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 glycerolipids and phospholipids were diglycosylacylglycerol (DGDG) and monoglycosylacylglycerol (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, oleoylinoleoinolein, palmitooylinoleooinoin, oleoylidinolein, and diniloleooinoim in increasing order. DGDG and MGDG contained six kinds of molecular species, in which dilinolein (LL) was the most abundant, followed by palmitooylinolein (PL), oleoylinolein, and linoleoylinolein 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 Raphalides 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 slage 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 sources 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, Yamashita 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 Ohihiro University of Agriculture and Veterinary Medicine. In parallel with those grains, one sample cultivated in Hokkaido (Japan) was also used in this study.

**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 Petkus 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 Petkus 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 Petkus 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 with 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). Monoglycosylacylglycerol (MGDG) and diglycosylacylglycerol (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.)
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 0.1N HCl had been added, the product was extracted three times with 1.5 ml of n-hexane. The combined extract was dried under an N₂ flow to remove pyridine; the residue was dissolved in 2.0 ml of n-hexane; and this solution was washed with equal volumes of 0.1N HCl (three times), 0.1N NaHCO₃, and 1N 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 x 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 (82:2, 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., Tokyo, 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-dimethyl 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% H₂SO₄. A CS-9000 two-wave chromatocanser (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 methanolation 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
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, monoglycosylmonocacylglycerol, N-acetylPE, PE, PI, and lysoPC were found as glycerolipids. Among them, the principal constituents were MGDG, PE, and PI, but no one of them exceed 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 Triglycerol from Rye Grain

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Molecular Species</th>
<th>Carbon Number of Acyl Chains</th>
<th>Total Double Bonds of Acyl Chains</th>
<th>Total Double Bonds ofacyl Chains</th>
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<td></td>
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</tr>
<tr>
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<td>SV 82018'</td>
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<td>25</td>
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</table>

*See Fig. 1.

**Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LLLn = linoleoyldiolenolen, LLLn = dioleoyldiolenolen, LLL = trilinolen, OLLn = oleoylinolenolenolen, PLLn = palmitoyldiolenolenolen, OLL = oleoyldiolenolen, PLL = palmitoyldiolenolen, OOL = oleoyldiolenolen, POL = palmitoyldiolenolen, OOO = triolenolen, PPO = palmitoyldiolenolen, PPO = palmitoylenolenolen.**

Values represent means of at least duplicate determinations.

### TABLE II

Molecular Composition (% of Monoglycosylacylglycerol (MGDG) and Diglycosylacylglycerol (DGDG) from Rye Grain

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Diacylglycerol Residues</th>
<th>MGDG</th>
<th>DGDG</th>
<th>MGDG</th>
<th>DGDG</th>
<th>MGDG</th>
<th>DGDG</th>
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<tr>
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<td>&lt;1</td>
<td>&lt;1</td>
<td>2</td>
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</table>

*See Fig. 2.

**Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LLn = linoleoyldiolenolen, LL = diilenolen, PL = palmitoyldiolenolen, OL = oleoyldiolenolen, PO = palmitoylolenolen, OO = oleoylenolenolen.**

Values represent means of at least duplicate determinations.

### TABLE III

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

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Diacylglycerol Residues</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
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</table>

*See Fig. 3.

**Abbreviations indicate fatty acids that are esterified with glycerol in the molecule where positional isomerism is not regarded. LnLn = linoleoyldiolenolen, LLn = linoleoyldiolenolen, LL = diilenolen, OLn = oleoylinolenolen, PLn = palmitoyldiolenolenolen, OL = oleoyldiolenolen, PO = palmitoyldiolenolen.**

Values represent means of at least duplicate determinations.

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**Fig. 3.** Separation of the molecular species of the dinitrobenzoylacylglycerol 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.
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: (Takana et al 1984, Maño et al unpublished work). The lipid contents of five rice varieties (three Japanese and two Indian types, all brown rice) were 2.2–2.6% (based on weight of dried grain), and the lipid content of maize (Pirika variety), the lipid content was 9.6% (based on weight of grain). 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 dilinoleoylolinein [LLOLn]) were predominant (69%) in rye grains grown in cold locations, but lower, unsaturated ones (palmitoyloleoylolinein, OLL, PLL and dioleoylolinein) 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 (OOL, LLL, OOL, PLO, 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 PL, LL, and PL types.

Last, regarding the DG molecules from PC, 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


Fig. 4. Comparison of the molecular species of typical representative glycerolipids from three kinds of cereal grains. Lipids: A, triacylglycerol (TG); B, diglycoylglycerol (DGDG); C, phosphatidylcholine (PC); D, phosphatidylinositol (PI). The symbols for the molecular species are the same for B, C, and D. LLL = dilinoleoylolinein, LLL = trilinolein, OLL = oleoylolinein, OLL = oleoylellin, PLL = palmitoylolinein, PLL = palmitoylolinein, LLL = linoleoylolinein, LL = dilinolein, OL = oleoylolinein, OO = diolein, PL = palmitoylolinein, PO = palmitoylolinein.

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 oleoylolinein (OLLn) was included, LLL plus OLL accounted for 21–27% of all the molecular species (Table I). Next were palmitoylolinein (PLL) at 16–20%, oleoylolinein (OLL) at 13–15%, and dilinoleoylolinein 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 Glycerolipids

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 palmitoylolinein (PL), oleoylolinein (OL), and linoleoylolinein (LLOLn), with a small amount of palmitoylolinein 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 LLL 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.


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