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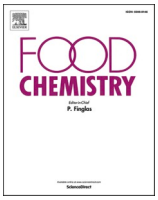

Determination of chloropropanol esters and glycidyl esters in instant noodles based on solid-phase microextraction with chitosan-β-cyclodextrin coated fiber

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ABSTRACT

We developed a method for the determination of chloropropanol esters and glycidyl esters (GE) in instant noodles using solid-phase microextraction with chitosan-β-cyclodextrin (CS-β-CD) coated fiber coupled with gas chromatography-tandem mass spectrometry. The developed low-cost fiber coating can improve the sensitivity of the method. Immobilized enzymes can improve operational stability and reusability compared to free enzymes, thereby reducing costs. The adsorption isotherm was modeled using the Langmuir model, while the adsorption kinetics followed the pseudo second-order model. The limit of detection was 0.3 ng/L. The method exhibited satisfactory recoveries for the analytes, ranging from 80.2 % to 105.3 %, with relative standard deviations < 9.9 %. Furthermore, the results of the exposure assessment showed that chloropropanol esters do not pose unacceptable risks to different age groups. However, the margin of exposure for GE suggested a potential health risk for populations between the ages of 3 and 12 years old.

1. Introduction

3-Chloro-1,2-propanediol (3-MCPD) esters, 1,3-dichloro-2-propanol (1,3-DCP) esters, and glycidol esters can be formed at elevated temperatures during the deodorization stage of the oil refining process or when heated in the presence of chlorine (Arisseto, Marcolino et al., 2017, Xu et al., 2020, Zhang et al., 2020). It has been reported that lipases present in the gastrointestinal system have the capability to convert chloropropanol esters and glycidyl esters (GE) into their free forms, which could potentially lead to renal toxicity (Wallace et al., 2016, Zheng et al., 2021). To date, 3-MCPD and 1,3-DCP have been considered as probably carcinogenic to humans (group 2B), and glycidol has been classified as a possible human carcinogen (group 2A) by the International Agency for Research on Cancer (IARC) (IARC, 2000). In 2018, the European Food Safety Authority (EFSA) established a tolerated daily intake (TDI) of 2 μg/kg body weight (bw) per day for 3-MCPD and its esters.

The primary route of exposure to chloropropanol esters and GE is believed to be dietary intake. Previous studies have provided evidence that edible oils, infant formula, and fried foods can contain high concentrations of 3-MCPD ester and GE (Li et al., 2022, Xu et al., 2020, Arisseto, Marcolino et al., 2017). Instant noodle, as a popular fast food item in Asian countries, are of particular interest. According to the World Instant Noodle Association (WINA), an estimated 1181.8 billion instant noodles were sold worldwide in 2021, with the Chinese market accounting for 439.9 billion, or 37.2 % of the total sales, securing the top position (WINA). It was reported that 1,3-DCP esters, 3-MCPD esters and GE have been found in instant noodles (Cui et al., 2021, Shimamura et al., 2021, Zhang et al., 2020).

Chloropropanol esters and GE were analyzed using LC–MS or GC–MS techniques (Custodio-Mendoza et al., 2019, Xu et al., 2020, Zheng et al., 2021). LC-MS methods allow for separate identification and quantification of 3-MCPD esters and GE without the need for derivatization (Custodio-Mendoza et al., 2019). However, not all quantification standards are commercially available. Indirect methods involve the hydrolysis of esters using acid/alkaline/enzymes to release free chloropropanols and glycidol (Cheng et al., 2017). Free glycidol released from GE is highly unstable and has a short lifespan. In order to quantify GE, glycidol is typically brominated to cleave the epoxy ring, forming 3-monobromopropanediol (3-MBPD), which is further derivatized for GC/

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MS analysis (Zheng et al., 2021). The determination of chloropropanol esters and GE contents is complicated by the complex conversion between chloropropanols and glycidol under alkaline or acidic conditions, as well as other unwanted side reactions (Tsai et al., 2021). Recently, the analysis of 3-MCPD esters in edible oils, fish oils, and oil-based products has been carried out using enzymatic hydrolysis (Custodio-Mendoza et al., 2023, Koyama et al., 2016, Miyazaki and Koyama, 2016, Miyazaki and Koyama, 2017). Immobilized enzymes, created by chemically or physically fusing a soluble enzyme with an insoluble carrier, can function as catalysts repeatedly within a specific area (Li et al., 2021). Immobilization technologies for enzymes have advanced significantly in recent years to achieve lower preparation costs, improved catalytic stability and efficiency, and easier operation. The search for new and superior carrier materials and appropriate immobilization techniques is a key focus in enzyme immobilization research (Li et al., 2020). Cellulose filter paper (CFP) has been chosen as a new carrier for enzyme immobilization due to its low cost, wide availability, large surface area, excellent biocompatibility, easy modification, and abundance of surface hydroxyl groups (Liu et al., 2019). After functional group modification, CFP provides binding sites for enzyme molecules. Chitosan, an amino-functionalization agent, can be physically coated on the surface of cellulose filter paper to introduce amino groups for the subsequent immobilization of enzymes (Zhao et al., 2020).

