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Comparison of γ-Oryzanol Contents in Crude Rice Bran Oils from Different Sources by Various Determination Methods

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Abstract: Although there are various determination methods for γ-oryzanol contained in rice bran oil by absorptiometry, normal-phase HPLC, and reversed-phase HPLC, their accuracies and the correlations among them have not been revealed yet. Chloroform-containing mixed solvents are widely used as mobile phases in some HPLC methods, but researchers have been apprehensive about its use in terms of safety for the human body and the environment.

In the present study, a simple and accurate determination method was developed by improving the reversed-phase HPLC method. This novel HPLC method uses methanol/acetonitrile/acetic acid (52/45/3 v/v/v), a non-chlorinated solvent, as the mobile phase, and shows an excellent linearity (y = 0.9527x + 0.1241, R^2 = 0.9974) with absorptiometry. The mean relative errors among the existing 3 methods and the novel method, determined by adding fixed amounts of γ-oryzanol into refined rice salad oil, were -4.7% for the absorptiometry, -6.8% for the existing normal-phase HPLC, +4.6% for the existing reversed-phase HPLC, and -1.6% for the novel reversed-phase HPLC method. γ-Oryzanol content in 12 kinds of crude rice bran oils obtained from different sources were determined by the four methods. The mean content of those oils were 1.75 ± 0.18% for the absorptiometry, 1.29 ± 0.11% for the existing normal-phase HPLC, 1.51 ± 0.10% for the existing reversed-phase HPLC, and 1.54 ± 0.19% for the novel reversed-phase HPLC method.

Key words: γ-Oryzanol, ferulic acid, rice bran oil, absorptiometry, normal-phase HPLC, reversed-phase HPLC

1 INTRODUCTION

The specific characteristic of rice bran oil is its large content of unsaponifiable components, as compared with common vegetable oils. Although crude vegetable oils generally contain less than 1% unsaponifiable components, crude rice bran oil contains more than 2% of those components, which is an especially large amount. The main unsaponifiable components contained in rice bran oil are γ-oryzanol (γ-Ory), phytosterols, tocopherols (Toc), tocotrienols, etc. γ-Ory is a physiologically active component which is rarely found in common vegetable oils. Kaneko and Tsuchiya reported in 1954 that rice bran oil showed absorption maxima at 230, 290 and 315 nm in heptane solvent[1)]. They isolated these components as a white crystal with a melting point of 137.5～138℃[2)] and named it "oryzanol." Afterwards, it was confirmed that γ-Ory is a mixture of ferulic acid ester with phytosterol or triterpene alcohol, and many specific phytosterols and triterpene alcohols have been reported as constituents of the alcoholic moiety[3,4)].

Although rice bran oil contains about 1.4-2.9% γ-Ory and corn oil contains about 0.5%, no γ-Ory is found in extracted oils from plants such as soybean, canola, palm and cotton. Tsuchiya, *et al.*[5)] have reported that the γ-Ory content in crude rice bran oil obtained from 42 different sources from all parts of Japan was 1.6-2.9% regardless of the source by

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the absorptiometry. The differences among the 42 sources not only depended on the cultivar, climate and cultivation conditions, but a higher γ-Ory content tended to be found in the oil extracted from older rice bran having a higher acid value (AV). On the other hand, it was reported that the γ-Ory contents in four crude rice bran oils produced in foreign countries were 1.4-1.9%[6], and that those oils have lower AV.

The quantitative determination of γ-Ory is very difficult to achieve because it is a mixture of many ferulic esters, such as ferulic acid with cycloartenol, 24-methylene cycloartanol, etc., and many determination methods have been proposed for γ-Ory. The most basic determination method for γ-Ory is the absorptiometry developed by Tsuchiya, *et al.*[5] in 1957, which is a method whereby the γ-Ory content in an oil sample is determined by measuring the absorbance at 315 nm, the absorbance maximum of γ-Ory.

