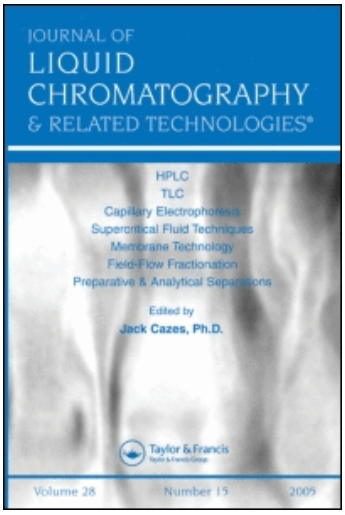

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A REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY/SCANNING DENSITOMETRIC METHOD FOR THE ANALYSIS OF GARDENIA YELLOW IN FOOD USING CROCETIN AS AN INDICATOR

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A REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY/SCANNING DENSITOMETRIC METHOD FOR THE ANALYSIS OF GARDENIA YELLOW IN FOOD USING CROCETIN AS AN INDICATOR

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ABSTRACT

In the present study, a TLC method for the analysis of gardenia yellow in foods using crocetin as an indicator was developed. Gardenia yellow was extracted from food samples with methanol or hydrous methanol, and after the extract was evaporated, the

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residue was dissolved in water and the pH was adjusted to 11 or above with 1 mol/L NaOH. The resultant mixture was occasionally stirred, then allowed to stand in a water bath at 50°C for 30 minutes. Subsequently, the pH of the mixture was slightly acidified using hydrochloric acid. It was then purified through a C18 cartridge before being subjected to the TLC analysis.

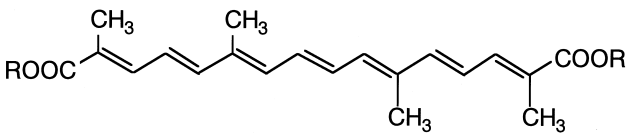
The TLC conditions were as follows: plate, RP-18F254S (Art. 15389, E. Merck); solvent system, acetonitrile-tetrahydrofuran-0.1 mol/L oxalic acid (7:8:7). The visible absorption spectrum of the color was measured using scanning densitometry without isolation of the color. In order to investigate the capability of the present method, 37 commercial foods were analyzed, and their chromatographic behaviors and spectra were observed.

The separation and the spectra obtained were not affected by coexisting substances in the foods, and the spots always gave the same Rf values and spectra as the standard, along with good reproducibility. The present method is considered to be useful for the rapid analysis of gardenia yellow in foods.

INTRODUCTION

Gardenia yellow is a yellow dye obtained by extracting or hydrolyzing the fruit of the *Gardenia augusta* MERR. var. *gardiflora* HORT. with water or ethanol, and is widely used for the coloring of noodles, candy, and candied chestnuts.(1-5) The yellow color of the dye is derived from the carotinoids crocin and crocetin (Fig. 1). Crocetin is the hydrolysis product of crocin.(6)

In our previous report,(7) we published the analytical method for gardenia yellow based on reversed-phase chromatography/scannin densitometry, using crocin as the indicator. However, when this method was applied to samples containing caramel or anthocyanins, their spots overlapped with that of crocin, which



Crocin R = β-D-gentiobiosyl
Crocetin R = H

Figure 1. Main components of gardenia yellow.

made it difficult to identify the gardenia yellow. Also, gardenia yellow added to Chinese noodles have been suggested to be present mostly as crocetin because crocin is hydrolyzed to crocetin during storage.(8) In this study, therefore, we investigated an analytical method using crocetin, generated by the hydrolysis of crocin in alkali, as an indicator for the identification of gardenia yellow in foods.

HPLC, which generally allows excellent separation of mixtures, is often used for the analysis of naturally occurring dyes. However, TLC, which allows the simultaneous analysis of a large number of samples, is widely used for actual analyses at Food Inspection Offices and Centers for Public Health, and is considered to also be more practical for the analysis of gardenia yellow using crocetin as an indicator. Therefore, in this study, we evaluated an analytical method for gardenia yellow based on reversed-phase TLC/scanning densitometry using crocetin as the indicator by hydrolyzing crocin extracted from food samples into crocetin.

