Development of New, Nonabsorbable Polymeric Antioxidants for Use in Foods[1]

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ABSTRACT

The metabolic fate of a new, oil soluble antioxidant, selected from a series representing a unique class of phenolic polymers, is discussed together with its activity in unsaturated vegetable oils as compared to butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butylhydroquinone (TBHQ). Using gel permeation chromatography, ^{14}C-radiolabeled polymer was isolated into discrete mol wt fractions and these administered as single oral doses to rats. Results indicate that while monomeric ^{14}C-TBHQ with a mol wt of 166 shows a total absorption of 88.3% of the administered dose, the absorption of polymeric antioxidant is vastly reduced with increasing mol wt; i.e., mol wt 760: 1.5%, mol wt 7300:0.44%, and mol wt 67,000: 0.34%. Functionality tests using the active oxygen method indicate that by increasing the phenolic constituents in the polymer composition the antioxidant activity can surpass that of certain traditional, monomeric food grade antioxidants. Stability tests using thermogravimetric analysis indicate the polymeric antioxidants are not depolymerized in the presence of air at temperatures up to 300 C. Further, the polymers are nonvolatile and under deep frying conditions result in nearly quantitative carry-through of antioxidant in that portion of oil absorbed by the food; a monomeric food grade antioxidant (TBHQ) shows losses due to volatilization.

INTRODUCTION

Food additives such as antioxidants serve no purpose entering man's diet once they have fulfilled their intended use as food processing aids or to extend shelf life. Admittedly, while those intended for direct use in foods have undergone extensive toxicological studies in animals or enjoy a relatively long history of use, it is impossible to assess their long-term toxic effects over the life span of man. Conceptually, the toxic risk to man can be considerably reduced if such additives are rendered nonabsorbable, thereby limiting contact to the gastrointestinal tract. This has now been effected by the development of polymeric antioxidants which are sufficiently high in mol wt to be virtually nonabsorbed and excreted intact via the feces. In this fashion, the antioxidant does not enter the circulatory system and is precluded from reaching possible internal target organs and tissues.

After nearly two decades of effective use in stabilizing lipid-containing foods, the long-term safety of monomeric good grade antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) is subject to controversy within the scientific community. As recently as February 1975, Branen (1) highlighted the issues when he reviewed the toxicology and biochemistry of BHT and BHA by surveying some 77 literature references, the bulk of which were published since 1965. This review, which critically evaluates studies conducted primarily in animals, clearly indicates the concern over observed pathological effects (2-8), carcinogenic potential (2,9-12), interactions with enzymes and lipids (2,3,8,13-23), effects on reproduction (5,13,25), and finally, the exact nature of the metabolic fate in man (1,26). Several investigators underscore that it is not clear whether the observed changes resulting from chronic feeding studies in animals represent normal physiological adaptive responses or pathological effects (8,27). Certainly, the manner in which various species handle monomeric antioxidants differ. For example, Daniel (28) concluded that the lack of appreciable enterohepatic circulation in man would permit BHT to accumulate to a greater extent than in rats.

More important, however, is the issue of using animal models with limited life spans (3-7 years) to predict what may occur in man after a 60-70 year history of daily consumption. It is doubtful that further animal studies with BHT and BHA will serve to clarify toxicological issues or be any more prophetic with respect to the consequences in man. However, it is important to recognize that a major step forward in reducing the potential risks in man could be achieved if antioxidants were precluded from interacting with target organs and tissues, i.e., if they were nonabsorbed.

The purpose of this study is to demonstrate that functionally active polymeric antioxidants can be prepared which are both useful in stabilizing dietary lipids and virtually nonabsorbed from the gastrointestinal tract.

