In vitro Digestibility

Literature studies have shown that sunflower protein is highly digestible and possesses a high biological value (2) and conforms to the value obtained for sunflower flour. Polyphenol-free concentrate showed much high value (90%) than that of the sunflower flour (84.7%).

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Phosphatidyl Ethanolamine as a Synergist for Primary Antioxidants in Edible Oils

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ABSTRACT

Dipalmitoyl phosphatidyl ethanolamine (DPE) is a potent synergist for a wide range of primary antioxidants in edible oils at elevated temperature, i.e., above 80 C. At lower temperatures it has very little synergistic action. At 120 C the synergistic effect increases progressively as the concentration of synergist increases from 0.025% to 0.25%. At a given level of synergist, its effect is proportionately greater at low rather than high levels of primary antioxidant.

INTRODUCTION

Though without significant antioxidant properties themselves, at elevated temperatures some of the naturally occurring classes of phospholipids (PL), in particular phosphatidyl ethanolamine (PE), greatly enhance the activity of primary antioxidants in edible oils with limited stability. Phosphatidyl choline and phosphatidyl serine are also effective, but markedly less so, and phosphatidyl inositol is without synergistic activity (1-3).

PE has been clearly shown to act synergistically in cooperation with various polyhydroxy flavones and flavanones (1), with polyhydroxy isoflavones (2) and with tocopherols and some of their derivatives (3). In these studies both lard, which contains no natural primary antioxidants, and soybean oil, which contains several tocopherols, have been studied. Assessment of stabilizing effects has been, as is customary, by measurement of induction periods under accelerated storage conditions, at 100-140 C.

From these earlier studies, the expectation that PE would be capable of providing synergistic effects with all primary antioxidants and polyunsaturated substrates seemed reasonable, but this needed to be demonstrated with a wider range of primary antioxidants. Further, some quantitative aspects, e.g., the relationships between concentration of synergist and the magnitude of the antioxidant effect, needed to be established. Finally, the influence of temperature on the antioxidant effect was obscure. The present communication reports the results of our studies on these themes.

EXPERIMENTAL PROCEDURES

Materials

Lard was donated by Messrs. Scot Bowyers Ltd., Trowbridge, England. It was unrefined, had not been chemically processed and was free from added antioxidants. Refined, low-erucic rapeseed oil was donated by Croda Edible Oils Ltd., Hull, England.

sn-Dipalmitoyl phosphatidyl ethanolamine (DPE) of 98% purity was purchased from Sigma (London) Ltd., England, and dl-α-tocopherol was kindly donated by Roche Products, Dunstable, England.

The other primary antioxidants were of best commercial quality when available. Propyl caffeate was prepared by direct esterification of caffeic acid with n-propanol in the presence of a trace of H_2SO_4. The product was recrystallized from aqueous ethanol. The preparation of 3,4-dihydroxy chalcone was as described in our earlier communication (4).

Methods

Induction periods were determined in the automated Rancimat (Metrohm AG, CH-9100 Herisau, Switzerland) at defined temperatures in the range 60-140 C. A continuous airstream is passed through the heated sample and the volatiles passed into a conductivity cell. Conductivities are

continuously monitored until a sudden rise signifies the end of the induction period.

RESULTS AND DISCUSSION

Relationship Between Synergist Concentration and Antioxidant Effect

Table I reports induction periods (Rancimat) obtained in the stabilization of lard at 120 C by the use of a combination of a series of primary antioxidants with varying levels of synergist (DPE). The primary antioxidants represent a range of different chemical classes, including mono-, di- and trihydric phenols, phenolic acids, esters and a chalcone. The last named was selected as representative of a highly active group of antioxidants on which we have recently reported (4). Synergistic efficiencies have been calculated from induction period data by the method of Bishov and Henick (5).

The data show clearly that, for a given concentration of primary antioxidant, induction periods and synergistic efficiencies are very dependent on the concentration of synergist. At low concentrations the synergist, in some cases, can have a negative effect. In general, the higher the concentration of synergist, at least up to 0.25%, the greater the synergistic efficiency, although considerable variation exists between primary antioxidants. DPE is particularly effective with α-tocopherol, in line with our earlier communication (3), with 3,4-dihydroxy chalcone (4) and with BHA. The negative synergism is particularly marked with propyl gallate, although even this compound acts cooperatively with DPE, provided the concentration of the latter is above 0.1%.

