Thermal Oxidation of Phospholipids 1,2-Dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine

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ABSTRACT

The purpose of this study was to compare thermal oxidation of phospholipids with that of triglycerides. Two simple saturated systems were chosen: 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine and tripalmitoylglycerol. Samples were heated at 150 C for 1 hr in air and the resulting volatile compounds analyzed qualitatively and quantitatively. Alkanes, ketones and lactones were major products in both cases. However, qualitative and quantitative differences were observed. Alkanals were absent in the oxidized phospholipid. This apparently is largely due to their reaction with the amine group as evidenced by thin layer chromatography. In addition, the phospholipid produced 2-pentanol, 2-methyl-1-propanol and ethyl hexadecanoate; smaller amounts of hydrocarbons and methyl ketones than with the triacylglycerol; and no 1-alkanols. Mechanisms which could be responsible for these observations are proposed.

INTRODUCTION

Phospholipids have been shown to easily oxidize or discolor during heating or prolonged storage (1). Phospholipids also have been incriminated as playing a major role in the development of warmed-over flavors in poultry, mutton, beef and pork (2). Soy phosphatidylcholine was found to develop rancid flavors and bitter taste during storage (3,4). In some foods, phospholipids oxidize before substantial oxidation of triglycerides (5,6). While model systems have been used in studying the volatile products of triglyceride thermal oxidation (7-11), to our knowledge no studies have been carried out in detail on the oxidative volatile decomposition products from phospholipids.

In this work, a simple model system, 1,2-dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine, was chosen. The volatile compounds produced were compared both qualitatively and quantitatively to those formed during the thermal oxidation of tripalmitoylglycerol.

It is understood that in nature phospholipids usually do not exist in bulk nor are they fully saturated. The model system selected here is to be considered a beginning step in discerning the mechanism by which a phospholipid breaks down when subjected to thermal and oxidative conditions.

EXPERIMENTAL PROCEDURES

Materials

1,2-Dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine (PE), > 99% pure, was purchased from Sigma Chemical Co. (St. Louis, MO), and reagent-grade tripalmitoylglycerol (TP) was purchased from Eastman Kodak Co. (Rochester, NY) and from Sigma Chemical Co. (St. Louis, MO). The purity of these substrates was further tested by collecting the volatiles by high-vacuum, cold-finger distillation at 80 C (12) and analyzing by gas chromatography (GC). PE contained no volatiles and was used as received. However, the TP from both sources contained trace amounts of volatiles which were removed by vacuum distillation, under the same conditions as just described, until no volatiles could be detected in the distillates. The TP purchased from Eastman Kodak (TP-E) was of less purity than that from Sigma (TP-S) as it required longer distillation time. No fatty acids other than palmitic were present. Phospholipid-aldehyde reaction products for thin layer chromatography (TLC) were formed according to Nakanishi and Suyama (13) by stirring 1.5 ml heptanal and 0.56 g PE in 12 ml hexane for 2 hr.

Heat Treatments

One-gram samples, in 200-ml round-bottomed flasks fitted with ground glass stoppers, were heated in an oil bath at 150 C for 1 hr. Air was left in the flasks during the period of heating. Samples were allowed to reach ambient temperature before further analysis.

Collection and Fractionation of Volatiles

The heated samples were distilled under vacuum as previously described (12). The distillations were carried out for 2 hr at 80 C and 10^{-3} torr, and the distillate was dissolved in pentane. Activated silica gel was stirred with the pentane-distillate solution to separate the polar from nonpolar compounds. The polar compounds were eluted with diethyl ether. Both fractions were concentrated under nitrogen for GC and mass spectrometric analysis. This technique of fractionation also provided useful information for chemical identification.

