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Triacylglycerol composition of dry peas (*Pisum sativum* L.)

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Abstract : Lipids in dry peas were extracted by the mixture of chloroform-methanol-water, and from the extracted lipids triacylglycerols(TG) were separated by thin layer chromatography. TG were separated into different fractions according to partition numbers by HPLC. Each of these collected fractions was analyzed on the basis of acyl carbon number by GLC, and their fatty acid compositions were also analyzed by GLC. From these results, the possible fatty acid combinations of TG in dry peas were estimated to be thirty three kinds and the major kinds were as follows : $C_{16:0}C_{18:2}C_{18:2}$(13.4%), $C_{18:1}C_{18:2}C_{18:3}$(9.3%), $C_{18:1}C_{18:2}C_{18:2}$ (9.2%), $C_{18:2}C_{18:2}C_{18:2}$(8.1%), $C_{18:2}C_{18:2}C_{18:3}$(6.4%) and $C_{18:0}C_{18:1}C_{18:2}$(5.4%).

Pea(*Pisum sativum* L.) is a kind of legume and is among the top ten vegetable crops of the world. Pea is usually used two forms, green(immature) peas and dry(mature) peas. Almost 4/5 of the world's pea crops is utilized in the form of dry beans and only about 1/5 as green peas. Dry peas may be utilized in the production of canned or dehydrated pea soups, or to a limited extent to make products such as instant pea soup mixes, quick-cooking dried peas, pea flour, pea protein concentrate, etc.[1] The lipid content of dry peas represents a small fraction compared with other components such as carbohydrate and protein. However, lipids have something to do with the functional properties of seed products during processing and storage. Especially the composition of triacylglycerols(TG) which are major component of seed lipids plays an important role in nutrition as well as in processing and storage. Accordingly many studies on the composition of TG in seeds such as soybean[2], cotton seed[3], corn[4], olive[5], rice bran[6], sesame[7], perilla[8], watermellon seed[9], kidney bean [10], etc. have been carried out. Very few papers, however, have been pulished on the composition of TG in dry peas. Therefore this study was performed to investigate the composition of TG in dry peas.

Materials and Methods

Materials

Dry peas were obtained from a culture in Wanju gun. The lipids of dry peas were extracted with chloroform-methanol-water mixture by the method of Bligh and Dyers.[11]

Key words : Triacylglycerols, dry peas.
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Isolation of triacylglycerols

The TG of dry peas were separated by thin layer chromatograpy(TLC, silica gel G, 0.5mm layer) using petroleum ether-diethyl ether-acetic acid at 90 : 10 : 1(V/V/V).

Bands of TG were detected by spraying 0.02% methanolic 2', 7'-dichlorofluorescein and by ultraviolet visulization. The bands were scraped from the plate, eluted with ether, filtered, and then the solvent was evaporated by rotatory evaporation.

HPLC of triacylglycerols

The isolated TG were separated into partition number(PN) fractions by HPLC on a column packed with μ-Bondapack C_{18}, using 2-propanol-acetone-methanol-acetonitrile mixture as a solvent. The other conditions for HPLC analysis were the same as in the previous study.[10]

Carbon number(CN) profiles of the triacylglycerol fractions

Carbon number profiles of TG frations were obtained by GLC. For this analysis, 1m × 3mm glass column packed with 2% OV-1 on chromossorb W were used and the other conditions were the same as in the previous study.[10] Each peak of the gas chromatogram was identified with standard TG.

Analysis of fatty acid methyl esters

Fatty acid methyl esters(FAME) were prepared by the procedure of Firestone et al.[12], using methanolic BF_3 solution. For this analysis, 2m × 3m stainless steel columns containing 15% DEGS on chromosorb W were used, and the other conditions were the same as in the previous study.[10] Each peak of the gas chromatogram was identified with standard FAMEs.

Results and Discussion

HPLC of triacylglycerols

Fig. 1 shows the chromatogram of TG in dry peas obtained by HPLC. As seen in the figure, TG in dry peas were clearly separated into five fractions, which were PN 40, PN 42, PN 44, PN 46, and PN 48.

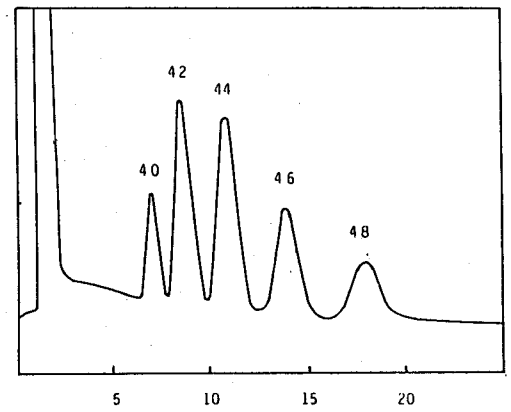

Fig. 1. HPLC chromatogram of triacylglycerols in dry peas.
Peak numbers indicate partition numbers.

The percentages of the TG fractions were calculated based on their peak areas on the chromatogram(Table 1) and they ranged from 10.0 to 29.9%.

GLC of triacylglycerol fractions

The five TG fractions were analyzed by GLC. Three acyl carbon(CN) groups, CN 50, CN 52 and CN 54 were revealed in the TG fractions. On the basis of the peak areas on the chromatograms obtained, the percentages of CN 50, CN 52 and CN 54 in each fraction were calculated and shown in Table 2.

