



Simultaneous quantification of four furfurals in cheeses by gas chromatography-triple quadrupole mass spectrometry

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ABSTRACT

Furfurals, including furfural (F), 5-hydroxymethylfurfural (5-HMF), 2-acetylfuran (FMC), and 5-methyl-2-furfural (MF), are important Maillard reaction intermediate products in dairy products. Due to the complex matrix of cheese samples and different polarity of four furfurals, it remains a major challenge to simultaneously determine these four furfurals in cheeses. A new method for simultaneous determination of four furfurals in cheeses by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) was established in the present study. The limits of detection (LODs) were in the range of 0.01–0.02 mg/kg, and the correlation coefficient (R^2) for each analyte was above 0.9986 with the calibration range established from 5 to 1000 ng/mL. The recoveries were ranged from 72.62% to 123.29% and relative standard deviations (RSDs) lower than 9.82%. Finally, the developed method was successfully employed for analyzing four furfurals in 30 cheese samples, and trace levels of the furfurals were found in cheeses.

1. Introduction

Due to the wide varieties and its nutritional and health properties, cheeses are popular worldwide. However, a range of reactions happened during cheese-making, including Maillard reaction, protein degradation, lipid oxidation, and lactose isomerization, which may have desired or unacceptable effects on the cheese (Corzo et al., 2000; Jaeger et al., 2010; Marilley, 2004).

Furfurals, including furfural (F), 5-hydroxymethylfurfural (5-HMF), 2-acetylfuran (FMC), and 5-methyl-2-furfural (MF), have been detected in many foods such as vinegars (Cocchi et al., 2011), honey, soy sauces, beers (Teixidó et al., 2011), raisins, milk powder and liquid milk (Cui et al., 2020; Sun et al., 2017). They have been recognized as indicators of heat damage and the extent of Maillard reaction in dairy products (Gómez-Narváez et al., 2019; Oral et al., 2014). Notably, once beyond a certain limit of human absorption, they will cause cytotoxicity, genotoxicity, and even carcinogenicity (Abraham et al., 2011; Farag et al., 2020; Li et al., 2021a). Two mechanisms of their formation have been proposed (Li et al., 2022): (i) the Maillard reaction product Amadori

compounds (mainly lactulosyl-lysine) from undergo 1,2-enolisation in acidic conditions (Arena et al., 2017; Jun et al., 2003), (ii) through lactose isomerized (Brands & van Boekel, 2001; Chávez-Servín et al., 2005), that is Lobry De Bruyn-Alberda-van Ekenstein transformation (L-A) and followed by degradation reactions (Morales & Jiménez-Pérez, 1998; Van Boekel, 1998).

The research on furfurals in cheeses is mostly limited to determination of them as aroma compounds by GC or GC-MS (Capozzi et al., 2020; Qian & Reineccius, 2002). In 2021, Li et al. (2021c) used HPLC to determine 5-HMF in three processed cheese samples, which is a common method for the determination of 5-HMF and F in various samples (Docheva, et al., 2020; Kamalabadi et al., 2015; Petisca et al., 2014; Rufian-Henares, Garcia-Villanova, Guerra-Hernandez, 2001). In recent years, simultaneous determination of four furfurals in milk-based formula, infant formulas, and liquid milk, etc. by HPLC were reported, but the samples need derivatization or acid hydrolysis, and the water-based extract is strongly acidic and extremely corrosive due to the use of trichloroacetic acid (Chávez-Servín et al., 2005; Ferrer et al., 2005; Xing et al., 2020). Cui et al. (2020) developed a GC-MS/MS method, in which

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four furfurals were simultaneously determined in liquid milk and milk powder samples with acetonitrile as extraction solvent and without the derivatization step. However, there is a lack of methods for the simultaneous determination of the four furfurals in high protein, high fat cheeses.

