Technical

Use of a Gas Chromatographic Reactor to Study Lipid Photooxidation

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ABSTRACT

A gas chromatographic reactor can be used to investigate the prooxidant activities of different light sources on polyunsaturated lipids, including linoleic acid and safflower oil. The rate of oxidation was followed from the early stages of oxygen uptake, using a thermal conductivity detector (TCD). The experiments were made at low O_2 concentrations and at a broad range of temperatures. To monitor the effect of light on the oxidation of linoleic acid, chlorophyll and methylene blue were used as sensitizers, and β-carotene was used as a reported inhibitor of oxidation. The simultaneous measurements of oxygen uptake and volatiles formation were also monitored using a TCD and flame ionization detector (FID). The method is presently being developed as a technique to predict the stability of fatty foods packaged in transparent or translucent materials.

INTRODUCTION

Oxidation of fresh or processed foods is mainly associated with the unsaturated moieties of the lipid portion, occurring by enzyme-catalyzed oxidation, autoxidation (self-catalyzed, free radical chain reactions), or photooxidation (1-3). Most packaged foods in supermarkets are exposed to some type of light, and are thus subject to photooxidation as well as autoxidation. The degree of photooxidation is influenced by the spectral emission of the light source and the transmission, absorption, and reflectance characteristics of the package and the product. Unsaturated fatty acids by themselves do not absorb visible light and normally are not subject to direct oxidation by ultraviolet (UV) light; however, oils and fatty foods do undergo accelerated oxidation when exposed to daylight or artificial lights. This is due to impurities present in vegetable oils and to colored substances in fatty foods which absorb light and thus may contribute to photosensitization (4-7).

The mechanism of photooxidation as a pathway for the production of hydroperoxides differs from that of autoxidation. In the latter (8), the initiation stage, represented by the lag phase of a measured reaction, includes the formation of the first hydroperoxides. The ensuing propagation phase involves the breakdown of these hydroperoxides to form free radicals, which further generate their own formation in an autocatalytic, chain reaction. The initiation stage of autoxidation has been considered of principle importance in determining the onset of rancidity in fatty foods (1) because it is during the early stages of oxidation that small molecular weight compounds causing off-flavors are formed. Off-flavors may arise from fats and oils oxidized to levels less than 0.2 ppm by weight (1).

In the photooxidation of lipids, the reaction is apparently not autocatalytic: the quantity of hydroperoxides formed is proportional to the total amount of light absorbed (2), and α-tocopherol, which interrupts chain reactions in the autoxidation mechanism, is ineffective in inhibiting photooxidation (2,9). Also in contrast to autoxidation, the hydroperoxides formed during photooxidation may be unconjugated (3). As they are formed, they undergo further oxidation by light to form free radicals (7,10), yet this does not give autocatalytic character to the photochemical oxidation. Pigments and dyes such as chlorophyll, methylene blue, and erythrosine (4,5,7) may act as photooxidation sensitizers by transferring their absorbed light energy to either molecular oxygen or a substrate (lipid) which then becomes a reactive intermediate (11). If an excited sensitizer reacts with molecular oxygen (relatively unreactive in its ground triplet state), singlet oxygen may be formed. Singlet oxygen is a reactive intermediate in the photooxidation of lipids and further, its participation as the primary source for the original hydroperoxide formation in the initiation of fatty acid autoxidation has been suggested (7,11).

Lipid oxidation can be measured by objective and subjective methods. Although widely used, subjective methods of assessing the stability of prepared foods are time-consuming, and taste panels are difficult to maintain. When objectively measured, levels of peroxide decomposition products and final reaction products are usually detectable only after the initiation of off-flavors in prepared foods (12). Gray (13) has commented on the need for a chemical method which correlates well with changes in organoleptic properties of oxidized lipids throughout the entire course of oxidation. The main objective of this paper is to introduce such a method. Additionally, the method may aid the theoretical study of lipid oxidation. The apparatus involved is a gas chromatographic reactor (GCR), which utilizes the principles of inverse gas chromatography (IGC). IGC is based on the interaction between a stationary phase ("unknown substrate") and a reactant ("known molecular probe") in the mobile phase (carrier gas, or solvent in HPLC). For a more detailed description of IGC theory, the reader is referred elsewhere (14).

