TABLE III

Comparison of Fatty Acids in Purified and Nonpurified[a] Triacylglycerols in Citrus Leaves

Triacylglycerol	Purified/ nonpurified[a]	Fatty acid 16:0	18:0	18:1	18:2	18:3	Total	TG purity[b]
		μg/g Fresh leaf						
Unhardened sour orange	P	23	1	13	42	60	139	
Unhardened sour orange	NP	90	9	41	89	125	354	39.3
Hardened sour orange	P	382	43	209	1193	633	2460	
Hardened sour orange	NP	333	45	260	1235	648	2521	97.6
Hardened Valencia	P	106	11	30	238	287	672	
Hardened Valencia	NP	150	12	42	357	257	818	82.2

[a]Nonpurified values from Table 1 of reference 3.
[b]Purity based on total fatty acids in column 8.

TG molecular species increase under a cold hardening regime does, however, lay the groundwork for future studies in which changes in lipids of citrus leaves subjected to freeze regimes will be examined.

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A Comparison of the Stability of Oils from Brazil Nut, Para Rubber and Passion Fruit Seeds

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ABSTRACT

The oxidation at 46 C of oils from Brazil nut, *Bertholletia excelsa* H.B.K.—Lecythidaceae (BNO), and from seeds of Para rubber, *Hevea brasiliensis*—Euphorbiaceae (PRO), and passion fruit, *Passiflora edulis*—f. flavicarpa—Passifloraceae (PFO), was followed over 115 days through the measurement of peroxide, acidity values, refractive indices, combustion energies and infrared (IR) spectra. The addition of 3 ppm Cu^{2+} to PFO oil shortened the induction period by 12%. The oxidation of BNO and PRO exhibited first-order kinetics in the production of hydroperoxide (RO_2H), up to the maximum values of the concentration of RO_2H. On the other hand, the oxidation of PFO and PFO + Cu^{2+} displayed first-order kinetics at higher concentrations of RO_2H and possibly half-order kinetics at low hydroperoxide concentrations in the first 15 days. Therefore, the 3 oils studied and PFO + Cu^{2+} did not show the same stability pattern over the 115 days of the experiment. The application of kinetic data, a side from the other parameters, allows the definition of 2 different stability patterns. From 0-15 days the oxidation rates led to the following order of stability: PFO + Cu^{2+} < PFO < BNO < PRO. From the 15th day to the end of the period corresponding to the maximum concentration of RO_2H, the rate constants led to the pattern: PFO + Cu^{2+} < BNO < PFO < PRO. Considering the whole period of the experiment, the changes in viscosity and the values of the induction periods point toward the first-mentioned stability pattern, demonstrating that without kinetic data these 2 parameters are insufficient to determine such patterns.

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INTRODUCTION

Bertholletia excelsa H.B.K.—Lecythidaceae and *Hevea brasilliensis*—Euphorbiaceae are native species of the Brazilian Amazon area, although they are cultivated in other topical countries. The fruit of the *Bertholletia* furnishes a kernel known as Brazil nut that produces a unstable clear yellow oil (1). The oil yield is ca. 70% (1,2). The fruit of *H. brasiliensis* (Para rubber) furnishes seeds that produce a dark red oil, with a high acidity and an oil yield of ca. 50% (3). *Passiflora edulis* f. flavicarpa—Passifloraceae produces a fruit (passion fruit), whose seeds are considered as a by-product of this fruit, is being processed in many countries in order to obtain juice. The seeds furnish a pale yellow oil with an average yield of 20% (4).

Nutritive products containing fats and vegetable oils, when stored, may yield undesirable organoleptic properties as a result of oxidation reactions that produce hydroperoxides (RO_2H) as principal products (5,6). The presence of natural or synthetic antioxidants in the above materials

may delay or diminish the oxidation rate. On the other hand, this reaction is known to be calatyzed by contaminant metals, which may be present at low levels of concentration (5). The autoxidation reaction of a given oil depends on the extent of contact with air (degree and kind of stirring) and processing temperature; additionally, the oil yield is influenced by its history, i.e., geographical origin, extraction method and storage time (7).

