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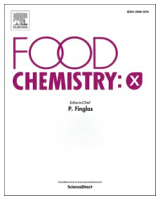

Optimization of QuEChERS cleanup for quantification of γ-oryzanol in vegetable oils by UHPLC-MS/MS

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ABSTRACT

This study was based on QuEChERS cleanup coupled with UHPLC-MS/MS for the determination of γ-oryzanol compounds in vegetable oils. Several parameters of QuEChERS and UHPLC-MS/MS were studied for purification and detection of γ-oryzanol compounds in oil samples. Under the optimized conditions, the whole pretreatment procedure could be accomplished within 10 min without tedious procedure, larger volume of organic solvent and complicated apparatus. The limit of detections and the limit of quantifications for γ-oryzanol compounds were ranging from 0.1-0.3 μg kg^{-1} and 0.4–1.0 μg kg^{-1}, respectively. Satisfactory recoveries of all analyts were ranging from 72.2 % to 101.3 %, and the intra-day and inter-day precision were less than 10.6 %. The validation indicated that rice band oil and corn oil were rich in 24-mCAF, CAF, β-SIF, CMF and STF. The QuEChERS-UHPLC-MS/MS simultaneously quantified five γ-oryzanol compounds in lipid matrices and assessed the nutritional and functional substances of vegetable oils.

1. Introduction

Vegetable oils are the most important ingredients for food cooking in restaurants and kitchens, and consist 75 % of all the edible oil and fat products consumed in the world (Yang et al., 2018). In 2021, about 246.7 million tons of vegetable oils were globally produced (Oilseeds: world market and trade, 2022). In Asia, vegetable oils are mainly soybean oil, rapeseed oil, peanut oil, sunflower oils, sesame oils, palm oils and these oil production keep stable development. Due to the growing consumption and healthy aspects, novel types of vegetable oils such as corn oil and rice bran oil widely appear in China, Japan, Thailand, and Korea. Those oils not only have high content of polyunsaturated fatty acids, but also are rich in functional substances including lipid-soluble vitamin, phytosterol, polyphenol, phytic acid and γ-oryzanol (Sakunpak et al., 2014). γ-Oryzanol is a complex mixture of ferulate esters as presented Fig. 1, which compose by hydroxycinnamic acids and phytosterol derivatives including campesteryl ferulate (CMF), β-sitosteryl ferulate (β-SIF), stigmasteryl ferulate (STF), cycloartenyl ferulate (CAF), 24-methylenecycloartanyl ferulate (24-mCAF) (D'Ambrosio, 2013). These substances have been reported to decrease cholesterol in serum and show potent antioxidant properties, such as preventing hypercholesterolemic, anti-inflammation, anti-angiogenesis, protecting nervous system (Qureshi et al., 2002; Miyazawa et al., 2009; Serbinova et al., 1991; Khanna et al., 2005). Therefore, it is important to propose a rapid, simple and accurate method to analyze the type and distribution of γ-oryzanol derivatives in vegetable oils.

Up until now, many analytic methods have been developed to detect the content of γ-oryzanol and its profile in food and related products by ultraviolet (UV) spectrophotometry and high performance liquid chromatography (HPLC) instrument. The total content of γ-oryzanol are analyzed by UV spectrum at fixed wavelength, second-derivative or multi-wavelength (Bucci et al., 2003). To determine the type and individual amount of γ-oryzanol derivatives, reversed-phase (RP) and normal-phase (NP) liquid chromatography have been used to separate analytes from complex matrice. NP-HPLC results in tedious equilibration, lower stability or poorer reproducibility. Hence, RP-HPLC coupled with UV (Thongchai and Liawruangrath, 2016), diode assay detector (Huang and Ng, 2011), MS (Stöggl et al., 2005), linear ion trap/orbitrap high resolution mass spectrometry (Zhu et al., 2017) and tandem mass spectrometry (MS/MS) (Waraksa et al., 2019) are accepted as the