The method being introduced involves continuous and simultaneous measurement of oxygen uptake and volatiles formation during a dynamic test monitored from the initial stages of lipid oxidation, under different light sources. The effects of sensitizers (chlorophyll and methylene blue) and of β-carotene as a quencher of singlet oxygen (5,9,15) during the photooxidation of linoleic acid and safflower oil were also investigated. Additionally, the efficiency of packaging materials as light barriers was studied.

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EXPERIMENTAL PROCEDURES

Apparatus

Figure 1 is a schematic diagram of the GCR used for the photooxidation experiments. The main parts of the GCR include the reactor (oxidation chamber), the condenser or trap (dry ice as the cooling medium), the detectors, the strip chart recorder, and an optional humidifier. The light sources used were a warm white fluorescent lamp (20 W), an incandescent lamp (40 W), and a "black light" (20 W–12/BLB). "Black light," a trademark of the General Electric Co., emits longer-wavelength UV rays, in the 315-380 nm range (16). More detail about the apparatus may be obtained elsewhere (17).

Materials

Purified linoleic acid, β-carotene, and methylene blue were obtained from Fisher Scientific Co., Springfield, NJ. Safflower oil (Hollywood brand, cold-pressed, no additives) was purchased from a local supermarket. Glass wool (borosilicate glass, free of heavy metals, Pyrex brand) and cotton, used as support material for the fatty sample packed in a quartz reaction column, were also obtained from Fisher Scientific. Chlorophyll was acetone-ether extracted from spinach leaves, not purified. Packaging materials (2 mils thickness) were supplied by ICI America, Inc., Stamford, CT.

Test Procedures

The sample (linoleic acid or safflower oil) was packed into a quartz column (30 cm length, 1 cm id) which contained cotton or glass wool as the inert support. The ratio of sample to inert support was 1:2 (w/w). Experimental tests were carried out isothermally throughout the entire reaction. Tests were begun only after the packed oxidation column had reached thermal equilibrium, i.e., after conditioning for ca. 1 hr before introduction of the reagent (oxygen). The total pressure for most of the oxidation isotherms was ca. 765 mm Hg (helium plus oxygen). Oxygen partial pressure was calculated by considering its mole fraction, given by the thermal conductivity detector (TCD) response and the total pressure as related by Dalton's Law.

When only oxygen uptake was being measured, the volatiles formed during oxidation were condensed at about -78 C by passing the carrier gas leaving the reactor through a trap containing dry ice and acetone as the cooling medium. However, when both oxygen uptake and volatiles were monitored, the carrier gas leaving the reactor was automatically sampled and analyzed by two detectors. The TCD was used to measure oxygen uptake and the flame ionization detector (FID) was used to measure total volatiles. Oxygen uptake was calculated directly from chromatograms using pure oxygen bypassing the reactor column as a standard; i.e., the oxygen absorbed was calculated by relating the area under the curve to the calibration area obtained when oxygen bypassed the reactor column (Fig. 2).

RESULTS AND DISCUSSION

The effect of light source on the photoxidation of linoleic acid as measured with the GCR method is shown in Figure 3. The rate of oxidation increased in the following order: control (dark) < fluorescent < incandescent < UV (black light) and showed a ratio of 1:2:3:6, respectively. The light-catalyzed oxidation of refined soybean oil has been demonstrated by Radtke et al. (18) to be much more dependent on the wavelength emitted and absorbed by the oil than the energy content of the emitted light. Because edible oils become more quickly oxidized using shorter wavelength light (19) and the quantity of hydroperoxides formed during photooxidation is directly proportional to the amount of light absorbed (2), greatest photooxidation of the linoleic acid sample in this study was to be expected using the black light. Both fluorescent and incandescent lamps do emit some UV light, but, of the total radiation in the visible and UV regions (wavelengths shorter than 760

