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Comparison of Triglyceride Composition between Red Pepper Seed Oils Harvested from the Chungsong and Youngyang Areas

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Abstract

A study was carried out to elucidate the triglyceride compositions of the red pepper seed oils harvested from two different areas. The oil was extracted from the red pepper seed with n-hexane. Each triglyceride of the oil was separated by thin layer chromatography (TLC) and fractionated by reverse phase high performance liquid chromatography (HPLC) on the basis of acyl carbon numbers, and partition number group (PN) and fatty acid composition of triglyceride were analyzed by gas liquid chromatography (GLC). From the results, it was found that the red pepper seed oils of the Chungsong and Youngyang areas consisted of 14 and 18 kinds of triglycerides, respectively. The red pepper seed oil of the Chungsong area consisted of ($C_{18:2}$, $C_{18:2}$, $C_{18:2}$ = 39.6%) and ($C_{16:0}$, $C_{18:2}$, $C_{18:2}$ = 37.1%), and that of the Youngyang area consisted of ($C_{18:2}$, $C_{18:2}$, $C_{18:2}$ = 41.0%), ($C_{16:0}$, $C_{18:2}$, $C_{18:2}$ = 36.3%) and ($C_{16:0}$, $C_{16:0}$, $C_{18:2}$ = 8.4%), as the major triglycerides.

Key words : red pepper seed oil, triglyceride composition

INTRODUCTION

Structurally, triglyceride is composed of three molecules of fatty acid and one molecule of glycerol. The variety of distribution of triglyceride has been known to be influenced by genotypic variation, season, environment, and maturity. The physicochemical properties of the lipid depend considerably on the distribution and position of fatty acids attached at glycerol[1]. The triglyceride composition and related characteristics of natural oil and fat have been determined using jointly HPLC and GLC by Plattener et al.[2,3] and Wada et al.[4,5] To give an example, Wada and Koizumi[6] reported that the triglyceride, having an unsaturated fatty acid linked at the 2-position of glycerol, was more stable towards oxidation than linked at the 1 or 3-position. In Korea, some researchers[7-15] have been reported to reveal the triglyceride compositions of vegetable oils, because there has been an increasing awareness of the influence of dietary fat on human health in recent years. The present paper was undertaken to compare the composition of triglyceride of the red pepper seed oil harvested from the Chungsong area with that of the seed oil from the Youngyang area, with a view to afford a useful knowdgement on how to use it efficiently as an edible oil source.

MATERIALS AND METHODS

Materials

The samples, red pepper seeds, were purchased from the Pujun market in Pusan.

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Extraction of the sample oil

After the sample was crushed in a pestle bowl, the oil was extracted from it with 5 volumes of n-hexane for 24 hours in a dark place, filtered, and concentrated with a rotary evaporator under the vacuum. After replacing the air with nitrogen gas, the oil was stored in a freezer at −20℃.

Analysis of triglyceride composition

Triglyceride was separated by TLC, divided to each PN and collected by HPLC, followed again to be analyzed into each carbon number by GLC. The compositions of fatty acid also were analyzed by GLC

Isolation of triglyceride by TLC

TLC plate of 20cm×20cm glass coated with a thickness of 0.25mm silica gel G was used. The plates were developed by the solvent system of petroleum ether : diethyl ether : acetic acid (145 : 55 : 1.5, v/v/v), dried, sprayed with 0.02% 2', 7'-dichloro-fluorescein solution, and visualized with UV light. The zones containing triglyceride were identified by comparison with the position of standard triolein, scraped from the plates, eluted with chloroform, filtered and freed of solvent in a stream of N_2 gas (Fig. 1).

