Thermal Stability of Some Commercial Synthetic Antioxidants

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ABSTRACT: Synthetic antioxidants are widely applied substances in human food and in animal feed industries. These products, which are mainly derived from phenolic structures, were developed to avoid or retard the oxidative rancidity of fats and oils when added either to raw material or to end-products. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroxyquinone (TBHQ), and ethoxyquin (EQ) are frequently applied during the cooking of the by-products (viscera, blood, and feathers) in the poultry feed industry. However, results in terms of oxidative prevention are unequal and usually modest. Because information about the thermal stability of synthetic antioxidants is scarce, we developed a laboratory model that simulates the cooking of poultry by-products to study the effectiveness of BHT, BHA, TBHQ, and EQ. The antioxidants were thermally treated at 100–200°C, over 1 or 2 h. The effectiveness of each antioxidant after the thermal treatment was assessed with the Rancimat test by measuring the modification of the induction period for the oxidation of sardine oil and comparing it to the oxidation kinetics of the oil without added antioxidants. Within our experimental conditions, all antioxidants assayed showed different degrees of thermal instability. BHT and TBHQ were effective as antioxidants at temperatures up to 175°C, exhibiting only 25 to 30% inactivation. However, BHA and EQ were inactivated by 70 and 60%, respectively, at 150°C. Heating time (1 and 2 h) at a given temperature did not significantly modify the behavior of the antioxidants assayed. EQ is the most frequently applied antioxidant to prevent oxidative rancidity in the cooking of poultry by-products. However, according to our results, EQ and BHA, which is another antioxidant frequently used by the poultry industry, are less suitable antioxidants for preventing oxidative rancidity.

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Oxidative rancidity of fats and oils may be avoided or retarded by using antioxidants. In theory, a substance may act as an antioxidant in a variety of ways, e.g., competitive binding of oxygen, retardation of the initiation step, blocking the propagation step by destroying or binding free radicals, inhibition of catalysts, stabilization of hydroperoxides, etc (1). Antioxidants can scavenge the active forms of oxygen involved in the initiation step of oxidation, or break the oxidative chain reaction by reacting with the fatty acid peroxy radicals to form stable antioxidant radicals, which are either too unreactive for further reactions or form nonradical products (2).

Synthetic antioxidants generally have been considered as relatively safe and are widely applied in a number of manufactured products including pharmaceuticals, cosmetics, human foods, and animal feed (3). The most popular are those derived from phenolic structures or those having a phenolic configuration within their molecular structure. Among the phenolic-derived structures, 2,6-di-tertiary-butyl-4-methyl phenol (BHT), tertiary-butyl-4-hydroxyanisole (BHA), which is a mixture of two isomers (2- and 3-BHA), tertiary-butylhydroquinone (TBHQ), and 6-ethoxy-1,2-dihydro-2,4-trimethylquinoline (ethoxyquin, EQ) are the most frequently used antioxidants (4).

Synthetic antioxidants are added to protect end products or raw materials; they are also routinely added when procedures involving high temperature and/or pressure have been applied in the manufacturing process. This is to avoid the deleterious action of both temperature and pressure on the antioxidant effectiveness. However, in the poultry feed industry synthetic antioxidants are frequently applied to avoid rancidity during the processing of raw material and/or by-products together with the application of high temperature and pressure. Results, in terms of oxidative prevention, are variable and frequently modest. Probably the most significant example of synthetic antioxidant application in the feed industry is in the cooking of bird by-products (viscera, blood, and feathers) after the slaughter-house laboring. The cooking is carried out in metal containers (iron manufactured or, much less frequently, stainless steel), at temperatures from 120–180°C applied directly (the most rustic procedure) or by steam-jacketed containers (used in the modern factories). Heat is applied during 60–120 min. The process, depending on the temperature applied and the heating time, develops pressures ranging from 1.2–1.6 kg/cm^2. At the end of the cooking, organic material is separated by filtration under pressure to obtain the liquid oily fraction named "chicken oil," and the remaining solid is

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dried to a powder texture to obtain a meal named "viscera meal" or "meat meal." Commercial synthetic antioxidants, singly or in mixtures of two or three, according to the supplier suggestions or the empirical experience of producers, are added to the by-products either before cooking, 10–15 min after heat application, or at the end of the heating when the cooked material is still between 100 and 80°C. Results, in terms of oxidative prevention (low peroxide values), are highly variable and frequently unsatisfactory for the producer's requirements (peroxide values lower than 2–5 meq/kg fat for the chicken oil and less than 1 meq/kg fat for the viscera meal).