Gas Chromatography-Mass Spectrometry (GC-MS)

A Carbowax 20 M capillary column, 500 ft x 0.02 in. id, was used in a Varian Aerograph Model 1200 equipped with a flame ionization detector. The temperature was programmed from 60 C to 180 C at 4 C/min. Twenty-five percent of the effluent was admitted via a heated line to a Biemann helium separator, both maintained at 200 C then to a Hitachi Perkin-Elmer model RMU-6A mass spectrometer. The ion source was operated at 70 eV, the accelerating voltage at 2500 V and the electron multiplier at 1400 V. Scanning time was 12 sec and the spectra were recorded with a Honeywell 1503 Visicorder.

Identification and Quantitation of Volatiles

The major products of thermal oxidation were quantitated using internal standards. Tridecene was used for the nonoxygenated fractions and 10-undecenal for the oxygenated fraction. PE (mp 195 C) was solid whereas TP (mp 65.5 C) remained liquid during distillation. Consequently, 25 ml of stripped corn oil were added to the heated PE and TP before distillation to bring about more comparable conditions for the collection of volatiles. PE did not, however, dissolve completely in the corn oil but was suspended in the fluid medium. A series of standard mixtures analogous to the identified volatile decomposition products were prepared and added, together with the internal standards already described, to 25 ml stripped corn oil. Cold-finger distillation, fractionation and concentration were carried out in the same manner as for the thermally oxidized samples. This procedure permitted the calculation of the appropriate recovery and response factors for each compound. With compounds for which authentic compounds were not available, correction factors of homologs differing by one or 2 carbons were used.

Separation and Analysis of Nonvolatile Compounds

Nonvolatile compounds were analyzed by TLC on glass plates coated with Adsorbosil-5 (400 μ) silica gel (Applied Science Laboratories, State College, PA). The plates were developed in chloroform/methanol/water (65:25:4, v/v/v). The following detection reagents were used: (a) ninhydrin-plus (Applied Science, State College, PA). The plate was heated in an oven for 5-10 min at 110 C to visualize compounds; (b) phospray (Supelco, Inc., Bellefonte, PA); (c) dichromate-sulfuric acid (Applied Science, State College, PA). After spots which were positive to ninhydrin-plus or Phospray were outlined, plates were sprayed with dichromatic-sulfuric acid and charred.

RESULTS

The compounds identified from thermal oxidation of TP and PE and the amounts of the individual components are given in Tables I and II. GC analyses for the nonpolar and polar fractions are shown in Figures 1 and 2, respectively. The TP used for this comparison was obtained from Eastman Kodak (TP-E). A discussion comparing the results of TP-E to those from Sigma Chemical (TP-S) will follow.

Both PE and TP decomposed to form series of alkanes, 2-alkanones and γ-lactones. Methyl hexadecanoate was also recovered from these substrates. Generally, the volatile compounds were recovered in greater amounts from TP than from PE.

Pentadecane was the major compound found in the nonpolar fraction of thermally oxidized TP. This is in accord with the Selke et al. data (10) in which C17 was the major *n*-hydrocarbon formed when tristearin was heated at 192 C. The C_{n-4} alkane was also formed in relatively large quantity but there was less of the tridecane than of the other hydrocarbons. Selke et al (10) found alkanes from C_4 to C_{17} in heated palmitate. The cold-finger distillation technique used in our present work provides efficient recovery of the

TABLE I

Quantitative Analysis (μg compd./g substrate) of Nonpolar Compounds Produced by the Thermal Oxidation of TP and PE

Compound carbon number	TP[a]	PE[b]	Peak no. in Fig. 1
n-Alkanes			
9	2.1 ± 0.0	— —	1
10	2.0 ± 0.1	0.41 ± 0.19	3
11	2.3 ± 0.2	0.65 ± 0.16	6
12	4.4 ± 0.9	0.59 ± 0.06	9
13	1.1 ± 0.2	0.82 ± 0.02	12
14	2.4 ± 0.9	0.64 ± 0.11	15
15	35 ± 4	0.67 ± 0.29	17
1-Alkenes			
9	0.33 ± 0.17	— — —	2
10	0.42 ± 0.22	— — —	5
11	0.86 ± 0.17	— — —	8
12	0.48 ± 0.22	— — —	10
14	0.64 ± 0.03	— — —	16
15	15 ± 6	0.24 ± 0.07	18

[a] Two determinations.