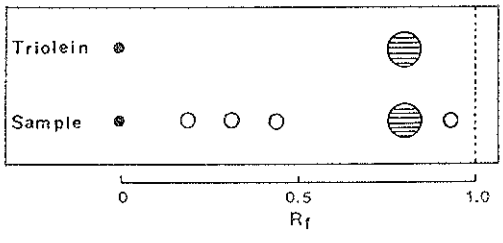

Fig. 1. TLC chromatogram of triglyceride in red pepper seed oil. Plate absorbant : silica gel G(250μm); Solvent system : petroleum ether-diethyl ether-acetic acid(145 : 55 : 1.5, v/v/v)

Fractionation by partition number (PN) of triglyceride

The triglycerides were fractionated into PN by HPLC under the analytical condition of Table 1. Each peak of HPLC chromatogram was compared with the retention time of standard materials. After concentration of each fraction it was maintained in a freezer, and used for GLC analysis.

Table 1. Condition for HPLC analysis of triglyceride

Instrument	Waters Associates (Model 440)
Column	μ-Bondapak C_{18} (10.0cm × 0.8cm i.d.)
Eluent	Acetonitrile : Acetone: Methanol : Chloroform (3:3:3:1)
Flow rate	1.0ml / min
Detector	RI
Chart speed	5mm / min
Temperature	25℃

Fractionation by acyl carbon number according to PN

The PN of triglyceride was separated into acyl carbon number (CN) by GLC with analytical condition(Table 2). Each peak on the gas chroma-togram was identified by comparision with standard materials.

Table 2. Condition for GLC analysis of triglyceride

Gas chromatograph	Shimadzu GC-RIA
Column	0.34m × 3.0mm i.d., glass
Packing material	1.5% OV-17 Silicon on 80-100 mesh Shimalite W
Column temperature	260-350℃ at 4℃/min
Chart speed	3 mm/min
Detector temperature	FID at 320℃
Carrier gas	100ml/min, nitrogen

Analysis of fatty acid composition for each PN

The PN of triglyceride was dissolved in benzene, added 14% BF_3-MeOH, and heated for 30 minutes at 80℃ in a water bath to be converted into its methylester. 20ml of distilled water and 30ml of petroleum ether were added to the methylester,

Table 3. Conditions for GLC analysis of fatty acid

Gas chromatograph	Shimadzu GC-RIA
Column	3.0 m × 3.0 mm i.d., glass
Packing material	10% 1,4-Butanediol Succinate on 60-80 mesh Shimalite W
Column temperature	195℃
Chart speed	5 mm/min
Injector temperature	250℃
Detector temperature	250℃, FID
Carrier gas	40 ml/min, nitrogen

which was again transferred into petroleum ether. After washing several times it was dehydrated by the anhydrous Na_2SO_4, followed by removal of the petroleum ether. Then it was dissolved in diethyl ether, and used for GLC analysis. The condition of the GLC analysis was shown in Table 3.

RESULTS AND DISCUSSION

Proximate composition and characteristics of the sample

The contents of crude lipid of the red pepper seed of the Chungsong and Youngyang areas were 26.71 % and 25.36%, respectively. Acid values of refined lipids were 1.1 and 1.3, and iodine values were 158.6 and 162.4, respectively (Table 4).

Table 4. Analytical data of red pepper seed oils

Sample item	Red pepper seed		Seed oils	
	Chungsong	Youngyang	Chungsong	Youngyang
Lipid(%)	26.71*	25.36	–	–
Moisture(%)	10.28	9.74	–	–
A.V.**	–	–	1.1	1.3
I.V.***	–	–	158.6	162.4

*Data represent means by three replications
**Acid value ***Iodine value

Fractionation by the PN of triglyceride

The triglyceride was fractionated into each PN by HPLC utilizing μ - Bondapak C_{18} column as shown in Fig. 2. Triglycerides of both red pepper seed oils of the Chungsong and Youngyang area showed such 5 peaks as PN 40, 42, 44, 46 and 48. The results have a tendency to be in accordance with reports of Chun et al[15]. on watermelon seed oil and safflower oil, as well as that of Choi et al[8]. on cotton seed oil, all of which were determined to be distributed with the same 5 peaks of PN 40, 42, 44, 46 and 48. The compositions of triglyceride calculated from peak areas are given in Table 5.

