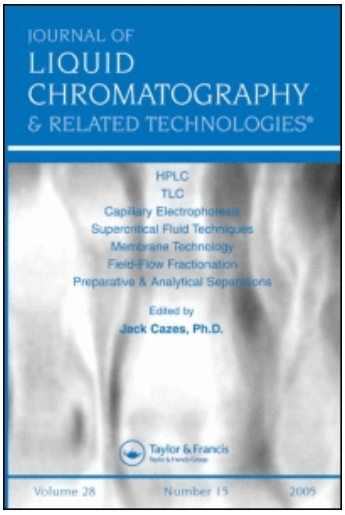

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Analysis of Natural Colorings in Foods by Thin Layer Chromatography

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Abstract: Natural colorings are frequently used in foods. In terms of food sanitation, the establishment of accurate and rapid analytical methods for natural colorings is required. Recently, the analytical methods of carotenoid colorings, quinoid colorings, flavonoid coloring, and anthocyanin coloring have been reported using reversed phase TLC with scanning densitometry. This paper reviews practical analytical methods of the above natural colorings in foods.

Keywords: Carotenoid coloring, Quinoid coloring, Flavonoid coloring, Anthocyanin coloring

INTRODUCTION

Natural colorings derived from natural materials have a wider variety than synthetic colorings, so that they are frequently used in foods in Japan.[1] In terms of food sanitation, the establishment of accurate and rapid analytical methods for natural colorings is required. Especially, for the carotenoid,

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quinod, flavonoid, and anthocyanin colorings that are used in various foods,[2] simple, rapid, and simultaneous analytical methods should be established. Synthetic colors are generally analyzed by thin-layer chromatography (TLC), because TLC allows for a faster analysis time and the simultaneous analysis of many samples. The establishment of a TLC method for natural colorings may be necessary to rapidly respond to sanitary food surveillance.

However, the identification of the separated components by TLC is difficult unless an appropriate spectrometric method such as ultraviolet-visible (UV-VIS) spectrophotometry is used. Furthermore, the stepwise operation including individual separation by TLC and measurement of the UV-VIS spectrum is laborious and time consuming, because it needs extra steps such as extraction of the desired compound from the TLC plate and elimination of adsorbents.

Recently, the analytical methods of annatto extract, orange color, gardenia yellow, paprika color, tomato color, marigold color, β-carotene, turmeric oleoresin (carotenoid colorings), lac color, cochineal color (quinoid colorings), carthamus yellow (flavonoid coloring), and red cabbage color (anthocyanin coloring) have been reported using reversed phase TLC with scanning densitometry.[3–9] In this paper, we introduce practical analytical methods of the above natural colorings in foods.

CAROTENOID COLORINGS

Tomato, Orange, and Marigold Colorings

The tomato color is obtained by extraction from the fruit of *Lycopersicon esculentum* MILL. and contains several components. The main component is lycopene (Fig. 1A).[10] This color is yellow-red and highly heat- and 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 ß-cryptoxanthin (Fig. 1B).[11] 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. 1C).[12] The color is strong yellow, relatively heat resistant and light resistant, and used for coloring beverages, confectionery, and fatty foods.

A TLC method for the analysis of tomato, orange, and marigold colors in foods was developed.[6] 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, 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