EXPERIMENTAL PROCEDURES

Materials

The monomeric antioxidants employed included food grade BHT, BHA, and tertiary butylhydroquinone (TBHQ) (Eastman Chemical Products, Kingsport, TN). These, together with each polymeric antioxidant, were incorporated into vegetable oils at various levels using 10-20% by wt concentrates of the antioxidants in antioxidant-free glycerol monooleate (GMO) obtained from Emery Industries (Cincinnati, OH). Unless otherwise indicated, the concentrates also contained propylene glycol and corn oil to codissolve citric acid used at 200 ppm in the vegetable oils as a heavy metal sequestrant. A typical concentrate consists of 10% antioxidant, 10% citric acid, 15% propylene glycol, and 65% GMO.

The vegetable oils employed as substrates to test for antioxidant activity included corn oil and a cottonseed-soybean oil blend free of added antioxidant; naturally occurring tocopherols were not removed.

The Poly-AO™ polymeric phenolic antioxidants

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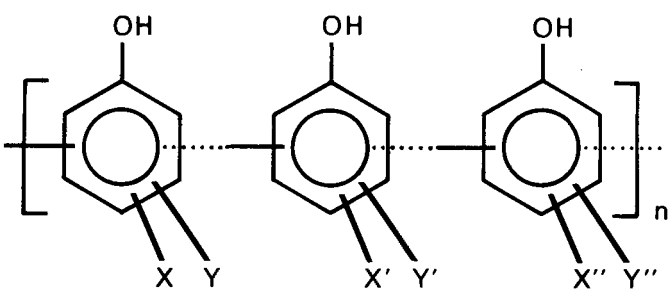

FIG. 1. General structure of polymeric antioxidants investigated.

studied are represented by the general structure shown in Figure 1. These are a series of hydroxyaromatics, covalently bonded by hydrocarbon-linking units shown in the formula by $\dashv \cdots]\cdot\cdot$. In our studies, we have examined phenol itself as the hydroxyaromatic as well as substituted phenols wherein x, y, x', y', x", and y" independently are hydrogen, lower alkyls, hydroxyl, lower alkoxyl, etc. The polymeric antioxicants referred to as Poly-AO I, Poly-AO II, and Poly-AO III contain a variety of these x- and y-substituted hydroxyaromatic mixtures. The chemistry and synthesis of the polymeric antioxidants employed in this study will be the subject of a forthcoming communication.

Radiolabeling

The TBHQ employed in biological (absorption) studies and for carry-through tests onto fried potato sections was uniformly ring-labeled with carbon-14. The polymeric antioxidant employed for carry-through studies was also prepared by carbon-14 ring-labeling the phenolic moiety prior to polymerization, while material employed for absorption studies was prepared by partially (10%) 0-methylating polymer fractions using [^{14}C]-methyl iodide.

Fractionation

All polymeric antioxidants were fractionated by nonsolvent precipitation (29) using tetrahydrofuran (THF) as the solvent with methanol and water employed as the nonsolvents. Mol wts were determined relative to polystyrene of known molecular size using gel permeation chromatography (GPC). For this purpose, the columns were packed with nominal 10^5-600 Å Styragel and operated at 400 psi using THF as the eluting solvent. The column eluants were simultaneously monitored utilizing UV (280 nm and 254 nm) and refractive index. In addition, fractions of radiolabeled polymers were monitored by liquid scintillation counting (Packard Tri-Carb Model 2420).

Antioxidant Activity

The antioxidant activity of BHT, BHA, TBHQ, and various polymeric antioxidants in vegetable oil was determined using the official active oxygen method (AOM) procedure (30). In addition, oils were aged at 80 C in a forced-draft oven and peroxide values (PV) determined (31). Endpoints in both instances were taken as the number of hours to reach 70 and 100 meq peroxide per kg of oil. Unless otherwise specified, the antioxidants were tested at levels up to 200 ppm in oil; polymeric antioxidants were also tested at equivalent (% by wt) aromatic hydroxyl content. In all instances, 200 ppm citric acid was added to the oils to chelate trace metals which act as prooxidant catalysts.

Antioxidant activity was also measured in an emulsified oil-water system using the hemoglobin peroxidation procedure described by Cort (32). In our modification of the Cort technique, the antioxidants were not added to preformed emulsions from alcohol stock solutions. Rather, the antioxidants were added to the oil phase from GMO concentrates, after which the system was emulsified as described.

