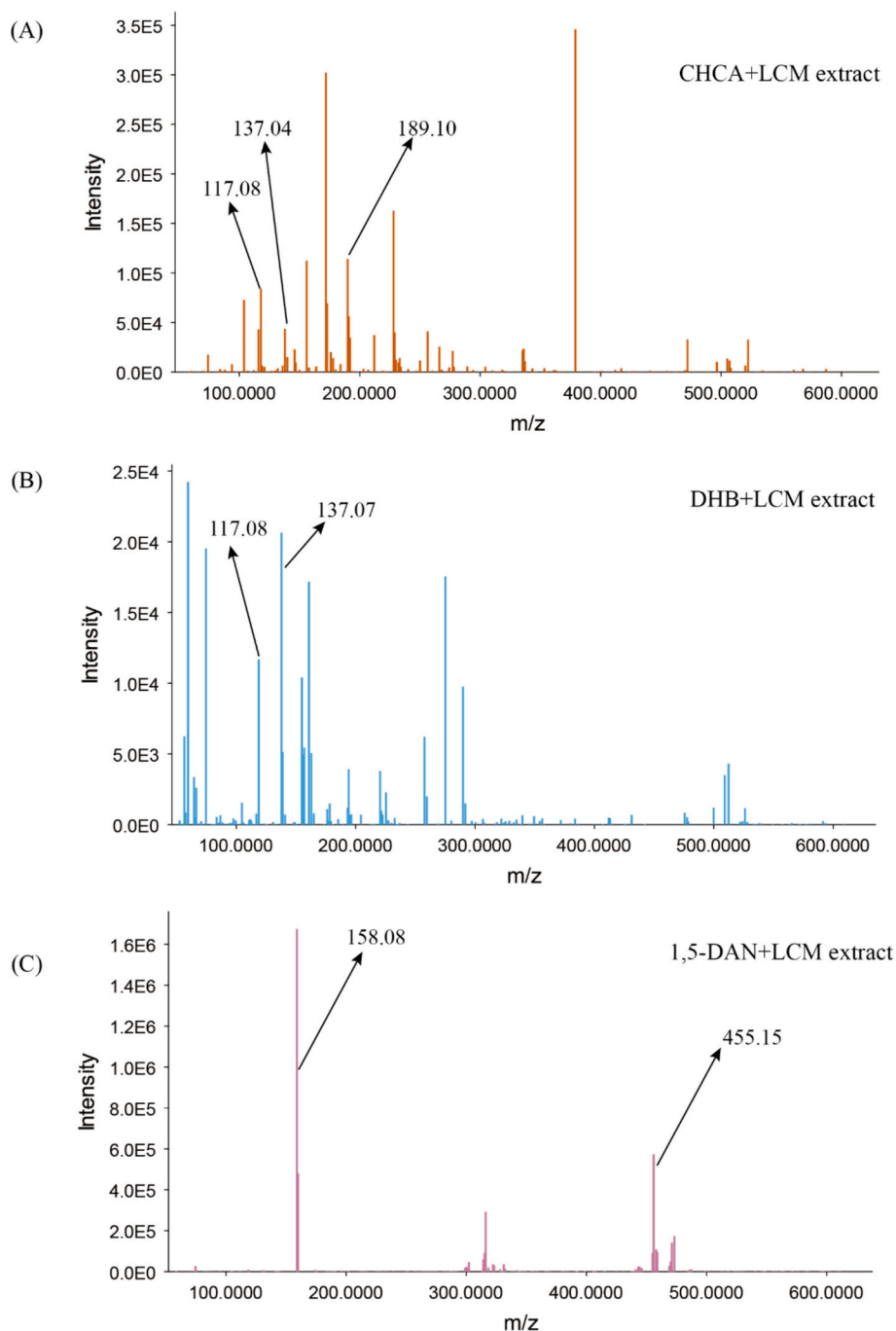

Fig. 2. MALDI-TOF MS spectra of LCM extract analyzed in positive ion mode by using (A) CHCA (B) DHB (C) 1,5-DAN.
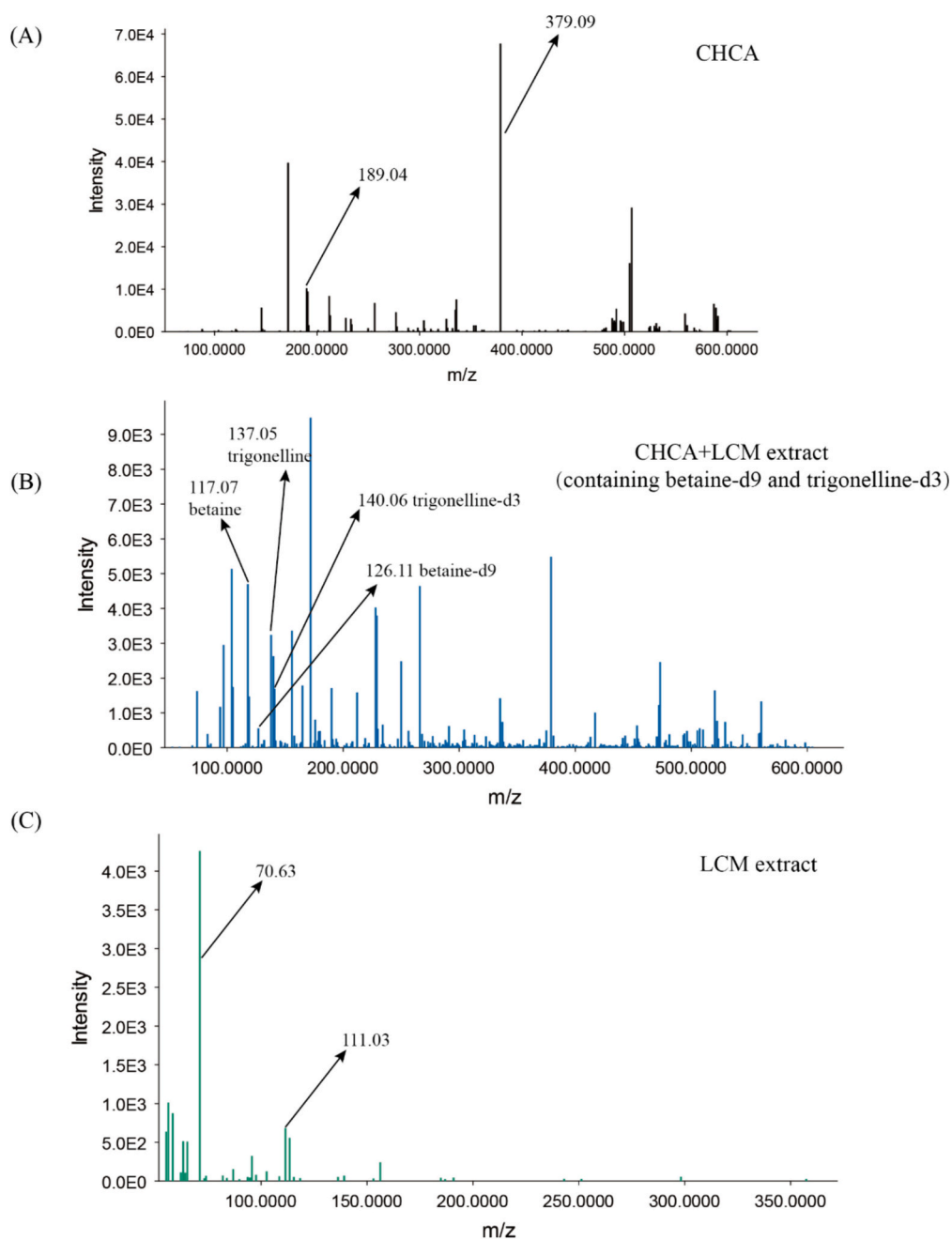
Fig. 3. MALDI-TOF MS spectra of (A) CHCA (B) CHCA+ LCM extract (C) LCM extract analyzed in positive ion mode.

Extraction Voltage Ratio 0.136; Laser Pulse Current 1.86 Amps; Source Voltage −19 kV; Sample Rate 800 ps; Motion Scanning Speed 0.50 mm/ s.

2.5. Method validation

Under the established optimal conditions, the performance of the MALDI MS strategy was assessed by the analysis of betaines in LCM and coffee extracts. The linearity, interday and intraday precisions, and relative recovery of the method were all evaluated (Dilmetz, Hoffmann, & Condina, 2021; Nie et al., 2020).

2.5.1. Calibration curves

To investigate the linearity of the approach, a series of standard mixture solutions containing both target analytes and IS. Namely, betaine and betaine-d9, as well as trigonelline and trigonelline-d3, were used to construct calibration curves, in which the IS were performed to normalize for matrix effects in the MALDI MS spectra efficiently.

