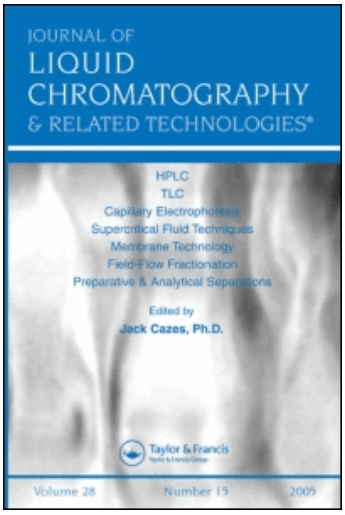

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A REVERSED-PHASE TLC/SCANNING DENSITOMETRIC METHOD FOR THE ANALYSIS OF TOMATO, ORANGE, AND MARIGOLD COLORS IN FOOD

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A REVERSED-PHASE TLC/SCANNING DENSITOMETRIC METHOD FOR THE ANALYSIS OF TOMATO, ORANGE, AND MARIGOLD COLORS IN FOOD

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ABSTRACT

In the present study, a thin-layer chromatography (TLC) method for the analysis of tomato, orange, and marigold colors in foods was developed. The colors were extracted from food samples with ethyl ether, and after the extract was evaporated, the residue was dissolved in methanol. For the analysis of the tomato color,

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after the addition of water to the methanol solution, it was then purified through a C_{18} cartridge before being subjected to the TLC analysis. With respect to the analyses of the orange and marigold colors, after adding 2 mL of a 5% sodium hydroxide–methanol solution to the methanol solution, the mixture was occasionally stirred, kept away from light, and then allowed to stand for 24 hours at room temperature. Subsequently, the pH of the mixture was adjusted to 4.5 or less using 1 mol/L hydrochloric acid. The mixture was then purified through a C_{18} cartridge before being subjected to the TLC analysis.

The TLC conditions were as follows: plate, RP-18F254S (Art. 15389, E. Merck); solvent system, acetonitrile–acetone–*n*-hexane (11 : 7 : 2) and acetone–water (9 : 1). The visible absorption spectra of the colors were measured using scanning densitometry without isolation of the colors. In order to investigate the capability of the present method, 95 commercial foods (33 for the tomato color, 38 for the orange color, and 24 for the marigold color) were analyzed, and their chromatographic behaviors and spectra were observed. The separation and obtained spectra were not affected by coexisting substances in the foods and the spots always gave the same *Rf* values and spectra as the standard with good reproducibility. The present method is considered to be useful for the rapid analysis of the tomato color, orange color, and marigold color in foods.

INTRODUCTION

Recently, natural colorings are being used more and more widely because consumers prefer them. Among them, there are many carotenoid colorings including annatto, gardenia yellow, saffron, carotene, paprika, tomato, orange, and marigold, which are used in a variety of foods.[1] Therefore, a simple, rapid, and reliable method for the analysis of these colors in food needs to be developed. In our previous studies,[2–6] we had already established simple, rapid, and reliable methods for the analysis of annatto, gardenia yellow, carotene, and paprika using reversed-phase thin-layer chromatography (TLC)/scanning densitometry. In this study, we decided to develop an analysis method for the tomato, orange, and marigold colors in foods using reversed-phase TLC/scanning densitometry.

The tomato color is obtained by extraction from the fruit of *Lycopersicon esculentum* MILL. and contains several components. The main component is lycopene (Fig. 1 top).[7] This color is yellow-red and highly heat- and