We are initiating an integrated research program that involves the physicochemical characterization of vegetable fatty oils from oil-producing plants of the Brazilian Amazon area. The oils from Brazil nut (BNO) (2), Para rubber (PRO) seeds (3) and passion fruit (PFO) seeds (4) show differences in such common characteristics as fatty acid composition, color, refractive index, acid and iodine value. This led us to carry out an investigation of their stabilities, particularly oxidation at moderate temperature (46 C). We carried out measurements of combustion energy in addition to the more common methods of measuring oxidation. The results are given in this paper.

EXPERIMENTAL

Materials

The 3 crude oil samples and PFO + Cu^{2+} used in this work were: Brazil nut oil; Para rubber seed oil; Passion fruit seed oil and Passion fruit seed oil plus 3 ppm of Cu^{2+} (salt of Copper [II] derived from cyclohexanebutyric acid, obtained from Continental Oil Company, Bonca City, Oklahoma, Conostan Division). All the oils were extracted in our laboratory in a soxhlet derive, of stainless steel, with comercial hexane (obtained from B. Herzog, São Paulo, S.P. Brazil) after distillation of the latter in our laboratory. The Brazil nut and passion fruit seeds were obtained, respectively, from Usina Progresso S.A. and Indústria Alimentícia Gelar S.A., Belém, state of Pará. The Para rubber seeds were collected at the shore of the Moju river, near Moju town, state of Pará.

Oil Oxidation

Ca. 1 kg of each oil was oxidized at 46 ± 0.5 C inside a transparent glass container, (16 cm high; 17 cm diameter) inside an electric oven (internal dimensions 60 × 80 × 50 cm). Aliquots of 40 mL from each homogenized oil were taken and placed in a 50 mL transparent ampoule and maintained during the experiment at -10 C, in nitrogen.

METHODS

Viscosity

Ca. 5 mL of oil were placed in a Connon-Fenske type viscometer, built and calibrated in our laboratories. The kinematical viscosity was calculated as the average of 4 measurements (ASTM-445-65), which were carried out daily at 40 C, 50 C, 60 C, 70 C and 80 C in a thermostatic bath (accuracy ± 0.01 C) built by Indústria e Comércio de Aparelhos de Precisão Ltda., INCOMAP, R.J., Brasil.

Refraction Index

These indices were determined daily at 30 C, 40 C, 50 C, 60 C and 70 C, using an Abbé Refractometer (Carl Zeiss), coupled to a thermostatic bath (accuracy ± 0.01 C).

Peroxide value and acidity value were determined in duplicate using the AOCS Official Methods Cd 8-53 and Cd 3a-63.

Iodine value and fatty acid composition measurements were carried out in duplicate on the first day. The iodine index was obtained using the conventional method of Wijs (AOCS Official Method Cd 1-25). The fatty acid composition was determined from methyl esters. These esters have been prepared by Klan's (8) procedure and analyzed on a capillary column SE-SY of 30 cm length in a gas chromatograph (GC)/mass spectrometer (MS)/Finnigan computer, model 4021, system.

Combustion

The gross heat combustion of oils (ΔU_C) was determined at 25 C using a PARR calorimeter with an isothermal jacket, model 1341; a PARR oxygen bomb, model 1108; a PARR thermometer covering the range 19-35 C. The measurements were done following the procedure described in ASTM method D-240-76 (1980). The calorimeter equivalent energy, E = (10340.14 ± 16.5) JK^{-1} was obtained (9) from 6 measurements and the ΔU_C values of samples from duplicate measurements. The correction in ΔU_C for the sulfuric acid formation was carried out determining the sulfur content by a combination of the ASTM method D-129-64 (1968) and of Vogel's method (10). The sample was burned in presence of 20 mL of H_2O_2 (6%) and the washings from the bomb were concentrated at ca. 100 C to 50 mL and, by adding 5 mL of $BaClO_4$ (≃ 12%), a precipitate was formed that was then dissolved in 2 mL of NaOH (2%) plus 50 mL of EDTA 0.01 M. The sulfure content was obtained by titrating, under pH = 10, the EDTA excess with $MgCl_2$ 0.01 M (10).