Information about the thermal stability of synthetic antioxidants is very scarce, mainly because these products were designed to protect fats and oils at ambient or relatively low temperatures. However, the heating applied to poultry by-products represents a thermal stress that can seriously affect the effectiveness of synthetic antioxidants such as BHA, BHT, TBHQ, or EQ when added before and/or during the cooking or processing. The aim of this work was the development of a laboratory-scale experimental model of the cooking of poultry by-products to assay the individual thermal stability of BHA, BHT, TBHQ, and EQ under conditions which mimic the heating conditions applied during processing. The effectiveness of the thermally treated antioxidants was assayed through the Rancimat test and using a highly purified fish oil as substrate for oxidation (5).

MATERIALS AND METHODS

Materials. Commercial BHT (99.5%), BHA (98%), and TBHQ (99.5%) as dry powders, and EQ (95%) adsorbed in vermiculite were obtained at the local market (Santiago, Chile) from different suppliers: BHT and BHA (Eastman Chemical Co.) were purchased from Molypak SA, TBHQ (Eastman Chemical Co.) was purchased from Quimica Alfa, and EQ (Monsanto Co.) was purchased from Novus. Solvents and salts of analytical grade were purchased from Merck Quimica Chilena (Santiago, Chile). Refined sardine oil was provided by CORPESCA SA (Mejillones, Chile).

Reaction vessel. The cooker is simulated by a 3.0 L capacity cylindrical reactor (50 cm, h × 20 cm, i.d.) manufactured of 316 stainless steel and opened at one side. The mouth of the reactor is hermetically closed by means of a 316 stainless steel lid, which is adjusted to the body of the reactor with five stainless steel screws. The lid has an inserted remote temperature measuring probe connected to a digital thermometer (Hanna Instruments model H.I. 9043 connected to a K-type probe; Padova, Italy). Hermetic adjustment of the lid to the body of the reactor is achieved by a temperature resistant silicone O-ring.

Methods. Assay of the thermal stability of each antioxidant was carried out by adding 1.5 L of distilled water to the reactor and 900 g of 5-mm glass beads to simulate the organic material to be cooked and to help the dispersion of the tested antioxidant. For each assay, 20 g of a finely powdered sample of the tested antioxidant was added to the reactor and suspended in the water by vigorous hand-stirring. Once the reactor was sealed with the lid, this was placed in an 82 L capacity electric oven (Fisher Isotemp 838-F, Cincinnati, OH) and heated at 100, 125, 150, 175, or 200°C (according to the temperature sensed by the probe) in periods of 1 and 2 h. At the end of each heating assay, the reaction vessel was allowed to cool to ambient temperature. Thereafter, the lid was opened, and the contents of the reactor (the antioxidant suspension together with the glass beads) were poured into a 1000 mL Buchner porcelain filter. The glass beads were additionally washed with 200 mL of cool (4°C) distilled water. The antioxidant suspended in the reaction mixture was recovered by filtration through glass fiber filters (borosilicate MFS GC-50; Advantec, Pleasanton, CA). Filters were dried at 40°C for 60 min and weighed to estimate the antioxidant recovered after the thermal treatment by comparison to the initial amount added to the reactor.

Rancimat assay. The effectiveness of each antioxidant was assayed with the Rancimat 679 (Metrohm, Herisau, Switzerland) oxidative stability test at 65°C with an air flow of 20 L/h, and in the induction period (IP) operation mode (6). Recently refined sardine oil with no added antioxidant was subjected to high-vacuum distillation as described by E. Dinamarca *et al.* (7) to reduce peroxides and other volatile compounds, and maintained under N_2 atmosphere in sealed brown glass bottles. Previous to the oxidation assay, each antioxidant was added to the oil from an ethanolic concentrated antioxidant solution to obtain a final antioxidant concentration of 500 ppm in the oxidation vessel. Results were calculated from the ratio of the IP (expressed in hours) obtained for the antioxidant assayed after the thermal treatment (IP_f) and the IP obtained before the thermal treatment (IP_i), and expressed as percentage of inactivation (inactivation %) with the equation:

$$\text{inactivation } \% = (1 - IP_f/IP_i) \times 100 \quad [1]$$

Statistical analysis. Results represent the average ± SD of six simultaneous assays carried out in the Rancimat. Statistical significance of the differences between mean values was assessed by analysis of variance coupled with Duncan's multiple-range test at the 5% level of significance (8).

