Analysis of the Molecular Species of Glycerolipids from Rye Grains by Reversed-Phase High-Performance Liquid Chromatography

YASUO MANO,[1] SAKANORI NISHIYAMA,[2] MICHIYUKI KOJIMA,[2] MASAO OHNISHI,[2] and SEISUKE ITO[2]

ABSTRACT

Cereal Chem. 68(3):280–284

Total lipids were extracted and fractionated from several varieties of rye grains harvested in Europe and in Japan. The molecular species of their principal glycerolipids were analyzed by reversed-phase high-performance liquid chromatography. The yields of the total lipids were 1.6–1.9%, in which triacylglycerol (TG) was over 40%. The major lipid classes of the glycolipids and phospholipids were diglycosyldiacylglycerol (DGDG) and monoglycosyldiacylglycerol (MGDG) in the first group and phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) in the second group. TG in rye grains was separable into at least 13 molecular species, among which the principal types (70% of total TG) were trilinolein, oleoyllinoleoyllinolenin, palmitoyldilinolein, oleoyldilinolein, and dilinoleoyllinolenin, in decreasing order. DGDG and MGDG contained six kinds of molecular species, in which dilinolein (LL) was the most abundant, followed by palmitoyllinolein (PL), oleoyllinolein, and linoleoyllinolenin in decreasing order. At least nine different types of molecular species were confirmed in PC, PE, and PI. The former two showed a similar pattern of molecular species, which were mainly composed of LL and PL. In the latter, the relative proportion of PL was over 40% of the whole. Comparison of the major lipid classes from rye grains with those from rice and maize showed a remarkable difference in the compositions of the molecular species in the TG from the different species. Slight distinctions were recognized in the constituents of the molecular species in the DGDG, PC, and PI derived from several species.

The studies on cereal lipids have been widely performed chiefly on wheat, rice, and maize, and very important information on processing and utilizing cereal grains has thus been obtained (Morrison 1978). In particular, the role of lipids in breadmaking (Zeringue et al 1981, Chung 1986) and the influence of lipids on the characteristics of starch grains (Karkalas and Raphaelides 1986, Takahashi and Seib 1988) are now being actively studied.

Rye (*Secale cereale* L.) is cultivated worldwide and is used as the raw material for brown bread and whisky. The cultivated areas are especially in the North European countries and regions in the Soviet Union. In Japan, rye is cultivated only on a small scale as material for whole crop silage and mulch, and there is only modest production for food uses. Rye can withstand low temperature (−25° C) and has sufficient ability to absorb nutrients from the soil that it can be cultivated in poor (sandy, peaty, and acidic) soils. To obtain food resources for future use in our country, it is important to promote the production of rye grains by using the cool climate of Hokkaido.

The lipid classes of rye grains, which constitute fatty acids and sterol compositions, have already been reported (Aylward and Showler 1962, Price and Parsons 1975, Morrison 1978, Zeringue and Feuge 1980). Nevertheless, they are very general, and detailed studies have not yet been done.

We recently presented a report concerning lipid contents, compositions of lipid classes, constituent fatty acids of the principal lipid classes, and the sterol compositions contained in four rye varieties harvested in Hokkaido, Japan (Mano et al 1990). A study of the molecular species of glycerolipids, which are the major lipid components in rye grains, has not yet been performed. Nowadays, the composition of molecular species based on the combination of constituent fatty acids of glycerolipids can be easily and accurately analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) (Plattner et al 1977, Yamauchi et al 1982, Demandre et al 1985). We have similarly reported on the chemical composition of glycerolipids from maize grain and have suggested the possibility of structural specificity in glycerolipids between cereal grains (Ohnishi et al 1989).

In this article, we discuss the molecular species from the six major glycerolipid groups in three varieties of rye grain, purchased from Europe and cultivated on the experimental farm of Obihiro University of Agriculture and Veterinary Medicine. In parallel with those grains, one sample cultivated in Hokkaido (Japan) was also used in this study.

