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# Thermal Degradation of Commercial Grade Sodium Copper Chlorophyllin

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Sodium copper chlorophyllin (SCC), a water-soluble commercial derivative of chlorophyll, has gained importance as a food colorant and dietary supplement with apparent chemopreventive activities. The thermal stability of SCC was studied to assess the potential application of this chlorophyll derivative for use in thermally processed foods and supplements. Thermal degradation of an aqueous 500 ppm SCC solution was monitored between 25 and 100 °C by a loss of absorbance at 627 nm. Decomposition was also followed by reversed phase $C_{18}$ HPLC with photodiode array detection to monitor the loss of Cu(II)Chlorin $e_4$, the major component of commercial grade SCC. The rate of thermal degradation of SCC was found to follow first-order reaction kinetics. HPLC analysis confirmed the ultraviolet and visible absorbance data and also demonstrated loss of the major SCC component, Cu(II)Chlorin $e_4$, at a rate faster than that of overall SCC. The activation energy was estimated using the Arrhenius equation and found to be $13.3 \pm 0.8$ and $16.0 \pm 2.1$ kcal/mol for the thermal degradation of SCC and Cu(II)Chlorin $e_4$, respectively. The observed temperature sensitivity of SCC was determined to be similar to that of natural chlorophyll and raises the possibility of color deterioration when used in food products where mild to severe thermal treatment is applied. Furthermore, the implication of rapid loss of Cu(II)Chlorin $e_4$, a reported bioactive component of SCC, upon heating may result in alteration of potential dietary benefits such as antimutagenic and antioxidant activity.

**KEYWORDS: Sodium copper chlorophyllin; Cu(II)Chlorin $e_4$; thermal degradation; UV−vis spectrophotometry; HPLC**

## INTRODUCTION

Sodium copper chlorophyllin (SCC), the bright green colorant derived from natural chlorophyll, has been marketed in the United States as a common dietary food supplement, in both liquid and powdered form, with functions ranging from internal deodorizer to natural wound healer and general promoter of kidney health (*1*). This mixture of water-soluble copper chlorophyll derivatives has gained notoriety as a potential chemopreventive agent (*2*−*5*). SCC has been found to have antimutagenic activity against a variety of known mutagens including aflatoxin $B_1$, benzo[*a*]pyrene, heterocyclic amines, 3-amino-1-methyl-5*H*-pyrido-[4,3-*b*]indole, and 2-amino-3-methylimidazo[4,5-*f*]quinolino (*6*−*10*). SCC has also been shown to have potential antioxidative properties because of its highly delocalized electron system with the ability to act as a radical scavenger (*9*, *11*). Most recently, SCC derivatives have demonstrated the ability to induce phase II enzymes such as quinone reductase (*12*). Together these studies have prompted considerable interest in SCC for its possible use in the prevention of chronic disease.

Commercial grade SCC is a mixture of numerous chlorin-type compounds derived from natural chlorophyll (*13*−*15*). Observed benefits most likely arise from action stemming from this mix of metalloporphyrins. Two main components have been identified in a complex and variable mixture (**Figure 1**), Cu(II)Chlorin $e_4$ and Cu(II)Chlorin $e_6$ (*13*, *15*, *16*). Numerous studies have demonstrated the thermal instability of natural chlorophylls and have characterized their major degradation products including metal-free pheophytin and pyropheophytins (*17*−*19*). Canjura et al. (*20*) studied the degradation kinetics of chlorophylls and chlorophyllides during thermal processing, demonstrating first-order reaction kinetics with activation energies between 15.0 and 22.8 kcal/mol, respectively. Ryan-Stoneham and Tong (*21*) more recently studied chlorophyll degradation in peas and found activation energies of 17.5 and 17.0 kcal/mol for the degradation of chlorophylls *a* and *b*, respectively. The structural similarity of SCC to natural chlorophylls makes this commercial preparation potentially labile to thermal treatment encountered during typical food-processing conditions. However, only a few studies have addressed the

