Food Chemistry 276 (2019) 390–396

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Simultaneous determination of sodium iron chlorophyllin and sodium copper chlorophyllin in food using high-performance liquid chromatography and ultra-performance liquid chromatography–mass spectrometry

Hee Sun Chong[a], Sol Sim[a], Tokutaro Yamaguchi[a], Juhee Park[b], Chan Lee[b], MeeKyung Kim[c], Gunyoung Lee[c], Sang Soon Yun[c], Ho Soo Lim[c], Hee-Jae Suh[a,*]

[a] Department of Food Science, Sun Moon University, Asan, Chungchengnam-do 31460, Republic of Korea
[b] Advanced Food Safety Research Group, BrainKorea21 Plus, Department of Food Science and Technology, Chung-Ang University, 4726, Seodong-daero, Anseong, Gyeonggi-do 17546, Republic of Korea
[c] Food Additives and Packaging Division, National Institute of Food and Drug Safety Evaluation, 187 Osongsaengmyeong 2-ro, Osong, Chungcheongbuk-do 28159, Republic of Korea

ARTICLE INFO

Chemical compounds studied in this article::
Sodium copper chlorophyllin (PubChem CID: 108006)
Sodium iron chlorophyllin (PubChem CID: 169450)

Keywords:
Sodium copper chlorophyllin
Sodium iron chlorophyllin
Colorant
UPLC–MS
HPLC–PDA

ABSTRACT

A simultaneous method for analyzing sodium iron chlorophyllin (SIC) and sodium copper chlorophyllin (SCC) using high-performance liquid chromatography was developed. This method employed an Inertsil ODS-2 column and diode array detection at 395 nm, using methanol–water (97:3 and 80:20, *v/v*) containing 1% acetic acid as the mobile phase. Liquid chromatography–tandem mass spectrometry was used to identify the main components of SIC and SCC as Fe-isochlorine e4 and Cu-isochlorine e4, respectively. The limits of detection and quantitation of SIC were 1.2 and 4.1 mg/kg, respectively, while those of SCC were 1.4 and 4.8 mg/kg, respectively. For intraday and interday tests, the SIC recoveries from candy ranged from 81% to 101%, while SCC recoveries ranged from 100% to 109%. The developed method can be applied to the rapid determination of SIC and SCC in candy.

1. Introduction

Sodium iron chlorophyllin (SIC) and sodium copper chlorophyllin (SCC) are non-tar colorants that are prepared by replacing Mg^{2+} ions at the center of tetrapyrroles in chlorophyll obtained from plant leaves with Fe^{3+} or Cu^{2+} ions (EFSA ANS Panel, 2015). SIC is a greenish brown pigment that dissolves in water, alcohols, and chloroform, but not ethers, while SCC is a dark green pigment with good solubility in water, alcohols, and ethers. SIC and SCC have superior processing stability to chlorophyll and are used as food colorings in many countries (KFDA, 2013; The Japan Food Chemical Research Foundation, 2016; Jie, 2015; Scotter, 2010). SIC is an allowed colorant in Korea and Japan, with use stipulated for all foods except meat, fish (including whale meat), shellfish, kelp, seaweed, vegetables, fruits, legumes, and tea leaves. SCC is an allowed food colorant in China, the United States, the European Union (EU), and CODEX, in addition to Korea and Japan. SCC is allowed for use in seaweeds, fruit preserves, vegetable storage, chewing gum, candy, and agar in Korea, and in similar food categories in Japan (KFDA, 2013; The Japan Food Chemical Research Foundation, 2016). In the European Union, SCC is managed as coloring in the Cu-chlorophyllin category (E141(ii)), in accordance with European Union (EC) Annex II (Regulation (EC) No. 1333/2008) (EFSA ANS Panel, 2015), and is allowed as a food colorant in candy, jam, jelly, drinks, and other products in the EU and CODEX (JECFA, 2017b). Although the toxicity of SIC has yet to be assessed, the toxicity of Cu-chlorophyllin (E141(ii)) has been assessed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) since 1969. EU and CODEX have set the acceptable daily intake (ADI) of the sum of both Cu-chlorophyll and Cu-chlorophyllin complexes as 0–15 mg/kg