Extracts of dairy products usually contain interfering substances such as fat, protein, and pigment, etc. that require effective clean-up prior to chromatographic analysis. Dispersive solid-phase extraction has the advantages of requiring relatively lower amounts of solvent and shorter pre-treatment time (Cui et al., 2020; Li et al., 2013; Xiong et al., 2018). Moreover, the sorbent primary secondary amine (PSA) and C18 are mentioned in the AOAC Official 2007.01 Method as being effective in removing matrix disturbances such as polar organic salts, fat and proteins from foods of animal origin (Liu et al., 2016; Park et al., 2020). PSA and C18 are also commonly used sorbent for dispersive solid-phase extraction applied for highly sensitive matrix removal, such as fat-rich foods (Huang et al., 2019b). Hence, it could be a good choice to select PSA and C18 for preparation approach removing impurities in cheeses.

This study aimed to establish a sensitive, accurate, and convenient method for simultaneous quantification of four furfurals in cheeses. The extraction solvent and extraction times were optimized to achieve better extraction and separation efficiency. Finally, the chosen method was verified and used for evaluating the contents of four furfurals in different categories of cheeses in Chinese market.

2. Materials and methods

2.1. Chemicals and materials

F (> 99.5% purity), 5-HMF (> 98.0% purity), MF (> 99.0% purity), and FMC (> 98.0% purity) were provided by Aladdin Holdings Group Co., Ltd (Beijing, China). Acetonitrile, and dichloromethane (HPLC grade) were acquired from Merck & Co., Inc (New Jersey, USA). NaCl, NaAc and MgSO₄ (analytical-grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bondesil C18 sorbent (40 µm) and bondesil PSA sorbent (40 µm) were obtained from Agilent Technologies Co., Ltd. (Santa Clara, CA, USA).

Thirty-one kinds of commercially available cheeses, including Manchego cheese (Anejo, 10–12 months), Manchego cheese (10 months), Manchego cheese (Curado, 5–6 months), Manchego cheese (3 months), Manchego cheese (Semicurado, 2–3 months), Monterey Jack, Parmesan cheese (24–36 months), Parmesan cheese (12 months), Gran Spicco cheese, Gran Moravia cheese, Grana Padano cheese, Pecorino Romano cheese, Kerrygold Reserve Cheddar, Kerrygold Cheddar (smooth and mild), Mild Cheddar, Kerrygold Dubliner, Gouda cheese, Amber cheese-long maturing distinctive Gouda, Ruby cheese-long maturing mild Gouda, Kerrygold Swiss cheese, Emmentaler cheese, Camembert cheese, Roquefort cheese, Suki Brie Cheese, Brie Au Bleu, Danish Blue cheese, White cheese, Buffalo Mozzarella (alpilatte), Natural Mozzarella (alpilatte), Mozzarella cheese, and Orange Cheddar (New Zealand), were obtained from Taobao online store and local supermarkets in Nanchang, Jiangxi, China. Detailed information about all cheeses is provided by [Supplementary Table 1](#). All samples were dehydrated with a lyophilizer and stored at −20 °C until analysis.

2.2. Preparation of standard solution

10 mg of 5-HMF, F, MF, and FMC were poured into four volumetric flask (10 mL), respectively, and then spiked with dichloromethane to make four final stock solutions (1 mg/mL). The stock solutions were diluted with dichloromethane to obtain a mixed working standard.

2.3. Preparation of sample

A total of 2.0 g of the lyophilized cheese powder was poured into a 50 mL centrifuge tube and 5 mL water (45 °C) was added to dissolve

completely. Then, dichloromethane (10 mL) was added and sonicated for another 20 min. Subsequently, 1 g of NaCl and 1 g of NaAc were added and sonicated for 20 min. The mixture was then centrifuged (Thermo Fisher Scientific, USA) at 8000 r/min for 5 min. After that, 1.4 mL of the above supernatant was added to 50 mg of C18 sorbent, 50 mg of PSA sorbent and 150 mg of MgSO₄, vortexed for 1 min, then centrifuged for 5 min at 8000 r/min. The supernatant was filtered again through a 0.22 µm filter and measured by GC-MS/MS in the end.