Thermogravimetric Analysis (TGA)

TGA was conducted on powdered samples of each antioxidant using the Rigaku instrument. The temperature programming was 5 C/min; analyses were conducted in air.

Carry-Through Tests

The carry-through characteristics of TBHQ and polymeric antioxidants onto potatoes during frying operations were evaluated using carbon-14 radiolabeled materials. Oils containing 200 ppm antioxidant (TBHQ=9.34 μCi/mg; polymeric antioxidant=1.22 μCi/mg) were used to fry potato sections measuring 1.0 cm in diameter x 0.65 cm thick for exactly 3 min at 375 F. After draining excess oil from the sections, the oil absorbed by the potato was exhaustively extracted with ether, the ether evaporated, and the residue weighed prior to radioassay. Subsequently, the residues were dissolved in 10 ml of Insta-Gel® (Packard Instruments, Inc., Downers Grove, IL) and counted in the Packard Tri-Carb Model 3385. In this fashion, the concentration of antioxidant in the frying bath was compared to that in the oil absorbed by the potato.

Absorption Studies

The following represents only the highlights of the biological procedures employed; details will be presented in a forthcoming publication by T.M. Parkinson et al. (Dynapol).

Groups of five female Sprague-Dawley derived rats (Simonsen Labs., Inc., Gilroy, CA) 7-8 weeks oil (175-195 g) were maintained on a diet of Simonsen white pellets and water ad lib. All animals received single doses of antioxidants in 0.75 ml of Wesson Oil (Hunt-Wesson Foods, Fullerton, CA) via stomach tube after an overnight fast (18-20 hr). Food was returned 8 hr after dosing.

Animals were housed in metabolism cages with urine and feces separators. Alternatively, urine was collected using an indwelling cannula (0.030 x 0.065 in. silicone rubber; Medical Grade Silastic®, Dow Corning, Midland, MI) surgically introduced into the urethra. Animals were allowed to recover from surgery for at least 3 days prior to dosing, and only those animals in good physical condition showing constant urine flows were selected for absorption studies.

Urine sample (usually 0.1 ml) were added directly to 10 ml of Instagel for liquid scintillation counting. Terminal blood samples were taken from the abdominal aorta, the serum was collected by centrifugation and counted in Instagel. Feces were homogenized 1:4 with water, transferred to Combustocones® (Packard Instruments), and dried overnight at 70 C. The dried samples were combusted in a Model 306 Tri-Carb Sample Oxidizer, and the CO_2 generated was absorbed by Carbsorb® and counted in Permafluor V® (Packard Instruments). Organs and tissues (e.g., liver, kidneys, spleen, periuterine fat, stomach, small intestine, caecum, and large intentine) were sampled and appropriately treated for combustion analysis and counting.

Net cpm for all samples were converted to dpm using automatic external standard quench curves of [^{14}C-toluene internal standards. Results are expressed in terms of percent administered dose of radioactivity.

For studies conducted with cannulated rats, the polymeric antioxidant had a sp act of 5.9 μCi/mg and peak mol wt by GPC of 67,000 g/mol. This material was dissolved in oil at a final sp act of 1.55×10^8 dpm/ml. For the polymer fractions, the sp act, including final dilutions in oil were: 760 g/mol, 2.1 μCi/mg, 4.53×10^7 dpm/ml; 7300 g/mol, 1.3 μCi/mg, 3.08×10^7 dpm/ml; 67,000 g/mol, 0.7 μCi/mg, 1.74×10^7 dpm/ml. Finally, the [^{14}C]-TBHQ had a sp act of 2.17 μCi/mg and was employed in oil at 14.48×10^6 dpm/ml.

