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EFFECT OF AMYLOSE CONTENT ON THE LIPIDS OF MATURE RICE GRAIN

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Abstract—Most of the nonstarch lipids in brown rice (*Oryza sativa*) of three rices differing in amylose content were contributed by bran, germ, polish and subaleurone layer. Nonstarch lipids consisted of 82–91% neutral lipids (of which 73–82% were triglycerides), 7–10% phospholipids and 2–8% glycolipids. Linoleic, oleic and palmitic acids were the major fatty acids. Nonwaxy (24 and 29% amylose) milled rice had proportionally more starch lipids and less nonstarch lipids than waxy (2% amylose) milled rice. Starch lipids were mainly lysophosphatidyl choline, lysophosphatidyl ethanolamine and free fatty acids. The major fatty acids were palmitic and linoleic acids, followed by oleic acid.

INTRODUCTION

Rice lipids occur mainly as spherosomes in the aleurone layer [1] and embryo [2], and also in association with membrane and protein bodies [3, 4] and starch granules [5]. Rice bran is mainly composed of the aleurone layer and embryo, and is rich in lipids [6]. Milled rice is mainly composed of endosperm and contains less lipids than rice bran. Fat-by-hydrolysis corresponds to the fatty-acid component of rice starch [7]. As part of our study of lipids of developing and mature rice grain [7], we studied the distribution and composition of lipids in milling fractions of brown rice of three rices whose starch differs in amylose content.

RESULTS

Nonstarch lipids

Bran, embryo, polish and subaleurone layer of endosperm contributed most of the 2.9–3.4% nonstarch lipids of the three brown-rice samples (Tables 1 and 2). The inner endosperm contributed less nonstarch lipids, considering that it is the major fraction of brown rice. Contents of nonstarch lipids in each milling fraction (except inner endosperm) of waxy and nonwaxy rices were similar (Table 2). The inner endosperm of waxy rice contained more nonstarch lipids than did that of nonwaxy rice. In the subaleurone layer, the corresponding values were 8.5% for IR4445-63-1, 7.1% for IR480-5-9 and 5.6% for IR42.

Neutral lipids were the major component of nonstarch lipids of brown rice and its milling fractions, followed by phospholipids and glycolipids (Table 2). The nonstarch lipids of inner endosperm, however, contained proportionally more glycolipids and phospholipids than the other milling fractions.

Triglycerides, the major components of neutral lipids, were predominantly present in nonstarch lipids in all milling fractions except the inner endosperm (Table 3). Nonstarch lipids of the inner endosperm contained the least triglycerides and the most free fatty acids, such that they were present in an almost 1 : 1 ratio. In addition, the inner endosperm contained more acyl sterol glycosides, sterol glycosides and diglycosyl diglycerides among glycolipids, and lysophosphatidyl ethanolamine and lysophosphatidyl choline among phospholipids than the outer milling fractions. Amylose content did not appreciably affect the composition of nonstarch lipids. Results were expressed as percentage of lipids, rather than micrograms of lipids per grain because of the lighter grain weight of IR42 (17 mg) compared with that of IR480-5-9 (24 mg) and IR4445-63-1 (19 mg).

Linoleic, oleic and palmitic acids were the major fatty acids in nonstarch lipids in all the milling fractions (Table 4). The inner endosperm lipids, however, had proportionally more palmitic and less oleic acid than the other milling fractions. Total nonstarch lipids and their neutral fraction had similar fatty-acid compositions. Glycolipids and phospholipids had proportionally more palmitic and less oleic acid than neutral lipids. Amylose content had little effect on the fatty-acid composition of nonstarch lipids.