RESULTS AND DISCUSSION

The low water-solubility of the antioxidants assayed (9) allows a 92–96% recovery for BHT, BHA, and TBHQ. Recovery of EQ (adsorbed in vermiculite) was 87–91%. Table 1 shows the IP_f obtained for each antioxidant after the thermal treatment at different temperatures, compared to the IP_i obtained for the same antioxidant in its untreated form after 1 and 2 h of heating, when assayed according the Rancimat test using sardine oil as a substrate of oxidation. The four untreated antioxidants show good inhibitory effect (high IP_i) against oxidation induced in sardine oil by the Rancimat conditions (control compared to control + antioxidant, Table 1). However, after application of temperature, differences are observed for the IP_f of each antioxidant when compared to its respective IP_i, BHT and TBHQ exhibited a higher thermal

TABLE 1
Induction Period (IP) for Sardine Oil Oxidation Assessed Through the Rancimat Test, After the Addition of BHT, BHA, TBHQ, or EQ Thermally Treated at Different Temperatures and During 1 and 2 h[a]

Antioxidant (500 ppm)	IP_i		IP_f									
			100°C		125°C		150°C		175°C		200°C	
	Control – Antiox.	Control + Antiox.	1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h
BHT	13.2 ± 0.8	35.8 ± 1.9	31.7 ± 2.2	30.4 ± 0.9[b]	28.5 ± 0.8[b]	26.5 ± 1.4[b]	26.3 ± 1.2[b]	24.8 ± 0.9[b]	20.9 ± 1.2[b]	19.1 ± 1.7[b]	17.4 ± 0.8[b]	15.2 ± 1.7[b]
BHA	13.3 ± 0.7	33.1 ± 1.2	25.7 ± 0.8[b]	22.1 ± 1.2[b]	18.7 ± 1.3[b]	16.5 ± 0.9[b]	11.1 ± 0.2[b]	11.4 ± 1.1[b]	13.6 ± 0.8[b]	12.1 ± 0.7[b]	13.2 ± 0.8[b]	12.2 ± 0.4[b]
TBHQ	13.2 ± 0.6	40.4 ± 0.8	35.9 ± 0.6	34.2 ± 1.2[b]	31.4 ± 1.1[b]	33.1 ± 0.8[b]	30.4 ± 0.7[b]	29.5 ± 0.8[b]	27.3 ± 0.6[b]	26.2 ± 0.4[b]	25.4 ± 1.2[b]	25.1 ± 1.6[b]
EQ	13.4 ± 0.6	39.2 ± 1.4	27.2 ± 1.4[b]	25.6 ± 0.8[b]	22.7 ± 0.9[b]	21.4 ± 0.3[b]	16.2 ± 0.7[b]	14.5 ± 0.6[b]	13.2 ± 0.7[b]	13.8 ± 0.4[b]	12.8 ± 0.5[b]	12.0 ± 0.2[b]

[a]Results represent the average ± SD of six assays.
[b]Different from control + antiox. for each antioxidant; $P < 0.05$. IP_i, IP before thermal treatment; IP_f, IP after thermal treatment; BHT, 2,6-di-tertiary-butyl-4-methyl phenol; BHA, tertiary-butyl-4-hydroxyanisole; TBHQ, tertiary butylhydroquinone; EQ, 6-ethoxy-1,2-dihydro-2,4-trimethylquinoline, Antiox., Antioxidant.

stability than BHA and EQ at each temperature assayed. It is remarkable that the heating period at each temperature does not substantially modify the antioxidant behavior, being that the temperature is the most crucial factor.

Figure 1 shows the results expressed as inactivation percentage. From this figure it is clear that the antioxidants assayed can be divided into two well-differentiated groups: those exhibiting higher thermal stability (BHT and TBHQ) and those showing lower thermal stability (BHA and EQ). At 100°C BHT and TBHQ show 11–12% inactivation, whereas BHA and EQ show 24 and 30% inactivation, respectively, at the same temperature. At 150°C, which is the temperature most frequently used for processing poultry by-products, BHT and TBHQ show around 25% inactivation. However, at the same temperature BHA and EQ are inactivated by 62 and 56%, respectively. It is interesting that up to 150°C BHA and EQ show no further inactivation (flattening curves, Fig. 1A and B). This is probably due to the formation of terminally stable structures having residual antioxidant activity. However, this speculation needs further chemical and structural confirmation, which are beyond the scope of this research. At the highest temperature assayed (200°C), all antioxidants showed variable