The concentrations of betaine and trigonelline in samples were determined from the calibration curve constructed by plotting the peak intensity ratios (analyte/IS) against the known betaine (1.0–100.0 μg/ mL) and trigonelline concentrations (0.01–100.0 μg/mL). In detail, for betaine calibration curve, standard betaine solutions containing 1.0, 5.0, 20.0, 40.0, 60.0, 80.0, 100.0 μg/mL, and a fixed concentration of betaine-d9 (25 μg/mL) were prepared, and the diluted 10 times of LCM were subjected to MALDI MS analysis for betaine determination. Identically, for the trigonelline calibration curve, standard trigonelline solutions containing 0.01, 0.1, 1.0, 10.0, 50.0, 80.0, 100.0 μg/mL, and a fixed concentration of trigonelline-d3 (5 μg/mL) were established. The LCM diluted 10 times and coffee extracts diluted 50 times were conducted to MALDI MS analysis for trigonelline determination.
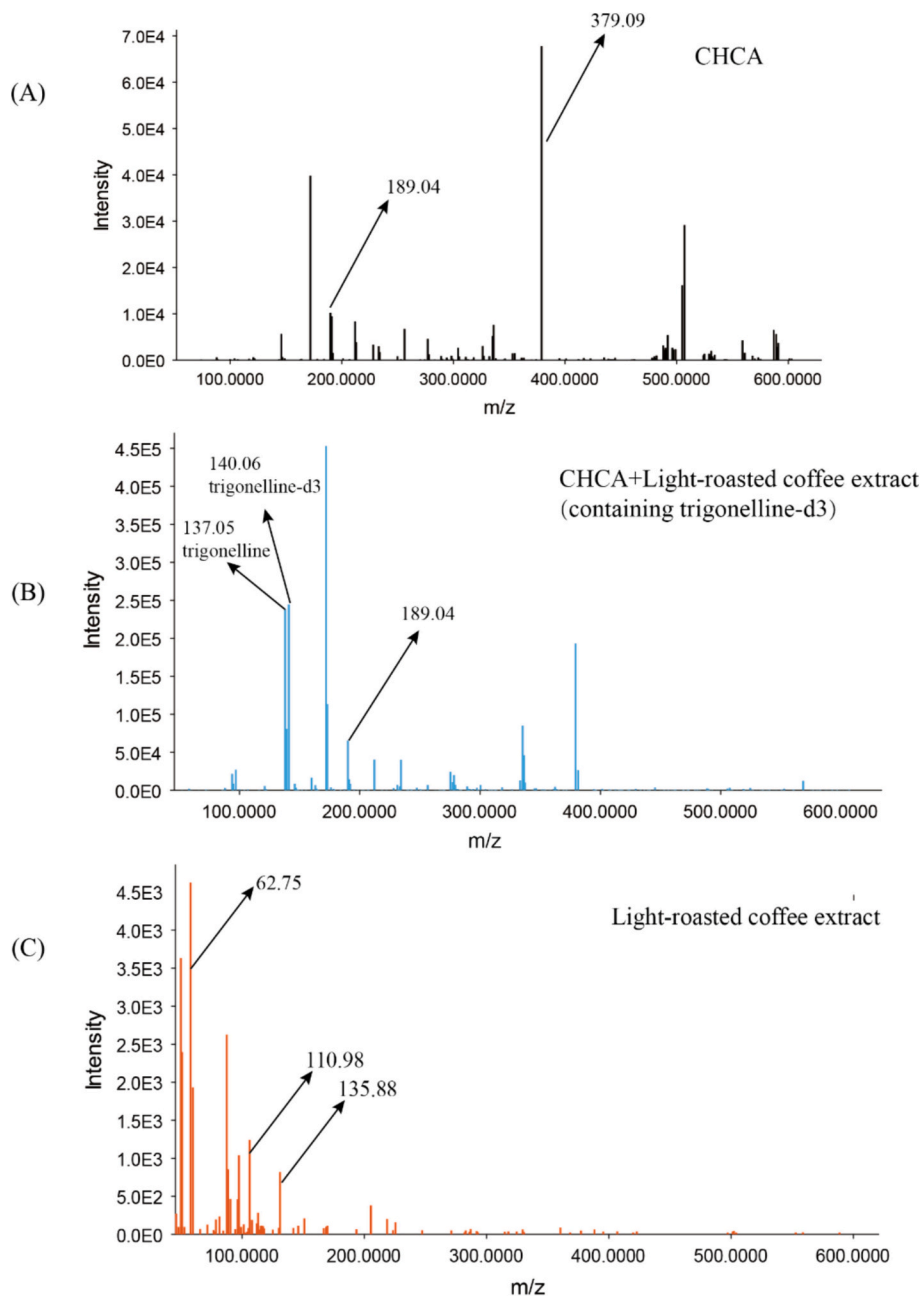
Fig. 4. MALDI-TOF MS spectra of (A) CHCA (B) CHCA+ light-roasted coffee extract (C) light-roasted coffee extract analyzed in positive ion mode.

2.5.2. Precision

The reproducibility of the proposed method was evaluated by interday and intraday RSD values. The intraday RSDs were obtained by analysis of the four prepared samples over 1 day, while the interday RSDs were determined by analyzing samples independently prepared over a continuous period of three days. Here, the intra-day and inter-day precision of betaine and trigonelline in LCM extract and trigonelline in light-roasted coffee extract were used to evaluate the method's precision.

2.5.3. Accuracy

Accuracy was determined in a recovery experiment where the sample was spiked at three different amounts–low, medium, and high–for each compound of interest. The recovery was acquired for betaine by adopting LCM extract diluted 10 times spiked with three different standard levels (20, 30, 40 μg/mL). The recovery of trigonelline was obtained using the light-roasted coffee extract spiked with 20, 30, and 40 μg/mL standard levels. Firstly, the endogenous concentrations of betaine in unspiked samples of the diluted LCM extract and the total concentrations of betaine in the spiked LCM extract diluted were calculated from the calibration curves. Then, the concentrations of added standard betaine in the spiked samples were calculated by subtracting the endogenous betaine concentration in the unspiked LCM extract from the total concentration of the spiked extract. Finally, the recovery values were obtained by comparing the calculated concentrations of standard betaine with the actual values employed in the spiked extract. The method of the recovery of trigonelline was the same as that of betaine.

