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J Rehabil Health Sci 2004; 2: 11-13

短 報

Gas Chromatographic Determination of γ-Linolenic Acid Content in Individual Seeds of Evening Primrose, *Oenothera biennis*

Akemi KINOSHITA, Kohei YAMAMOTO, Shuichi MIYATANI, and Akira SHIBAHARA†

Department of Clinical Nutrition, Osaka Prefecture College of Nursing, 7-30 Habikino 3-chome, Habikino 583-8555, Japan

Received October 30, 2004; accepted November 29, 2004

Key words: γ-linolenic acid; individual seed; evening primrose; *Oenothera biennis*; gas chromatography

1 Introduction

In a previous paper,[1] we presented our method for determining fatty acid composition of oil from a single seed of rape by gas chromatography (GC). The method consists of two steps: 1) using rapid methanolysis directly on seed tissue and 2) employing high-speed GC. This method does not require extracting the oil from the seeds, which is usually done. Therefore, even a single seed of rape, which contains the C22 monoenoic acid (erucic acid), can be tested in less than ten minutes. In this paper, we apply this method to the analysis of individual seeds of evening primrose (*Oenothera biennis*, belonging to the family Onagraceae).

Evening primrose seed oil contains a unique fatty acid, γ-linolenic acid [18:3(*n*-6)] (GLA), as one of the acyl components of its oil.[2] GLA is a double-bond positional isomer of α-linolenic acid [18:3(*n*-3)] which is commonly present in higher plants' seeds, leaves, etc. After Riley's report[3] on GLA in evening primrose seed oil published in 1949, the screening for GLA in higher plants began. Those results (including the mechanism for GLA bioformation[4] in higher plants) were reviewed by Gunstone.[2] In humans, GLA is biosynthesized from linoleic acid [18:2(*n*-6)] by the action of Δ-6 desaturase, and GLA serves as a precursor for the formation of dihomo-γ-linolenic acid [20:3(*n*-6)] plus its subsequent conversion to arachidonic acid [20:4(*n*-6)]. It has been suggested that the etiology of certain diseases may develop from a relative deficiency of GLA.[5] This may arise from an inadequate bioconversion of linoleic acid to GLA by Δ-6 desaturase. So oils containing GLA are increasing in demand due to the research which indicates that a link exists between the lack of GLA and the risk of developing diseases such as cardiovascular disorders, premenstrual syndrome, atopic eczema, rheumatic arthritis, alcoholism, etc.[5]

†Corresponding author. E-mail: shibahar@osaka-hsu.ac.jp

2 Materials and Methods

2.1 Seeds and chemicals

Seeds of evening primrose grown in England were supplied by the General Testing Research Institute of Japan Oil Stuff Inspectors' Corporation (Kobe, Japan). Some seeds of the same species were collected near our campus. GLA (purity 99%) was obtained from Wako Pure Chemical Industries (Osaka, Japan) and methylated with 14% BF_3/methanol.[6] Our laboratory standard mixture, composed of several kinds of fatty acid methyl esters (FAMEs), was also used as a reference substance for peak identification in GC.[7] Other chemicals were of the highest reagent grade available, and all solvents were distilled before use.

2.2 Preparation of FAMEs from individual seeds of evening primrose

A single seed was cut in half with scissors, put into a small glass tube, and then crushed with a glass rod. After pouring 2 mL hexane into the tube (at the same time, rinsing the glass rod), 0.2 mL 2 mol/L KOH/methanol was added. The tube was vigorously vortexed at room temperature for 2 min.[8] The mixture was allowed to stand for a few minutes, and then the upper phase containing the resultant FAMEs was transferred to a vial and evaporated to dryness under a stream of nitrogen. A small volume of hexane was added, and an aliquot of the hexane solution was withdrawn and injected directly into the GC system.

2.3 GC

The FAMEs were analyzed on a glass column (2 m × 3 mm i.d.) packed with 10% SP-2340 on 100-120 mesh Chromosorb W AW DMCS (Supelco, Bellefonte, PA) in a Shimadzu GC-14B equipped with a flame ionization detector and a data-processor, Shimadzu Chromatopac C-R7A. The column temperature was isothermally held at 200℃. The carrier gas was nitrogen at a flow rate of 35 mL/min.