Tanabe, *et al.*[7] reported the normal-phase HPLC method for γ-Ory determination; this method uses cholesterol ferulate as the external standard, Zipax-BOP as the column, hexane/diethyl ether (85/15 v/v) as the mobile phase, and 315 nm as the detection wave length. Their HPLC methods can accurately determine the values, with very small standard deviation of 0.03%. It was later confirmed that the data obtained by the normal-phase HPLC method gives determination values which are lower by 0.23-0.33% than those from absorptiometry, based on a comparison of the γ-Ory contents obtained from 13 kinds of crude rice bran oils: the γ-Ory contents in crude rice bran oils were 1.31-1.70%, as determined by normal-phase HPLC and 1.54-2.03% by absorptiometry. They thought that the differences between the two methods arose from the fact that all the substances had an absorbance maximum at 315 nm were determined in the absorptiometry, while the normal-phase HPLC method separates γ-Ory from the other components before determination.

Other normal-phase HPLC methods have also been developed by using butyl hydroxyl anisole (BHA) as the internal standard, an aminopropyl-bonded silica column, hexane/dichloromethane/acetic acid (70/30/1 v/v/v) as the mobile phase, and 290 nm[9] as the detection wave length. Ohsumi[8] has examined this method by adding various amounts of γ-Ory into medicine containing 10 mg of γ-Ory, 300 mg of *dl*-α-Toc acetate and 140 mg of wheat germ oil, and he has reported that γ-Ory could be precisely determined by means of a calibration curve with excellent linearity in the range of 0.01-0.09 mg/mL of γ-Ory and peak areas or heights without obstruction from the Toc and the wheat germ oil. In addition, he has also reported that a highly precise recovery of γ-Ory could be achieved, at an average of 101.1%.

Moreover, purified rice salad oils containing 0.5-2.0% γ-Ory were examined by the normal-phase HPLC method and absorptiometry, and the results obtained from the two methods were nearly the same, with an excellent correlation coefficient (above 0.999) between the added and the determined amounts[9].

Reversed-phase HPLC methods have also been proposed. In these methods, an ODS column, chloroform/methanol (1/4 v/v) as the mobile phase, and BHA[9] as the internal standard or cycloartenol ferulate[10] as the external standard are used. In the reversed-phase HPLC method with cycloartenol ferulate as the external standard, the recovery from edible oils of various amounts of added γ-Ory was 86-98%[10].

Rogers, *et al.*[11] have developed for the simultaneous separation and quantitation of tocopherols, tocotrienols, and γ-Ory present in rice bran oil by a reversed-phase HPLC. In this method, an ODS column, acetonitrile/methanol/2-propanol/water (45/45/5/5 v/v) as the mobile phase are used and cycloartenyl, 24-methylene cycloartanyl, campesteryl, β-sitosteryl, and cycloartanyl ferulates as the major γ-Ory were separated.

Terashima, *et al.*[12] have proposed a reversed-phase HPLC method which determines the ferulic acid formed after the saponification of γ-Ory. The analytical conditions of this HPLC method were as follows: a gel ODS column, methanol/water (95/5 v/v), and *o*-coumaric acid as the internal standard. The results of the determination of the γ-Ory content in commercially available medicines by the reversed-phase HPLC method of Terashima, *et al.* and absorptiometry showed that the determination values obtained by the HPLC method were 8-26% higher than those by absorptiometry, and that the values ranged from 70 to 123% of the indicated amounts for the medicines examined. Chen, *et al.*[13], Stoggl, *et al.*[14] and Britz, *et al.*[15] have also separately reported the determination methods for γ-Ory content in rice bran oil by using reversed-phase HPLC with non-chlorinated solvent mixture.

Although various HPLC methods of γ-Ory determination have been proposed and examined as described above, many HPLC methods use chlorinated solvents as the mobile phase; this raises the concern about their safety and impact on the environment. In addition, there are still many unknown factors involved in the precision and accuracy of those methods, as well as the correlations between them.

