NOTE

Distribution of Lipids in Embryo and Bran-Endosperm Fractions of Riso 1508 and Hiproly Barley Grains

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In two previous publications (Bhatty and Rossnagel 1979, 1980), we reported the lipid and fatty acid contents of Riso 1508 (Riso) and a normal Canadian barley (Bonanza). We suggested that the higher (88%) lipid content of Riso be used in improving the lipid and hence digestible energy content of feed barley. Earlier, Munck (1976), Tallberg (1977), and Shewry et al (1979) also reported a higher lipid content in Riso, and Welch (1978) reported higher lipid contents in Riso and in Hiproly, another high-lysine genotype of barley. The higher lipid content in Riso was ascribed to its larger embryo (Tallberg 1977).

The objective of the present study was to compare the distribution of lipids in the embryo and bran-endosperm fractions of Riso and Hiproly barley grains. In addition, data were collected on the fatty acid composition of triglycerides isolated from meal and from embryo and bran-endosperm fractions of the two genotypes.

MATERIALS AND METHODS

Riso and Hiproly barleys were grown in 1979 on experimental plots at the University of Saskatchewan, Saskatoon, from seed stocks of the two genotypes maintained by B. Rossnagel of this department.

Riso was dehulled by treatment with sulfuric acid (Whitemore 1960). Cleaned, whole seeds of Riso and Hiproly were weighed and soaked in distilled water at 25° C for 3 hr; the soak water was then decanted off and the seed blotted dry. The embryo including the scutellum (hereafter called embryo) was manually excised from the seed under a magnifying glass. The excised embryo and the bran-endosperm fraction (the portion of seed remaining after removal of the embryo) were gently dried (overnight at 30° C). The partially dried embryo fraction was ground in a Krups coffee grinder; the bran-endosperm and the whole seed (unsoaked) were each ground in a Unimac mill to pass through a 1.0-mm screen. The moisture content of the three samples was determined by heating subsamples at 110° C for 2 hr. All data are reported on a moisture-free basis and are means of at least duplicate determinations.

Lipids were extracted from the three samples of each genotype of barley with petroleum ether in a Goldfisch extraction apparatus or by the procedure of Bligh and Dyer (1959).

Silicic acid (Sigma, 60–200 mesh) was washed according to Hirsch and Ahrens (1958) and packed into an 18.0 × 2.5-cm column equilibrated with diethylether. An aliquot of the lipids (about 200 mg) extracted by the procedure of Bligh and Dyer (1959) was applied to the column. The nonpolar lipids (NPL) were eluted with 250 ml of diethylether and the polar lipids (PL) with 250 ml each of acetone and methanol. The solvents were removed from each fraction on a rotary evaporator; the lipids were weighed and stored at −20° C.

NPL were fractionated by thin-layer chromatography on Redi-glass plates (Fisher Scientific), 20 × 20 cm, precoated with silica gel G (250 μm). The lipid sample (2–3 mg in 10 μl of diethylether) was spotted on the plates, which were then eluted with petroleum ether:diethylether:acetic acid, 85:20:1 (Rao et al 1978). After elution, the plates were sprayed with 50% H_2SO_4 and charred at 130° C for 10 min. Authentic samples of monoolein, 1,2- and 1,3-diolein, free cholesterol, trilinolein, and cholesteryl palmitate were eluted from the plates under identical conditions. The developed plates were observed under ultraviolet light. The triglyceride spots corresponding to trilinolein were scraped from plates not sprayed with H_2SO_4 and transferred into separate Erlenmeyer flasks, each containing 50 ml of methanol:diethylether, 2:1 (Price and Parsons 1980). The triglyceride was separated from the silica gel by filtration through glass fiber filter paper and freed of the solvent.

Fatty acid composition of the isolated triglyceride fractions was determined immediately by gas-liquid chromatography. The preparation of methyl esters and operating conditions for the gas-liquid chromatography have been previously described (Bhatty and Rossnagel 1980).

RESULTS AND DISCUSSION

A total of 113.0 g of Riso seed and 176.2 g of Hiproly seed was hand-dissected to obtain embryo and bran-endosperm fractions. In Riso, seed weights ranging from 10.9 to 17.7 g yielded 4.5–4.8% of the dry matter content of the seed as embryo and 88.9–92.9% as bran-endosperm. In Hiproly, seed weights with a range of 14.7–22.2 g yielded 3.4–3.9% of the dry seed weight as embryo and 90.9–92.3% as bran-endosperm. Thus, manual separation of embryo and bran-endosperm fractions from barley seed was highly repeatable with low standard deviation. In Riso, the mean embryo and bran-endosperm weights were 4.7 and 90.1% and in Hiproly, 3.8 and 91.7%, respectively. Therefore, on soaking, 5.2% of the seed weight in Riso and 4.5% in Hiproly was lost, partly attributable to washing the grain and partly to leaching from the grain. On a 100.0% recovery basis, the embryo constituted 5.0% and the bran-endosperm 95.0% of the dry seed weight in Riso and 4.0% and 96.0%, respectively, in Hiproly (Table I). Thus, the embryo in Riso was 25.0% larger than the embryo in Hiproly.

