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LIPIDS OF RICE BRAN*

A. I. Glushenkova, N. T. Ul'chenko, M. Talipova, Kh. S. Mukhamedova, N. P. Bekker, and I. Tolibaev

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The class and fatty-acid compositions of the total lipids and phospholipids and the types of triacylglycerols of rice bran oil have been studied. It has been shown that the main lipid class consists of the triacylglycerols; in them the 16:0, 18:1, and 18:2 acids predominate. The main phospholipids are the PCs, PIs, and PEs. The predominating TAGs are the trisaturated and the monosaturated-diunsaturated types, which are present in equal amounts.

In the processing of rice grain, a considerable amount of bran is formed that contains 10-14% of protein and is therefore used at the present time mainly in the mixed-feed industry [1]. At the same time, it contains 13-15% of lipids possessing a high biological activity: they improve the carbohydrate metabolism, promote hair growth, and oppose the development of microorganisms [2]. In addition, rice bran contains about 40% of essential fatty acids.

However, rice bran also contains a very active hydrolytic enzyme that hydrolyzes the oil, increasing its content of free fatty acids (FFAs) and mono- and diacylglycerols (MAGs and DAGs). This leads to sharp falls in the yield and in the quality of the refined oil, which is used as a foodstuff. With the aim of retaining the native nature of the lipids, therefore, the enzymes have been inactivated before their extraction.

We have studied rice bran provided by the Tashkent rice factory. To inactivate the enzymes, the bran was treated either with a steam–water mixture or with a solution of sodium hydroxide. Hexane and chloroform–methanol (2:1) were used as solvents. The yield of hexane extract amounted to 13.2%. The acidity of the oil was 15%, while without preliminary treatment of the bran it amounted to 61.1 mg KOH [3].

To determine the composition of the lipids, the oil was separated into individual factions by CC on silica gel in solvent systems 1-6. The fractions obtained were checked in a thin layer of silica gel in systems 5 and 7. Fractions containing two or more classes of lipids were additionally separated by PTLC in the same systems. The lipids were identified on the basis of their migrational characteristics, by comparison with model specimens, and by the use of qualitative reactions for epoxide rings with picric acid and for steroids with 50% H_2SO_4. Their composition is given in Table 1.

The main components of the oil were triacylglycerols and FFAs. In addition, incompletely acylated glycerols (mono- and diacylglycerols) were detected.

The compositions of the total fatty acids of the TAGs and of the fatty acids present in the 2-position of the TAGs were determined (Table 2). The main acids were the 16:0, 18:1, and 18:2 types.

The localization of the acids in the TAGs was determined by Coleman's method [4] and it was established that 90.3% of their *sn*-2 positions were occupied by unsaturated acids, which agrees with literature information for plant TAGs and is traditional. The position-species composition of the TAGs was calculated from the composition of the total fatty acids and of the acids in their *sn*-2 positions (Table 3).

The amounts of the individual types of TAGs were as follows, % by weight: SSS – 1.2; SSU – 2.8; USS – 6.5; SUS – 10.3; SUU – 34.7; USU – 5.2; UUU – 39.3 (where U is the sum of the unsaturated and S the sum of the saturated acids).

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 302-305, May-June, 1998. Original article submitted February 23, 1998.

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TABLE 1. Composition of the Rice Oil Lipids, % on the Total Weight

Lipid	Amount
Hydrocarbons	1.3
Esters	0.8
Triacylglycerols	44.3
Free fatty acids	27.7
Epoxyacyldiacylglycerols	2.5
Hydroxyacyldiacylglycerols + triterpenols	5.9
Diacylglycerols + sterols	15.5
Monoacylglycerols	2.0

TABLE 2. Composition of the Total Fatty Acids in the *sn*-2 Position of the Rice-Oil TAGs, % GLC

Acids	Triacylglycerols	*sn*-2-position
12:0	3.6	0.9
14:0	1.4	–
16:0	18.8	8.8
16:1	3.1	2.3
18:0	4.8	–
18:1	30.3	39.4
18:2	38.0	48.6
$\Sigma_{saturated}$	28.6	9.7
$\Sigma_{unsaturated}$	71.4	90.3

Triunsaturated and diunsaturated-monosaturated TAGs were present in equal amounts (39.3 and 39.9%), while disaturated-monounsaturated species made up 19.6%, including 10.3% of TAGs in which the *sn*-2 position was occupied by an unsaturated acid and the *sn*-1 and *sn*-3 positions by saturated acids.