Several sample pretreatment methods combined with GC have been developed to analyze 3-MCPD esters in instant noodles (Cui et al., 2021). An efficient and more sustainable method of sample preparation for GC–MS analysis is solid-phase microextraction (SPME), which allows for direct adsorption of analytes onto an SPME fiber without the need for prior extraction with organic solvents (Li et al., 2022). In SPME applications, the composition and microporous structure of the material coated on the extraction head are crucial. Various functional materials have been utilized as innovative coating materials for the pre-enrichment of various analytes in complex samples (Liao et al., 2022). Chitosan (CS), a hydrophilic, non-toxic and biocompatible linear polysaccharide, contains numerous $–NH_2$ and –OH groups that can serve as hydrogen bond sites for pollutants (Liu et al., 2011). β-cyclodextrin (β-CD), generated from starch hydrolysis with a macrocycle of seven glucose units, is safe for human consumption and widely available. β-CD can form stable host–guest inclusion complexes with various chemical, inorganic, and biological guest molecules due to its unique structure, which features a hydrophobic cavity and a hydrophilic exterior (Chen et al., 2018). Several studies have reported the use of CS grafted β-CD for the removal of heavy metal ions and organic compounds, demonstrating its remarkable adsorption capability (Chai and Ji, 2012, Mashile et al., 2021, Tsiepe et al., 2018).

In this study, cellulose filter paper (CFP) was employed as the carrier for immobilized enzymes responsible for hydrolyzing the chloropropanol esters and GE in instant noodles. Subsequently, CS-β-CD was utilized as SPME coating for the extraction of the 3-MCPD esters, 1,3-DCP esters and GE hydrolysis products, and then derivatized with 1-(Heptafluorobutyryl) imidazole (HFBI) and detected by gas chromatography tandem mass spectrometry (GC–MS/MS). The immobilized enzyme conditions, SPME parameters, and derivatization conditions were optimized. In addition, the adsorption isotherms, kinetics and adsorption mechanisms were investigated in detail. Finally, the health risks associated with exposure to chloropropanol esters and GE through consumption of instant noodles were evaluated for children and adults.

2. Materials and methods

2.1. Chemicals and materials

3-Chloro-1,2-propanediol (3-MCPD) was bought from Dr. Ehrenstorfer (Augsburg, Germany). 3-MCPD-1,2-dipalmitoyl ester (PP-3-MCPD) were purchased from Alta Scientific (Tianjin, China). 3-Monobromopropanediol (3-MBPD) was supplied by CATO Research Chemicals Inc (Guangzhou, China). Lipase (from Candida rugosa, > 700 U/mg), glutaraldehyde (GA, 50 % w/v) and chitosan were obtained from Sigma-Aldrich (Shanghai, China). The medium-speed qualitative cellulose filter papers (CFP) were purchased from Hangzhou Fuyang North Wood Pulp and Paper Co. LTD (Hangzhou, China). Sodium bromide was purchased from Biorbyt Chemical Trading Co., LTD (Tianjin, China). 1,3-Dichloro-2-propanol (1,3-DCP), methyl *tert*-butyl ether (MTBE), ethanol and *n*-hexane were bought from Aladdin (Shanghai, China). *N*-Heptafluorobutyrylimidazole (HFBI) (≥97 %) was purchased from J&K SCIENTIFIC LTD (Beijing, China).

β-CD was purchased from Macklin (98 %, Shanghai, China), and sodium hydroxide (NaOH) were supplied by Sinopharm Chemistry Reagent Co. Ltd (Shanghai, China). Supelco (Bellefonte, PA, USA) supplied the SPME manual holder with 50/30 μm-divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fiber.

2.2. Sample collection

A total of 22 samples of instant noodles were collected from domestic brands available in retail stores in Tianjin, China. The samples were handled according to the manufacturer's specified storage instructions and they were analyzed before their respective expiration dates following the collection. This ensures that the samples were representative of the products available in the market and were within the recommended shelf life at the time of analysis.

2.3. Immobilization of lipase on CFP

Firstly, 100 mg of CS were mixed in 10 mL of 1 % (v/v) acetic acid solution, and the mixture was then thoroughly dissolved by ultrasound. A piece of CFP was immersed in the CS solution for 12 h, and then repeatedly washed with ultrapure water. The remaining liquid on CFP/CS was dried off by a fresh CFP. Then the obtained CFP/CS was punched with a paper punch into small pieces with 6 mm diameter. Secondly, 1 mL of 10 % GA solution was used to immerse the CFP/CS in a 1.5 mL Eppendorf tubes. The CFP/CS with GA-modified (CFP/CS/GA) was produced and repeatedly washed with ultrapure water after being shaken for 3 h at 40 °C. Thirdly, in order to immobilize lipase on CFP, the remaining liquid on CFP/CS/GA was dried off by a fresh CFP, which was then dipped into 1 mL of lipase solution and shaken at 35 °C for 4 h. Finally, the resulting CFP was then dried at 35 °C after being washed with phosphate buffer several times. The circular CFP/CS/GA/lipase was kept in a glass vial at 4 °C for further research.

2.4. Preparation of CS-β-CD and fabrication of the CS-β-CD coated fiber

The preparation of CS-β-CD followed a previous reported procedure (Chai and Ji, 2012). Briefly, 0.5 g of chitosan was dissolved in 25 mL of 0.1 M HCl solution. This chitosan solution was then added to a 25 mL of β-CD aqueous solution containing 3.0 g of β-CD. The mixture was stirred for 1 h at 25 °C. Then, 3 mL of 25 % glutaraldehyde solution was gradually added over the course of 30 min, and the reaction was then allowed to continue at 70 °C with stirring for 6 h. Subsequently, 8.75 mL of 1 M NaOH aqueous solution was added to the reaction solution to precipitate the product. The precipitate was washed with water and acetone, and dried overnight. Fabrication of the CS-β-CD coated fiber details are shown in the Supplementary Material.

2.5. Adsorption experiments

The adsorption capacity of the CS-β-CD was investigated using the adsorption isotherm and adsorption kinetic. 1,3-DCP was chosen as the model compound. Isotherm and kinetic studies were performed with the bulk CS-β-CD in ultrapure water.