RESULTS AND DISCUSSION

Before reporting the results of functionality and absorption studies, it is important to comment on certain handling characteristics viewed desirable for a food grade antioxidant. As described in the Experimental Procedures section, both monomeric and polymeric antioxidants explored in this study were added to oils from solution concentrates containing GMO. This was not performed merely for procedural expediency in the laboratory. To the contrary, traditional food grade antioxidants are currently marketed as convenient liquid concentrates containing as much as 20% by wt antioxidant (e.g., Tenox® brand products; Eastman

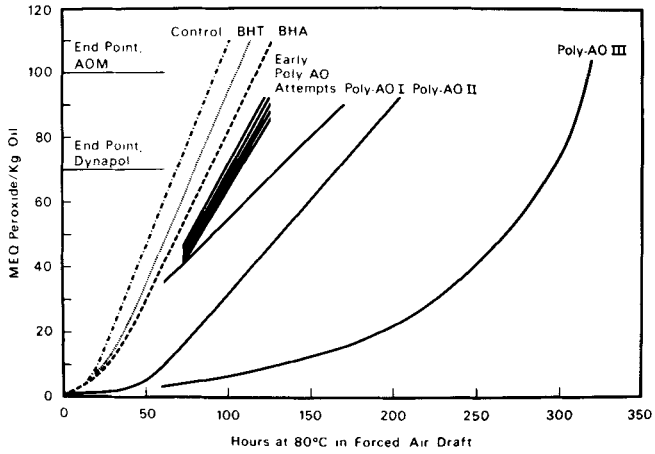

FIG. 2. Comparative activity of monomeric and polymeric antioxidants at 200 ppm in cottonseed-soybean oil in the presence of 200 ppm citric acid. Poly-A0 = polymeric antioxidant, AOM = active oxygen method, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene.

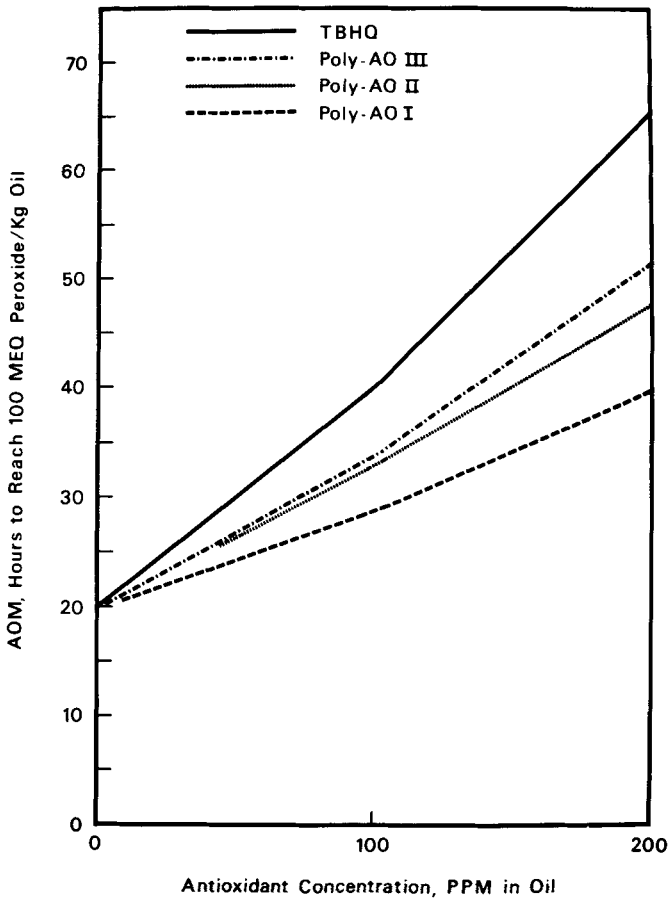

FIG. 3. Comparative activity of tertiary butylhydroquinone (TBHQ) and polymeric antioxidants (Poly-A0 in cottonseed-soybean oil on an equal wt basis; 200 ppm citric acid added. AOM = active oxygen method.