[b] Five determinations.

TABLE II

Quantitative Analysis (μg compd./g substrate) of Polar Compounds Produced by the Thermal Oxidation of TP and PE

	TP[a] (average μ/g)	PE[b] (average μ/g)	Peak no. in Fig. 2
Alkanals			
6	16[c] ± 4	–	9
7	15[c] ± 2	–	14
8	11 ± 1	–	18
9	13 ± 1	–	23
10	20[c] ± 5	–	27
11	15[c] ± 2	–	33
12	10[c] ± 1	–	40
13	23 ± 2	–	45
2-Alkanones			
4	9.8 ± 2.6	5.1[c] ± 3.9	1
5	10[c] ± 3	5.1[c] ± 3.9	2
6	16[c] ± 4	4.8 ± 4.0	8
7	15[c] ± 2	2.4 ± 0.5	13
8	2.9 ± 0.8	4.2 ± 0.7	17
9	5.3 ± 0.4	4.9 ± 1.0	22
10	6.3 ± 1.2	5.5 ± 0.7	26
11	7.5 ± 1.8	4.3 ± 0.6	32
12	7.0 ± 1.4	5.1 ± 0.8	39
13	3.6 ± 1.3	2.2 ± 0.5	44
14	29 ± 5	1.2 ± 0.3	48
15	240 ± 50	31 ± 5	53
Alkenones			
5	3.2 ± 1.0	–	6
6	9.7 ± 2.8	–	11
7	15[c] ± 1	–	15
8	3.9 ± 0.7	–	19
9	18 ± 2	–	24
10	20[c] ± 5	–	28
11	15[c] ± 2	–	34
12	10[c] ± 1	–	41
3-Alkanone			
15	1.8 ± 0.3	–	51
4-Alkanone			
15	4.5 ± 1.0	–	50
1-n-Alcohols			
4	6.1 ± 1.8	–	12
5	4.0 ± 1.1	–	16
6	5.2 ± 0.9	–	21
7	6.0 ± 2.0	–	25
8	5.8 ± 1.9	–	30
9	4.2 ± 1.6	–	36
10	3.9 ± 1.1	–	42
11	3.7 ± 1.1	–	46
12	5.1 ± 1.0	–	49
2-Methyl-1-propanol	–	1.2 ± 0.6	7
2-Pentanol	–	12 ± 1.0	10
2-Methyl-2-Butanol	10[c] ± 3	5.1[c] ± 3.9	3
γ-Lactones			
6	8.7 ± 2.2	–	38
7	11 ± 4	1.2 ± 0.4	43
8	13 ± 4	2.1 ± 0.6	47
9	8.6 ± 0.5	–	52
10	9.8 ± 3.2	1.8 ± 0.5	54
11	16 ± 5	2.9 ± 1.4	56
12	11 ± 4	1.9 ± 1.0	58
Ethyl ester			
16	–	6.0 ± 2.4	57

[a] Three determinations.

[b] Five determinations.

[c] This value includes other components with the same retention time.

hydrocarbons with carbon chains C_9-C_{18} (12). No attempt was made to study gases, extremely volatile compounds, dimers or polymers. With the oxidized PE, tridecane was formed in slightly greater amounts than the other hydrocarbons and the *n*-alkanes were present in substantially lesser amounts than in oxidized PE; many of the alkanes were absent.

2-Pentadecanone was the major compound in the oxygenated fraction of both TP and PE. 2-Tetradecanone and 2-pentadecanone from TP were each significantly greater than the corresponding 2-alkanones formed during PE decomposition, whereas 3- and 4-pentadecanone were absent in the oxidized PE.

