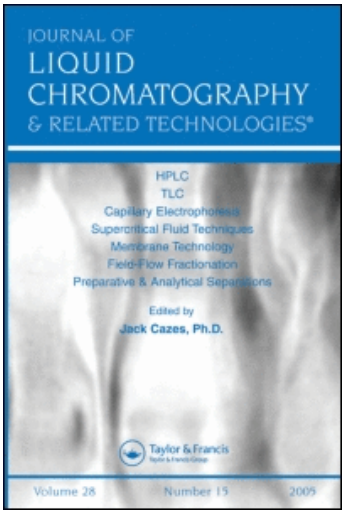

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A REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY/SCANNING DENSITOMETRIC METHOD FOR THE ANALYSIS OF RED CABBAGE COLOR IN FOOD

Yuko Itakura,[1] Naoko Ozeki,[1] Hisao Oka,[2,*] Yuko Ito,[2] Eiji Ueno,[2] Tomomi Goto,[2] Tomoko Hayashi,[3] Hitomi Ohno,[1] Yasuhisa Sasaki,[1] Masakuni Mukoyama,[1] Hiroshi Matsumoto,[2] and Hisamitsu Nagase[4]

[1]Okazaki Public Health Center, Aichi Prefecture, 3-30, Kosei-dorinishi, Okazaki, Aichi 444-8545, Japan
[2]Aichi Prefectural Institute of Public Health, 7-6, Nagare, Tsuji-machi, Kita-ku, Nagoya 462-8576, Japan
[3]Ichinomiya Public Health Center, Aichi Prefecture, 1-3, Kogane-machi, Ichinomiya, Aichi 491-0867, Japan
[4]Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan

ABSTRACT

A technique for the analysis of red cabbage color using reversed-phase TLC and scanning densitometry is described.

*Corresponding author. E-mail: hisaooka@alles.or.jp

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The technique involves the following three steps: 1) clean up of the color with a C18 cartridge, 2) separation of the colors on the reversed-phase C18-TLC using acetonitrile-0.2 mol/L trifluoroacetic acid (1 : 2) as the solvent system, and 3) measurement of visible absorption spectra of the color using scanning densitometry without isolation of the color. In order to investigate the capability of the present method, 45 commercial foods were analyzed, and their chromatographic behaviors and spectra were observed. The obtained separation and the spectra were not affected by coexisting substances, including grape skin color, elderberry color, perilla color, and cochineal color in the foods, and the spots always gave the same Rf values and spectra as the standards with good reproducibility. The present method is considered to be useful for the rapid analysis of red cabbage color in foods.

INTRODUCTION

In recent years, public concern over the use of synthetic colors in foods has grown rapidly and consumer preference has led to greater use of natural colors in foods. Anthocyanins, represented by the red cabbage color, are widely used as food colors. However, a simple, reliable, and rapid analysis method of the anthocyanins, which allows the simultaneous analysis of a large number of samples, has not been reported.

Red cabbage color is a red dye obtained by extracting or hydrolyzing the red leaf of the *Brassica oleracea* LINNE var. *capitata* DC. with water under weak acidic conditions.[1,2] The red color of the dye is derived from derivatives of cyanidin acylglucoside.[3,4] This color shows a red-purple color under acidic conditions, and is highly heat- and light-resistant, especially at pHs lower than 3.0. By making use of these properties, it is widely used for the coloring of juice, candy, jelly, chewing gum, fruit wine, etc.[1]

In our previous studies,[5–8] we have already established simple, rapid, and reliable methods for the analysis of various natural colors, including annatto, gardenia yellow, carotene, turmeric oleoresin, lac, cochineal, and paprika using reversed-phase thin-layer chromatography/scanning densitometry. Therefore, in this study, we decided to develop a simple, reliable, and rapid analysis method for the red cabbage color in foods using reversed-phase thin-layer chromatography/scanning densitometry, and we describe the techniques for the separation and identification of this color in foods.

EXPERIMENTAL

Samples

Foods available on the Japanese market, including jelly, candy, juice, marshmallow, and fruit wine were used.

Standards and Chemical Reagents

Red cabbage color, grape skin color, and cochineal color were from Kanto Chemical (Tokyo, Japan), perilla color from Aizen (Tokyo, Japan), and elderberry color from San-Ei Gen FFI (Osaka, Japan) were used. The C18 cartridges used in the study were Sep-Pak C18 Vac 3cc (500 mg) 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-0.2 mol/L trifluoroacetic acid (TFA) (1 : 2).