Chemical Products). Liquid concentrates permit users to rapidly incorporate antioxidants into fats and oils without the need to employ on-site dissolving operations. In most instances of bulk use, the concentrates are merely pumped. Further, concentrates provide a means by which complementary additives, such as citric acid, can be conveniently introduced into fats and oils. Since the choice of lipid-compatible food grade solvents is limited, it is important to recognize that a system achieving the proper solubilizing

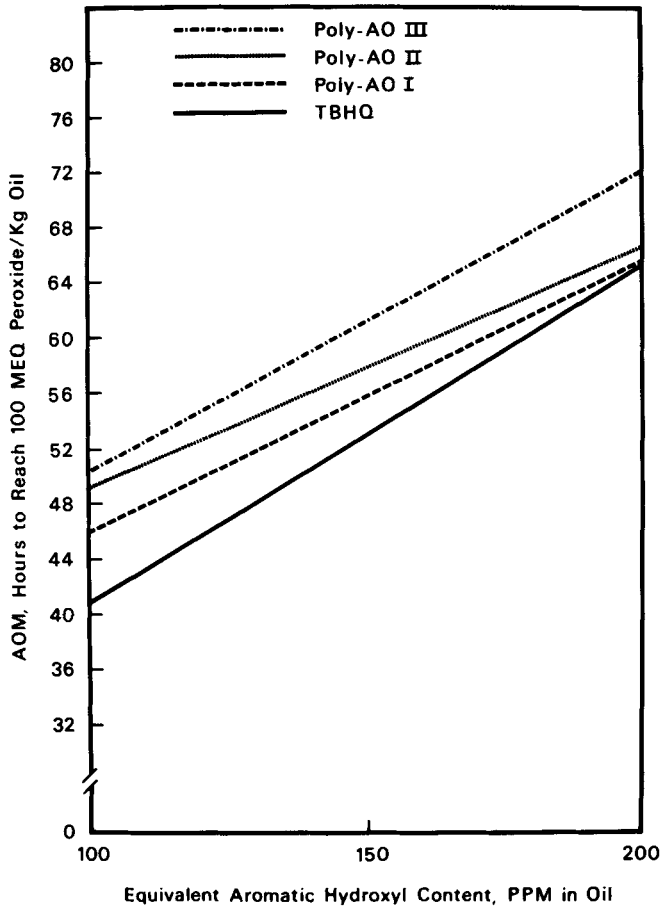

FIG. 4. Comparative activity of tertiary butylhydroquinone (TBHQ) and polymeric antioxidants (Poly-A0) in cottonseed-soybean oil on equivalent aromatic hydroxyl content; 200 ppm citric acid added. AOM = active oxygen method.

characteristics at high levels for both an oil soluble antioxidant and a water soluble adjuvant, e.g., citric acid, is a technically sophisticated achievement. Such is the case when GMO, propylene glycol, and a vegetable oil, e.g., corn oil, are used as cosolvents. Consequently, the ability to prepare similar formulations using certain polymeric antioxidants is a feature worth stressing.

As indicated in Figure 2, even polymeric antioxidants prepared in early attempts show parity with BHT and BHA when tested in vegetable oils at 200 ppm in the presence of 200 ppm citric acid. In this instance, a cottonseed-soybean oil blend was employed, but the relative activity is not affected when othe roil substrates, e.g., corn oil, safflower oil, etc., are employed or, as snown later, when the higher temperature and air sparging conditions of the AOM is employed. Similarly, the activity in vegetable oils is not seriously influenced by the choice of endpoints; i.e., whether one employs the AOM endpoint of 100 meq/kg oil or a more conservative value of 70 meq/kg oil.