Fractionation by acyl carbon number according to PN

The PN of triglyceride was again fractionated into acyl carbon number by GLC. As shown in Fig. 3, the chromatogram of the fractions was able to be separated by GLC and the composition ratios were shown in Table 6.

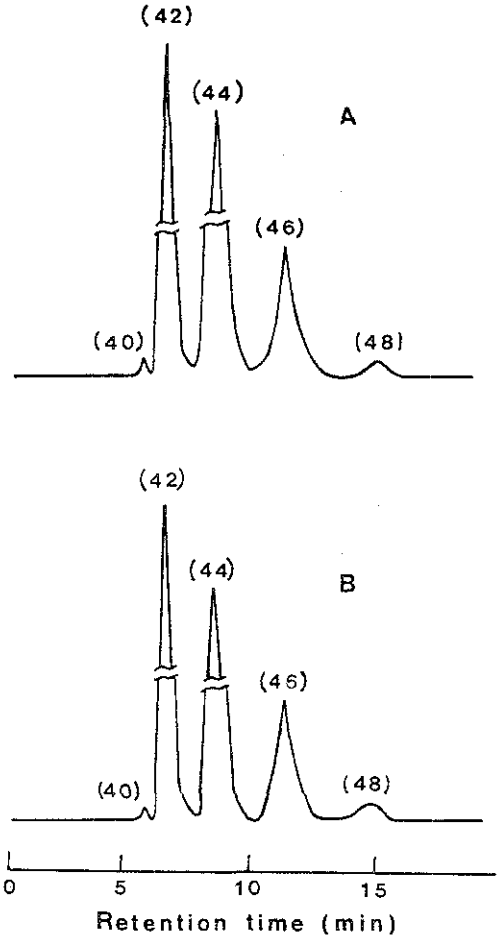

Fig. 2. HPLC chromatograms of triglycerides separated according to partition number (PN) in the red pepper seed oil harvested from the Chungsong (A) and Youngyang areas (B). Numbers of parenthesis indicate partition numbers.

Table 5. Triglycerides separated according to partition number in HPLC for red pepper seed oil (Area %)

Fraction No	Partition No.	Composition	
		A*	B**
1	40	0.6 ***	0.2
2	42	42.7	42.7
3	44	40.4	42.4
4	46	13.5	13.6
5	48	2.8	1.1

* Chungsong area ** Youngyang area
*** Data represent means by three replications

The ratios of the red pepper oil from the Chungsong area were as follows. PN 42 was composed of simply the acyl carbon number 54, PN 44 was also only the numbers 52, and the composition ratios were 100%. PN 46 was 50, 52 and 54, their ratios 50.8%, 45.2% and 4.0%, respectively. On the other hand, the composition ratios of the seed oil from the Youngyang area were as follows : PN 42 was composed of just the acyl carbon number 54, and PN 44 also was just 52, and the ratios of them were 100%. PN 46 was the numbers 50, 52 and 54, and their ratios were 83.3%, 16.7% plus the trace amount. PN 48 was composed of the acyl numbers 50, 52 and 54, and their ratios were 18.1%, 63.6% and 18.3%, respectively. From PN and acyl carbon numbers, the triglyceride compositions were estimated. Results are given in Table 7.