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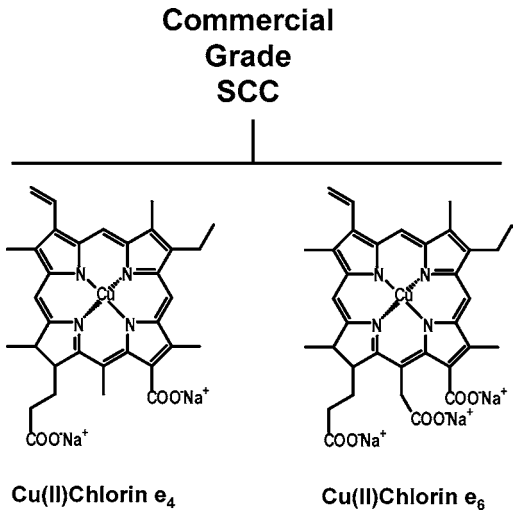


**Figure 1.** Structures of major chlorophyllin derivatives present in commercial grade SCC. Cu(II)Chlorin $e_4$ was identified as the major chlorin component (>85%) in SCC preparations utilized in this study.

potential sensitivity of SCC. Pentillä et al. (*22*) investigated photobleaching of chlorophyllin, demonstrating a biphasic aerobic degradation. Salin et al. (*23*) further reported that photobleaching of SCC is temperature dependent and proceeds by a multistep pseudo-first-order process with a potential oxidative component. As its use as a food colorant and food additive is expanding, it is critical to better understand the impact of traditional food processes on SCC.

The purpose of this study is to gain a better understanding of the temperature sensitivity of commercial grade SCC. Utilizing an aqueous model system, degradation kinetics of crude SCC solutions were assessed by both ultraviolet and visible (UV−vis) spectrophotometry and high-performance liquid chromatography (HPLC). Results of these experiments will aid in assessing the potential usefulness of this mixture of copper chlorophyll derivatives for food grade applications that involve thermal processing.

## MATERIALS AND METHODS

**Chemicals and Standards.** Commercial grade sodium copper chlorophyllin was purchased form Sigma-Aldrich (St. Louis, MO). The purity of commercial SCC was calculated to be 47.8% based on a 4.5% copper content, specified by the manufacturer, with respect to the main component, Cu(II)Chlorin $e_4$. These findings agree with previously published values for commercial grade SCC (*13*, *15*). SCC concentrations for experiments were adjusted on the basis of this level of purity. Authentic standards of Cu(II)Chlorin $e_4$ were purchased from Frontier Scientific (Logan, UT) and determined to be >95% pure by HPLC analysis.

**Assessment of SCC Thermal Stability.** All experiments and manipulations were conducted under subdued light to minimize photooxidative degradation of SCC. Approximately 250 mg of commercial grade SCC was solubilized in deionized water to a final volume of 500 mL, yielding a 500 ppm working solution. Two milliliter aliquots of the SCC working solution were placed into 2 mL cryogenic vials and tightly sealed. Thermal treatments were applied using a shaking water bath (Thelco Precision Scientific, Chicago, IL) set between 25 and 100 °C. Sample vials were exposed to thermal treatment by submersion into the water bath and collected at predetermined time intervals over 120 min. Analysis was performed by monitoring SCC's electronic absorption spectrum with a Hewlett-Packard model 8453 UV−vis spectrophotometer (Avondale, PA). Spectral data were collected between 250 and 700 nm. SCC degradation was assayed as a loss of absorbance at 627 nm, corresponding to both the observed and published red absorption maxima of SCC (*13*). This method has been previously determined to be in close agreement with data calculated from elemental analysis (*24*).

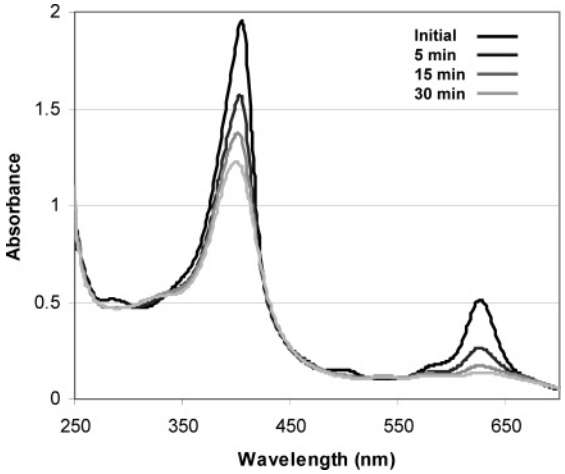


**Figure 2.** Ultraviolet and visible absorbance spectra of aqueous SCC (500 ppm) exposed to 100 °C treatment for 0, 5, 15, and 30 min.

