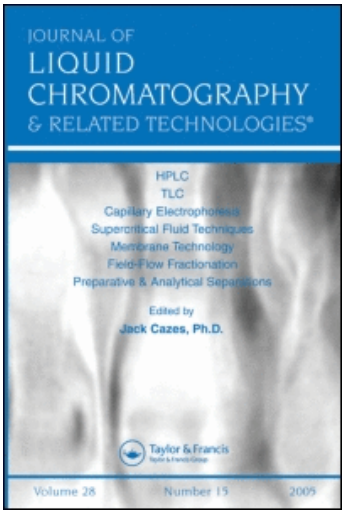

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ABSTRACT

The aim of the present study was to examine the applicability of liquid chromatography (LC) with photodiode array detection (PAD) and mass spectrometry (MS) for the determination of natural food colorants such as safflower yellow in foods. The main coloring constituents in safflower yellow as investigated by LC/PAD, were safflomin A (SF-A) and safflomin B (SF-B). The development of an accurate, sensitive, and selective analytical method was attempted by using LC/MS with an electrospray

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ionization interface. In addition, food prepared by solid-phase extraction (SPE) was evaluated. Cartridges with only the reversed-phase mode (C_{18}, C_8, and OASIS-HLB) and the mixed-phase mode (reversed phase and anion exchange: Bond Elut Certify 2) were examined in terms of recovery. The recoveries of SF-A and -B were above 70% when the mixed-phase mode cartridge was used. Therefore, the mixed-phase mode phase was found to be more suitable for food sample recovery than the other modes. The detection levels of SF-A and -B in foods were very low, and they could not be detected by LC-UV. However, the combination of LC/MS-selected ion monitoring (SIM) and SPE enabled the successful detection of trace amounts of safflower yellow in food samples.

Key Words: Safflower yellow; Safflomin; Liquid chromatography mass spectrometry (LC-MS); Solid phase extraction; Food.

INTRODUCTION

Safflower, *Carthamus tinctorius* L., is a well-known herb and natural colorant matter. Its flowers produce red and yellow pigments, the demand for which is, respectively, 12 and 160 t/year in Japan.[1] The yellow safflower pigment has been used as a natural food colorant for a long time. It was reported that the yellow pigment has numerous components, with safflomin A (SF-A) and safflomin B (SF-B) being the major ones.[2–4] The structure of SF-A, the major coloring constituent in safflower yellow, was corrected, as shown in Fig. 1, mainly on the basis of NMR and HPLC photodiode-array detection (PAD) data.[5] On the other hand, the structure of SF-B was also corrected, as shown in Fig. 1.[5]

Recently, five kinds of natural colors, including safflower yellow, in processed foods were detected by thin-layer chromatography (TLC).[6] The yellow pigment of a safflower sample was analyzed by capillary zone electrophoresis to identify and separate major components.[7] In addition, use of HPLC in the investigation of pigments was reported.[8] However, few methods are available for the identification and determination of safflower yellow in foods. Therefore, the development of a sensitive, selective, and accurate method for the evaluation of natural pigments in food is required. In the present study, we describe the development of a simple, accurate, sensitive, and selective analytical technique for the determination of safflower yellow in foods, using HPLC with PAD and electrospray-mass spectrometry (MS) prior to solid-phase extraction (SPE). First, the major color components are identified by LC–PAD and MS. Then, the major compounds comprising yellow pigments in foods are analyzed by LC–PAD and MS. This approach is useful for the evaluation of natural pigments in foods.