Poly-AO I, II, and III are related polymeric antioxidants differring principally in the amount of armoatic hydroxyl content incorporated in the molecule. The functionally active and hydrocarbon-linking species are the same for each, as are the average peak mol wts (ca. $3\text{-}5\text{x}10^3$). Figure 2 clearly demonstrates the higher activity of all three polymers at 200 ppm as compred to equivalent levels of BHT and BHA. However, when compared on an equal wt basis to TBHQ (Fig. 3), the best of these (Poly-AO III) shows less activity. This is quite understandable when one considers the dilution effect one may expect from a hydrocarbon-linking moiety lacking functionally active sites to abstract oxidative initiators. A priori, one would select a

TABLE I

Effect of Aromatic Hydroxyl Content of Monomeric and Polymeric Antioxidants on Activity in Cottonseed-Soybean Oil (200 ppm Citric Acid Added)[a]

Antioxidant	% by wt aromatic OH	AOM (hours to reach 70 & 100 meq peroxide/kg oil) 70 meq: 100 ppm eq aromatic OH	70 meq: 200 ppm eq aromatic OH	100 meq: 100 ppm eq aromatic OH	100 meq: 200 ppm eq aromatic OH
BHT	15.7		9.0		12.0
TBHQ	20.4	38.0	62.0	41.0	64.5
Poly-A0 III	10.7	47.5	69.0	50.5	72.0
Poly-A0 II	10.3	46.0	63.5	49.5	66.5
Poly-A0 I	9.0	43.0	61.5	46.0	64.0
Control		7		9	

[a]AOM = active oxygen method, BHT = butylated hydroxytoluene, TBHQ = tertiary butylhydroquinone, Poly-A0 = polymeric antioxidant.

backbone contributing the least unit wt, but this must be judiciously counterbalanced by choosing a structure that will impart the desired physical-chemical characteristics for the intended use. However, when the concentrations of the polymeric antioxidants in oils are adjusted to deliver an aromatic hydroxyl content equivalent to TBHQ, all three polymeric antioxidants show superior activity (Fig. 4).

The effect of aromatic hydroxyl content on the activity of polymeric antioxidants is illustrated in Table I. In each instance, the % by wt aromatic hydroxyl for BHT and TBHQ is greater than for Poly-A0 I, II, III. In the case of TBHQ, the aromatic hydroxyl content is twice that of the polymeric antioxidant. Again using either 70 or 100 meq/kg as endpoints, the polymeric antioxidants are either equivalent or superior in performance to TBHQ but always more active than BHT. In terms of actual wt of antioxidant employed in oil, we stress that some 375 ppm of Poly-A0 III with half the aromatic hydroxyl content of TBHQ provides somewhat greater activity than 200 ppm TBHQ.

Equally important, seemingly small increased in the aromatic hydroxyl content of the polymeric antioxidants can result in marked improvement in activity. We are hesitant to claim synergism even though the hydrocarbon-linking units in Poly-A0 I, II, and III are identical in each case and void of functional groups to which an antioxidant mechanism can be ascribed. Nonetheless, once aromatic hydroxyl equivalency among the polymeric antioxidants has been taken into account, there is an apparent increase in activity; i.e., Poly-A0 III > Poly-A0 II > Poly-A0 I. We can only suspect that the nature of the aromatic hydroxyl moieties and the manner in which the hydrocarbon-linking units are attached favorably affects the activity of the polymers.

The behavior of antioxidants in pure fats and oils is quite different compared with their behavior in complex food systems such as emulsions (sauces, dressings, and spreads). In emulsions of the oil-in-water type, the oil phase is surrounded by an oxygen-rich environment. While oxidation can proceed from initiators residing within the oil phase, in emulsions, oxidation can also take place by reactions at the interface.

The partioning of an antioxidant between oil and water phases can be expected to influence the overall stability of the system. In the method described by Cort (32), antioxidants are screened by adding them directly to pre-formed emulsions from ethanol solutions. While this technique is adequate for screening monomeric antioxidants, the addition of an oil soluble polymeric antioxidant to an emulsion containing upwards of 90% water causes coacervation with precipitation of the polymeric antioxidant. This is consistent with the expectation that, due to the molecular size