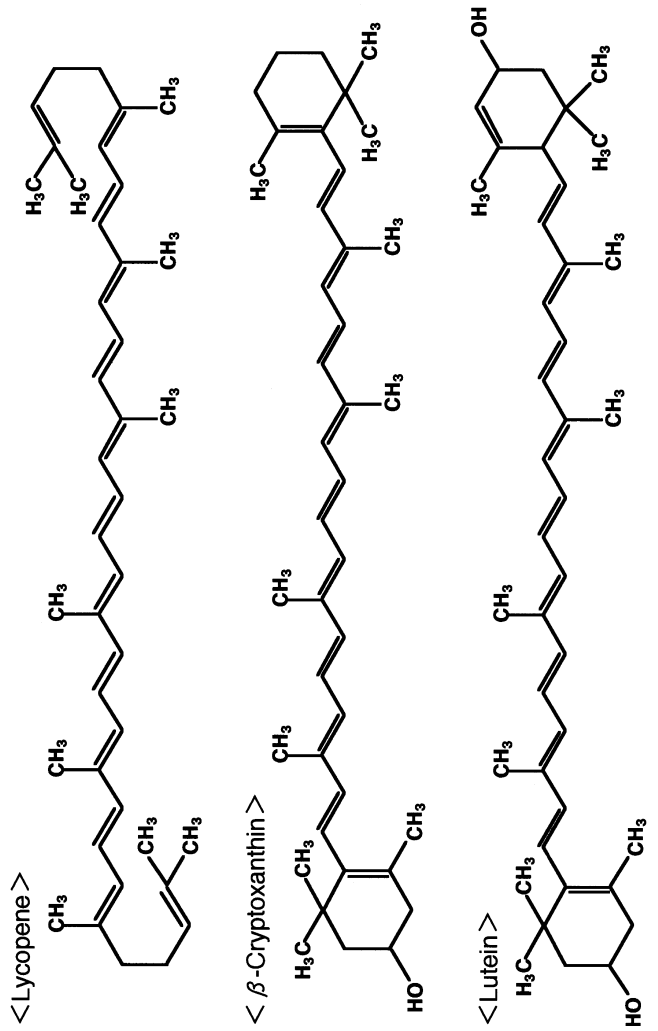

Figure 1. Structures of lycopene, β-cryptoxanthin, and lutein.

light-resistant, but readily oxidizes. This color is used for coloring tomato processed foods, processed marine products, jelly, and candy.

The orange color is obtained from the fruit or rind of *Citrus sinensis* OSBECK and contains several components. The main component is a fatty acid ester of β-cryptoxanthin (Fig. 1 middle).[8] This color is yellow-orange and used for the coloring of citrus fruit beverages, confectionery, and sherbet.

The marigold color is obtained by extraction from the flower of *Tagetes erect* WILLD. and contains several components. The main component is a fatty acid ester of lutein (Fig. 1 bottom).[9] The color is strong yellow, relatively heat resistant and light resistant, and used for coloring beverages, confectionery, and fatty foods.

In the present paper, we describe the techniques for the separation and identification of the tomato, orange, and marigold colors in foods using reversed-phase TLC/scanning densitometry.

EXPERIMENTAL

Samples

Foods available on the Japanese market including candies, juices, sherbets, marmalades, spaghetti sauces, ketchups, and noodles were used.

Standards and Chemical Reagents

Lycopene from Sigma-Aldrich (Steinheim, Germany), β-cryptoxanthin from Extrasynthese (Lyon, France), and lutein from DHI Water and Environment (Hørsholm, Denmark) were used. The C_{18} cartridges used in the study were Sep-Pak C18 Vac 3cc (500 mg) from Waters (Milford, MA). All the other reagents were of analytical grade from Wako (Osaka, Japan) and Kanto Kagaku (Tokyo, Japan).

Thin-Layer Chromatographic Conditions

The TLC plate was an RP-18F254S (Art. 15389, E. Merck, Darmstadt, Germany), and the solvent systems were (I) acetonitrile–acetone–*n*-hexane (11 : 7 : 2) and (II) acetone–water (9 : 1).

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

Tomato Color

This color was directly applied to a C_{18} cartridge from liquid foods such as juices, but from water-soluble foods such as candies and jellies after dissolution and dilution to a sugar concentration close to that of the juice. The solid samples were homogenized with ethyl ether and water, and then centrifuged. After centrifugation, the supernatant was collected, the ethyl ether removed by evaporation in a vacuum, and methanol added to the residue. After the addition of 20 mL of water to the methanol solution, the solution was loaded onto a C_{18} cartridge that had been activated with methanol and water (5 mL each) in advance. The cartridge was then washed with 10 mL of water, the color was eluted with 20 mL of *n*-hexane, and a test solution was obtained by concentrating the eluate.