IR Absorptions

The absorption in the IR region was measured in a Perkin-Elmer spectrophotometer, model 727B, using an oil film between the NaCl windows.

Metals

The estimate of iron and copper contents was done using HCl as an extraction agent, through a modification of Persmark and Toregard's (11) method and using a Zeiss atomic absorption spectrophotometer, model FMD 4. One hundred g of each oil (repeated 3 times) was mixed with 100 mL of HCl 3.5 M and magnetically stirred for 2 hours. The aqueous phase was separated and analyzed.

RESULTS AND DISCUSSION

Composition of the Sample Oils

The fatty acid composition and the iodine value are given in Table I. The PFO oil contains more unsaturated fatty acids than PRO and BNO oils. The PFO oil is also the richest in linoleic acid. In both PRO and BNO oils, oleic acid is the main component.

TABLE I

Characteristics of Oils from Para Rubber (PRO) Seed, Brazil Nut (BNO) and Passion Fruit (PFO) Seed

Characteristics	Samples		
	PRO	BNO	PFO
Iodine value (Wijs)	130.2	95.4	133.5
Fe, ppm	0.65	2.24	0.30
Cu, ppm	0.41	1.40	0.25
Fatty acid composition (100% basis)			
16:0	7.7	12.0	8.0
18:0	10.0	10.4	2.2
18:1	49.9	41.2	12.6
18:2	32.3	36.1	77.2
Unsaturated fatty acid (%)	82.2	77.3	89.8

Changes in the IR Spectra

Changes in IR spectra were interpreted as described by O'Connor (12). All samples exhibited an intensity enhancement in the hydroperoxide band, 3475 cm^{-1} (2.88 μm), an intensity decrease in the band at 3025 cm^{-1} (7.3 μm), associated with the substitution of hydrogen of the double bonds by other radicals, and an increase of a weak band at 990 cm^{-1} (10.3 μm) pointing to a *cis, trans* isomerization. The PRO oil displayed the least significant intensity enhancement at 3475 cm^{-1} (2.88 μm) and intensity decrease at 3025 cm^{-1} (7.3 μm).

Changes in Peroxide Value and Acid Value and Metal Analyses

In Figure 1, we give the changes in the peroxide values. The PFO oil displayed the fastest hydroperoxide formation in the early stages of oxidation; this can be related to its greater linoleic acid content (13). The initial peroxide values for all oils was between 20-30 meq/kg; this led us to conclude that they has already accquired some rancidity in the first days of the tests (14). This is probably the result of the oil-producing process, which was not controlled to prevent oxidation. The curves for BNO, PFO and PFO + Cu^{2+} oils showed the usual characteristics (15), with miximum peroxide values close to each other. The PRO oil showed low peroxide formation over the autoxidation period.

In Figure 2 are the curves of acidity change in the oils. As recognized before (16), we found among the products of lipid oxidation the presence of acids which result by the fission of fatty hydroperoxide. Up to nearly 60 days of storage, the acidity of all oils ramained fairly constant. The PRO oil having a high initial acidity, showed small enhancement compared with the other oils, after being stored 115 days. The lower initial and final acidity of PFO + Cu^{2+} oil can be related to dilution effects caused by the addition of Cu^{2+} dissolved in a mineral oil. The greatest free acidity yield was displayed by BNO and PFO oils. By the end of the storage period, BNO and PFO showed enhancements of 7.2 units and 6.4 units.