EXPERIMENTAL

Samples

Foods available on the Japanese market including Chinese noodle, chocolate, jelly, candy, pickles, mustard, grated *wasabi*, and candied chestnut were used.

Standards and Chemical Reagents

Crocetin from Sigma (St. Louis, MO, USA) and crocin from Wako (Osaka, Japan) were used. The C18 cartridges used in the study were Sep-Pak C18 Vac 3cc (500mg) from Waters (Milford, MA, USA). All the other reagents were of analytical grade from Wako and Kanto Kagaku (Tokyo, Japan).

TLC Conditions

The TLC plate was an RP-18F254S (Art. 15389, E. Merck, Darmstadt, Germany), and the solvent system was acetonitrile-tetrahydrofuran-0.1 mol/L oxalic acid (7:8:7).

Scanning Densitometric Conditions

The scanning densitometer used in the study was a CS-9000 from Shimadzu (Tokyo, Japan). The measurement conditions were as follows: wave-

length scanning range, 370–700 nm; slit size 0.4 × 0.4 mm; method, reflecting absorption

Electrospray Mass Spectrometric Conditons

The mass spectrometer used was a Quattro II (Micromass, Altrincham, UK) with an electrospray ion source and the instrument was operated in the negative mode. The capillary voltage was 3.0 kV, cone voltage was 30-35 kV, the ion source temperature was 130°C, and the desolvation temperature was 350°C.

Preparation of Test Solutions

Dyes were extracted directly from liquid foods such as juice, but from water-soluble foods such as candy and jelly after dissolution and dilution to a sugar concentration close to that of juice. Solid samples such as cake and pickles were homogenized with methanol or hydrous methanol and centrifuged. Uncooked noodles were boiled in a small volume of water, washed well with water, and treated similarly to other solid samples with the addition of methanol. After centrifugation, the supernatant was collected, methanol was removed by evaporation in a vacuum, water was added to the residue, and the solution was used as a dye extract. The dye extracts obtained above were hydrolyzed as follows: After the pH was adjusted to 11 or above with 1 mol/L NaOH, the extracts were allowed to stand in a water bath at 50017°C for 30 minutes. After cooling, they were slightly acidified with 1 mol/L HCl and loaded into a C18 cartridge. The cartridge was washed with 20 mL of water, dyes were eluted with 5 mL of methanol, and a test solution was obtained by concentrating the eluate.

RESULTS AND DISCUSSION

Hydrolysis Conditions of Crocin

In order to examine the optimal conditions, 200 μg/20 mL of standard crocin solution was hydrolyzed by varying the pH of the solution, temperature, and incubation time, and the degree of hydrolysis was followed by reverse-phase TLC after purification, using the C18 cartridge described in the Experimental section.

The pH of the standard solution was serially varied from 7 to 12 by adding NaOH at 0.1 mol/L or 1 mol/L, the solution was allowed to stand in a water bath at 50°C for 30 minutes, and the appearance of the crocin and crocetin spots on

the TLC plates was studied. As shown in Fig. 2, the crocetin spots appeared under all the experimental conditions, but 3 crocin spots clearly remained at pH7 and also faintly at pH9, suggesting an insufficient hydrolysis. At pH10 or above, all the crocin was hydrolyzed to crocetin.

Next, the pH of the solution was fixed at 11, but the solution was incubated in a water bath at 50°C for 5 to 30 minutes, and the appearance of the crocin and crocetin spots on the TLC plates was studied as above. As shown in Fig. 3, the crocin spots were still observed after 10 minutes, but they disappeared, and only crocetin spots were observed after 15 minutes or longer.