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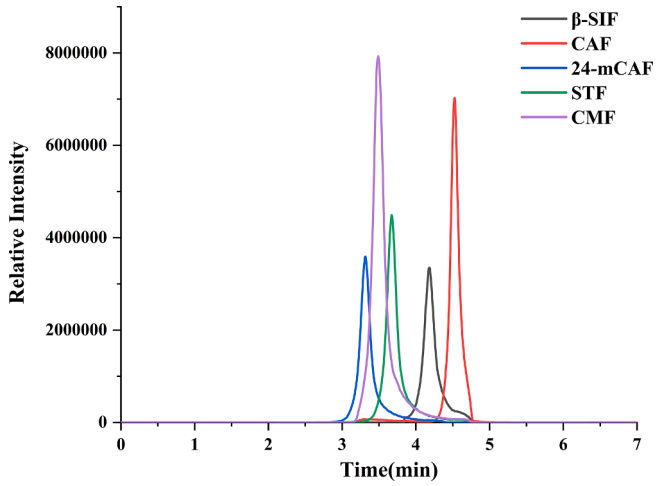

Fig. 1. Typical MRM chromatograms of γ-oryzanol spiked at 2 μg kg^{-1} in rice bran oil.

routine technique for the determination of γ-oryzanol. Among those hyphenated instruments, UHPLC-MS/MS is the most reliable and efficient method in official laboratory owing to high sensitivity, excellent selectivity and short separation time.

To minimize matrix effect and improve HPLC resolution, several preparation methods were applied to extract and purify analytes in foods including thin lay chromatography (Sakunpak et al., 2014), liquid to liquid extraction (D'Ambrosio, 2013), ultrasound-assisted extraction (Waraksa et al., 2019), mixed-mode anion exchange solid-phase extraction (Li et al., 2022), molecular imprinted polymer extraction (Thongchai and Liawruangrath, 2016), and supercritical CO_2 (Sookwong et al., 2016). Most of these reported pretreatment methods used larger amount of extraction solvent, required time-consuming procedure or complicate instruments. Therefore, it is of great importance to optimize the extraction and purification procedure of γ-oryzanol from complicated samples, which were mainly consisted of triglycerides, free fatty acids and lipid-soluble matrices.

Quick, easy, cheap, effective, rugged and safe (QuEChERS) method, which mainly consists of two steps involving liquid to liquid extraction & dispersive solid phase extraction (LLE-dSPE), has been widely utilized to isolate and enrich pesticides (Enia et al., 2022), mycotoxins (Junsai et al., 2021), chemical additives (Gan and Zhu, 2022), organic contaminants (Sun et al., 2022) in vegetable oils. Enia et al. found that the proposed combination of extraction solvents and cleanup sorbents were ethyl acetate/ACN and Z-Sep sorbents, which detected 222 pesticides in corn oils coupling with GC–MS/MS. Junsai et al. analyzed the contents of aflatoxin B_1, B_2, G_1, G_2, beauvericin, ochratoxin A, zearalenone, fumonisin B_1 and B_2 in vegetable oils using QuEChERS LC-MS/MS. For chemical additives, QuEChERS with supercritical fluid chromatography (SFC) had been proposed to simultaneously extract and purify antioxidants, photoinitiators, plasticizers and ultraviolet absorbers and quantified by SFC-MS/MS. The combination of *n*-hexane saturated with ACN and C_{18}-Z-Sep + were optimized to extract and purify phthalates, polychlorinated biphenyls and polycyclic aromatic hydrocarbons by LLE-dSPE *via* freezing-liquid precipitation, and further semi-quantified with GC–MS and NAGINATA2.0 screening software.

This study was developed a rapid, simple and accurate method to analyze the content of γ-oryzanol compounds in vegetable oils by QuEChERS-UHPLC-MS/MS. All the target compounds were enrichment and cleanup from vegetable oil by QuEChERS procedure, and analyzed by UHPLC-MS/MS. The content and distribution of five γ-oryzanol were all detected for the evaluation of functional nutritional components in edible oils and the quality development for high content of γ-oryzanol in lipid products.