In the present work, we focused on the establishment of a novel reversed-phase HPLC method to determine γ-Ory without using a chlorinated solvent as the mobile phase. We also made a comparison of the precision and accuracy of our novel method and those of the current methods (absorptiometry, normal-phase HPLC and reversed-phase HPLC), and investigated the correlations between them. In addition, γ-Ory contained in crude rice bran oils from different sources were determined by the exisiting methods and the novel method.

2 EXPERIMENTAL

2.1 Samples and reagents

In our examination of the existent γ-Ory determination methods, we used crude rice bran oils from 12 different sources: Niigata, Saitama, Shizuoka, Kagoshima, Yamagata, Tokyo, Mie, Okinawa, China, Vietnam, Thailand and Brazil. These oils were kindly donated by Boso Oil & Fat Co., Ltd. (Funabashi, Japan). A rice salad oil from Boso Oil & Fat Co., Ltd. and a canola oil from Nisshin OilliO Group, Ltd. (Tokyo, Japan) were used as reference samples. A pure sample of γ-Ory was purchased from Tokyo Chemical Industry Co., Ltd. All the reagents used were of special grade or first grade, as commercially available, and were used without further purification, unless otherwise specified.

2.2 Characterization of the sample oils

2.2.1 Analysis of the lipid composition

The lipid compositions of crude rice bran oil, rice salad oil and canola oil were determined by TLC-FID analysis. One μL of a 1% chloroform solution of the sample oil was loaded on a chromarod with a length of 100 mm; it was then developed with a mixed solvent of benzene/chloroform/acetic acid (35/25/1 v/v/v) and determined by Iatroscan model MK-5 of Mitsubishi Kagaku Iatron, Inc. (Tokyo, Japan).

2.2.2 Analysis of the fatty acid composition

The fatty acid compositions of the crude rice bran oil, rice salad oil, and canola oil were determined by GLC analysis. The constituent fatty acids of the sample oils were methyl-esterified by the method of Jham *et al.*[16], and were determined by GLC analysis performed by a GC-18A (Shimadzu Corporation, Kyoto, Japan) equipped with a bonded capillary column, HR-SS-10 (0.25 mm I.D.× 25 m) of Shinwa Chemical Industries, Ltd. (Kyoto, Japan) at 200℃ and a flame ionization detector (FID) using helium as the carrier gas.

2.3 Determination methods for γ-Ory

2.3.1 Absorptiometry[5]

The γ-Ory content in canola oil, to which various amounts of pure γ-Ory were added, was determined by the measurement of the absorption at 315 nm in hexane solution. The quantification performance of the absorptiometry was examined by comparing the added amount with the measured amount. The analysis was conducted by using a spectrophotometer, model Ubest-50, equipped with a thermal plotter recorder, model RTL-396, of JASCO Co. (Tokyo, Japan).

2.3.2 Normal-phase HPLC method[8,9]

The γ-Ory content of the samples prepared as described in 2.3.1 was determined by means of an HPLC equipped with an intelligent HPLC pump, model 880-PU, a normal-phase Finepak SIL NH_2 column (4.6 mm ID × 250 mm), and a UV detector, model 875-UV (JASCO Co., Tokyo, Japan). The determination was carried out at detection wavelength of 300 nm and by running hexane/chloroform/acetic acid (70/30/1) at flow rate of 1 mL/min as the mobile phase. The quantification performance of the normal-phase HPLC method was determined by comparing the added amount with the measured amount, obtained by determining the γ-Ory content from the peak area ratio between γ-Ory and the BHA used as the internal standard sample.

2.3.3 Reversed-phase HPLC method I (chloroform/methanol system)[9]

By using the samples prepared as described in 2.3.1, a reversed-phase HPLC analysis was conducted by using the same HPLC apparatus equipped with a Crestpak C18S column (4.6 mm I.D.× 150 mm) and running chloroform/methanol (4/1 v/v) at the flow rate of 1 mL/min. The quantification performance of reversed-phase HPLC method I was evaluated in the same way as described in 2.3.2.