2.6. UHPLC-MS/MS system conditions

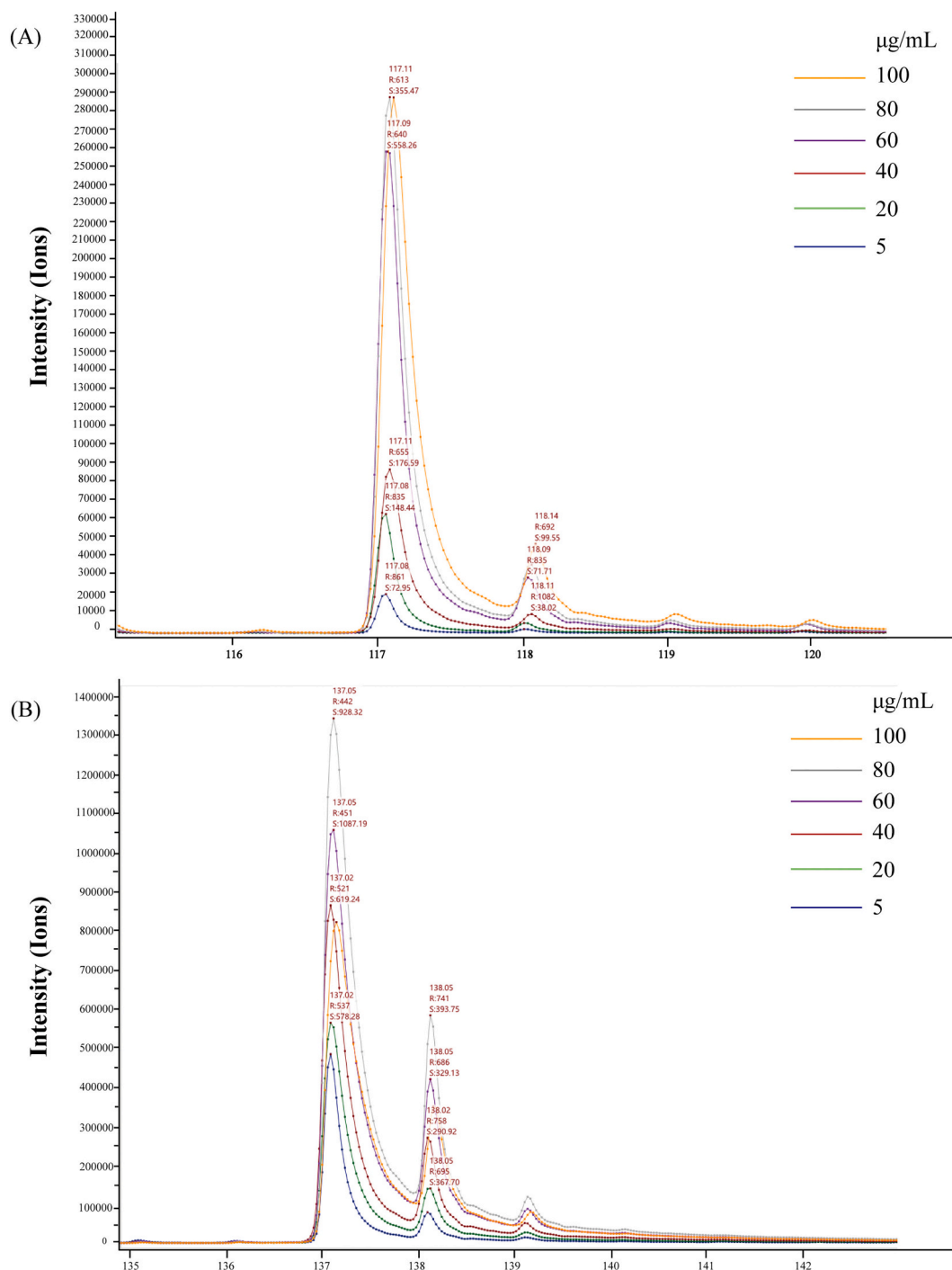In the metabolomics analysis of coffee beans of varying degrees of

Fig. 5. MALDI-TOF MS spectra of different gradient concentrations of (A) betaine and (B) trigonelline standard without the corresponding isotope internal standard.

roasting, chromatographic separation was conducted on a Thermo Dionex Ultimate 3000 UHPLC system hyphenated with a Thermo Q-Exactive Orbitrap High-Resolution. Chromatographic peaks were separated on a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) at a flow rate of 0.20 mL/min with water containing 0.1% formic acid (*v*/v) (A) and acetonitrile (B) Mass Spectrometry. The gradient elution was as follows: 0.0–1.0 min, 5% B; 1.0–9.0 min, 5%–100% B; 9.0–12.0 min, 100% B; 12.0–12.1 min, 100%–5% B; A Q-Exactive high-resolution mass spectrometry was tandem to the UHPLC system employing an electrospray ionization (ESI) ion source, and the optimized parameters of mass spectrometry were as follows: Spray voltage: +3.5 kV or 3.2 kV; Capillary temperature: 320 °C; Sheath gas