[1]Obihiro Ohtani Junior College, Otofuke-cho, Hokkaido 080-01, Japan.
[2]Department of Bioresource Chemistry, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Hokkaido 080, Japan.

MATERIALS AND METHODS

Rye Samples

Rye varieties SV 82018 and SV 83019 were cultivated in Sweden and variety Kustro in West Germany, and grains from these were harvested and brought to Japan. Seeds of the same three varieties were also grown on the university's experimental farm from September 1987 to August 1988 and then harvested. A Petokus sample harvested in Japan in 1988 was obtained from the Kyowa-cho Agricultural Cooperatives (Hokkaido, Japan). These three varieties from Europe and Japan and the Petokus sample harvested in Japan were used as the experimental materials. The protein contents of the four varieties were determined by the Kjeldahl method (N × 5.83%).

Protein contents for the three European rye varieties were 13.9% for SV 83019, 11.8% for Kustro, and 8.4% for SV 82018. The Petokus sample contained 9.6% protein. The values (percent of grain weights) represent means of at least duplicate determinations.

Extraction and Fractionation of Total Lipids

A portion (100–200 g) of each rye grain was crushed to powder and steamed under atmospheric pressure for 3 min to inactivate lipolytic enzymes. Powdered rye grain, after steaming, was extracted three times with four volumes of a chloroform-methanol (2:1, v/v) mixture with stirring at room temperature. The respective extracts were combined and washed using Folch's method to obtain total lipids (Folch et al 1957). The total lipids were applied to a silicic acid column and successively eluted with chloroform to obtain the neutral lipid fraction, with acetone for the glycolipid fraction, and with methanol for the phospholipid fraction (Rouser et al 1967).

Isolation of Glycerolipids

Triacylglycerol (TG) was isolated and purified with the combination of silicic acid column chromatography and silicic acid thin-layer chromatography, according to a method previously described (Miyazawa et al 1978). Monoglycosyldiacylglycerol (MGDG) and diglycosyldiacylglycerol (DGDG) were isolated from the glycolipid fraction by repeated silicic acid column chromatography using a chloroform-acetone solvent system (Fujino and Sakata 1973). The phospholipid fraction was fractionated into neutral and acid fractions using column chromatography with diethylaminoethyl (DEAE)-Toyopearl 650M (Tosoh Co., Ltd.)

(Toriyama et al 1988). The DEAE-Toyopearl 650M, suspended in the mixed solvent of chloroform-methanol (1:4, v/v), was packed in a column (1 × 4 cm) and loaded with about 50 mg of phospholipid fraction dissolved in the same solvent. The neutral phospholipid fraction was eluted with chloroform-methanol (1:4, v/v), and the acidic one with chloroform-methanol-10*M* ammonium acetate (20:80:0.2, v/v). The former fraction was fractionated by silicic acid thin-layer chromatography with a solvent system consisting of chloroform-methanol-water (65:25:4, v/v) to obtain purified phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The latter was washed using Folch's method (Folch et al 1957) and was subjected to silicic acid thin-layer chromatography, as described above, to obtain phosphatidylinositol (PI).

Preparation of Dinitrobenzoyl Derivatives of Diacylglycerols from Phospholipids

Isolated and purified PC, PE, and PI were decomposed to diacylglycerols (DGs) with phospholipase C (Ito et al 1979) and converted to dinitrobenzoyl (DNB) derivatives (Kito et al 1985). The DGs (prepared from 0.5 mg of the phospholipids) and 3,5-DNB-chloride (25 mg) were dissolved in dry pyridine (0.5 ml) and heated in a sealed vial at 60°C for 10 min. Then 0.5 ml of water was added, and the solution was heated at 60°C for a further 10 min. After 2.0 ml of 0.1*N* HCl had been added, the product was extracted three times with 1.5 ml of *n*-hexane. The combined extract was dried under an N_2 flow to remove pyridine; the residue was dissolved in 2.0 ml of *n*-hexane; and this solution was washed with equal volumes of 0.1*N* HCl (three times), 0.1*N* $NaHCO_3$, and 1*N* NaCl. Then 2.0 ml of water was added, and the product was extracted with 2.0 ml of *n*-hexane.

