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LIPIDS IN DEVELOPING AND MATURE RICE GRAIN

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Key Word Index—*Oryza sativa*; Gramineae; brown rice; nonstarch lipids; starch lipids; hull lipids; neutral lipids; glycolipids; phospholipids; fat-by-hydrolysis; fatty acid composition.

Abstract—The neutral fraction of nonstarch lipids in developing brown rice (*Oryza sativa* L., cv IR42) was accumulated up to 16 days after flowering (DAF), but phospholipids and glycolipids increased only up to 8 DAF. Fatty acids accumulated in nonstarch lipids until 12 DAF. However, the proportion of linolenic acid in the lipid fraction decreased and that of oleic acid increased during this period. Accumulation of fat-by-hydrolysis in the brown rice occurred until 20 DAF and followed closely that of starch. The proportion of linolenic acid decreased and that of linoleic acid increased until 16 DAF. The fatty acid composition of fat-by-hydrolysis and starch lipids were identical and fat-by-hydrolysis accounted for 48% by weight of starch lipids. Nonstarch lipids were mainly composed of triglycerides and were located in the bran and embryo of mature brown rice. Starch lipids were mainly composed of lysophosphatidyl choline, free fatty acids and lysophosphatidyl ethanolamine, and were located in the endosperm.

INTRODUCTION

Although much work on the composition of lipids in mature rice grain has been done, studies on the changes of properties (content and composition) of lipids during grain development have so far been neglected. Such studies have been done on wheat [1–4], corn [4–7], and other cereals [8]. We studied properties of lipids in developing IR42 brown rice to see if similar changes also take place in rice in relation to known morphological development of the tissues in the grain.

RESULTS

Lipid accumulation during grain development

IR42 brown rice accumulated only 74 μg nonstarch lipids per grain at 4 days after flowering (DAF) (Fig 1). At this stage of grain development, lipids were rich in polar lipids containing 22 μg glycolopids, 20 μg phospholipids and only 32 μg neutral lipids. During grain development, neutral lipids increased 11-fold during the period of 4–16 DAF and remained constant thereafter. Glycolipids and phospholipids only doubled in amount during the period of 4–8 DAF and then became stationary. In terms of percentage of nonstarch lipids, neutral lipids increased from 42% at 4 DAF to 84% at 28 DAF. In contrast, glycolipids constituted 32% of nonstarch lipids at 4 DAF but dropped to 5% at maturity. Phospholipids dropped from 25 to 11% of nonstarch lipids during grain development.

Fat-by-hydrolysis accumulated in brown rice up to 20 DAF and its accumulation essentially followed that of starch (Fig. 1). Fat-by-hydrolysis constituted 0.15% of brown rice at 4 DAF but increased to 0.36% of the mature brown rice.

Dehulling was performed because hull lipids can greatly alter the ratio of lipid fractions of brown rice, particularly in immature grain (Tables 1 and 2). In contrast to those of brown rice, glycolipids and phospholipids of green hull decreased during senescence while neutral lipids remained constant. Differences in composition between lipids from hull and those from brown rice of mature rice grain have also recently been reported [9].

Triglycerides, the major components of nonstarch lipids of brown rice, showed the greatest accumulation during grain development (Table 2). Acyl sterol glycosides, sterol glycosides, and diglycosyl diglycerides in glycolipids, and phosphatidyl choline and phosphatidyl ethanolamine in phospholipids also increased per grain. Lysophosphatidyl choline and lysophosphatidyl ethanolamine in nonstarch phospholipids and monoglycosyl monoglycerides in nonstarch glycolipids also increased quantitatively up to 8 DAF, but decreased afterwards.

In addition to triglycerides and free fatty acids, other neutral nonstarch lipids, sterol and monomethyl sterols, and in lesser amounts, 1,3- and 1,2-diglycerides, and trace levels of monoglycerides were detected in brown rice [10]. Other minor nonstarch glycolipids were monoglycosyl diglycerides, diglycosyl monoglycerides, ceramide glycosides and ceramides, whereas the other minor nonstarch phospholipid was phosphatidyl myo-inositol.