2. Materials and methods

2.1. Chemical and materials

Campesteryl ferulate (CAS: 20972-07-0), β-sitosteryl ferulate (CAS: 4952-28-7), stigmasteryl ferulate (CAS: 20972-08-1), cycloartenyl ferulate (CAS: 21238-33-5), 24-methylenecycloartanyl ferulate (CAS:469-36-3) with purity ≥ 98 % were purchased from Zhenzhun Biotechnology Co., Ltd. (Shanghai, China). Methanol (MeOH), acetonitrile and ammonium acetate were purchased from Thermo Fisher Scientific (MO, USA). *n*-Hexane and isopropyl alcohol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). C18 (40 - 60 μm), *N*-Propylethylenediamine (PSA, 40 ~ 63 μm), were purchased from Aladdin (Shanghai, China). Graphitized Carbon Black (GCB, 100 ~ 300 μm) were purchased from Xianfeng Nano Co., Ltd. (Jiangsu, China). Anhydrous magnesium sulfate and sodium chloride were purchased from Xilong Chemical Co., Ltd. (Hubei, China).

2.2. Preparation of vegetable oils

Vegetable oils including rice brand oil ($n = 5$), corn oils ($n = 5$), sunflower oils ($n = 5$), sesame oils ($n = 5$) and soybean oils ($n = 5$) were collected from supermarkets and stored at 25 ℃ before analysis. The detail information of type, geographical origin and production year related to vegetable oils were listed in Table S1.

2.3. QuEChERS cleanup

Firstly, vegetable oil (1.00 ± 0.01 g) was accurately weighted into a centrifuge tube, and mixed with 0.5 mL of ultrapure water and 9 mL of acetonitrile. After vortex for 2 min, 1.2 g of anhydrous magnesium sulfate and 0.3 g of sodium chloride were added to the centrifuge tube and mixed for 2 min vortex. When centrifuged with 4500 rpm for 5 min, 4.5 mL of the supernatant was drawn into a centrifuge tube, and mixed with 0.3 g of anhydrous magnesium sulfate and 55 mg of C18 sorbents for 2 min. After centrifuging at 4500 rpm for 5 min, the supernatant was collected and evaporated under a gentle stream of N_2. The resulting residue was re-dissolved with 500 μL of MeOH and subjected to UHPLC-MS/MS.

2.4. UHPLC-MS/MS condition

UHPLC-MS/MS detection was conducted by a Shimadzu 8050 triple quadrupole mass spectrometer with a Shimadzu LC-30AD chromatography system (Kyoto, Japan). The separation of 24-mCAF, CAF, β-SIF, CMF and STF was performed by a Hypesil gold C18 column (100 mm × 2.1 mm, 3 μm, Thermo, USA) at 40 ℃ with the flow rate at 400 μL min^{-1}. The mobile phase was 5 mmol/L ammonium acetate aqueous solution (solvent A) and acetonitrile (solvent B). The linear gradient program was set as followed: 0 min, 40 %A; 1.1 min, 40 % A; 2.5 min, 5 % A; 3.5 min, 5 % A; 4.5 min, 40 % A; 7 min, 40 % A. Finally, 1 μL of the re-solved sample was injected into UHPLC-MS/MS.

With an electrospray ionization (ESI) source, the MS analysis was performed in multiple reaction monitoring (MRM) under positive mode. The MS parameter for quantification were nebulizing gas (N_2) 3.0 L min^{-1}, heating gas (N_2) 10.0 L min^{-1}, heating block temperature 400 ℃, DL temperature 250 ℃. All the analytes were confirmed by the MRM ion pair and the retention time. Maximum permitted tolerances for relative ion intensities were provided in Table S2, and MRM transitions and retention time in Table S3. All the data of UHPLC-MS/MS were acquired and analyzed by Shimadzu workstation (version 5.89, Kyoto, Japan).

2.5. Validation

QuEChERS-UHPLC-MS/MS was validated in the laboratory by European Commission Decision 2002/657/EC (European Commission,

2002) and Joint Research Center Technical Reports (Wenzl et al., 2016). The calibration curve, sensitivity, accuracy, precision and matrix effect of the developed method were performed. γ-Oryzanol compounds were spiked in vegetable oils to evaluate the recoveries of the developed method.

