Fatty Acid Composition of Selected Buckwheat Species by Fluorometric High-Performance Liquid Chromatography

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ABSTRACT

The fatty acid composition of buckwheat seeds, including the tartary species, was analyzed by high-performance liquid chromatography (HPLC) using 9-bromomethylacridine (9-Br-Ma) as a fluorescent labeling reagent for the carboxyl group. In a comparison with the common species, tartary was characterized in relation to the amount of C18:3 fatty acids and those with 20 or more carbons. The values obtained by combining fluorometry and HPLC were compared with those obtained by gas chromatography. The values of both methods were in good agreement, although slight differences were found in the C16:0 and C24:0 fatty acid contents. These results suggest that the combination of fluorometry and HPLC is a highly sensitive, simple procedure for determining the fatty acid composition of buckwheat seeds.

The lipids in buckwheat seeds have been studied from nutritional and botanical aspects by many investigators (Obara and Yuasa 1969, Obara and Miyata 1969, Dorrell 1971, Oka et al 1972, Kusano et al 1974, Mazza 1988). Since most of the lipids are present in the embryo, they can easily be preserved without loss during the milling procedure. The fatty acid composition of buckwheat seeds has been reported in detail (Dorrell 1971, Kusano et al 1974, Taira et al 1986, Mazza 1988) and was found to be similar to that of rice bran oil, particularly in relation to the long-chain saturated and monounsaturated fatty acids, such as C20:0 (arachidic, C20:1 (gadoleic), C22:0 (behenic), and C24:0 (lignoceric). On the other hand, little information is available on wild species, such as *Fagopyrum tartaricum* and *F. cymosum*, since they are seldom used as food materials.

Gas chromatography (GC) has been a conventional method for determining fatty acid composition. Recently, high-performance liquid chromatography (HPLC) combined with highly sensitive fluorometric derivatization reagents for fatty acids has been used to analyze fatty acids (Lawrence 1979). Several fluorescent labeling reagents for the carboxylic group have been developed, such as 9-anthryldiazomethane (Nimura and Kinoshita 1980). 4-bromomethyl-7-methoxycoumarin (Dungs 1977), 4-bromomethyl-1,7-dimethoxycoumarin (Farinotti et al 1983), 4-bromomethyl-7-acetoxycoumarin (Tsuchiya et al 1982) and 9-aminophenanthrene (Ikeda et al 1984). We reported that 9-bromomethylacridine (9-Br-Ma) was a fluorescent and UV-labeling reagent for determining the fatty acid composition of triacylglycerides (Akasaka et al 1990). The fluorometric method is highly sensitive and allows for determinations at picomol levels.

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In the present study, the fatty acid composition of seeds of several buckwheat species was determined by combining fluorometry and HPLC with 9-Br-Ma as a fluorescent labeling reagent for the carboxylic acid. The values obtained were compared with those obtained by GC. Because of the increasing use of wild types of buckwheat seeds and the need to identify new biologically active components, we investigated the fatty acid composition of tartary buckwheat and compared it with that of common species.

MATERIALS AND METHODS

Materials and Reagents

Thirty-six buckwheat samples were used (Table I). Buckwheat seeds of the same size were selected, and their pericarp was removed manually. All solvents for HPLC of analytical or superpure grade were purchased from Wako Pure Chemical Co. (Osaka, Japan) and Kanto Chemicals (Tokyo) and used without further purification. Standard samples of fatty acids were obtained from Sigma Chemical Co. (St. Louis, MO). 9-Bromomethylacridine and tetraethylammonium carbonate were prepared according to the method described previously (Akasaka et al 1987).