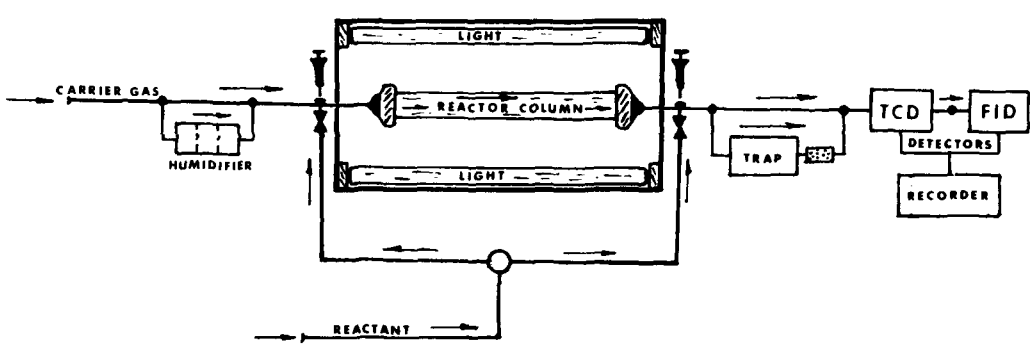

FIG. 1. Diagram of the gas chromatographic reactor (GCR) used for oxidation studies (17). U.S. Patent pending by J.A.F. Faria, 1981.

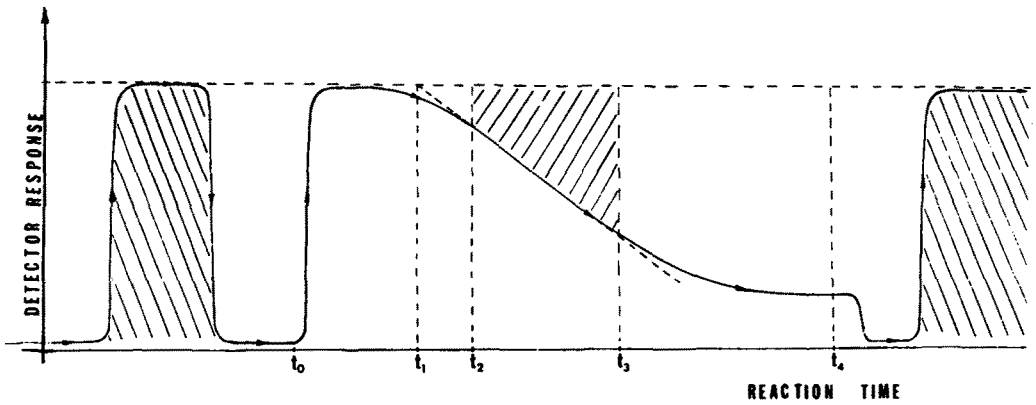

FIG. 2. Typical chromatogram obtained during lipid oxidation, using the GCR method.

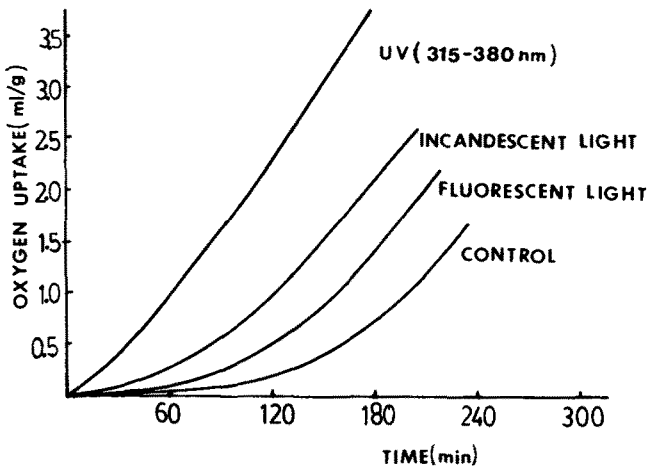

FIG. 3. Effect of light source on the oxidation of linoleic acid at 65 C and 55 mm Hg of O_2. Control = dark; UV = black light.

