Fate of Propyl Gallate and Diphosphatidylethanolamine in Lard during Autoxidation at 120 °C

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The breakdown of propyl gallate and diphosphatidylethanolamine, in lard, during autoxidation at 120 °C, has been studied. When propyl gallate is present alone concentration gradually falls during the accelerated stability test. When propyl gallate can no longer be detected, the end of the induction period is reached. When diphosphatidylethanolamine is simultaneously present, the "life" of the propyl gallate is extended and longer induction periods are observed.

INTRODUCTION

Synergists (secondary antioxidants) are compounds that show little or no antioxidant efficiency per se but enhance the efficiency of primary antioxidants present in the system. Phospholipids, in particular diphosphatidylethanolamine (DPE), exert marked synergistic effects (Hudson and Lewis, 1983; Hudson and Ghavami, 1984) with a wide range of primary antioxidants (Dziedzic and Hudson, 1984a). The ratio of synergist to primary antioxidant is important: the higher the ratio, the greater the synergistic effect. The reaction is also temperature dependant, being at its maximum in the 100–140 °C range and probably absent below 80 °C (Dziedzic and Hudson, 1984a).

Our interest has centered around the mechanism of the synergistic effect. To this end, we were interested in following the fate of a primary antioxidant in the presence and absence of a synergist and of the synergist itself, in the hope of obtaining information to assist in the elucidation of the mechanism of the reaction.

MATERIALS AND METHODS

Freshly dried rendered lard, free from additives was kindly donated by Bowyers Ltd., Trowbridge. sn-Dipalmitoyl-DL-α-phosphatidylethanolamine (98%; DPE) was purchased from Sigma Ltd., London. The lard had a total phospholipid content of 0.03%.

Induction Periods (IP). Induction periods were determined in the Automated Rancimat (Metrohm AG, CH-9100 Herisau, Switzerland) at 120 °C. In this test, a continuous airstream is passed through the heated sample and the volatiles are absorbed in a conductivity cell. Conductivities are continuously monitored until a sudden rise signifies the end of the IP.

Our method employed a minor modification to the recommended procedure. The Rancimat test usually employs a fat sample of 2–5 g. In our experiments, to minimize any variation, samples (25 g) containing 0.02% added propyl gallate (PG) were heated in two Rancimat tubes, to one of which was added 0.3% DPE. At regular intervals 1.5-g subsamples were withdrawn from both tubes. These were used for the assays of PG and DPE.

Assay of Propyl Gallate (PG) by HPLC. Lard (1 g) was weighed into a glass stoppered test tube that was placed in an oven at 70 °C until the lard melted. Methanol (about 9.5 mL) was added and the mixture vigorously mixed on a vortex mixer. This was then cooled in a refrigerator until the lard had solidified. The methanol supernatant was filtered through Whatman No. 4 paper into a 10-mL volumetric flask and made to volume. In-

jections of 5 or 10 μL of extract were made. A range of standard solutions of PG in methanol was also prepared.

A Gilson Model 302 pump and Data Master integrator (Anachem Ltd., Luton, U.K.) and a fixed-wavelength UV monitor, Model 750/11, were used (HPLC Technology, Macclesfield, U.K.; formerly Applied Chromatography Sytems, Luton, U.K.). Injection was achieved through a Rheodyne valve, model 7125 (fitted with a 20-μL loop), onto a Spherisorb -5-ODS 1 (Phase Separations, Queensferry, Clwyd, Wales) column (200 × 4.6 mm i.d.) that had been slurry packed from acetone in our own laboratories. Elution was with methanol/water/acetic acid (70:30:1, v/v/v) at a flow rate of 1 mL min⁻¹. Detection was at 280 nm with 0.02 AUFS. Triplicate determinations were made with standard solutions to establish a calibration curve. Duplicate analyses were carried out on samples. The calibration was linear within the range 0.915–91.5 μg injected (correlation coefficient 0.9999; number of standards 7). Recovery was determined to be 99.7%.

Assay of Dipalmitoylphosphatidylethanolamine (DPE) by HPLC. Lard (0.5 g) was heated in an oven at 70 °C until melted. It was then quantitatively transferred as a solution in chloroform to a 5-mL volumetric flask and made to volume at room temperature. Standard solutions of DPE were prepared by solution in warm chloroform/methanol (2:1, v/v) and made to volume at room temperature.

