



Simultaneous determination of capsaicin and dihydrocapsaicin for vegetable oil adulteration by immunoaffinity chromatography cleanup coupled with LC–MS/MS

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ABSTRACT

Capsaicin and dihydrocapsaicin were selected as adulteration markers to authenticate vegetable oils. In this study, a method of immunoaffinity chromatography (IAC) combined with liquid chromatography–tandem mass spectrometry was established for the determination of capsaicin and dihydrocapsaicin in vegetable oils. In this method, immunosorbents were obtained by covalently coupling highly specific capsaicinoid polyclonal antibodies with CNBr-activated Sepharose 4B, and then packed into a polyethylene column. In this paper, the major parameters affecting IAC extraction efficiency, including loading, washing and eluting conditions, were also investigated. The IAC column displayed high selectivity for capsaicin and dihydrocapsaicin with the maximum capacity of 240 ng. The limit of detection (LOD) and limit of quantification (LOQ) for capsaicin were calculated as 0.02 and 0.08 $\mu\text{g kg}^{-1}$, and for dihydrocapsaicin were 0.03 and 0.10 $\mu\text{g kg}^{-1}$. The recoveries of capsaicin and dihydrocapsaicin in oil samples were in the range of 87.3–95.2% with the relative standard deviation (RSD) of less than 6.1%. The results indicated that capsaicinoid compounds could not be found in edible vegetable oils. Therefore, the proposed method is simple, reliable and adequate for routine monitoring of capsaicinoid compounds in vegetable oils and has an excellent potential for detection of adulteration with inedible waste oil.

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1. Introduction

Waste oil, consisting of cooking oil collected from food wastes, waste cooking oils recycled from drains or grease traps, residual oils extracted from animal fats, deep frying oils, and other inedible oils, have caused serious food safety scandals in developing countries when they are illegally sold and used as edible oils [1]. These

inedible oils are generally contaminated by hazardous compounds such as condiments, heavy metal ions, bio-toxins, polycyclic aromatic hydrocarbons (PAH), 3-monochloropropane-1,2-diol (3-MCPD) and its esters, phthalates, dioxins and polychlorinated biphenyls (PCBs) [2–9] or degradation products of oxidation process. In the illicit refining industry, the complete removal of contaminants or additives is difficult. Parts of them still remain in recycled used oils though they are treated by degumming, neutralizing, washing, drying, bleaching, filtering and deodorizing processes [10]. As a result, those oils can adversely impact human health due to oxidation products and other hazardous materials from the draining and reprocessing processes [11].

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Generally, sensory evaluation was used to detect adulteration of inedible waste oils. However, sensory analysis depends on the experience of analysts, and the subjective judgment may cause false negative results. In this case, many analytical methods were developed to detect and quantify the adulterants. As rapid analytical methods, NMR, NIR and Raman could characterize oil samples in the whole spectrum [12–14]. Recently, the metabolite profiles of fatty acids, triacylglycerols and volatile components were used to analyze inedible oils and detect potential adulteration [15–17]. During data processing, the optimized model needs many standards and possible adulterated samples and might not be effective for samples out of the training set. Compared with the preceding methods, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is a promising approach to assess adulteration and quality of vegetable oils since it satisfies routine analysis requirements for rapid determination and identification of contaminants or additives, which can be used as marker compounds [18,19].

With the sensory attributes of pungency, aroma and color, hot peppers are popular food additives and have been consumed in large quantities throughout China and the world. The most abundant capsaicinoids are capsaicin and dihydrocapsaicin (the molecular structures of these chemicals are present in Fig. 1), which constitute nearly 90% of the total pungency of pepper fruits [20]. Previous researches indicated that capsaicin and dihydrocapsaicin were lipophilic and stable during the refining process of inedible waste oils with high boiling points. Therefore these compounds could be selected as specific biomarkers to detect and authenticate edible vegetable oils [21].

Considering the complexity of lipid matrix and low concentration of capsaicinoids in inedible oils, the analytical methods usually use thin layer chromatography (TLC) or solid phase extraction (SPE) to extract and enrich the analytes [22–24]. The procedures generally consist of liquid–liquid extraction, saponification and neutralization, which provide non-specific retention and are time-consuming, laborious, low sensitivity and consuming large volumes of organic solvents. Immunoaffinity chromatography (IAC) is a separation method which takes advantage of the specific interaction between antibodies and antigens. It is a simple and selective technique to purify analytes without tedious pretreatment and can save organic solvents during pretreatment [25,26]. Many reports have been found for the determination of toxins, pesticide residues, veterinary drugs and illegal additives using IAC cleanup [27–29]. To the best of our knowledge, IAC cleanup has not been used to determine capsaicin and dihydrocapsaicin in combination with LC–MS/MS.

