antimicrobial activity. In practice, a compound would only be used in an environment in which it was effective.

Two types of compounds are represented in Table I: (a) diesters in which the ester groupings are in close proximity, being separated by only a methylene group; and (b) triester amides in which the amide moiety and one of the ester groupings are separated by a methylene group, and the other two ester groupings are further removed from the amide moiety. Both types of compounds had examples that inhibited two or more of the microorganisms. The most inhibitory compound under the test conditions was the diester of adipic acid with methyl glycolate (No. 5 in Table I). It was strongly inhibitory against all of the organisms. The long chain compounds might be more effective in nonaqueous media than under the aqueous conditions of the screening test. The test results, however, show that all of the compounds have some inhibitory effect on the four microorganisms used in the test, and six of them inhibited two or more in the area of application of the compound. Thus, some of the compounds merit further testing as biostatic agents.

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Quantitative Analysis of Fatty Acids and Sterols in Malagasy Rice Bran Oils

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ABSTRACT

The fatty acid and sterol compositions of six Malagasy rice bran oils were evaluated. Investigation by gas liquid chromatography (GLC) using Carbowax 20 M revealed 10 fatty acids, mainly palmitic (16-20%) oleic (41-44%) and linolenic (31-37%) acids. An OV 17 column was used to separate eight sterols, mainly β-sitosterol (53-59%), campesterol (16-26%) and stigmasterol (10-13%). No significant variation for the fatty acid and sterol contents was observed among the rice varieties studied.

INTRODUCTION

Rice bran is an interesting raw material that could be used for edible oil production in Madagascar: paddy rice crops yearly exceed 2,000,000 t and the need for a rice bran oil extraction plant is being evaluated. Feasibility studies previously were reported (1-4) and extraction plants already exist in various countries.

Our laboratories are working on the composition and properties of the oil extracted from some local rice varieties. We studied 6 varieties which are widely grown in Madagascar: japonica, vory, lava, alicumbo, angika and makalioka. Preliminary results were obtained for laboratory neutralized and bleached oils.

EXPERIMENTAL PROCEDURES

Fatty acid methyl esters were prepared by saponification of triglycerides and acid catalyzed methylation according to Wolff (5). A Girdel Model 30 gas chromatograph equipped with a flame ionization detector was used for the analyses. The column employed was a 100-m-long, 0.25-mm-i.d. glass capillary column coated with Carbowax 20 M. The oven temp was kept at 200 C.

TABLE I

Moisture, Fat and Unsaponifiable Matter Content in Rice Bran

Rice variety	Moisture content (%)	Fat content (%)	Unsaponifiable matter (%)
Japonica	15.8	13.9	5.2
Vory	13.7	14.6	5.3
Lava	12.3	11.2	5.7
Alicumbo	15.4	14.9	6.0
Angika	16.8	11.9	5.5
Makalioka	10.3	10.0	6.0

TABLE II

Fatty Acid Analysis of Various Rice Oils (%)

Fatty acid	Variety					
	Japonica	Vory	Lava	Alicumbo	Angika	Makalioka
14:0	0.4	0.2	0.3	0.2	0.3	0.2
16:0	16.4	18.5	17.9	19.0	18.6	20.4
16:1ω7	0.1	0.1	0.1	0.1	0.1	0.1
18:0	1.8	2.2	1.9	1.9	2.1	2.0
18:1ω9	41.6	42.0	48.1	44.6	43.2	44.3
18:1ω7	0.9	1.0	1.1	1.0	1.0	1.0
18:2ω6	37.2	34.3	29.0	31.4	34.2	30.6
18:3ω3	1.3	1.0	1.0	1.0	1.8	1.0
20:0	0.4	0.4	0.3	0.4	0.6	0.3
20:1ω9	0.2	0.2	0.3	0.2	0.1	0.1
Ratio 18:1/18:2	1.15	1.27	1.69	1.42	1.27	1.48

TABLE III

Sterol Analysis of Various Rice Oils

Sterol	Relative retention	Variety				
		Japonica	Vory	Lava	Alicumbo	Angika
Cholesterol	0.59	1.1	0.4	0.8	1.5	1.5
Campesterol	0.78	25.6	21.0	25.6	18.6	16.7
Stigmasterol	0.86	13.2	11.2	12.6	12.2	10.3
Ergosterol	0.92	0.5	0.5	0.5	0.5	0.5
β-Sitosterol	1.00	53.0	59.0	54.7	57.0	58.6
Δ-5 Avenasterol	1.09	2.1	2.7	1.5	3.8	5.5
Δ-7 Stigmastenol	1.15	2.6	3.0	2.8	4.0	4.5
Δ-7 Avenasterol	1.30	1.6	1.7	0.8	2.0	2.3

Sterols were separated from unsaponifiable matter by thin layer chromatography (TLC) (6). The sample was analyzed by depositing a 5% unsaponifiable carbon tetrachloride (CCl_4) solution (150 μl) on H F_{254} silica gel and using an ethyl ether-$CHCl_3$ mixture (90:10) as elution solvent. The rhodamine B-vaporized plate was examined under 254 nm ultraviolet (UV) light. The sterol band (Rf = 0.45) was traced and sterols were quantitatively recovered and then treated with *N,O-bis*-(trimethylsilyl)-trifluoroacetamide (BSTFA). Silyled components were then injected at 245 C, using a solid-type injector, into a 1 mm x 50 m glass capillary column filled with OV 17 (phenyl methyl silicone).

RESULTS AND DISCUSSION

The rice brans investigated were obtained fresh from rice mills and stored at room temperature in darkness to prevent lipid autoxidation (7). However, some samples developed 5-7% free fatty acids (FFA) before they were treated for lipase destruction (8).

Table I shows the oil, moisture and unsaponifiable contents as determined by other researchers, (5-6).

Methyl esters were identified using olive oil as a standard and comparing samples with Flanzy's work (9) on the equivalent chain length. Results are reproduced in Table II. We investigated 10 fatty acids, but focused on palmitic (16-20%), oleic 41-44%) and linoleic (31-37%) acids. These results agree with other previously studied rice bran oils (10,11,12). No significant differences were found among varieties. However, if we consider the ratio 18:1/18:2 (Table II), differences exist between the japonica and lava varieties, in which the ratios are 1.15 and 1.69, respectively. Fatty acid differentiation in other varieties is difficult and gives less conclusive results.

Itoh et al. (13,14) established the presence of 8 sterols. The retention times were expressed against β-sitosterol (Table III). In the 5 varieties analyzed, 0.4-1.5% cholesterol was detected. The most valuable sterols were β-sitosterol (53-59%), campesterol (16-26%) and stigmasterol (10-13%), whereas brassicasterol was not detected. The sterol composition for these different varieties was somewhat similar and thus made their differentiation by this analytic method difficult to attain.

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