Relationship Between Primary Antioxidant Concentration and Antioxidant Effect at a Fixed Synergist Level

Figure 1a-b shows, at 2 temperatures, that the effect of the synergist DPE is proportionately greater at low levels of the primary antioxidant α-tocopherol. Hence, synergistic efficiency falls steadily as the concentration of α-tocopherol increases. This is because, although induction periods in the absence of synergist continue to rise as α-tocopherol concentrations increase above 0.01%, at least as far as 0.5%, induction periods in the presence of synergist do not rise very significantly or regularly in this concentration range. The effect of temperature, as between 80-120 C, does not appear to be significant.

Relationship Between Synergistic Efficiency and Temperature

The Rancimat equipment is capable of operating over the temperature range 60-140 C. Synergistic effects with the model-system lard–0.025% α-tocopherol–with 0.1% DPE were therefore evaluated over this range and are recorded in Table II. Induction periods in a simple substrate can be very roughly correlated with operating temperature in an Arrhenius-type relationship, and this can be seen in the recorded data. However, though synergistic efficiency is high at 100-140 C, below 100 C it falls rapidly and at 60 C (and presumably below 60 C) is virtually nonexistent.

This rather unexpected finding called for confirmation through the investigation of a less artificial system. Accordingly, the effect of DPE as a synergist for refined rapeseed oil was studied over the same temperature range. The results are reported in Table III. Because rapeseed oil devoid of primary antioxidants is not available as a control, calculating synergistic efficiencies is not possible. Protective factors are calculated instead, and that the same conclusions can be reached as in the case of the data in Table II is immediately obvious. DPE does not act as a synergist at 60 C, although it has some activity at 80 C and quite a marked effect at 100-140 C.

Is There Synergism at Ambient Temperature?

Stability in edible oils and fats is commonly determined by accelerated deterioration tests, sometimes as a result of the action of light or UV radiation but much more commonly at elevated temperatures. Indeed, such tests are often highly relevant to the conditions to which oils and fats are subject, as in production processing, food manufacture or domestic use. In quality control, however, information on long-term deterioration on storage at ambient temperatures is usually of decisive importance. Results obtained under the much more rapid and convenient accelerated storage conditions are often extrapolated to provide guidance on the probable behavior of the same products on ambient storage.

TABLE I

Effect of Concentration of Dipalmitoyl Phosphatidyl Ethanolamine (DPE) on Its Synergistic Action in Cooperation with Primary Antioxidants Added to Lard at 120 C

Primary antioxidant at 0.025%	Induction periods (hr) at 120 C (concentration [%] of DPE)					Percentage of synergistic efficiency[a] (concentration [%] of DPE)			
	Nil	0.025	0.05	0.10	0.25	0.025	0.05	0.10	0.25
None	0.3	0.45	0.50	0.65	1.7	–	–	–	–
Gallic acid	16.3	13.6	17.1	21.9	39.8	−21	+4	+42	+56
Propyl gallate	12.7	9.1	11.1	13.1	21.9	−43	−17	0	+36
Caffeic acid	14.0	13.1	15.4	17.0	29.4	−8	+8	+16	+48
Propyl caffeate	8.3	9.3	11.5	15.0	24.5	+9	+27	+43	+61
3,4-Dihydroxy chalcone	10.0	9.0	11.1	15.0	37.6	−13	+8	+32	+70
dl-α-Tocopherol	4.5	5.9	7.1	10.8	20.7	+22	+35	+57	+78
BHA	2.6	3.5	4.8	5.0	12.6	+23	+56	+44	+70
BHT	0.7	0.9	1.1	1.5	2.1	+8	+25	+37	0
TBHQ	3.9	3.9	7.1	7.7	9.4	−4	+42	+45	+45

[a]Percentage of synergistic efficiency $= \frac{100[(I_M - I_L) - (I_A - I_L) - (I_S - I_L)]}{(I_M - I_L)}$

Where: I_L = Induction period of the substrate (in this case, lard).
I_A = Induction period of the substrate + primary antioxidant.
I_S = Induction period of the substrate + synergist.
I_M = Induction period of the substrate + primary antioxidant + synergist.