2.4. GC-MS/MS analysis

Simultaneous quantification of four furfurals was performed on triple quadrupole GC-MS/MS system (Agilent 7890B-7000D) equipped with a DB-35 ms column (30 m × 0.25 mm, 0.25 µm). The injection volume was 2 µL in splitless mode, and the solvent delay was 4 min. The column head pressure was set at 17 psi at a constant flow rate of 1.5 mL/min, using high-purity helium as carrier gas. The temperature of transfer line and ion source was 260 °C and 230 °C, respectively. The injector temperature was 240 °C. The oven temperature program was: 60 °C for 2 min, to 110 °C at 10 °C/min, then to 240 °C at 20 °C/min (hold for 10 min). The mass spectrometer was operated with an electron ion source (EI) at 70 eV in multiple reaction monitoring (MRM) mode. The argon was used as the collision gas, the pressure in the collision cell was set at 7.8 V and 0.8 V, respectively. The optimized MS parameters of the four furfurals were summarized in [Table 1](#). MassHunter Workstation software was performed for data acquisition and analysis.

2.5. Validation of the method

The precision of the method was validated by measuring sample added with standard solutions of low, medium and high concentration (10, 50 and 90 µg/kg for F, MF, FMC and 50, 250 and 450 µg/kg for 5-HMF) within a day and each concentration measured six times. The influence of sample matrices on the quantitative results was tested, and matrix effect (ME) was calculated by the formula $ME (\%) = B/A \times 100$ (A: Response values for analytes in pure solvents, B: Response values for the same level of analyte added to the sample matrix). The recovery was confirmed by analyzing sample added with standard solutions of low, medium and high concentration (10, 50 and 90 µg/kg for F, MF, FMC and 50, 250 and 450 µg/kg for 5-HMF), and each recovery measurement was repeated three times. The formula for calculating the recovery rate is as follows: $Recovery (\%) = (\text{amount of analytes tested} / \text{amount of analytes added}) \times 100$. Calibration curves were obtained from standard concentrations of 5, 10, 20, 50, 100 and 200 ng/mL (six concentration levels) for F, MF and FMC and of 25, 50, 100, 250, 500 and 1000 ng/mL (six concentration levels) for 5-HMF. Quantitative analysis was carried out using the above calibration curves.

2.6. Statistical analysis

Statistical analysis was carried out utilizing SPSS Statistics 17.0 software with LSD and Duncan tests, and one-way analysis of variance

Table 1
Mass spectrometric settings for four furfurals.

Furfurals	Precursor ion	Product ion ^a	Collision Energy (eV)	Retention time
5-HMF	126	69(C) 97(Q)	6 6	11.59
MF	110	53(C) 109(Q)	10 10	8.05
FMC	95	37(C) 39(Q)	10 18	7.12
F	96	39(C) 95(Q)	16 10	5.91

^a Q, quantification ion; C, confirmation ion.

(ANOVA) was applied to detect whether there are any statistically significant differences ($p < 0.05$). All experiments were repeated three times.

3. Results and discussion

3.1. Optimization of GC-MS/MS conditions

In this study, GC-MS/MS was applied to simultaneously measure 5-HMF, F, FMC, and MF. A set of arguments (such as ion pairs, monitoring reaction ion pairs and collision energy) must be optimized to improve the detection sensitivity and ensure the accuracy of qualitative and quantitative analysis. Firstly, each standard solution was individually sampled in full scan mode to find the possible precursor ions. The ions with a high m/z ratio and a high relative abundance were selected as the precursor ions. Then product ion scan was conducted for each target analyte to obtain the optimal ion pairs. The collision energy for each ion pair was optimized in MRM mode. In the end, the ion pair with the strongest response was used for quantification and the other was qualitative one. The optimized parameters are detailed in 2.4.

Since the target compounds have carbonyl groups, three GC columns with different polarities (HP-INNOWax, HP-5 and DB-35 ms) were chosen to separate the four furfurals. HP-INNOWax is coated with polyethylene glycol (PEG) stationary phase and is the recommended column for the detection of furfurals. HP-5 is non-polar column coated with 5%-phenyl-methylpolysiloxane. DB-35 ms is coated with 35%-phenyl-methylpolysiloxane, which is an intermediate polarity column with very low bleed, and is ideal for GC/MS. The results showed that HP-INNOWax worked with large column bleeding and thus did not match the GC-MS/MS equipment. The tailing on HP-5 is serious and the separation efficiency is significantly poor, which may be the slightly greater polarity of the functional groups contained in the target compounds. Finally, the peak shape, resolution as well as the column bleeding on DB-35 ms is all satisfactory, therefore, a DB-35 ms column (30 m \times 0.25 mm, 0.25 μ m) was selected to simultaneously separate the four furfurals. The total ion current chromatogram (TIC) for a 200 ng/mL analytical standard solution were shown in Fig. 1.