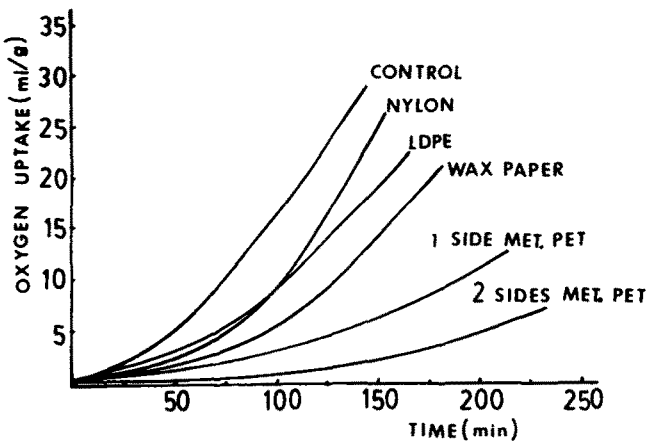

FIG. 4. Performance of packaging materials as light barriers during the oxidation of safflower oil. Light source = black light.

nm), a tungsten filament at 3,000 K emits about 1.4% as UV below 380 nm (16), and fluorescent lamps radiate only about 0.5% near the UV (3). In Figure 3, the control followed autoxidation in the dark; it can be seen that the lag phase (initiation stage) became less prominent as greater photooxidative activity occurred. With the UV light, the rate of oxidation appeared almost entirely linear. This affirms that photooxidation is of a noncatalytic nature; there is no build-up of reactive intermediates which decompose into reactive species, as observed in the autoxidation of lipids.

Figure 4 shows the performance of different packaging materials as light barriers during the oxidation of safflower oil, using the GCR method. For such studies, the clear reactor column, containing the oil over cotton support, was covered with one layer of the packaging material and held under black light. The degree of protection against photooxidation followed the order: two-side metallized polyester > one-side metallized polyester > wax paper > low density polyethylene > nylon > control. The results correlated well with the spectral transmissions of the packaging materials in the black light range, as shown in Figure 5. This approach for evaluating packaging materials for light-sensitive foods has the main advantage of being fast and reproducible compared to conventional methods, such as peroxide value determination and iodometry (20,21).

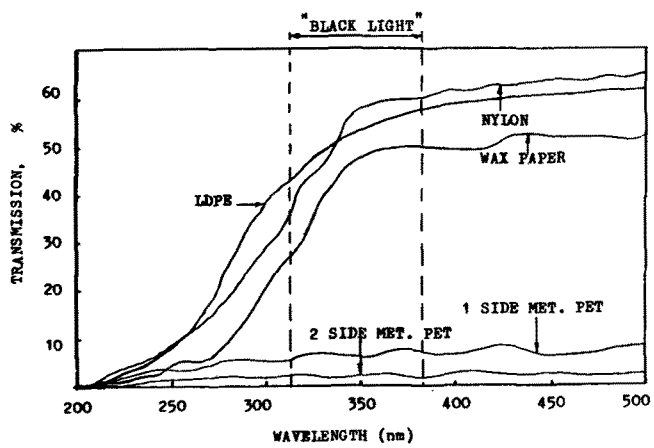

FIG. 5. Transmission spectra of different packaging materials.

Use of the GCR method as a valid means to study dye-sensitive photooxidations of organic substrates was also investigated. Chlorophyll has been considered among the most effective sensitizers (5), i.e., to enhance the generation of singlet oxygen (7). The effect of its addition to linoleic

acid exposed to black light, as shown in Figure 6, might have been that of a sensitizer; however, the observed increase in oxygen uptake could also have been due to the degradation of the pigment itself, as it was found to be bleached after the reaction. Although methylene blue is also recognized as a photosensitizer (5,11), its inclusion with linoleic acid decreased oxygen uptake (Fig. 7). This was probably due to reflectance of the light employed, since black light approaches the blue wavelength region.

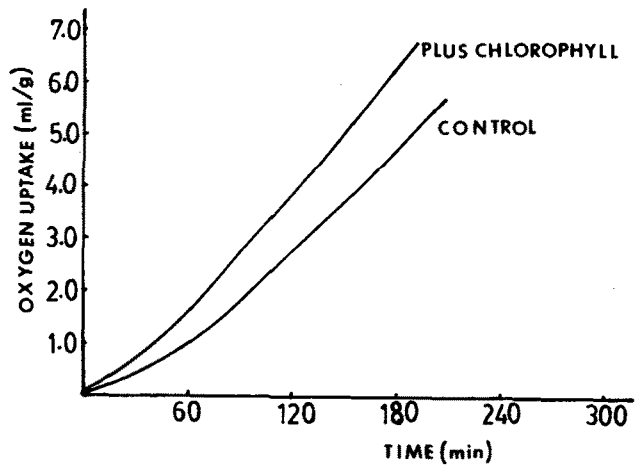

FIG. 6. Effect of chlorophyll on the oxidation of linoleic acid under black light. 40 C, 55 mm Hg O_2.