Based on these results, as all the crocin in the standard solution was considered to be hydrolyzed by incubation at 50° for 15 minutes, the dye extracts from foods were analyzed under these conditions. However, due to the presence of contaminants such as carbohydrates in the dye extracts from the foods, hydrolysis did not progress as smoothly as in the standard solution, and the crocin spots did not disappear after the 15-minute incubation in many samples. Therefore, we

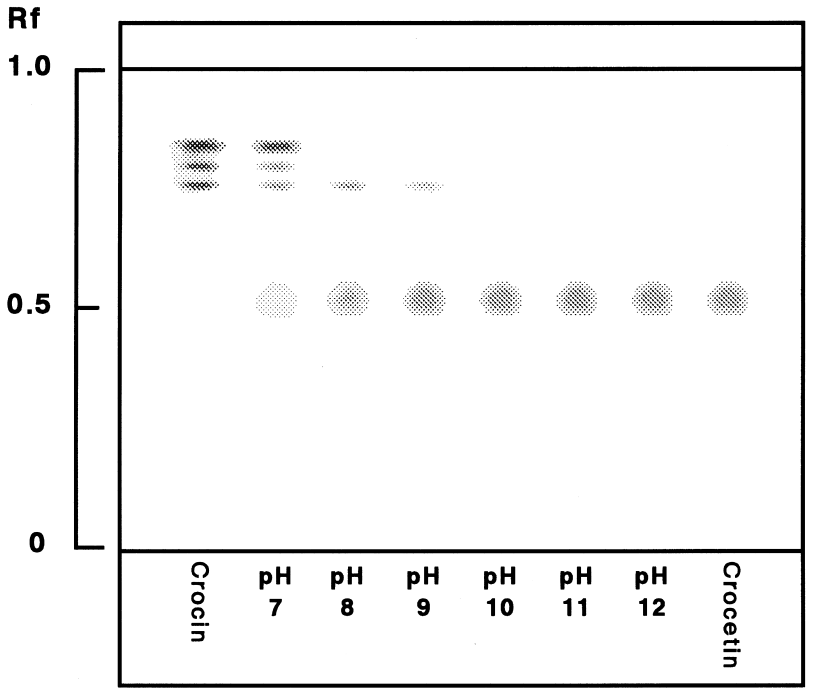


Figure 2. Effect of pH of the extract on the hydrolysis of crocin. Temperature: 50°C, standing time: 30 min. TLC conditions: see Experimental section.

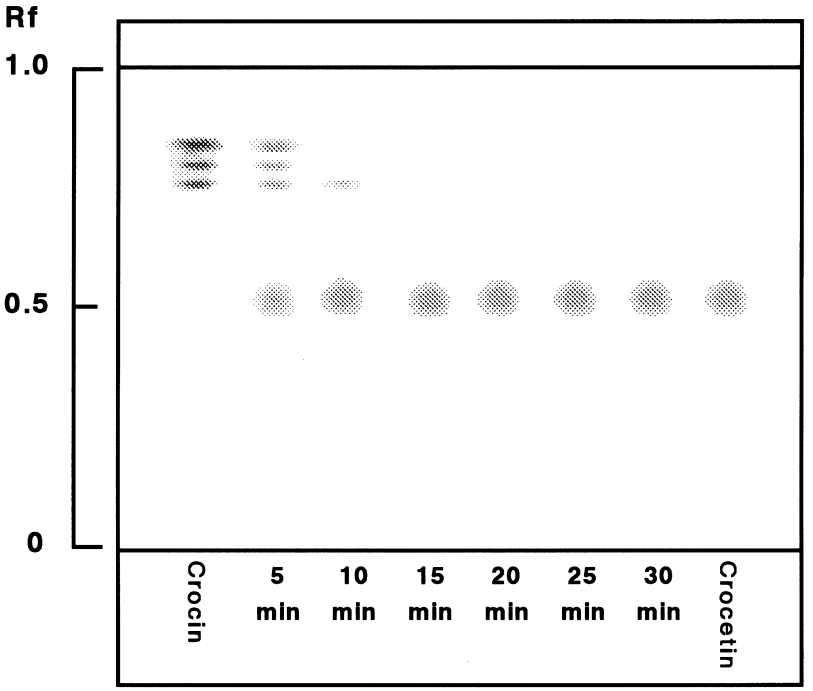


Figure 3. Effect of the standing time of the extract on the hydrolysis of crocin. Temperature: 50°C, pH: 11. TLC conditions: see Experimental section.

decided to hydrolyze the samples by adjusting the pH to 11 or above and incubating them at 50°C for 30 minutes.