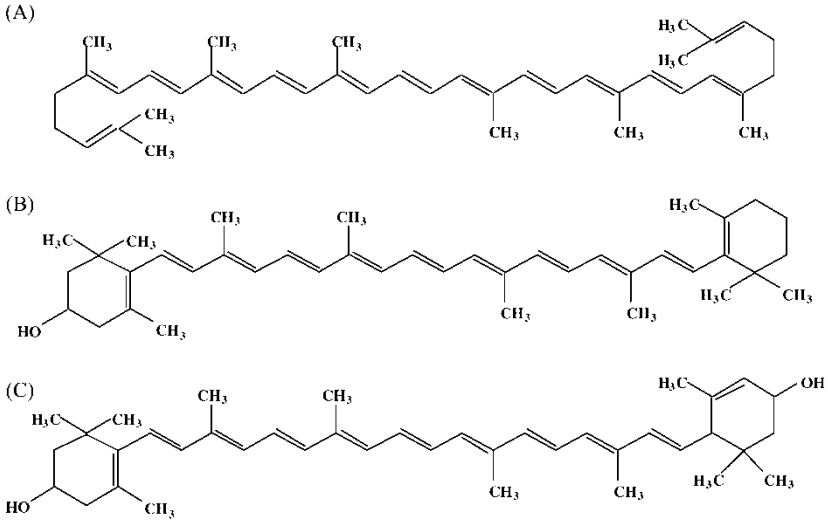

Figure 1. Structures of lycopene (A), β-cryptoxanthin (B), and lutein (C).

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 (Fig. 2, Table 1). The present method is considered to be useful for the rapid analysis of the tomato color, orange color, and marigold color in foods.

β-Carotene and Paprika Coloring

β-Carotene is one of the orange dyes found in most green leaves, and in carrots and is used in foods as a coloring (Fig. 3A).[13] It is sometimes added to foods for its anti-oxidant effects and is sometimes added to foods or vitamin supplements as a nutrient.

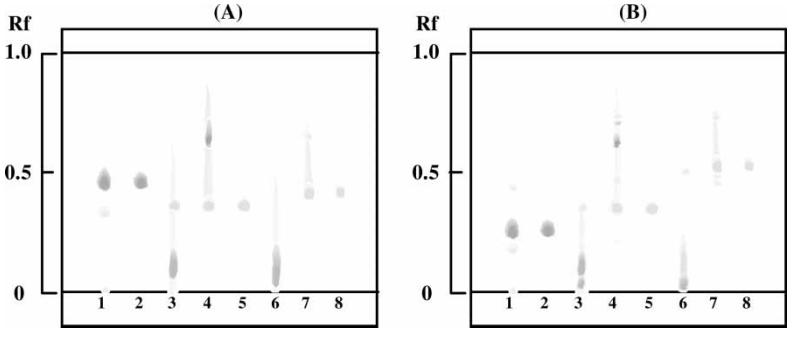

Figure 2. Thin-layer chromatograms of tomato, orange, and marigold colors. 1) Tomato color; 2) Lycopene; 3) Orange color (before saponification); 4) Orange color (after saponification); 5) β-Cryptoanthin; 6) Marigold color (before saponification; 7) Marigold color (after saponification); 8) Lutein. TLC conditions: Plate: RP-q8F 254s (E. Marck); Solvent system: (A) Acetonitrile-acetone-n-hexane = 11-7-2; (B) Acetone-water = 9–1.

Paprika color is obtained by extraction from the fruit of red peppers (*Capsicum annuum*) and contains capsanthin and its esters, such as lauric acid, myristic acid, and palmitic acid, in large amounts as its color components (Fig. 3B).[14] Commercially available paprika colors are known to have different compositions of these color components depending on the material the paprika color is extracted from, which makes the determination of

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

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

[a]see Fig. 2.
[b]Ratio of *Rf* (sample)/*Rf* (standard).
[c]Juice, spaghetti sauce, ketchup, etc.
[d]Candy, jelly, sherbet, marmalade, etc.
[e]Noodle, juice, etc.

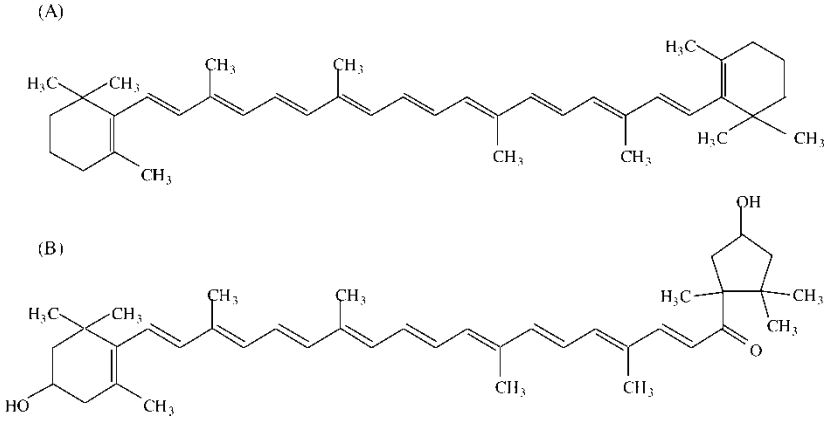

Figure 3. Structures of β-carotene (A) and capsaithin (B).

paprika color based on the analysis of the color components impossible, causing difficulty in developing a simple, rapid, and reliable method for the analysis of the paprika color in foods.