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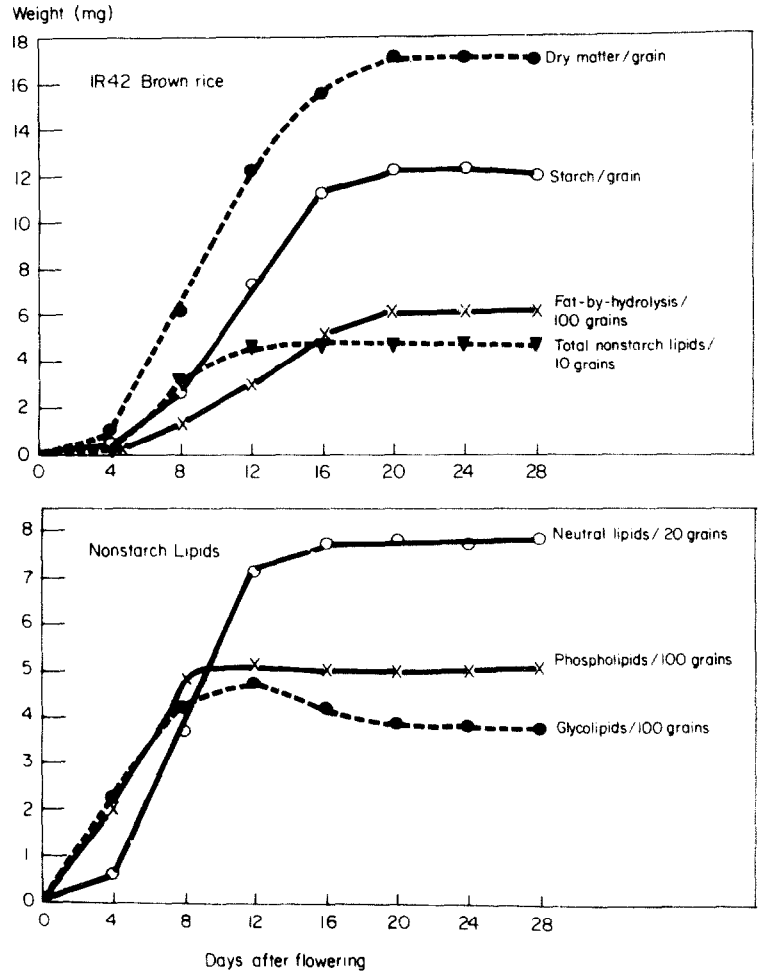

Fig. 1. Accumulation of dry matter, starch, nonstarch lipids, fat-by-hydrolysis and fractions of nonstarch lipids in developing IR42 brown rice.

Lipid distribution in mature brown rice

A study of the distribution of lipids in milling fractions of IR42 mature brown rice revealed that most (74%) of the nonstarch lipids were in the outer layers of brown rice but the starch lipids were mainly located in the milled rice (subaleurone layer plus inner endosperm) (Table 3). Neutral lipids were mainly in the bran, embryo and polish. In contrast, more than half of the total glycolipids and phospholipids were in the milled rice. Similar lipid distribution in wheat, corn, and other cereal grains has been reported [4, 8].

Table 1. Changes in lipid fractions of IR42 hull during grain development

Days after flowering	Lipid fraction (μg/grain)			
	Total lipids	Neutral lipids	Glycolipids	Phospholipids
0	39	12	15	12
4	38	11	16	11
8	38	12	16	10
12	35	11	15	9
16	25	11	9	5
20	21	12	6	3
24	21	12	6	3

In terms of specific lipid species, triglycerides were confirmed to be the major fraction of bran, embryo, and polish nonstarch lipids (Table 4). The same was true for milled rice, but contents of free fatty acids were higher in the inner endosperm than in the nonstarchy milling fractions. Free fatty acids were the second major lipid fraction in most milling fractions except the embryo. Acyl sterol glycosides were the principal nonstarch glycolipids in all milling fractions. Phosphatidyl choline and phosphatidyl ethanolamine, along with nonstarch lysophosphatidyl ethanolamine, were the principal nonstarch phospholipids in all milling fractions.