Table I gives the results of metal analysis (copper and iron), indicating that all oils contained metals before the oxidation process. These concentration values may have arisen from 2 distinct sources, contamination during extraction and assimilation of metals by the plant. Iron and copper, like other transition metals, are oxidation catalysts (5) and thus, in all the oils examined, there can be some catalytic activity from metallic ions. For this reason, 3 ppm of Cu^{2+} were added to the PFO oil, giving the PFO + Cu^{2+} oil and, as can be seen in Figure 1, this was the oil with the fastest hydroperoxide formation. The PRO oil showed an unexpected behavior, considering its fatty acid composition and metallic ion content; this may be related to the effect of some natural antioxidant in the oil, which would compensate for the catalytic action of metals.

Changes in Combustion Energy

In Table II are the values of the gross heat of combustion. Keffler (17) has determined the heat of combustion for elaidic acid, oleic acid and methylic esters from these acids; this author also observed that the heat of combustion decreases with time. According to Keffler (17), this decrease is possibly caused by an equilibrium being established between the monomolecular oleic acid and a stable or metastable polymer. In this work, no major change in heat combustion values for each oil were observed during 50 days of storage. In the final period of storage, a decrease in ΔU_C did occur, leading to the following percentage changes from the initial value: 4.8%, 8.4%, 7.2% and 5.7% for BNO,

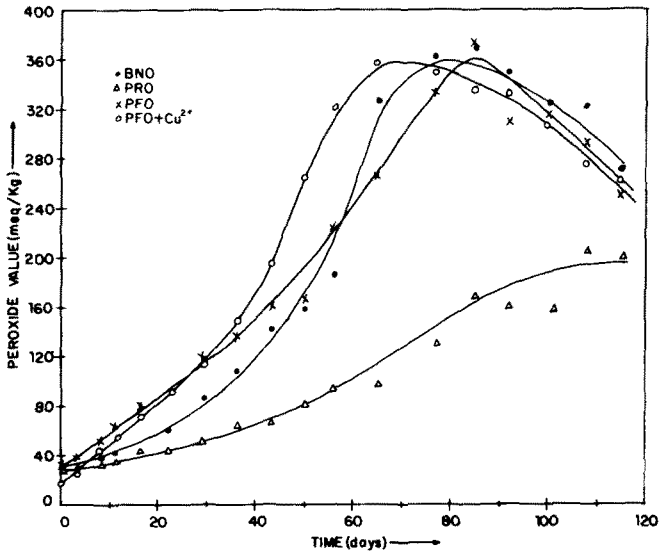

FIG. 1. Changes in the peroxide values of oils from Para rubber (PRO) seed, Brazil nut (BNO), passion fruit (PFO) seed and passion fruit seed plus Cu^{2+} (PFO + Cu^{2+}).

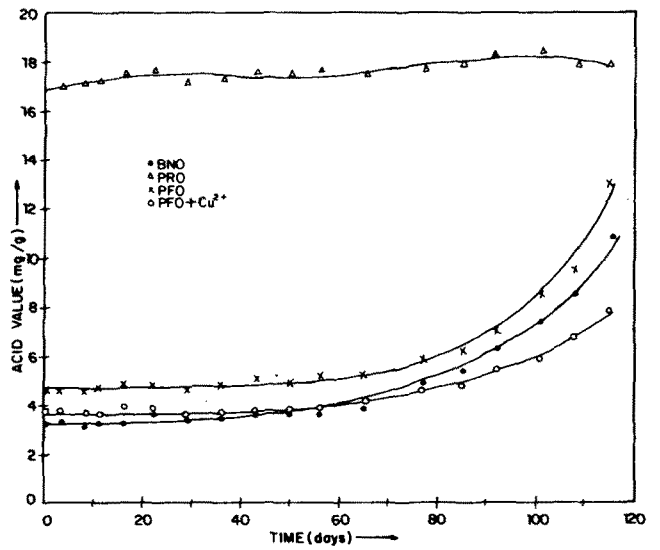

FIG. 2. Changes in acidity values of oils from Para rubber (PRO) seed, Brazil nut (BNO), passion fruit (PFO) seed and passion fruit seed plus Cu^{2+} (PFO + Cu^{2+}).