Dansyl chloride (DNS) derivatives were prepared as described by Chen et al. (1981) except that 5, 10, or 20 μL of chloroform (or chloroform/methanol) solution was used (depending upon the approximate amount of DPE expected) and that these chloroform solutions were not evaporated to dryness prior to derivatization.

A Gilson Model 302 pump (Anachem, Luton, U.K.) together with a Model 3000 fluorescence spectrophotometer (Perkin-Elmer, Beaconsfield, U.K.) was used. Excitation and emission wavelengths were 340 and 500 nm, respectively, and slit widths of 5 mm were used. Integration was with a Model 3390A integrator (Hewlett-Packard, Winnersh, U.K.). Injection was achieved through a Rheodyne valve, Model 7125 (fitted with a 20-μL loop), onto a Partisil 5-μm (Phase Separations, Gwent, U.K.) column (250 × 4.6 mm i.d.) that had been slurry packed from dry methanol in our own laboratories. Elution was with dichloromethane/methanol/15 M ammonia (360:36:4, v/v/v). Injection volumes were between 2.5 and 10 μL. All samples and standard were analyzed in duplicate.

The calibration was linear within the range 0.00175–1.75 ng injected (correlation coefficient 0.9993; number of standards 8).

RESULTS AND DISCUSSION

The interaction between primary antioxidant and synergist can be seen in Figure 1. Lard shows an IP of 0.7

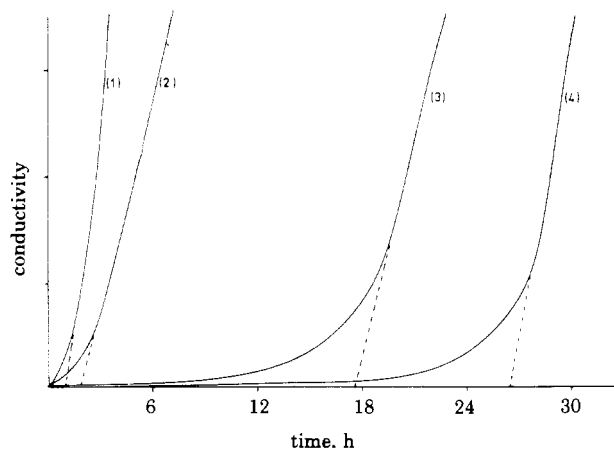Department of Food Science, University of Reading, Whiteknights, Reading, RG6 2AP U.K.

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Figure 1. Induction periods at 120 °C of lard with additives: 1, no additive; 2, 0.32% DPE added; 3, 0.02% PG added; 4, 0.02% PG and 0.32% DPE added.
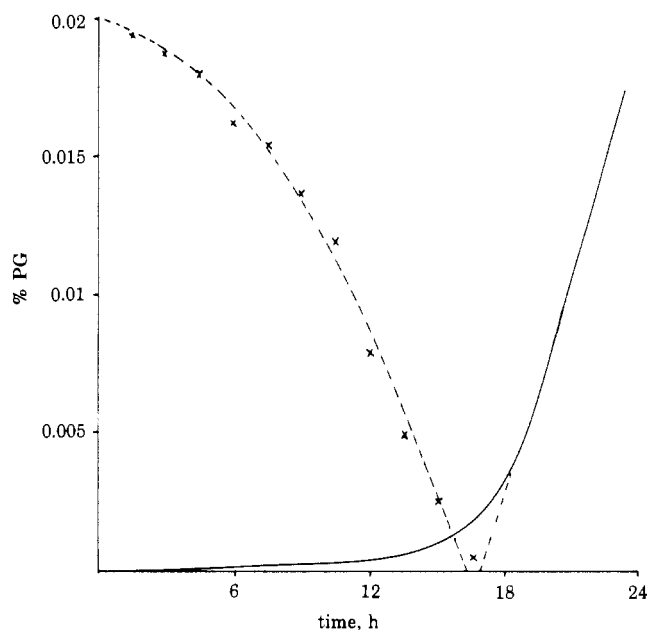

Figure 3. Progress of oxidation of lard with added PG (0.02%) and DPE (0.32%). Key: —, induction period at 120 °C; ×, percent PG; ○, percent DPE.