RP-HPLC

RP-HPLC was done under the following conditions. An LC-6A high-performance liquid chromatograph (Shimadzu Co., Ltd., Kyoto, Japan) was used, with an ERC-ODS-1282 column (6 × 250 mm). The mobile phase was acetone-acetonitrile (64:36, v/v) for TG analysis at a flow rate of 1.5 ml/min, methanol-water (95:5, v/v) for MGDG and DGDG analyses at a flow rate of 1.5 ml/min, and acetonitrile-isopropanol (8:2, v/v) for analysis of the DNB derivatives at a flow rate of 1.0 ml/min. The column temperature was 30°C for TG analysis, 50°C for MGDG and DGDG analyses, and 40°C for DNB derivative analyses. Dissolution of lipids used TG in chloroform, MGDG and DGDG in chloroform-methanol (1:1, v/v), and DNB derivatives in *n*-hexane. The detector was an ERC-7520 differential refractometer (Erma Optical Co., Ltd., Toyko, Japan) for TG, MGDG, and DGDG and a Shimadzu SPD-2A ultra-violet detector (254 nm) for DNB derivatives.

Densitometry

The neutral lipid, glycolipid, and phospholipid fractions were analyzed using silicic acid thin-layer chromatography. The neutral lipid fraction was developed with *n*-hexane-diethyl ether-acetic acid (80:20:1, v/v), the glycolipid fraction with chloroform-methanol-water (65:16:2, v/v), and the phospholipid fraction with chloroform-methanol-water (65:25:4, v/v). All the plates were subjected to densitometry to obtain the approximate compositions of the respective lipid classes after spraying with 50% H_2SO_4. A CS-9000 two-wave chromatoscanner (Shimadzu Co., Ltd.) was used for the densitometric analysis.

Gas Chromatography

For the identification of material in the peaks detected in the RP-HPLC analysis, samples were recovered and concentrated to dryness. After mathanolysis of these samples with 5% HCl in methanol for 2 hr at 100°C, the resulting fatty acid methyl esters were analyzed by gas chromatography according to a previously described method (Ohnishi et al 1986). The TGs obtained from the HPLC analysis were directly subjected to gas chromatography using a Diasolid ZT packed column (Miyazawa et al 1978).

RESULTS

Lipid Yields

The lipid contents of the three rye varieties harvested in Europe (SV 83019, SV 82018, and Kustro) were 1.6–1.9%. The values represent means (percent of grain weights) of at least duplicate determinations. No significant difference in the lipid contents was found among the three of them. The four rye varieties cultivated in Japan (SV 83019, SV 82018, Kustro, and Petokus) also contained 1.6–1.9% lipid. The compositions of the rye lipid classes harvested in Europe showed little difference from those cultivated at our experimental farm (Mano et al 1990). For total lipids, TG was the principal lipid class, occupying 41–49% of total lipids, and was most abundant in the Petokus variety in spite of there

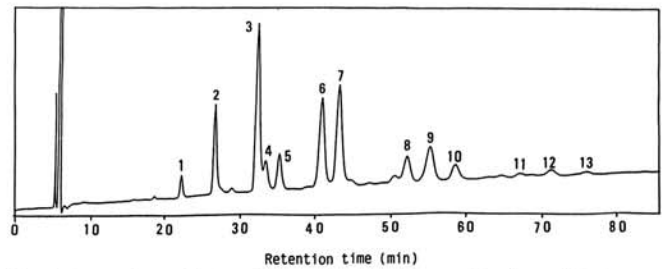

Fig. 1. Separation of triacylglycerol molecular species from Petokus rye grain by reversed-phase high-performance liquid chromatography using an ERC-ODS-1282 column. The column temperature was 30°C. The mobile phase was acetone-acetonitrile (64:36, v/v) at the flow rate of 1.5 ml/min. Peaks were monitored with a refractive index detector. See Table I for peak identification.