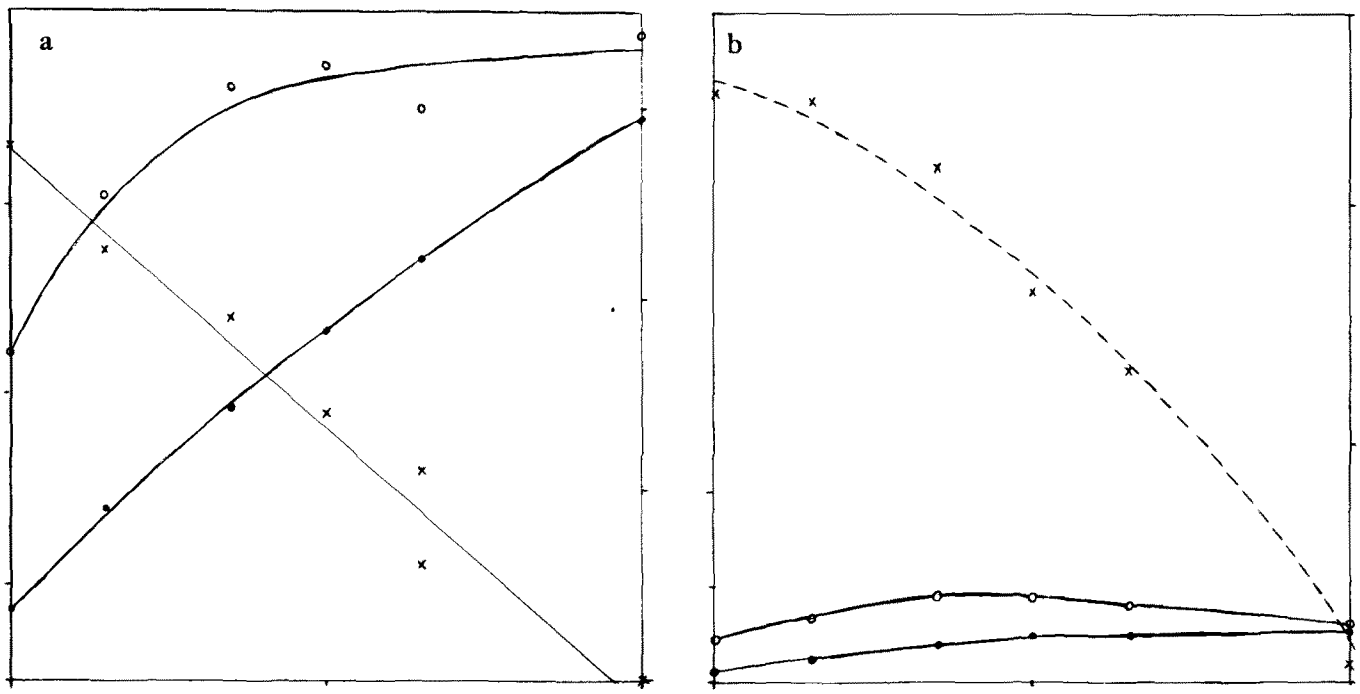

FIG. 1. Effect of variation of primary antioxidant (α-T) *concentration on synergism* between α-T and a fixed level of DPE (0.1%) in lard at (a) 80 C and (b) 120 C.

TABLE II

Effect of Temperature on Synergistic Efficiency of DPE Added to Lard Stabilized with α-Tocopherol (α-T)

Operating temperature (C)	Induction periods (hr)				Synergistic efficiency (%)
	Lard alone	Lard + 0.025% α-T	Lard + 0.1% DPE	Lard + 0.025% α-T + 0.1% DPE	
60	16.1	122	18.4	125	+1
80	4.1	39.1	5.6	63.8	+38
100	1.2	15.5	1.7	31.5	+51
120	0.35	4.0	0.65	9.4	+56
140	0.1	1.0	0.15	2.5	+60

TABLE III

Effect of Temperature on Synergistic Efficiency of DPE Added to Refined Deodorized Rapeseed Oil

Operating temperature (C)	Induction periods (hr)		Increase (%) in induction period	Protective factor[a]
	Rapeseed oil	Rapeseed oil + 0.1% PE		
60	153	152	−0.7	0.99
80	42.5	49.8	17	1.17
100	15.6	20.5	31	1.31
120	4.4	6.7	52	1.5
140	1.2	1.7	42	1.4

[a]Synergistic efficiency as understood in Table II cannot be applied here as the primary antioxidants are an integral part of the rapeseed oil. "Protective factor" (IP of synergized oil/IP of untreated oil) gives an idea of the extent of synergism.