Figure 2. Progress of oxidation of lard with added PG (0.02%). Key: —, induction period at 120 °C; ×, percent PG.
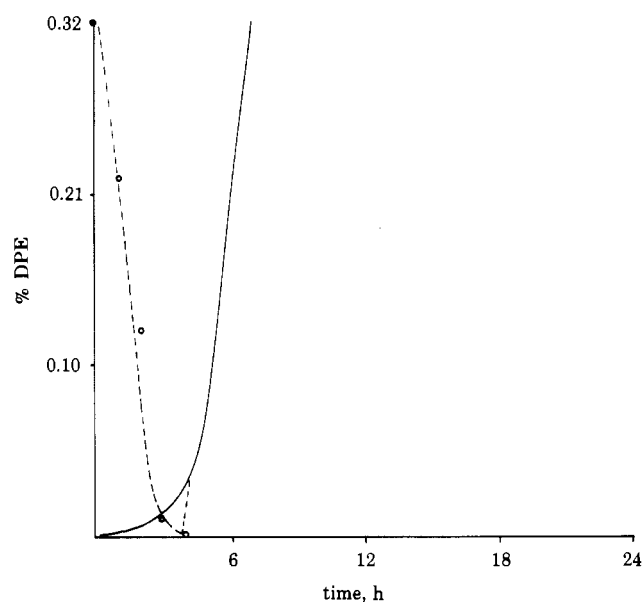
Figure 4. Progress of oxidation of lard with added DPE (0.32%). Key: —, induction period at 120 °C; ○, percent DPE.

h, which is increased to 1.3 h by the addition of 0.3% DPE. PG has a marked effect on the stability of lard, increasing the IP to 17.5 h. The addition of DPE can be clearly seen to synergize the primary antioxidant present, increasing the IP to 26.5 h. Calculated by the method of Bishov and Henick (1972), the synergism is 31.4%.

It will be noted that during the autoxidation process the level of PG progressively declines (Figure 2). The rate of loss of PG increases with time, a feature of classical free-radical chain reactions. At the point when PG is no longer detectable, there is a rapid increase in the autoxidation of the lard, corresponding to the IP.

Figure 3 shows that when PG and DPE are simultaneously present, the loss of PG is much slower than when DPE is absent, as long as DPE is still present. It would appear that the breakdown of PG follows, kinetically, a typical zero-order "decay" reaction in which PG, under these conditions, has a half-life of about 9 h (compare Figure 2 with Figure 3).

Figure 4 shows that when DPE only is present in the system, it is degraded quite rapidly, the decomposition products showing little or no antioxidant effect.

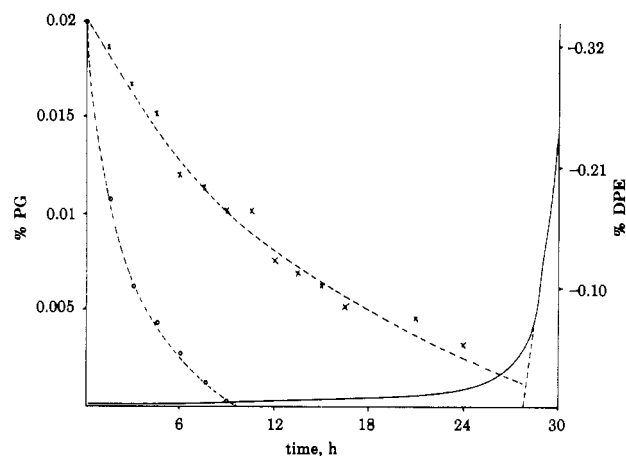It is of interest to speculate on the products resulting from the degradation of PG and DPE. In both the pres-
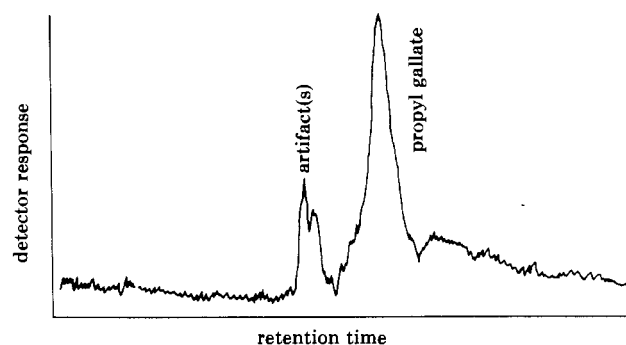

Figure 5. HPLC response for PG and artifact(s) in late stage of induction period.

ence and absence of DPE, PG forms a minor but increasing amount of an artifact with a shorter retention time than PG itself (see Figure 5, a chromatogram obtained for the antioxidant components about 2 h before the total disappearance of PG when the IP has been reached). This product is the only significant artifact observed.