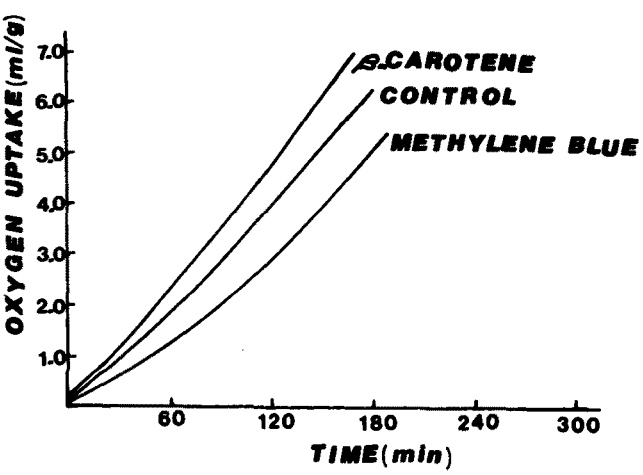

FIG. 7. Effect of methylene blue and β-carotene on the oxidation of linoleic acid under black light. 40 C, 55 mm Hg O_2.

The absorbance spectra of methylene blue and of β-carotene do not overlap, and the latter has been considered to work in a manner opposite to that of photosensitizers: β-carotene is a quencher of singlet oxygen (9,15). Yet β-carotene added to linoleic acid increased oxygen uptake under the given experimental conditions (Fig. 7). According to El-Tinay and Chichester (22), the degradation of β-carotene by oxygen alone may occur through an activated dipole association complex by overall zero-order kinetics. This reaction, like lipid photooxidation, is not autocatalytic. The activation energy for β-carotene oxidation is 10.2 kcal/mol, 5 kcal/mol less than that for linoleic acid oxidation (22); oxidation of the pigment in addition to that of linoleic acid might explain the increase in oxygen uptake when β-carotene was added to the control (linoleic acid), as shown in Figure 7; however, this increase may not have been merely additive. β-Carotene degradation by oxygen alone is not autocatalytic, but it may have some free radical character, as El-Tinay and Chichester (22) reported a rate-enhancement and a difference in the reaction mechanism of β-carotene oxidation in the presence of free radical initiators. When free radical inhibitors were used, these researchers observed a lag period, indicative of the initiation stage of a free radical mechanism. Moreover, they reported the enhancement of β-carotene oxidation upon the addition of stearic acid to β-carotene in the presence of a free radical initiator. Increases in the rate of oxidation of fats containing β-carotene have been reported by Emanuel and Lyaskovskaya (23), but current studies focus on the role of carotenes as quenchers of singlet oxygen (9,15) in acting as photoprotectors. It nevertheless seems plausible to suggest that during the oxidative degradation of β-carotene with a polyunsaturated lipid undergoing photooxidative or autoxidative degradation, carotenes might also participate in free radical reactions, possibly enhancing the degradation rates of both. The use of this method to investigate the efficiency of β-carotene as a singlet oxygen quencher in a degrading lipid system, in combination with different light sources and packaging materials, might be a subject for further studies.

The GCR method was additionally used to investigate the photooxidation of polyunsaturated lipids by the simultaneous monitoring of oxygen uptake and formed volatiles, as shown in Figure 8. A profile of linoleic acid photooxida-

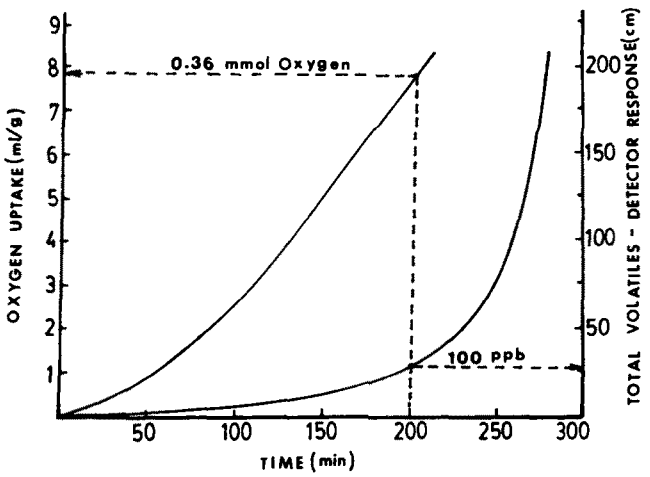

FIG. 8. Profile of oxygen uptake and volatiles formation during linoleic acid oxidation under black light. 55 C, 55 mm Hg O_2. Acetone was used as the standard for calculating volatiles formation.