3.2. Optimization of sample preparation

This study aimed to seek a suitable extraction solvent to extract four furfurals from cheeses and compatible with the subsequent GC-MS/MS analysis. Acetonitrile and methanol were first considered as extraction solvents, which have been used in previous studies (Cui et al., 2020; Huang et al., 2019a; Sun et al., 2017). However, the polar solvents such

as methanol and water behave very poorly on GC or GC-MS because their expansion volumes are quite large (methanol, 567:1; H₂O 1277:1, inlet: 250 °C, pressure: 13 psi), which will expand beyond the volume of the injection port and possibly deposit some of analytes in the liners. On the contrary, ethyl acetate, and dichloromethane are polar solvents, but expansion volumes are acceptable (ethyl acetate, 235:1; dichloromethane 360:1, inlet: 250 °C, pressure: 13 psi). The U. S. Food and Drug Administration (FDA) state that hexane is good injection solvent for GC-MS/MS because the vapor volume is low and would not over-flow the injector liner. Therefore, the extraction efficiency of several solvents of different polarity such as acetonitrile, ethyl acetate, dichloromethane, and n-hexane, was explored. The results showed that n-hexane had the poorest extraction efficiency, which may be due to the fact that furfurals especially 5-HMF are polar analytes, so their solubility will be reduced in non-polar solvents due to no H-bonding with the nonpolar solvent such as hexane. It also showed that it is not advisable to decrease with n-hexane in this method. Secondly, the extraction efficiency of acetonitrile was unsatisfactory, this may be due to the fact that acetonitrile is soluble in water and water in cheese samples might be co-extracted. Once water is introduced into GC-MS, it reduces the ionisation efficiency and detector sensitivity (Aeppli et al. 2008; Yu et al., 2014). Besides, the results showed that although ethyl acetate extracts furfurals better, its parallelism is unsatisfactory. Dichloromethane not only extracted furfurals, but the parallelism and recovery was within acceptable limits. This may be due to the fact that dichloromethane is not readily soluble in water and that dichloromethane is less polar than ethyl acetate and acetonitrile, and it belongs to medium polarity relatively. Therefore, dichloromethane was used as the extraction solvent.

To ensure a good extraction efficiency, the effect of extraction solvent volume and times (10 mL, 1 time (a); 5 mL, 2 times (b)) was also investigated, and the results are shown in Fig. 2. 10 mL, 1 time represents sample was extracted once with 10 mL of dichloromethane for GC-MS/MS detection; 5 mL, 2 times means sample was extracted twice with 5 mL of dichloromethane and solutions were combined for GC-MS/MS detection. By comparing the signal intensities of both, we found that the highest intensities of target analytes were obtained by using one 10 mL of dichloromethane, especially for FMC and 5-HMF. We then extracted twice with 5 mL of dichloromethane and analyzed each of the two extracts (5 mL, First, and 5 mL, Second) separately. Interestingly, the first extraction has extracted most of the target analytes (58.92% ~ 89.11%), especially F (87.19%), MF (88.35%) and FMC (89.11%). Therefore, one extraction with 10 mL of dichloromethane was sufficient to meet the analytical requirements.

3.3. Method validation

The analytical performance of the proposed assay for four target compounds was validated under optimal conditions. Considering the different levels of four furfurals in various samples, mixed standard solutions of four furfurals in different linear ranges was prepared. The limits of detection (LODs), linear calibration ranges, linear equations, and correlation coefficients of furfurals were summarized in Table 2. The calibration curves showed good linearity in wide ranges (5–1000 ng/mL) with correlation coefficients (R^2) from 0.9986 to 0.9995. The LODs and limits of quantification (LOQs) refer to the concentration on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LODs and LOQs were ranged from 0.01 to 0.02 mg/kg and 0.03–0.07 mg/kg, respectively, which showed this method was more sensitive than that of Chávez-Servín (2005), Ferrer (2005) and Xing et al. (2020).