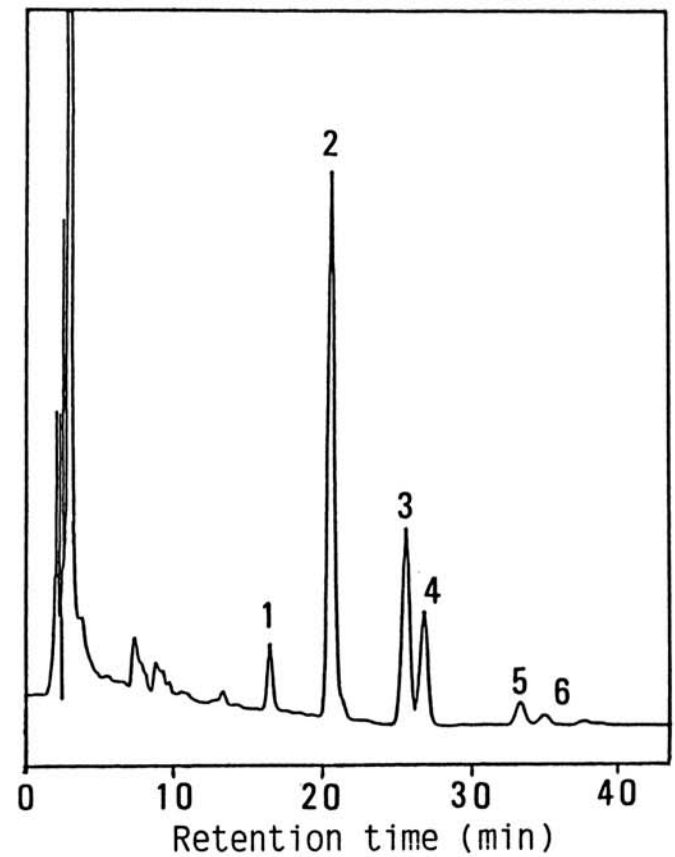

Fig. 2. Separation of diglycosyldiacylglycerol molecular species from Petokus rye grain by reversed-phase high-performance liquid chromatography using an ERC-ODS-1282 column. The column temperature was 50°C. The mobile phase was methanol-water (95:5, v/v) at the flow rate of 1.5 ml/min. Peaks were monitored with a refractive index detector. See Table II for peak identification.

being little difference in the TG content among the other three. Therefore, no differences due to growth environment were detected. The lipid classes next to TG were PC and DGDG, which comprised 12–13% and 4–5% of the total lipids, respectively. Besides these three lipid classes, DG, MGDG, monoglycosylmonoacylglycerol, *N*-acylPE, PE, PI, and lysoPC were found as glycerolipids. Among them, the principal constituents were MGDG, PE, and PI, but no one of them exceeded 3% of the total lipids. These figures were calculated from the relative proportions of neutral lipid, glycolipid, and phospholipid fractions, and their respective densitograms. The determinations were at least duplicated, and the values represent means of the results.