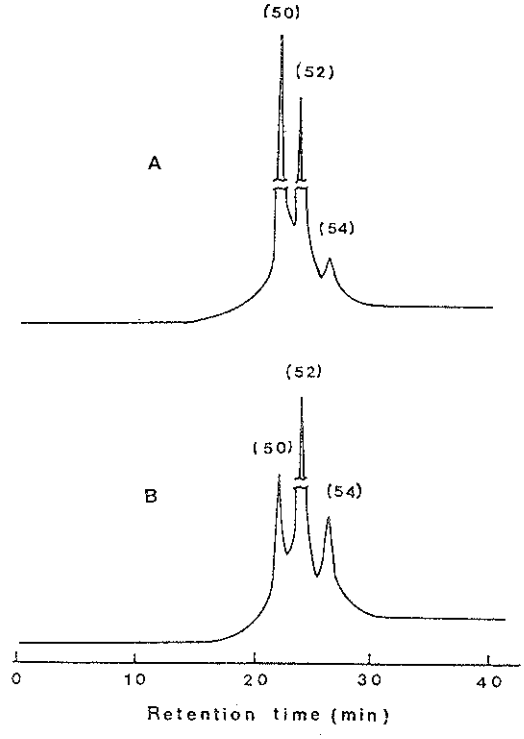

Fig. 3. GLC chromatograms of PN 46(Chungsong area, A) and PN 48 (Youngyang area, B) fractioned on the basis of acyl carbon number (CN).

Table 6. Triglycerides of CN fractions according to PN in the red pepper seed oil (Area %)

	CN \ PN	40	42	44	46	48
	50	–	–	–	50.8***	14.2
A*	52	–	–	100	45.2	57.4
	54	–	100	100	4.0	28.4
	48	4.5	–	–	–	–
B**	50	–	–	–	83.3	18.1
	52	50.7	–	100	16.7	63.6
	54	44.8	100	–	trace	18.3

*, **,***: See the footnotes in Table 5

Table 7. Triglycerides of CN fractions according to PN in the red pepper seed oil (Area %)

	CN \ PN	40	42	44	46	48
	50	–	–	–	6.9***	0.4
A*	52	–	–	40.4	6.1	1.6
	54	–	42.7	–	0.5	0.8
	48	trace	–	–	–	–
B**	50	–	–	–	11.3	0.2
	52	0.1	–	42.4	2.3	0.7
	54	0.1	42.7	–	–	0.2

*, **,***: See the footnotes in Table 5

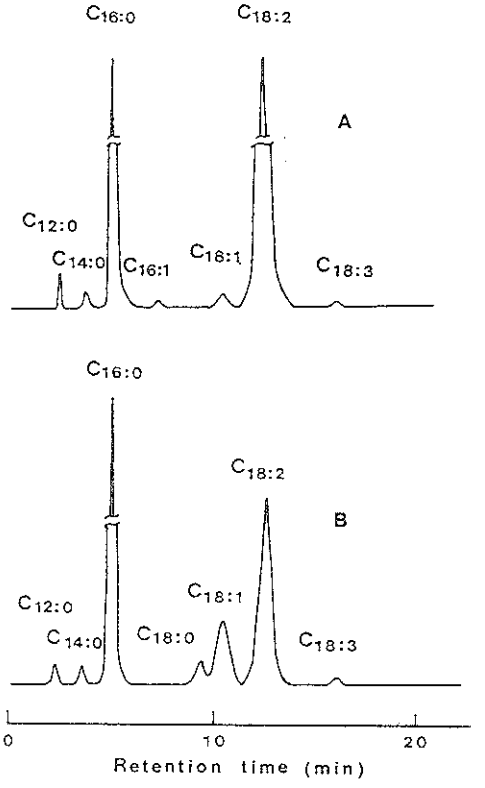

Fig. 4. GLC chromatograms of fatty acids in PN 44 (Chungsong area, A) and PN 46(Youngyang area, B).

The composition of fatty acid for each PN

The GLC chromatograms of fatty acid are shown in Fig. 4 and the composition ratios are displayed in Table 8.