Scanning Densitometric Conditions

The scanning densitometer used in the study was a CS-9000 from Shimadzu (Tokyo, Japan). The measurement conditions were as follows: wavelength scanning range, 370–700 nm; slit size 0.4×0.4 mm; method, reflecting absorption.

Preparation of Test Solutions

Using a C18 cartridge preconditioned with methanol and 0.1% TFA, the color was directly extracted from the liquid foods such as juice, but was extracted from water-soluble foods, such as candy and jelly, after dissolution was extracted with 0.1% TFA and dilution to a sugar concentration close to that of the juice. The cartridge was washed with 10 mL of 0.1% TFA, the color was eluted with 5 mL of methanol-0.1% TFA (9 : 1), and a test solution was obtained by concentrating the eluate.

RESULTS AND DISCUSSION

Separation by Reversed-Phase

After various experiments, the best results were achieved using acetonitrile-0.2 mol/L TFA (1 : 2) as the solvent system on the C18 TLC plate. As shown in Figure 1A, the red cabbage color standard was separated into four spots and the Rf values of the two main spots were 0.39 and 0.34.

The various experimental results to obtain the optimal separation conditions are discussed below.

Addition of Acid

The red cabbage color standard showed extreme tailing on the C18 TLC plate using an aqueous solution of acetonitrile or methanol as the solvent systems. It is well known that acidic solvent systems have good separation of anthocyanin colors without tailing, because anthocyanin colors give stable structures under acidic conditions. However, the anthocyanin colors are easily hydrolyzed in the presence of mineral acids, such as hydrochloric acid and sulfuric acid.[9] Therefore, we decided to acidify the solvent system using TFA.

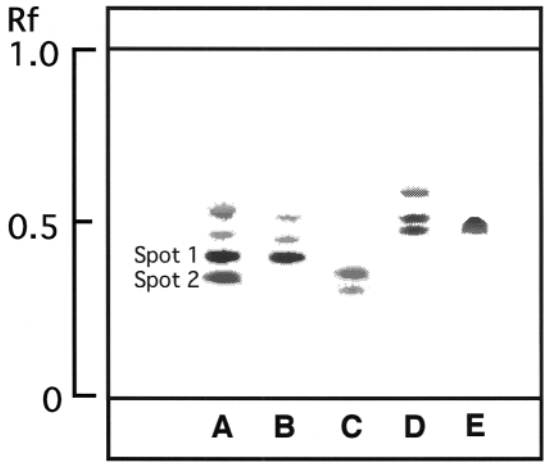

Figure 1. Thin-layer chromatograms of red cabbage, grape skin, perilla elderberry, and cochineal color standards. A) Red cabbage color; B) Grape skin color; C) Perilla color; D) Elderberry color; E) Cochineal color. TLC conditions: see Experimental.

Using acetonitrile-TFA (1 : 2) as the solvent system, the influence of the acid concentration was examined. The resolution of the spots increased upon increasing the acid concentration, so that good resolution was obtained above 0.2 mol/L. Therefore, we used 0.2 mol/L TFA in the subsequent experiments.

Combination of Acetonitrile and TFA

Various combinations of acetonitrile and 0.2 mol/L TFA were tested to determine the suitable solvent system for use with the C18 TLC plates. As shown in Figure 2, the concentrations of acetonitrile higher than 40% in the solvent system resulted in unsatisfactory separation of the red cabbage color components (3 spots). The best separation was obtained below 40%. Because the Rf values were too low using any concentration below 30% acetonitrile, we recommend acetonitrile-0.2 mol/L TFA (1 : 2) as the solvent system.

Influence of Other Coexisting Natural Colors

The red cabbage color is frequently used together with grape skin color, elderberry color, perilla color, and cochineal color. We investigated the chromatographic behaviors of these colors under the optimal TLC conditions