TABLE II

Values of Gross Heat Combustion ($\Delta Uc/KJg^{-1}$) of Oils from Para Rubber (PRO) Seeds, Brazil Nut (BNO), Passion Fruit (PFO) Seed and Passion Fruit Seed plus Cu^{2+} (PFO + Cu^{2+})

Storage at 46 C (days)	Samples			
	PRO	BNO	PFO	PFO + Cu^{2+}
0	40.101	39.955	40.130	39.921
50	39.715	30.733	39.533	39.577
115	38.168	36.601	37.222	37.630

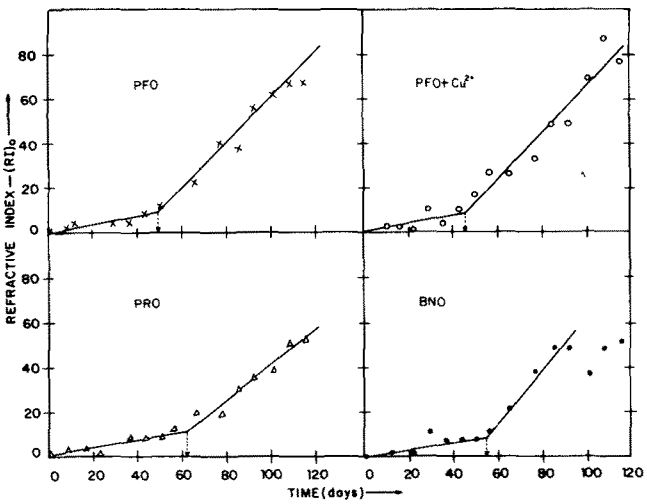

FIG. 3. Changes in refractive indices of oils, measured at 40 C, from Para rubber seed (PRO), Brazil nut (BNO), passion fruit seed (PFO) and passion fruit seed + Cu^{2+} (PFO + Cu^{2+}). RI_0 was the refractive index at day zero. The arrows indicate the end of the induction period.

PFO, PFO + Cu^{2+} and PRO oils. As expected, because of its greater unsaturation, the PFO oil gave the largest decrease in ΔU_C; on the other hand, the catalytic effect of Cu^{2+} was practically nothing. The observed changes may also be attributed to the formation of polymeric species (17) that lower the temperature change during combustion. Data from Table II do not establish a stability pattern.

Changes in Refractive Index and Viscosity

Changes in refractive indices, measured at 40 C, as a function of time are given in Figure 3. We observed, after measurements at several temperatures, that the highest values were reached during the oxidation period for the PFO oil, followed by the values of PRO oil and, finally, BNO oil. The values of the refractive indices for day zero, RI_0, at 40 C, were 1.4674, 1.4672, 1.4670 and 1.4631 for PFO, PFO + Cu^{2+}, PRO and BNO. The presence of unsaturation, conjugated double bonds (18) and carbonyls (15) causes an increase in the refractive index. The conjugation is known to be lowered during oxidation (7) but conversely, more carbonyls are formed, providing an overall increase in refractive indices of the oil as oxidation proceeds. Our data agree with these facts. The induction periods were obtained from the changes in refractive index va time (15,19). For each temperature measurement (30 C, 40 C, 50 C, 60 C and 70 C), the induction period was determined. Small random fluctuations characterized each value. The values indicated in Table III are averages and are very close to the ones indicated by arrows in Figure 3. The induction period for PFO + Cu^{2+} was observed to be 12% shorter than the corresponding period for PFO.