**Assessment of SCC Degradation by Liquid Chromatography.** High-performance liquid chromatography was used to monitor the loss of Cu(II)Chlorin $e_4$, the main component of SCC. The method of Ferruzzi et al. (*15*) was utilized with minor modification. A Hewlett-Packard model 1100 (Santa Clara, CA) equipped with a model 1100 diode array detector was used. A Beckman Ultrasphere $C_{18}$ analytical scale (4.6 mm i.d. × 150 mm) reversed phase column with a $C_{18}$ stationary phase guard column was also used. Gradient elution parameters determined for the separation of chlorophyllin derivatives were based on a binary mobile phase of methanol/water/acetic acid in reservoir A (75:24.5:0.5) and ethyl acetate in reservoir B. Initial conditions were set at 100% A with a linear gradient to 50:50 A/B over 20 min, followed by a 5.0 min linear gradient back to 100% A for a final chromatographic run time of 25 min. Detection and identification Cu(II)Chlorin $e_4$ components were accomplished by cochromatography with an authentic standard and comparison of electronic absorption spectra obtained from in-line diode array detection with previously published electronic absorption spectra of major SCC derivatives (*15*, *16*).

**Data Analysis.** Data were analyzed using SAS 9.1.3 (SAS Institute, Cary, NC). Descriptive statistics including mean and standard error of mean (SEM) were calculated for each spectrophotometric measurement. HPLC assessment of Cu(II)Chlorin $e_4$ represents an average of two independent observations. Reaction rate constants were calculated using linear regression analysis based on apparent first-order models with significance determined by analysis of variance. Activation energy was estimated within the range of 25−100 °C using Arrhenius parameters.

## RESULTS AND DISCUSSION

Thermal degradation of SCC was investigated at temperatures between 25 and 100 °C by following the electronic absorption spectra between 250 and 700 nm (**Figure 2**). Reduction in absorbance at 627 nm was chosen to monitor the degradation of total copper chlorophyllin complexes as loss of metal ions from chlorophyll derivatives has been shown to significantly reduce absorbtivity in the Q region (*25*). The specific loss of Cu(II)Chlorin $e_4$ was followed by HPLC. The degradation of both total SCC and Cu(II)Chlorin $e_4$ was found to be temperature dependent following first-order reaction kinetics as depicted by the linear relationship in the thermal degradation curves (**Figures 3** and **4**). Reaction rate constants were subsequently calculated on the basis of apparent first-order models within the range of 25−100 °C. Calculated $R^2$ values (**Table 1**) indicated significant ($p < 0.05$) linear behavior for SCC and the Cu(II)Chlorin $e_4$ in SCC degradation at all temperatures. This apparent linearity

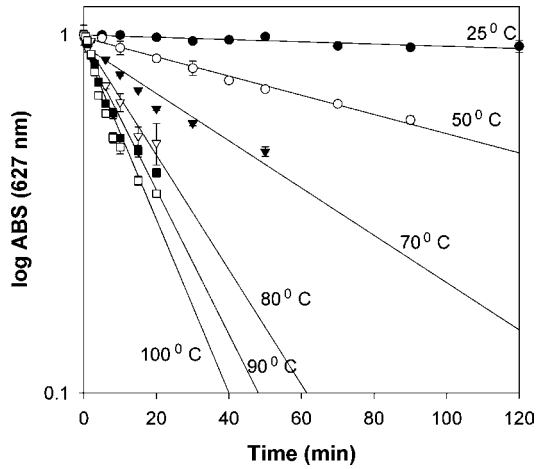


**Figure 3.** First-order thermal degradation of crude SCC in aqueous solution. Samples were transferred to 2 mL vials and exposed to thermal treatments by submersion into the appropriate water bath temperature. Each point for crude SCC represents the mean ± SEM for triplicate spectrophotometric measurements as described under Materials and Methods. Linear regression and analysis of variance indicated significant linear behavior for SCC degradation at 25 °C ($p < 0.05$), 50 °C ($p < 0.01$), 70 °C ($p < 0.05$), 80 °C ($p < 0.05$), 90 °C ($p < 0.05$), and 100 °C ($p < 0.05$).