Orange and Marigold Colors

These colors were directly saponified from the liquid foods such as juice, but from water-soluble foods such as candies and jellies, after dissolution and dilution to a sugar concentration close to that of the juice. The solid samples were homogenized with ethyl ether and water, and then centrifuged. After centrifugation, the supernatant was collected, the ethyl ether removed by evaporation in a vacuum, and methanol added to the residue. It was then saponified in the following manner: after adding 2 mL of a 5% sodium hydroxide (NaOH)–methanol solution, the mixture was placed in a tightly stoppered container, and allowed to stand for 24 hours at room temperature, occasionally stirred and kept away from light. Subsequently, 20 mL of water was added and the pH of the mixture was adjusted to 4.5 or less using 1 mol/L hydrochloric acid. The mixture was loaded onto a C_{18} cartridge that had been activated with methanol and water (5 mL each) in advance. The cartridge was then washed with 10 mL of water, the colors eluted with 20 mL of *n*-hexane and 5 mL of acetone, and a test solution obtained by concentrating the eluate.

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RESULTS AND DISCUSSION

Reversed-Phase Thin-Layer Chromatography

As shown in Fig. 2A, reversed-phase TLC of the standard tomato color yielded a red main spot at an *Rf* value of 0.46 under TLC conditions I and at an *Rf* value of 0.27 under conditions II described in the Experimental section. Under both conditions, the main spot was identical with the spot of the lycopene standard in terms of *Rf* value, color, and shape (Fig. 2B). These results suggest that the tomato color can be identified using lycopene as an indicator.

Reversed-phase TLC of the standard orange color and standard marigold color revealed many continuous spots, thus showing poor separation (Fig. 2C and F). This appeared to be due to the many types of esters contained in this color. Therefore, as with the paprika color in our previous study,[10] this color was hydrolyzed into carotenoid and fatty acid by saponification and then subjected to TLC. The main spot of saponified orange color was clearly observed at an *Rf* value of 0.37 under TLC conditions I and at an *Rf* value of 0.36 under conditions II (Fig. 2D). Under both conditions, its *Rf* value, color, and shape were consistent with those of β-cryptoxanthin (Fig. 2E).

In TLC of the standard saponified marigold color, a yellow main spot was clearly observed at an *Rf* value of 0.43 under TLC conditions I and at an *Rf* value of 0.55 under conditions II (Fig. 2G). Under both conditions, the *Rf* value, color, and shape were identical with those of the lutein standard (Fig. 2H).

These results strongly suggest that the orange color and marigold color can be identified using β-cryptoxanthin and lutein, respectively, after saponification as an indicator.

Measurement of Visible Absorption Spectra by Scanning Densitometry

The reflection absorption spectra of the spots separated by reversed-phase TLC under the above conditions were measured using a scanning densitometer at a scanning wavelength of 370–700 nm. As shown in Fig. 3, from the spot of each color, clear visible absorption spectra were obtained and the maximum absorption wavelength of the main spot was 470 nm for the tomato color, 455 nm for the saponified orange color, and 450 nm for the saponified marigold color. There was complete agreement in the visible absorption spectra between the main spot of the standard tomato color and the standard lycopene, between the main spot of the saponified standard orange color and the standard β-cryptoxanthin, and between the main spot of the saponified standard marigold color and the standard lutein.