To study the phospholipids (PhLs), the oil was extracted from the bran by Folch's method [5] and was separated by CC on silica gel into neutral lipids and PhLs. In total, the amount of PhLs was 7%, their phosphorus content being 1.7%.

A dried extract of the total PhLs was readily soluble in chloroform, forming a clear greenish yellow solution.

According to two-dimensional TLC in systems 8 (direction 1) and 9 (direction 2), the total PhLs consisted of seven components, the main ones quantitatively being, %: phosphatidylcholines (PCs) – 32.5; phosphatidylinositols (PIs) – 23.5; and phosphatidylethanolamines (PEs) – 20.8; while the minor components of the PhLs were represented by lyso-PCs – 6.8; N-acyl-PEs – 6.9; N-acyl-lyso-PEs – 6.9; and phosphatidylserines (PSs) – 2.6.

In addition, we isolated waxes, which precipitated when the oil was cooled to 5°C.

EXPERIMENTAL

GLC was conducted on a Chrom-41 instrument using a column (4 × 2000 mm) filled with 15% of Reoplex 400 on Chromaton N-AW at 198°C, with helium as the carrier gas.

Solvent systems: hexane–diethyl ether – 1) 9:1, 2) 8:2, 3) 7:3, 4) 6:4, 5) 1:1; 6) diethyl ether; 7) hexane–diethyl ether–acetic acid (8:2:0.1); 8) first direction – chloroform–methanol–ammonia (65:35:5); and 9) second direction – chloroform–methanol–water (65:35:3).

To determine the optimum conditions for inactivating the enzymes, rice bran was treated with a mixture of steam and water or with 0.1% potassium hydroxide solution. It was brought to moisture contents of 10, 15, 17, 20, and 23%, after which it was left for 0.5 h to achieve a uniform distribution of moisture throughout the thickness of the layer. The resulting mass was granulated and was dried in a drying cabinet at 115-120°C to a moisture content of 7-7.5%. The optimum moisture content proved to be 15-16%, at which sufficiently strong granules were obtained that did not disintegrate during the extraction of the oil with shaking, which promoted their free separation on filtration.

The oil was extracted with hexane three times with shaking at room temperature for 6 h each time.

The PhLs were extracted by Folch's method [5]. The phosphorus content was determined by the combustion method.

Fatty acids were extracted as in [3] and were methylated with diazomethane in diethyl ether.

Acid numbers were determined as in [3].

TABLE 3. Position-Species Composition of the Triacylglycerols, % on the Total Mass of TAGS*

TAG species	Amount	TAG species	Amount	TAG species	Amount	TAG species	Amount
PPP	0.8	PSP	0.2	OPP	3.5	SLP	0.8
PPS	0.1	OSP	0.3	LOP	3.0	OLP	4.4
PPO	0.8	LSP	0.4	KOS	0.6	LLP	4.8
PPL	1.0	OSS	0.1	SOS	0.1	PLS	0.8
SPP	0.1	LSS	0.1	OOS	0.7	SLS	0.2
OPS	0.2	PSO	0.3	LOS	0.8	OLS	0.9
LPS	0.2	OSO	0.3	POP	3.5	LSS	0.8
OPP	0.8	LSO	0.4	SOO	0.7	PLO	4.4
SPO	0.2	LSL	0.3	OOO	3.8	SLO	0.8
OTO	0.9	SSL	0.1	LOO	5.2	OLO	4.7
LPO	1.0	OSL	0.3	POL	3.8	LLO	5.0
LPP	0.9	PLL	4.4	SOL	0.8	SLL	0.9
SPL	0.1	OLL	5.2	OOL	4.1	LLL	5.7
OPL	1.1	POP	3.2	LOL	5.6		
LPL	1.2	SOP	0.6	PLP	4.0		

*P) Palmitic; S) stearic; O) oleic; L) linoleic acid.

The column chromatography of the lipids and qualitative reactions were carried out as described by Kates [6]. The enzymatic hydrolysis of the triacylglycerols was achieved by Coleman's method [4].

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