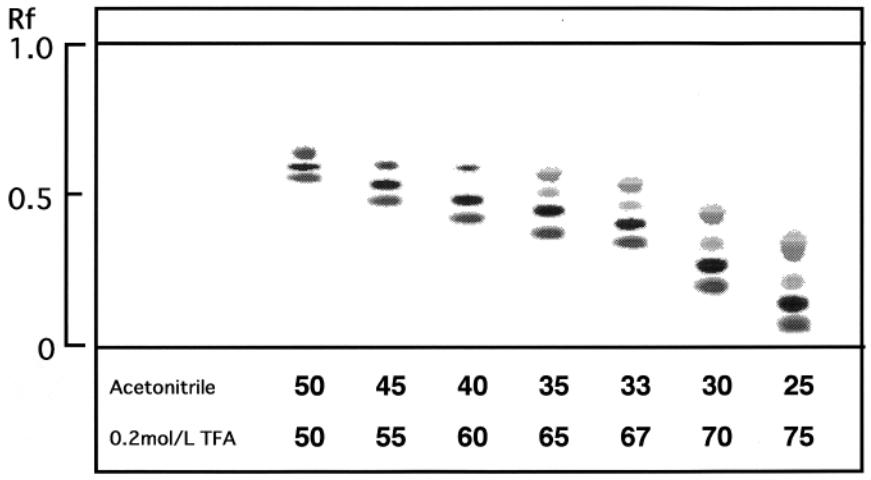

Figure 2. Effect of ratio of acetonitrile and 0.2 mol/L trifluoroacetic acid on the separation of red cabbage color. TLC conditions: see Experimental.

described in the experimental section. As shown in Figure 1, the main spots, 1 and 2 (Rf = 0.39 and Rf = 0.34), of the red cabbage color completely separated from the elderberry color and cochineal color, so we could identify the red cabbage color with these coexisting two colors. On the other hand, spots of grape skin color and perilla color overlapped spots 1 and 2, respectively. However, as described below, measuring the visible absorption spectra of the spots on the TLC plate made it possible to identify the red cabbage color, even if the grape skin color and perilla color exist in the test solution. Therefore, we chose the TLC conditions described in the experimental section.

Measurement of Visible Spectrum by Scanning Densitometry

Reflection spectra of the main spots, 1 and 2 (Rf = 0.39 and Rf = 0.34), of the red cabbage color standard on the TLC plates separated under the conditions described in the experimental section, were measured at scanning wavelengths of 370–700 nm. Figure 3 shows the obtained visible spectra, and the maximum absorption wavelengths were 546 nm and 528 nm for spots 1 and 2, respectively.

Using scanning densitometry, we tried to identify the red cabbage color with the coexisting grape skin color that gave unsatisfactory separation on the TLC plate. After the separation of a mixture of the red cabbage and grape skin color standards on the C18 TLC plate (Figure 4B), the visible spectra of spots 1 and 2 were measured. The spectrum of spot 1 did not agree with that of spot 1 of only the red cabbage color (Figure 4A), due to the influence of the grape skin color. However, as shown in Figure 4 right, the spectrum of spot 2 completely agreed with that of spot 2 of only the red cabbage color (Figure 4A).

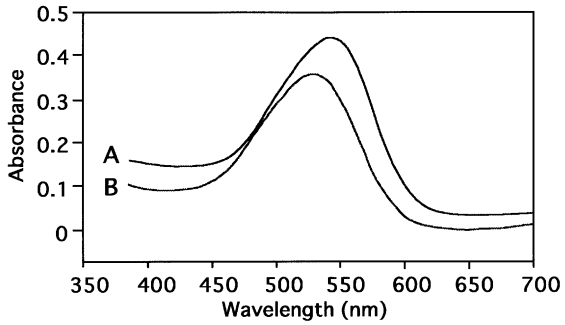

Figure 3. Visible spectra of red cabbage color standard under TLC/scanning densitometry. A) Spot 1; B) Spot 2. TLC conditions: see Experimental.