* Corresponding author.
E-mail addresses: yamaguchi@sunmoon.ac.kr (T. Yamaguchi), chanlee@cau.ac.kr (C. Lee), mkim@korea.kr (M. Kim), grasia1969@korea.kr (G. Lee), yss0520@korea.kr (S.S. Yun), hslim70@korea.kr (H.S. Lim), suhhj@sunmoon.ac.kr (H.-J. Suh).

https://doi.org/10.1016/j.foodchem.2018.10.015
Received 27 May 2018; Received in revised form 28 September 2018; Accepted 2 October 2018
Available online 04 October 2018
0308-8146/

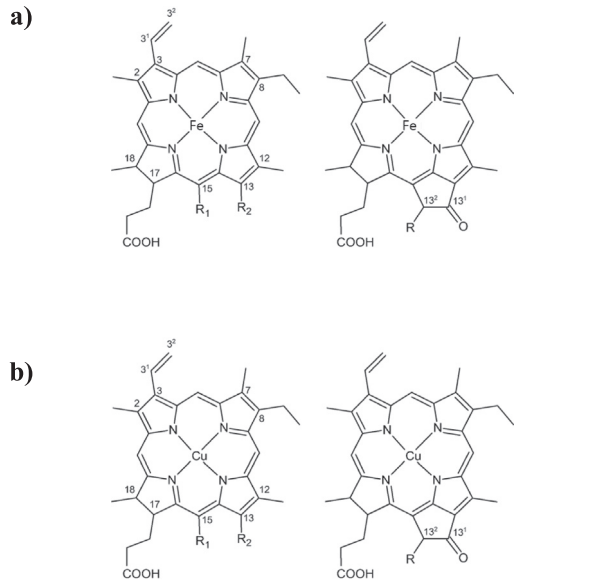

Fig. 1. Structures of iron chlorins and copper chlorins.

bw/day based on these toxicity data (EFSA ANS Panel, 2015), while the United States Food and Drug Administration (US FDA) has allocated an ADI of 7.5 mg/kg/day for sodium/copper chlorophyllins (FDA, 2002).

As shown in Fig. 1, SIC and SCC are not single substances, but composed of various derivatives, and can form different derivatives depending on the functional groups present at R, R1, and R2. The derivatives of SIC are Fe-chlorine e6, Fe-chlorine e4, Fe-isochlorin e4, and Fe-pheophorbide a, while the derivatives of SCC are Cu-chlorin e6, Cu-chlorin e4, Cu-isochlorin e4, and Cu-pheophorbide a. However, metallo-chlorine e6 and metallo-pheophorbide a are known to be degraded under certain pH and heat treatments during food manufacturing processes (Mortensen & Geppel, 2007; Wood, Foster, Damant, & Key, 2004).

SIC and SCC analyses have been reported using reversed-phase and high-performance liquid chromatography (HPLC) since the 1990s (Amakawa, Ogiwara, Takeuchi, Ohnishi, & Kano, 1993; Gandul-Rojas, Roca, & Gallardo-Guerrero, 2012; Inoue et al., 1994; Raymond, Chernomorsky, Sahai, & Poretz, 1997; Scotter, Castle, & Roberts, 2005; Tsunoda, Inoue, Tachibana, & Aoyama, 1993; Yasuda et al., 1995). An analysis of SCC was reported by Amakawa et al. (1993) using HPLC with a C18 column and an ultraviolet detector (PDA) at 623 nm using methanol (MeOH), water, and acetic acid as mobile phase (Amakawa et al., 1993). Inoue et al. (1994) analyzed SCC using a C18 column (Inertsil ODS-2) and HPLC-PDA at 407 nm and 423 nm with MeOH and water containing 1% acetic acid as the mobile phase (Inoue et al., 1994). Yasuda et al. (1995) analyzed SCC at 405 nm using a HPLC method similar to that of Inoue (Inoue et al., 1994) after extracting green matter from bracken, agar, and gum using diethyl ether (pH 3–4). Scotter et al. (2005) extracted SCC from commercial foods, including candy, jelly, and biscuits, using citric acid/phosphate buffer (pH 2.6) and ethyl acetate/acetone (5:1, v/v), with a Vydac 201TP54 column and HPLC-PDA used to analyze SCC at 650 nm and MeOH, 0.1 M ammonium acetate, and acetone as the mobile phase (Scotter et al., 2005). However, previous studies report little research into methods to simultaneously extraction these two pigments from foods or HPLC method validation. Furthermore, no studies have simultaneously analyzed SIC and SCC using HPLC or SIC and SCC derivatives using liquid chromatography–mass spectrometry (LC–MS).