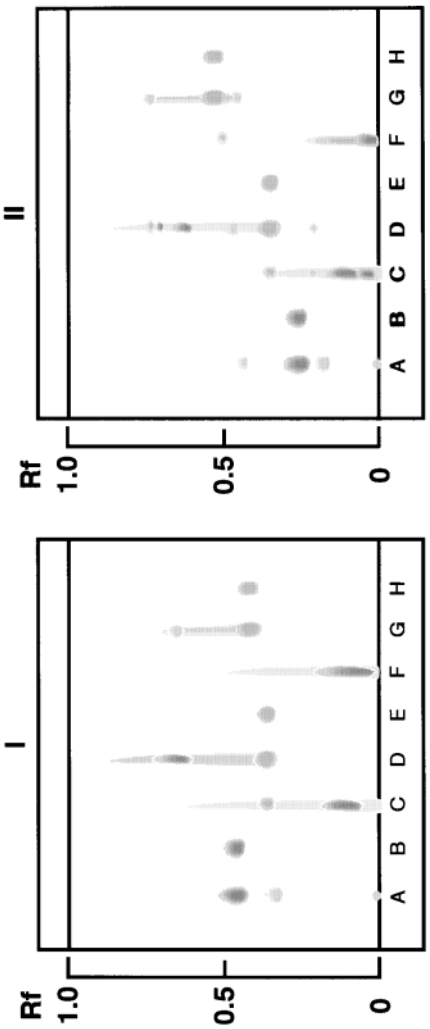

Figure 2. Thin-layer chromatograms of tomato, orange, and marigold colors. A) Tomato color. B) Lycopene. C) Orange color (before saponification). D) Orange color (after saponification). E) β-Cryptoxanthin. F) Marigold color (before saponification). G) Marigold color (after saponification). H) Lutein. TLC conditions: Plate: RP-18F 254s (E. Merck). Solvent system: I: acetonitrile–acetone–*n*-hexane = 11 : 7 : 2. II: acetone–water = 9 : 1.

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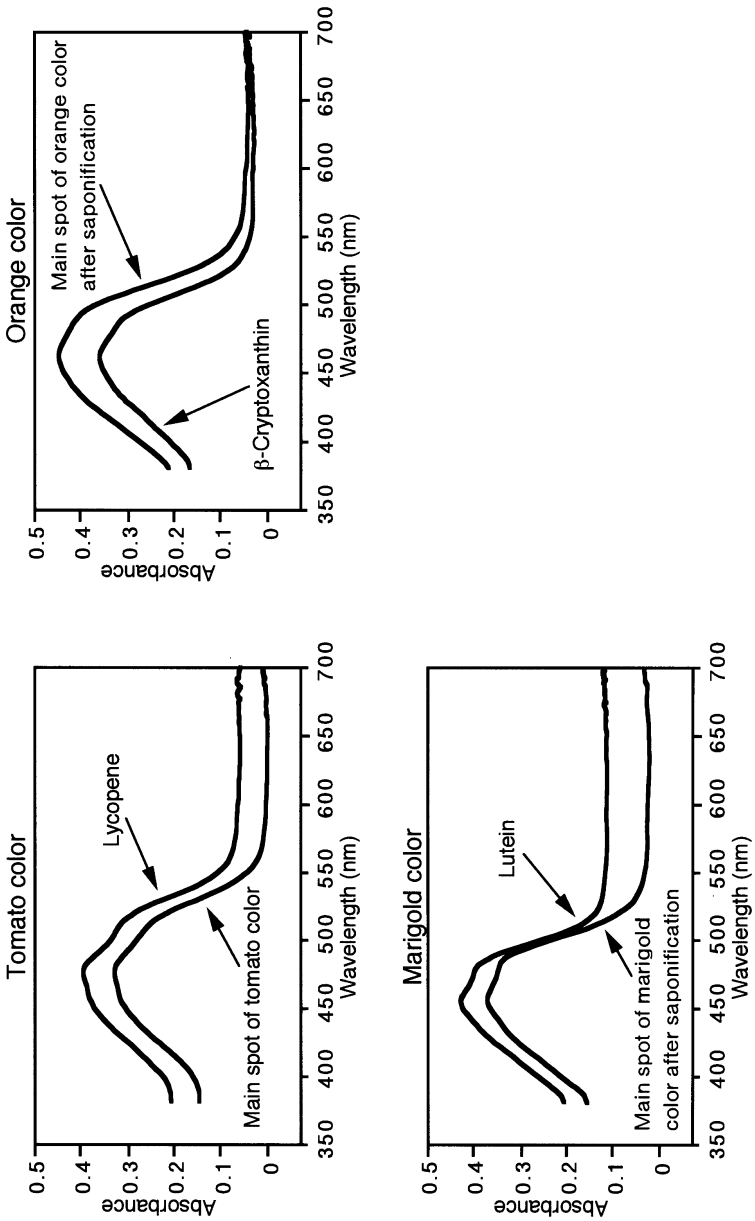

Figure 3. Visible spectra of main spots of tomato, orange, and marigold colors after saponification. Thin-layer chromatography/scanning densitometric conditions: Solvent system I. The others: see Experimental section.