TABLE I
Molecular Composition (%) of Triacylglycerol from Rye Grain

Peak Number[a]	Molecular Species[b]	Carbon Number of Acyl Chains	Total Double Bonds of Acyl Chains	Varieties						
				SV 83019[c]		SV 82018[c]		Kustro[c]		Petokus[c]
				Europe	Japan	Europe	Japan	Europe	Japan	Japan
1	LLnLn	54	8	2	3	3	3	1	3	2
2	LLLn	54	7	10	11	12	12	7	11	12
3 + 4	LLL + OLLn	54	6	25	26	27	26	21	27	26
5	PLLn	52	5	7	7	6	6	9	6	5
6	OLL	54	5	14	14	14	13	14	14	15
7	PLL	52	4	20	20	20	20	20	19	16
8	OOL	54	4	5	4	5	5	6	4	5
9	POL	52	3	8	7	7	7	10	7	9
10	PPL	50	2	4	3	3	3	4	3	3
11	OOO	54	3	1	...	1	1	1	...	1
12	POO	52	2	2	1	1	1	2	2	2
13	PPO	50	1	1	1	1	1	1	1	1
	Others	...	...	1	3	...	2	4	3	3

[a]See Fig. 1.
[b]Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LLnLn = linoleoyldilinolenin, LLLn = dilinoleoyllinolenin, LLL = trilinolein, OLLn = oleoyllinoleoyllinolenin, PLLn = palmitoyllinoleoyllinolenin, OLL = oleoyldilinolein, PLL = palmitoyldilinolein, OOL = dioleoyllinolein, POL = palmitoyloleoyllinolein, PPL = dipalmitoyllinolein, OOO = triolein, POO = palmitoyldiolein, PPO = dipalmitoylolein.
[c]Values represent means of at least duplicate determinations.

TABLE II
Molecular Composition (%) of Monoglycosyldiacylglycerol (MGDG) and Diglycosyldiacylglycerol (DGDG) from Rye Grain

Peak Number[a]	Diacylglycerol Residues[b]	SV 83019[c]			SV 82018[c]			Kustro[c]			Petokus[c]	
		MGDG	DGDG		MGDG	DGDG		MGDG	DGDG		MGDG	DGDG
			Europe	Japan		Europe	Japan		Europe	Japan		
1	LLn	10	9	8	9	6	8	9	7	8	4	5
2	LL	78	58	57	73	52	59	73	54	59	60	54
3	PL	5	20	22	8	23	21	9	20	21	14	23
4	OL	7	10	11	9	14	9	9	13	9	20	13
5	PO	<1	3	2	1	4	2	<1	4	2	2	3
6	OO	<1	<1	<1	<1	2	1	<1	2	1	<1	2

[a]See Fig. 2.
[b]Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LLn = linoleoyllinolenin, LL = dilinolein, PL = palmitoyllinolein, OL = oleoyllinolein, PO = palmitoylolein, OO = diolein.
[c]Values represent means of at least duplicate determinations.

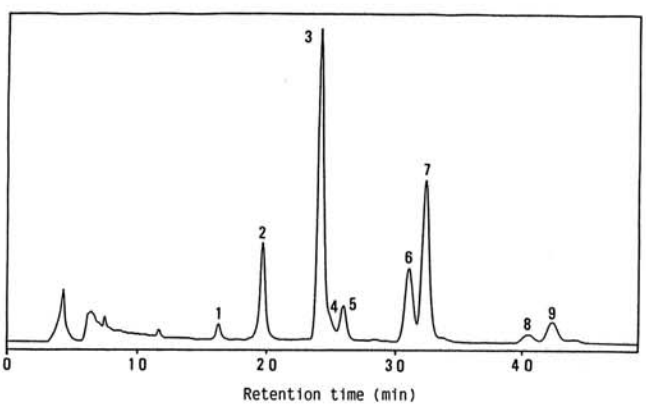

Fig. 3. Separation of the molecular species of the dinitrobenzoyldiacylglycerol derivatives prepared from phosphatidylcholine of Petokus rye grain by high-performance liquid chromatography using an ERC-ODS-1282 column. The column temperature was 40°C. The mobile phase was acetonitrile-isopropanol (8:2, v/v) at the flow rate of 1.0 ml/min. Peaks were monitored with an ultraviolet detector (254 nm). See Table III for peak identification.