The behavior of the viscosity, a function of temperature and time for PRO, can be seen in Figure 4. The arrow in this figure indicates the induction period, from which were obtained, by interpolation, the viscosity values corresponding to each test temperature. The same procedure was employed with the other oils, whose behaviors were very similar to PRO. The viscosity values for each oil for the first day, the induction period and the last day are given in Table II. In this table, the activation energies obtained from Arrhenius plot by linear regression from data of viscosity vs temperature are presented. Also, the catalytic effect of Cu^{2+} in the oxidation of PFO produced a greater difference in activation energies (20.2 units) between the last and the first days. According to several authors (7,15), the viscosity increase during the oxidation involves molecular dimerization and polymerization; so, according to the data of Table III, the rate of oxidative polimerization is in the order: PFO + Cu^{2+} > PFO > BNO > PRO.

TABLE III

Kinematic Viscosity (cSt) vs Temperature and Time, Activation Energy ($KJmol^{-1}$) of oils from Para Rubber (PRO) Seed, Brazil Nut (BNO), Passion Fruit (PFO) Seed and Passion Fruit Seed + Cu^{2+} (PFO + Cu^{2+})

	Samples (days)											
	PRO			BNO			PFO			PFO + Cu^{2+}		
t/c	0	63[a]	115	0	54[a]	115	0	50[a]	115	0	44[a]	115
40	37.0	44	190.1	36.1	43	218.2	31.1	36	698.1	29.4	38	723.0
50	27.8	32	135	24.8	30	146.1	22.6	26	403.3	23.1	26	418.5
60	21.5	24	88.2	20.5	23	88.0	17.4	18	242.8	17.8	20	243.9
70	16.6	18	60.3	15.8	16	60.1	13.6	14	157.1	13.9	13	163.0
80	13.6	15	43.0	12.3	14	44.3	10.9	11	106.7	10.5	11	108.0
Activation energy	23.41(1.00)[b]	24.7	34.8(0.98)	24.3(0.99)	26.8	37.6(0.99)	24.9(0.99)	27.9	43.4(0.99)	23.9(0.99)	29.4	44.1(0.99)

[a]Induction periods obtained from curves of refractive index vs time.
[b]The figures in parentheses are the correlation coefficients of the corresponding Arrhenius plots. All viscosity values for induction period were obtained by extrapolation as explained in the text.

Rates of Oxidation

The oxidation reaction of BNO and PRO oils, at 46 C, was of the first order in the production of RO_2H, from the early stages of oxidation to the maximum concentration of RO_2H. On the other hand, the oxidation of PFO and PFO + Cu^{2+} oils showed a different behavior, curving toward the origin in the plot of peroxide value vs time at low peroxide concentration range (Fig. 5). These 2 distinct behaviors have already been described in the literature. Bolland (20) studied the oxidation of ethyl linoleate at 45 C and 100 mm of oxygen pressure and found that the reaction was of the first order in RO_2H; Lau (21) observed the same order for the oxidation of corn oil at 28 C, randomized corn oil and methyl esters of corn oil. Bateman (22) mentions the autoxidation of cyclohexene at 45 C and 728 mm of oxygen pressure was first order in the production of RO_2H, and the autoxidation of tetralin, at 75 C and 180 nm and of methylcyclohexene, at 65 C and 350 mm, ½ order during the early atages of oxidation (low concentration of RO_2H) and first order at high concentrations. The oxidations of PFO and (PFO + Cu^{2+}) have rate curves similar to the second case mentioned above; they are of first order at high hydroperoxide concentrations and possibly ½ order at low concentrations (Fig. 5). Table IV are given the first-order rate constants, calculated by linear regression using the data relative to the linear regions of the curves. In this table are also given the correlation coefficients r and the half-lives. The BNO oxidized 1.8 times faster than PRO.

Comparison of Oil Stabilities

If the peroxide values of the crude oils were zero meg/kg, the induction period would be longer than the values given in Table III, and consequently the changes in other properties studied in the present work would be different. However, we think that these changes would be proportional to the ones we have observed.

