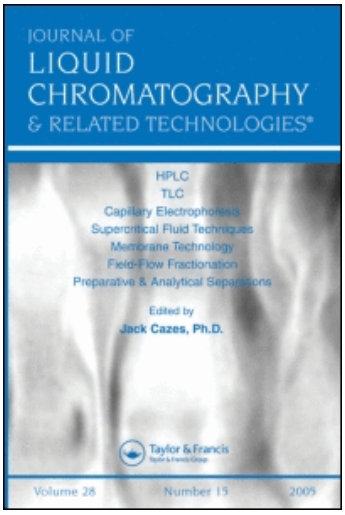

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AN HPLC METHOD FOR THE ANALYSIS OF PAPRIKA COLOR IN FOOD USING CAPSANTHIN AS AN INDICATOR

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ABSTRACT

In the present study, an HPLC method for the analysis of paprika color in food using capasanthin as an indicator was developed. Paprika color was extracted from food samples with ether, and after the extract was evaporated, the residue was dissolved in methanol, to which 5% sodium hydroxide–methanol solution was

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then added. The resultant mixture was occasionally stirred, then allowed to stand for 24 hours at room temperature in a tightly sealed container kept away from light. Subsequently, distilled water was added and the pH of the mixture was adjusted to be 4.5 or less using hydrochloric acid. It was then purified with a C18 cartridge before being subjected to the HPLC analysis.

The HPLC conditions were as follows: column, TOSOH TSK gel ODS-80Ts (5 μm, 4.6 × 150 mm); column temperature, 40°C; mobile phase, acetonitrile–water (3:1); flow rate, 0.8mL/min; detection wavelength, 460nm. According to the present method, the average recoveries of the paprika color when the fortified concentrations were 0.25, 0.50, and 1.00 mg/g were over 81.9% from sherbet, over 81.4% from snack foods, and over 85.1% from pickles.

The coefficients of variation were 8.1% or less. Thirty-two samples from commercially available foods, such as sherbet that had a label stating the use of paprika color were analysed, and the detected capsanthin concentrations ranged from 0.04 to 27.30 μg/g.

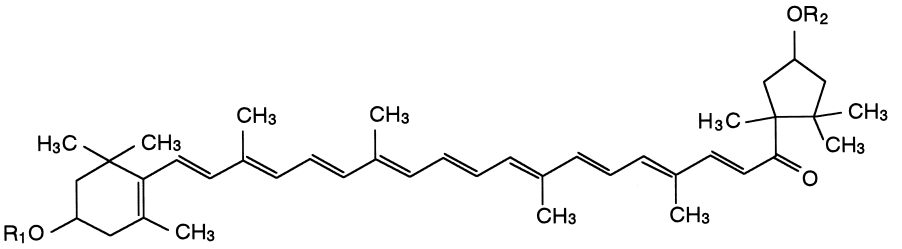
INTRODUCTION

Recently, natural colorings are being used more and more widely because they are preferred by consumers. Among them, there are many carotenoid colorings including annatto, orange, gardenia yellow, saffron, carotene, paprika, tomato, and marigold, and they are used in a variety of foods. In particular, paprika color is used worldwide; thus a simple, rapid, and reliable method for the analysis of the paprika color in food needs to be developed.

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. 1)(1-6). 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 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.

In a previous study,(7) we developed a TLC method for analysing paprika color extracted from various foods, namely, the extracted paprika color was saponificated and then purified using a C18 cartridge before being identified by reversed phase TLC/scanning densitometry. The main product of the saponification was identified as capsanthin as will be described later.

In the present study, an HPLC method for the analysis of paprika color using capsanthin, which is a main product of saponification, as an indicator was investigated.



Capsanthin : R 1 , R 2 = H

Esterified capsanthins : R 1 = H , lauroyl, myristoyl, palmytoyl
R 2 = lauroyl, myristoyl, palmytoyl

Figure 1. Structures of capsanthin and its esters.

EXPERIMENTAL

Samples

Foods available on the Japanese market including snack foods, sherbet, ice cream, salted fish guts, spiced cod roe, seasonings, and pickles were used.