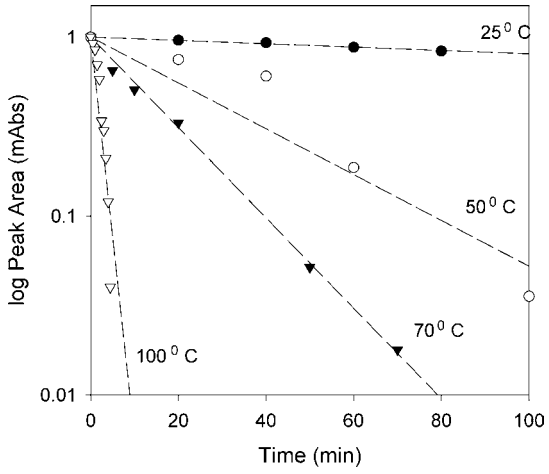


**Figure 4.** First-order thermal degradation of SCC component Cu(II)Chlorin $e_4$ in aqueous solution. Samples were transferred to 2 mL vials and exposed to thermal treatments by submersion into the appropriate water bath temperature. Cu(II)Chlorin $e_4$ was determined by HPLC as described under Materials and Methods, and each point represents an average of two independent measurements. Linear regression and analysis of variance indicated significant linear behavior for degradation of Cu(II)chlorine $e_4$ in SCC at 25 °C ($p < 0.01$), 50 °C ($p < 0.05$), 70 °C ($p < 0.05$), and 100 °C ($p < 0.05$).

indicates an appropriate use of first-order models and allows for the calculation of activation energy ($E_a$) from the slopes of Arrhenius plots (**Figure 5**).

Experimental rate constants and calculated $E_a$ for both crude SCC and the Cu(II)Chlorin $e_4$ component of crude SCC are depicted in **Table 1**. Cu(II)Chlorin $e_4$ was determined to degrade at a rate between 3 and 10 times faster than that of crude SCC. Accumulation of specific SCC thermal degradation products was not observed under the chromatographic conditions employed in this study. However, the formation of unique compounds should not be discounted as observed alteration of SCC absorption spectra, combined with visible olive brown discoloration, allows for the possibility that degradative components

**Table 1.** Reaction Rate Constants and Calculated Activation Energy for Commercial Grade SCC Degradation[a,b]

| temp (°C) | UV–vis $k$ ($min^{-1}$) | $R^2$ | HPLC $k$ ($min^{-1}$) | $R^2$ |
|---|---|---|---|---|
| 25 | $3.20 \times 10^{-4}$ | 0.91 | $9.72 \times 10^{-4}$ | 0.99 |
| 50 | $2.80 \times 10^{-3}$ | 0.97 | $1.49 \times 10^{-2}$ | 0.91 |
| 70 | $6.60 \times 10^{-3}$ | 0.87 | $2.47 \times 10^{-2}$ | 0.93 |
| 80 | $1.60 \times 10^{-2}$ | 0.92 | | |
| 90 | $2.01 \times 10^{-2}$ | 0.88 | | |
| 100 | $2.94 \times 10^{-2}$ | 0.85 | $2.81 \times 10^{-1}$ | 0.97 |
| $E_a$ = | 13.3 ± 0.8 kcal/mol | | 16.0 ± 2.1 kcal/mol | |

[a] Rate values obtained from regression analysis of thermal degradation data (**Figures 3** and **4**). [b] Activation energy values obtained from the slopes of Arrhenius plots (**Figure 5**).

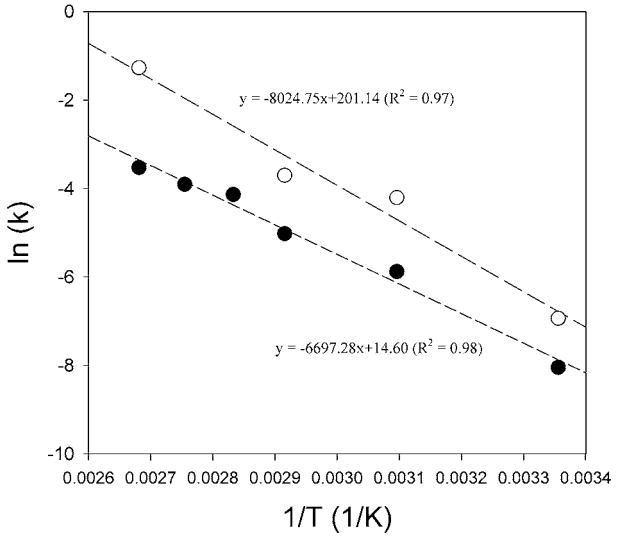


**Figure 5.** Arrenhius plots for crude SCC (●) and Cu(II)Chlorin $e_4$ (○). Plots were constructed from thermal degradation data obtained as described under Materials and Methods. Results of linear regression analysis utilized for calculation of $E_a$ are inset.

