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Analytical Methods

Simultaneous determination of red and yellow artificial food colourants and carotenoid pigments in food products

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ABSTRACT

A method for simultaneously determining four artificial food colourants [Red Nos. 2 (R2) and 40 (R40), Yellow Nos. 5 (Y5) and 6 (Y6)] and three carotenoids [lycopene, lutein, and β-carotene] was developed. They were successfully separated by the developed high pressure liquid chromatography (HPLC) method combined with a photo diode array detector. The detection limit (at signal to noise > 4) was from the lowest of 0.2 ng/mL for lutein to the highest of 50.0 ng/mL for R40. With a two-phase solvent and ultrasound-assisted extraction, the recoveries of the artificial and natural pigments in fifteen different types of food products were between 80.5–97.2% and 80.1–98.4%, respectively. This HPLC method with the ultrasound-assisted extraction protocol could be used as a sensitive and reliable analysis technique in simultaneously identifying and quantifying the reddish and yellowish pigments in different foods regardless of they are artificial food colourants or/and natural carotenoids.

1. Introduction

Food colour is an important attribute of food quality and affects consumer acceptance. It is usually imparted by naturally occurring pigments or colourants added during food preparation. Carotenoids are one group of the natural pigments responsible for yellow, orange and red colours in many fruits and vegetables. Among them, three carotenoids, lutein, β-carotene and lycopene are prevalent in our daily diets. In addition to the colour attribute, they have been confirmed to possess some health benefit functions, such as supplying vitamin A to the body, reducing the damage of the eye retina from exposure to near-ultraviolet light, preventing the risk of prostate cancer, etc. (Rodriguez-Amaya, 2010). The level of carotenoids has been an important index of healthy foods in the market. However, these carotenoids are not stable and readily degraded upon exposure to light, heat, or severe pH during food storage and processing (Kopec, Cooperstone, Cichon, & Schwartz, 2012). The degradation causes not only the fading of their original colour intensity but also the loss of the nutrition value.

As yellow and red artificial colourants have similar tint to carotenoid pigments, they have been used totally or partially to replace the natural pigments in food products (Kiseleva, Pimenova, & Eller, 2003). Their high stability and solubility made them widely used in various drinks and processed food products. However, the main chemical structure of artificial food colourants consists of azo groups and aromatic rings which may have potential toxicity to human health, especially when they are excessively consumed daily (Minioti, Sakellariou, & Thomaidis, 2007). Recently, it has also been reported that artificial colourants may be responsible for the children's attention deficit disorder (ADD), immune system problem, and certain allergic reactions (Kanarek, 2011). Thus, the level of the added artificial colourants which were used to enhance food colour intensity or mimic the colour of carotenoids in food materials and products needs to be restrictively controlled.

Although several simultaneous determination of food colourants have been reported, the colourants in these methods were only limited to food banned artificial Sudan dyes or a high level of natural dyes (Bessonov, Perederyaev, Vedishcheva, Yu., & Bogachuk, 2010; Daood & Biacs, 2005; Dixit, Khanna, & Das, 2008). A reliable and efficient method for simultaneously identifying and quantifying both red and yellow allowed artificial colourants and carotenoids has not been reported. In this study, an HPLC method was developed for simultaneously monitoring the levels of artificial colourants and naturally occurring carotenoids in raw food materials and final food products. Also, fifteen different types of commercial food products, such as drinks, pastes, canned foods, oily dressing, etc. were used to examine the recovery and reliability of our developed analysis method. The developed method could

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provide an efficient technique in identifying and quantifying the food artificial and natural carotenoid pigments responsible for red, yellow, or orange colour in various food products.

2. Materials and methods

2.1. Chemicals and reagents

Standards of Y5 (Tartrazine), Y6 (Sunset yellow), R2 (Amaranth), R40 (Allura red), lycopene, β-carotene, and lutein were purchased from Sigma–Aldrich (St. Louis, MO, USA). Each of them was prepared to serve as a stock solution (100 μg/mL) in methanol and stored in dark at 4 °C before use. HPLC grade acetone was purchased from Marcron Avantor Performance Materials Inc (Phillipsburg, NJ, USA). HPLC grade methanol was purchased from EMD Millipore Corporation (Billerica, MA, USA). Ammonium acetate was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Fifteen commercial food stuffs were purchased from local food grocery stores (Baton Rouge, LA, USA).