flow rate (Arb): 40 in positive mode or 38 in negative mode; collision energy values were 20, 40, and 60 eV; Aux gas pressure: 10 arb. The data was respectively acquired in the positive and negative modes by full scan/ddms2 scan patterns from 80 to 1200 *m/z* at the mass resolving power of 17,500 in MS/MS. All data collected in profile mode were acquired and processed using Thermo Xcalibur 4.0.27 software (Thermo Scientific, San Jose, USA).
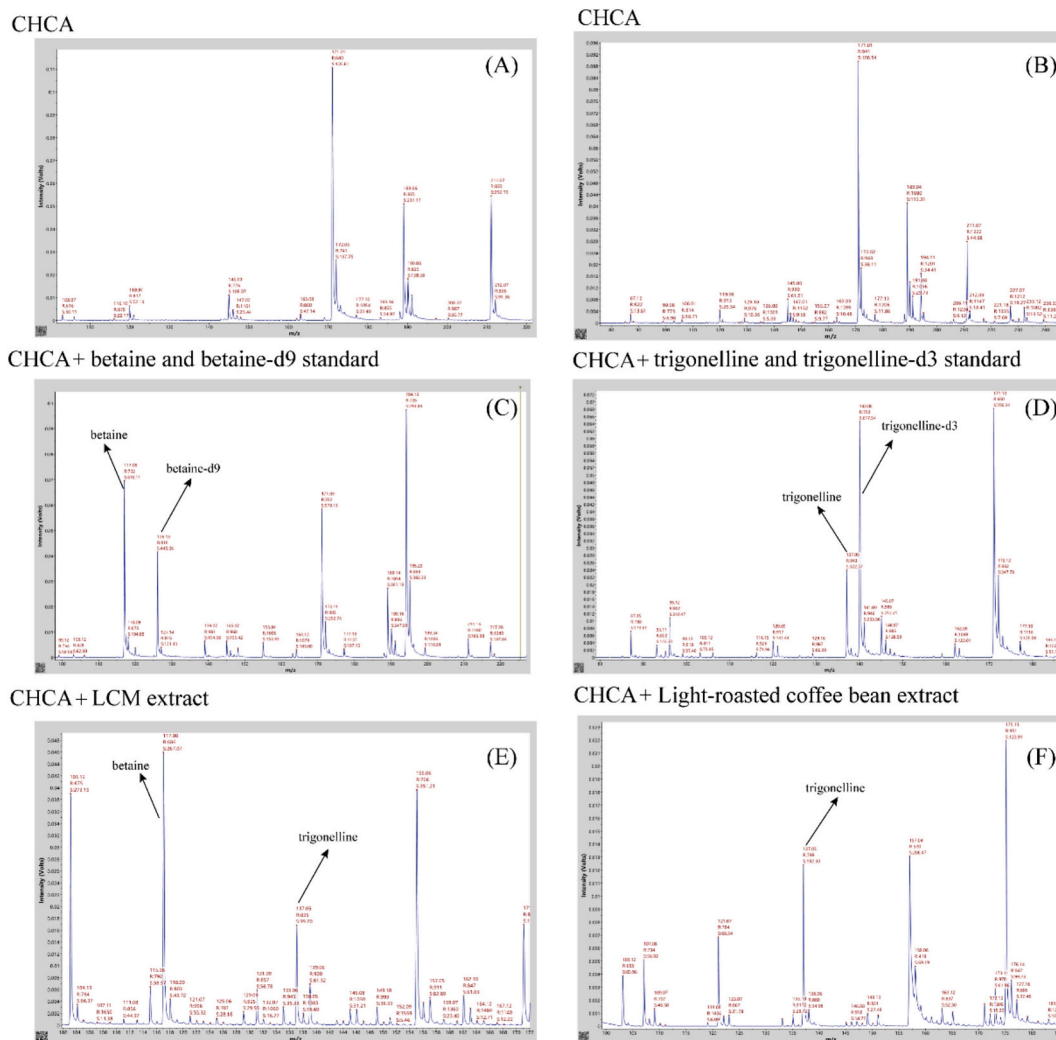
Fig. 6. The mass spectral peaks of (A-B) the blank CHCA matrix, (C) betaine and betaine-d9 standards, (D) trigonelline and trigonelline-d3 standards, (E) LCM extract with CHCA, (F) light-roasted coffee bean extract with CHCA in MALDI MS.

3. Results and discussion

3.1. MALDI MS detection performance for betaines

The feasibility of the proposed MALDI MS strategy for betaines analysis was preliminarily evaluated. Since betaine and trigonelline are both present in LCM extract, the methodological feasibility study of matrix screening was carried out to analyze the LCM extract as the analyte to be measured. Firstly, to achieve the highest efficiencies of betaine and trigonelline, different matrices mixed with analytes were used to analyze the response intensity of the target substance peaks. 1,5-DAN (10 mg/mL) dissolved in ACN/H_2O/FA (3.5:1.49:0.01, $v/v/v$), DHB (10 mg/mL) and CHCA (10 mg/mL) dissolved in ACN/0.1%TFA (7:3, v/v) were successively tried as MALDI matrices to measure. The

dried-droplet sample preparation method was conducted as follows: LCM extract diluted 10 times was deposited on the stainless steel; after the analytes were dried, 1,5-DAN, DHB, and CHCA substrate solutions were introduced to mix for crystallization and analyzed in positive mode. As shown in Fig. 2, the LCM extract mixed with CHCA showed better response intensity at m/z values of 117.08 and 137.04 with no matrix interference peaks compared to DHB. Similarly, betaine-(tri-methyl-d9) and trigonelline-d3 (chloride), at m/z values of 126.11 and 140.06, respectively, are also free of matrix interference peaks. For 1,5-DAN, the target peaks are less responsive than CHCA and DHB. For further comparison, the MALDI MS spectra of a blank sample, CHCA, and the mixed analyte with CHCA were recorded in Fig. 3 and Fig. 4, demonstrating the appearance of betaines peaks relies on matrix-assisted laser desorption/ionization assisted matrix to desorption/ioni-zation. In Fig. 5, the response peaks of betaine and trigonelline standard at different gradient concentrations were displayed; however, the peak response values do not increase linearly in proportion with increasing concentration due to matrix effects, so in the present work, betaine-d9 and trigonelline-d3 isotopes were employed to correct for matrix effects.