Standards and Chemical Reagents

Capsanthin from Extrasynthese (Lyon, France) was used as the capsanthin standard, and capsanthin from paprika (vegetable oil solution) from Tokyo Kasei (Tokyo, Japan) was used as the paprika color standard. The C18 cartridges used in the study were Sep-Pak C18 Vac 3cc (500 mg) from Waters (MA, USA). All the other reagents were of analytical grade from Wako (Osaka, Japan) and Kanto Kagaku (Tokyo, Japan).

Analytical Conditions

TLC

The TLC plate was an RP-18F254S (Art. 15389, E. Merck, Darmstadt, Germany), and the solvent system was acetonitrile–acetone–n-hexane (11:7:2).

HPLC

The HPLC used in the study was from Hitachi, Ltd. (Tokyo, Japan): Detector, L-4200 UV-VIS; column oven, L-7300; autosampler, L-7200: pump, L-6000; integrator, D-2500. The HPLC conditions were as follows: column, TOSOH TSK gel ODS-80Ts (5 μm, 4.6 × 150 mm); column temperature, 40°C; mobile phase A, acetonitrile–acetone–n-hexane (11:7:2); mobile phase B, acetonitrile–water (3:1); flow rate, 0.8 mL/min; detection wavelength, 460 nm.

LC/MS

The LC/MS used in the study was a PE Sciex API 300 from P E Sciex (Thornhill, Canada). The LC/MS conditions were as follows: column, TOSOH TSK gel ODS-80Ts (5 μm, 4.6 × 150 mm); column temperature, 40°C; mobile phase, acetonitrile–water (3:1); flow rate, 0.8 mL/min; ion source, APCI; nebulizer temperature, 425°C; capillary temperature, 400°C; scan mode, positive.

Scanning Densitometer

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

A 50 mL of water was added to 10 g of the sliced and homogenized food samples. The colored species were extracted from the food samples with 30 mL of ether, and after the extract was evaporated, the residue was dissolved in 20 mL of methanol. It was then saponificated in the following manner: after adding 2 mL of 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 into a C18 cartridge that had been activated with methanol and water (5 mL each) in advance. The cartridge was then washed with 20 mL of n–hexane, and the colors were eluted with 5 mL of acetone and collected in a 5 mL volumetric flask. The obtained test solution was subjected to an HPLC analysis.

Measurement of Color Values

The color values were measured using acetone solutions according to the color value measurement as defined in the Official Method for Food Additives, 7th edition(8).

RESULTS AND DISCUSSION

Saponification Conditions of Paprika Colors

When a paprika color standard before saponification was subjected to reversed phase TLC, a number of overlapping spots were observed, and a satisfactory separation could not be obtained (Fig. 2A). This was probably because the paprika color contains a large number of esters. Paprika color is known to be hydrolyzed into carotenoid and a fatty acid when saponificated under mild conditions(3-6). Thus, a paprika color standard after saponification was subjected to TLC. It was found that the paprika color standard after saponification was satisfactorily separated into a main spot having an Rf value of 0.50 and two sub-spots having Rf values of 0.60 and 0.75 (Fig. 2B). The main spot was identical with the spot of the capsanthin standard in terms of its Rf value, color, and shape (Fig. 2C).

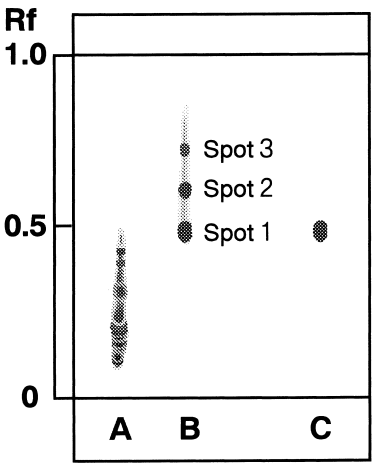


Figure 2. TLC chromatograms of paprika color with and without saponification and capsanthin. Plate: RP-18 F254S TLC (E. Merck, Art. 15389 and solvent system: acetonitrile-acetone-n-hexane (11:7:2). A: Paprika color before saponification, B: paprika color after saponification, and C: standard of capsanthin.

As described above, it was suggested that the paprika color is hydrolyzed into carotenoid and a fatty acid by saponification under mild conditions. Next, the saponification conditions were investigated. Various volumes (0.1, 0.2, 1.0, 2.0, 5.0, 7.0, and 10 mL) of 5% NaOH–methanol solution were added to the paprika color standard methanol solution (100 mg/100 mL) and the mixtures were placed in tightly sealed containers, and allowed to stand for 0.5, 1, 3, 6, 16, and 24 hours at room temperature, occasionally stirred and kept away from light, for saponification.