In the red pepper seed oil from the Chungsong area, the high contents of main fatty acids by PN were as follows : For PN 42, the composition ratio of $C_{18:2}$ was 92.7%. For PN 44, the ratios were $C_{16:0}$ = 31.9% and $C_{18:2}$ = 61.1%. For PN 46, they were $C_{12:0}$ = 7.4%, $C_{14:0}$ = 7.7%, $C_{16:0}$ =19.8%, $C_{18:0}$ = 11.0%, $C_{16:1}$ = 16.5%, and $C_{18:2}$ = 37.6%. For PN 48, they were $C_{12:0}$ = 7.3%, $C_{14:0}$ = 5.6%, $C_{16:0}$ = 14.2%, $C_{18:0}$ = 9.8%, $C_{18:1}$ = 25.3% and $C_{18:2}$ = 37.9%. In the red pepper seed oil from the Youngyang area, the high contents of main fatty acids by PN were as follows : For PN 40, the composition ratios were $C_{12:0}$ = 8.0%, $C_{14:0}$ = 11.4%, $C_{16:0}$ = 6.7%, $C_{16:1}$ = 6.6%, $C_{18:0}$ = 4.6%, $C_{18:1}$ = 5.9%, $C_{18:2}$ = 43.9%, and $C_{18:3}$ = 12.9%. For PN 42, the ratio of $C_{18:2}$ was 96.2% and the others were insignificant For PN 44, the ratio of $C_{16:0}$ was 28.5% and $C_{18:2}$ was 63.6%. For PN 46, they were $C_{16:0}$ = 45.6%, $C_{18:1}$ = 10.8% and $C_{18:2}$ = 36.9%. For PN 48, the high contents of main fatty acids were $C_{16:0}$ = 19.3%, $C_{18:0}$ = 9.7%, $C_{18:1}$ = 21.1% and $C_{18:2}$ = 44.7%.

Estimation of triglyceride composition

From the above results, the combinations of triglyceride are shown in Tables 9 and 10. The calculation was performed by the method of Plattner[2, 3]. It was found that there were 14 and 18 kinds of triglycerides in the red pepper seed oils of the Chungsong and Youngyang areas, repectively. The combinations of the main triglycerides were : The triglyceride of ($C_{18:2}$, $C_{18:2}$, $C_{18:2}$) was 39.6% and that of ($C_{16:0}$, $C_{18:2}$, $C_{18:2}$) was 37.1% for the oil of the Chungsong area, while the triglyceride of ($C_{18:2}$, $C_{18:2}$, $C_{18:2}$) was 41.0%, that of ($C_{16:0}$, $C_{18:2}$, $C_{18:2}$) was 36.3%, and that of ($C_{16:0}$, $C_{16:0}$, $C_{18:2}$) was 8.4% in the oil of the Youngyang area, respectively. On the other hand, it was reported that the trilinolen contents of triglyceride were shown to be 50.6% in safflower oil[15], while they were 2.3% in rice bran oil[11], 27.6% in watermelon seed oil[15] and 15.5% in cotton seed oil[8]. From these results, it was found that there are high contents of trilinolen, which is known to have an important nutritive value, in the triglyceride of red pepper seed oil.

Table 8. Fatty acid compositions of the triglyceride fractioned by PN in the red pepper seed oil (Area %)

	Fatty acid \ PN	40	42	44	46	48
A*	$C_{12:0}$	–	–	2.5***	7.4	7.3
	$C_{14:0}$	–	1.9	1.5	7.7	5.6
	$C_{16:0}$	–	2.0	31.9	19.8	14.2
	$C_{16:1}$	–	–	trace	trace	–
	$C_{18:0}$	–	2.5	–	11.0	9.8
	$C_{18:1}$	–	0.9	2.9	16.5	25.3
	$C_{18:2}$	–	92.7	61.1	37.6	37.9
	$C_{18:3}$	–	trace	trace	trace	trace
B**	$C_{12:0}$	8.0	1.6	1.1	1.0	1.3
	$C_{14:0}$	11.4	2.2	3.0	2.1	3.9
	$C_{16:0}$	6.7	trace	28.5	45.6	19.3
	$C_{16:1}$	5.9	–	–	–	–
	$C_{18:0}$	4.6	–	–	3.6	9.7
	$C_{18:1}$	6.6	trace	3.8	10.8	21.1
	$C_{18:2}$	43.9	96.2	63.6	36.9	44.7
	$C_{18:3}$	12.9	trace	trace	trace	trace