2.2. Ultrasound-assisted solvent extraction

The solid samples were ground in a mortar, weighed and transferred to a clean test tube. One milliliter of methanol was mixed with 0.50 mL of liquid sample or 0.20 g of paste or ground solid sample in a 15 mL test tube. An ultrasonic probe (60 Sonic Dismembrator, Fisher Scientific Inc., Fair Lawn, NJ) was immersed in the sample mixture to perform ultrasound-assisted solvent extraction for 15 min. The supernatant layer was separated by centrifugation, collected and transferred to another test tube. The methanol extraction was repeated three times. All supernatant layers were combined. The precipitated residue was extracted using 1 mL of acetone and repeated three times as well. The acetone layers were combined and evaporated using a centrifuge vacuum evaporator (CentriVap Mobile System, Labconco, Kansas, MO, USA). The methanol supernatant collected in the methanol extraction was also evaporated after it had been mixed with the dried extract from the acetone extraction. The final dried extract was re-dissolved by 2000 μL of methanol, filtered using 0.45 μm microporous film and transferred to an HPLC vial.

2.3. Chromatographic analysis

The four artificial food colourants and three carotenoids pigments were analyzed using a high performance liquid chromatography system (2690, Waters, Torrance, USA) with a C18 column (id 250 × 4.60 mm 5 micron, Phenomenex, Torrance, USA) and a photodiode array detector. The HPLC mobile phase was controlled by a gradient program of maintaining 100% of ammonium acetate solution (1%) for 5 min, then linearly ramping methanol from 0% to 50% from 5 to 23 min, changing to 50% methanol and 50% acetone from 23 to 24 min, and holding for 18 min. The flow rate was kept constant at 1.0 mL/min. The composition of the mobile phase was changed back to 100% of ammonium acetate solution (1%) to equilibrate the column for 13 min before the next injection. The maximum absorption wavelength of each pigment with the corresponding mobile phase composition at that retention time was used for quantification (Table 1). The concentration of each pigment was calculated using a calibration curve obtained from its standard.

2.4. Recovery study for different samples

The artificial colourant and carotenoid standards were spiked to each type of the tested food products. The spiking amount of each artificial colourant or carotenoid was 2 μg per gram or per milliliter of the sample. For the sample in which the level of colourant or carotenoid was over 100 μg/g or μg/mL, the spiking amount of the colourant or carotenoid standard was increased to 20 μg per gram or per milliliter of the sample. The spiked samples were homogenized and subject to the same extraction procedure as mentioned in Section 2.2. The recovery percentage was calculated by dividing the difference of the artificial or carotenoid pigment level in the samples with and without the spiked standards by the spiked level of the artificial or carotenoid followed and then multiplying by 100.

2.5. Data analysis

The analysis experiment for each sample was independently performed in triplicate. The means and standard deviations of the sample were calculated using Microsoft Excel Software (Redmond, WA, USA).

3. Results and discussion

The chemical structures of artificial colourants (Y5, Y6, R2, and R40) and carotenoids pigments (lutein, lycopene and beta-carotene) are illustrated in Fig. 1. Although both types of the pigments exhibit red or yellow colour, their chemical structures are very different. The artificial colourants mainly consist of azo groups, benzene or naphthalene rings and Na^+, while the core structure of carotenoids is a long carbon chain which is linked by a number of isoprene basic units. Therefore, the artificial colourants are more hydrophilic than the carotenoids. HPLC technique is usually used to determine the artificial colourants or carotenoids pigments (Breithaupt, 2004; Kiseleva et al., 2003; Marinova & Ribarova, 2007). However, a method of simultaneously identifying and quantifying both types of the pigments has not been reported. In our study, an optimized composition and gradient program of HPLC mobile phase successfully separated all seven pigments in the standards as well as in the corn chip spiked with these standards (Fig. 2). They were eluted in the sequence of Y5, R2, Y6, R40, lutein, lycopene, and β-carotene. The order of the elution also reflected their hydrophilic properties. Due to the relatively higher hydrophilicity of benzene ring than naphthalene and other polar functional groups such as hydroxyl and sulfonate groups in Y5 and R2, it resulted in their shorter retention time than Y6 or R40 in a reversed phase column. This was in agreement with the results of the previous studies of Vidotti, Costa, and Oliveira (2006) and Yoshioka and Ichihashi (2008). In order to improve the resolution and separation of the charged artificial colourants, an ion-pair chemical was recommended to add in mobile phase as a modifier in a previous study (Kiseleva et al., 2003). In our study, it was found that the mobile phase mixed with the ion-pair could problematically cause the overlapping problem of the retention times of Y6 or R40 and carotenoids in the HPLC chromatogram, because the ion-pair extended the retention time of Y6 and R40 to the time of eluting the carotenoids. In this method, ammonium acetate buffer was used to replace the ion-pair mobile phase for achieving the separation of the hydrophilic artificial colourants before the retention time of carotenoids. As the lower hydrophilicity of carotenoids, the mobile phase of ammonium acetate and methanol was not able to efficiently separate them in a short range of retention time and resulted in poor separation and sensitivity of the carotenoids. Thus, in this study, the composition of the mobile phase was changed to methanol and acetone after the artificial pigments had been eluted. Among the three carotenoids, lutein had two hydroxyl groups which lowered its hydrophobicity and contributed to the shorter retention time than lycopene or β-carotene in the HPLC