As described in the Experimental section, the saponificated paprika color was then purified using a C18 cartridge, and the progress of saponification was observed by subjecting it to reversed phase TLC. When a 5% NaOH–methanol solution was added in an amount of 0.2 mL or less, no change was observed by TLC, even 24 hours after the initiation of saponification. Likewise, no change was observed when the mixture was allowed to stand for 3 hours or less, even when 10 mL of 5% NaOH–methanol solution was added. Only slight and unclear changes in the spots were observed when the mixture was allowed to stand for 6 hours with 5% NaOH–methanol solution being added in an amount of over 1 mL, or when it was allowed to stand for 16 hours with 1 mL of 5% NaOH–methanol solution being added.

In contrast, when the mixture was allowed to stand for 16 hours with 2 mL or more of 5% NaOH–methanol solution being added, or when it was allowed to stand for 24 hours with 1 mL or more of 5% NaOH–methanol solution being added, the spots were satisfactorily separated into three spots as shown in Fig. 2B. Of the three spots, it is considered that spot 1 is capsanthin and that spots 2 and 3 are analogous compounds according to their Rf values. Based on these findings, the following saponification conditions were selected: stand time, 24 hours; amount of 5% NaOH–methanol solution, 2 mL.

Identification of the Main Product After Saponification of Paprika Color

TLC/Scanning Densitometry

The separated spots, obtained by subjecting a paprika color standard after saponification to reversed phase TLC under the conditions described in the Experimental section, were then subjected to scanning densitometory. The visible absorption spectra were measured in the range of a 370-700 nm scanning wavelength, and excellent visible absorption spectra were obtained. The spectrum of the main spot (spot 1) of the paprika color after saponification showed its maximum absorption wavelength at 480 nm, with a small shoulder around 510 nm, which identically matches the spectrum of the capsanthin standard.

HPLC

Fig. 3 shows chromatograms of the paprika color before saponification, after saponification, and of the capsanthin standard obtained by subjecting them to HPLC under the conditions described in 2.3.2. As shown in Fig. 3A(1), the paprika color standard before saponification showed a number of peaks in mobile phase A, while the paprika color standard after saponification showed a peak at the retention time of 2 minutes as shown in Fig. 3A(2). Thus, it is considered that sufficient saponification of the paprika color was carried out. The product showed the same retention time as the capsanthin standard as shown in Fig. 3A(3), however, the retention time was as short as 2 minutes. Therefore, another HPLC analysis was performed using mobile phase B so that the peak of the capsanthin standard would be detected at the retention time of around 30 minutes.

In mobile phase B, as shown in Fig. 3B(1), the paprika color standard before saponification showed no clear peak before the retention time of 70 minutes. In contrast, as shown in Fig. 3B(2), the chromatogram of the paprika color after saponification showed its main peak at the retention time of 38 minutes. The main peak was confirmed to be the main spot by isolation and purification using the TLC described above. Moreover, the retention time of this peak completely agreed with that of the capsanthin standard as shown in Fig. 3B(3).

LC/MS

The paprika color standard after saponification and the capsanthin standard were analysed under the LC/MS conditions described in the Experimental section. Fig. 4 shows the mass spectrum of the main peak of the paprika color after saponification taken at the top of the peak at the retention time of 38 minutes. $[M+H]^+$ and $[M+H-H_2O]^+$ were clearly observed at M/Z 585 and at M/Z 567, respectively, and the mass spectrum was found to be identical with the spectrum of the capsanthin standard.

Accordingly, the main product obtained after saponification and purification of the paprika color was confirmed to be capsanthin.

