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Review

Quantitative determination of carmine in foods by high-performance liquid chromatography

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ABSTRACT

A simple and rapid method has been developed and validated for the determination of carmine in foods. Samples were homogenised and extracted with 0.05 M NaOH, followed by centrifugation. The resulting solution was filtered and injected to HPLC. Carmine was separated by HPLC using an NovaPak C_{18} column coupled to a photodiode array detector. The contents of carmine were finally quantified using corresponding calibration curves over ranges of 1.0–100 μg ml^{-1}, with good correlation coefficients (r^2 = 0.9999). The recoveries of carmine from foods spiked at levels of 10, 50, and 100 μg g^{-1} which ranged from 90.4% to 96.2% with relative standard deviations between 2.8% and 6.8%. Limit of detection and limit of quantification of carmine were 0.4 and 1.0 μg ml^{-1}, respectively. This method was found to be useful to distinguish carmine from carminic acid, a major component of cochineal extract. The method has been successfully applied to various foods.

Contents

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1. Introduction

Synthetic food dyes are food additives that can impart colour when added to foods. These substances must be preapproved by the Korea Ministry of Food and Drug Safety and listed in Korea Food Additives Code (MFDS, 2013a) in order to be legally used in food products retailed in Korea. The MFDS lists 18 permitted synthetic colour additives in synthetic substances part of the Code. The use of food colours is strictly controlled by laws and regulations (MFDS, 2013b). Carmine, one of the synthetic food dyes, is authorised for use in Korea, USA (FDA, 2012), and European Union (European Commission, 1994) except for Japan. The acceptable daily intake (ADI) value of carmine, which is formulated by Joint FAO/WHO Expert Committee on Food Additives, is 5 mg kg^{-1} based on weight (JECFA, 2001). As the Committee's conclusion, carmine in foods may initiate or provoke allergic reactions in some individuals.

Carmine is a lake pigment used in many different products such as juice, candies, and confectioneries. It is usually produced by precipitating carminic acid in aluminium hydroxide in the form of aluminium or aluminium-calcium salt. Carminic acid is obtained from dried bodies of the female insect *Cocus cacti* L., which lives on various cactus plants (Feller, 1986). Carminic acid also is a major component of cochineal extract, which is used as a natural food colour in Korea. Carmine and cochineal extract permitted in Korea should not be used for the food items as followings: natural food (meat, seafood (whale meat included), fruits, vegetables, marine algae, bean and their simply processed food (peeled or cut)); tea and coffee; red pepper powder or shredded red pepper; *Kimchi*; *Gochujang* (fermented red pepper pastes) or seasoned *Gochujang* (seasoned soybean paste with red peppers); vinegars; spice products (limited to products containing red pepper or red pepper powder). In addition, the permissible level is not established in Korea Food Additive Code.

Carmine might be present as a non-permitted colour additive in food products imported to Korea and illegally added to food products by manufacturers to improve appearance and colour. Cochineal extracts also may be marked on food products where carmine was actually used. Accordingly, monitoring of carmine in foods is necessary to ensure food safety and consumer confidence.

Most research has been related to the analysis of carminic acid, a major component of cochineal extract, using high-performance liquid chromatography (Ishikawa, Shigeoka, Nagashima, Takahashi, & Kamimura, 2003; Lancaster & Lawrence, 1996; Merino, Edberg, & Tidriks, 1997), capillary electrophoresis (Liu et al. 1995), and spectrophotometric methods (Tripathi, Khanna, & Das, 2004). Recently, a method used to determine carmine itself in foodstuffs by stripping voltammetry was reported (Alghamdi, Alshammery, & Ahdalla, 2009). This method had an advantage that is suitable for the analysis of very diluted samples and decrease the real time of determination. However, the proposed method was applied to determine carmine only in spiked commercial ice cream and soft drinks. This electrochemical procedure is known to be influenced by factors such as temperature, pH, and organic substances (Kalvoda & Kopanica, 1989). The stripping voltammetry is also not widely used in the analysis of food additives.

To the best of our knowledge, no chromatographic method which can separate carmine from carminic acid in foods, has been reported in the literature for the determination of this dye by high-performance liquid chromatography. This is due to insolubility in water and organic solvents. For this reason, carmine cannot be effectively extracted from food samples with usual liquid extraction. Thus, the development of a specific method for the quantification of carmine in foods is required.

In the study described in this paper, we have developed a simple and quantitative method for the analysis of carmine in food products by high-performance liquid chromatography coupled to a diode array detector. The new method separates carmine from carminic acid in food samples. The procedure is quantitatively characterised and linearity, accuracy, limits of detection and quantification are satisfactory.

2. Materials and methods

2.1. Chemicals and reagents

Carmine and carminic acid used as standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in extraction and preparation of mobile phase, such as sodium hydroxide, sodium phosphate dibasic, and sodium phosphate monobasic were of analytical reagent grade and also supplied by Sigma–Aldrich. Water for all applications in our study was obtained from an Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA) with resistivity equal to or higher than 18.2 MΩ cm.

2.2. Preparation of standard solutions

The stock solution (1000 μg ml^{-1}) of carmine was prepared by transferring 100 mg of carmine into a 100 ml beaker and adding 0.05 M NaOH solution to give a final volume of 100 ml. Calibration standard solutions at serial concentrations of carmine were obtained by mixing subsequent dilution (1–100 μg ml^{-1}) with water.

2.3. Sample collection

A total of 124 samples were purchased from retail stores in big cities including Seoul, Incheon, Daejeon, Gwangju, Daegu and Busan in South Korea. The samples were categorised into 16 food types. Processed milk (4), confectionery (50), functional food (20), processed fruit vegetable product (4), tea (1), sugaring product (5), meat product (4), fish meat product (7), processed cheese (4), beverage (10), fermented soybean paste (2), salted seafood (5), seasoned food (4), *Kimchi* (2), chocolate (1) and coffee (1) in commercial products were purchased after checking the labels and colours written on the products. All samples were stored at 4 °C.

2.4. Preparation of samples

A 5.0 g of ground food samples was transferred into a 100 ml beaker and 0.05 M NaOH solution was added to give a final volume of 50 ml. After it was mixed by a homogenizer for 2 min, the sample was shaken mechanically for 10 min with a shaking rate of 300 min^{-1}. The extract was centrifuged for 5 min at 5000 rpm. The 10 ml supernatant was then filtered with 0.45 μm membrane filter for injection into HPLC.

2.5. High performance liquid chromatography

HPLC analysis was performed on an Agilent HPLC 1200 series (Santa Clara, CA, USA) coupled to a photodiode array detector. The LC system consisted of degasser, binary pump, autosampler, and column oven. An NovaPak C_{18} column (150 × 3.9 mm, 5 μm) purchased from Waters Corporation (Milford, MA, USA) was used for chromatographic separation. All separations were carried out isocratically at room temperature with a mobile phase consisting of methanol-phosphate buffer (pH 6.0) at ratios of 15:85 (v/v). The flow-rate was maintained at 0.8 ml min^{-1} and a 20 μl sample volume was injected into HPLC.

Carmine, eluted from the column, was monitored by photodiode array detector set at 281 nm. The absorption spectra of carmine