Application to Commercially Available Foods

The above experimental results using standards suggested that this method is applicable to the analysis of these three colors in commercially available foods. Therefore, test solutions were prepared by the method using the C_{18} cartridges described in Experimental section from a total of 95 food samples (33 for the tomato color, 38 for the orange color, and 24 for the marigold color).

Reproducibility of *Rf* Values by Reversed-Phase Thin-Layer Chromatography

To evaluate the effects of impurities from the samples on the *Rf* value, the *Rf* values of the spots obtained by reversed-phase TLC were compared with those of the standards. As previously reported,[11] the difference between the *Rf* value of the color in the samples and that of the standard color on the TLC plates was expressed as the ratio of the *Rf* value of the color in the sample (*Ra*) to that of the standard color (*Rs*). The reproducibility was evaluated according to the coefficient of variation of this *Ra*/*Rs* ratio. These results are shown in Table 1. Under the two TLC conditions, the average *Ra*/*Rs* ratios were 0.99 and 1.01, and the coefficients of variation were 1.5% and 2.9% for the tomato color. For the saponified orange color, the average *Ra*/*Rs* ratio of the main spot was 1.01 for each condition, and the coefficients of variation were 2.1% and 2.9%. For the saponified marigold color, the average *Ra*/*Rs* value was 1.02 for each condition, and the coefficients of variation were 1.3% and 2.4%. Both the difference in the *Rf* value and its reproducibility were satisfactory, thus suggesting that the three colors can be reliably identified without any influence by impurities.

Identification by Reversed-Phase Thin-Layer Chromatography/Scanning Densitometry

After reversed-phase TLC of the test solutions, visible absorption spectra of color spots on the TLC plates were measured using a scanning densitometer. The typical TLC chromatograms and spectra are shown in Figs. 4–6. The spectra of the main spots were measured after saponification for the orange and marigold colors, but without any treatment for the tomato color. The maximum absorption and pattern of the visible absorption spectra of the main spots were consistent with those of the standards, suggesting that each color can be reliably and readily identified.

These results suggest the excellent applicability of the present reversed-phase TLC method for the examinations of the tomato, orange, and marigold

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Table 1. *Ra/Rs* Values of *β*-Cryptoxanthin, Lycopene, and Lutein in Foods on Reversed Phase TLC

Color	Solvent System[a]	*Rf* Value	Average *Ra/Rs* Value[b]	C.V. (%)	*n*
Lycopene (Tomato color)	I	0.46	0.99	1.5	33[c]
	II	0.27	1.01	2.9	
β-Cryptoxanthin (Orange color)	I	0.37	1.01	2.9	38[d]
	II	0.36	1.01	2.1	
Lutein (Marigold color)	I	0.43	1.02	1.3	24[e]
	II	0.55	1.02	2.4	

[a]see Experimental section.
[b]Ratio of *Rf* (sample)/*Rf* (standard).
[c]Juice, spaghetti sauce, ketchup, etc.
[d]Candy, jelly, sherbet, marmalade, etc.
[e]Noodle, juice, etc.