Capsanthin Obtained from the Paprika Color Standard

Correlation and Reproducibility

The paprika color standard was dissolved in 50 mL of methanol in the amounts of 2.5, 5.0, 10.0, 25.0, and 50 mg. By using these mixtures, test solutions were prepared according to the method described in the Experimental sec-

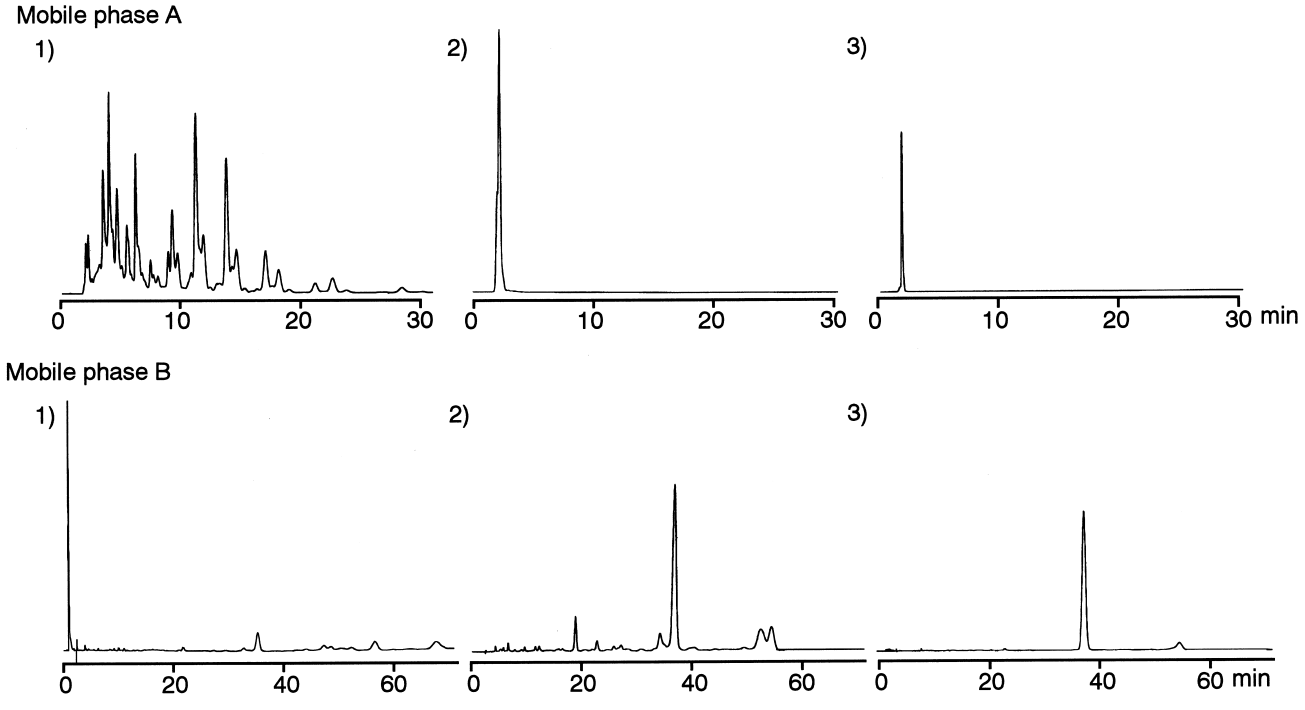


Figure 3. HPLC chromatograms of paprika color with and without saponification and standard of capsanthin. Column: TOHSOH TSK gel ODS-80Ts 5μm (4.6X150mm), column temperature: 40°C, detection: 460 nm, flow rate: 0.8 mL/min, mobile phase A: acetonitrile-acetone-n-hexane (11:7:2), and mobile phase B: acetonitile-water (3:1). 1) Paprika color before saponification, 2) paprika color after saponification, and 3) standard of capsanthin.