TABLE III
Molecular Composition (%) of Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), and Phosphatidylinositol (PI) from Rye Grain

Peak Number[a]	Diacylglycerol Residues[b]	Kustro[c]			Petokus[c]		
		PC	PE	PI	PC	PE	PI
1	LnLn	1	1	1	1	1	...
2	LLn	8	10	7	8	11	4
3	LL	30	33	18	38	36	22
4	OLn	5	2	3	3	3	...
5	PLn	5	3	7	2	4	3
6	OL	13	11	9	18	11	16
7	PL	28	34	44	23	28	41
8	OO	3	2	1	2	2	4
9	PO	7	4	8	5	4	10

[a]See Fig. 3.
[b]Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LnLn = dilinolenin, LLn = linoleoyllinolenin, LL = dilinolein, OLn = oleoyllinolenin, PLn = palmitoyllinolenin, OL = oleoyllinolein, PL = palmitoyllinolein, OO = diolein, PO = palmitoylolein.
[c]Values represent means of at least duplicate determinations.

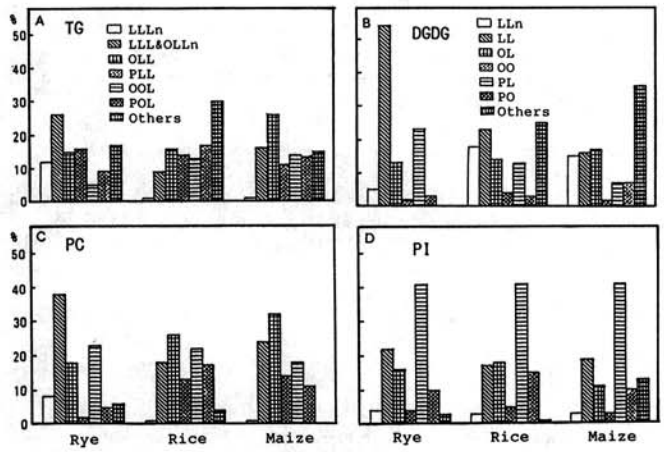

Fig. 4. Comparison of the molecular species of typical representative glycerolipids from three kinds of cereal grains. Lipids: **A,** triacylglycerol (TG); **B,** diglycosyldiacylglycerol (DGDG); **C,** phosphatidylcholine (PC); **D,** phosphatidylinositol (PI). The symbols for the molecular species are the same for B, C, and D. LLLn = dilinoleoyllinolenin, LLL = trilinolein, OLLn = oleoyllinoleoyllinolenin, OLL = oleoyldilinolein, PLL = palmitoyldilinolein, OOL = dioleoyllinolein, POL = palmitoyloleoyllinolein, LLn = linoleoyllinolenin, LL = dilinolein, OL = oleoyllinolein, OO = diolein, PL = palmitoyllinolein, PO = palmitoylolein.

TG Molecular Species

The TG isolated from the Petokus sample was subjected to RP-HPLC; the chromatogram is shown in Figure 1. All the TGs from the four rye varieties were separable into at least 13 molecular species, among which the most plentiful type was trilinolein (LLL). When the shoulder peak of oleoyllinoleoyllinolenin (OLLn) was included, LLL plus OLLn accounted for 21–27% of all the molecular species (Table I). Next were palmitoyldilinolein (PLL) at 16–20%, oleoyldilinolein (OLL) at 13–15%, and dilinoleoyllinolenin at 7–12%. These five molecular species made up about 70% of the total. With regard to the present analysis, a quantitative difference in rye TG molecular species based on their growing locations was scarcely found.

The Molecular Species of Glyceroglycolipids

At least six kinds of molecular species were determined by RP-HPLC from DGDG that originated from the Petokus variety (Fig. 2). Among them, the major type was entirely dilinolein (LL), which composed 52–59% of the diacylglycerol (DG) residues (Table II). In addition to LL were palmityollinolein (PL), oleoyllinolein (OL), and linoleoyllinolenin (LLn), with a small amount of palmitoylolein and diolein. When MGDG from the four rye varieties harvested in Japan were similarly analyzed, DG residues having a close similarity to the combination of fatty acids of DGDG were found (Table II). The principal DG residue of MGDG was LL, which contained 60–78% of the DG residues and was proportionately higher than the LL of DGDG in every variety. Even though there was little difference in the ratio of LLn to OL between MGDG and DGDG, the ratio of PL to OL was considerably higher in DGDG than in MGDG.