In starch lipids, the principal fraction was not triglycerides but free fatty acids, lysophosphatidyl choline and lysophosphatidyl ethanolamine (Table 4). Free fatty acids were the principal neutral lipids. More than 50% of starch glycolipids were nonlipid substances that appeared as three unknown spots on TLC plates. Monoglycosyl monoglycerides, acyl sterol glycosides and sterol glycosides were the principal true

Table 2. Accumulation of major components of nonstarch lipids in IR42 brown rice during grain development

Days after flowering	Total lipids	Major lipid components* (μg/grain)									
		Neutral lipids		Glycolipids				Phospholipids			
		TG	FFA	ASG	SG	DGDG	MGMG	PC	PE	LPE	LPC
4	74	23	3	10	3	4	5	5	4	4	3
8	272	155	16	17	5	6	9	13	10	10	8
12	460	321	14	21	7	7	7	16	13	8	5
16	470	335	13	20	6	7	4	19	15	5	—
20	472	336	15	19	6	7	4	21	16	4	—
24	464	329	17	19	6	7	4	20	16	4	—
28	468	332	20	19	6	7	4	22	15	4	—

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

Table 3. Distribution of lipids in milling fractions of IR42 mature brown rice

Type of lipids and milling fractions	Wt % of brown rice	Lipid content (% dry basis)	Wt % of brown rice lipids	Lipid fraction (μg/grain)		
				Neutral lipids	Glyco-lipids	Phospho-lipids
A. Nonstarch lipids						
Brown rice	100	2.9	100	404	29	43
Bran	6.2	20.0	41	173	8	13
Embryo	1.3	36.5	18	76	2	6
Polish	4.3	10.2	15	61	4	6
Subaleurone layer	4.9	5.6	12	41	4	5
Inner endosperm	84.6	0.45	14	44	12	11
B. Starch lipids						
Brown rice	100	0.66	100	29	19	58
Inner endosperm	84.6	0.55	69	19	12	40
Total lipids of brown rice	100	3.56	100	433	48	101

Table 4. Distribution of lipid fractions in milling fractions of IR42 mature brown rice

Type of lipids and milling fractions	Lipid component* (μg/grain)										
	Neutral lipids		Glycolipids				Phospholipids				Others
	TG	FFA	ASG	SG	DGDG	MGMG	PC	PE	LPE	LPC	
A. Nonstarch lipids											
Brown rice	335	22	20	6	7	4	19	17	5	2	42
Bran	147	4	6	2	4	1	6	5	1	—	18
Embryo	65	2	2	tr	tr	tr	3	3	tr	—	9
Polish	45	5	3	1	1	1	3	2	1	—	10
Subaleurone layer	29	3	3	1	1	1	2	2	1	tr	8
Inner endosperm	23	9	6	2	1	2	3	3	2	2	14
B. Starch lipids											
Brown rice	5	23	2	1	tr	4	6	4	20	23	18
Inner endosperm	1	15	1	1	tr	3	4	3	14	15	14
Total lipids of brown rice	340	45	22	7	7	8	25	21	25	25	60

*See Table 2 for abbreviations.

glycolipids. Minor starch neutral lipids were 4-monomethyl sterols and sterols plus traces of diglycerides and monoglycerides [10]. Minor starch glycolipids were diglycosyl diglycerides, monoglycosyl diglycerides and diglycosyl diglycerides, plus trace amounts of ceramides and ceramide glycosides. Lysophospholipids predominated over phospholipids. Phosphatidyl myo-inositol was also present in starch phospholipids.

The solvent system used for extracting nonstarch lipids before starch lipids is probably not mutually exclusive particularly for inner endosperm. The composition of nonstarch lipids of inner endosperm was between that of the other nonstarch lipids of other milling fractions and that of starch lipids (Table 4). Some starch lipids, principally free fatty acids, are extracted during the 1 hr extraction with water-saturated *n*-BuOH. Earlier studies indicated that 95% of the lipids of α-amylase destarched cooked milled rice (mainly cell walls, membranes and protein bodies) are extracted by $CHCl_3$–MeOH and a 5 min extraction with water-saturated *n*-BuOH (84% + 11%). The lower content of starch lipids of the inner endosperm, compared with that of brown rice, suggests that some starch lipids may have contaminated the nonstarch lipid extract.