2.5.1. Adsorption isotherm

The 5 mg of CS-β-CD were immersed into 5 mL of 1,3-DCP solution at different concentrations (0.1–700 mg/L) and then shaken for 4 h. The supernatant was centrifugated at 1254 g for 3 min and extracted with ethyl acetate. The ethyl acetate layer was derivatized with HFBI for analysis by GC–MS/MS. The equilibrium adsorption capacity (Q_e, mg/g) of 1,3-DCP on CS-β-CD was calculated by the Eq. (1):

$$Q_e = (C_i - C_e)\ v/m \tag{1}$$

C_i and C_e represent the concentrations at initial and equilibrium, respectively. v (mL) is the volume of solution. m (mg) is the mass of the CS-β-CD.

To further process the data, Langmuir Eq. (2) and Freundlich Eq. (3) were applied. The expressions of the two models are as follows:

$$C_e/Q_e = 1/(K_L Q_{max}) + C_e/Q_{max} \tag{2}$$

$$\ln Q_e = \ln C_e/n + \ln K_F \tag{3}$$

Q_{max} (mg/g) is Langmuir theoretical maximum adsorption capacity and K_L (L/mg) is the Langmuir adsorption equilibrium constant. K_F and n are the Freundlich constants.

2.5.2. Adsorption kinetics

The kinetic adsorptions of 1,3-DCP with the concentrations of 200 mg/L were carried out by immersing the 5 mg of CS-β-CD in solution and then shaken for different time (5–240 min). The supernatant was centrifugated at 1254 g for 3 min and extracted with ethyl acetate. The ethyl acetate layer was derivatized with HFBI for analysis by GC–MS/MS. The quantity (Q_t, mg/g) of 1,3-DCP bound to CS-β-CD at time t was calculated according to the Eq. (4):

$$Q_t = (C_i - C_t)\ v/m \tag{4}$$

C_i and C_t represent the concentrations at initial (200 mg/L) and time t, respectively. v is the volume of solution.

The pseudo first-order (5), pseudo second-order (6), and intra-particle diffusion (7) models were used to further analyze the data:

$$\ln (Q_e - Q_t) = -k_1 t + \ln Q_{e1} \tag{5}$$

$$t/Q_t = t/Q_{e2} + 1/k_2 Q_{e2}^2 \tag{6}$$

$$q_t = k_i\ t^{0.5} + c \tag{7}$$

Q_{e1} and Q_{e2} represent adsorption capacity at equilibrium. k_1 (min^{-1}), k_2 (g mg^{-1} min^{-1}), and k_i (mg/g·$min^{1/2}$) are the pseudo first-order, pseudo second-order, and intra-particle diffusion rate constants, respectively.

2.6. Instrumentations

The Fourier transform infrared (FT-IR) analysis was performed on a Thermo Scientific Nicolet IS50 spectrometer (Thermo Fisher Scientific, USA). Morphologies of CS-β-CD and CS-β-CD coated fiber were determined by a field-emission scanning electron microscope (FE-SEM) (JSM-IT300LV, JEOL, Japan). X-ray diffraction (XRD, D/max-2500 diffractometer, Rigaku, Japan) patterns were recorded in the 2θ region of 5° to 80°. The Thermo SCIENTIFIC ESCALAB 250Xi was used for the X-ray photoelectron spectroscopy (XPS) analysis.

An Agilent 7890B GC coupled with Agilent 7000C mass spectrometer system (Palo Alto, USA) was used for GC–MS/MS analysis. The Supplementary Materials and Table S1 include information on instrument parameters and detection details.

2.7. Sample pretreatment

2.7.1. Extraction of fats from samples

The extraction of fats from samples followed a previous reported method (Miyazaki et al., 2022). Briefly, 0.4 g of instant noodles were weighed after grinding and shifted over to a screw-capped 15 mL centrifuge tube, and then 1 mL of ethanol and 3 mL of *n*-hexane/MTBE (1:2, v/v) were added. After vortexed a few seconds, the tube was held at 60 °C in a water bath for 5 min. Without allowing the tube to cool, it was removed from the water bath, and shaken on a shaker for 10 min at room temperature. The mixture was vortexed a few seconds and centrifuged at 1254 g for 5 min after 4 mL of 30 % (w/v) sodium bromide solution was added. The residual aqueous layer was then given 3 mL of *n*-hexane/MTBE (1:2, v/v) after the organic layer was moved to a fresh tube. The tube was centrifuged at 1254 g for 5 min after being vortexed. The organic layer was merged in both processes and was evaporated through a rotary evaporator.

2.7.2. Immobilized enzyme and SPME

A volume of 0.5 mL MTBE was used to dissolve the residue, and 3 mL of sodium bromide solution (pH 5.0) containing the CFP/CS/GA/lipase was added (Miyazaki and Koyama, 2017). Chloropropanol esters and GE were hydrolyzed by shaking the tube at room temperature (about 25 °C) for 30 min before taking out the filter paper. Then the tube was heated in a water bath at 50 °C for 15 min to produce chloropropanols and 3-MBPD completely. The organic phase was discarded after the addition of 3 mL of *n*-hexane in order to eliminate fatty acid methyl esters and other impurities, this process was repeated twice. To prepare the aqueous solutions for extraction and derivatization, they were put into 20 mL sample vials. The fiber coated by CS-β-CD was then placed in the headspace of the vials above the aqueous solutions for 30 min at 50 °C and then transferred into a 1.5 mL vial containing 30 μL of HFBI for derivatization at 70 °C for 30 min. Finally, the fiber was placed into the inlet of GC at 250 °C for 8 min for desorption. To prevent background contamination, the fiber was preconditioned at 250 °C for 30 min in the GC injection port with a nitrogen atmosphere before use. Blanks were regularly run in order to lessen or totally eliminate the carryover impact.