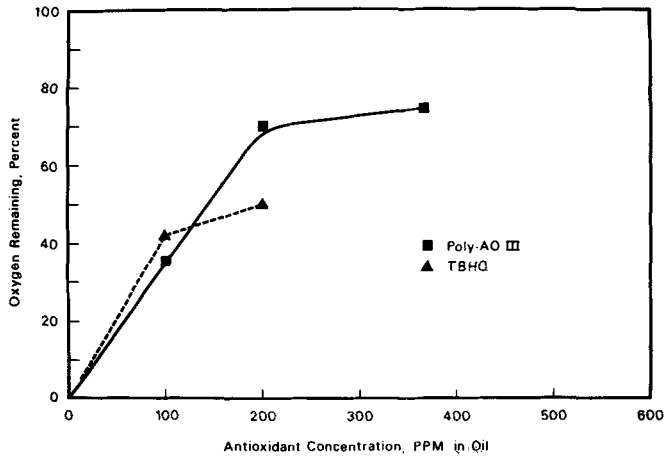

FIG. 5. Antioxidant activity of tertiary butylhydroquinone) and Poly-A0 111 (polymeric antioxidant) in oil-in-water emulsion. Antioxidants predissolved in oil phase; oil to water ratio is 1:9.

of polymers, diffusion is hindered across the oil-water interface. Further, one can envision that the emulsifier acts as a membrane which regulates the diffusion process and once disturbed by the presence of a nonpermeating polymer ceases to function as a stabilizer. Low mol wt antioxidants of similar polarity, i.e., oil soluble, can be transported into the oil phase due to their much higher diffusivity. However, by presolubilizing the polymeric antioxidant in the oil phase (as would be the case industrially) and then forming the emulsion, the antioxidant is confined within the oil globule and coacervation is avoided. Under these conditions, it is interesting to note that Poly-A0 III displays greater antioxidant activity on an equal wt basis than TBHQ (Fig. 5). This is in clear contrast to the relative activities of Poly-A0 III and TBHQ in pure vegetable oils as measured by the AOM. We are confident that the same properties which preclude the diffusion of polymeric antioxidants into the oil phase, when added to an oil-in-water emulsion, prevent its partitioning into water once presolubilized in the oil.

In numerous instances, antioxidants are subjected to high temperature processing conditions such as frying and baking. Certainly, it is important that antioxidants survive such stress if they are to impart process stability and later, shelf life. As indicated in Figure 6, thermogravimetric analysis (TGA) indicates polymeric antioxidants of the type described are exceptionally stable as compared to BHT, BHA, and TBHQ at elevated temperatures. Significantly, depolymerization is not apparent even in the presence of air at temperatures up to 300 C while BHT, BHA, and TBHQ

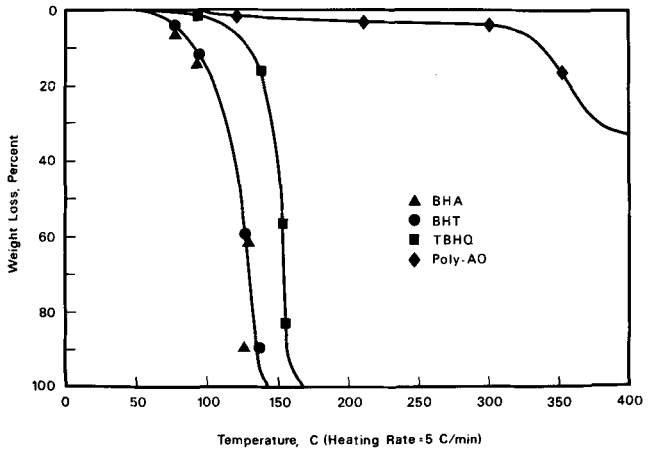

FIG. 6. Comparative thermogravimetric analysis (TGA) of monomeric and polymeric antioxidants in air. BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, TBHQ = tertiary butylhydroquinone, Poly-A0 = polymeric antioxidant.