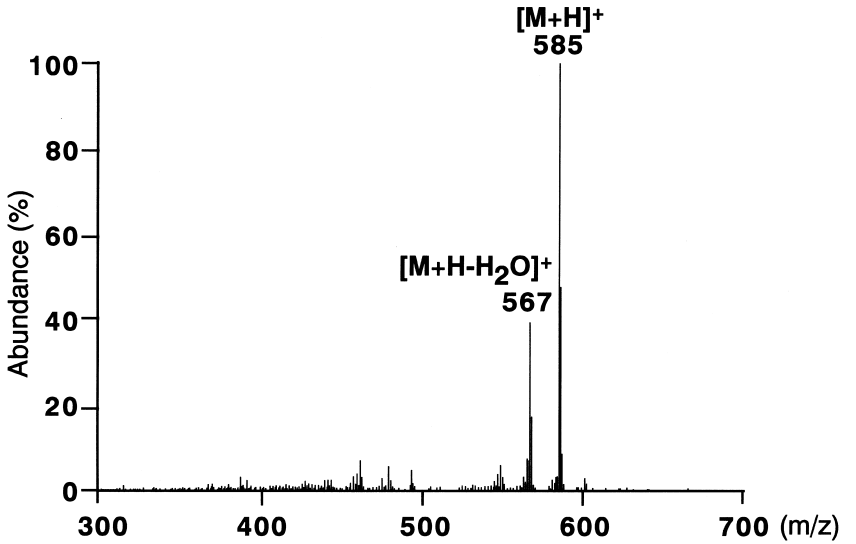


Figure 4. Mass spectrum of paprika color with saponification under APCI LC/MS conditions.

tion. The capsanthin concentrations in three samples for each of the three concentrations (9 samples in total) were determined under the HPLC conditions (mobile phase B) described in the Experimental section. These results are shown in Fig. 5. When the paprika color concentration varied in the range of 0.05-1.00 mg/mL, the capsanthin concentration was found to range from 2.5 to 74.9 μg/mL. A satisfactory correlation was obtained between the paprika color concentration and the capsanthin concentration with the coefficient of correlation being 0.999. Also, an excellent reproducibility with the coefficient of variation being 6.9% or less was obtained for each of the paprika color concentrations.

Stability

The stability of capsanthin obtained from the paprika color standard was studied. Test solutions were prepared in the same manner as described, using methanol solutions containing the paprika color in the amount of 0.05-1.00 mg/mL. The capsanthin concentrations in the test solutions were determined by HPLC. The remaining refrigerated capsanthin concentrations for each storage time, with respect to the capsanthin concentration immediately after purification

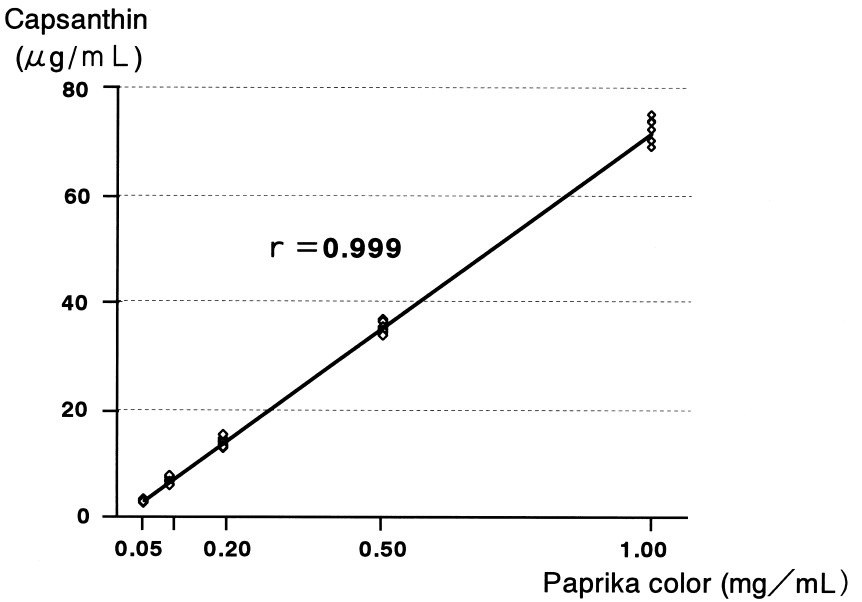


Figure 5. Correlation between paprika color and capsanthin concentrations.

(100%), were represented as percentages (Table 1). When the storage time was 5 hours, the remaining capsanthin concentration was over 95% at any concentration, and over 81% even when the storage time was as long as 5 days, except when the concentration of the paprika color was 0.05 mg/mL. Thus, an excellent stability was obtained.

Table 1. Stability of Capsanthin Obtained from Paprika Color

Paprika Color (mg/mL)	Remaining Capsanthin (%)						
	0 hr.	5 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
0.05	100	95	89	79	77	78	78
0.10	100	99	99	89	87	87	86
0.20	100	99	99	96	90	87	85
0.50	100	99	99	93	88	88	81
1.00	100	99	98	94	87	84	84

n=5.