Starch lipids

Brown rice of nonwaxy rices had 0.66 or 0.76% starch lipids (Table 2). The inner endosperm seemed to contain less starch lipids than brown rice. Starch lipids were richer in phospholipids than nonstarch lipids. They had only 28% neutral lipids and 18–20%

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Table 1. Properties of milling fractions of three brown-rice samples differing in amylose content*

Milling fraction	Wt % of brown rice	Starch (%)†	Protein (%)†	Nonstarch lipids (% of total)	Starch lipids (% of total)
Brown rice	100	70–73	8–11	100	100
Bran	5.9– 6.4	15–18	12–14	39–41	—
Embryo	1.3– 1.5	—	—	14–18	—
Polish	4.1– 4.4	30–32	13–17	15–21	—
Subaleurone layer	4.9– 5.2	47–51	13–18	12–14	—
Inner endosperm	82.5–83.8	72–76	6– 9	12–19	48–71

* IR42 (29% amylose), IR480-5-9 (24% amylose) and IR4445-63-1 (1.7% amylose).
† At 12% moisture.

glycolipids. Lysophosphatidyl choline and lysophosphatidyl ethanolamine were the major components in phospholipids (Table 3). Free fatty acids were the major components of neutral lipids. More than 50% of glycolipids were again nonlipid substances [7]. True major glycolipids were monoglycosyl monoglycerides together with acyl sterol glycosides and sterol glycosides.

Palmitic, linoleic and oleic acids were also the major components of starch lipids (Table 4). However, starch lipids had proportionally more palmitic and less oleic acid than nonstarch lipids. Neutral starch lipids had proportionally less palmitic and more linoleic acid than starch glycolipids and phospholipids. The fatty-acid composition of starch phospholipids and glycolipids was more or less similar to that of total starch lipids.

The brown rice and inner endosperm of the waxy IR4445 line had only 0.21 and 0.12% starch lipids, respectively (Table 2). They had proportionally more neutral lipids and less phospholipids than nonwaxy rices. Free fatty acids, lysophosphatidyl choline and lysophosphatidyl ethanolamine were also their major lipid species. Starch lipids of waxy rice had proportionally more oleic and less linoleic acids than starch lipids of nonwaxy rices.

DISCUSSION

The amylose–amylopectin ratio of starch had little effect on the content and distribution of lipids in milling fractions of brown rice except in the starchy endosperm itself (Table 1). The recessive *waxy* gene is known to be expressed only in the pollen and starchy

Table 2. The content of nonstarch and starch lipids in the milling fractions of three brown-rice samples differing in amylose content*

Type of lipids and milling fractions	Total lipids (% dry basis)	Lipid fractions (% of total) Neutral lipids	Glyco-lipids	Phospho-lipids
A. Nonstarch lipids				
Brown rice	2.9– 3.4	85–87	4–6	8–9
Bran	19.4–25.5	88–90	4–5	7–8
Embryo	34.1–36.5	91–92	2–3	6–7
Polish	10.2–15.0	86–88	4–5	8–9
Subaleurone layer	5.6– 8.5	82–86	5–7	8–12
Inner endosperm				
IR42	0.45	66	18	16
IR480-5-9	0.41	66	17	17
IR4445-63-1	0.81	76	12	12
B. Starch lipids				
Brown rice				
IR42	0.66	28	18	54
IR480-5-9	0.76	28	20	52
IR4445-63-1	0.21	41	21	37
Inner endosperm				
IR42	0.55	27	17	56
IR480-5-9	0.57	26	15	59
IR4445-63-1	0.12	47	29	24

* Ranges are presented for properties in which the three samples had similar values.

Table 3. Composition of nonstarch and starch lipids in the milling fractions of three brown-rice samples differing in amylose content (as % of total nonstarch/starch lipids)

Type of lipids and milling fractions	Lipid fraction and species* (% of nonstarch or starch lipids)										
	Neutral lipids			Glycolipids			Phospholipids				
	TG	FFA	ASG	SG	DGDG	MGMG	PE	PC	LPE	LPC	Others
A. Nonstarch lipids†											
Brown rice	69–71	6– 7	2–3	<1	<1	tr	3–4	4	<1	—	8–11
Bran	75–76	4– 5	2	<1	<1	tr	3	3–4	<1	—	8– 9
Embryo	77–79	4	1	<1	<1	tr	3–4	3–4	<1	—	8– 9
Polish	70–74	5– 8	2–3	1	<1	tr	3	3–4	<1	—	9–12
Subaleurone layer	58–62	13–17	2–4	1–2	1	tr	3–4	4	1–2	1–2	11–12
Inner endosperm	30–37	27–29	5–6	2–3	1–2	tr	3–5	3–5	2–4	2–4	15–17
B. Starch lipids											
Brown rice											
IR42	4	21	2	1	tr	2	4	5	21	23	17
IR480-5-9	4	20	2	1	tr	3	4	5	21	21	19
IR4445-63-1	5	28	3	1	tr	3	3	5	15	18	18
Inner endosperm											
IR42	1	22	1	1	tr	3	4	5	22	24	17
IR480-5-9	2	21	2	2	tr	2	4	4	23	24	17
IR4445-63-1	5	37	3	2	tr	3	3	3	12	13	16