2.3.4 Reversed-phase HPLC method II (methanol/acetnitrile/acetic acid system)

To establish a novel determination method using no chlorinated mobile phase in the HPLC analysis, the same apparatus and system as that described in 2.3.3 were used, except for the mobile phase; that is, the ratio of methanol/acetnitrile/acetic acid of the mobile phase was changed and the separation and retention time of γ-Ory were examined. The effectiveness of various internal standard samples for the determination of γ-Ory was also examined, and the quantification performance of the novel method was evaluated in the same way as described in 2.3.2.

2.3.5 Determination of the γ-Ory contained in crude rice bran oil by four different methods

The γ-Ory contents in the crude rice bran oils from 12 different origins were determined by absorptiometry, normal-phase HPLC, reversed-phase HPLC I, and reversed-phase HPLC II. The γ-Ory content was calculated based on each calibration curve and the values obtained by each method were compared.

3 RESULTS AND DISCUSSION

3.1 Characterization of the sample oils

3.1.1 Analysis of the lipid composition

The lipid compositions of the sample oils were determined by TLC-FID analysis, and the results are shown in Table 1. Rice salad oil and canola oil were composed of 100% triglyceride (TG), whereas all crude rice bran oils used in the present experiment contained about 2.8% DG and 1.7% highly polar components, which are considered to be unsaponifiable substances.

3.1.2 Analysis of the fatty acid composition

The fatty acid compositions of the sample oils are shown

Table 1 Lipid Compositions of the Sample Oils (%).

	Sources of sample oils	TG	DG	Unsaponifiable
Rice bran oils	Niigata	95.5	2.7	1.8
	Yamagata	96.7	1.9	1.4
	Saitama	93.4	5.1	1.5
	Tokyo	94.1	3.2	2.7
	Shizuoka	95.8	2.2	2.0
	Mie	96.8	2.0	1.2
	Kagoshima	96.8	2.4	0.8
	Okinawa	95.0	2.9	2.1
	China	92.4	4.8	2.8
	Thailand	96.9	1.7	1.4
	Vietnam	96.4	2.8	0.8
	Brazil	97.2	1.3	1.5
Rice salad oil		100.0	0.0	0.0
Canola oil		100.0	0.0	0.0

Table 2 Fatty Acid Compositions of the Sample Oils (%).

	Sources of sample oils	C16:0	C18:0	C18:1	C18:2	C18:3
Rice bran oils	Niigata	18.6	1.6	42.9	35.6	1.3
	Yamagata	19.1	1.4	41.6	36.4	1.5
	Saitama	17.3	1.3	44.2	35.8	1.4
	Tokyo	19.0	1.6	41.3	36.7	1.4
	Shizuoka	19.1	1.6	42.1	35.8	1.4
	Mie	18.7	1.8	41.1	36.4	2.0
	Kagoshima	17.5	1.8	40.9	38.5	1.3
	Okinawa	20.0	1.6	40.7	36.3	1.4
	China	23.0	1.4	37.1	37.0	1.5
	Thailand	21.7	2.0	40.7	34.1	1.5
	Vietnam	22.1	1.8	40.3	34.2	1.6
	Brazil	20.6	1.6	39.7	36.0	2.1
Rice salad oil		19.2	1.5	42.6	35.5	1.2
Canola oil		3.9	1.7	65.1	20.4	8.9

in Table 2. The main constituent in crude rice bran oil and rice salad oil was oleic acid, and its content was around 41% regardless of the source; these oils also contained about 36% linoleic acid. The foreign crude rice bran oils tended to contain somewhat more saturated fatty acids as compared with the domestic oils. The main constituent fatty acid of canola oil was also oleic acid.

3.2 Determination methods for γ-Ory

3.2.1 Absorptiometry

The mean relative error of the γ-Ory content in eight canola oil samples to which 1.2-2.8% γ-Ory standard sample was added was -4.67 ± 1.14% when the γ-Ory content was determined by absorptiometry. Although the measurements were carried out in triplicate and each standard deviation was less than 3%, the data obtained by absorptiometry gave lower values than the actual added amounts.