Correlation Between the Color Value and the Capsanthin Concentration

It was confirmed that the capsanthin obtained by saponification and purification of the paprika color standard exhibited an excellent reproducibility and stability, and that a high correlation was obtained between the capsanthin and paprika color concentrations. However, because paprika colors have different compositions of capsanthin and its esters depending on the material the paprika color is extracted from, the paprika color concentration and the capsanthin concentration obtained after saponification vary depending on the material the paprika color is extracted from. Thus, the color values of the paprika color standards were measured in order to investigate the correlation between the color values and the capsanthin concentrations obtained by saponification of the paprika colors.

The color values of 8 types of paprika color standards obtained from 8 different manufacturers, were measured according to the color value measuring method, and the capsanthin concentrations obtained after saponification of the paprika colors were also determined under the HPLC conditions (mobile phase B) described in the Experimental section. Each of the paprika color standards and their capsanthin concentrations after saponification was determined 5 times. These results are shown in Fig. 6.

The color values of the paprika color standards varied in the range of 500 to 1370, and the capsanthin concentrations obtained after saponification of the paprika colors ranged from 85.0 to 219.2 μg/mL, with an excellent coefficient of correlation of 0.962. Thus, it is considered that the present analysis method using capsanthin as an indicator can be applied to paprika colors having different compositions of capsanthin and its esters.

Application to Commercially Available Foods

Next, the present method was applied to the analysis of paprika color in commercially available foods, since the results obtained using a paprika color standard suggest that the method may be satisfactorily applicable to an analysis of the paprika color in foods.

Recovery Test of Paprika Color

Sherbet, rice crackers, and pickles were selected as typical foods containing the paprika color. After confirming that no paprika color was detected in any of the foods, a recovery test was performed in the following manner. A paprika color standard was added to 10 g of each sample to make the concentration 0.25,

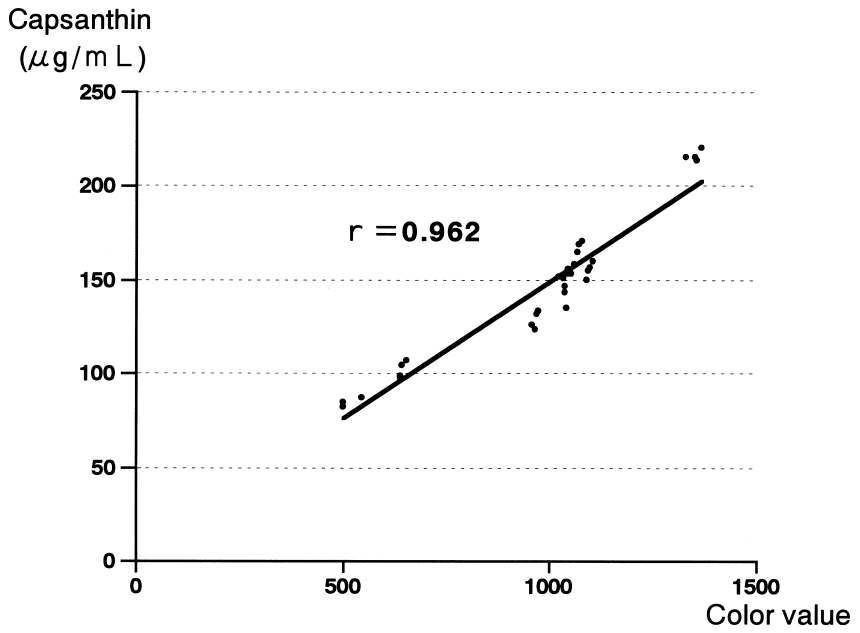


Figure 6. Correlation between color value and capsanthin concentration.

0.50, and 1.00 mg/g. The paprika color concentrations were determined 5 times at each concentration level from the capsanthin concentrations as described in the Experimental section. Table 2 shows the average recoveries of the paprika color from the various foods with respect to the capsanthin concentration obtained from the paprika color standard (100%) and the coefficients of variation. The average recoveries from the sherbet, rice crackers, and pickles were over 81.9%,

Table 2. Recovery of Paprika Color from Various Foods

Fortified Concentration (mg/g)	Recovery, % (C.V., %)		
	Sherbet	Rice Cracker	Pickles
0.25	81.9 (0.2)	91.9 (8.1)	95.6 (3.1)
0.50	90.8 (4.2)	81.4 (5.5)	97.0 (3.1)
1.00	93.2 (7.7)	84.7 (2.0)	85.1 (2.9)

n=5.