2.8. Method validation

The method's sensitivity, linearity, accuracy and precision were all validated. Limits of detection (LOD) and limits of quantification (LOQ) were used to assess the sensitivity of the approach. A signal-to-noise ratio (S/N) equal to 3 and 10 were used to calculate the LOD and LOQ, respectively. To assess the linearity test, standards spiked at levels over the LOQ-100 μg/L range were employed. The samples were spiked at 0.1, 1 and 10 μg/L for each analyte in order to evaluate the accuracy and precision. Three successive injections spiked samples were used to calculate the precision.

2.9. Exposure assessment

In order to obtain the estimated exposure of chloropropanol esters and GE, the estimated daily intake (EDI) (μg/kg bw per day) was assessed according to the following Eq. (8):

$$EDI = \frac{C \times M}{bw} \tag{8}$$

where C is concentration of chloropropanol esters or GE in this study (μg/kg); M is consumption of consumers from instant noodles (kg/day). The national survey of the 2012 China Total Dietary Study provided the consumption data (Cui et al., 2021); bw is body weight of consumers (kg). The exposures were estimated for each age class of population, including 3–6 years, 6–9 years, 9–12 years, 12–15 years, 15–18 years, 18–44 years, 45–59 years, 60–79 years, and > 80 years (Wallace et al., 2016). Assuming that all of the esters are converted to their free forms, the intakes of chloropropanol esters and GE were estimated.

In order to estimate the carcinogenic risks of 1,3-DCP and glycidol, the following equations (9) and (10) were used to calculate the margin

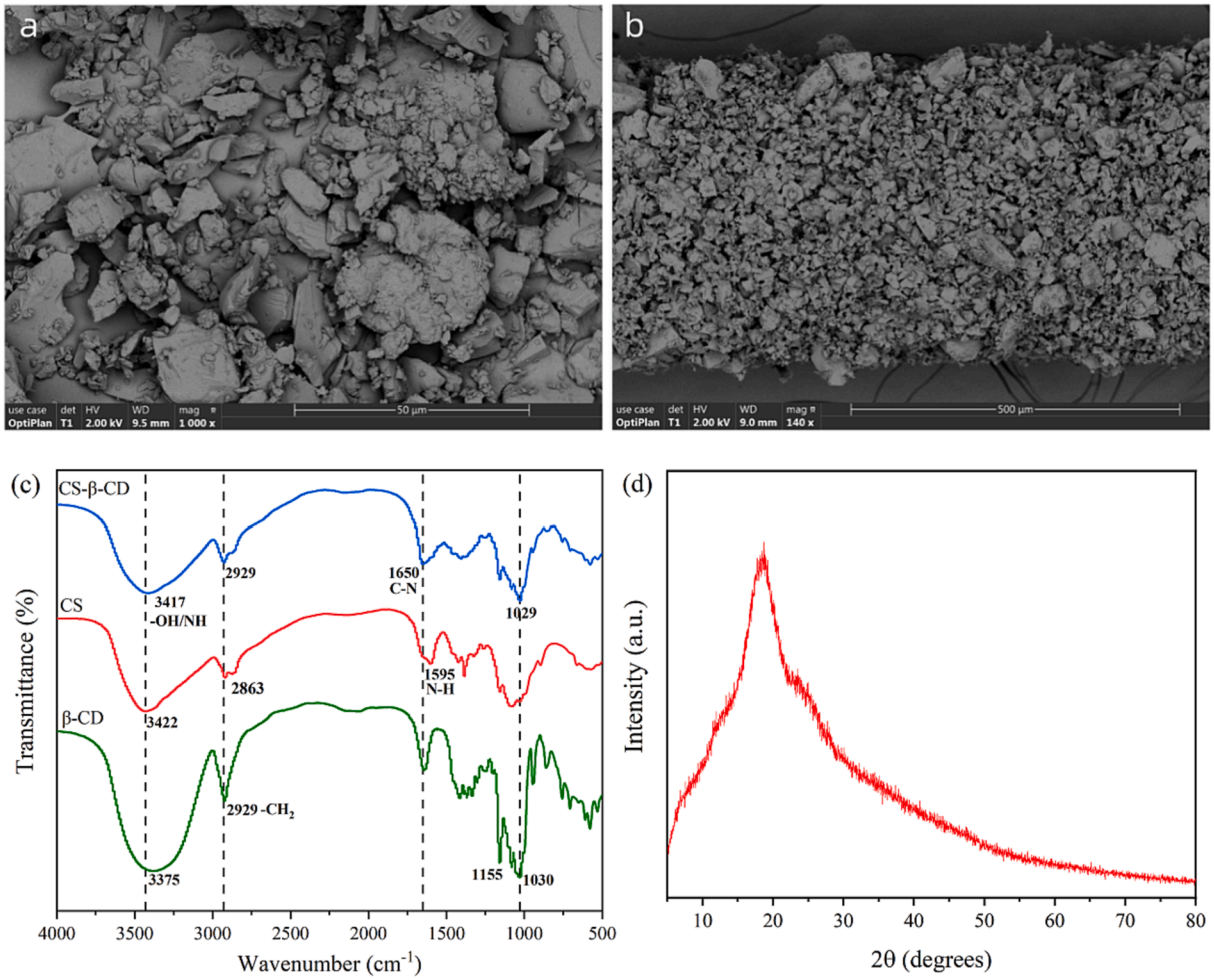

Fig. 1. (a) SEM images of CS-β-CD and (b) CS-β-CD coated fiber, (c) FT-IR spectra of CS-β-CD and (d) XRD pattern.

of exposure (MOE), respectively:

$$\mathrm{MOE} = \frac{\mathrm{BMDL}_{10}}{\mathrm{EDI}} \tag{9}$$

$$\mathrm{MOE} = \frac{\mathrm{T}_{25}}{\mathrm{EDI}} \tag{10}$$

where the value of BMDL_{10} was 3.3 mg/kg bw per day recommended by Expert Committee on Food Additives and Contaminants (JECFA) (JECFA, 2007) and the T_{25} for peritoneal mesothelioma in male rats was 10.2 mg/kg bw per day provided by EFSA (Wallace et al., 2016).