Starch lipids, fat-by-hydrolysis and free fatty acids

IR42 brown rice, freed of nonstarch lipids, had 0.66% starch lipids and 0.41% fat-by-hydrolysis (Table 5). These two lipids had similar fatty acid composition, showing that fatty acids of fat-by-hydrolysis originated from starch lipids. A fraction of starch lipids amounting to 0.99% residual fat-by-hydrolysis could not be extracted from the residue of IR42 brown rice, free of nonstarch lipids, by our extraction method for starch lipids. Fatty acids of fat-by-hydrolysis constituted 48% of extracted starch lipids of brown rice in nonwaxy rice.

Free fatty acids of nonstarch lipids and starch lipids have similar composition (Table 5), showing that free fatty acids of both lipids originated from the same pool. For the reasons discussed earlier, the free fatty acids are probably located mainly in the endosperm in normal mature brown rice, mainly in starch lipids, as previously reported for corn [4] and rice starch [11].

Changes in fatty acid composition

The major fatty acids of nonstarch lipids in developing IR42 brown rice were linoleic, palmitic, oleic and linolenic acids (Table 6). The accumulation of fatty acids in nonstarch lipids followed total lipid accumulation and was practically completed by 12 DAF. At 4 DAF, nonstarch lipids were very rich in linolenic acid. The proportion of linolenic acid decreased and that of oleic acid increased during grain development. There was also a slight increase in the proportion of linoleic acid from 4 to 8 DAF. The proportion of palmitic acid remained essentially stationary throughout grain development.

The accumulation of fatty acids and the changes in their composition in the neutral fractions of nonstarch lipids were almost similar to those in total nonstarch lipids (Table 6). However, free fatty acids, which are also part of neutral lipids, did not change in composition.

Fatty acid accumulation in glycolipids and phospholipids was completed by 8 DAF (Table 6). The proportion of linolenic acid decreased also in these two fractions of lipids. In glycolipids, the proportion of linoleic acid increased from 4 to 8 DAF, that of palmitic acid increased from 12 to 20 DAF and that of oleic acid increased from 8 to 12 DAF. In phospholipids, the proportion of palmitic acid increased from 4 to 8 DAF and that of oleic acid increased from 4 to 20 DAF. The proportion of linoleic acid in phospholipids remained practically stationary throughout grain development.

Like nonstarch lipids, fat-by-hydrolysis also had palmitic, linoleic, oleic, and linolenic acids as major fatty acids (Table 6). However, in the mature grain, fat-by-hydrolysis had proportionally higher palmitic and lower oleic acid content than nonstarch lipids. During grain development, the proportion of linolenic acid decreased and that of linoleic acid increased in fat-by-hydrolysis. The proportion of palmitic and oleic acid remained stationary throughout grain development.

Fatty acid analysis of nonstarch lipids of milling fractions of IR42 mature brown rice showed similar composition for brown rice, bran, embryo and polish nonstarch lipids with linoleic, oleic and palmitic as principal fatty acids (Table 7). Subaleurone layer and inner endosperm had lower oleic and more palmitic

Table 5. Content and fatty acid composition of starch lipids, fat-by-hydrolysis and free fatty acids of IR42 brown rice previously defatted with $CHCl_3$–MeOH and H_2O-satd *n*-BuOH for 1 hr

Lipid fraction	Lipid content* (%)	Fatty acid composition (wt % of total) 16:0	18:1	18:2	Others†
Starch lipids	0.66	46	13	37	4
Fat-by-hydrolysis	0.41	42	15	38	5
Residual fat-by-hydrolysis‡	0.09	38	18	41	3
Free fatty acids					
Nonstarch lipids	—	22	24	46	8
Starch lipids	—	24	23	48	5

*As % of defatted brown rice dry matter.

†Included 0–1% 14:0, 2–3% 18:0 and 1–3% 18:3.

‡After defatting brown rice with $CHCl_3$–MeOH (2:1) for 8 hr and H_2O-satd *n*-BuOH for 23 hr at 25°.