Extraction of Lipids and Determination by Fluorometric HPLC

The lipids were purified according to the method of Folch et al (1957). The lipids were extracted from about 300 mg of powdered seeds with 20 ml of a solvent consisting of chloroform and methanol (2:1) at room temperature overnight. After the precipitate was removed by filtration, 4 ml of 0.8% $CaCl_2$ was added to the solution, which was then shaken vigorously. An aliquot of 2.0 ml of the chloroform-methanol solution was transferred to test tubes. The solvent was completely removed under a nitrogen gas stream. The residue was dissolved in 1 ml of 0.2*N* NaOH-methanol and heated at 70°C for 90 min. After saponification, the reaction mixture was allowed to stand at room temperature. After acidification by the addition of 1 ml of 0.3*N* HCl, the free fatty acids were extracted with hexane. The extract was evaporated to dryness under reduced pressure at 40°C and dissolved in a mixture of 100 μl of 10 m*M* 9-Br-Ma-dimethyl formamide and 100 μl of 5 m*M* tetraethylammonium carbonate-dimethyl formamide. The labeling reaction was performed at room temperature. The reaction mixture was stored in the dark and refrigerated until analysis. The fluorescent-labeled fatty acids were separated by HPLC, equipped with a pump (Jasco model PU-880, Japan Spectroscopic Co. Ltd., Tokyo) and a loop type sample injector (Reodyne). The separation column used (TSKgel ODS 80TM, Toso Manufacturing Co., Tokyo) was 150 mm × 4.6 mm i.d. Detection was done with a Jasco FP-820 spectrofluorometer. A portion of the sample (10 μl) was injected and eluted with a solvent mixture of ethanol, acetonitrile, and water (95:95:10) at a flow rate of 0.8 ml/min. Detection was done by monitoring the fluorescence intensities at 425 nm (excitation at 365 nm). Data processing was performed with an integrator (Chromatopack C-R1B, Shimadzu Seisakusho, Kyoto).

TABLE I
Buckwheat Seeds

Sample No.	Species	Harvest Year	Location
1	*Fagopyrum tartaricum rotundatum*	...	China
2	*F. tartaricum tuberculatum*	...	Japan
3		...	Nagano, Japan
4		...	Canada
5		...	Miyazaki, Japan
6		...	Himalayas
7		...	Nepal
8		1987	Soviet Union
9		1987	Soviet Union
10		1985	Soviet Union
11	*F. cymosum*	1987	Iwate, Japan
12		1987	Miyazaki, Japan
13	*F. esculentum* cv. Saint Seglen	1987	France
14		1985	Mongolia
15		1986	China
16		1986	Canada
17		1987	Nagano, Japan
18		1986	Hokkaido, Japan
19		1985	China
20		1986	China
21		1985	Mongolia
22		1985	China
23		1986	Canada
24	Miyazakiotsubu	1986	Miyazaki, Japan
25		1986	Miyazaki, Japan
26		1987	North Dakota,U.S.
27	Botansoba	1987	Japan
28		1987	Nagoya, Japan
29		1987	China
30	Skorospelyl '81	1987	Soviet Union
31	Hokkai No. 1	1986	Japan
32	Emkens-selita	1987	Poland
33	Hokkai No. 1	1985	Japan
34	Mancan	1987	Canada
35	Hokkai No. 1	1987	Japan
36	Bogatal	1987	Soviet Union

Analysis by GC

For the methylation of the fatty acids, 5 ml of a 3% HCl-methanol solution in benzene was added to the lipids extracted from buckwheat seeds. After incubation of the mixture at 70°C for 2 hr, the resultant methylesters were extracted with 5 ml of hexane. An aliquot of the hexane was applied for analysis by GC. The gas chromatograph used (model GC-8APF, Shimadzu Seisakusho, Kyoto) was equipped with a hydrogen flame ionization detector, using a glass column (200 cm × 0.3 cm i.d.) packed with 17% diethyleneglycol succinate on 80–100 mesh (Chromosorb-W AW DMCS) at programmed temperatures of 160–230°C, (4°C/min) under a nitrogen stream (40 ml/min). The data were processed with the integrator (Chromatopack C-R1A, Shimadzu Seisakusho, Kyoto).

RESULTS AND DISCUSSION

Determination of Standard Fatty Acids by HPLC

The HPLC separation pattern of the standard fatty acids is shown in Figure 1. Twelve standard fatty acids in this aliquot consisted of 10 pmol each of C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0, C22:1, and C24:0. The retention times of three of them (C14:0, C16:1, and C18:3) were similar, and the peaks of these three could not be separated under the experimental conditions. However, results obtained by GC indicate that buckwheat seeds contain a very small amount of C14:0 and C16:1 fatty acids. In a previous experiment, the fluorescent-labeled fatty acids were well separated by HPLC using a solvent of methanol and water (Akasaka et al 1987). Under these conditions, the derivatives of the fatty acid C18:0 were eluted at 55 min. In the present study, the retention time of the same fatty acids was 15 min using the solvent of ethanol, acetonitrile, and water, since the separation of fatty acids C14–C16 was not required. Thus, this method was suitable for determining the saturated long-chain fatty acids in buckwheat seeds.