3. Results and discussion

3.1. Characterization

In the morphology images of CS-β-CD depicted in Fig. 1a, it is evident that CS and β-CD are clearly aggregated together in CS-β-CD. As shown in Fig. 1b, CS-β-CD had been successfully adhered to the surface of fibers, which was conducive to its further application in SPME. The infrared peak of β-CD, CS and CS-β-CD are illustrated in Fig. 1c. A peak at 3417 cm^{-1} which is the stretching vibration of –OH/N—H in CS-β-CD, and the peak at 1650 cm^{-1} confirms the stretching vibration of C—N in the spectra of CS-β-CD, which is attributed to amino groups in the grafted CS (Chai and Ji, 2012). Besides, the stretching vibration peak of $-\mathrm{CH}_2-$ belonging to β-CD molecule appears at 2929 cm^{-1}. These results showed that CS-β-CD were successfully prepared. X-ray power diffraction (XRD) was used to acquire additional proof that CS-β-CD was formed, as shown in Fig. 1d. The amorphous CS-β-CD characters were visible in the XRD pattern.

3.2. Comparison of free and immobilized lipase activities

For evaluating the hydrolysis ability of free and immobilized lipase, the ester standard solution was pretreated with free lipase and immobilized lipase under the same conditions, respectively. The signal response of 3-MCPD derivative was used to compare the hydrolysis ability of free and immobilized enzymes. As it can be seen from Fig. S1, the catalytic activities of immobilized enzyme and free enzyme are basically consistent without significant difference.

3.3. Optimization of immobilization conditions

The impact of immobilized lipase concentration in the range of 0.05–0.6 $\mathrm{mg{\cdot}mL^{-1}}$ on signal response of 3-MCPD derivative was investigated. As shown in Fig. S2a, the signal response of the target analyte initially increased and then decreased with increasing enzyme concentration. The highest signal response was recorded at a concentration of 0.4 $\mathrm{mg{\cdot}mL^{-1}}$, indicating that the enzyme loading had reached saturation. To immobilize the lipase, GA, acting as a cross-linking agent, was utilized to bind the lipase to the amino-functionalized CFP by reacting with the amino group grafted onto CFP and the amino group of an enzyme molecule via the Schiff base reaction. The impact of crosslinking time on enzyme activity was assessed by varying the GA crosslinking time from 2 to 5 h. As depicted in Fig. S2b, the signal response increased as the crosslinking time increased up to 3 h. However, when the

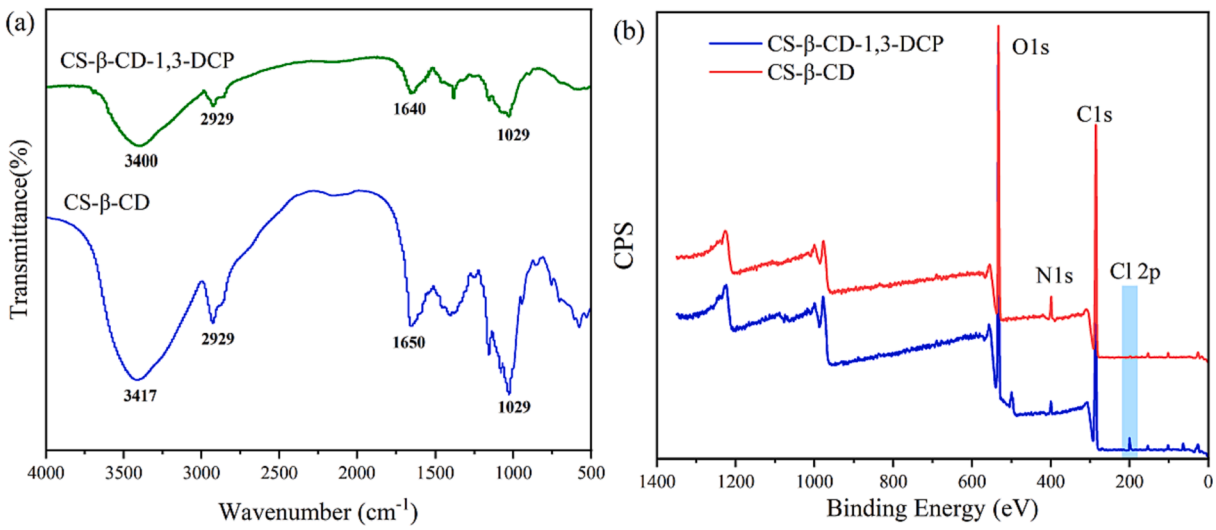

Fig. 2. (a) FTIR, (b) XPS spectra of CS-β-CD before and after adsorption of 1,3-DCP.

crosslinking duration exceeded 3 h, the signal response decreased due to the presence of less reactive GA. Therefore, a crosslinking time of 3 h was chosen. A sufficient immobilization time is required to prepare immobilized lipase. As depicted in Fig. S2c, the activity of immobilized enzyme increased and then decreased within the investigation range of 0.5–8 h. The peak activity of the immobilized lipase was observed at an immobilization time of 4 h. This could due to the fact that as immobilization time increased, the amount of immobilized enzyme also increased. However, excessive enzyme crowding led to agglomeration and coverage of the active sites, resulting in decreased enzyme activity. Therefore, an immobilization time of 4 h was chosen to achieve higher enzyme activity.