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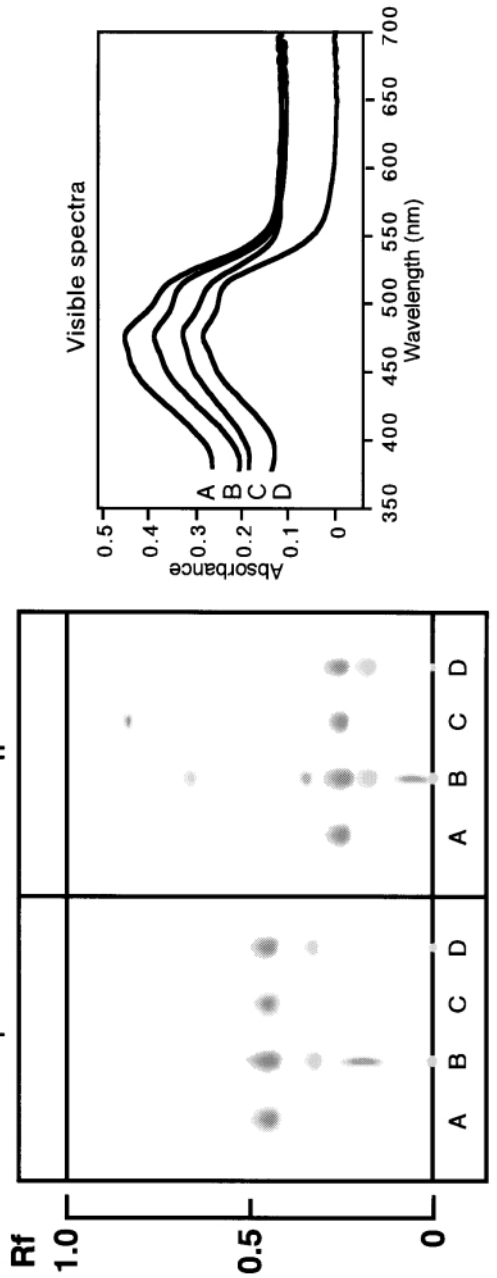

Figure 4. Thin-layer chromatograms and visible spectra of the extract of various foods under TLC/scanning densitometry. A) Lycopene. B) Juice. C) Spaghetti sauce. D) Ketchup. Thin-layer chromatography/scanning densitometric conditions: see Experimental section.

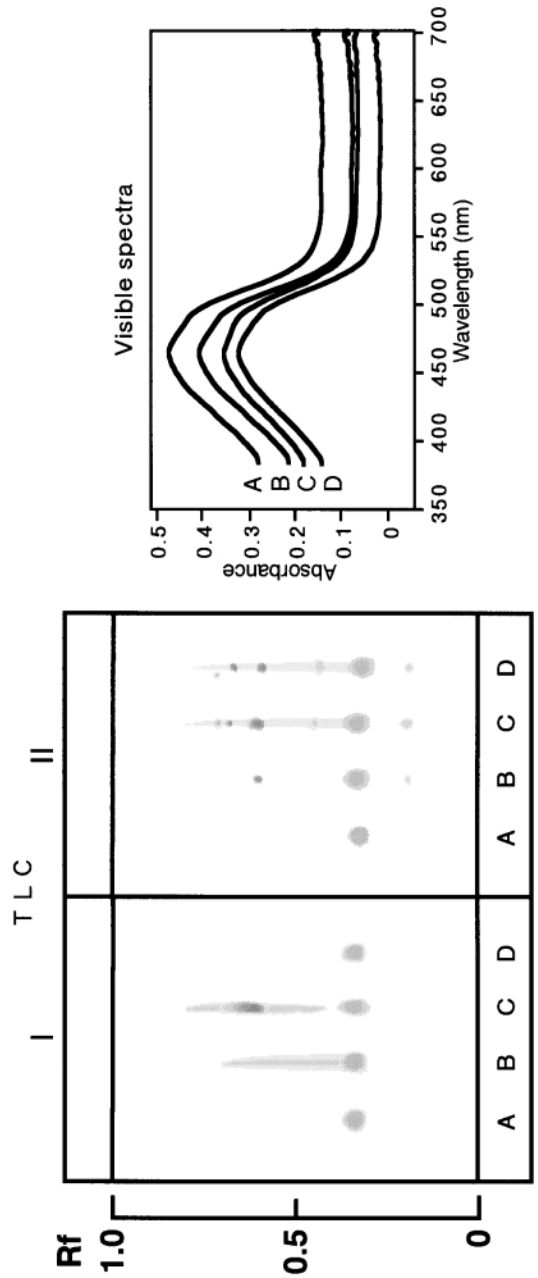

Figure 5. Thin-layer chromatograms and visible spectra of the saponified extract of various foods under TLC/scanning densitometry. A) β-Cryptoxantin. B) Jelly. C) Orange juice. D) Orange sherbet. Thin-layer chromatography/scanning densitometric conditions: see Experimental section.