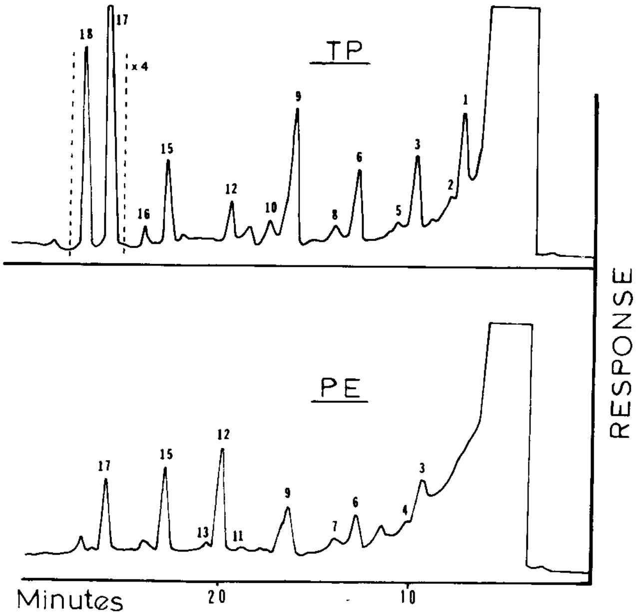
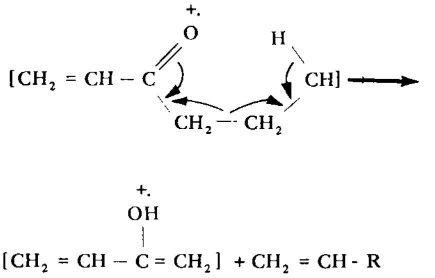


FIG. 1. Gas chromatographic trace of the nonpolar volatiles from oxidized TP and PE. Peak numbers correspond to compounds listed in Table I.

A homologous series not previously reported was recovered from TP but not from PE and identified as the $C_{5\text{-}12}$ 1-alkene-3-ones. On the Carbowax column, members of this series eluted immediately following the *n*-alkanals having the same carbon number. Their mass spectra (Fig. 3) featured a molecular ion of 2 mass units less than that of the corresponding alkanals, strong peaks at m/e 55 and 70, and fragment ions at M-15 and M-29. For the $C_{5\text{-}8}$ members of this series the base peak was that observed at m/e 55 whereas the ion at m/e 70 accounted for the base peak for the $C_{9\text{-}12}$ compounds. The ion $CH_2{=}CH{-}C{\equiv}O^+$ resulting from cleavage alpha to the carbonyl group is responsible for the peak at m/e 55 and a McLafferty rearrangement, involving transfer of a γ-hydrogen to the carbonyl oxygen with concomitant β-fission gives the ion at m/e 70:



$$[CH_2=CH-\overset{\overset{+\cdot}{OH}}{C}=CH_2] + CH_2=CH-R$$

Such a cyclization mechanism would not be possible, however, in the short-chain member 1-pentene-3-one. Indeed, the ion at m/e 70 was absent in the spectrum of this compound (Fig. 4).

A major difference between the oxidation products of TP and PE is the complete absence of the *n*-alkanal and the 1-alkanol series in the phospholipid. On the other hand, 2-pentanol, 2-methyl-1-pentanol and ethyl hexanoate were found in the oxidation PE but not TP.

The same series of γ-lactones were found in both TP and PE decomposition products. Quantitatively, however, the γ-lactones from PE were recovered in significantly lesser amounts compared to those from TP thermal oxidation. For both substrates, the C_{11} γ-lactone was produced in relatively large amounts.