In their study of the degradation of PG under irradiation with UV in ethanolic solution, Kurechi and Kunugi (1983) also observed the formation of an artifact, which they identified as ellagic acid. This is an autoxidation product,

with much reduced antioxidant activity, resulting from the coupling of two PG free radicals. We (Dziedzic and Hudson, 1984b) found ellagic acid to be inactive in the Rancimat test. It may well be the same compound as we observed as an artifact in our PG degradation.

GENERAL CONCLUSIONS

There has been much controversy as to whether phospholipids are pro- or antioxidants and by what mechanism they exert their effects. The subject has been reviewed in some detail by Brandt et al. (1973). The work presented here sheds some light on this area of uncertainty. Phospholipids, as exemplified by DPE, present in an oil, will evidently degrade, whether or not primary antioxidants are also present, but much more rapidly in their absence than in their presence. It is possible that the mechanism of their degradation may differ in the two situations: we have already shown that the magnitude of their synergistic effects is dependent on the structures of the antioxidants used (Dziedzic and Hudson, 1984a). We therefore propose that the phospholipid or possibly its breakdown compounds interact with the free radicals from the primary antioxidant by regenerating the primary antioxidant and thus prolonging its useful life. The phospholipid must have this capability by virtue of its ability to generate hydrogen radicals or protons.

Phospholipids are partially removed during commercial oil refining by the "degumming" process. This is considered essential since their presence is often associated with undesirable flavors, odors, colors, and surface activity effects. On subsequent bleaching and deodorization the oil will be heated for a period of time at temperatures at or above of 100 °C. Such a system will, with the previous incomplete removal of phospholipid, promote the initial breakdown of the primary antioxidant. Thus, any subsequent storage, due to the lower level of antioxidant, will show a decreased stability and a prooxidant effect of the phospholipid. Such mechanisms may well be relevant to the controversy to date regarding pro- and antioxidant effects of phospholipids.

The growing concern over the safety of synthetic antioxidants has highlighted a need for new antioxidants and antioxidant systems. Phospholipid degradation products are presumably present in foods, e.g. in frying oils, and their incorporation may therefore greatly enhance the effect of an added or naturally present antioxidant.

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Chirality Evaluation of 2-Methyl-4-propyl-1,3-oxathiane from the Yellow Passion Fruit

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In order to determine the chirality of 2-methyl-4-propyl-1,3-oxathiane (MPO), an essential trace component of the yellow passion fruit, a flavor concentrate of fresh yellow passion fruit was prepared by solvent extraction of the neutralized pulp with pentane–dichloromethane (2:1, v/v) and subsequent silica gel LC preseparation using a pentane–diethyl ether mixture (9:1, v/v). The concentrated eluate was further fractionated by repeated HPLC on Spherisorb CN (pentane–diethyl ether, 9:1 and 99:1, v/v), and the fractions were analyzed by coupled capillary gas chromatography–mass spectrometry using multi-ion detection. From the MPO-containing fraction, which still displayed a complex composition of volatiles, MPO was separated by Tenax-GC collection using capillary gas chromatography on Carbowax 20 M. After solvent desorption and concentration, finally, complexation gas chromatography on a chiral nickel(II) bis[3-(heptafluorobutyryl)-1(R)-camphorate] capillary column revealed the (2R,4S)-configurated cis-MPO by comparison of its retention time with that of the synthesized reference MPO.

Due to its delicate flavor the volatiles of yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) have been extensively studied (Hiu and Scheuer, 1961; Winter and Klöti, 1972; Huet 1973; Winter et al., 1976; Demole et al., 1979; Casimir et al., 1981; Chen et al; 1982; Engel and Tressl, 1983a, b).

Sulfur-containing components and some unusual aliphatic esters have been reported to play important roles in the unique flavor of this tropical fruit. In particular, Winter et al. (1976) stressed the importance of cis- and trans-2-methyl-4-propyl-1,3-oxathiane (MPO), whose mixture showed, according to these authors, a strong and natural fruity odor with a green and slightly burnt note. Both MPO isomers have been found in yellow passion fruit in a 10:1 mixture with the cis-configurated compound as the main product.

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