Therefore, herein, we have studied SIC and SCC pre-treatment methods, and developed an HPLC method for simultaneous analysis of the two pigments and verified its validity. Furthermore, SIC and SCC were analyzed using ultra performance LC–MS (UPLC–MS) to identify the main derivatives of the two pigments.

2. Materials and methods

2.1. Reagents

Phosphate buffer was purchased from Sigma Aldrich Co. (St. Louis, MO). HPLC-grade water, methanol, and acetonitrile were obtained from Honeywell Co. (Seoul, Korea). Hydrochloric acid (0.1 N) and acetic acid were purchased from Samchun Chemical Co. (Seoul, Korea) and Avantor (Center Valley, PA), respectively. Oasis HLB 6 mL (200 mg) extraction cartridges (Waters, Dublin, Ireland) and 0.2-μm polyvinylidene fluorides (PVDF) filter (Whatman, Maidstone, UK) were also obtained.

2.2. Calibration standards

SIC and SCC standards of high purity (> 95%) could not be purchased. Instead, commercial grade SIC and SCC (trisodium copper chlorophyllin) were purchased from Waco Pure Chemical Inc. (Tokyo, Japan) and Sigma Aldrich (Gillingham, UK), respectively. The calibration solution for HPLC analysis was prepared according to the Korean Food Additives Code (KFDA, 2015) as follows: SIC and SCC (approx. 0.1 g) were dissolved in water and diluted to exactly 100 mL in a volumetric flask; exactly 1 mL of this solution was diluted to 100 mL using phosphate buffer (1 M, pH 7.5); the absorbance was measured quickly at 398 nm for SIC and 405 nm for SCC against a blank 1 M phosphate buffer using a UV–Vis spectrophotometer (Vision, Seoul, Korea), which was repeated ten times. Using the following formula, the absorbance at the corresponding wavelength was measured and concentration determined (expressed as total SIC and SCC) using extinction coefficient ($E_{1\mathrm{cm}}^{1\%}$) values of 400 for SIC (Eq. (1)) (KFDA) and 565 for SCC (Eq. (2)) (KFDA, 2015; JECFA, 2017a; Scotter et al., 2005).

$$\text{Concentration } (mg/L) = \frac{Absorbance\ at\ \lambda max \times dilution}{400} \quad (1)$$

$$\text{Concentration } (mg/L) = \frac{Absorbance\ at\ \lambda max \times dilution}{565} \quad (2)$$

Individual stock standard solutions of SIC and SCC were prepared in MeOH at 1000 mg/L and stored at −4 °C until use. Standard solutions of mixtures of both pigments were prepared using MeOH at appropriate concentrations. Dilution of both standards was used to prepare concentrations of 10, 25, 50, 100, 300, and 500 mg/L. All dilution standards were used immediately after preparation.

2.3. Sample preparation

Methods for extracting SIC and SCC from samples were developed and optimized. To extract SIC and SCC contained in the candy, colorless candy samples were purchased from a local supermarket and recovery experiments were conducted. The fortified samples were prepared by spiking SIC and SCC into colorless candy samples at five concentrations of the calibration curve to assess accuracy. Solid SIC and SCC-fortified candies were finely crushed, about 5–10 g samples measured precisely and placed in a centrifuge tube. After adding 0.1 N hydrochloric acid (5 mL), the mixture was ultrasonicated at 50 °C for 10 min and diluted to 20 mL with methanol (MeOH). After vortex mixing and centrifugation at 10,000 rpm for 10 min, the upper layer was collected. The solution was then filtered with a 0.2-μm membrane filter, with the filtrate used as a test solution for HPLC analysis. If the test solution was lightly