3.4. Comparison of CS-β-CD coated fiber with commercial fiber

The CS-β-CD coated fiber was compared with commercial 50/30 μm-divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fiber used for the extraction of chloropropanols and 3-MBPD. As shown in Fig. S3, CS-β-CD coating had an extraction efficiency that was 5–8 times higher than commercial fiber.

3.5. Optimization of SPME and derivatization conditions

Effect of extraction time was performed from 10 to 50 min. As depicted in Fig. S4a, the peak areas of analytes grew as the extraction period increased from 10 to 30 min, and then declined after 30 min, suggesting that a longer extraction time might lead to volatilization or redissolution of the analytes. Based on these results, 30 min was maintained for further experiments. The mass transfer rate of target analytes from solution into fiber is controlled by the extraction temperature. The results of extraction temperature from 30 to 70 °C were shown in Fig. S4b. The responses peaked at 50 °C, and declined as the temperature increased further, most likely due to excessive temperature causing the decrease of the partition coefficients of analytes between the fiber coating and the sample solution. Therefore, 50 °C was chosen as the extraction temperature. The effects of 10–50 μL of HFBI on derivatization were investigated and showed in Fig. S4c. The responses peaked with the volume of 30 μL. Therefore, the volume of HFBI was set as 30 μL for further study.

3.6. Adsorption isotherms and kinetics of CS-β-CD

The adsorption isotherms of CS-β-CD for 1,3-DCP was studied at room temperature. As illustrated in Fig. S5a, the adsorption capacity for 1,3-DCP was constantly increased as the initial concentration and then reached adsorption equilibrium. The maximum adsorption capacity was 362.2 mg/g (Table S2), which showed a higher adsorption capacity for 1,3-DCP compared to the study of using this adsorbent for the adsorption of other compounds (Chai and Ji, 2012). The adsorption isotherms parameters were presented in Table S2. The findings revealed that the adsorption process of 1,3-DCP on CS-β-CD was well fitted with Langmuir adsorption model than Freundlich adsorption model, suggesting that the monolayer adsorption played a predominant role.

To evaluate the adsorption rates and behavior of 1,3-DCP by CS-β-CD, the adsorption was further determined under 1,3-DCP of 200 mg/L. The adsorption equilibrium achieved with 50 min (Fig. S5b), revealing the fast adsorption kinetics of CS-β-CD for 1,3-DCP. The related parameters about pseudo first-order pseudo, second-order pseudo, and intra-particle diffusion models were presented in Table S3. The findings indicated that 1,3-DCP adsorption on CS-β-CD was well-fitted the pseudo second-order kinetic model, with a higher value of R^2 than the pseudo first-order kinetic model, which indicated that chemisorption was mainly responsible for the adsorption process. In addition, it can be inferred that the adsorption process was controlled by both outer diffusion and intra-particle diffusion.

3.7. Adsorption mechanisms

To identify the possible adsorption mechanisms of CS-β-CD for compounds, FTIR and XPS analysis of CS-β-CD before and after the adsorption of 1,3-DCP was performed. FTIR spectra was depicted in Fig. 2 (a), the band at 1650 cm^{-1} attributed to C—N stretching vibration moved to 1640 cm^{-1} after 1,3-DCP adsorption. This occurs as a result of the 1,3-DCP molecules being adsorb by the amine group in CS-β-CD, which causes a reduction in the density electron cloud (Wei et al., 2023). The peak at 3417 cm^{-1} corresponding to N—H/O—H shifted to 3400 cm^{-1} demonstrating that hydrogen bond was involved (Du et al., 2019, Gong et al., 2022).

As shown in Fig. 2 (b), the peak of Cl2p at 199.9 eV indicated the successful adsorption of 1,3-DCP on CS-β-CD (Wei et al., 2023). C1s, N1s, and O1s spectra were examined in order to comprehend the impact of functional groups of CS-β-CD on adsorption of 1,3-DCP. Fig. 3 (a) and (b) indicated that after loading 1,3-DCP, the C1s splitting C—N and C—O peaks of CS-β-CD shifted from 287.5 eV and 286.2 eV to 288 eV and 286.6 eV, demonstrating the presence of electron transport between CS-β-CD and adsorbate, which influencing the density of the electron cloud and the binding energy (Gong et al., 2022, Wei et al., 2023). As shown in Fig. 3 (c) and (d), after loading 1,3-DCP, the C—O and –OH peaks of CS-