As has been shown above in the special case of DPE synergism, results obtained at, say, 100-140 C cannot be extrapolated to 60 C, let alone ambient temperatures. An accelerated ambient storage test was therefore developed to evaluate the activity, in a reasonable time, of synergists in edible oils. In this test, exactly 2 g of oil or fat is exposed to atmosphere in a Petri dish with 90 mm i.d. for predetermined periods. Peroxide values (PV) are determined, using the whole sample. Table IV shows the effect of time on PV development under these conditions. As a result of the incorporation of 0.1% DPE in rapeseed oil there is only a very slight antioxidant effect and the progress of autoxidation follows the same course whether the synergist is present or not.

From the evidence presented, the phospholipid DPE acts as a synergist for primary antioxidants over the temperature range 80-140 C. Below 80 C the synergistic activity is small or negligible. When synergists are under consideration, stabilizing effects demonstrated by test methods relying on elevated temperatures cannot be extrapolated to low, including ambient, temperatures.

These observations raise again the unsettled question of the mechanism by which synergists afford their stabilizing activity. Two conclusions can be drawn in this context. In view of the quantitative relationships demonstrated between activity and concentration, synergism is not a catalytic process: the synergist must itself take part in a chemical reaction. Further, the mechanisms of autoxidation are probably different between low and high temperatures, the high temperature mechanism permitting the intervention of the synergist.

TABLE IV

Effect of Time on PV Development in Rapeseed Oil Exposed to Atmosphere in a Thin Layer

Period of exposure (days)	Peroxide value	
	Rapeseed oil	Rapeseed oil + 0.1% DPE
0	0.5	0.5
1	3.5	2.7
3	13.5	12.5
6	29.7	27.2
10	100	85
27	295	210

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A Rapid Quantitative Method for Determination of Astaxanthin Pigment Concentration in Oil Extracts

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ABSTRACT

Power models were developed to demonstrate relationships between absorption maxima (λmax) and specific extinction coefficients ($E^{1\%}_{1cm}$) for the carotenoid astaxanthin and astacene in different oils and organic solvents. An accurate and rapid determination of concentration of astaxanthin-enriched oil can be achieved by using the predicted $E^{1\%}_{1cm}$ value based on absorption maxima in the visible light spectrum. Several vegetable or fish oils have been shown to be comparatively efficient in extracting astaxanthin pigment from crustacean waste in a pilot plant.

INTRODUCTION

Astaxanthin, 3,3′-dihydroxy-4,4′-diketo-β-carotene, is the most prevalent carotenoid in various animals, e.g., crustacea, fish and tropical birds, e.g., flamingos. Documentation is increasing on improved consumer acceptance of hatchery-raised fish and crustacea by including astaxanthin in dietary formulations, which is ultimately concentrated in the pigment in the integument and flesh of the aquatic species (1-3). Recently, heat-processed waste from Louisiana crawfish *(Procambarus clarkii)* has been identified as a significant source of biologically active astaxanthin pigment (4) based on the production and availability of as much as 30 million lbs of waste per year with noteworthy pigment concentration of 153 μg/g wet material (5). Current efforts include pilot plants for efficient pigment extraction using a vegetable or fish oil for economical recovery of the oil-soluble pigment. Accurate determination of pigment concentration in such oil extracts is critical in assessing process parameters and in determining the final market value of the pigmented oil.

The standard method for quantitatively determining carotenoids is by chromatography and spectrophotometry. An isolated carotenoid can be identified by the positions of the absorption maxima (λmax) and its spectral characteristics, described by molar (ε) or specific ($E^{1\%}_{1cm}$) extinction coefficient. The absorption spectra of carotenoids are recognized as depending on the functional groups of the polyene chromophore as well as on the particular solvent used (6,7). The absorption spectrum of astaxanthin in the visible light region is characterized as a broad single peak, with lowest maxima in petroleum ether (467-470 nm) and highest in carbon disulfide (502-505 nm) (8,9).

Because most of the published $E^{1\%}_{1cm}$ values for astaxanthin are in organic solvents, statistical models were used here to attempt to establish a relationship between the λmax and $E^{1\%}_{1cm}$ values based on data from the literature. The predicted $E^{1\%}_{1cm}$ values were compared with experimental results to develop a more accurate and rapid method for quantitative determination of astaxanthin pigment concentrations in oil.

MATERIALS AND METHODS

Determination of Specific Extinction Coefficient

Accurately weighed crystalline racemic astaxanthin pigment (Hoffman LaRoche) was dissolved in a known volume of a