3.2.2 Normal-phase HPLC method

As described in 3.2.1, eight canola oil samples to which various amounts of γ-Ory standard sample were added were analyzed by the normal-phase HPLC method. As a result, two peaks of γ-Ory were detected, and mean relative error for the γ-Ory amounts added was -6.79 ± 2.70%. Although the measurements were carried out in triplicate and each standard deviation was less than 2%, the determination values given by the normal-phase HPLC method were still lower than those given by absorptiometry.

3.2.3 Reversed-phase HPLC method I

The γ-Ory content was determined by reversed-phase HPLC method I in eight canola oil samples to which 1.4-2.3% of γ-Ory standard sample was added. γ-Ory was detected as one peak, and the mean relative error was +4.57 ± 2.14%. Although the measurements were carried out in triplicate and each standard deviation was less than 3%, the determination values given by reversed-phase HPLC method I were higher than the actual added amounts.

3.2.4 Reversed-phase HPLC method II

i) Examination of the mobile phase and the internal standard

Since every current HPLC method uses chloroform as a component of the mobile phase, there has been some concern about the destruction of the environment. So, mixed solvent systems using methanol/acetnitrile/acetic acid were examined as the mobile phase in a reversed-phase HPLC equipped with the widely used ODS column.

As a result, it was confirmed from the evaluation based on the resolution (Rs) and the retention time of γ-Ory that γ-Ory could be separated very well by adding acetic acid into the mobile phase when the ratio of methanol/acetnitrile/acetic acid was altered. The best mobile-phase ratio for γ-Ory separation, in which γ-Ory was separated completely (Rs > 1.5) at the appropriate retention time, was methanol/acetnitrile/acetic acid = 52/45/3 (v/v/v).

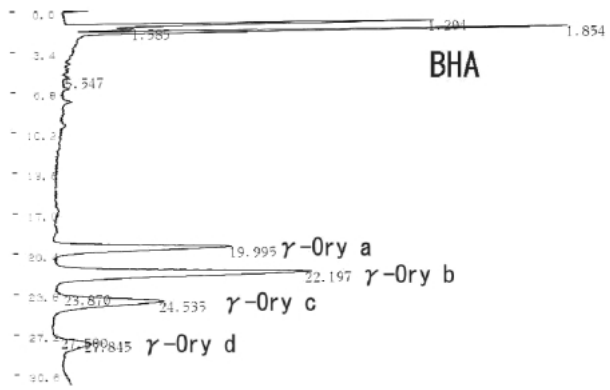

Fig. 1 A Novel Reversed-Phase HPLC Chromatogram for γ-Oryzanol Determination.

Methyl *p*-hydroxy benzoate (MHB), propyl *p*-hydroxy benzoate (PHB), ethyl 3,4-dihydroxy benzoate (EDHB), BHA, butyl hydroxyl toluene (BHT), propyl gallate (PG) and chlorogenic acid were examined as the internal standard. It was found that MHB and PHB were inadequate as internal standards because of their absorption maxima at 255 nm, which is very different from the absorption maximum of γ-Ory (315 nm). Ultimately, BHA was found to be the best internal standard, because the BHA peak in the HPLC was sharp and did not overlap with the peaks of γ-Ory.

ii) Examination of the quantification performance

In the case of the analysis by reversed-phase HPLC method II (see 2.3.4), γ-Ory was separated into four peaks as shown in Fig. 1, and the mean relative error of the total quantified value of the four peaks was -1.61 ± 2.38%.

Although eight canola oil samples with an additional 0.8-3.0% γ-Ory standard sample were analyzed in triplicate and the standard deviations were less than 2%, reversed-phase HPLC method II gave slightly lower values than the added amounts. However, the present novel method gave an excellent quantification as compared to current methods such as absorptiometry, normal-phase and reversed-phase HPLC.