The Molecular Species of Glycerophospholipids

The RP-HPLC chromatogram of DNB derivatives of PC showed at least nine peaks (Fig. 3). Table III presents the combinations of the DG residues from the PC, PE, and PI isolated from Kustro and Petokus varieties harvested in Japan. Their characteristics were as follows. The DG residues of PC and PE, which are both neutral phospholipids, resembled each other; their main residues were LL, PL, and OL, especially the former two types. These three residues made up over 70% of the residues found in both varieties. On the other hand, the DG of PI, an acidic phospholipid, contained mostly PL (41–44%) and then LL (18–22%), which was the reverse of the order found in the neutral ones.

DISCUSSION

In this study, the difference among the molecular species of the principal glycerolipid groups in the respective rye grains and their growing locations was investigated using RP-HPLC. The representative lipid classes of the three glycerolipids were TG in neutral lipids, MGDG and DGDG in glycolipids, and PC, PE, and PI in phospholipids. Also, their major molecular species were as follows, LLL and PLL in TG, LL and PL in MGDG and DGDG, and LL and PL in PC, PE, and PI.

In our previous report (Mano et al 1990), about 60% of the constituent fatty acids of the total lipids from rye grains was linoleic acid, followed by palmitic acid at about 15%. TG also showed the same tendency, but MGDG, DGDG, PC, and PE contained 61–79% linoleic acid and 8–20% palmitic acid. In the case of PI, the former was 41–54% and the latter 29–43%.

Subsequently, the molecular species of TG, DGDG, PC, and PI from rye grains were compared with those of other cereals (rice and maize); the results are illustrated in Figure 4. The lipid values obtained for the two cereals chosen for the comparison with rye grain were as follows (Tanaka et al 1984, Mano et al *unpublished work*). The lipid contents of five rice varieties (three Japonica and two Indica types, all brown rices) were 2.2–2.6% (based on grain weight). In the case of maize (Pirika variety), the lipid content was 9.6% (based on grain weight). These values represent means of at least duplicate determinations.

First, in TG, the pattern of molecular species between rye and rice showed a significant contrast. Higher, unsaturated molecular species (LLL, PLL, OLL, and dilinoleoyllinolenin [LLLn]) were predominant (69%) in rye grains grown in cold locations, but lower, unsaturated ones (palmitoyloleoyllinolein, OLL, PLL and dioleoyllinolein) were in the majority (60%) in rice cultivated originally in Southern Asia. The molecular species from maize grains, which were initially grown in Mexico or Central America and are widely cultivated throughout the world, were composed of intermediate degrees of unsaturation (OLL, LLL, OOL, POL and PLL, 83%) compared with the other two cereals.

Second, in the case of DGDG, LL residues were overwhelming (more than 50%) in rye grains, and five other kinds of DG molecules were detected. Rice and maize grains differed quantitatively in their constituent molecular species.

Third, in the case of PC, rye grains contained mainly LL and PL residues, as described above. Rice and maize showed similar patterns composed principally of OL, PL, and LL types.

Last, regarding the DG molecules from PI, rye, rice, and maize grains had in common an abundant amount (over 40%) of PL residues, but the ratios of the other DG molecules differed among the grains.

ACKNOWLEDGMENTS

We thank the Foundation for Promoting Food Science, Japan, which partly supported this work.

LITERATURE CITED

AYLWARD, F., and SHOWLER, A. J. 1962. Plant lipids. IV. The glycerides and phosphatides in cereal grains. J. Sci. Food Agric. 13:492-497.