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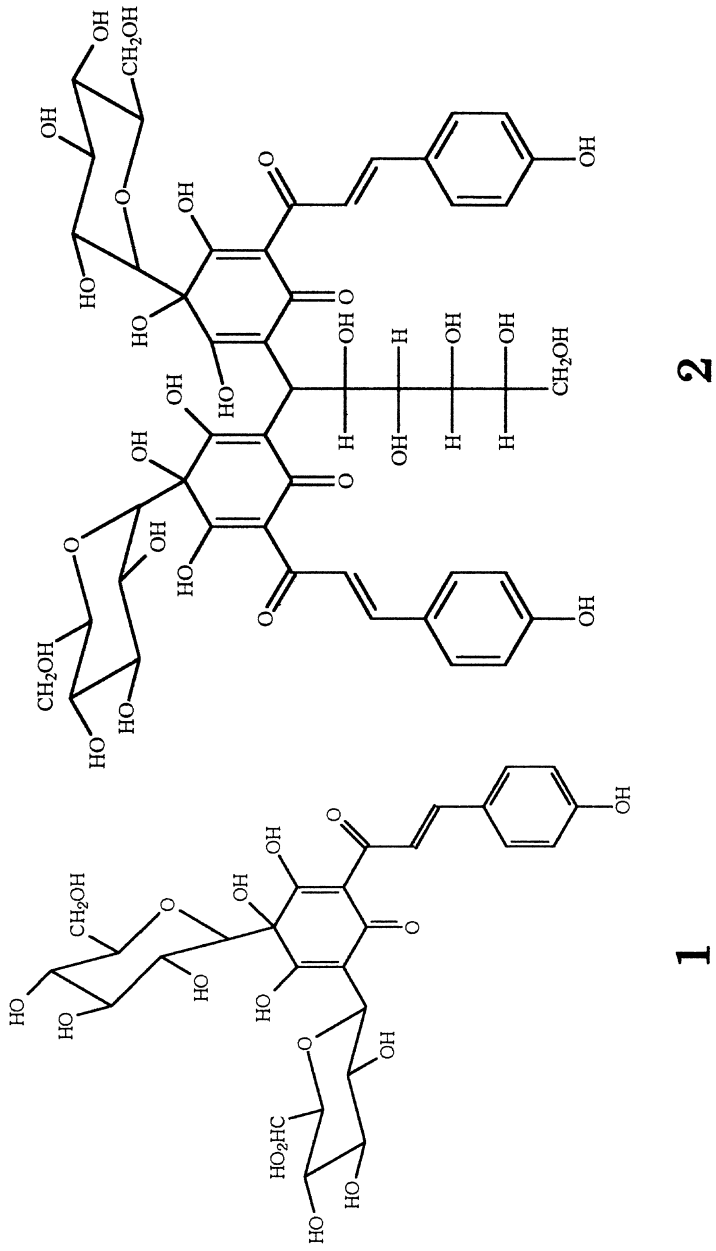

Figure 1. Structural formulae of the main compounds in Carthamus yellow. (1) Safflomin A (SF-A); (2) Safflomin B (SF-B).

EXPERIMENTAL

Reagents and Samples

Methanol, HPLC grade, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Water, purified by the Milli-Q water purification system (Millipore, Bedford, MA) was used. Carthamus yellow (Safflower yellow) was a kind gift from San-Ei Gen F.F.I., Inc. (Osaka, Japan). Other Carthamus standards were purchased from Kanto Chemical Industries Ltd. (Tokyo, Japan).

Concentrated solutions (1.0 mg/mL) of Carthamus yellow were prepared in methanol, and dilutions were made at 0.1–200 μg/mL as required by the addition of methanol.

Soft drinks and candy samples were obtained from various supermarkets and convenience stores in Japan.

Apparatus and Instrument Conditions

The LC–PAD and MS were performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). The PAD system was set at 200–500 nm. A Mightysil RP-18 GP (2×250 mm, 5 μm) reversed-phase column (Kanto Chemical Co., Ltd., Tokyo, Japan) was used. Samples volumes of 5.0 μL were injected. Liquid Chromatographic separation was carried out using 1.0% formic acid in water (mobile phase A) and 1.0% formic acid in methanol (mobile phase B). The gradient mode was as follows: 0 min at 25% mobile phase B, followed by 0–30 min of a linear increase from 25 to 60% mobile phase B. The flow rate was 0.2 mL/min. The working conditions for electrospray-MS were as follows: the drying nitrogen gas temperature was set at 350°C and the gas was introduced into the capillary region at a flow rate of 12 L/min; the capillary was held at a potential of 3500 V relative to the counter electrode in the negative-ion mode. The fragmentor voltage was fixed at 170 V for SF-A and -B during the chromatographic run. When working in the selected ion monitoring (SIM) mode, m/z 611 and 1043 ions were monitored.