3. Results and discussion

3.1. Optimization of UHPLC-MS/MS conditions

To obtain good separation and minimize chromatography time, different types of mobile phase were performed to optimize the chromatography condition including pure water as phase A and acetonitrile/MeOH as phase B. Compared with MeOH, acetonitrile could lower the pressure of C18 column and obtain shorter separation time owing to the viscosity of the entire mobile phases. Additionally, ammonium acetate ranging from 1 mmol/L to 10 mmol/L was added as chromatography addictive to increase the proton efficiency and further improve the sensitivity during MS/MS analysis. When the concentration of ammonium acetate was increased from 1 mmol/L to 5 mmol/L, the peak area of all analytes increased with symmetrical peak shape and satisfactory chromatography separation. However, the peak areas gradually reduced from 5 mmol/L to 10 mmol/L. Hydroxyl group of γ-oryzanol compounds could significantly dissociate when mobile phase was at low pH (Taarji et al., 2023), and 5 mmol/L of ammonium acetate promoted the analytes dissociation and increased the ionization efficiency during MS analysis. Therefore, the mobile phase A and B were fixed at 5 mmol/L ammonium acetate for aqueous solution and acetonitrile respectively.

To achieve high intensity and sensitivity, the standard solution of 24-mCAF, CAF, β-SIF, CMF and STF was separately injected into MS detector at 0.5 μg mL^{-1} with a automatic needle pump. Firstly, full scan mode was set to obtain the patent and product ions of all analytes under negative ESI mode. The characteristic parent ions of 24-mCAF, CAF, β-SIF, CMF and STF were the molecule ions $[M-H]^-$ in negative mode. The maximum permitted tolerances and the ion transition of MRM were optimized with collision energy as listed in Table S2 and S3. The product ions for quantification and qualification were [M−H−15] and 133.1, which were the loss of methyl group and triterpene alcohol ester. The identification points (IPs) of all analytes were equal to 4 which were consistent to European Commission Decision 2002/657/EC for the analytic method for routine detection (European Commission, 2002).

3.2. Optimization of QuEChERS procedure

To optimze QuEChERS procedure, various parameters related to extraction and purification were evaluated including the type, material to liquid ratio and time of extraction, the type and amount of purification sorbents. 24-mCAF, CAF, β-SIF, CMF and STF spiking at 50 μg kg^{-1} in rice band oils were utilized to improve the efficiency of QuEChERS procedure. The experiment were all performed by triplicate and calculated as the mean value.