A technique for the analysis of β-carotene and paprika colors in foods has been established using reversed phase TLC and scanning densitometry.[4] β-Carotene was directly extracted with ethyl ether from foods, and paprika color was extracted with ethyl ether after saponification with sodium hydroxide-methanol. Both extracts were cleaned up with a C_{18} cartridge. Separation of both colors was achieved on the reversed phase C_{18} TLC plate using n-hexane:acetone:acetonitrile (2:7:11) as the solvent system (Fig. 4). The visible absorption spectra of the colors were measured using scanning

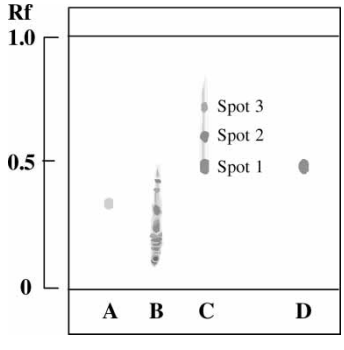

Figure 4. TLC of standards of β-carotene (A), paprika color before saponification (B), paprika color after saponification (C), and capsanthin (D). TLC conditions: Plate: RP-18F 254s (E. Merck); Solvent system: Acetonitrile-acetone-n-hexane = 11-7-2.

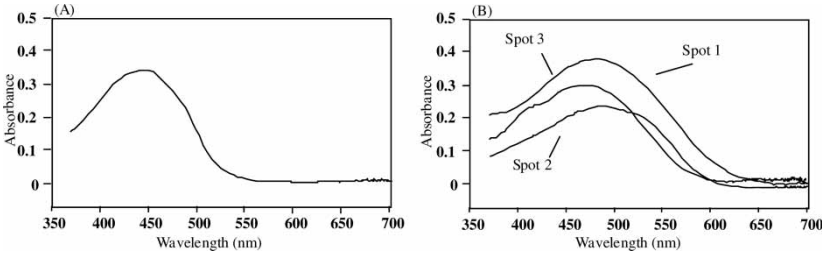

Figure 5. Visible absorption spectra of β-carotene (A) and paprika color after saponification (B).

densitometry without isolation of the colors (Fig. 5). In order to investigate the capability of the present method, 77 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 standards with good reproducibility (Fig. 6).

Turmeric Oleoresin, Gardenia Yellow, and Annatto Extract

Turmeric Oleoresin is a yellow pigment obtained by extracting the tubers of *Curcuma longa* LINN, with volatile solvents and concentration to remove

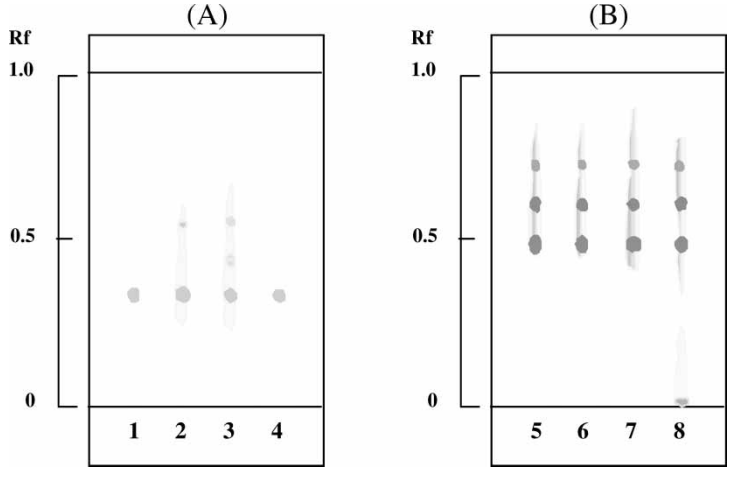

Figure 6. TLC of β-carotene (A) and paprika color (B) extracted from various foods. 1) Standard of β-carotene; 2) Candy; 3) Jelly; 4) Chocolate; 5) Standard of paprika color; 6) Cuttlefish and Alaska pollack roe in red pepper; 7) Korean pickles; 8) Rice cracker.