Sample Preparation

The pretreatment of food samples involves various extraction procedures. However, these procedures are difficult and require a large amount of organic solvent. In this study, SPE was carried out using an extraction cartridge, because the SPE method is very easy and uses a small amount of organic

solvent. The major compounds comprising yellow pigments in food samples were pretreated using four different SPE cartridges: C_{18}-based solid, (Bond Elut C_{18}; size: 500 mg/3 mL, Quantity 50, Varian Co., USA), C_8-based solid, (Bond Elut C_8; size: 500 mg/3 mL, Quantity 50, Varian Co., USA), *N*-vinylpyrrolidone and divinylbenzene polymer, (OASIS-HLB; size: 60 mg/3 mL, Waters Co., USA), and mix C_8-based and SAX (Bond Elut Certify 2, size: 200 mg/3 mL, Varian Co., USA). The SPE cartridges for pretreatment of Carthamus yellow were Bond Elut C_{18}, C_8, and OASIS-HLB for aqueous sample. Before extracting the samples, C_{18}, C_8, and OASIS-HLB SPE cartridges were conditioned by eluting 3.0 mL of acetonitrile followed by 3.0 mL of distilled water. Six milliliter of an aqueous sample (3 mL of food sample + 3 mL of water) was eluted through the SPE cartridges. Then, the cartridges were washed with 3.0 mL of water. Acetonitrile (5.0 mL) was added, at a low flow rate to elute compounds that were retained on the cartridges.

The solutions were evaporated to dryness under a stream of nitrogen at 40°C. Then, the samples were adjusted with 1.5 mL of methanol and measured by LC/MS. On other hand, the protocol of Bond Elut Certify 2 SPE cartridge for pretreatment of Carthamus yellow was as follows. Before extracting the samples, this cartridge was conditioned by eluting 3.0 mL of methanol followed by 3.0 mL of 0.1% sodium bicarbonate in distilled water. Six milliliter of an aqueous sample (3 mL of food sample + 3 mL of 0.1% sodium bicarbonate in distilled water) was passed through the SPE cartridge. Then, the cartridge was washed with 3.0 mL of water, followed by 3 mL of methanol. Hydrochloric acid (1 M, 5.0 mL) in methanol was added at a low flow rate to elute the compounds that were retained on the cartridge. The solutions were evaporated to dryness under a stream of nitrogen at 40°C. Then, the samples were adjusted with 1.5 mL of water. The obtained samples were measured by LC/MS.

The extraction of Carthamus yellow from a solid sample was carried out by using water. Carthamus yellow was extracted from 0.1 g of a solid sample by adding 10 mL of water and supersonication for 10 min (ULTRA sonic 104X 340 W, Yucaipa, CA). Then, the sample was subjected to the same SPE method as above.

RESULTS AND DISCUSSION

Liquid Chromatography/Photodiode Array Detection and Mass Spectrometry Detection and Identification of Carthamus Yellow

The absorption and mass spectral investigation of Carthamus yellow solution (10 μg/mL) by LC/PAD and MS in the SCAN mode revealed two

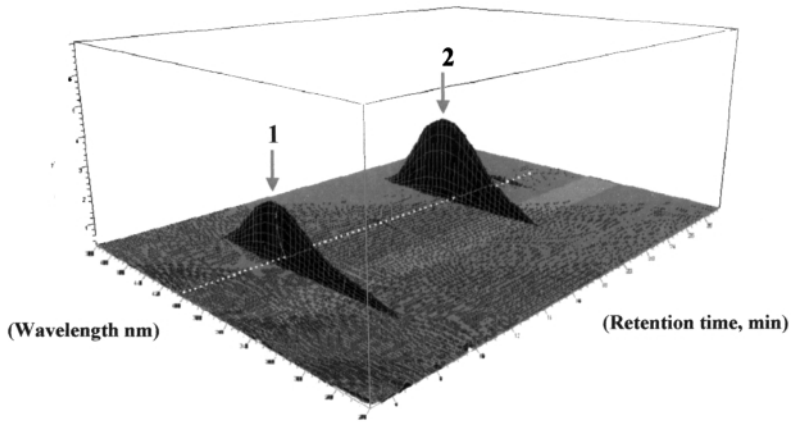

Figure 2. Liquid chromatography/Photodiode array detection chromatogram of Carthamus yellow (10 μg/mL).

peaks (Fig. 2). The absorption maximum peak at 405 nm is due to the major compound in Carthamus yellow. In addition, mass spectra were obtained by LC/MS-SCAN (m/z 200–1500) (Fig. 3). The peaks were identified to be those of SF-A (1) and SF-B (2) from Ref.[5] The electrospray mass spectrum (Fig. 3) of SF-B shows a major molecular ion at m/z 1043. In this ionization, m/z 1043 may be assigned to [M (SF-B molecular weight: 1062)-H_2O—H]— or anhydrous SF-B molecule in the negative ion mode. Therefore, we should develop a novel evaluation method for the quantification of SF-A and -B. It was considered that the integrated evaluation was possible for determining the concentrations of SF-A and -B per Carthamus yellow standard.