The precision of the proposed method was estimate by considering relative recoveries and the relative standard deviations (RSDs) ($n = 6$) using Orange Cheddar (New Zealand) sample spiked at low, middle and high concentrations (Table 2). The recoveries of four furfurals ranged from 72.62% to 123.29%, with precision (RSDs) ranged from 0.17% to 9.82%. The ME was 71.40~100.44%, indicating that the cheese sample

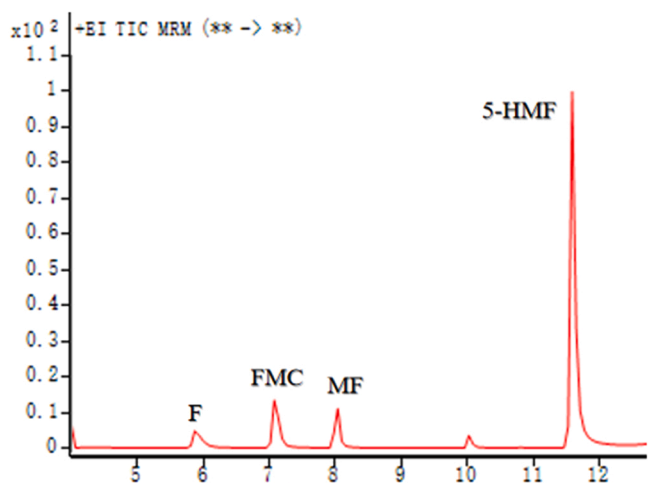


Fig. 1. GC-MS/MS chromatograms of analyte standards.