3 Results and Discussion

Fig. 1 shows a gas chromatogram taken from the

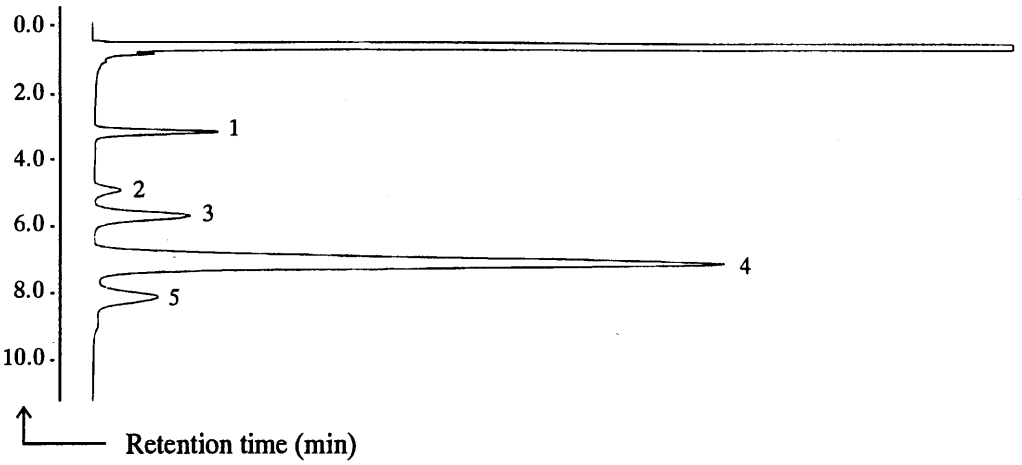

Fig. 1 Gas chromatogram of fatty acid methyl esters prepared from a single seed of evening primrose grown in England (cf. Table 1, seed no. 1).
1=palmitic acid (16:0), 2=stearic acid (18:0), 3=oleic acid [18:1(*n*-9)]+a small amount of *cis*-vaccenic acid [18:1(*n*-7)], 4=linoleic acid [18:2(*n*-6)], 5=γ-linolenic acid [18:3(*n*-6)].

analysis of the FAMEs prepared from a single seed of evening primrose grown in England (cf. Table 1, seed no. 1). All the peaks numbered in Fig. 1 were confirmed by comparing their retention times to those of our laboratory standard mixture. Furthermore, peak 5 eluted exactly at the position of authentic GLA methyl ester. In addition, α-linolenic acid, which usually occurs in plant seed oils but not in evening primrose seed oil,[2,9-11] was not detected in this sample.

According to previous research,[12] which traced the fatty acid composition of oils from evening primrose seeds during maturation, both α-linolenic acid and GLA were present at the early stage of maturation; but at the later stage of maturation (and also in mature seeds), α-linolenic acid was absent. This means the sample we analyzed did not contain any seeds at the early stage of maturation (cf. Tables 1 and 2).

Table 1 shows the fatty acid composition of the seed oil from evening primrose grown in England. Thirty analyses were carried out with thirty seeds (one analysis per one seed). Varying amounts of GLA were observed; the maximum value was 13.5% (seed no. 27), and the minimum value was 5.5% (seed no. 12). The amounts of other fatty acids similarly varied; and especially, the level of oleic acid (which included a small amount of *cis*-vaccenic acid [18:1(*n*-7)]) widely varied, ranging from 7.3% to 34.9%. We averaged the analytical values, and the GLA content in thirty seeds amounted to 7.8%. This value and also the averaged amounts of the other fatty acids indicate that there was not a distinct difference between our data and the data on evening primrose previously reported.[2,9]

Table 2 summarizes the fatty acid composition of the seed oil from evening primrose grown in Japan. Seeds in sample A were collected from one plant, and seeds in sample B were collected from another plant

Table 1 The fatty acid composition of the oil from individual seeds of evening primrose grown in England