Measurement of Visible Spectrum by Scanning Densitometry and Electrospray Mass Spectrum

Reflection spectra of the spots on the TLC plates separated under the conditions described in the Experimental section were measured at scanning wavelengths of 370-700 nm. Figure 4 shows the visible spectra obtained, and the maximum absorption wavelengths were 435 nm and 460 nm, being in complete agreement with the visible absorption spectrum for the standard preparation of crocetin. To further confirm that crocetin was generated by the hydrolysis of crocin, the electrospray mass spectrum was measured. As shown in Fig. 5, a deprotonated molecule ($[M-H]^-$) was detected at m/z327, and $[M-H-CO_2]^-$ was detected at m/z287, which were results identical with those in the mass spectrum

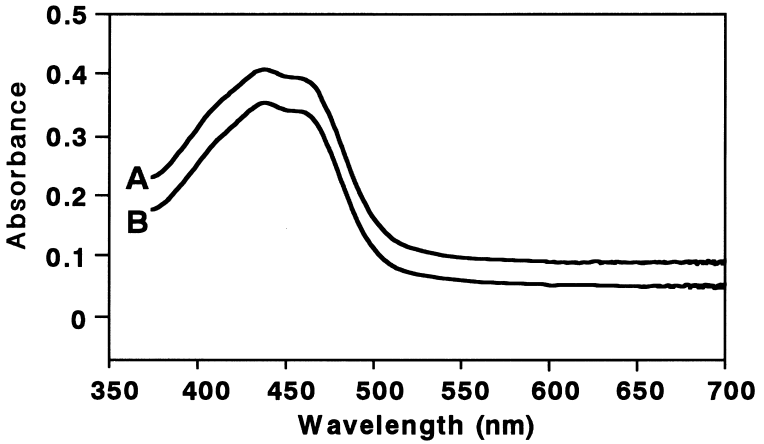


Figure 4. Visible spectra of crocetin and the crocin hydrolyzed under TLC/scanning densitometry. A) Crocetin, B) the crocin hydrolyzed. TLC/scanning densitometric conditions: see Experimental section.

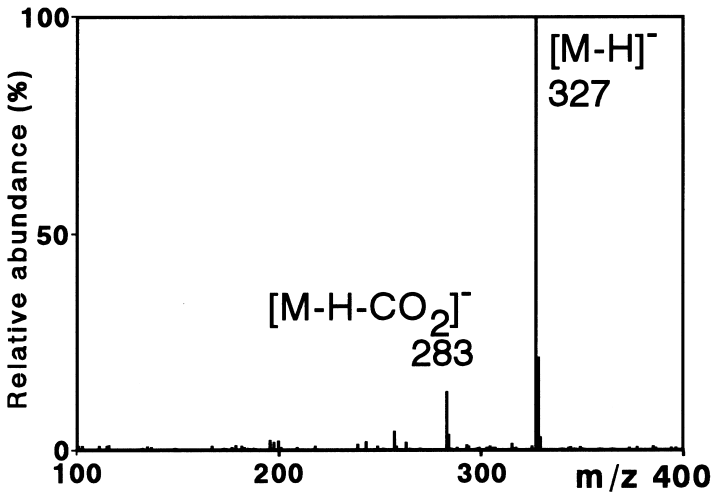


Figure 5. Electrospray mass spectrum of the crocin hydrolyzed. ESI MS conditions: see Experimental section.