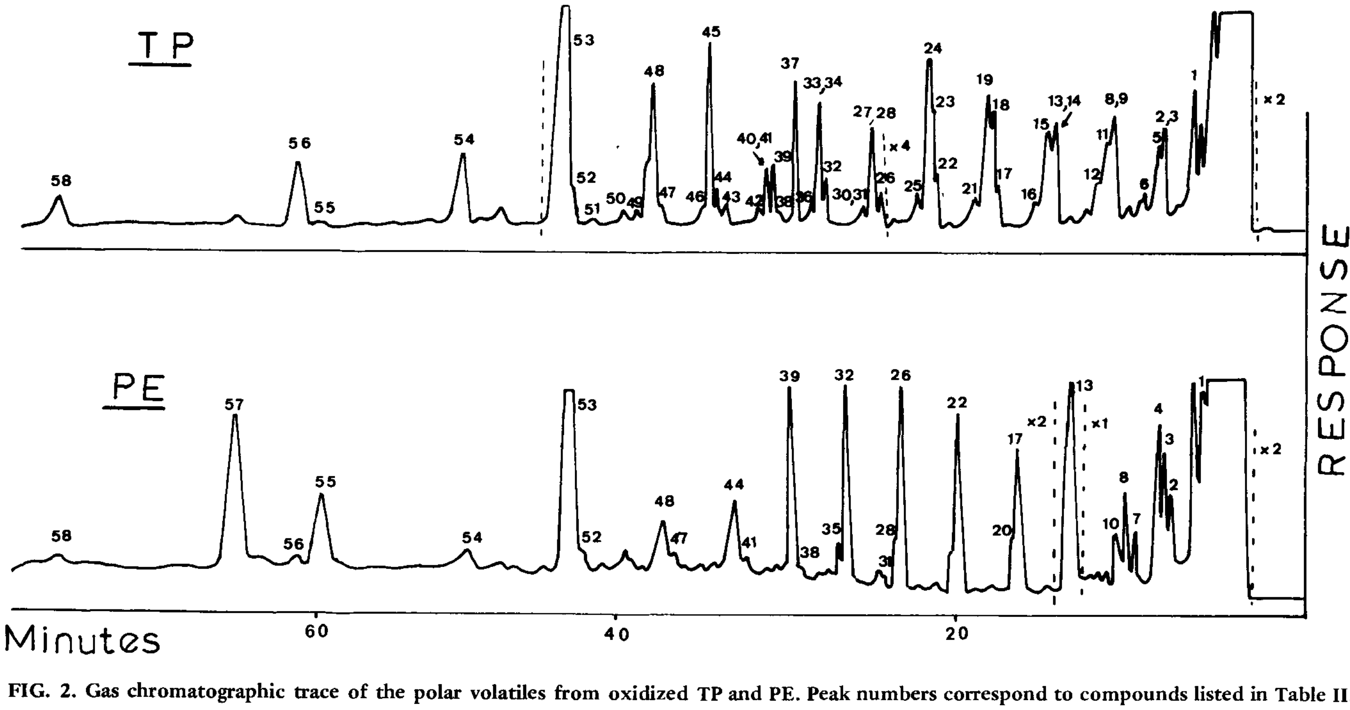


FIG. 2. Gas chromatographic trace of the polar volatiles from oxidized TP and PE. Peak numbers correspond to compounds listed in Table II.

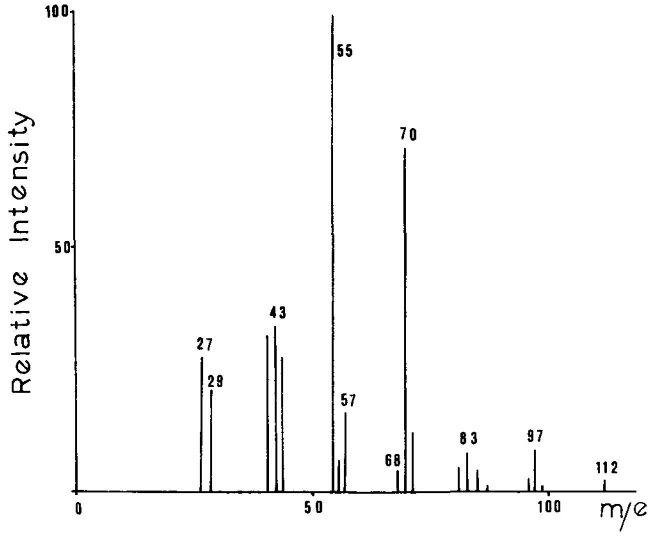


FIG. 3. Mass spectrum of compound tentatively identified as 1-hepten-3-one.