3.2.5 Comparison of the four determination methods

To compare the values determined by the four methods using canola oil samples with additional γ-Ory, the relative linearities between each added amount (x) and the determination value (y) were calculated as follows: $y = 0.9890 - 0.0739$ ($R^2 = 0.9963$) for the absorptiometry, $y = 0.9897 - 0.1096$ ($R^2 = 0.9885$) for the normal-phase HPLC method, $y = 0.9543 + 0.1575$ ($R^2 = 0.9931$) for reversed-phase HPLC method I, and $y = 0.9714 + 0.0203$ ($R^2 = 0.9980$) for

reversed-phase HPLC method II.

In addition, the correlation coefficient between the straight lines was excellent ($R^2 > 0.98$). Moreover, the determination values among the four methods agreed quite well. On the other hand, the determination value given by reversed-phase HPLC method I was slightly higher than those given by the other methods. Therefore, it was confirmed that absorptiometry, the normal-phase HPLC method and reversed-phase HPLC method II gave nearly the same determination values.

3.2.6 Determination of γ-Ory contained in crude rice bran oil by the four determination methods

The γ-Ory contents in 12 kinds of crude rice bran oil were determined in triplicate by the four determination methods, and the mean values are listed in Table 3. It was found that all the crude rice bran oils analyzed contained about 1.5% γ-Ory, although the individual γ-Ory content was different according to the source of the oil. Furthermore, the correlations between the values determined by absorptiometry (x) and those by the three HPLC methods (y) were calculated as follows: $y = 0.3828x + 0.6117$ ($R^2 = 0.3714$) for the normal-phase HPLC method, $y = 0.3761x + 0.8509$ ($R2 = 0.4054$) for reversed-phase HPLC method I, and $y = 0.7877x + 0.1551$ ($R^2 = 0.5898$) for reversed-phase HPLC method II. As seen in these results, the correlation coefficients shown above had a large dispersion: R^2=0.37-0.59. However, it was considered that reversed-phase HPLC method II, which allows the strong separation of γ-Ory, was almost unaffected by perturbing factors, since the correlation coefficient ($R^2 = 0.5898$) calculated from the values obtained by absorptiometry and reversed-phase HPLC method II was larger than those given by the other two HPLC methods.

On the other hand, the correlations between the γ-Ory content in the canola oils with various additional amounts of γ-Ory, as determined by absorptiometry (x) and by the other three HPLC methods (y), were calculated as follows: $y = 0.9887x - 0.0062$ ($R^2 = 0.9828$) for the normal-phase HPLC method, $y = 0.9652x + 0.2283$ ($R^2 = 0.9973$) for reversed-phase HPLC method I, and $y = 0.9527x + 0.1241$ ($R^2 = 0.9974$) for reversed-phase HPLC method II. It was obvious that excellent correlations ($R^2 > 0.98$) were obtained between the absorptiometry and each of the three HPLC methods.

From the above description, it was considered that crude rice bran oil contained some components which can affect the determination of γ-Ory in any of the four methods examined.

However, it was confirmed that reversed-phase HPLC method II was better suited for the determination of γ-Ory than the current methods. Our novel method has the following five merits: 1) it uses non-chlorinated solvents, 2) it uses the versatile ODS column, 3) it can separate the γ-Ory very well, 4) it is unaffected by perturbing factors, 5) it has the smallest relative mean error (-1.61%) according to the quantitative addition test.

Table 3 γ-Ory Content of the Crude Rice Bran Oils (%).