Values Obtained by Fluorometric HPLC and GC

The results obtained by fluorometric HPLC were compared with those obtained by GC using the seeds of Botan buckwheat, a common variety in Hokkaido. The seeds were milled and fractionated in a five-step procedure during processing into flour. The extracted lipids were used for determination by the two methods (Table II). The values obtained by HPLC agreed with those obtained by GC, except for palmitic acid (C16:0). The content of C16:0 determined by HPLC was about 10% higher than that determined by GC. Using GC, the C18:3 fatty acids could not be separated from other fatty acids. Although the separation between fatty acids C18:3 and C20:0 by GC was incomplete under these conditions, the total content agreed with

the sum of the contents of C18:3 and C20:0 fatty acids obtained by HPLC. No significant differences were found in the composition of the fatty acids among the fractions in the milling process, except for C18:2 fatty acids. The quantity of C18:2 fatty acids reported by Kusano et al (1974) was confirmed by this result.

Fatty Acid Composition of Buckwheat Species

The chromatographic pattern of the buckwheat variety Hokkaido-Botan as an example is shown in Figure 2. Each peak could be identified by comparison with the peaks of standard fatty acids. The fatty acid composition of 36 samples of buckwheat seeds is presented in Table III. The data for the same species that were statistically analyzed are shown in Table IV. Because the number of samples was small, *F. cymosum* species were not analyzed statistically. The small standard deviation (0.05–1.93) suggests that the fatty acid composition is characteristic for each species, and the values were similar to those reported previously (Kusano et al 1974), except for the C18:3 fatty acids.

The concentration of fatty acids such as C18:0, C18:1, and C18:2 was higher in the tartary buckwheat species than in common buckwheat, whereas the concentration of C16:0 fatty acids was lower. Significant differences were observed for C18:3 and fatty acids with longer chains (20 or more carbons). The contents of

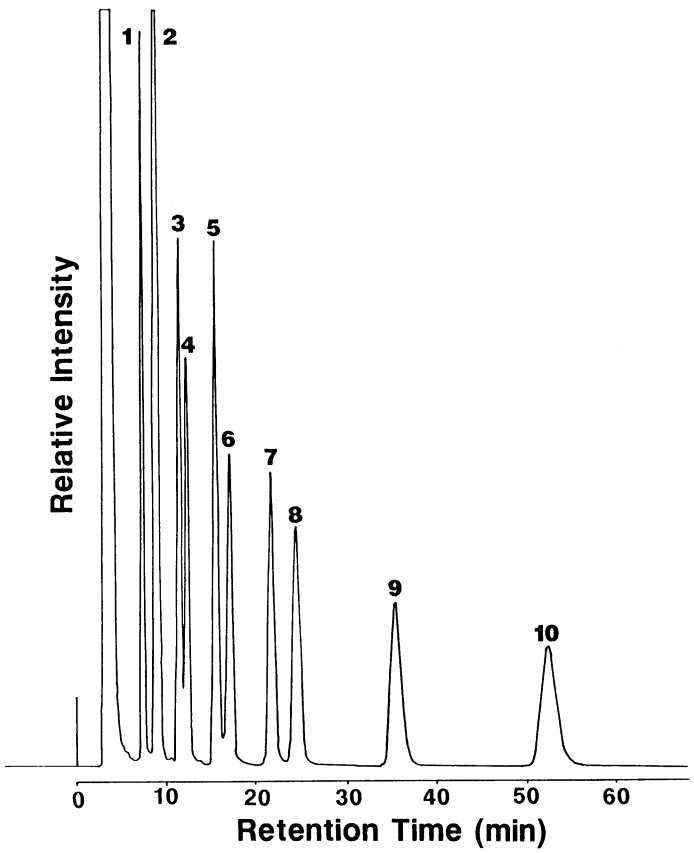

Fig. 1. High-performance liquid chromatogram of the 9-Ma-Br derivatives of standard fatty acids. 1 = C14:0 + C16:1 + C18:3, 2 = C18:2, 3 = C16:0, 4 = C18:1, 5 = C20:1, 6 = C18:0, 7 = C22:1, 8 = C20:0, 9 = C22:0, 10 = C24:0.