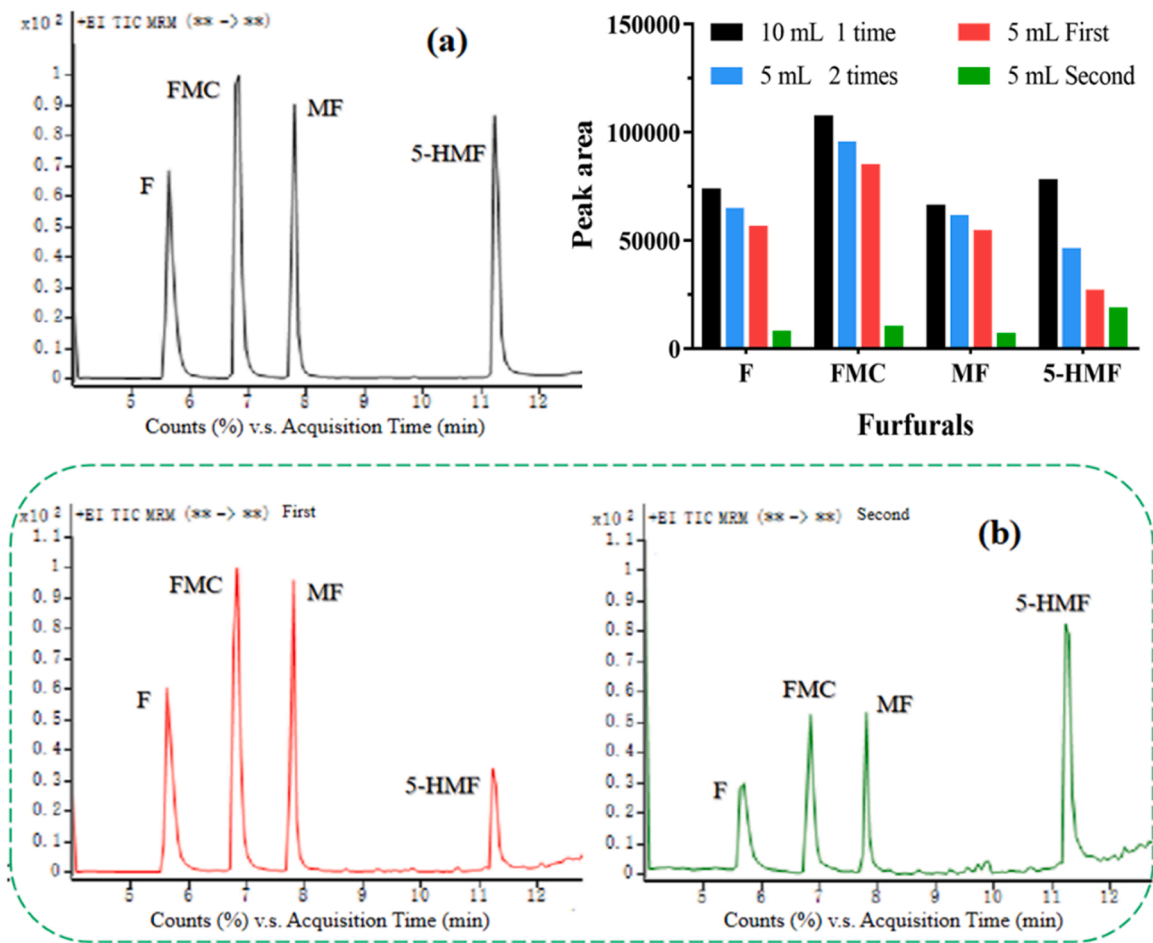


Fig. 2. GC-MS/MS chromatograms and peak areas of standards obtained with different extraction ways (10 mL, 1 time represents sample was extracted once with 10 mL of dichloromethane for GC-MS/MS detection; 5 mL, 2 times means sample was extracted twice with 5 mL of dichloromethane and then two 5-mL were combined for detection; 5 mL, First means sample was extracted twice with 5 mL of dichloromethane and the first 5-mL was used for detection; 5 mL, Second means sample was extracted twice with 5 mL of dichloromethane and the second 5-mL were used for detection).

Table 2
Method validation of four furfurals determined simultaneously with GC-MS/MS.

Furfurals	Calibration range (ng/mL)	Linear equations	Correlation coefficient (R2)	LODs (mg/kg)	LOQs (mg/kg)	Concentration (µg/kg)	RSD (%) (n = 6)	Recovery (%) (n = 6)
5-HMF	25–1000	$y = 127.96 x - 223.00$	0.9995	0.01	0.04	50	1.56	82.55
						250	0.49	82.74
						450	0.17	83.99
						10	1.44	73.53
F	5–200	$y = 66.72 x + 92.75$	0.9991	0.02	0.07	50	3.06	72.62
						90	2.30	82.30
						10	4.71	76.73
MF	5–200	$y = 65.67 x + 150.64$	0.9995	0.01	0.03	50	2.80	86.42
						90	1.21	88.08
						10	9.82	108.49
FMC	5–200	$y = 90.11 x + 176.19$	0.9986	0.01	0.03	50	1.11	123.29
						90	2.02	91.75
						10		

LODs: the limits of detection; LOQs: the limits of quantification; RSD: relative standard deviations.

matrix had an acceptable level of influence on the quantitative determination of furfurals in this method. Therefore, the established method had good linearity, sensitivity, precision, stability and recovery, and satisfied the determination of four furfurals in cheeses.

In previous studies (Table 3), most chromatographic methods are more focused on the determination of 5-HMF in food (Bignardi et al., 2014; Gökmen and Şenyuva, 2006; Teixidó et al., 2006). Moreover, only 5-HMF was also detected in infant formulae by spectrophotometric

methods, and the sensitivity was not satisfactory (Vella and Attard, 2019). Furthermore, previous studies have found that despite spectrophotometric methods to be simple and inexpensive when applied to dairy products, the analytical results are inaccurate due to drawbacks such as its strong matrix effect and low selectivity (Espinosa Mansilla et al., 1993). HPLC method can simultaneously determine two (5-HMF and F) or three furfurals (5-HMF, F and MF), but sample requires derivatization or acid hydrolysis with trichloroacetic acid (Kamalabadi

Table 3

Comparisons of the previous reported method for furfurals analysis.