The most important parameters affecting LC/MS are the fragmentor voltage and the concentrations of acid and water in the mobile phase. In order to establish the optimum fragmentor voltage for the detection of SF-A and -B, the signals at m/z 611 and 1043 were investigated at various fragmentor voltages. From this, it was revealed that the optimal fragmentor voltage was 170 V (Fig. 4). On the other hand, the water/formic acid/methanol mobile phase was used in the LC separation of Carthamus yellow. Good separation of Carthamus yellow solution was achieved with 1.0% formic acid in water and methanol as the mobile phase. Therefore, this condition was useful for the detection of SF-A and -B by MS. Based on these results, we decided to use the conditions for LC/PDA/MS for the analysis of Carthamus yellow.

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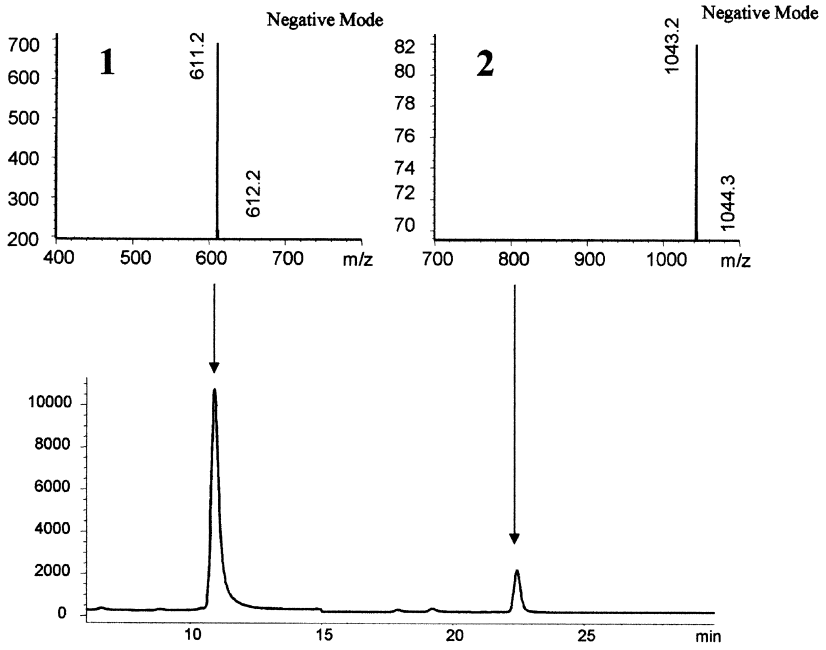

Figure 3. Liquid chromatography/Mass Spectrometry chromatogram and mass spectra of Carthamus yellow (10 μg/mL).

Evaluation of the Calculated Value

The limit of quantification (LOQ) was calculated using the equation: $10\,S_b = A_s - A_b$ (where A_s is the average of the sample signal, A_b is the average of the blank signal and S_b is the standard deviation of the blank signal). The LOQ of Carthamus yellow standard (SF-A and B) solution was 0.1 μg/mL. Using this method, the sensitive and selective determination of Carthamus yellow was realized.

In this study, the evaluation of Carthamus yellow in foods was achieved by LC/MS detection of SF-A and -B. When Carthamus yellow was subjected to LC/MS-SIM, well-separated peaks of SF-A and -B were observed. For the purpose of this study, calculation of the average recoveries of SF-A and -B was useful. Therefore, we evaluated Carthamus yellow in foods by means of individual calibration curves (the linearity of these compounds is within 0.5 ng to 1.0 μg of Carthamus yellow). A positive correlation was observed: SF-A

