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A new method research for determination of natural pigment crocin yellow in foods by solid-phase extraction ultrahigh pressure liquid chromatography

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ABSTRACT

Crocin yellow was determined in soft drinks, sausages and sauces by ultra performance liquid chromatography coupled with ultraviolet detector and analyzed within 5 min using a short analytical column ACQUITY UPLC HSS T3 2.1 × 100 mm 1.8 μm) with gradient elution. An innovative pretreatment method based on homemade macroporous resin solid-phase extraction (SPE) column was established. The SPE column packed with macroporous resins could simplify the sample preparation of multi-matrices and be reused by regeneration steps. The recoveries of crocin yellow added to soft drinks, sausages and sauces at three levels ranged from 81.3% to 106.2%, and relative standard deviations (RSDs) were within 8.8%. The limits of quantitation of soft drinks, sausages and sauces were 0.5 mg/kg, 5 mg/kg and 5 mg/kg, respectively.

1. Introduction

In recent years, the abuse of food additives, especially synthetic food colors, has drawn more and more attention in the world. Synthetic food colors, which are widely used in food industry, cause potential human health concerns; many kinds of synthetic colors are even not permitted to be used in foods on account of the related legal issues [1]. However, the use of food colors is important in food industry because they improve the appearance of foods. Hence the solution is to use natural colors instead, which are much safer and healthier. Crocin yellow is one of the important natural colors [2].

Crocin yellow, which is extracted from *Gardenia jasminoides Ellis*, is widely used as a natural food colorant in Asian countries, while Gardenia extract has been used in Chinese traditional medicine (CTM) for curing a number of ailments [3]. These crocetin derivatives, which are different from most families of carotenoids, are known for their coloring properties owing to their peculiar water-soluble behavior [4]. Numerous studies have dealt with the component structures of yellow pigment extracts isolated from gardenia fruits [4–8], their spectroscopic characterization and radical-scavenging activity [4,9], data concerning the concentration of major components for the determination of optimal time of harvest and extraction process [10,11]. However, reports on the determination of crocin yellow in food products, to our knowledge, are limited in number to date. With the development of food industry and the improvement of people's health consciousness, natural colors will be more and more widely used in food products. Therefore, it is very necessary to establish a simple, fast and accurate method as the industrial standard to detect the addition of natural colors in food products.

A large number of analytical methods for synthetic food colors have been proposed, such as thin-layer chromatography (TLC) [12,13], spectrophotometry [14,15], capillary electrophoresis (CE) [16,17], ion chromatography [18] and high-performance liquid chromatography (HPLC) [19,20]. In HPLC methods, polyamide absorption column was used as the pretreatment method of food samples [21]. The detection methods of synthetic colors are becoming more perfect and standardized. However, the determination methods for natural food colors still need to be improved. We have tested the feasibility of the use of polyamide adsorbent in the sample pretreatment method of natural color detection. Due to the differences in structures and characteristics of natural and artificial colors, polyamide has strong ability to absorb natural color. Strong alkaline ammonia solution is harmful as the common eluent of polyamide adsorbent has destructive effort to most of natural color [22], and the elution rates of some other organic eluents such as methanol and ethanol aqueous solution are low. Therefore, the

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Table 1
Physical characteristics of macroporous resins.

Grade	Surface area (m^2/g)	Ave. pore diam. (Å)	Polarity
X-5	500–600	290–300	Non-polar
AB-8	480–520	130–140	Weak-polar
D-101	480–550	12–15	Non-polar

result was far from ideal. Based on a number of reports stating that macroporous resins could be used to separate and purify natural products by adsorption–desorption process [23–25], we initiatively made solid-phase extraction (SPE) column by using macroporous resins as the stationary phase to pretreat food samples.

Macroporous resin is a new type of nonionic high-molecular-polymer adsorbent mainly using styrene and divinylbenzene as raw materials. This material has the properties of selective adsorption. The adsorption of macroporous resin is caused by Van der Waals' forces or hydrogen bonding between molecules. Simultaneously, the porous structure and various surface functional groups available cause different sized objects to have different adsorption characterstics with the resins. By eluting, different substances are separated. Therefore, the enrichment and filtration of macroporous resins are achieved based on the fact that adsorption performance varies in different substances.

In the present study, the most suitable SPE packing was selected by comparing the recoveries of crocin yellow on different macroporous resins. The studies on the relationship between regenerated times and adsorption–elution efficiency were also conducted.

This paper describes an analytical method for the determination of crocin yellow in drinks, meat products and flavorings in 5 min by ultrahigh pressure liquid chromatography (UHPLC) equipped with UV detector using a short analytical column. The detection method has advantages of short analysis time, high repeatability and accuracy. Compared with commercial SPE columns, the sample pretreatment method of SPE column using macroporous resins as the stationary phase to adsorb crocin yellow is simple with a wide range of applications.