Table I shows that Riso contained 61% more lipids than Hiproly (4.2 vs 2.6%), further confirming its high lipid content. The lipid value obtained for Hiproly was closer to that of regular Canadian barleys. The Hiproly embryo contained only 4.0% more (21.4 vs 20.5%) lipids than the Riso embryo, and this difference may not be significant. However, Riso bran-endosperm contained 88.9% more lipids than in the Hiproly bran-endosperm. The lipid values for Riso seed, embryo, and bran-endosperm fractions were generally similar to those obtained previously, and the values for Hiproly seed, embryo, and bran-endosperm fractions were close to those of Fairfield barley (Bhatty and Rossnagel 1980). In Riso, the embryo contributed 24.0% and in Hiproly 33.2% of the total seed lipids; the latter value was higher than that reported by MacLeod and White (1961) for Proctor (six-row) barley (30.0%). A major portion of Riso lipids (76.0%) was in the bran-endosperm; the amount was 66.8% in Hiproly. The distribution of lipids in dehulled Prilar barley has been reported by Price and Parsons (1979); the embryonic axis contained 17.9% and the endosperm 77.1% of the grain lipids. The later value is almost identical to that obtained in Riso bran-endosperm. The distribution of lipids in Riso, a high lipid barley, is unlike that in corn, in which a major portion of the lipids (76–83%) is in the germ. High-lipid cultivars usually have larger embryos (Tan and Morrison 1979).

Riso meal and embryo had lower NPL and higher PL than the similar fractions of Hiproly (Table II). Only Riso bran-endosperm had higher NPL and lower PL than Hiproly endosperm. The values obtained for NPL of Riso and Hiproly meal (68.6–70.5%) were close to the mean of 71.0% for the neutral lipid fraction reported for

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TABLE I
Distribution of Lipids[a] in Embryo and Bran-Endosperm Fractions of Riso and Hiproly Barleys[b]

Genotype	Size of Fraction (% of seed)			Lipid in Fraction (% of fraction)			Lipid Distribution, %		
	Seed	Embryo	Bran-Endosperm	Seed	Embryo	Bran-Endosperm	Seed	Embryo	Bran-Endosperm
Riso	100.0	5.0	95.0	4.2	20.5	3.4	100.0	24.0	76.0
Hiproly	100.0	4.0	96.0	2.6	21.4	1.8	100.0	33.2	66.8

[a]Adjusted to 100% recovery.
[b]Lipids extracted with petroleum ether.

TABLE II
Distribution[a] of Nonpolar and Polar Lipids of Riso and Hiproly Barleys[b] Fractionated on a Silicic Acid Column

Genotype	Fraction	Nonpolar Lipids	Polar Lipids
Riso	Meal	68.6	31.4
	Embryo	86.9	13.1
	Bran-endosperm	67.9	32.1
Hiproly	Meal	70.5	29.5
	Embryo	90.2	9.8
	Bran-endosperm	64.5	35.5

[a]Adjusted to 100% recovery.
[b]Lipids extracted by the procedure of Bligh and Dyer (1959).

TABLE III
Fatty Acid Composition of Triglycerides Isolated from the Nonpolar Lipids of Meal and Embryo and Bran-Endosperm Fractions of Riso and Hiproly Barleys

Fraction	Genotype	Fatty Acid[a] (%)				
		C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Meal	Riso	21.8	1.7	20.5	51.2	4.8
	Hiproly	19.1	1.1	21.2	54.2	4.4
Embryo	Riso	23.9	trace	17.6	51.4	7.1
	Hiproly	20.9	1.3	18.0	51.7	8.1
Bran-endosperm	Riso	21.7	2.0	22.9	51.3	2.1
	Hiproly	19.3	1.9	22.2	52.5	4.2

[a]16, palmitic; 18, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

six genotypes of barley by Price and Parsons (1974) but lower than the neutral lipid fraction of different cereals species (except wheat and triticale) reported later by Price and Parsons (1975). Nevertheless, in barley, as in other cereals, NPL are the major lipids, and an increase in the total lipid content of barley can come mostly through an increase of this fraction.

Thin-layer chromatography of the NPL showed triglyceride to be the major component of the barley lipids. The NPL fractions of meal, embryo, and bran-endosperm of the two genotypes showed, in addition to triglycerides, faint spots (visible under ultraviolet light) of monoglycerides, 1,2-diglycerides, 1,3-diglycerides (very little in the embryo fractions), free sterol, unidentified compounds, free fatty acids, and steryl esters. No attempt was made to quantitate these components.

The fatty acid composition of the triglycerides of meal and of embryo and bran-endosperm fractions of the two genotypes was generally similar (Table III). Linoleic was the major fatty acid, followed by oleic and palmitic acids. The triglycerides also contained trace quantities of myristic acid (C14:0) and probably of lauric acid (C12:0), but no attempts were made to quantitate these. Riso meal, embryo, and bran-endosperm contained slightly more palmitic acid but less oleic and linoleic acids (except in bran-endosperm) than did the corresponding grain fractions from Hiproly. However, Hiproly bran-endosperm contained 100% more linolenic acid than did Riso bran-endosperm. The levels of palmitic, oleic, and linoleic acids in the fractions were within the ranges reported for Kearney and Prilar barley by Price and Parsons (1980).

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