of SCC may include copper-free porphyrins or cleavage products similar to those encountered during the degradation of natural chlorophylls (*26*). Electronic absorption spectra of fully degraded SCC maintained a depressed absorbance in the Soret region (406 nm), whereas the absorbance in the Q region was almost completely lost (**Figure 2**). Degradation of the porphyrin structure would result in significant depression of Soret absorbance consistent with previously published results (*23*). Loss of absorbance in the Q region is similar to the spectral shifts associated with chlorophyll to pheophytin transformation (pheophytinization) wherein the central magnesium atom is replaced by two hydrogens (*25*), resulting in an olive brown discoloration encountered during the thermal processing of green vegetables (*19*). Further thermal treatment of chlorophyll compounds may result in a complete deterioration of the tetrapyrrole structure and the formation of colorless porphyrin breakdown products including organic acids (*23*, *25*).

Activation energies determined from the Arrhenius plots were 13.3 ± 0.8 and 16.0 ± 2.1 kcal/mol for crude SCC and Cu(II)Chlorin $e_4$, respectively (**Table 1**; **Figure 5**). These values are similar to those previously reported for natural chlorophyll derivatives (*19*−*21*, *27*) and are indicative of only a minor temperature sensitivity for these copper chlorophyll derivatives. Other degradative food reactions have activation energies similar to those observed for SCC. Specifically, oxidation of vitamin C and lipids occurs with activation energies between 10 and 30 kcal/mol (*28*−*29*). Bleaching of SCC in the dark was

previously shown to be highly aerobic and linked to active oxygen intermediates including peroxides (*22*). Salin et al. (*23*) reported a pseudo-first-order, temperature-dependent rate of SCC photobleaching and concluded that both oxidative and photochemical components were responsible for overall SCC degradation. As oxygen was not excluded from the experimental condition prior to thermal treatments, it is possible that an oxidative component to SCC thermal degradation does exist in this system.

The crude nature of commercial grade SCC allows for the inclusion of a large portion of non-chlorin material, which has not been fully characterized. With the high batch to batch variability often encountered with these commercial preparations, non-chlorin components may comprise as much as 40−50% of total SCC (*13*, *16*). The extent to which these components contribute to SCC degradation is unclear and remains to be investigated.

Although affecting sensorial properties such as color, the thermal degradation of SCC potentially modulates associated biological activity. Loss of the central copper ion from both chlorophyll and chlorin porphyrin backbones is known to significantly reduce measurable antioxidant activity while having minimal impact on in vitro antimutagenic activity (*9*). The antimutagenic activity of SCC has focused on molecular complexation of dietary mutagens through porphyrin−mutagen interactions, which would ultimately reduce the bioavailability of the mutagen (*3*, *30*). Central to this bioactivity is the integrity of the porphyrin macrocycle (*2*, *9*). Therefore, the thermally induced degradation of SCC, which affects the porphyin structure, evidenced by a decrease in absorbance at 407 nm, may negatively affect the antimutagenic activity of SCC and warrant careful consideration and control in formulations.

In the United States, SCC is marketed primarily as a liquid or powdered dietary supplement, whereas in Europe and Asia, SCC is formulated into finished consumer foods including ready-to-drink (RTD) liquid beverage products. Although both are exposed to a certain degree of thermal processing, RTD beverages will be subjected to significant thermal treatment including high-temperature−short-time or ultrahigh−temperature processing, raising the potential for significant SCC degradation. Thermal treatment of aqueous SCC results in temperature- and time-dependent first-order degradation of copper chlorin components. Cu(II)Chlorin $e_4$, a major functional component comprising >85% of the total chlorin fraction of crude SCC utilized in our investigation, was found to degrade between 3 and 10 times faster than crude SCC. Loss of major SCC components such as Cu(II)Chlorin $e_4$ through typical food processing and preparation must be considered as it affects both the color and potential functional properties of this commercial grade chlorophyll preparation. Furthermore, although specific end products of SCC thermal degradation were not identified in this study, future characterization efforts should be considered in light of the potential accumulation of these compounds in processed foods and to better understand the thermal process behavior of SCC.

## ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; SCC, sodium copper chlorophyllin; $E_a$, activation energy; UV−vis, ultraviolet and visible.

## ACKNOWLEDGMENT

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