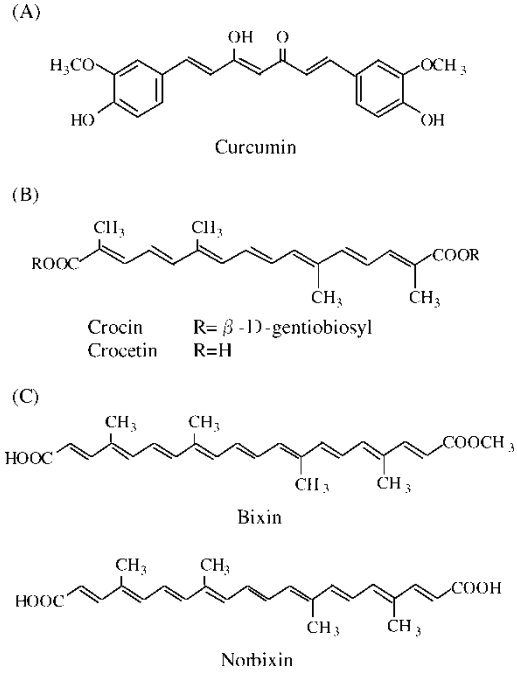

Figure 7. Main components of turmeric oleoresin (A), gardenia yellow (B), and annatto extract (C).

the solvent. The main component is curcumin (Fig. 7A).[15] This color is used for the coloring of pickle and curry powder.

Gardenia yellow extracted from gardenia fruit (*Gardenia jasminoides* Ellis) is a yellow natural food additive and is widely used for coloring foods. It is known that the yellow color is derived from a water soluble pigment including crocin and crocetin (Fig. 7B) as major components.[16]

Anatto extract is yellow-orange pigment produced from the seeds of *Bixa orellana*, a small tree which grows in Central and South America. The main components are bixin and norbixin (Fig. 7C). It is used for colouring butter, cheese, and varnishes.[15]

An analytical method of food colors, turmeric oleoresin, gardenia yellow, and annatto extract (including annatto, water soluble) in foods has been established using reversed-phase thin-layer chromatography/scanning densitometry.[4] The colors were directly extracted with water or methanol from foods, and extracts were cleaned up with a C_{18} cartridge after evaporation of methanol. Separation of the colors was achieved on the reversed phase C_{18} TLC plate using acetonitrile-tetrahydrofuran-0.1 mol/L oxalic acid (7:8:7) as a solvent system (Fig. 8), and measurement of visible absorption spectra of the colors was carried out

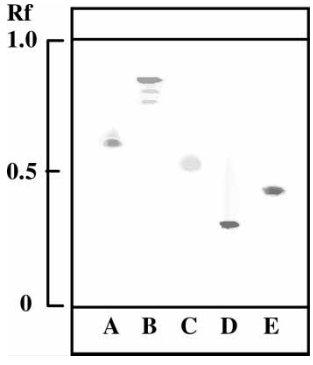

Figure 8. TLC of turmeric oleoresin, gardenia yellow, and annatto extract. A) Curcumin; B) Crocin; C) Crocetin; D) Bixin; E) Norbixin. TLC conditions; Plate: RP-18F_{254s} (E Merck, 15389). Solvent System: Acetonitrile-tetrahydrofuran-0.1 mol/L oxalic acid (7:8:7).

using scanning densitometry without isolation of the colors (Fig. 9). In order to investigate the capability of the present method, 89 commercial foods were analyzed (Fig. 10), and their chromatographic behaviors and spectra were observed. The separation and the spectra obtained were not affected by coexisting substances in the foods. 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 turmeric oleoresin, gardenia yellow, and annatto extract including water-soluble annatto in foods.