3.2. Method validation

3.2.1. The selectivity

With appropriate optimizations and peak detection setting of the

Table 1
Calibration parameters for quantification of betaine and trigonelline.

Analyte	Calibration range (μg/mL)	Regression equation	R^2	LOD (ng/mL)	LOQ (ng/mL)
Betaine	1.0–100.0	Y = 0.0445× + 0.2195	0.9962	60.0	200
Trigonelline	0.01–100	Y = 0.0973× + 0.2852	0.9946	1.8	5.9

Table 2

Intraday and interday precisions of betaine and trigonelline detection in LCM extract and light roasted coffee extract determined by the proposed MALDI MS method.

Analyte	Intraday precision (RSD %, n = 4)	Interday precision (RSD %, n = 4)
Betaine (LCM extract)	0.7	2.1
Trigonelline (LCM extract)	4.8	8.3
Trigonelline (light-roasted coffee beans extract)	1.1	2.8

Table 3

The relative recoveries of betaine and trigonelline spiked in LCM extract and light-roasted coffee extract.

Compound	Added (µg/mL)	Recovery ($n = 6$, %)	RSD (n = 6, %)
Betaine (LCM extract)	20	99.1	1.56
	30	99.1	1.32
	40	97.5	0.47
Trigonelline (LCM extract)	1	107.6	7.66
	2	114.1	2.22
	3	116.0	1.75
Trigonelline (light-roasted coffee beans extract)	20	99.2	0.77
	30	108.6	2.41
	40	92.2	5.07

Table 4

The detected concentrations of analyte in samples.

Compound Samples	Betaine (%)	Trigonelline (%)
LCM extract	0.90	0.0046
Green coffee beans extract	–	1.71
Light-roasted coffee beans extract	–	1.35
Dark-roasted coffee beans extract	–	0.53

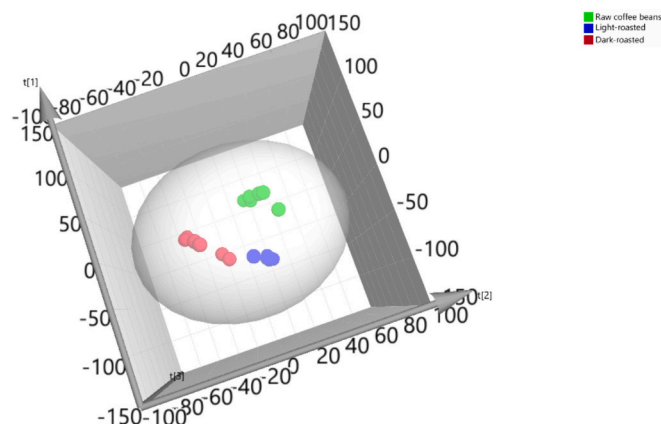**Fig. 7.** 3D plot of PCA score for the raw coffee beans, light-roasted and dark-roasted coffee beans in ESI$^+$ mode.

experimental conditions, In Fig. 6, the results show the mass spectral peaks of the blank CHCA matrix, the betaine, betaine-d9, trigonelline, and trigonelline-d3 standards, and the samples to be measured were well detected, which did not constitute an interference for the analytes. In addition, the internal standards for the substance were also employed to eliminate the matrix effects in the work. It can be concluded that the selectivity of the proposed method is good.

3.2.2. The calibration curves, LOD, and LOQ

As shown in Table 1, for betaine and trigonelline, the method was linear in the concentration range of 1.0–100.0 µg/mL (for betaine) and 0.01–100 µg/mL (for trigonelline) with an acceptable regression coefficient (r^2) of 0.9962 for betaine, and 0.9946 for trigonelline. The limit of detection (LOD) of betaine and trigonelline, defined as the amount of analyte at a signal-to-noise ratio (SNR) of 3, was 60.0 ng/mL and 1.8 ng/mL, respectively. The LOQs of betaine and trigonelline for each analyte were determined as the concentration equivalents to 10 times, which were 0.2 µg/mL and 5.9 ng/mL, respectively, suggesting good sensitivity for the method. The results showed that the curves in the given dynamic response range exhibited good linearity, and R^2 is >0.99, which is acceptable in the MALDI spectrum (Ares et al., 2022; Mahale et al., 2022). In the present study, trigonelline displayed a lower detection line with a broader mass range, and the response in the MALDI-TOF spectrum was superior to the intensity of the betaine response.

3.2.3. The repeatability

To evaluate the precision of the data obtained by the proposed method, the determination of inter- and intra-day precision of betaine in LCM samples and trigonelline in light-roasted coffee was carried out. As shown in Table 2, for betaine and trigonelline, good repeatability in all four spike levels was obtained, demonstrating good repeatability in the method. In Table 2, the RSD of these measurements was <8.3% ($n = 4$), and the associated reproducibility for all analytes met the acceptable level of RSD ≤ 10% (Qin, Ding, Yu, Zhou, & Feng, 2021). These results indicated that the reported method had good reproducibility.