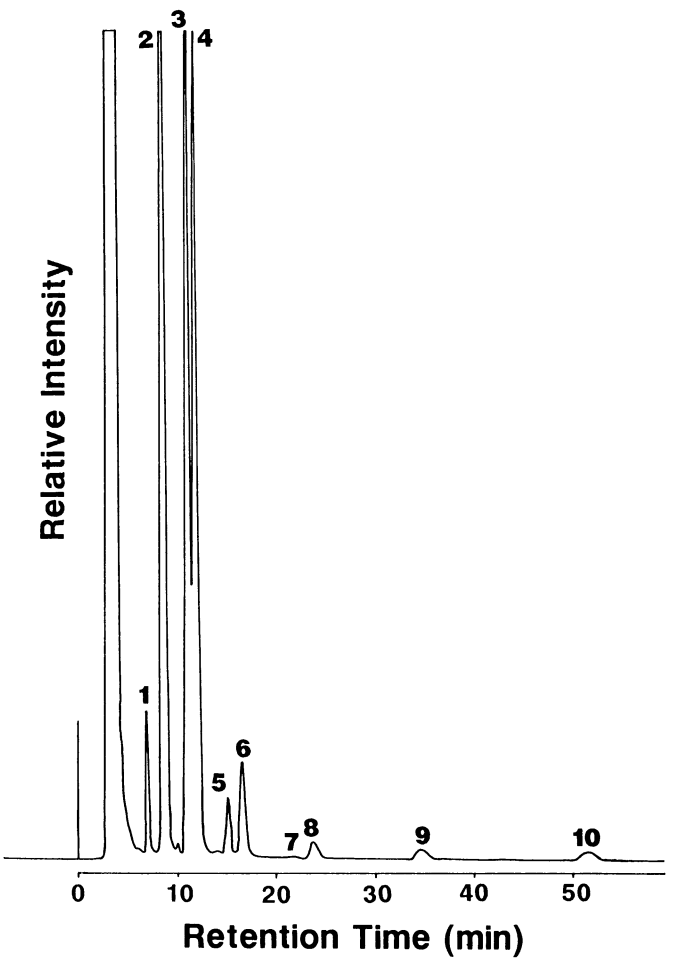

Fig. 2. High-performance liquid chromatogram of the 9-Ma-Br derivatives of fatty acids of buckwheat seeds. 1 = C14:0 + C16:1 + C18:3, 2 = C18:2, 3 = C16:0, 4 = C18:1, 5 = C20:1, 6 = C18:0, 7 = C22:1, 8 = C20:0, 9 = C22:0, 10 = C24:0.

TABLE II
Methods for Determining Fatty Acid Composition of Buckwheat Seeds Fractionated by Milling

	Fraction									
	First		Second		Third		Fourth		Fifth	
Fatty Acid	HPLC[a]	GC	HPLC	GC	HPLC	GC	HPLC	GC	HPLC	GC
C14:0	···	0.1	···	0.1	···	0.1	···	0.1	···	0.1
C16:0	21.4	17.5	19.2	15.3	18.6	14.9	18.6	14.6	18.7	15.6
C16:1	···	0.2	···	0.3	···	0.3	···	0.5	···	0.4
C18:0	2.9	2.9	2.3	2.1	1.9	2.0	1.9	2.2	2.1	2.2
C18:1	34.4	34.2	37.0	37.4	36.9	37.7	36.8	36.9	34.7	34.5
C18:3	2.2	3.7[b]	1.7	3.5[b]	2.1	3.5[b]	1.9	3.6[b]	2.0	4.1[b]
C20:0	0.8		1.4		1.3		1.5		1.5	
C20:1	1.7	1.8	3.1	2.9	3.1	2.9	3.2	3.0	3.0	3.5
C22:0	0.7	1.0	1.4	1.6	1.3	1.5	1.5	1.6	1.6	2.1
C22:1	···	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C24:0	0.9	1.5	1.0	1.2	0.8	1.3	0.9	1.4	1.1	2.1

[a] HPLC = fluorometric high-performance liquid chromatography, GC = gas chromatography.
[b] Sum of fatty acids C18:3 and C20:0.

TABLE III
Fatty Acid Composition of Buckwheat Seeds (%)