The aim of this study was to establish IAC using polyclonal antibodies (pAb) covalently immobilized on CNBr-activated Sepharose-4B for simultaneous determination of capsaicin and dihydrocapsaicin in vegetable oils, which were further quantified by LC–MS/MS. The extraction conditions of the IAC column for capsaicinoid compounds were optimized and the IAC column was characterized in terms of binding capacity, extraction recovery and reproducibility. Under the optimized conditions, the analytes in lipid matrix were successfully quantified and used to detect oils adulterated with inedible waste oil.

2. Experimental

2.1. Reagents and materials

CNBr-activated Sepharose 4B was purchased from GE Healthcare (Uppsala, Sweden). Capsaicin and dihydrocapsaicin standards were obtained from Sigma–Aldrich (St. Louis, MO, USA). Capsaicin- d_3 and dihydrocapsaicin- d_3 standards were obtained from Toronto Research Chemicals (North York, Canada). Formic acid, methanol (MeOH) and acetonitrile of HPLC grade were from Sigma–Aldrich

(Shanghai, China). Sodium acetate (NaAc), sodium chloride (NaCl), sodium dihydrogen phosphate, disodium hydrogen phosphate, glacial acetic acid, *tris*(hydroxymethyl) aminomethane and sodium azide (NaN_3) were from Sinopharm Chemical Reagent (Shanghai, China). Hydrochloric acid (HCl), caprylic acid, hexane, ammonium sulfate, sodium bicarbonate and potassium dihydrogen phosphate were obtained from Kermel Chemicals (Tianjin, China). All other chemicals and organic solvents were of analytical reagent grade. Ultra-pure water (18 m Ω) was obtained from a Milli-Q water purification system (Milford, MA, USA). Nonspecific rabbit immunoglobulins were produced in our laboratory.

The stock solutions of capsaicin, dihydrocapsaicin, capsaicin- d_3 and dihydrocapsaicin- d_3 were separately prepared by accurately weighing 1 mg of the reference materials and dissolving them in 20 mL methanol. A series of standard solutions were prepared by diluting the stock solutions with methanol. All standard solutions were sealed with parafilm, covered with aluminum foil, and stored in the dark at 4 °C until use.

2.2. Instrumentation

A Thermo lyophilizer (Savant, England) was used to freeze dry antibodies. A shaker (Chinese Academy of Sciences Scientific Instrument, Wuhan) was used to prepare the immunosorbents. Centrifugation of the vegetable samples was performed on a centrifuge (Hitachi CF 16RX), and a homogenizer (IKA Laboratory Equipment, Germany) was used for sample preparation. Sample analysis was performed in selected reaction monitoring (SRM) mode on the Accela HPLC system coupled to TSQ Quantum Ultra EMR (Thermo Fisher Scientific, USA). Separation was performed at 35 °C on a Thermo Scientific C₁₈ column (Hypersil Gold, 100 mm \times 2.1 mm, 3.0 μm). Mobile phase A consisted of 0.1% aqueous formic acid and mobile phase B acetonitrile. A linear gradient elution program was applied as follows: initial B was linearly increased from 33.5% to 83.5% in 10 min, held for 1 min, and returned to 33.5% B in 3 min, which was held for 4 additional minutes for re-equilibration, giving a total run time of 18 min. The mobile phase flow rate was 250 $\mu\text{L min}^{-1}$, and an aliquot of 10 μL sample was injected into the HPLC system. The mass spectra were obtained by Thermo TSQ Quantum Ultra EMR triple quadrupole (Thermo Scientific, USA) coupled with electrospray interface (ESI). The MS/MS conditions were set as follows: spray voltage, 3.5 kV in positive mode; capillary temperature, 350 °C; sheath gas pressure (N_2), 30 units; auxiliary gas pressure (N_2), 5 units; collision gas (Ar), 1.5 mTorr; scan time, 0.1 s. As shown in Table 1, two parent-to-product transitions for each analyte were simultaneously monitored in SRM mode.

All the experiments were conducted in triplicate, and the average values \pm standard deviations (SD) were reported. LC–MS/MS data were processed using Xcalibur 2.0.7 SP1. Statistical analyses were performed with the @Risk 5.5.1 software package (Palisade, Australia).

2.3. Production and purification of polyclonal antibodies

Polyclonal antibodies (pAb) against capsaicinoids were produced according to the procedure described in our previous study [30]. As illustrated in Fig. 2, the hydroxyl group of capsaicin molecules was changed to the ester group under bromination reaction, and then the esters were alkaline hydrolyzed to the carboxyl group which was covalently coupled to the carrier protein (bovine serum albumin, BSA) using the modified active ester method. The capsaicin-BSA conjugate was dialyzed and then used as an immunogen to immunize New Zealand white rabbits for polyclonal antibody production. The obtained antiserum was further purified according to the modified saturated ammonium sulfate caprylic

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