Sources of sample oils	γ-Ory content (%)			
	Absorptiometry	Normal phase HPLC	Reversed phase HPLC I	Reversed phase HPLC II
Niigata	1.77 ± 0.03	1.26 ± 0.02	1.59 ± 0.02	1.61 ± 0.04
Yamagata	1.83 ± 0.01	1.30 ± 0.01	1.54 ± 0.04	1.62 ± 0.02
Saitama	1.95 ± 0.01	1.41 ± 0.01	1.55 ± 0.02	1.65 ± 0.02
Tokyo	1.75 ± 0.01	1.31 ± 0.02	1.60 ± 0.08	1.57 ± 0.01
Shizuoka	1.77 ± 0.01	1.25 ± 0.01	1.44 ± 0.02	1.45 ± 0.03
Mie	1.47 ± 0.01	1.08 ± 0.03	1.33 ± 0.02	1.20 ± 0.04
Kagoshima	1.58 ± 0.02	1.30 ± 0.01	1.50 ± 0.02	1.54 ± 0.02
Okinawa	1.91 ± 0.02	1.43 ± 0.01	1.67 ± 0.03	1.65 ± 0.03
China	1.93 ± 0.01	1.17 ± 0.04	1.46 ± 0.04	1.47 ± 0.01
Thailand	1.64 ± 0.01	1.40 ± 0.02	1.58 ± 0.04	1.34 ± 0.01
Vietnam	2.02 ± 0.01	1.39 ± 0.01	1.57 ± 0.08	1.95 ± 0.02
Brazil	1.49 ± 0.01	1.12 ± 0.01	1.32 ± 0.01	1.44 ± 0.03
Mean ± SD	1.75 ± 0.18	1.29 ± 0.11	1.51 ± 0.10	1.54 ± 0.19

Table 4 Composition of Each Peak Constituting the γ-Ory Contained in Crude Rice Bran Oil, as Detected by Reversed-phase HPLC Method II (%).

Sources of sample oils	γ -Ory (a)	γ -Ory (b)	γ -Ory (c)
Niigata	25.5	37.0	26.3
Yamagata	21.4	32.0	26.4
Saitama	22.7	36.8	29.6
Tokyo	19.1	32.4	29.4
Shizuoka	19.2	36.1	35.9
Mie	16.2	36.5	39.2
Kagoshima	27.2	36.1	25.7
Okinawa	25.6	34.6	24.2
China	22.6	36.6	23.4
Thailand	16.2	40.7	32.4
Vietnam	21.6	37.8	28.3
Brazil	23.0	40.5	28.3

Each crude rice bran oil used in the present experiment gave four peaks of γ-Ory on the chromatogram of reversed-phase HPLC method II, as shown in Fig. 1; the calculated compositions of these peaks are shown in Table 4. Although each peak named γ-Ory (a), (b) of the main component, (c), and (d) could not identify, but the four peaks will be identified in a future study.

4 SUMMARY

In the determination of γ-Ory in canola oil containing additional γ-Ory, the mean relative errors for the added canola oil amounts and the determination amounts were as follows: -4.67 ± 1.14% for the absorptiometry, -6.79 ± 2.70% for the normal-phase HPLC method, +4.57 ± 2.14% for reversed-phase HPLC method I, and -1.61 ± 2.38%, the smallest value, for reversed-phase HPLC method II. It was found that the absorptiometry, the normal-phase HPLC method and reversed-phase HPLC method II errored mostly on the negative side, although reversed-phase HPLC method I also gave a slightly high positive value. On the other hand, all correlations between the determination value and the added amount of γ-Ory were very high, and the correlation coefficients were as follows: R^2=0.9963 for the absorptiometry, R^2=0.9885 for the normal-phase HPLC method, R^2=0.9931 for reversed-phase HPLC method I, and R^2=0.9980 for reversed-phase HPLC method II. It was confirmed that the absorptiometry, the normal-phase HPLC method, and reversed phase HPLC-method II gave nearly the same determination values throughout the series. The correlation of the determination value for each crude rice bran oil by the four methods showed a large dispersion because of the correlation coefficient of R^2=0.37-0.59. However, high correlation coefficients for the determination values obtained from canola oil with additional γ-Ory between the absorptiometry and each of the three HPLC methods were observed. Although it was considered that rice bran oil contained some components which affected the determination of γ-Ory, we could not confirm those components. They will be examined in a future study.

However, it was confirmed that reversed-phase HPLC method II was better suited for the determination of γ-Ory than the current methods. Our novel method has the following five merits: 1) it uses non-chlorinated solvents, 2) it uses the versatile ODS column, 3) it allows the strong separation of γ-Ory, 4) it is almost unaffected by perturbing factors, 5) it gives the smallest mean error (-1.61%) according to the quantitative addition test.

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