Sample No.	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
1	16.8	2.3	38.6	34.9	1.3	1.2	2.2	1.1	0.5	0.7
2	17.5	2.2	35.5	37.4	1.5	1.2	2.2	1.1	0.6	0.6
3	17.1	2.1	37.3	36.3	1.4	1.2	2.2	1.1	0.5	0.6
4	17.2	2.2	37.6	35.8	1.3	1.2	2.2	1.1	0.5	0.6
5	16.8	2.1	37.5	36.2	1.5	1.2	2.3	1.1	0.5	0.6
6	17.2	2.0	36.5	36.8	1.6	1.2	2.2	1.1	0.5	0.6
7	17.5	1.8	33.5	39.7	2.6	0.9	1.9	0.9	0.5	0.5
8	17.3	2.1	39.3	33.9	1.3	1.2	1.9	1.1	0.5	0.6
9	16.0	1.9	35.4	37.8	1.9	1.0	2.1	1.0	0.6	0.6
10	17.5	1.8	35.4	37.8	1.9	1.0	2.1	1.0	0.6	0.6
11	18.5	1.9	37.6	34.7	3.1	1.1	1.3	0.8	0.2	0.7
12	20.4	2.4	22.4	45.0	4.1	1.3	1.3	1.0	0.3	0.9
13	18.6	1.7	34.6	36.6	2.1	1.2	2.8	1.3	0.1	0.9
14	19.1	1.8	35.0	34.9	2.3	1.4	2.9	1.5	0.2	0.9
15	19.4	2.0	35.5	33.5	2.3	1.5	3.0	1.5	0.2	1.0
16	18.4	2.1	37.1	33.6	2.0	1.4	2.9	1.4	0.2	0.9
17	19.1	2.1	38.0	32.0	1.9	1.5	2.8	1.5	0.1	0.9
18	17.9	1.9	36.6	34.4	2.1	1.4	3.0	1.5	0.2	0.9
19	19.3	1.8	35.1	33.2	2.4	1.5	3.7	1.6	0.2	1.1
20	18.5	2.0	34.7	34.9	2.6	1.4	3.1	1.5	0.2	1.0
21	18.8	1.9	35.4	34.2	2.4	1.4	3.1	1.5	0.2	0.9
22	18.8	2.0	35.6	33.9	2.2	1.5	3.1	1.5	0.2	0.9
23	19.3	2.0	35.2	33.9	2.2	1.5	3.1	1.5	0.2	0.9
24	16.9	1.9	39.5	32.8	2.2	1.3	3.1	1.3	0.2	0.8
25	18.3	2.0	36.8	33.2	2.6	1.4	3.1	1.3	0.2	0.8
26	18.7	2.0	37.8	32.6	1.9	1.5	3.0	1.4	0.2	0.9
27	18.8	1.8	35.5	34.8	2.2	1.3	2.9	1.5	0.2	0.9
28	18.9	1.7	33.6	36.6	2.8	1.3	2.4	1.4	0.2	0.9
29	18.9	1.9	33.6	35.6	2.3	1.4	3.2	1.6	0.2	1.1
30	17.2	1.7	35.0	37.6	2.3	1.2	2.6	1.3	0.1	0.9
31	18.4	1.8	37.0	33.7	2.1	1.4	2.9	1.5	0.2	0.9
32	17.1	1.7	35.6	36.6	2.5	1.3	2.7	1.3	0.2	0.8
33	19.2	2.2	38.0	31.9	1.8	1.6	2.5	1.5	0.1	0.9
34	18.7	1.8	36.1	34.7	2.1	1.3	2.9	1.4	0.2	0.8
35	19.2	1.8	35.5	34.5	2.1	1.4	2.9	1.4	0.2	0.9
36	18.1	1.8	34.1	36.4	2.4	1.4	3.0	1.4	0.2	1.0

TABLE IV
Fatty Acid Composition of Tartary and Common Buckwheat (%)

Fatty Acids	Tartary		Common	
	Average	SD	Average	SD
C16:0	17.09	0.46	18.57	0.70
C18:0	2.05	0.17	1.89	0.14
C18:1	36.70	1.71	35.87	1.47
C18:2	36.88	1.93	34.42	1.53
C18:3	1.56	0.42	2.24	0.24
C20:0	1.13	0.12	1.40	0.10
C20:1	2.12	0.14	2.95	0.26
C22:0	1.06	0.07	1.44	0.09
C22:1	0.52	0.04	0.18	0.04
C24:0	0.60	0.05	0.91	0.08

the fatty acids C18:3, C20:1, C20:0, and C22:0 of tartary buckwheat were 70, 81, 82, and 84%, respectively, of those of the common species. Tartary buckwheat was characterized by the presence of C22:1 and C24:0 fatty acids. Tartary buckwheat contained three times as much C22:1 and two thirds as much C24:0 as did common buckwheat, as reported by Dorrell (1971). Among the two samples of *F. cymosum* seeds, remarkable differences were observed in the main fatty acids such as C18:1 and C18:2 (Table IV). In particular, the seeds of sample 12 contained a higher concentration of C18:2 and a smaller concentration of C18:1 fatty acids than did the others. The wild buckwheat species had more C18:3 and less C20:1 fatty acids than did the tartary and common buckwheat species.

We conclude that the combination of HPLC and 9-Br-Ma is an appropriate method for determining fatty acid composition and concentration in buckwheat seeds and other cereals. In particular, this method can be used to analyze long-chain or polysaturated fatty acids.

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