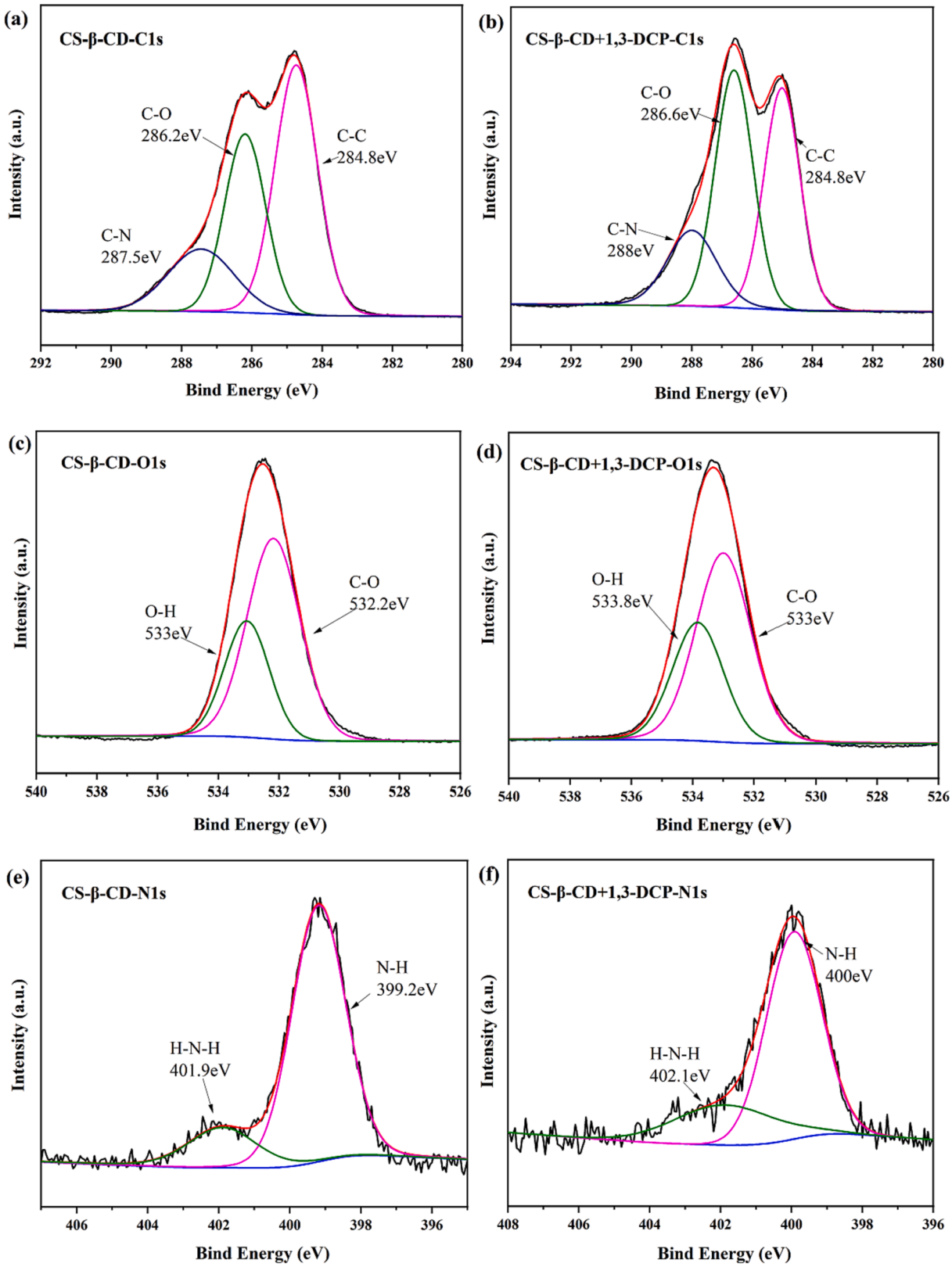

Fig. 3. C1s, O1s and N1s XPS spectra of CS-β-CD (a, c, and e) before and (b, d, and f) after the adsorption of 1,3-DCP.

β-CD shifted from 532.2 eV and 533 eV to high binding energies of 533 eV and 533.8 eV. This may be the result of hydrogen bonds forming between the O moieties of CS-β-CD, the electron donor and the hydroxyl group in 1,3-DCP, the electron acceptor (Cui et al., 2019, Han et al., 2021, Wei et al., 2023). Similarly, after loading 1,3-DCP, the splitting N1s peaks of N—H and H—N—H shifted from 399.2 eV and 401.9 eV to high binding energies of 400 eV and 402.1 eV as shown in Fig. 3 (e) and (f). The results showed that N moieties of CS-β-CD exhibited electron transfer characteristics similar to those of O moieties because the O/N moieties in CS-β-CD act as electron donors to create a hydrogen bond

Table 1
Linear ranges, LOD and LOQ of 1,3-DCP, 3-MCPD and 3-MBPD and repeatability, reproducibility of CS-β-CD coated fibers.

Analytes	Linear range (μg/L)	R^2	LOD (μg/L)	LOQ (μg/L)	RSD (%)	
					One fiber (n = 3)	Fiber to fiber (n = 3)
1,3-DCP	0.001–100	0.9987	0.0003	0.001	9.5	6.2
3-MCPD	0.001–100	0.9994	0.0003	0.001	4.9	9.8
3-MBPD	0.001–100	0.9982	0.0003	0.001	7.3	6.8

Table 2
Accuracy and precision of the proposed method.

Analytes	Spiked amount (μg/L)	Recoveries (%)	RSD (%)
1,3-DCP	0.1	103.7 ± 3.8	3.7
	1	90.9 ± 9.0	9.9
	10	97.8 ± 7.0	7.1
3-MCPD	0.1	96.7 ± 7.3	9.5
	1	105.3 ± 11.1	4.6
	10	89.0 ± 8.1	9.1
3-MBPD	0.1	80.2 ± 2.8	5.3
	1	94.0 ± 7.4	7.9
	10	96.3 ± 9.4	9.8

Table 3
Concentrations (μg/kg, range, mean, median and P95) and detection frequency (DF, %) of target analytes in instant noodles samples.

Analytes	DF	Range	Mean	Median	P95
1,3-DCP esters	100	0.14–27.4	7.5	4.5	22.4
3-MCPD esters	72.7	< LOD-377.8	22.2	0.3	26.9
GE	100	0.2–947.2	95.5	1.1	789.0

with 1,3-DCP.