Analysis of Figure 5 and the data of Table IV suggests that much of these data are oxidation sensitive and from the observed kinetic behavior 2 different periods must be considered for stability comparisons: from zero to fifteen days and from fifteen days to the time corresponding to the maximum RO_2H concentration for all oils. In the first case, oxidation rates were obtained the following order: (PFO + Cu^{2+})<PFO<BNO<PRO, and in the second case, using the rate constants and corresponding half-lives: (PFO + Cu^{2+})<BNO<PFO<PRO. On the other hand, using only changes in 3 parameters, viz., viscosity, activation energy and induction period (Table III), over the the entire storage period, one would conclude a stability order of: (PFO + Cu^{2+})<PFO<BNO<PRO. If one also considers the kinetic data the latter stability pattern is maintained for BNO and PRO. However, for PFO + Cu^{2+} and PFO, no such agreement is found. Using the 3 mentioned indicators independently from kinetic data for the prediction of a particular oil stability is inadvisable. In fact, this change in reaction order during oxidation for those last oils (Fig. 5) has prevented us from obtaining only a single stability pattern for the 4 systems.

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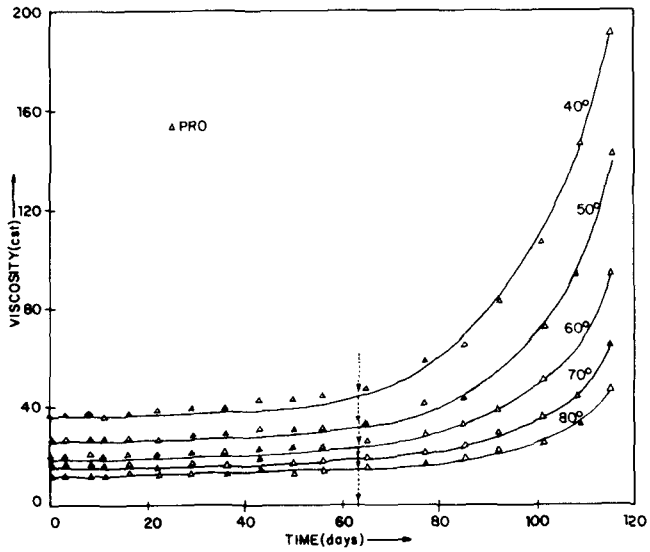

FIG. 4. Changes in the viscosity of oil from Para rubber (PRO) seed. The arrows indicate the end of the induction period obtained from the curves of refractive index vs time.

TABLE IV

First-Order Rate Constant (K) Half-lives Formation of Hydroperoxide of the Oils from Para Rubber (PRO) Seed, (BNO), Passion Fruit (PFO) Seed and Passion Fruit Seed + Cu^{2+} (PFO + Cu^{2+})

Samples	K X 10^3 ($days^{-1}$)	r[a]	Half-lives (days)
PRO	8.6	0.99	80
BNO	15.3	0.99	45
PFO	9.9	0.99	69
PFO + Cu^{2+}	16.1	0.99	42

[a]Correlation coefficient.

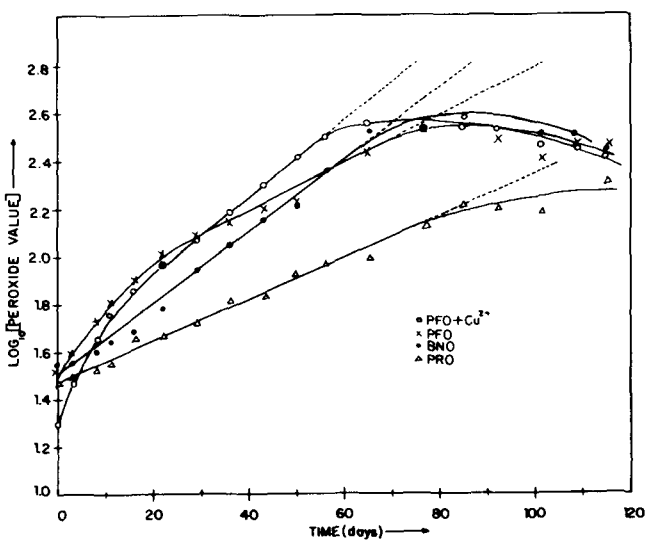

FIG. 5. Plot of logarithm of peroxide values vs time for the oils from Para rubber (PRO) seed, Brazil nut (BNO), passion fruit (PFO) seed and passion fruit seed plus Cu^{2+} (PFO + Cu^{2+}).