3.2.1. Effect of extraction solvent

Owing to the diversity of molecular structure, different type of

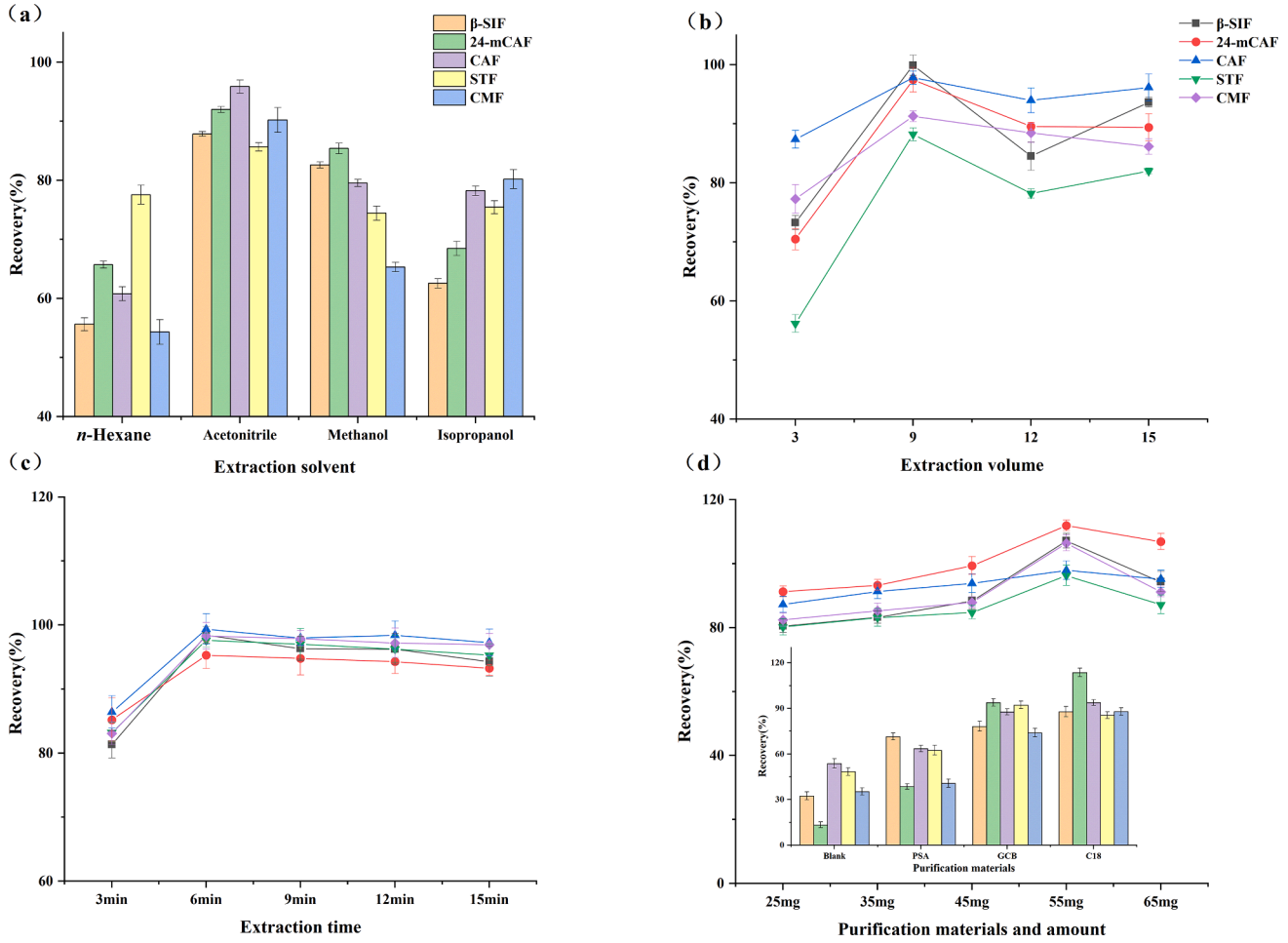

Fig. 2. Optimization of QuEChERS conditions: (a) extraction solvent, (b) extraction volume, (c) extraction time, (d) purification materials and amount. The oil samples were spiked with γ-oryzanol compounds at 50 μg kg^{-1}.

organic solvents including *n*-hexane, acetonitrile, methanol and isopropanol were used to improve the efficiecny of extraction step. As presented in Fig. 2a, the recoveries were acetonitrile (polarity of 0.895) > MeOH (polarity of 0.857) > isopropanol (polarity of 0.847) > *n*-hexane (polarity of 0.519) (Catalán et al., 1995). When the polarity of extraction solvent increased, the recoveries of γ-oryzanol compounds were gradually increased and achieved highest recoveries ranging from 85.7 % to 95.9 %. The phenomenon could attribute to the molecualr groups such as hydroxyl, methoxy and ester bonds, which were easily soluble in polar organic reagents such as acetonitrile. Therefore, acetonitrile was used as the extraction solvent.

3.2.2. Effect of extraction volume

The volume of extraction sovlent plays a vital role in the extraction efficiency. To study the effect of extraction volume, different volume of acetonitrile ranging from 3 to 15 mL was performed for the QuEChERS of γ-oryzanol compounds from vegetable oils in Fig. 2b. The recoveries of all analytes were increased from 3 to 9 mL, and the recoveries of STF and β-SIF significantly decreased from 88.2 % to 99.9 % to 78.2 % to 84.5 % when the extraction volume was increased from 9 to 15 mL. Thus, 9 mL was selected as the extraction volume of further experiments.

3.2.3. Effect of extraction time

In QuEChERS, extraction time is vital to obtain extraction equilibrium between extraction solvent to analytes from oil samples (Zheng et al., 2022). This equilibrium was achieved dependence to time, and different extraction time was evaluated including 3, 6, 9, 12 and 15 min. As shown in Fig. 2c, the extraction efficiency of all γ-oryzanol compounds were significantly increased along with the extraction time increasing from 3 to 6 min. When kept prolonging the extraction time, the extraction efficiency kept almost constantly. As a results, the extraction time was fixed at 6 min.