tion was followed during the initial stages. After 4 hr of dynamic oxidation, 1 g linoleic acid reacted with about 8 ml O_2. On a molar basis, this corresponded to 3.6 mmol linoleic acid and 0.36 mmol O_2; hence, the sample was about 10% oxidized. Total volatiles at this time had reached about 100 ppb (calibrated with acetone as the internal standard). Considering that, of the total volatiles arising from the breakdown of linoleic acid, 66 mol% accounts for hexanal (24), the sample at this point would have been unacceptable on a consumer market level based on an olfactory perception threshhold for some lipid-containing foods. The drastic change in the rate of total volatiles

formed as decomposition shifted from a monomolecular to bimolecular basis (1) may be attributed to the photolysis of hydroperoxides by UV light (10). Therefore, light is harmful to fatty foods packaged in transparent materials not only as an initiator for photooxidation reactions, but also during the propagation step by enhancing free radical formation ($ROOH \xrightarrow{UV} RO\cdot + HO\cdot$) and in the onset of objectionable off-flavors.

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REFERENCES

1. Labuza, T.R., CRC Crit. Rev. Food Technol. 2:355 (1972).
2. Lundberg, W.O., in Symposium on Foods: Lipids and Their Oxidations, edited by H.W. Schultz, E.A. Day and R.O. Sinnhuber, AVI, Westport, CT, 1962, p. 31.
3. Sattar, A., and J.M. deMan, CRC Crit. Rev. Food Sci. Nutr. 47:13 (1975).
4. Chan, H.W.-S., JAOCS 54:100 (1976).
5. Foote, C.S., and R.W. Denny, J. Am. Chem. Soc. 90:22 (1968).
6. Korycka-Dahl, M.B., and T. Richardson, CRC Crit. Rev. Food Sci. Technol. 84:209 (1978).
7. Rawls, H.R., and P.J. Van Santen, JAOCS 47:121 (1970).
8. Bateman, L., Q. Rev. 8:147 (1954).
9. Carlsson, D.J., T. Suprunchuk and D.M. Wiles, Can. J. Chem. 52:3728 (1954).
10. Hiatt, R.R., CRC Crit. Rev. Food Sci. Nutr. 47:1 (1975).
11. Foote, C.S., Acc. Chem. Res. 1:104 (1968).
12. Erickson, D.R., and R.H. Bowers, in Objective Methods for Food Evaluation, National Academy of Science, Washington, DC, 1976, p. 133.
13. Gray, J.I., JAOCS 55:540 (1958).
14. Senich, G.A., Chemtech 11:360 (1981).
15. Rodgers, M.A.J., and A.L. Bates, Photochem. Photobiol. 31:533 (1980).
16. General Electric Company, literature upon request (1968).
17. Faria, J.A.F., Ph.D. Thesis, Rutgers University, Univ. Microfilms, Ann Arbor, MI (1980).
18. Radtke, R., P. Smits and R. Heiss, Fette, Seifen, Anstrichm. 72:497 (1970).
19. Paul, G., R. Heiss, K. Becker and R. Radtke, Ibid. 74:120, 359 (1972).
20. Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1 and 2, 3rd edn., AOCS, Champaign, IL, 1964 (revised to 1972), Method Cd 8-53.
21. Wills, E.D., Biochem. J. 123:983 (1971).
22. El-Tinay, A.H., and C.O. Chichester, J. Org. Chem. 35:2290 (1970).
23. Emanuel, N.M., and Y.N. Lyaskovskaya, in The Inhibition of Fat Oxidation Processes, Pergamon Press, New York, NY, 1967, p. 105.
24. Schieberle, P., and W. Grosch, JAOCS 58:602 (1981).

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