CHUNG, O. K. 1986. Lipid-protein interaction in wheat flour, dough, gluten, and protein fractions. Cereal Food World, 31:242-256.

DEMANDRE, C., TREMOLIERES, A., JUSTIN, A. M., and MAZLIAK, P. 1985. Analysis of molecular species of plant polar lipids by high performance and gas liquid chromatography. Phytochemistry 24:481-485.

FOLCH, J., LEES, M., and SLOANE STANLEY, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

FUJINO, Y., and SAKATA, S. 1973. Glyceroglycolipids in rice grains. Cereal Chem. 50:379-382.

ITO, S., SATO, S., and FUJINO, Y. 1979. Internal lipid in rice starch. Starch/Staerke 31:217-221.

KARKALAS, J., and RAPHAELIDES, S. 1986. Quantitative aspects of amylose-lipid interactions. Carbohydr. Res. 157:215-218.

KITO, M., TAKAMURA, H., NARITA, H., and URADE, R. 1985. A sensitive method for quantitative analysis of phospholipid molecular species by high-performance liquid chromatography. J. Biochem. 98:327-331.

MANO, Y., OHNISHI, M., SATO, H., NAKANISHI, H., MAEMOTO, M., and ITO, S. 1990. Composition of lipid classes, fatty acids and sterols in domestic rye grains (in Japanese). Nippon Shokuhin Kogyo Gakkaishi 37:338-345.

MIYAZAWA, T., TAZAWA, H., and FUJINO, Y. 1978. Molecular species of triacylglyceride in rice bran. Cereal Chem. 55:138-145.

MORRISON, W. R. 1978. Cereal lipids. Adv. Cereal Sci. Technol. 2:221-348.

OHNISHI, M., OBATA, S., ITO, S., and FUJINO, Y. 1986. Composition and molecular species of waxy lipids in wheat grain. Cereal Chem. 63:193-196.

OHNISHI, M., YASUI, Y., MANO, Y., ITO, S., and FUJINO, Y. 1989. Fatty acid distribution and characterization of 1,2-diacylglycerol residues in glycerolipids from maize seeds. Agric. Biol. Chem. 53:565-567.

PLATTNER, R. D., SPENCER, G. H., and KLEIMAN, R. 1977. Triglyceride separation by reverse phase high performance liquid chromatography. J. Am. Oil Chem. Soc. 54:511-515.

PRICE, P. B., and PARSONS, J. G. 1975. Lipids of seven cereal grains. J. Am. Oil Chem. Soc. 52:490-493.

ROUSER, G., KRITCHEVSKY, G., SIMON, G., and NELSON, G. J. 1967. Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids 2:37-40.

TAKAHASHI, S., and SEIB, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. Cereal Chem. 65:474-483.

TANAKA, H., OHNISHI, M., and FUJINO, Y. 1984. On glyco-lipids in corn seeds (in Japanese). Nippon Nogeikagaku Kaishi 58:17-24.

TORIYAMA, S., HITANA, K., NISHIDA, I., and MURATA, N. 1988. Prominent difference of glycerolipids among anther walls, pollen grains and leaves of rice and maize. Plant Cell Physiol. 29:88-93.

YAMAUCHI, R., KOJIMA, M., ISOGAI, M., KATO, K., and UENO, Y. 1982. Separation and purification of molecular species of galactolipids by high performance liquid chromatography. Agric. Biol. Chem. 46:2846-2849.

ZERINGUE, H. J., Jr., and FEUGE, R. O. 1980. A comparison of the lipids of triticale, wheat and rye grown under similar ecological conditions. J. Am. Oil Chem. Soc. 57:373-376.

ZERINGUE, H. J., Jr., SINGH, B., and FEUGE, R. O. 1981. Triticale lipids: Composition and bread-making characteristics of triticale flours. Cereal Chem. 58:351-354.

[Received May 1, 1990. Accepted December 14, 1990.]