3.2.4. The accuracy

The mean recoveries for six analytes in each spike level are displayed in Table 3. Using the calibration curves of betaine and trigonelline, the relative recoveries ranged from 92.2% to 116.0%, within 10% from unity were found for the compounds of interest in the three spike levels, which indicated satisfactory accuracy for the proposed method. Accuracy, calculated as the agreement (%) of measured and calculated concentration, was 97.5–99.1 for betaine in LCM extract, 107.6–116.0, and 92.2–108.6 for trigonelline in LCM and light-roasted coffee bean extract, respectively, with an acceptably relative standard deviation of <20%, meeting the acceptance criteria for accuracy of the International Conference on Harmonization (ICH) guidelines (da Silva et al., 2021; Keighley, Ramwell, Werner, & Sinclair, 2021). The data demonstrate the proposed method of MALDI-TOF MS as a reliable tool enabling a rapid and accurate quantitative determination of betaine and trigonelline in LCM and coffee extracts.

3.3. Analysis of LCM samples and coffee samples with different roast levels

In order to assess the applicability of the method in the work, LCM samples were used to determine the content of betaine and trigonelline, as well as raw, light-roasted, and dark-roasted coffee beans were used to determine the content of trigonelline. As shown in Table 4, the proportion of betaine and trigonelline in LCM was 0.9% and 0.046%. Trigonelline was the highest in raw coffee beans, with 1.71%, followed by light-roasted and dark-roasted coffee beans, with 1.35% and 0.53%, respectively. It has been found that increasing the degree of roasting of coffee beans reduces the amount of trigonelline. This is due to the decomposition of trigonelline during the roasting process, and the extent of the decomposition is strongly dependent on the roasting time and temperature (Duarte, Pereira, & Farah, 2010; Farah, Monteiro, Calado, Franca, & Trugo, 2006). In the process of coffee roasting, trigonelline can be degraded to produce two important compounds, pyridine and nicotinic acid (Dong et al., 2023), which is why trigonelline has a great deal to do with the aroma and taste of coffee, and trigonelline content varies a lot depending on the degree of roasting and the origin of coffee.

Fig. 8. The volcano plot of *t*-test with FC for the(A) raw coffee beans and light-roasted beans group, (B) light-roasted beans and dark-roasted beans group in ESI⁺mode.
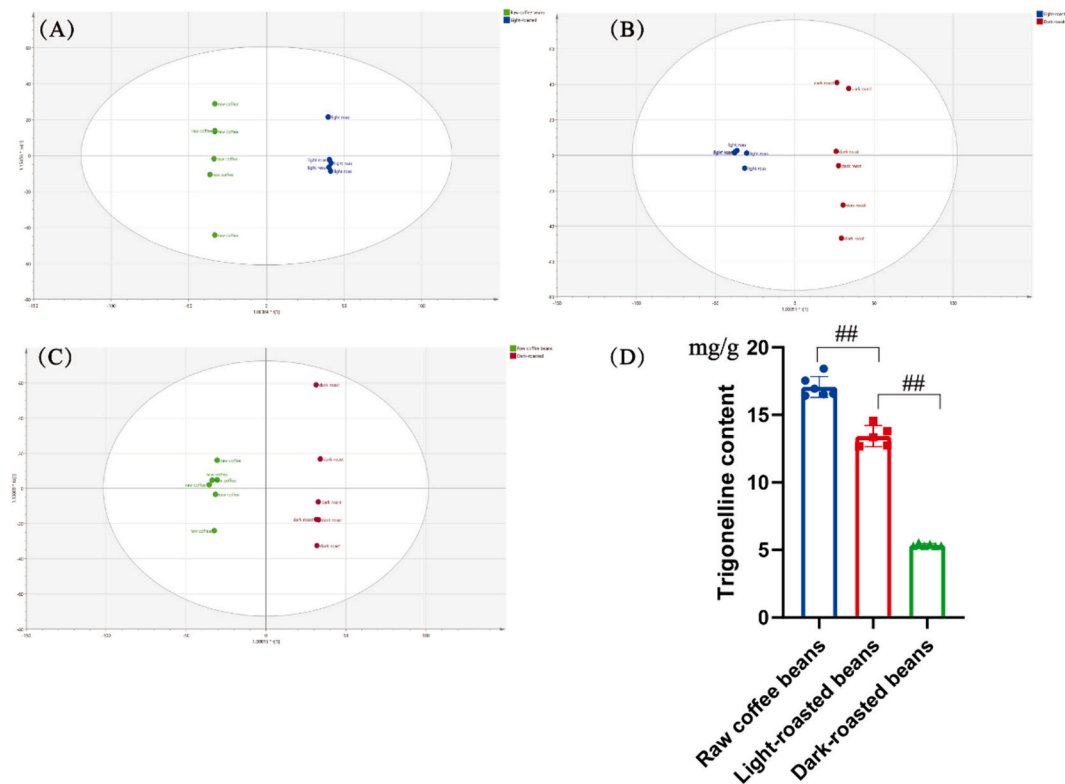
Fig. 9. Multivariate data analyses in the samples of different roasted coffee beans groups in ESI⁺ mode (A) score plot of OPLS-DA analysis of the raw coffee beans group and light-roasted beans group, (B) score plot of OPLS-DA analysis of the dark coffee beans group and light-roasted beans group, (C) score plot of OPLS-DA analysis of the raw coffee beans group and dark-roasted beans group, (D) analysis of trigonelline content in raw coffee beans, light-roasted beans, and dark-roasted beans extracts.