Table 1. Percentage of triacylglycerol fraction separated by HPLC

Fraction No.	Partition No.	Composition (%)
1	40	10.0
2	42	27.5
3	44	29.9
4	46	20.0
5	48	12.6

Table 2. Triacylglycerol composition of the fractions separated by HPLC

(mol %)

CN	PN				
	40	42	44	46	48
50	—	6.3	—	16.1	—
52	2.7	16.6	52.2	46.0	44.3
54	97.3	77.1	47.8	37.9	55.7

PN 40, PN 44 and PN 48 fration revealed two peaks corresponding to those of CN 52 and CN 54 TG on the chromatograms. PN 42 and PN 46 fraction contained another shorter chain length TG, CN 50, in addition to CN 52 and CN 54 TG.

Fatty acid composition of triacylglycerol fractions

Each TG fraction collected by HPLC was subjected to GLC after transesterfication with BF_3-MEOH, in order to examine its fatty acid composition. All TG fractions were composed of six fatty acids such as $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. But PN 48 fraction contained trace amounts of $C_{18:3}$(Table 3).

Table 3. Fatty acid composition of triacylglycerol fraction separated by HPLC

(mol %)

Fatty acid	PN				
	40	42	44	46	48
16 : 0	5.9	6.2	21.4	15.4	16.1
16 : 1	16.2	6.8	2.2	14.5	10.7
18 : 0	7.6	4.6	4.7	6.7	11.5
18 : 1	10.7	13.2	20.8	38.3	33.5
18 : 2	39.4	55.9	44.8	18.2	24.4
18 : 3	20.2	13.3	6.1	6.9	trace

Estimation of triacylglycerol composition

Based on the data shown in Table 1, 2 and 3 the possible TG composition of each fraction was calculated and the results are shown in Table 4. The total TG composition of dry peas were composed of 33 kinds and the major kinds were P L L(13.4%), O L Le(9.3%), O L L(9.2%), L L L(8.1%), L L Le(6.4%) and S O L(5.4%). Compared with another kinds of legume, the major kinds of TG in dry peas differed from those in kidney bean[10] which were L Le Le(26.6%), L LLe(18.5%), Le Le Le (14.9%) and O L Le(11.3%). But the major kinds of TG in dry peas were similar to those in soybean[2] which were L L L(17.5%), O L L(13.6%), P L L(12.6%), P O L(11.6%) and L L Le(8.0%), although there was a difference in their contents.

Table 4. Triacylglycerol composition of lipids in dry peas

Fraction No.	Fatty acid composition	PN	CN	DBN	Composition In each fraction (mol %)	Composition Total (%)
1	P Le Le	40	52	6	0.7	0.1
	Po L Le				2.0	0.2
	O Le Le		54	7	33.1	3.3
	L L Le				64.2	6.4
2	P Po Le	42	50	4	4.5	1.2
	Po Po L				1.8	0.5

Table 4. (Continued)

	P L Le		52	5	10.0	2.8
	Po O Le				3.6	1.0
	Po L L				3.6	1.0
	L L L		54	6	29.4	8.1
	S Le Le				33.8	9.3
	O L Le				33.8	9.3
3	P L L	44	52	4	44.7	13.4
	Po Po Le				0.9	0.3
	Po S Le				6.6	2.0
	S L Le		54	5	7.5	2.2
	O O Le				9.4	2.8
	O L L				30.0	9.2
4	P P L	46	50	2	3.8	0.8
	P Po O				10.2	2.0
	Po Po S				2.1	0.4
	P S Le		52	3	6.5	1.3
	P O L				19.4	3.9
	Po S L				7.1	1.4
	Po O O				13.0	2.6
	S O Le		54	4	11.9	2.4
	S L L				4.5	0.9
	O O L				21.5	4.3
5	P S L	48	52	2	17.3	2.2
	P O O				16.9	2.1
	Po S O				10.1	1.3
	O O O		54	3	12.5	1.6
	S O L				42.9	5.4

P : $C_{16:0}$, P_0 : $C_{16:1}$, S : $C_{18:0}$, O : $C_{18:1}$, L : $C_{18:2}$, Le : $C_{18:3}$
DBN : double bond number

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완두의 트리아실글리세롤 조성

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초록 : 완두의 트리아실글리세롤의 조성을 구명하기 위하여 TLC로 트리아실글리세롤을 분리한 후 HPLC를 이용하여 PN별로 분획하고 GLC로 분석하였다. 이들 결과를 종합하여 트리아실글리세롤의 조성을 추정한 결과 건조된 완두의 트리아실글리세롤은 33종류였으며 주요 트리아실글리세롤은 $C_{16:0}C_{18:2}C_{18:2}$(13.4%), $C_{18:1}C_{18:2}C_{18:3}$(9.3%), $C_{18:1}C_{18:2}C_{18:2}$(9.2%), $C_{18:2}C_{18:2}C_{18:2}$(8.1%), $C_{18:2}C_{18:2}C_{18:3}$(6.4%) 및 $C_{18:0}C_{18:1}C_{18:2}$(5.4%)이었다.