Simultaneous Analysis of Carotenoid Colorings

A simultaneous analytical method by TLC for carotenoid colorings (annatto extract, orange color, gardenia yellow, paprika color, tomato color, marigold color, and β-carotene) in foods has been reported.[8] Reversed phase C_{18} TLC using the solvent systems of acetonitrile-acetone-n-hexane = 11-7-2 and acetone-water = 9–1, and normal phase silica gel TLC using the solvent systems of n-hexane-diethyl ether-acetic acid = 4-1-1 and benzene-ethyl acetate-methanol = 15-4-1, yielded well delineated spots with good separation (Table 2). These TLCs were applied to the analysis of a total of 294 commercially available foods, and the Rf value of each color spot was evaluated under the four TLC conditions. The difference in the Rf value was slight between each color extracted from the food samples and the standard color, and the coefficient of variation was small, indicating excellent reproducibility. The present method is considered to be useful for the rapid analysis of the carotenoid colorings.

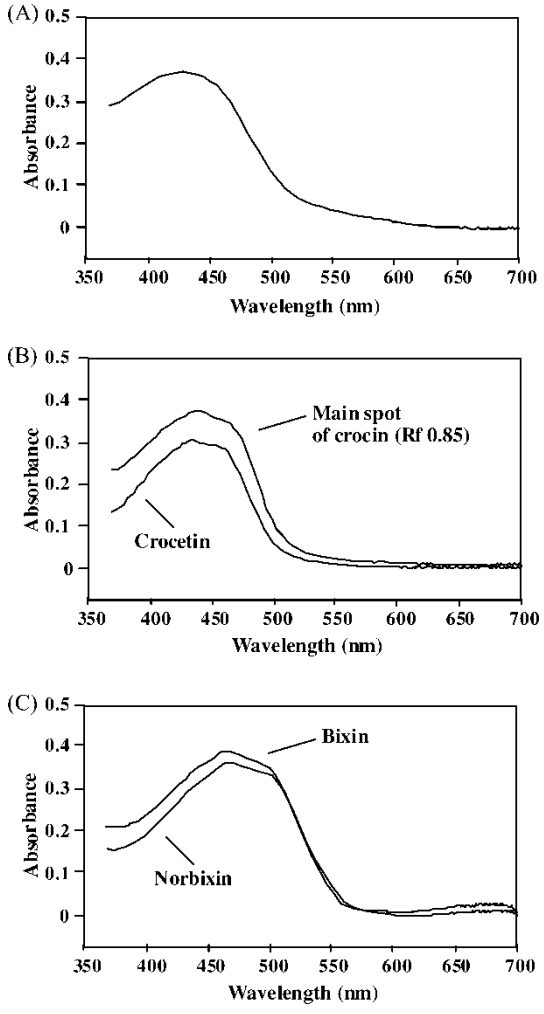

Figure 9. Visible absorbance spectra of turmeric oleoresin (A), gardenia yellow (B), and annatto extract (C) by scanning densitometry.

QUINONE COLORING

Lac and Cochineal Colorings

Lac dye is a natural food additive extracted from a stick lac, which is a secretion of the insect *Coccus laccae* (*Laccifer lacca* Kerr) and is widely used for coloring food.[17–19] It is known that the red color is derived from a water-soluble pigment including laccaic acids A, B, C, and E (Fig. 11A).