3.8. Method validation

The LODs were 0.3 ng/L for all the analytes in samples (Table 1). The analytes had good linearity over the concentration range of 0.001–100 μg/L with coefficients of determination (R^2) ranged from 0.9982 to 0.9994. The repeatability obtained from one fiber for three replicated experiments with the relative standard deviations (RSDs) ranged from 4.9 % to 9.5 %. The RSDs of reproducibility in fiber-to-fiber (n = 3) ranged from 6.2 % to 9.8 %. In addition, the analytes extraction efficiency of the CS-β-CD coated fiber was not significantly reduced after using 60 times with RSDs less than 12.5 %, demonstrating that the developed method for SPME fiber coated by CS-β-CD was repeatable and reproducible. A recovery study was used to assess the accuracy of the optimized approach, as presented in Table 2. The recoveries were between 80.2 % and 105.3 %, and the RSDs values for precision obtained were < 9.9 %. The developed method exhibited super sensitivity, great recoveries and excellent precision. To our knowledge, the LODs of these analytes in the current method behave lower than previous reports (Table S4). These results demonstrated the suitability of the method for the determination of chloropropanol esters and GE using CS-β-CD coated fiber.

3.9. Real sample analysis and risk assessment

3.9.1. Occurrence of chloropropanol esters and GE in instant noodles samples

The three compounds showed detection frequencies at 72.7 % for 3-MCPD esters, 100 % for 1,3-DCP esters and 100 % for GE in 22 instant noodles samples. The levels from not detected (ND) to 377.8 μg/kg (mean 22.2 μg/kg), 0.14 to 27.4 μg/kg (mean 7.5 μg/kg) and 0.2 to 947.2 μg/kg (mean 95.5 μg/kg) for 3-MCPD esters, 1,3-DCP esters and GE, respectively (Table 3). Recent investigations have consistently identified the presence of these compounds in instant noodles. The findings regarding 3-MCPD esters in this research were in agreement with previous study (Jiang et al., 2018). However, the concentrations of 3-MPCD esters reported in previous study in China (Zhao et al., 2020), ranging from < LOD to 1.95 mg/kg were higher than the concentrations in this study. Similarly, a study conducted in Japan reported lower concentrations of 3-MCPD esters, ranging from 13.8 to 86.4 μg/kg (Shimamura et al., 2021). The detection frequencies and concentrations of 1,3-DCP esters in this study are higher compared to another study (Jiang et al., 2018). On the other hand, the detection frequencies and the concentrations of GE in this study were consistent with the results reported by Shimamura et al (Shimamura et al., 2021). The variations observed among these investigations could be attributed to differences in the selection of raw materials and processing technologies employed in the production of instant noodles. These factors can influence the levels of these compounds in the final products.

3.9.2. Exposure and risk assessment for chloropropanol esters and glycidyl esters

The estimated exposures for the general population aged 3 years and above were calculated using the mean and 95th percentile (P95) exposures for each age group (Jiang et al., 2018). The estimated daily intake of 3-MCPD esters from instant noodles ranged from 0.001 to 0.033 μg/kg bw/day, as shown in Table S5. The results were below the TDI value of 2 μg/kg bw/day established by EFSA, and consistent with a previous study (Zhao et al., 2020). However, a prior investigation in China found that the consumption of instant noodles contaminated with 3-MCPD esters may pose a health risk (Jiang et al., 2018). Using equation (8), the MOE for 1,3-DCP ranged from 119,334 to 513,994 for the high exposure levels (P95) in the population. An MOE value < 10,000 is considered of high concern, indicating a need for risk management actions and exposure reduction (Korte et al., 2022). Therefore, the resulting MOE values indicated that the estimated exposures to 1,3-DCP for high exposure populations of different age were of low risk for human health. In addition, regarding GE, the MOE values for the high exposure population (P95) ranged from 10,470 to 45,098, with values less than 25,000 for the 3 to 12 years old population. Human exposure to GE is considered as a health risk when the MOE is less than 25,000 (Arisseto, Silva et al., 2017, Wallace et al., 2016). These results indicated that GE poses a potential risk to high exposure population of 3 to 12 years old through the consumption of instant noodles.

4. Conclusion

In this study, a novel method was proposed and validated for the extraction and quantification of chloropropanol ester and GE using immobilized enzymes combined with GC–MS/MS and CS-β-CD coated SPME. The catalytic activities of immobilized enzyme and free enzyme were found to be essentially consistent under the same conditions. The maximum adsorption capacity of CS-β-CD for 1,3-DCP was 362.2 mg/g, and the adsorption isotherm followed the Langmuir model. The kinetic model showed that the adsorption process followed a pseudo second-order kinetic model. The superior performance of the home-made CS-β-CD fiber, compared to a commercial fiber, in terms of adsorption for the target analytes can be attributed to the hydrogen bonding interaction between target analytes and CS-β-CD. Utilizing this approach, the chemical profiles of 3-MCPD esters, 1,3-DCP esters and GE in 22 instant noodles were obtained and consumers exposure levels to these chemicals were assessed. The results proved that GE in instant noodles samples may pose a potential health risk for the high exposure population of 3 to 12 years old.

CRediT authorship contribution statement

Linlin Bian: Writing – original draft, Validation, Methodology, Investigation. **Xue Ge:** Formal analysis, Investigation, Conceptualization. **Senwei Feng:** Formal analysis. **Guangxuan Chen:** Resources. **Kefeng Li:** Writing – review & editing. **Xu Wang:** Writing – review & editing, Supervision.

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

The data that has been used is confidential.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138419.

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