3.2.4. Effect of purification materials and amount

To avoid the matrix effect and to improve sensitivity, the purification materials C18, GCB and PSA, which have the adsorption effect on oils, pigments and free fatty acids, were selected to evaluate the effect on the recoveries of the analytes. As shown in Fig. 2d, when the spiked concentration was 50 μg kg^{-1}, the recoveries of the C18 material for five γ-oryzanol compounds ranged from 85.6 % to 113.7 %, which was able to satisfy the detection requirements for analyte quantification. Compared without using the sorbents, the GCB and PSA purification materials could reduce the matrix effect and adsorb the impurity components during the mass detection, with the recoveries of 24-mCAF and STF more than 80 %.

In addition, the effect of C18 amount (25, 35, 45, 55, 65 mg) on analyte recovery was compared. It can be seen from Fig. 2d that the recovery increased from 80.3 % to 111.9 % when the amount of C18 was increased from 25 mg to 55 mg. When the amount of C18 was increased to 65 mg, the recoveries of 24-mCAF, β-SIF, CMF and STF decreased significantly. Therefore, the purification material was 55 mg of C18.

3.3. QuEChERS UHPLC-MS/MS validation

3.3.1. Matrix effect

To assess the suppression or enhancement of MS signal, matrix effect (ME) were investigated in spiked oil samples. The ME of all analytes were calculated by comparing the MS response of chromatography peak at different concentrations, which was measured as a blank oil spiked with the standard solutions ($Area_{matrix+std}$), the blank oil ($Area_{matrix+std}$), and the pure standard solution ($Area_{std}$). The matrix effect of γ-oryzanol were separately calculated as the following equation:

$$ME(\%) = \left[\frac{Area_{matrix+std} - Area_{matrix}}{Area_{std}}\right] \times 100\%$$

When the absolute values of ME were more than 15 %, the matrices-matched calibration or internal standard method was used to improve the accuracy and sensitivity of IMSPE UHPLC-MS/MS.

As presented in Table 1, the ME were ranging from −11.4 % to −5.5 %, which were acceptable as insignificant owing to the range from −20 % to 20 %. The results indicated that the QuEChERS procedure could effectively purify the target compounds from complicated samples, and avoid the lipid-soluble interference during the MS analysis.

3.3.2. Calibration curve and sensitivity

To evaluate the robustness of the proposed experimental parameters, different amount of γ-oryzanol compounds in pure solution were analyze by UHPLC-MS/MS, and the linear regress equations were established by the MS peak area of the standard solution. As summarized in Table 1, the correlation coefficient (R^2) of all analyte equations were ranging from 0.9939 to 0.9999. The sensitivity of this optimized method were estimated by the limits of detection (LOD) and quantification (LOQ) with the analyte in the pure solutions, which were determined as the signal-to-noise ratios (S/N) equal or above of 3 and 10 to the corresponding concentration of the analytes. The LODs and LOQs of 24-mCAF, CAF, β-SIF, CMF and STF were ranging from 0.1-0.3 μg kg^{-1} and 0.4–1.0 μg kg^{-1}, respectively.

3.3.3. Accuracy and precision

Accuracy were studied as trueness (systematic error) by spiking γ-oryzanol compounds at three concentration (5, 10 and 50 μg kg^{-1}). The mean recoveries were calculated using the ratio of (total detecting concentration − original concentration) to spiking concentration. As presented in Table 2, the recoveries of rice bran oil were ranging from 72.2 % to 101.3 % for the quantification of all analytes in oil samples, and it satisfied the requirement for the UHPLC-MS/MS analysis. In addition, the precision were expressed as the intra-day and inter-day precisions by detecting the standard solutions within the same day and independent days. All the experiments were analyzed by conducting in different concentrations of the standards by sextuplicate. Satisfactory precision was achieved with the relative deviation (RSD, %) less than 10.6 %, which was acceptable for the current European Commission Decision 2002/657/EC for the analytic method for routine detection (European Commission, 2002).