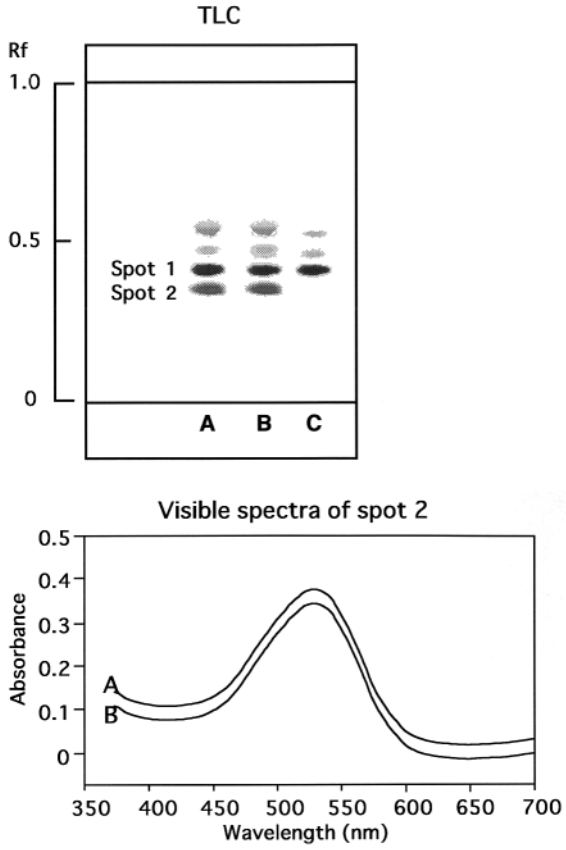

Figure 4. Thin-layer chromatograms and visible spectra of red cabbage color containing grape skin color under TLC/scanning densitometry. A) Red cabbage color standard; B) Red cabbage color standard containing grape skin color standard; C) Grape skin color standard. TLC/scanning densitometry conditions: see Experimental.

For the identification of the red cabbage color with the coexisting perilla color, the measurement of visible absorption spectrum of spot 1 was effective, although that of spot 2 was affected by the coexisting perilla color (Figure 5).

Thus, using the present method, we can identify the red cabbage color even if grape skin color, elderberry color, perilla color, and cochineal color coexist with red cabbage color by the combined use of C18 TLC and scanning densitometry.

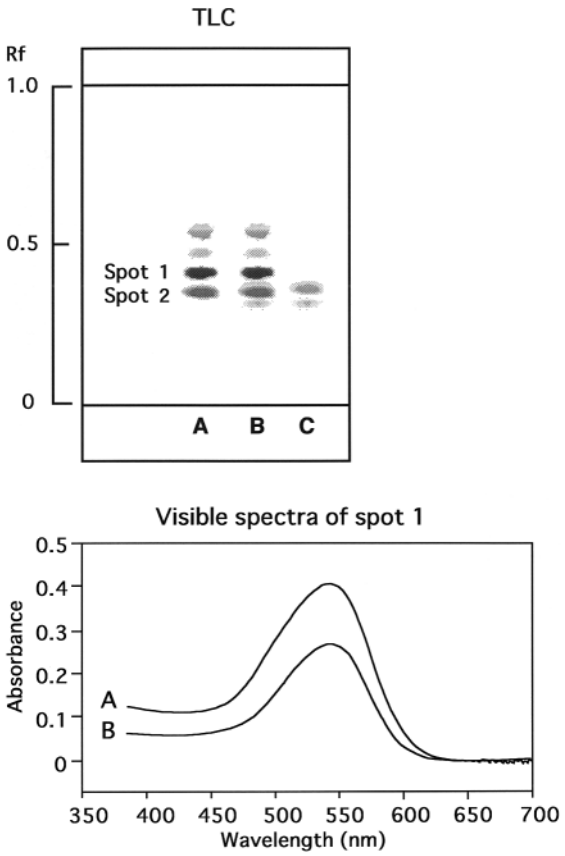

Figure 5. Thin-layer chromatograms and visible spectra of red cabbage color containing perilla color under TLC/scanning densitometry. A) Red cabbage color standard; B) Red cabbage color standard containing perilla color standard; C) Perilla color standard. TLC/scanning densitometry conditions: see Experimental.

Application to Commercial Foods

Reproducibility of the Rf Value by Reverse-Phase TLC

To examine the effects of the contaminants contained in the sample on the Rf value, 45 commercial foods were purified by the method described in the experimental section 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.[10] As shown in Table 1, the average Ra/Rs values were 1.00 with the coefficient of variation of 2.6% and 0.99 with 1.7% for spots 1 and 2, respectively. These results suggest that the main spots, 1 and 2, extracted from the samples, appear nearly at the same positions as those of the red cabbage standard without being affected by contaminants in the sample, and that the identification of the color is reliable and reproducible. Therefore, the method