Table 6. Changes in composition of major fatty acids in brown rice lipids during development of IR42 rice grain

Lipid fraction	Days after flowering	Fatty acid accumulation (% of 20-day grain)	Fatty acid composition (wt % of total) 16:0	18:1	18:2	18:3
Total	4	10	19	14	39	26
nonstarch	8	50	20	22	44	12
lipids	20	100	21	28	43	4
Nonstarch	4	8	15	22	42	20
neutral	8	46	17	29	43	9
lipids	20	100	19	31	43	4
Nonstarch	4	20	20	26	46	5
free fatty	8	106	20	27	45	5
acids	20	100	22	24	46	5
Nonstarch	4	45	18	8	29	43
glycolipids	8	97	18	9	43	27
	12	99	17	12	45	24
	20	100	30	14	42	12
	24	100	31	15	43	7
Nonstarch	4	45	25	10	44	18
phospholipids	8	100	30	12	45	10
	20	100	29	21	44	3
Fat-by-	4	3	42	18	26	11
hydrolysis	8	18	43	18	32	4
	12	49	43	15	35	3
	20	100	42	15	38	2

*Also included 0–1% 14:0 and 2–3% 18:0.

acid than the outer layers of brown rice, which were intermediate between the other nonstarch lipids and starch lipids of brown rice and inner endosperm. These results support the suggestion that nonstarch lipids of milled rice are probably contaminated by starch lipids. However, contamination of brown rice starch lipids by nonstarch lipids was not reflected in the fatty acid analysis.

Lipase and lipoxygenase

Lipase activity increased during the early period of grain development, reached a maximum by 12 DAF but decreased and levelled off to about 50% of the maximum activity by 24 DAF (Fig. 2). In contrast, lipoxygenase activity increased progressively from 4 to 20 DAF and then levelled off. Activities of the two enzymes in the developing and mature grain were

Table 7. Fatty acid composition of lipids in milling fractions of IR42 mature brown rice (wt % of total)

Milling fraction	16:0	18:1	18:2	Others*
A. Nonstarch lipids				
Brown rice	24	32	40	4
Bran	23	36	37	4
Embryo	23	37	36	4
Polish	24	36	37	4
Subaleurone layer	28	28	40	4
Inner endosperm	33	20	41	6
B. Starch lipids				
Brown rice	48	13	35	4
Inner endosperm	46	13	37	4

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

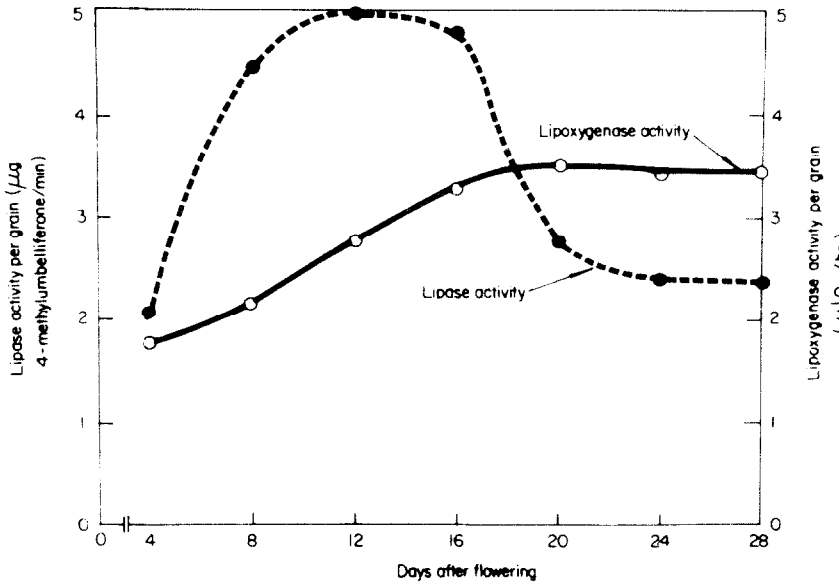

Fig. 2. Changes in lipase and lipoxygenase activity in developing IR42 brown rice.

lower than those of bran lipoxygenase [12] and lipase in the germinating grain [13].

DISCUSSION

In the developing IR42 brown rice, the sequence of synthesis of lipid fractions was phospholipids, glycolipids, neutral lipids and fat-by-hydrolysis (Fig. 1). The early synthesis of phospholipids and glycolipids is consistent with the earlier synthesis of cell walls [14] and membrane-bound organelles, including starch granules and protein bodies [15, 16]. Neutral lipids are mainly in oil bodies or spherosomes in the aleurone layer and embryo [17, 18], whose development in the aleurone layer is completed by 15 DAF and in the embryo by 20 DAF [19]. Starch lipids, represented by fat-by-hydrolysis, were accumulated last; its accumulation followed closely that of starch in the developing grain. These observations are similar to those in developing corn endosperm (embryoless) [4] in which phospholipids and glycolipids accumulated ahead of neutral lipids. Much less degradation of lipid fractions occurred in rice than in corn during grain desiccation.