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Elucidation of the Chemical Structure of a Novel Antioxidant, Rosmaridiphenol, Isolated from Rosemary

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ABSTRACT

A novel antioxidant compound has been isolated and identified from the leaves of the *Rosmarinus officinalis L.* The compound, named rosmaridiphenol, is a diphenolic diterpene. When tested in lard, the antioxidant activity of this compound was superior to BHA. Structural elucidation of rosmaridiphenol was accomplished by infrared spectroscopy (IR), mass spectroscopy (MS), ^{1}H-NMR (nuclear magnetic resonance) and ^{13}C-NMR spectroscopy.

INTRODUCTION

In the 1950's, Chipault and coworkers (1-3) evaluated the antioxygenic properties of several herbs. Although these tests were performed using a variety of fat products, 2 herbs, rosemary (*Rosmarinus officinalis L.*) and sage (*Salvia officinalis L.*) consistantly demonstrated superior antioxidative effects.

Over the years, several reports have appeared on the preparation of rosemary extracts, which were very effective in retarding lipid autoxiation (4-7).

In addition to the production of extracts, several studies have been aimed at isolating and identifying active antioxidant compounds in rosemary. In 1964, Brieskorn et al. (8) isolated a phenolic diterpene, carnosol, from rosemary leaves. Later, Wu et al. (9), using a different isolation method, also identified carnosol from the leaves of the *Rosmarinus officinalis L.* plant. They reported that when carnosol was added to lard, its antioxidative effectiveness was comparable to BHT (9). Recently, Inatani et al. (10) isolated another antioxidant compound, rosmanol, from the leaves of the same plant. Rosmanol was also a phenolic diterpene and possessed a structure closely related to that of carnosol. In a subsequent study, Inatani et al. (11) reported that rosmanol was a fine antioxidant in several fat substrates with activity similar to that of carnosol.

The present paper reports the isolation and characterization of a new antioxidant, rosmaridiphenol, from rosemary leaves.

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EXPERIMENTAL

Isolation of the New Antioxidative Compound

A rosemary antioxidant extract was obtained from dried, ground rosemary leaves following a procedure described by Wu et al. (9). Following a vacuum steam distillation process, this extract was fractionated using a 5 cm × 122 cm glass column packed with activated silicic acid. Activation of this adsorbent was accomplished by a procedure set forth by Sahasrabudhe and Chapman (12). The column was eluted by step-by-step gradient using 100% hexane as the initial eluent and then employing the following solutions of diethyl ether in hexane (E/H): 5% E/H, 10% E/H, 15% E/H, 25% E/H, 50% E/H, and 75% E/H. The final eluent of this separation was 100% methanol. A total of 15 fractions resulted from this elution pattern.

Spectroscopic Procedures

The infrared (IR) spectrum of this antioxidant compound was obtained using a KBr pellet on a Beckman Acculab 4 Infrared Spectrophotometer. A mass spectrum (MS) was acquired using a duPont 21-490 Mass Spectrometer. The source temperature was held at 200 C with the ionization voltage at 70 eV. All of the proton and carbon-13 NMR (nuclear magnetic spectra) spectra was obtained using a Bruker WM-250 NMR Spectrometer. Quantitative elemental analysis of carbon and hydrogen was performed by Galbraith Laboratories, Inc., Knoxville, TN.

Antioxidant Activity Analysis

The antioxidant activity of each compound tested was based on its ability to prevent the formation of peroxides in prime steam lard samples. The samples were kept at 60 C without light for 4 weeks. Peroxide values were determined by Official Method Cd 8-53 of the American Oil Chemists' Society (13).