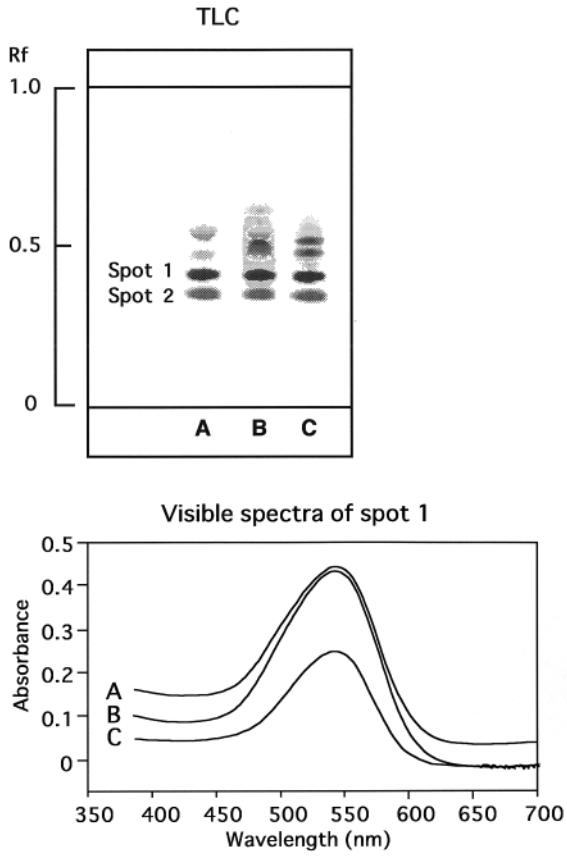

Figure 6. Thin-layer chromatograms and visible spectra of the extracts from various foods under TLC/scanning densitometry. A) Red cabbage color standard; B) Juice containing red cabbage and cochineal colors; C) Candy containing red cabbage and unknown anthocyanin colors. TLC/scanning densitometry conditions: see Experimental.

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 main spots of the red cabbage color on the C18 TLC plates, for which the reproducibility of the Rf value had been evaluated, were measured using a scanning densitometer. Figures 6 and 7 show the typically

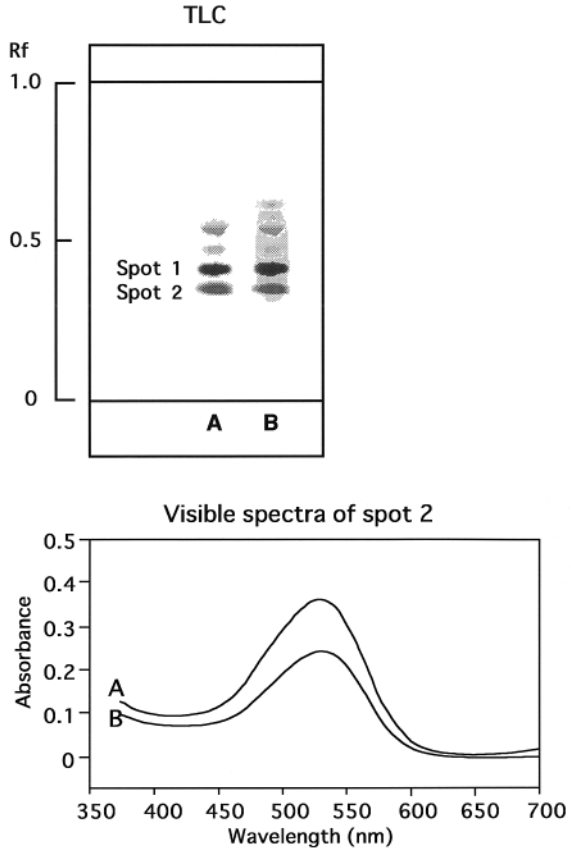

Figure 7. Thin-layer chromatograms and visible spectra of the extracts from juice under TLC/scanning densitometry. A) Red cabbage color standard; B) Juice containing red cabbage and grape skin colors. TLC/scanning densitometry conditions: see Experimental.

obtained TLC chromatograms and spectra obtained on spot 1 that coexists with the cochineal color in juice, and on spot 2 that coexists with the grape skin color in juice, respectively. The spectra of the color purified from foods were in good agreement with that of the standard color, and the identification reliability was then established.

CONCLUSIONS

Using a reversed-phase TLC/scanning densitometry, we established a simple, rapid, and reliable method for the analysis of red cabbage color in foods and the following results were obtained.

(1) The combined use of the C18 TLC plate and acetonitrile-0.2 mol/L TFA (1 : 2) as a solvent system gave the best results for the separation of the red cabbage color.
(2) When the red cabbage color spots that appeared on the TLC plates were analyzed by scanning densitometry, satisfactory visible spectra were obtained, and the spectra of the color spots contained in commercial foods were in complete agreement with those of the spots of the red cabbage color standard.
(3) When 45 commercial foods were analyzed by the present method, the spots of the red cabbage color were consistently observed on the TLC plates, and their Rf values were highly reproducible.
(4) The present method could be successfully applied to the identification of the red cabbage color in foods with coexisting colors such as cochineal color, grape skin color, perilla color, and elderberry color.

Based on these results, reverse-phase TLC/scanning densitometry was shown to be effective for the analysis of the red cabbage color.

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