Triglycerides were mainly in the nonstarch lipids, mostly localized in the bran and embryo (Table 4). In contrast, free fatty acids were mainly associated with endosperm and considered as starch lipids by the classification of Morrison [8]. Diglycerides probably have the same localization as triglycerides in the spherosomes of the aleurone layer and embryo.

The nonstarch triglycerides in rice endosperm are probably associated mainly with protein bodies and membranes. Purified rice starch granules contain lysophosphatidyl choline, free fatty acids and lysophosphatidyl ethanolamine as associated lipids [20].

Starch and nonstarch lipids differed in palmitic and oleic acid contents but had similar linoleic acid content (Table 6). The increase in oleic acid content of nonstarch lipids during grain development is consistent with the increased proportion of neutral lipids over glycolipids and phospholipids in the developing rice grain (Fig. 1). The reduction in linolenic acid content in all lipid fractions is consistent with the early synthesis of membrane lipids which are rich in linolenic acid. Similar results have been reported for other developing cereal grains [2, 4, 5, 7, 21].

EXPERIMENTAL

Samples. The variety IR42 was used in the study. The panicles were tagged in the field on the day of flowering and collected at 4-day intervals starting on the 4th day after flowering until the 28th day. The samples were kept in plastic bags under ice immediately after harvest. In the laboratory, the upper half of the panicles were cut and quickly frozen at −40° and freeze-dried. The samples were stored at 4° in sealed plastic bags. The samples collected at 4, 8, 12, and 16 DAF were dehulled by hand. Older samples were dehulled with a Satake THU-35A test husker. They were ground in a Wig-L-Bug amalgamator or a Udy Cyclone mill with 40-mesh sieve and immediately after, used for lipid extraction. Milling fractions of mature brown rice (200 g) were prepared using a Satake grain testing mill TM-05 at 1450 rpm with abrasive roller mesh No. 36. The first fraction containing the outer 0–6% (w/w) of brown rice was termed as bran, the second fraction containing the outer 6–10% (w/w) of brown rice as polish, the third fraction containing the outer 10–15% (w/w) of brown rice as subaleurone layer, and the remaining endosperm as inner endosperm. The bran, polish and subaleurone layer were passed through a 40-mesh sieve to remove the brokens and germs (embryos). The germs were separated from the brokens by flotation by making a water slurry of the mixture and tabling the mixture on a 25 × 14 cm glass tray. The percentage of each milling fraction was calculated after the separation.

Lipid extraction. Nonstarch lipids were extracted from rice powder (40 mesh) with $CHCl_3$–MeOH (2:1) for 8 hr at 25°, then with H_2O-satd *n*-BuOH for 1 hr, also at 25° according to ref. [20]. Starch lipids from the residue, free of nonstarch lipids, were extracted with H_2O-satd *n*-BuOH at

25° for 23 hr according to the procedure of ref. [22] with modification. Fat-by-hydrolysis was liberated with HCl and extracted with $CHCl_3$ at 25° according to ref. [23] from the residue of brown rice powder after nonstarch lipid extraction.

Lipid fractionation and analysis. Nonstarch lipids were separated into neutral lipids, glycolipids and phospholipids by Si gel CC [24]. The neutral fraction contained pigments, which were removed by treating the fraction with bleaching earth. The fractions of nonstarch lipids were separated and identified by TLC and quantified with the densitometer [1]. The fatty acid composition of nonstarch lipids and fat-by-hydrolysis was determined by GLC [1]. Fatty acid methyl esters for this purpose were prepared according to the procedure of ref. [25].

Enzyme assays. Lipase activity of brown rice flour was determined by the method of ref. [26] as employed in ref. [13] with 4-methylumbelliferone butyrate as substrate. Lipoxygenase activity of the flour was determined from an extract by O_2-uptake measurement using a Clark O_2 electrode according to ref. [27].

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