Samples	Extraction method Sample preparation method Column	LOD of 5-HMF (mg/kg)	LOD of F (mg/ kg)	LOD of MF (mg/ kg)	LOD of FMC (mg/ kg)	Analytical methods	Reference
Jam, Honey, Orange juice, Bakery products	ENV+ cartridges Derivatisation (N,O-bis-trimethylsilyltrifluoroacetamide) DB-5MS	0.006	-	-	-	GC-MS	Teixidó et al. (2006)
Milk-based foods, Cereal-based foods	aqueous extraction solid-phase extraction (HLB) Zorbax Bonus RP Narrow Bore	0.005	-	-	-	LC-MS (APCI)	Gökmen and Şenyuva (2006)
Cereal-based baby foods, Coffee, Soft beverages, Vinegars	Trichloroacetic acid Capillary electrophoresis	0.03	-	-	-	CE-MS ²	Bignardi et al. (2014)
Infant formulae	sodium bisulfite	0.11	-	-	-	Spectrophotometric	Vella and Attard (2019)
Baby formula	Headspace solid phase microextraction (dodecylbenzenesulfonate-doped polypyrrole coating) Ion mobility spectrometry ODS column	0.005	0.006	-	-	RP-HPLC-UV	Kamalabadi et al. (2015)
Baby formula	Dispersive liquid-liquid microextraction (ethanol, 1-octanol) ODS column	0.002	0.001	-	-	RP-HPLC-UV	Madani-Tonekaboni et al. (2015)
Honey, Sugars, Vinegars	Direct immersion solid-phase microextraction ZB-WAX	0.009	0.001	0.007	-	GC-FID	Gaspar and Lopes (2009)
Bovine milk powder	Methanol Derivatisation 4-((hydroxyamino)butyl)- 7-hydroxycoumarin Eclipse XDB-C ₁₈	0.8 nM	0.2 nM	0.1 nM	-	RP-HPLC-FLD	Sun et al. (2017)
Infant formulas (adapted, follow-up)	Trichloroacetic acid Spherisorb ODS2C ₁₈	0.067	0.133	0.133	0.133	RP-HPLC-UV	Ferrer et al. (2005)
Milk-based formula	Trichloroacetic acid Tracer ODS-2 C ₁₈	0.321	0.003	0.186	0.057	RP-HPLC-DAD	Chávez-Servín et al. (2005)
Liquid milk, Milk powder	Acetonitrile QuEChERS Rtx-WAX	0.02	0.002	0.002	0.002	GC-MS/MS	Cui et al. (2020)
Liquid milk	Trichloroacetic acid Atlantis T3	0.17	0.21	0.18	0.22	RP-HPLC-DAD	Xing et al. (2020)

LOD: the limit of detection.

et al., 2015; Madani-Tonekaboni et al., 2015; Sun et al., 2017). In addition, some scholars have simultaneously determined four furfurals in food by HPLC, but those methods have LODs higher than 0.02 mg/kg (Chávez-Servín et al., 2005; Ferrer et al., 2005; Xing et al., 2020). In contrast, the LODs of our method are lower, in the range between 0.01 and 0.02 mg/kg, meanwhile, our sample preparation method was simpler, without any acid hydrolysis or derivatization. Compared to GC-MS and HPLC-MS, GC-MS/MS offers higher sensitivity and selectivity, and provides an efficient tool for the qualitative and quantitative

analysis of trace target analytes in various matrices (Rodríguez-Carrasco et al., 2014). Although the GC-MS/MS method established by Cui et al. (2020) can simultaneously determine four furfurals in liquid milk and milk powder samples with low LODs, they used acetonitrile as extraction solvent, which could lead to column bleeding and contamination of the mass spectrometry system. On the contrary, our method selected dichloromethane as the extraction solvent and achieves the goal of simultaneous determination of four furfural compounds in cheese.

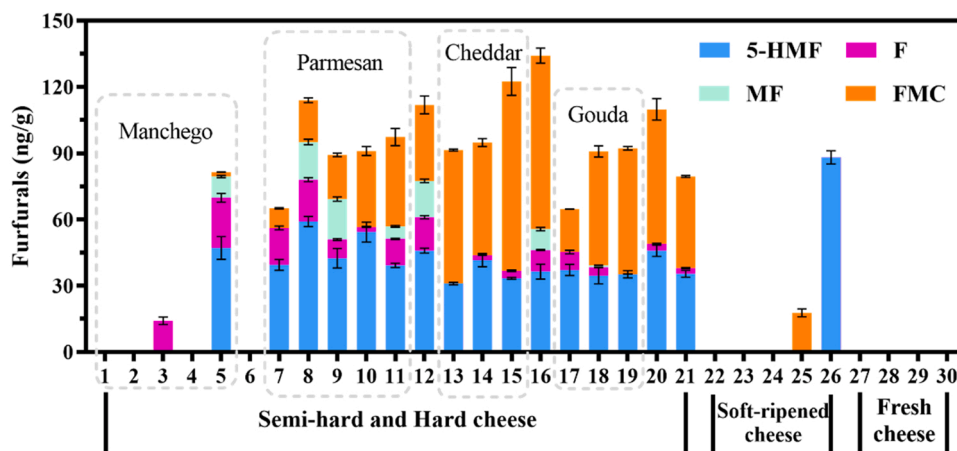


Fig. 3. The levels of four furfurals in 30 commercially available cheeses (For interpretation of the references to sample ID in this figure, the reader is referred to the Supplementary Table 1).