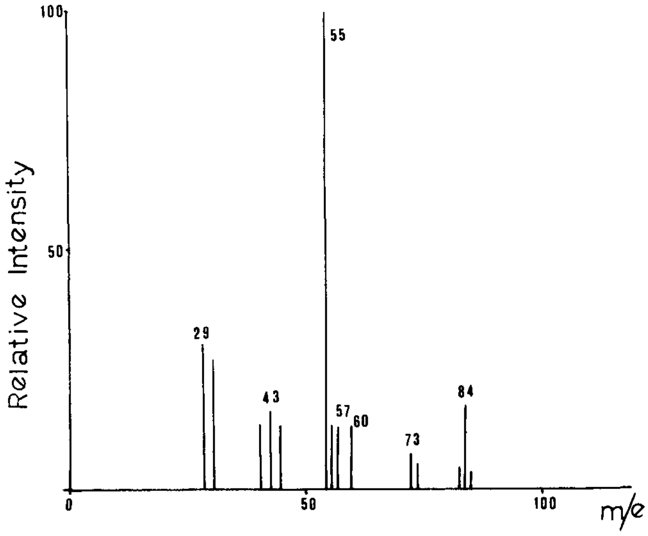


FIG. 4. Mass spectrum of compound tentatively identified as 1-penten-3-one.

TLC of the oxidized PE gave rise to a spot not found in oxidized TP, which has an R_f value greater than PE and was positive to both nitrogen and phosphorus detecting reagents. It is speculated that the spot represents a carbonyl-amino addition product.

DISCUSSION

It is evident from the results of this study that a preferential oxidative attack does take place at the β-carbon for both PE and TP. Monohydroperoxide formation and decarboxylation (7) would lead to the formation of 2-pentadecanone, which was the compound recovered in greatest amounts from the volatile fractions of thermally oxidized TP and PE. Oxidative attack at the β-carbon could also result in the formation of tetradecanal and tridecane. Preferential oxygen attack may occur at the γ-carbon of the fatty acid and would produce the γ-lactones, 3-pentadecanone, 2-tetradecanone, tridecanal, dodecane, dodecene and dodecanol.

The *n*-hydrocarbons and *n*-alcohols present in TP could be formed by the reaction of alkyl free radicals with hydrogen and hydroxy free radicals, respectively. The reduced amounts or absence of these compounds in thermally oxidized PE suggest that a different mechanism is involved. Pokorny et. al. (14) found that hydroperoxides formed as primary reaction products were relatively unstable in phosphatidylethanolamine compared to their stability in alkanol esters. Tai et. al. (15) suggested that the presence of bound phosphoric acid prevents the accumulation of hydroperoxides in oxidized phospholipids. Proton attack at the hydroperoxide could result in expulsion of water, subsequent oxidation with the formation of a ketone and regeneration of the proton. This mechanism could account for the lack of aldehydes, alcohols and alkenes. The proton which is present in the phospholipid might also provide an explanation for the formation of 2-pentanol. If 1-pentanol were originally formed, protons present could induce carbonium ion formation and rearrangement resulting in the formation of the secondary alcohol. The presence of 2-pentanol in oxidation products from PE suggests possible. selective splitting between acyl carbons 11 and 12. It is notable that in the PE, the C11 γ-lactone was formed in relatively large quantities.