*, **,***: See the footnotes in Table 5

Table 9. Triglyceride compositions of each fraction in the red pepper seed oil of the Chungsong area

Fraction No.	Fatty acid composition			Triglyceride composition	
				Mole % in each fraction	% in whole triglyceride
2	18:2	18:2	18:2	92.7 *	39.6
3	16:0	18:2	18:2	91.8	37.1
4	14:0	18:0	18:2	16.2	1.1
	14:0	18:1	18:1	6.9	0.5
	16:0	16:0	18:2	12.6	0.9
	16:0	18:1	18:2	33.0	2.0
	18:0	18:2	18:2	1.8	trace
	18:1	18:1	18:2	1.2	trace
5	14:0	18:0	18:1	4.2	trace
	16:0	18:0	18:1	3.9	trace
	16:0	18:0	18:2	16.8	0.3
	16:0	18:1	18:1	17.7	0.3
	18:0	18:1	18:2	8.4	0.1
	18:1	18:1	18:1	7.8	0.1

*Data represent means by three replications

Table 10. Triglyceride compositions of each fraction in the red pepper seed oil of the Chungsong area

Fraction No.	Fatty acid composition			Triglyceride composition	
				Mole % in each fraction	% in whole triglyceride
1	12:0	18:2	18:2	3.3 *	trace
	12:0	18:1	18:3	0.3	trace
	14:0	16:1	18:3	0.3	trace
	18:1	18:2	18:3	11.1	trace
	18:2	18:2	18:2	18.2	trace
	18:2	18:2	18:3	23.4	0.1
2	18:2	18:2	18:2	96.0	41.0
3	16:0	18:2	18:2	85.5	36.3
4	14:0	18:0	18:2	3.0	0.4
	14:0	18:1	18:1	3.3	0.5
	16:0	16:0	18:2	61.5	8.4
	16:0	18:1	18:2	13.4	1.8
5	14:0	18:0	18:1	2.7	trace
	16:0	16:0	18:1	8.1	0.1
	16:0	18:0	18:2	23.2	0.3
	16:0	18:1	18:1	11.6	0.1
	18:0	18:1	18:2	2.3	trace
	18:1	18:1	18:1	8.2	0.1

* Data represent means by three replications

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청송 · 영양산 고추씨 기름의 Triglyceride 조성의 비교

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요 약

식용자원의 하나인 고추씨 기름에 관하여 식품화학 및 유화학적인 기초자료를 얻기 위한 연구의 일환으로 고추씨 기름의 triglyceride조성을 분석하고, 중요한 고추 생산지 두 곳을 선정하여 산지별에 따른 그 차이를 비교 검토하였다. 즉 청송산 및 영양산 고추씨 기름의 triglyceride 조성을 밝히기 위하여 시료유를 TLC에 의하여 triglyceride를 분석하고, HPLC로써 PN별로 분획한 후, 각 획분을 분취하여 GLC로써 acyl carbon number별로 분획하였다. 또한 PN별 획분을 GLC에 의하여 지방산 조성을 분석하였다. 그리고 이들 분석된 결과로 부터 triglyceride조성을 산정하였는데, 청송산 고추씨 기름은 14종류, 영양산은 18종류의 triglyceride로 구성되어 있었으며, 주요triglyceride는 청송산 고추씨기름이 $(3\times C_{18:0})$가 39.6%, $(C_{18:0}\times C_{18:0})$가 37.1%이었고, 영양산의 경우는 $(3\times C_{18:0})$가 41.0%, $(C_{18:0}\times C_{18:0})$가 36.3%이었다.