	Fatty acid (wt%)					
Seed no.	16:0	18:0	18:1(*n*-9)[a]	18:2(*n*-6)	18:3(*n*-6)	Others
1	6.8	1.6	10.4	72.8	8.4	0.0
2	7.4	1.9	19.4	62.4	8.4	0.5
3	6.5	2.0	13.1	70.7	7.7	0.0
4	6.5	1.5	14.5	70.2	7.3	0.0
5	6.5	1.5	19.1	64.9	7.4	0.6
6	7.5	1.6	17.1	65.6	8.1	0.1
7	7.5	1.8	11.6	69.9	8.9	0.3
8	6.0	1.6	13.0	70.3	8.4	0.7
9	7.3	1.6	7.3	73.5	10.1	0.2
10	5.9	1.8	16.4	68.1	7.1	0.7
11	6.5	1.7	12.2	72.3	7.1	0.2
12	5.8	1.6	34.9	51.6	5.5	0.6
13	7.6	2.1	12.2	70.8	6.6	0.7
14	6.2	1.7	13.8	70.5	6.8	1.0
15	5.4	1.8	20.7	66.0	5.7	0.4
16	6.1	1.7	13.2	71.4	7.1	0.5
17	6.3	1.6	11.5	71.5	7.6	1.5
18	6.5	2.3	20.3	64.0	6.5	0.4
19	6.4	1.8	14.2	70.2	6.8	0.6
20	7.4	1.1	9.6	68.4	12.8	0.7
21	7.9	2.2	16.7	65.9	6.9	0.4
22	6.5	2.1	15.1	69.3	6.2	0.8
23	6.9	1.7	14.2	69.5	7.3	0.4
24	5.9	1.6	12.4	72.0	8.0	0.1
25	5.5	1.6	15.6	68.4	7.1	1.8
26	6.3	2.3	10.0	73.4	7.7	0.3
27	15.3	4.7	15.4	51.0	13.5	0.1
28	6.1	1.7	7.6	75.8	8.4	0.4
29	7.6	2.2	15.1	67.8	7.3	0.0
30	6.7	1.6	16.4	68.2	6.8	0.3
Mean	6.9	1.9	14.8	68.2	7.8	0.5

The fatty acid names matching the abbreviations are in the legend in Fig. 1.
[a] Including a small amount of *cis*-vaccenic acid.

Table 2 The averaged fatty acid composition of the oil from individual seeds of evening primrose grown in Japan[a]

	Fatty acid (wt%)					
	16:0	18:0	18:1(*n*-9)[b]	18:2(*n*-6)	18:3(*n*-6)	Others
Sample A[c]						
Mean	7.9	2.2	7.8	74.4	7.3	0.5
SD	0.6	0.3	0.7	1.0	0.4	0.5
Max	9.3	2.8	10.0	75.8	8.2	2.5
Min	7.1	1.8	6.4	72.5	6.3	0.0
Sample B[c]						
Mean	7.0	2.1	8.1	75.3	7.1	0.5
SD	0.8	0.3	0.6	0.9	0.7	0.5
Max	7.9	2.7	9.4	77.6	9.4	1.8
Min	4.7	1.4	7.2	74.0	6.1	0.0

The fatty acid names matching the abbreviations are in the legend in Fig. 1.

[a] Each value is an average of the data from thirty analyses carried out with thirty seeds (one analysis per one seed; thirty seeds per sample).

[b] Including a small amount of *cis*-vaccenic acid.

[c] Seeds in sample A were collected from one plant, and seeds in sample B were collected from another plant nine days after sample A was collected.

nine days after sample A was collected. Each value is an average of the thirty analyses carried out with the thirty seeds (one analysis per one seed; thirty seeds per sample). The amounts of GLA and also of the other fatty acids in the seeds from sample A and sample B did not vary as much as those observed in Table 1. The fatty acid composition of oil from each seed from one plant seemed to remain constant.

It is known that the GLA level in evening primrose seed oil changes depending on seed yield, growth temperature, autumn- or spring-sown seed, growing location, soil moisture level, and seed maturation.[2,12,13] The data supporting the above were probably obtained by a usual analytical method in which researchers analyzed FAMEs prepared from oils from a batch of seeds from many evening primrose plants. From our data in Tables 1 and 2, we think that there may exist some plants of evening primrose which produce a higher level of GLA than other evening primrose plants grown in the same controlled conditions. This means that high-yielding GLA evening primrose plants can be defined. Growers of evening primrose can have their plants' seeds easily tested with our simple method, presented in this paper, and begin growing high-yielding GLA evening primrose plants for commercial use.

In Japan, evening primrose seed oil is imported and sold as a health food supplement under the name *tsukimi-so-yu* (oil), but *tsukimi-so* is not evening primrose. *Tsukimi-so* (*Oenothera tetraptera*) is the name of a plant with white flowers that is so rare that botanical gardens around the world desire it for their collections. Evening primrose has yellow flowers and grows wild in fields throughout Japan. The high cost of evening primrose oil would be greatly reduced if high-yielding GLA evening primrose were cultivated for commercial use in this country.

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