TABLE III

Antioxidant Activity of Rosmariquinone and Other Commercial Antioxidants

Additive (0.02%)	Peroxide value (Meq/kg) at 60 C after days: 7	14	21	28
Control, no additive	4.70	10.08	29.93	119.67
BHT	1.26	1.86	2.71	3.37
BHA	2.72	6.54	12.10	17.01
Rosmariquinone	3.28	3.81	4.52	5.10

prime steam lard at a concentration of 0.02%. The peroxide value of the lard was determined when the samples were fresh and after being aged at 60 C in the dark for 7, 14, 21 and 28 days, respectively. The decrease in the rate of formation of peroxide was used as a measurement of the antioxidant activity of the sample. The peroxide values obtained from rosmariquinone and other standards appear in Table III. The antioxidant activity of rosmariquinone was superior to BHA, but it was slightly less than BHT.

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Comparative Studies of Three Solvent Mixtures for the Extraction of Soybean Lipids

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ABSTRACT

Soybean seeds were extracted with chloroform-methanol (2:1), methylene chloride-methanol (2:1) and hexane-isopropanol (3:2) mixtures. The seed lipids were then fractionated by column chromatography. Neutral lipids were further separated by thin layer chromatography (TLC) and quantified by acid charring method. Fatty acid methyl esters were prepared and analyzed by gas liquid chromatography (GLC). Our results show that methylene chloride-methanol (2:1) was as good as chloroform-methanol (2:1) for the extraction of soybean lipids, but hexane-isopropanol (3:2) was somewhat inferior.

INTRODUCTION

Chloroform-methanol (2:1) mixture has been widely accepted as the most exhaustive solvent mixture and has been used extensively for the extraction of plant and animal lipids (1). However, the extensive usage of chloroform has caused great concern recently because chloroform is hepatoxic and a suspected carcinogen (2,3). In view of this, two other solvent mixtures, hexane-isopropanol (3:2) (4) and methylene chloride-methanol (2:1) (5), which avoid the use of chloroform, recently have been suggested. These new solvent mixtures have not been widely tested under laboratory conditions. In this communication, we compare the effectiveness of the three solvent mixtures for the extraction of soybean seed lipids under the same laboratory conditions.

EXPERIMENTAL

Materials

Soybean seeds were obtained from Lam Soon Oils and Soaps Manufacturing Limited, Petaling Jaya, Selangor, Malaysia. The seeds were ground mechanically to a fine powder before lipid extraction.

All organic solvents were of analytical grade, and chloroform was redistilled before use.

Methods

Lipids were extracted with chloroform-methanol (2:1) according to Folch et al. (6), with methylene chloride-methanol (2:1) according to Chen et al. (5) and hexane-isopropanol (3:2) according to Hara and Radin (4). Total lipids were fractionated into neutral lipid, glycolipid and phospholipid fractions by HCl-treated Florisil column chromatography (7). Neutral lipids were separated further by TLC using hexane-diethyl ether-formic acid (80:20:2) as the developing solvent system and quantified by sulphuric acid charring method (8). Fatty acid methyl esters (FAME) were prepared and analyzed as described before (9).

RESULTS AND DISCUSSION

Our results (Table I) show that chloroform-methanol (CM) and methylene chloride-methanol (MM) extracted comparable amounts of total seed lipids, whereas hexane-isopropanol (HI) extracted much less seed lipids. The difference between the MM and HI extracted seed lipids is statistically significant ($P<0.05$).

Fractionation of the soybean total lipids in HCl-treated Florisil column showed that the HI method extracted much less phospholipids compared to those extracted by the CM and MM methods (Table I). Sahasrabudhe and Smallbone (10) also reported that the HI method extracted much less polar lipids from beef compared to the CM and MM methods.

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TABLE I

Soybean Lipids Extracted by Three Different Solvent Mixtures[a]

Extraction methods[b]	Total lipids	Neutral lipids	Glycolipids	Phospholipids
CM	17.8	14.7	0.3	2.6
MM	18.5	15.8	0.3	2.0
HI	13.9	13.2	0.2	0.5

[a]Each value is the average of 2 or 3 analyses and is expressed as wt. %.

[b]CM = chloroform-methanol; MM = methylene chloride-methanol; and HI = hexane-isopropanol.

TABLE II

Neutral Lipids of Soybean Seeds

Extraction methods	Neutral lipids (wt. %)[a]			
	TG	FA	DG	ST
CM	81.7	12.4	3.3	2.6
MM	80.4	13.7	3.0	2.8
HI	86.6	6.9	4.0	2.3

[a]TG = triacylglycerol; FA = fatty acid; DG = diacylglycerol, and ST = sterol.

TABLE III

Fatty Acid Profiles of Soybean Lipids (area %)

Fatty acids	Extraction methods		
	CM	MM	HI
12:0	t[b]	t[b]	t[b]
14:0	t[b]	t[b]	t[b]
16:0	6.6	6.4	6.2
16:1	0.1	0.1	0.1
18:0	2.1	2.1	2.1
18:1	11.2	11.2	11.3
18:2	61.1	61.0	60.4
18:3[a]	18.6	18.8	19.4
20:0	0.1	0.1	0.1
22:1	0.3	0.2	0.3

[a]18:3 overlaps with 20:1.

[b]t = trace, less than 0.1%.

Quantification of the neutral lipids after TLC by acid charring method (8) showed that, other than the fatty acid levels, there was no marked difference in the neutral lipid classes extracted by the three different methods (Table II). The higher level of fatty acids in the neutral lipid fraction of the CM extracted seed lipids compared to the HI extracted seed lipids could be due to chloroform, which activated, and isopropanol, which inhibited lipolytic enzymes during the extraction process (11). The fact that the fatty acid level in the MM extracted seed lipids was as high as that of the CM extracted seed lipids indicates that methylene chloride also could activate lipolytic enzymes during the extraction process.

Analysis of the fatty acid composition of the soybean lipids obtained by different extraction methods by GLC showed no major difference in the fatty acid profiles (Table III). From the above results it may be concluded that in the process of extraction of seed lipids methylene chloride functions very much like chloroform and hence it can effectively replace chloroform in the extraction procedure. As methylene chloride is much less toxic than chloroform (2,5), the use of methylene chloride in place of chloroform should be encouraged.

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