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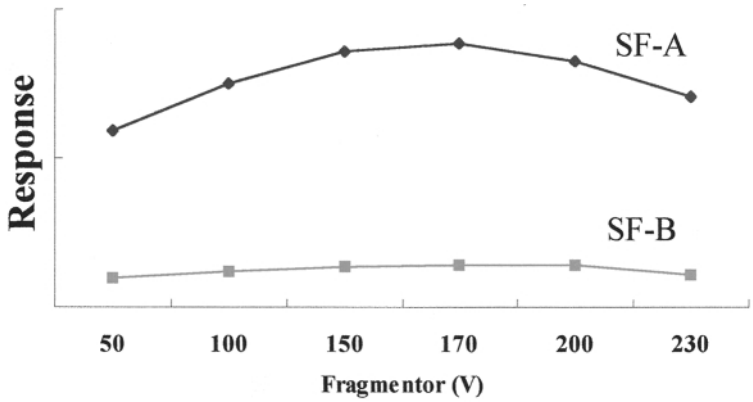

Figure 4. Effect of fragmentor voltage on the peak responses of SF-A and SF-B.

($r = 0.9999$: Area $= 1244.2 *$ Amount $+ 55.5$) and -B ($r = 0.9996$: Area $=$ $190.7 *$ Amount $- 80.9$). This analytical performance was found to be reliable, sensitive, and suitable for routine analysis. It was possible to separate and determine these compounds in a single run of 30 min by using LC/MS with SIM. The method enables the precise determination of standards and may be used in the detection of trace amounts of Carthamus yellow in food samples.

Solid-Phase Extraction for Pretreatment of Carthamus Yellow in Foods

Solid-phase extraction with the reversed-phase mode and the mixed-phase mode (reversed-phase and anion-exchange) cartridges was examined in terms of recovery. The recoveries of SF-A and -B were performed using soft drink samples spiked with a solution of Carthamus yellow. The extractions using the SPE cartridges were performed according to the above-described methods. The recoveries of SF-A and -B were above 70% when the mixed-phase mode cartridge was used (Table 1). The mixed-phase mode cartridge showed higher recoveries for samples spiked with SF-A and -B than the reversed-phase mode cartridge. Therefore, the mixed-phase mode cartridge is more suitable for food samples than the reversed-phase mode cartridges. The recoveries using mixed-phase mode SPE cartridge in aqueous food samples were determined. Good recoveries and relative standard deviations (RSD) for food samples spiked with SF-A and -B are shown in Table 2. Thus, we decided to use this cartridge for the simple and selective pretreatment of Carthamus in food samples.

Table 1. Recoveries of SF-A and -B from soft drink samples using various SPE cartridges.

	Av. recovery (%)	
SPE cartridge	SF-A	SF-B
C_{18}	49.8	22.0
C_8	22.1	43.7
OASIS-HLB	19.9	24.5
Certify 2	79.4	90.8

Note: $N = 3$.

Table 2. Recoveries of SF-A and -B from soft drink samples using certify 2 cartridge.

Carthamus yellow compound	Av. recovery (%) [RSD, %]
SF-A	81.0 [2.7]
SF-B	76.6 [2.7]

Note: 50 ppm Carthamus yellow in aqueous food samples ($N = 4$).

Table 3. Residual Carthamus yellow in foods.

		Conc. (ppm); Carthamus yellow	
No.	Sample	SF-A	SF-B
1	Soft drink	10.6	8.6
2	Soft drink	60.7	19.4
3	Soft drink	103.1	124.9
4	Soft drink	235.6	159.6
5	Soft drink	110.5	208.2
6	Soft drink	401.9	529.9
7	Candy	4.6	<0.15
8	Candy	1.2	25.8
9	Candy	112.9	322.7
10	Candy	<0.15	<0.15