* TG = triglycerides, FFA = free fatty acids, ASG = acyl sterol glycosides, SG = sterol glycosides, DGDG = diglycosyl diglycerides, MGMG = monoglycosyl monoglycerides, PE = phosphatidyl ethanolamine, PC = phosphatidyl choline, LPE = lysophosphatidyl ethanolamine, LPC = lysophosphatidyl choline, tr = trace.

† Ranges of values for IR42, IR480-5-9 and IR4445-63-1 rices are presented as they were similar.

endosperm, which are haploid tissues [8]. The milling fractions of the three samples differing in amylose content showed a similar distribution of nonstarch lipids. The bran, embryo and polish were richest in nonstarch lipids (Table 1) and had almost the same ratio of lipid fractions, major lipid species and fatty-acid composition as nonstarch lipids of brown rice (Tables 2–4). Miyazawa *et al.* [9] reported a similar composition of phospholipids in rice bran of nonwaxy and waxy rice. The distribution in the rice grain of nonstarch lipids [10] was similar to that in other cereal grains [11–13].

The inner endosperm and, to some extent, the subaleurone layer, of waxy rice IR4445-63-1 contained

Table 4. Fatty-acid composition of free and bound lipids, and their fractions in the milling fractions of three brown-rice samples differing in amylose content (wt % of total)*

Type of lipids and milling fractions	Total lipids			Neutral lipids			Glycolipids			Phospholipids		
	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
A. Nonstarch lipids†												
Brown rice	23–24	32–37	36–40	22–24	35–37	35–39	30–33	24–26	36–38	25–29	30–34	34–38
Embryo	23–25	36–38	35–38	23–24	34–36	37–38	28–29	32–34	35–36	22–24	36–37	36–37
Bran	22–25	36–37	36–37	22–23	38–41	37–41	25–27	30–31	35–39	20–25	36–43	32–36
Polish	23–25	36–39	37–39	22–25	35–37	37–39	26–30	26–28	35–39	25–26	34–37	33–35
Subaleurone layer	26–28	26–28	40–41	22–24	29–31	38–45	35–38	18–20	36–39	34–36	24–28	35–36
Inner endosperm	32–34	20–21	38–41	21–27	17–22	45–56	47–49	10–12	34–38	44–51	10–12	34–40
B. Starch lipids												
Brown rice												
IR42	48	13	35	24	23	48	55	7	26	54	9	32
IR480-5-9	43	12	40	23	20	53	54	7	32	45	10	39
IR4445-63-1	45	17	29	38	22	30	53	12	26	50	14	30
Inner endosperm												
IR42	46	13	37	25	22	48	54	9	26	50	15	30
IR480-5-9	44	10	42	21	21	54	56	7	31	46	16	31
IR4445-63-1	44	18	30	35	20	38	53	16	23	56	15	23

* Trace–3% 14:0, 2–4% 18:0 and 1–2% 18:3.

† Ranges of composition for IR42, IR480-5-9 and IR4445-63-1 rices are presented as they were similar.

more nonstarch lipids than those of nonwaxy rice (Table 2). In addition, the waxy endosperm nonstarch lipids contained more neutral lipids and less glycolipids and phospholipids than the nonwaxy endosperm nonstarch lipids. Nonstarch lipids from the inner endosperm contained less triglycerides, lysophosphatidyl choline and lysophosphatidyl ethanolamine than those from bran, polish and embryo (Table 3). The effect of a higher content of nonstarch lipids of waxy milled rice on fat rancidity during storage is worthwhile investigating.