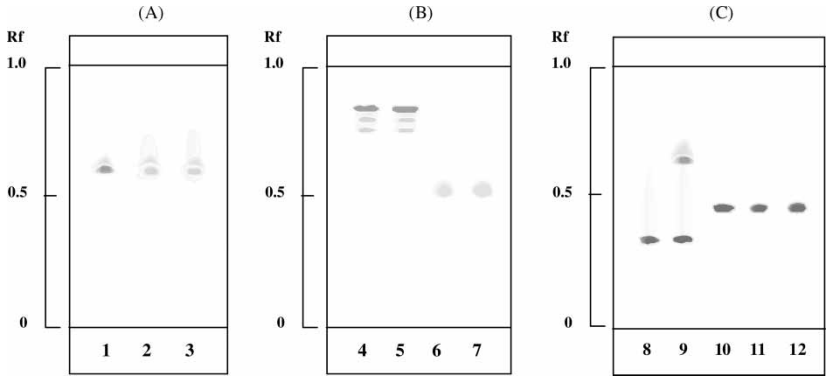

Figure 10. TLC of turmeric oleoresin (A), gardenia yellow (B), and annatto extracted from various foods. 1) Turmeric oleoresin standard; 2) Candy; 3) Pickles; 4) Gardenia yellow standard (crocetin); 7) Unboiled chinese noodle; 8) Annatto extract standard (Bixin); 9) Rice cracker; 10) Annatto extract standard (Norbixin); 11) Candy; 12) Chewing gum.

Table 2. Rf values of Bixin, Norbixin, β-cryptoxanthin, Crocin, Crocetin, Capsanthin, Lycopene, Lutein, and β-carptene on TLC

	Rf value			
Color	TLC condition 1[a]	TLC condition 2[b]	TLC condition 3[c]	TLC condition 4[d]
Bixin	0.80	0.73	0.38	0.35
Norbixin (Annato extract)	0.83	0.80	0.37	0.10
β-cryptocanthin (Orange color)	0.37	0.36	0.40	0.68
Crocin	0.00	0.89	0.00	0.00
Crocetin (Gardenia yellow)	0.86	0.84	0.39	0.22
Capsanthin (Paprika color)	0.50	0.64	0.26	0.32
Lycopene (Tomato color)	0.46	0.27	0.87	0.93
Lutein (Marigold color)	0.43	0.55	0.36	0.37
β-carotene	0.34	0.18	0.89	0.91

[a]TLC condition1: C18 TLC using the solvent systems of acetonitrile-acetone-n-hexane = 11-7-2.

[b]TLC condition2: C18 TLC using the solvent system of acetone-water = 9–1 Fig. 12.

[c]TLC condition3: silica gel TLC using the solvent systems of n-hexane-diethyl ether-acetic acid = 4-1-1.

[d]TLC condition4: silica gel TLC using the solvent systems of benzene-ethyl acetate-methanol = 15-4-1.

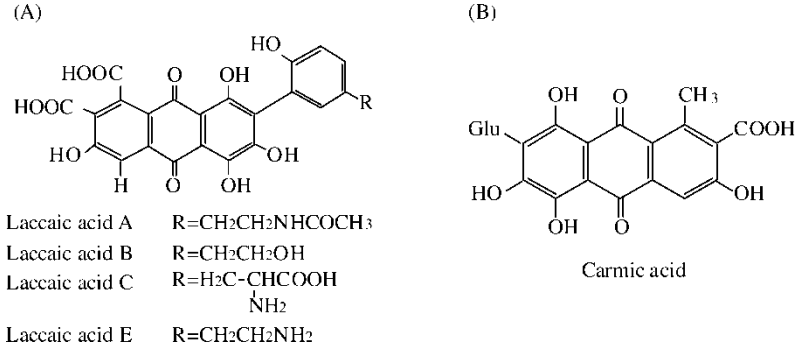

Figure 11. Main components of lac (A) and cochineal colors (B).

Cochineal Coloring is a red pigment obtained from the insect *Coccus cacti*, that lives on various cactus plants. The insect is native to tropical South and Central America and produces the pigment as a deterrent against other insects. The main component is carmic acid (Fig. 11B).[20–22] This color is used for the coloring of candy and jelly.

A technique for the analysis of lac and cochineal colors using reversed phase TLC and scanning densitometry has been reported.[3] The colors were directly extracted with 0.1 mol/L oxalic acid; 80% methanol from foods and extracts were cleaned up with a C_{18} cartridge after evaporation of methanol. Separation of the colors was achieved on the reversed phase C_{18} TLC plate using methanol-0.5 mol/L oxalic acid(5.5:4.5) as a solvent system (Fig. 12), and measurement of visible absorption spectra of the colors was carried out using scanning densitometry without isolation of the colors (Fig. 13). In order to investigate the capability of the present method, 122 commercial foods were analyzed,

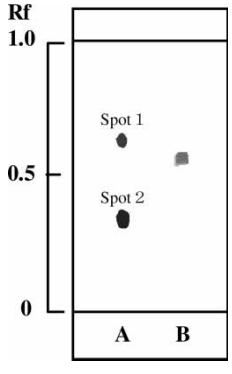

Figure 12. TLC of lac (A) and cochineal colors (B). TLC conditions: Plate: RP-18 TLC (Merck, 15389); Solvent system: Methanol-0.5 mol/L oxalic acid (5.5:4.5).