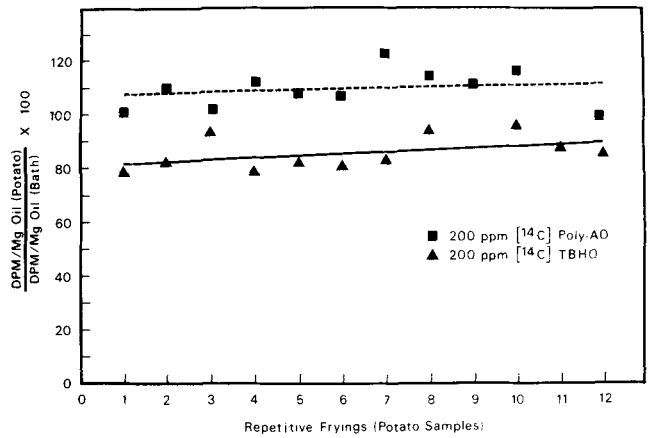

FIG. 7. Carry-through of tertiary butylhydroquinone (TBHQ) and polymeric antioxidant (Poly-A0) onto potato sections from deep fryings in vegetable oil; antioxidant concentration 200 ppm.

show very rapid and complete wt loss at temperatures of ca. 150-170 C. However, it is quite conceivable that the polymeric antioxidant, while not depolymerized at 300 C in the presence of air, may have reacted sufficiently with air (oxygen) under the severe stresses of TGQ to render it inactive. While such stresses are not common to food processing practices, studies in this vein are planned.

Of considerable importance to food processors is the loss of antioxidant during frying operations. Ideally, the oil absorbed by food during frying should contain the same concentration of antioxidant as the oil in the cooker. This is not the case when BHT, BHA, and TBHQ are employed, since the water vapor generated at the surface of the food product during frying codistills with significant portions of antioxidant. This is demonstrated in Figure 7 where frying oils were prepared with 200 ppm antioxidant using radiolabeled TBHQ and Poly-A0 III. Repetitive fryings of potato sections for 3 min at 374 F resulted in some 15% loss of TBHQ due to steam distillation while the nonvolatile nature of Poly-A0 111 produced nearly quantitative carry-through. While additional studies are in progress, it is reasonable to assume from published vapor pressure data that losses of BHT and BHA will be even more severe than for TBHQ (33,34). Organoleptic studies indicating that the polymeric antioxidant has been delivered to food products from frying oils at 375 F in an active form demonstrating carry-through performance will be reported in a subsequent communication.

Turning to biological absorption, it is clear from the data in Table II that polymeric antioxidants show two orders of magnitude less absorption from the gastrointestinal tract following oral administration than a typical monomeric antioxidant (TBHQ). Using radiolabeled TBHQ as the model monomeric antioxidant, some 88% of the oral dose is absorbed as compared to only 0.44% for Poly-A0 III having a mol wt of 7.3 x 10^3. Further, the relationship of mol wt to absorption is demonstrated. Clearly, these results indicate that increasing the mol wt appreciably reduces absorption. A major reduction in absorption occurs as the mol wt approaches 1000 g/mol; e.g., 88% absorption at 166 g/mol vs. 1.5% absorption at 760 g/mol. Increaseing the mol wt still further to 7300 g/mol and 67,000 g/mol is reflected by additional reductions in absorption to 0.44% and 0.34%, respectively. However, even these low levels of absorption can be accounted for by the presence of small quantities of low mol wt components in the administered dose. Our observations that polymeric antioxidants show reduced absorption with increasing mol wt is consistent with the published report for a series of monomeric antioxidants of increasing mol wt (35).

ACKNOWLEDGMENTS

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TABLE II

Summary of Biological Absorption Data

Antioxidant[a]	Mol wt	Radioactivity (% of oral dose Δ 96 hr) Urine	Bile	Organs, tissues, etc.	Feces	Total absorption
TBHQ	166	73.4	6.9		8.0	88.3
Poly-A0, Fraction 1	760	0.752		0.806	86.81	1.5
Poly-A0, Fraction 2	7300	0.170		0.270	102.64	0.44
Poly-A0, Fraction 3	67000	0.166		0.178	92.84	0.34

[a]TBHQ = tertiary butylhydroquinone, Poly-A0 = polymeric antioxidant.

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