The exact mechanism for the formation of the alkenone series found in TP has not yet been elucidated. When Nichols and Holman (16) pyrolyzed trioctadecanoin, they found allyl ester to be the fourth most abundant product. They proposed that allyl stearate might arise by direct loss of 2 acyloxy groups from the triglyceride. Perhaps an analogous compound was formed during the thermal oxidation of TP and was followed by subsequent cleavage of the acyloxy bond. The resultant allyl-oxy compound might react with an alkyl radical to form the alkenone.

Some differences were observed in the oxidative behavior of the TP-S which was of higher purity. After thermal oxidation, it has a lighter color and a more crystalline appearance than the oxidized TP-E. The alkenones and *n*-alcohols which were found in the decomposition products of TP-E were absent from the volatiles of TP-S whereas the only hydrocarbons detectable in the TP-S were pentadecane and pentadecene. In addition, the $C_{5\text{-}14}$ 2-alkanones were recovered in greater amounts in TP-S than TP-E whereas 2-pentadecacone was found to be in greater abundance in TP-E. It is possible that the alkenones found may be derived from impurities in TP-E or from a more extensive oxidation in samples of less purity. Brodnitz et. al. (17), investigating the initial products of autoxidation, found 99.5% pure methyl palmitate to be more susceptible to oxidation at 60 C than if the substrate was 99.99% pure. Thaler and Saumweber (18) also observed that the rate of autoxidation of saturated fatty acids at room temperature was a function of their purity.

The most striking difference between the oxidative decomposition of PE and that of TP is the complete absence of alkanals in the PE. Several researchers have proposed mechanisms by which the nitrogen of phosphatidylethanolamine reacts either with carbonyl compounds or hydroperoxides (14,15,19). Nakanishi and Suyama (13) heated phosphatidylethanolamine with alkanals and identified compounds which would indicate that 3 molecules of alkanal could react with 1 molecule of phosphatidylethanolamine. The absence of alkanals in the volatile decomposition products of PE may result from the immediate reaction of the alkanals produced from the fatty acid moieties with the amino group of ethanolamine. The TLC data support this conclusion.

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REFERENCES

1. Lea, C.H., J. Sci. Food Agric. 8:1 (1957).
2. Wilson, B.R., A.M. Pearson and F.B. Shorland, J. Agric. Food Chem. 24:7 (1976).
3. Sessa, D.J., K. Warner and D.H. Hong, J. Food Sci. 39:69 (1974).
4. Sessa, D.J., K. Warner and J.J. Rackis, J. Agric. Food Chem. 24:16 (1976).
5. Roubal, W.T., JAOCS 44:325 (1967).
6. Hornstein, I., P.F. Crowe and M.J. Heimberg, J. Food Sci. 26:581 (1961).
7. Crossley, A., T.D. Heyes and B.J.F. Hudson, JAOCS 39:9 (1962).
8. Endres, J.G., V.R. Bhalerao and F.S. Kummerow, Ibid. 39:118 (1962).
9. Endres, J.G., V.R. Bhalerao and F.S. Kummerow, Ibid. 39:159 (1962).
10. Selke, E., W.K. Rohwedder and H.J. Dutton, Ibid. 52:232 (1975).
11. Selke, E., W.K. Rohwedder and H.J. Dutton, Ibid. 54:62 (1977).
12. Nawar, W.W., J.R. Champagne, M.F.Dubravcic and P.R. LeTellier, J. Agric. Food Chem. 17:645 (1969).
13. Nakanishi, R., and K. Suyama, Agric. Biol. Chem. 38:1141 (1974).
14. Pokorny, J., P. Rai and G. Janicek, Z. Lebensm.Unters. Forsch. 153:322 (1973).
15. Tai, T., J. Pokorny and G. Janicek, Ibid. 156:256 (1974).
16. Nichols, P.C., and R.T. Holman, Lipids 7:773 (1972).
17. Brodnitz, M.H., W.W. Nawar and I.S. Fagerson, Ibid. 3:59 (1968).
18. Thaler, H., and W. Saumweber, Fette Seifen Anstrichm. 63:945 (1961).
19. Pokorny, J., P. Tai and G. Janicek, Nahrung 20:149 (1976).