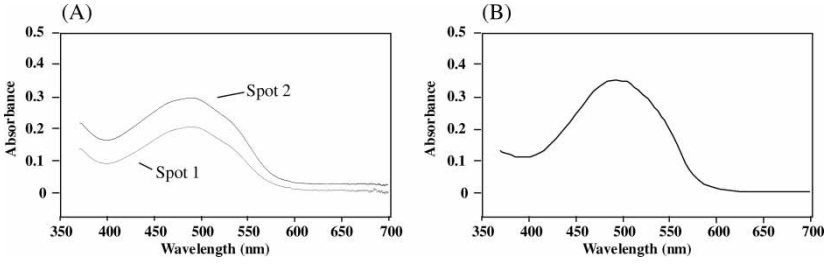

Figure 13. Visible absorption spectra of lac (A) and cochineal color (B) standards measured by scanning densitometry.

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 standards with good reproducibility. The present method is considered to be useful for the rapid analysis of lac and cochineal colors in foods.

FLAVONOID

Carthamus Yellow

Carthamus yellow is a yellow dye obtained by extracting the flower of the *Carthamus tinctorius* LINNE with water. The yellow color of the dye is

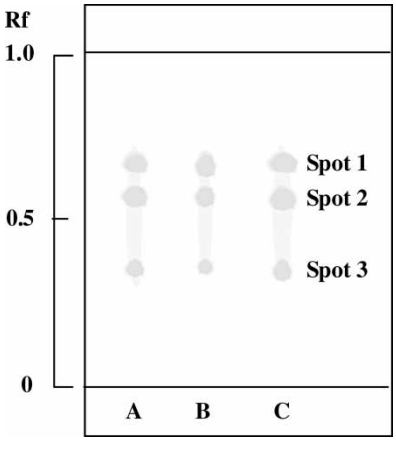

Figure 14. Thin-layer chromatograms of the extracts from foods iner TLC/scanning densitometry. A) Carthamus yellow standard; B) Candy; C) Jelly. TLC conditions: Plate: RP-18F 254s (E. Merck). Solvent system: 2-Butanone-methanol-5% sodium sulfate-5% acetic acid (3:2 :5:5).

mainly derived from the flavonoids, saffronin A and saffronin B. This color shows a yellow color under acidic conditions and a reddish yellow color under basic conditions, and is highly heat- and light-resistant. By making use of these properties, it is widely used for the coloring of juice, candy, jelly, chewing gum, fruit wine, chocolate, etc.[23–25]