81.4%, and 85.1%, respectively, and the coefficients of variation of the sherbet, rice crackers, and pickles were less than 7.7%, 8.1%, and 3.1%, respectively. Thus, excellent recoveries and reproducibilities were obtained.

The Analytical Results of Paprika Color in Commercially Available Foods

The paprika color concentrations in 32 samples from commercially available foods that had a label stating the use of paprika color were determined, three times each, from the capsanthin concentrations. The results are shown in Table 3. The detected capsanthin concentrations were as follows: 0.04-11.9 μg/g from 7 samples of ice cream and sherbet, 0.06-6.81 μg/g from 8 samples of snack foods including potato crisps, rice-cake cubes, and rice crackers, 1.85-6.36 μg/g from 5 samples of pickles such as kimchi, 0.05-27.3 μg/g from 10 samples of seasonings, 0.09 μg/g from 1 sample of salted fish guts, and 0.13 μg/g from 1 sample of spiced cod roe. The coefficients of variation were 9.8% or less; thus, an excellent reproducibility was obtained. Accordingly, the present method is considered to be applicable to the analysis of paprika color in commercially available foods. The limit of determination was 0.02 μg/g as capsanthin.

We have developed an HPLC method for the analysis of paprika color in food using capsanthin as an indicator. Capsanthin is obtained as the main product of saponification of the paprika color followed by purification using C18 cartridge. Based on the results described above, it was confirmed that the present method is satisfactory for practical use.

CONCLUSIONS

An HPLC method for the analysis of paprika color in food using capsanthin as an indicator was developed and the following results were obtained.

1) It was confirmed that the main product obtained by saponification of a paprika color standard followed by purification using a C18 cartridge was capsanthin.

2) The capsanthin obtained by the saponification and purification of a paprika color standard exhibited excellent reproducibility and a high correlation was obtained between the capsanthin and paprika color concentrations. Also, the refrigerated capsanthin remained stable for as long as 5 days.

3) When 8 types of paprika color standards having different color values were saponificated and the resultant capsanthin concentrations were determined, an excellent correlation was obtained between the color values and the capsanthin concentrations.

Table 3. Analytical Results of Paprika Color in Foods on the Market

Food	n	Determination*	
		Mean (μg/g)	C.V. (%)
Ice cream	3	0.07	9.5
Sherbet A	3	0.04	6.4
Sherbet B	3	0.12	6.8
Sherbet C	3	0.28	2.8
Sherbet D	3	10.71	2.4
Sherbet E	3	11.72	1.6
Sherbet F	3	11.90	2.3
Rice-cake cubes A	3	0.06	8.4
Rice-cake cubes B	3	0.41	1.3
Rice-cake cubes C	3	1.66	7.3
Rice cracker A	3	0.28	9.4
Rice cracker B	3	0.72	8.1
Rice cracker C	3	0.98	4.2
Potato chips A	3	2.67	5.5
Potato chips B	3	6.81	0.5
Pickles A	3	1.85	8.8
Pickles B	3	2.74	6.3
Pickles C	3	3.01	3.6
Pickles D	3	5.57	5.8
Pickles E	3	6.36	7.5
Seasoning A	3	0.05	4.0
Seasoning B	3	0.41	1.2
Seasoning C	3	1.05	6.0
Seasoning D	3	1.95	1.7
Seasoning E	3	3.37	8.7
Seasoning F	3	3.61	2.7
Seasoning G	3	5.55	8.7
Seasoning H	3	7.07	4.1
Seasoning I	3	9.34	0.9
Seasoning J	3	27.30	5.7
Salted fish gut	3	0.09	9.8
Spiced cod roe	3	0.13	9.5

*As capsanthin.

4) The results of the recovery tests of the paprika color in typical foods exhibited excellent recoveries and reproducibility. Thus, the present method using capsanthin as an indicator for paprika color was found to be applicable to an analysis of the paprika color in commercially available foods.

Based on these findings, the present method was found to be useful for the quantitative analysis of the paprika color in foods.

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