3.4. Validation of QuEChERS-UHPLC-MS/MS to analyze γ-oryzanol in vegetable oils

To validate the applicability of the proposed method, various types of vegetable oils including rice band oil, corn oil, sunflower oil, sesame oil and soybean oil were collected to determine the content of γ-oryzanol compounds by QuEChERS-UHPLC-MS/MS. As presented in Fig. 3, only cereal vegetable oils including rice band oil and corn oil were detected all types of γ-oryzanol compounds. Rice band oils, which is prepared from rice bran (the outer layer of the rice kernel), had the total amount of γ-oryzanol higher than corn oils. The content and distribution of γ-oryzanol higher in rice band oils were 24-mCAF (1.9×10^4-5.0×10^4 mg kg^{-1}) > CAF (1.4×10^4-3.9×10^4 mg kg^{-}) > CMF (0.6×10^3-1.6×10^4 mg kg^{-1}) > β-SIF (0.4×10^3-1.1×10^4 mg kg^{-1}) > STF (0.1×10^3-0.3×10^3 mg kg^{-1}). Corn oils, which were pressed from corn germ also found γ-oryzanol in all samples, mainly consisted of β-SIF (0.08×10^3-0.1×10^3 mg kg^{-1}) and CMF (0.1×10^3-0.2×10^3 mg kg^{-1}). The results illustrated that rice band oil and corn oil were rich in 24-mCAF, CAF, β-SIF, CMF and STF, which could satisfied the urgent requirement of consumers for high quality and nutritional vegetable oils as the supplement food of γ-oryzanol compounds.

The proposed method had been compared with the reported methods in the literature for the detection of γ-oryzanol compounds in cereal crops and its products. Table 3 was presented the analytic parameters including extraction, cleanup, pretreatment time, the determination technique, and LOQs. The results indicated that QuEChERS-UHPLC-MS/

Table 1
The linear range, calibration equations, LODs, LOQs and matrix effects for γ-oryzanol compounds.

Analyte	Linear range (μg kg^{-1})	Calibration equation	R^2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Matrix effects (%)[a]		
						5 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}
β-SIF	1.0–200	Y = 35553.0X + 398760	0.9996	0.1	0.4	−6.4	−9.6	−11.3
24-mCAF	1.0–200	Y = 63192.9X-117956	0.9999	0.2	0.7	−7.4	−6.5	−9.2
CAF	1.0–200	Y = 70875.5X + 404817	0.9989	0.2	0.7	−6.3	−8.5	−10.1
STF	1.0–200	Y = 49215.8X-436310	0.9939	0.3	1.0	−10.3	−7.9	−6.7
CMF	1.0–200	Y = 96592.7X + 36578.5	0.9976	0.1	0.4	−5.5	−7.6	−11.4

[a] Matrix effects were analyzed using the rice oils spiked at 5 μg kg^{-1}, 10 μg kg^{-1} and 50 μg kg^{-1}, respectively.

Table 2
Recoveries and precisions for the determination of γ-oryzanols in oil samples[a].

Analyte	Recovery (%, n = 6)			Intra-day precision (RSD%, n = 6)			Inter-day precision (RSD%, n = 6)		
	5 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}	5 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}	5 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}
β-SIF	75.1	87.2	95.2	4.1	5.3	3.2	8.4	9.5	9.03
24-mCAF	72.2	86.3	93.2	3.1	5.3	4.6	9.4	10.4	9.87
CAF	76.3	85.6	101.3	5.3	4.6	6.5	9.0	9.4	9.75
STF	81.3	88.3	97.5	4.3	5.4	5.3	9.6	10.6	10.35
CMF	74.1	84.4	98.8	4.5	6.4	5.5	9.3	10.3	8.45

[a] Recoveries, intra-day and inter-day precisions were evaluated as mean values in sextuplicate analysis.

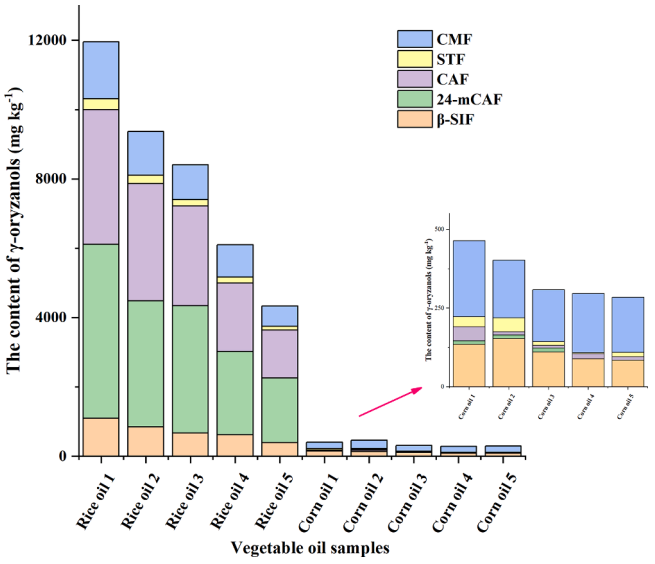

Fig. 3. Types and content of γ-oryzanol compounds in vegetable oils.