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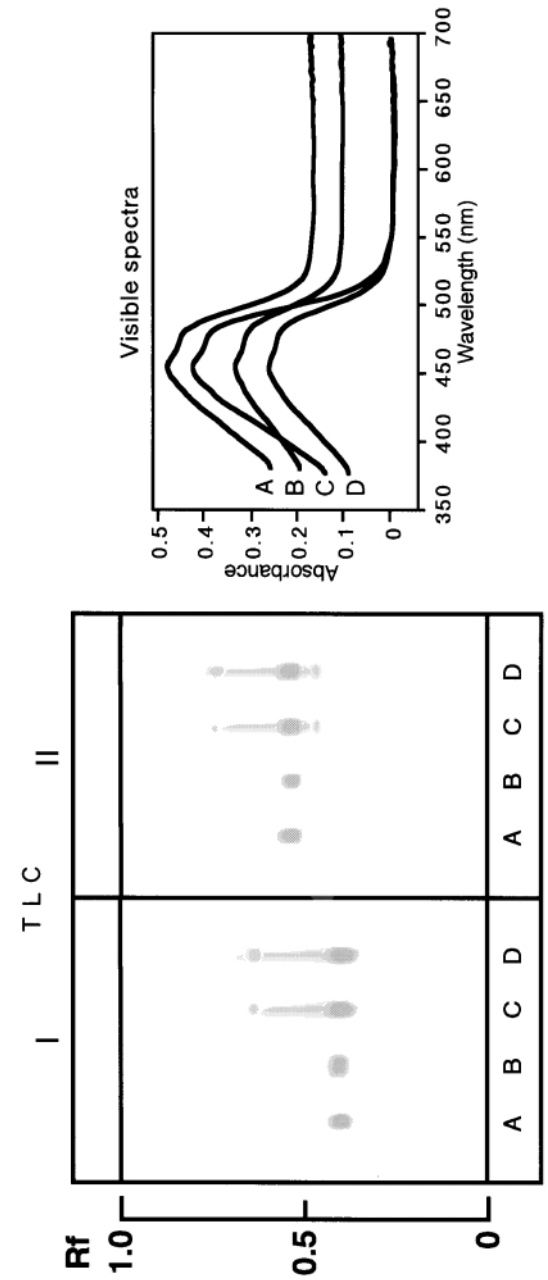

Figure 6. Thin-layer chromatograms and visible spectra of the saponified extract of various foods under TLC/scanning densitometry. A) Lutein. B) Juice. C) Noodle A. D) Noodle B. Thin-layer chromatography/scanning densitometric conditions: see Experimental section.

colors, such as in public health centers. In addition, the present scanning densitometric method, which allows reliable identification of colors without isolation, was confirmed to be applicable to the three colors.

CONCLUSIONS

For the reliable identification of the tomato, orange, and marigold colors in foods, we developed a reversed-phase TLC/scanning densitometric method and obtained the following results.

1) Reversed-phase C_{18} TLC of an untreated tomato color and saponified orange and marigold colors yielded clear spots using solvent systems of acetonitrile–acetone–*n*-hexane (11 : 7 : 2) and acetone–waterß (9 : 1).

2) The C_{18} cartridge method was useful for clean up of the tomato, orange, and marigold colors.

3) Measurement of visible absorption spectra of color spots on TLC plates using a scanning densitometer showed satisfactory results. The spectra of the color spots extracted from commercially available foods were in complete agreement with those of the standards.

4) The reproducibility of the *Rf* value by this method was evaluated using 95 samples of commercially available foods (33 for the tomato color, 38 for the orange color, and 24 for the marigold color). The difference in the *Rf* values between the samples and the standard color was slight, and the coefficient of variation was low, which suggested a high reproducibility.

Therefore, the present method using reversed-phase TLC/scanning densitometry is effective for the analysis of the tomato, orange, and marigold colors in foods.

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