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Evaluation of Hydrogenation Catalyst Activity

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ABSTRACT

The AOCS Recommended Practice for testing activity of hydrogenation catalysts was used to compare activity and properties of a number of commercial catalysts with the AOCS standard catalyst. Four of five commercial catalysts tested were similar to the standard but one commercial catalyst was markedly more active and more selective. It also was very difficult to filter after hydrogenation. Selectivity of the catalysts in hydrogenation of soybean oil was determined from change in fatty acid composition. The most selective catalyst produced the highest level of *trans* isomers and the highest dropping point. Solid fat contents measured after 30 and 40 min of hydrogenation time were determined by wide-line nuclear magnetic resonance. The Recommended Practice and standard catalyst were useful tools in evaluating activity and selectivity of hydrogenation catalysts.

INTRODUCTION

During the hydrogenation of oils, the progress of the reaction is influenced by process conditions as well as the characteristics of the catalyst used (1). A catalyst can be described in terms of activity, which refers to the amount of catalyst needed to hydrogenate a batch of oil in a reasonable time, and its selectivity, which refers to the ratio of the reaction constants of 2 of the participating fatty acid species (2). According to Allen (1), activity may be defined as the decrease in iodine value (IV) per unit of time under specific conditions of hydrogenation. To enable easier comparison of different catalysts, the American Oil Chemists' Society has issued a Recommended Practice (3) for evaluation of the activity of catalysts. This involves hydrogenation of soybean oil with a standard catalyst and comparison with the results obtained with other catalysts. The effect of particle size of the catalyst on the activity has been studied by Dafler (4), who concluded that the catalysts with the smallest particle size are not necessarily the most active. Catalyst particle size is important in filtration of the hydrogenated oil as oils containing extremely small particles are difficult to filter and result in high nickel content.

This study was undertaken to evaluate a number of commercial hydrogenation catalysts using the recommended practice and the AOCS standard catalyst.

EXPERIMENTAL

Refined and bleached soybean oil was supplied by Canada Packers Ltd. of Toronto, Ontario. The hydrogenation catalysts were commercially available and were the dry-reduced type, except one was wet-reduced. The standard hydrogenation catalyst was obtained from the American Oil Chemists' Society. Hydrogenations were carried out in a Parr pressure reaction apparatus, series 4500, using a 2-ℓ bomb and a charge of 1 ℓ of oil. The hydrogenation conditions selected were: temperature 176 C, hydrogen pressure 200 kPa (15 psig) and catalyst concentration 0.2% (corresponding to 0.05% nickel). Agitation speed in the reactor was 610 rpm; an IV of 80 was reached with soybean oil and the standard catalyst within 40 ± 4 min as required by the AOCS recommended practice.

Iodine values were determined by the Wijs method (AOCS Cd 1-25). *Trans* fatty acids were determined by the infrared spectrophotometric method (AOCS Cd 14-61). Fatty acid composition was determined by gas liquid chromatography (GLC) of the methyl esters on a 125-cm column packed with 15% DEGS on Chromosorb RZ-60/80 mesh operated at 185 C and using a model 402 Hewlett Packard instrument with dual flame ionization detectors. Dropping point of hydrogenated fats was determined with the Mettler FP3 automatic dropping point apparatus as described by Mertens and deMan (5). Ease of filtration was evaluated by heating the hydrogenated oils with 0.1% diatomaceous earth for 10 min at 150 C and filtering them under vacuum (1.35 kPa or 10 mm Hg) through a Whatman No. 40 filter with a filter area of 12.5 cm^2. The time required for 250 ml of oil to pass through the filter

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