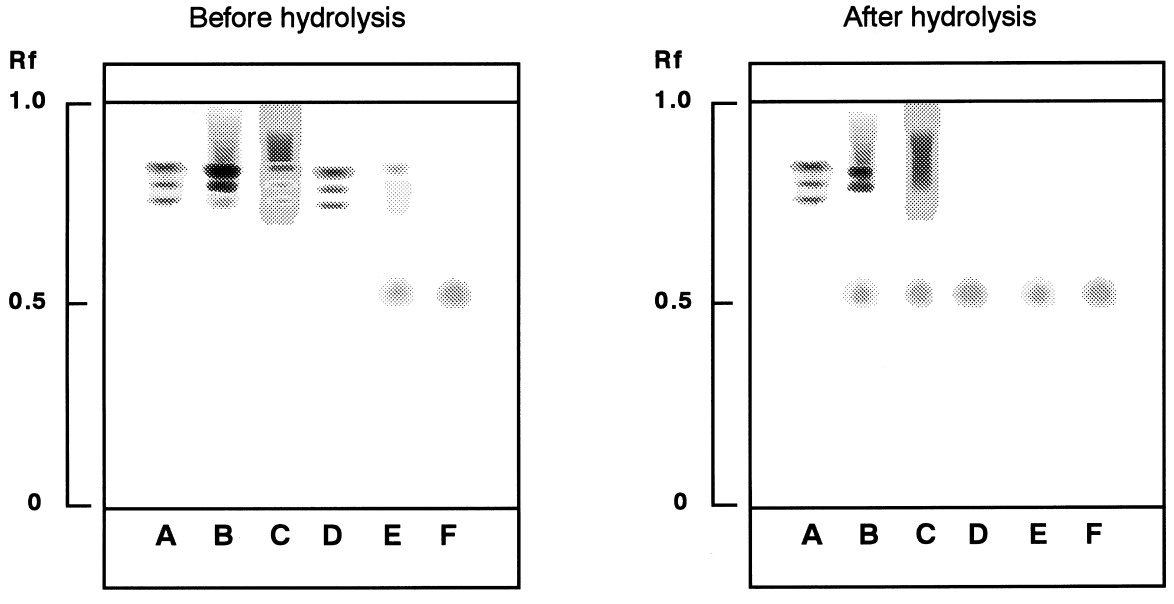


Figure 6. Effect of hydrolysis on the analysis of gardenia yellow. A: Crocin, B: Candy, C: Pickles, D: Chocolate, E: Chinese noodle, F: Crocetin. TLC conditions: see Experimental section.

of the standard preparation of crocetin. Therefore, the hydrolysis product of crotin was identified as crocetin.

Application to Commercial Foods

Application to Foods for Which Crocin Was Difficult to Use as an Indicator

Foods that contained caramel or anthocyanins and Chinese noodles, for which the identification of gardenia yellow was impossible by the analytical method using crocin as an indicator due to the appearance of interfering spots at the same positions as the spots of crocin on the reverse-phase TLC plates (Fig. 6 left), were analyzed by the present method. As shown in Fig. 6 (right), crocetin spots were not observed before hydrolysis, but appeared as clear spots after hydrolysis. Also, in Chinese noodles, the spots of both crocin and crocetin were observed before hydrolysis, but all spots became crocetin after hydrolysis. Moreover, the shape and Rf value of each spot were in close agreement with those of the standard preparation, so that gardenia yellow was able to be identified by using crocetin as the indicator.

Reproducibility of the Rf Value by Reverse-Phase TLC

To examine the effects of the contaminants contained in the sample on the Rf value, 37 commercial foods were purified by the above method and analyzed by reverse-phase TLC. The obtained Rf values of the spots were then compared. The difference between the Rf value of the standard dye and the Rf value of the dye in the sample was expressed as the ratio between the Rf value of the dye in the sample (Ra) and the Rf value of the standard dye (Rs), and the reproducibility was evaluated according to the coefficient of variation of this ratio.(9,10)

As shown in Table 1, the mean value of Ra/Rs was 0.99, and the coefficient of variation was 2.5%. These results suggest that the spots of crocetin generated

Table 1. Ra/Rs Values of Crocetin in Foods on Reversed Phase TLC

	Rf Value	Average Ra/Rs Value[a]	C.V. (%)	n
Crocetin	0.55	0.99	2.5	37[b]

[a]Ratio of Rf (sample)/Rf (standard).
[b]Candy, pickles, jelly, mastard, grated wasabi, Chinese noodle, chocolate, sweet-boiled Japanese chestnut, etc.