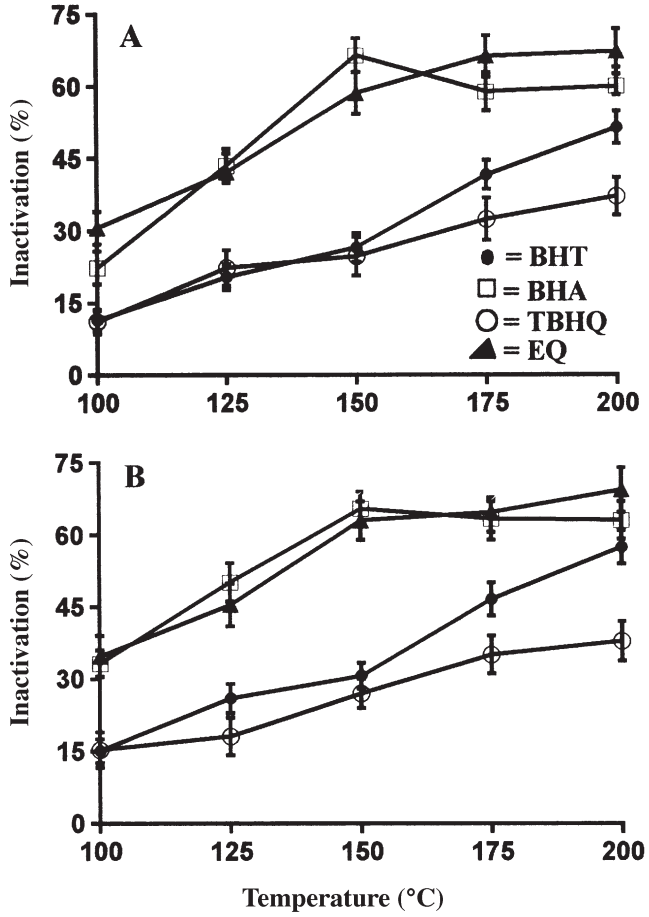

FIG. 1. Thermal inactivation (%) of synthetic antioxidants after 1 (A) and 2 h (B) of treatment. Results represent the average ± SD of six assays. BHT, 2,6-di-tertiary-butyl-4-methyl phenol; BHA, tertiary-butyl-4-hydroquinone; TBHQ, tertiary butyl-hydroquinone; EQ, 6-ethoxy-1,2-dihydro-2,4-trimethylquinoline.

but significant inactivation; TBHQ exhibited the highest thermal stability, and EQ exhibited the lowest thermal stability. BHA is commercially expended as a mixture of 2- and 3-isomers of tertiarybutyl-4-methoxy phenol (10). Because of the tertiarybutyl group ortho or meta to the hydroxyl group, BHA is referred to as a hindered phenol. This steric hindrance is believed to be responsible for the relative ineffectiveness of BHA as an antioxidant in vegetable oils because the tertiary butyl group interferes with the antioxidant activity of the phenolic group. This structural steric hindrance must also be responsible for the low thermal stability observed for this antioxidant. EQ is also thermally unstable according to our assay. The heating period at each temperature produced only a small reduction in the activity of the antioxidants assayed; therefore, this parameter may be considered less relevant than the temperature.

Our results demonstrate that temperature can drastically affect the antioxidant effectiveness of some frequently used commercial antioxidants. In our experimental model, BHA and EQ show a higher susceptibility to thermal inactivation (50 and 70% inactivation, respectively) at temperatures up to 175°C. This is very important because EQ is the most frequently applied antioxidant in the poultry feed industry and in poultry by-product processing because of its availability and low cost. TBHQ showed the highest thermal stability. However, in spite of its great effectiveness as antioxidant, TBHQ is not used by poultry by-product processing factories because the antioxidant is three to four times more expensive than EQ. BHT, which is also thermally stable, may be suitable to replace EQ in poultry by-product processing because its low price. Therefore, according to our results, replacement of EQ by TBHQ, or by another less expensive antioxidant such as BHT, might be considered with priority when the oxidative stability of the product is a concern. Viscera meal and/or chicken oil, having high levels of oxidation when mixed with the other components of the feeding formula, may significantly reduce the shelf life of the feed (11). Also, birds that are fed with oxidized by-products develop different metabolic abnormalities, such as encephalomalacia (crazy chick disease) (12), and other diseases which result in slow growth, increased susceptibility to infections, and off-flavored meats (13). To our knowledge, no other study on thermal stability of synthetic antioxidants is available in spite of the relatively abundant information about the effect of heating on some natural antioxidants such as lycopene (14), lutein (15), β-carotene (16), and flavonoids (17).

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