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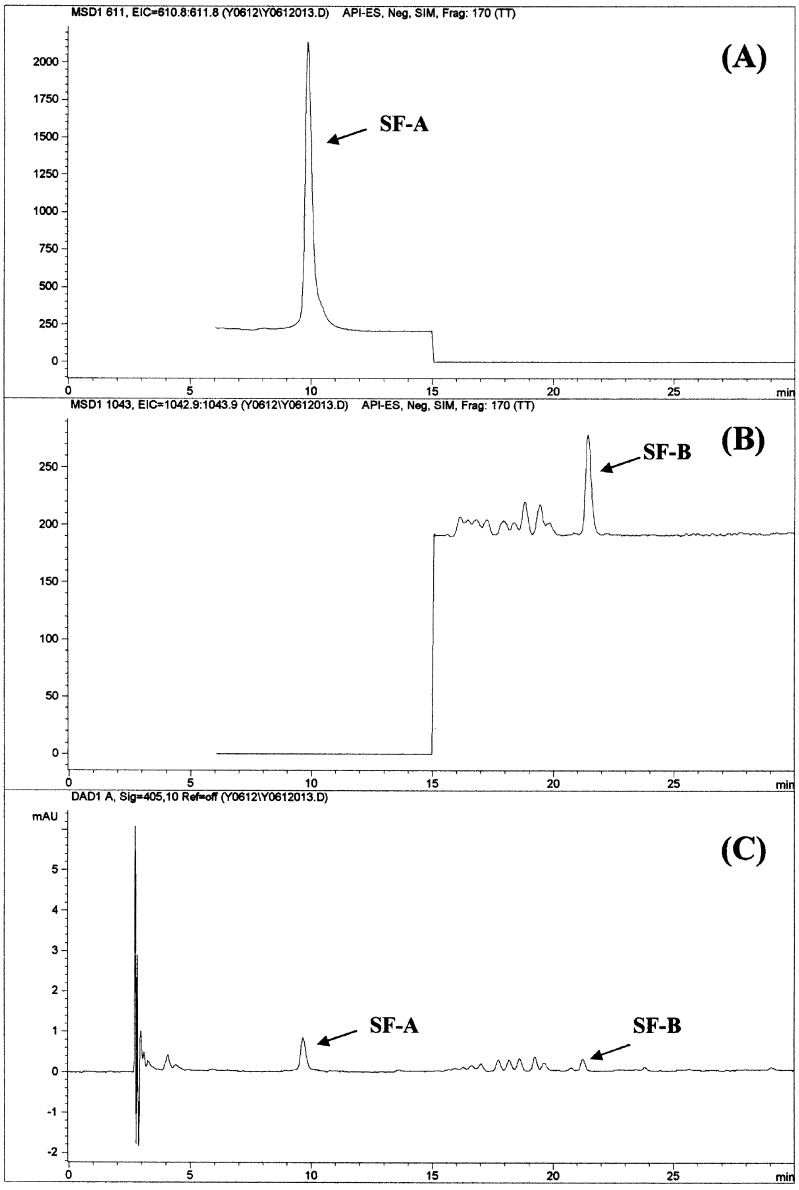

Figure 5. Chromatograms of Carthamus yellow in food sample (No. 2). (A) SIM (m/z 611); (B) SIM (m/z 1043); (C) PDA (405 nm).

Application

A total of 10 samples of soft drinks and candies were analyzed by the present method. Safflomin A and -B were detected from almost all samples, as shown in Table 3. The detection levels of SF-A and -B in foods were very low. Residual SF-A and -B were not detected by LC-UV. However, the combination of LC/MS-SIM and SPE enabled the successful detection of trace amounts of Carthamus yellow in food samples (Fig. 5).

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REFERENCES

1. Nishiyama, K. Food Chem. Jpn. **2002**, *4*, 19.
2. Sato, M.; Murata, T. Food Chem. **1994**, *51*, 307.
3. Sato, S.; Obara, H.; Onodera, J.; Endo, A.; Matsuba, S. Bull. Chem. Soc. Jpn. **1992**, *65*, 452.
4. Takahashi, Y.; Miyasaka, N.; Tasaka, S.; Miura, I.; Urano, S.; Ikura, M.; Hikichi, K.; Matsumoto, T.; Wada, M. Tetrahedron Lett. **1982**, *23*, 5163.
5. Goda, Y.; Suzuki, J.; Maitani, T. Jpn. Food Chem. **1997**, *4*, 54.
6. Hirokado, M.; Kimura, K.; Suzuki, K.; Sadamasu, Y.; Katsuki, K.; Yasuda, K.; Nishijima, M. J. Food Hyg. Soc. Jpn. **1999**, *6*, 488.
7. Watanabe, T.; Hasegawa, N.; Yamamoto, A.; Nagai, S.; Terabe, S. Biosci. Biotech. Biochem. **1997**, *61*, 1179.
8. Nakano, K.; Sekino, Y.; Yomo, N.; Wakayama, S.; Miyano, S.; Kusaka, K.; Daimon, E.; Imaizumi, K.; Totsuka, Y.; Oda, S.; Yamada, Y. J. Chromatogr. **1988**, *438*, 61.

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