Table 5
Comparisons of the analytical characteristics of the proposed betaine method developed with other reported methods.

Detection technique	Sample preparation	Processing time (min)	LOD (s)	Reference
HPLC-UV	SLE	> 95	1.46 µg/g	(Hefni, McEntyre, Lever, & Slow, 2015)
HILIC-MS/MS	ASE/SPE	>8	19 µg/g	(Rivoira, Studzińska, Szultka-Młyńska, Bruzzoniti, & Buszewski, 2017)
HILIC-MS/MS	SLE	>40	2–5 µg/g	(Xiong et al., 2012)
LC–MS/MS	SPE	>15	5.6 µg/g	(MacKinnon, Hiltz, Ugarte, & Craft, 2009)
HILIC-ELSD	SPE	>17	115.5–150 µg/g	(Shin et al., 2012; Zhao et al., 2013)
MALDI-TOF MS	–	<1	60 ng/mL	This work

Table 6
Comparisons of the analytical characteristics of the proposed trigonelline method developed with other reported methods.

Detection technique	Sample preparation	Processing time (min)	LOD (s)	Reference
HPLC-DAD	SPE	> 70	75.5–188.5 µg/g	(Rodrigues & Bragagnolo, 2013)
HPLC-UV	SPE	>10	375 µg/g	(Martín, et al., 1997)
UPLC-MS/MS	SPE	>5	60 ng/mL	(Wadhwa et al., 2021)
HILIC-MS/MS	SPE	>2.5	10 ng/mL	(Szczesny et al., 2017)
HPLC-VWD	SPE	>25	5 µg/g	(Caprioli et al., 2014)
MALDI-TOF MS	–	<1	1.8 ng/mL	This work

In this regard, a metabolomics approach based on UHPLC-HRMS was performed to reveal metabolite profiles in different roasted coffee beans. The results revealed that notable metabolic changes occurred in the raw coffee beans group, light-roasted group, and dark-roasted group in the 3D plot of PCA cluster analysis (shown in Fig. 7). Furthermore, the volcano maps with the $P < 0.05$ and FC > 2 or FC < 0.5 values were performed to find the significant metabolites. From the result, trigonelline was one of the metabolites with highly significant differences, especially in the result of the raw coffee beans group and the light-roasted group, as illustrated in Fig. 8 (A). In addition, score plots of OPLS-DA analysis of different roasted coffee bean groups in ESI$^+$ mode were displayed in Fig. 9.

3.4. Comparisons of the analytical characteristics of the proposed method developed with other reported methods

Nowadays, several investigations have been conducted to determine the betaine and trigonelline in samples, and various methods have been introduced for this purpose. Although these analytical methods are sensitive and accurate, they are expensive, tedious operations that require the separation of the analytes, which lengthens the analysis time. In this work, the separation of the betaine and trigonelline determined were not required, and betaine-d9 and trigonelline-d3 were added to eliminate matrix effects in the MALDI MS spectra efficiently during analysis. The experiment has been methodologically validated, and the samples can be determined quickly and easily, which fully satisfies the daily beverage detection in <1 min per sample. The method's advantages include its speed, simplicity, versatility, minimum sample preparation needs, high sensitivity, good accuracy, and reproducibility. Literature on the detection of betaine and trigonelline by other methods

was summarized, and the advantages of the research method proposed in this work were reflected thoroughly through comparative analysis (shown in Table 5 and Table 6).

4. Conclusions

Results obtained in this work show the potential of MALDI MS analysis in the quality control of LCM and coffee. Validation data showed that the method yielded reliable and reproducible results. In this work, the rapid analysis of trigonelline and betaine in LCM and trigonelline in different levels of roasting coffee was proposed by matrix-assisted laser desorption/ionization mass spectrometry for the first time. Extensive experimental results demonstrated high sensitivity and throughput by realizing the accurate detection of trigonelline and betaine within 1 min per sample. The proposed method is selective and sensitive and enables the elimination of the separation step, greatly shortening the time of analysis and detection of trigonelline and betaine, which is in line with daily demand. The work reveals the MALDI MS approach is promising further applications for routine analysis of these compounds in various industries for LCM quality control and the flavor and quality of coffee samples.

CRediT authorship contribution statement

Peipei Zhou: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Di Chen:** Writing – review & editing, Funding acquisition, Conceptualization. **Chang Liu:** Investigation, Formal analysis, Data curation. **Liwei Liu:** Resources, Investigation. **Tianyuan Zheng:** Validation, Software, Resources. **Wenbo Cheng:** Software, Resources. **Yunyu Duan:** Visualization, Validation, Resources. **Yifei Wang:** Visualization, Resources. **Lihua Zuo:** Supervision. **Zhi Sun:** Supervision, Project administration.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled "Matrix-assisted laser desorption/ionization mass spectrometry for the rapid and high throughput analysisof betaine and trigonelline in *Lycium chinense* Mill. and trigonelline in coffee".

Data availability

Data will be made available on request.

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