MS was more simple and accuracy than the analytic methods in the literature. The whole pretreatment could be accomplished within 10 min without tedious procedure, larger volume of organic solvent and complicated apparatus. Additionally, the QuEChERS kit achieved satisfactory recoveries and sensitivity with low cost (about 60 % reducing of the reported SPE column). The proposed method could be used as a rapid, sensitive and routine method for the determination of five γ-oryzanol compounds in lipid matrices.

4. Conclusion

A novel QuEChERS-UHPLC-MS/MS method was successfully established for extraction and purification of γ-oryzanol compounds from vegetable oils. The parameters of QuEChERS and UHPLC-MS/MS were optimized for isolation and cleanup γ-oryzanol compounds from oil samples. Under the optimized experimental conditions, the whole pretreatment procedure could be accomplished within 10 min without tedious procedure, larger volume of organic solvent and complicated apparatus. The LODs and LOQs of γ-oryzanol compounds were ranging from 0.1 – 0.3 μg kg^{-1} and 0.4 – 1.0 μg kg^{-1}, respectively. The recoveries of all analyts in rice bran oils were ranging from 72.2 % to 101.3 %, and the intra-day and inter-day precisions were less than 10.6 %. The validation indicated that rice band oil and corn oil were rich in 24-mCAF, CAF, β-SIF, CMF and STF, which could satisfy the urgent requirement of consumers for high quality and nutritional vegetable oils

Table 3
Comparison of sample pretreatment procedure and LOQs among the reported methods.

Agri-product	Analytes	Extraction	Cleanup sorbents	Pretreatment time (min)	Determination technique	LOQs (μg kg^{-1})	Ref.
Rice	24-mCAF, CAF, CMF	50 mL of *n*-hexane mixed and stored for 12 h, and ultrasonicated for 20 min, N_2 evaporated and re-dissolved	Mixed-mode anion exchange sorbents	20	UHPLC–MS/MS	2.0 – 3.5	Li et al., 2022
Rice, rice bran, maize, maize germ	24-mCAF, CAF	6 mL of *n*-hexane with ultrasonication for 25 min	Silica sorbents	30	UHPLC–MS/MS	24-mCAF, CAF were 1.0	Lv et al., 2023
Rice oil	β-SIF, 24-mCAF, CAF, STF, CMF	Diluted and adjusted the volume to 5 mL with *n*-hexane	Sep-Pak silica sorbents	25	HPLC-UV	0.5×10^3 – 0.6×10^3	Lu et al., 2014
Vegetable oil	β-SIF, 24-mCAF, CAF, STF, CMF	Diluted with dichloromethane and adjusted the volume to 1 mL	–	5	HPLC-DAD-FLD	0.632 – 2.166	Pokkanta et al., 2019
Vegetable oil	β-SIF, 24-mCAF, CAF, STF, CMF	0.5 mL of ultrapure water and 9 ml of acetonitrile with vortex for 2 min	C18 sorbents	10	UHPLC–MS/MS	0.4 – 1.0	This work

as the supplement food of γ-oryzanol compounds. Therefore, it is a rapid, simple, efficient and cost-effective method to evaluate the nutritional and functional aspects of γ-oryzanol compounds in lipid matrices.

CRediT authorship contribution statement

Shaowei Li: Validation, Resources, Methodology, Investigation. **Yuting Yuan:** Writing – original draft, Visualization, Resources, Data curation. **Liangxiao Zhang:** Software, Formal analysis. **Fei Ma:** Writing – review & editing, Visualization, Investigation, Funding acquisition, Conceptualization. **Peiwu Li:** Supervision, Project administration, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101467.

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