3.4. Analysis of cheese samples

The method developed in this research allowed to determine the contents of four furfurals in different types of cheese from the Chinese market. The levels of four furfurals in thirty popularly consumed cheeses, including Cheddar, Gouda, Manchego, Parmesan, Brie, Mozzarella, Spicco, Moravia, Padano, Romano, Swiss, Emmentaler, Camembert, Roquefort, and White cheese, were analyzed (Fig. 3). The furfurals contents of semi-hard and hard cheese were higher, while soft-ripened cheese and fresh cheese was less. Interestingly, samples 25 (Brie Au Bleu) and 26 (Danish Blue cheese) are the only soft-ripened cheeses that contained FMC and 5-HMF, which might be due to their processing technology. Comparing sample 24 (Suki Brie Cheese) with sample 25 (Brie Au Bleu), we found that sample 24 (white mold) was not detected, whereas sample 25 (Penicillium) contained trace amounts of FMC, which may be due to the different strains they contain. Moreover, the total levels of four furfurals in various cheeses ranged from < LOD to 134.23 ng/g, and the overall variation trend of four furfurals contents are as follows: 5-HMF > FMC > F > MF. Ripening is a necessary step in the processing of ripened cheese. Additionally, we analyzed furfurals levels in cheeses with different ripening time. To be more specific, for Manchego cheese, sample 5 (2–3 months) contained the highest contents of furfurals, sample 3 (5–6 months) contained only F, whereas no any furfurals were detectable in samples 1 (10–12 months), 2 (10 months), 4 (3 months). For Parmesan cheese, furfurals were detected, with the highest content in sample 8 (12 months) and the lowest in sample 7 (24–36 months). For Cheddar cheese, the furfurals content in samples 13 (Reserve) - 15 (Mild) gradually increased, and Gouda cheese (samples 17–19) also followed the same trend. This indicated that the contents of furfurals gradually dropped with the prolongation of ripening time, which may be due to the run out of lactose in cheese (Corzo et al., 2000), and furfurals may be further converted into other substances (Li et al., 2021b; Li et al., 2022). In addition, the quality of those common cheeses based on their total furfurals content decreased in the order: Brie, Blue, Mozzarella, Manchego, Gouda, Parmesan, and Cheddar cheese. Although Cheddar cheese provided consumers with a great taste experience, our results showed that their contents of total furfurals were relatively high.

4. Conclusion

In this study, a GC-MS/MS method was established to simultaneously measure the contents of four furfurals in cheese samples, including 5-HMF, F, MF, and FMC. Dichloromethane (10 mL, 1 time) was proved to be the most effective extraction solvent. The validation data demonstrated that the method achieved the simultaneous determination of four furfurals in cheese with low LODs and was appropriate for routine analysis with acceptable sensitivity, recovery and precision. Finally, this method was successfully applied to the simultaneous quantification of the four furfurals in commercially available cheeses of different types, ripening times and raw materials, and the results revealed that furfurals were mainly detected in semi-hard and hard cheeses and were rarely present in soft-ripened and fresh cheeses. Moreover, the contents of furfurals were influenced by cheese type and ripening time.

CRedit authorship contribution statement

Mingyu Li: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Aiping Yan:** Resources, Methodology, Data curation. **Yousheng Huang:** Editing, Supervision, Project administration. **Jingnan Lu:** Investigation, Analysis. **Jian Zhang:** Editing. **Yanli Du:** Visualization. **Jianhua Xie:** Editing, Supervision. **Mingyue Shen:** Writing – review & editing, Supervision, Project administration. **Mingyong Xie:** Project administration, Funding acquisition.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105213.

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