Starch lipids were mainly located in the endosperm (Table 2) where starch was mainly found in the mature brown rice [14]. Waxy milled rice had less starch lipids than nonwaxy milled rice, but correspondingly had more nonstarch lipids. Total lipids in the inner endosperm was 0.93% for IR4445-63-1, compared with 0.98% for IR480-5-9 and 1.00% for IR42. Starch lipids of waxy rice had more neutral lipids and glycolipids but less phospholipids than starch lipids of nonwaxy rice. Waxy-rice starch lipids contained more free fatty acids and less lysophospholipids than nonwaxy starch lipids (Table 3). These differences were reflected in the higher oleic- and lower linoleic-acid content of waxy starch lipids (Table 4).

In nonwaxy rice, starch lipids were richer in phospholipids and glycolipids, and poorer in neutral lipids than nonstarch lipids (Table 2). The major neutral lipids were free fatty acids instead of triglycerides, monoglycosyl monoglycerides instead of diglycosyl diglycerides in the glycolipid fraction, and lysophospholipids instead of phospholipids (Table 3). These differences in the ratio of polar to neutral lipids are reflected in the higher content of palmitic acid and the lower content of oleic acid in starch lipids, which are characteristic of polar lipids (Table 4).

In a related study of rice starch granules prepared by sodium dodecyl benzene sulfonate extraction of protein from eight milled rices [15], starch lipids were present only in trace levels in waxy rice but at 0.2–0.4% in nonwaxy starch, and at maximum levels in intermediate- and high-amylose rices. In the present study, the inner endosperm of IR480-5-9 (24% amylose) and that of IR42 (29% amylose) had similar starch lipid levels.

Starch–lipid binding must involve the amylose fraction of starch granules since waxy rice has almost no starch lipids. Fatty acids and lysophospholipids readily complex with amylose [11, 16, 17]. Our values of 0.55 or 0.57% for nonwaxy rices are the minimum since the water-saturated BuOH solvent at room temperature removed only 80% of the total starch lipids on the basis of fat-by-hydrolysis data [7] and some starch lipids are extracted during the 1 hr extraction with water-saturated BuOH after $CHCl_3$–MeOH extraction [7].

The composition of nonstarch lipids of the inner endosperm was between that of starch lipids and that of nonstarch lipids of the outer milling fractions (Tables 2–4). This verified our results with IR42 [7], reflecting that some starch lipids are extracted during the 1 hr extraction with water-saturated BuOH. Destarched endosperm is a preferable material for preparing nonstarch lipids, and purified starch granules treated with alkaline protease is the preferred material for the extraction of starch lipids to minimize cross-contamination between nonstarch and starch lipids in the rice endosperm. The reported composition of starch lipids with more than 0.1% N probably suffers from contamination with nonstarch lipids [18, 19].

EXPERIMENTAL

Samples. The rice variety IR42 and lines IR480-5-9 and IR4445-63-1 were used in the study. Defatted brown rice (refluxing 95% EtOH) of IR42 had 29%, IR480-5-9 24% and IR4445-63-1 2% amylose, respectively, by the method of ref. [20]. Milling fractions were prepared by milling 200 g brown rice in a Satake grain testing mill TM-05 (Satake Engineering Co., Japan) at 1450 rpm with abrasive roller mesh No. 36. The first fraction containing the outer 0–6% (w/w) of brown rice was termed bran, the second fraction containing the outer 6–10% (w/w) of brown rice was termed polish, the third fraction containing the outer 10–15% (w/w) of brown rice was termed subaleurone layer, and the remaining endosperm was termed inner endosperm. The milling fractions were passed through a 40-mesh sieve to separate the brokens and germs (embryos) from bran, polish and subaleurone layer. The percentage of milling fractions was calculated after separation of brokens and germs. The nonstarch lipids were extracted; the extracted lipids were fractionated into neutral lipids, glycolipids and phospholipids; and the lipid species and fatty-acid composition in starch and nonstarch lipids were determined according to procedures described earlier [7]. Starch lipids were extracted from the nonstarch–lipid-free rice powder with H_2O-saturated BuOH (5 ml/g rice) for 2×12 hr at 25°. The extract was dried in the rotary evaporator, redissolved in $CHCl_3$ and filtered through a medium-porosity sintered glass filter. The filtrate was dried and washed with H_2O according to the procedure of ref. [21]. The quantity of lipids was determined gravimetrically.

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