2. Experiment

2.1. Chemicals and reagents

Crocin yellow high-purity extracts were purchased from Tokyo Chemical Industry Co., LTD. The extracts were dissolved in deionized water to give a concentration of 0.5 mg/L. Acetonitrile of HPLC grade was purchased from Fisher Scientific, Fairlawn, NJ (USA). Ethanol and acetic acid were of analytical grade from Beijing Chemical Reagents Co. (Beijing, China). Deionized water was purified by a Milli-Q Water Purification system (Millipore, MA, USA).

Macroporous resins including AB-8, D-101 and X-5 were purchased from Nankai University (Tianjin, China). Their physical properties are listed in Table 1. The resins were pretreated by 4% HCl and NaOH solutions successively to remove the monomers and other agents trapped inside the pores during the synthesis process. Treated resins were soaked in ethanol and subsequently washed by deionized water thoroughly before each use.

2.2. Apparatus and instrumental parameters

All UHPLC measurements for crocin yellow determination were done using a Waters ACQUITY^{TM} 1100 UPLC system equipped with a quaternary solvent delivery system, an autosampler and a UV detector. Separation was achieved on a Waters ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm) column. Solvent A was acetonitrile and solvent B was water, which were applied in the gradient elution as follows: 0–3 min, linear gradient from A–B (60:40, v/v) to A–B (80:20, v/v); 3–3.5 min, linear gradient to A–B (98:2, v/v), which was held for 1 min; and 4.5–5 min, linear gradient to A–B (60:40, v/v). The column temperature was set at 30 °C. The flow rate was set at 0.25 mL/min, and the injection volume was 5 μL. The detection wavelength of UV detector was 440 nm.

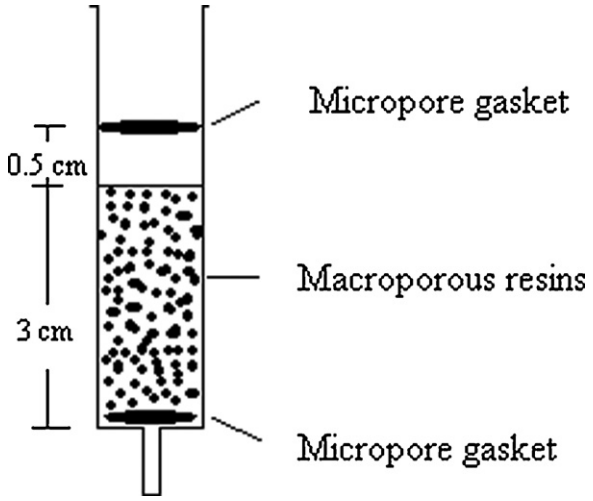

Fig. 1. Diagrammatic drawing of homemade SPE column.

Mass spectrometry was performed on Waters Micromass®-Quattro Premier XE operating in ESI$^+$ modes. The nebulization gas was set to 600 L/h at a temperature of 400 °C, the cone gas was set to 50 L/h, and the source temperature was set to 110 °C. The capillary voltage was set to 3.5 kV, and the cone voltage was set to 40 V. All of the raw data were analyzed by Masslynx V4.1.

2.3. Sample preparation

2.3.1. Preparation of macroporous resin SPE column

Treated hydrated resins (3 cm) were put into the glass column measuring 5 cm in length and 1 cm in diameter. Micropore gaskets were fixed in the column. The void of 0.5 cm was reserved because the resin swells during the elution (Fig. 1).

2.3.2. Optimization of SPE conditions

AB-8, D-101 and X-5 resins were selected to investigate the possibility of their use for the SPE column packing by comparing the recoveries of crocin yellow after adsorption–elution test. According to the Section 2.3.1, different kinds of macroporous resin SPE columns were made. Different loading quantities, pH values, ratios of eluent and velocities of flow were optimized in the process. Accordingly the best macroporous resins and SPE conditions were chosen.

2.3.3. Sample preparation

For drinks, a 5 g amount of sample was weighed accurately in a beaker. If the sample was carbonated, it was degassed by ultrasonication for 5 min. The content was adjusted to approximately pH 4 with 36% acetic acid.

For sausages and sauces, a 0.5 g amount of crushed sample was weighed accurately in a 10 mL centrifuge tube. Five milliliters of acetic acid solution (pH 4) was added to extract the colors. The supernatant was collected and mixed after centrifugation at 12,000 revolutions per minute (rpm). The extraction step was repeated three times.

The sample solution was applied to the column, and then the column was washed with pH 4 acetic acid solutions in order to remove the additives such as inorganic salts, followed by 15 mL of