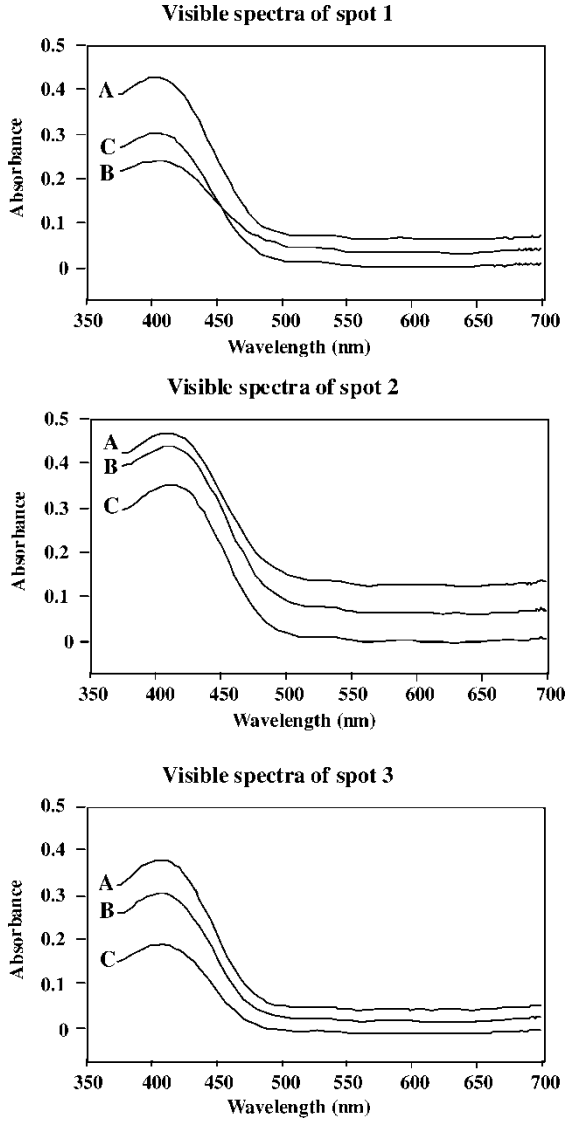

Figure 15. Visible spectra of the extracts from foods under TLC/scanning densitometry. A) Carthamus yellow standard; B) Candy; C) Jelly.

A technique for the analysis of carthamus yellow using reversed-phase TLC and scanning densitometry has been described.[9] The colors were directly extracted with water from foods and extracts were cleaned up with a C_{18} cartridge. Separation of the colors was achieved on the reversed phase C_{18} TLC plate using 2-butanone:methanol:5% sodium sulfate:5% acetic acid (3:2:5:5) (Fig. 14), and measurement of visible absorption spectra of the colors was carried out using scanning densitometry without isolation of the colors (Fig. 15). In order to investigate the capability of the present method, 35 commercial foods were analyzed, and their chromatographic behaviors and spectra were observed. The obtained separation and the spectra were not affected by coexisting substances 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 carthamus yellow in foods.

ANTHOCYANIN

Red Cabbage Color

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.[26,27] The red color of the dye is derived from derivatives of cyanidin acylglucoside.[28,29] This color shows a red-purple color under acidic conditions, is highly heat- and light-resistant, and 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.[26]

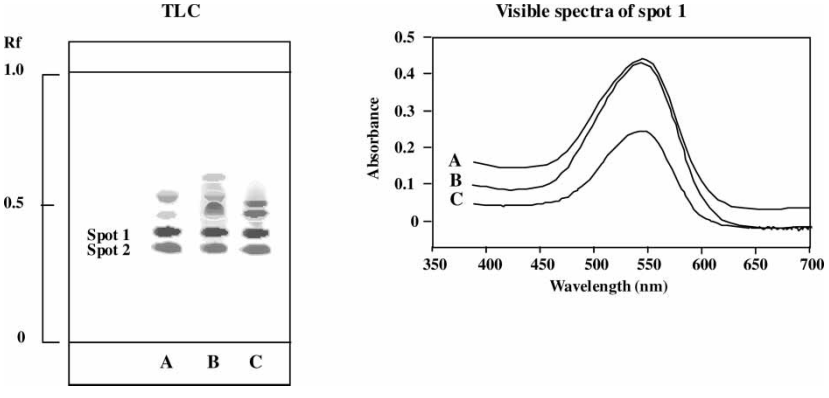

Figure 16. TLC and visible spectra of the extracts from various foods under TLC/scanning densitometry. A) Red cabbage color standard; B) Juice containing red cabbage and cochineal colors; C) Candy containing red cabbage and unknown anthocyanin colors.

A technique for the analysis of red cabbage color using reversed-phase TLC and scanning densitometry has been developed.[7] The color was directly extracted with 0.1% trifluoroacetic acid from foods and extract was cleaned up using a C_{18} cartridge with 5 mL of methanol-0.1% trifluoroacetic acid (9:1). Separation of the colors was achieved on the reversed phase C_{18} TLC plate using acetonitrile-0.2 mol/L trifluoroacetic acid (1:2), and measurement of visible absorption spectra of the colors was carried out using scanning densitometry without isolation of the colors. 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 (Fig. 16), 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.

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