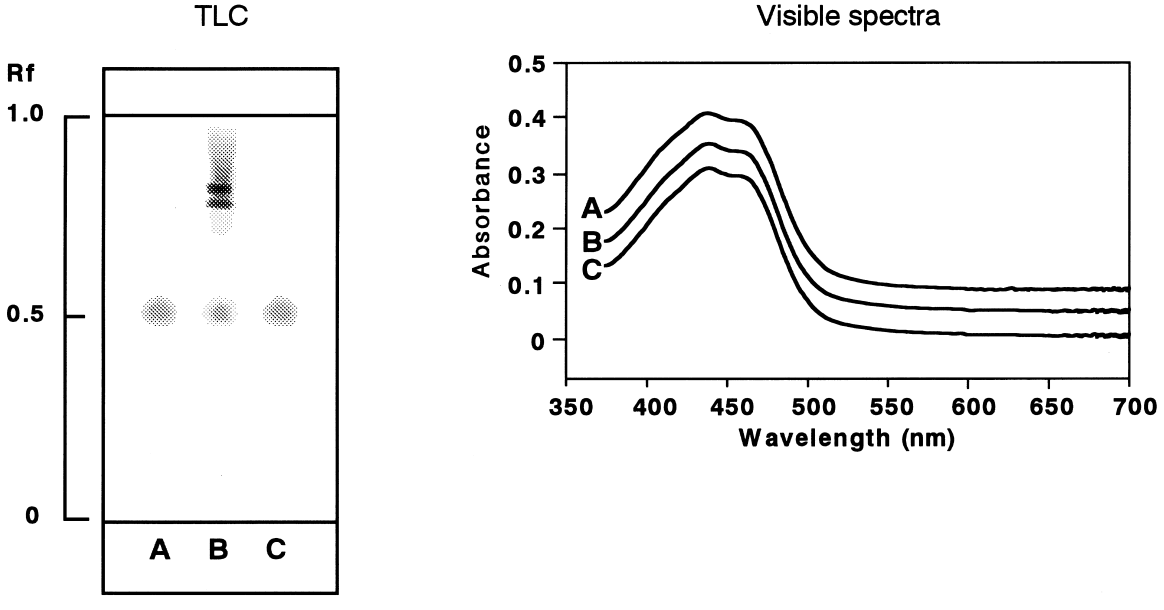


Figure 7. Thin-layer chromatograms and visible spectra of the hydrolyzed extract of various foods under TLC/scanning densitometry. A) Crocetin, B) Candy, C) Chocolate. TLC/scanning densitometric conditions: see Experimental section.

by hydrolysis appear nearly at the same positions as those of the standard dye, without being affected by contaminants in the sample, and that the identification of the dye is reliable and reproducible. Therefore, the method is considered to be sufficiently applicable to routine analyses at facilities such as the Centers of Public Health and the Food Inspection Office.

Identification by Reverse TLC/Scanning Densitometry

The visible spectra of the crocetin spots on the reverse-phase TLC plates, for which the reproducibility of the Rf value had been evaluated, were measured using a scanning densitometer. Figure 7 shows the typically obtained TLC chromatograms and spectra obtained. The spectra of the dyes purified from foods were in close agreement with that of the standard dye, and the identification reliability was then established.

CONCLUSIONS

A method for analysis of gardenia yellow using crocetin as an indicator was evaluated, and the following results were obtained:

(1) Crocin was hydrolyzed to crocetin by adjusting the pH of the dye extract to 11 or above and incubating the extract in a water bath at 50°C for 30 minutes.

(2) When the crocetin spots that appeared on the TLC plates were analyzed by scanning densitometry, satisfactory visible spectra were obtained, and the spectra of the spots of the dye contained in commercial foods were in complete agreement with those of the spots of the standard preparation.

(3) When 37 commercial foods were analyzed by this method, the crocetin spots were consistently observed on the TLC plates, and their Rf values were highly reproducible.

From these results, reverse-phase TLC/scanning densitometry is shown to be effective for the analysis of gardenia yellow.

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