12

Antioxidants: Regulatory Status

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1. INTRODUCTION

Oxidation of unsaturated lipids is a major cause of food quality deterioration by giving rise to the development of off-flavor compounds and loss of nutritional value of food products (1). Although it has been known for a long time that lipid oxidation can be induced by catalytic systems such as light, temperature, enzymes, metals, and metalloproteins; the mechanism of oxidation reactions remained uncertain until the 1940s when free radicals and reactive oxygen species were found to be involved in oxidation processes by the pioneering work of Farmer et al. (2), Bolland and Gee (3), and Bateman et al. (4–6). Furthermore, antioxidants were found to protect lipids against oxidation either by quenching free radicals or scavenging oxygen, among others (6). Antioxidants are substances that, when present in foods at low concentrations compared with that of an oxidizable substrate, markedly delay or prevent the oxidation of the substrate (7). Antioxidants that fit in this definition include free radical scavengers, inactivators of peroxides, and other reactive oxygen species (ROS), chelators of metals, and quenchers of secondary lipid oxidation products that produce rancid odors (8). Antioxidants have also been

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used in the health-related area because of their ability to protect the body against damage caused by ROS as well as reactive nitrogen species (RNS) and those of reactive chlorine species (RCS) (9).

Antioxidants can be broadly classified by their mechanism of action as primary antioxidants, which break the chain reaction of oxidation by hydrogen donation and generation of more stable radicals, and secondary antioxidants, which slow the oxidation rate by several mechanisms, including chelation of metals, regeneration of primary antioxidants, decomposition of hydroperoxides, and scavenging of oxygen, among others. These substances may occur naturally in foods, such as tocopherols and ascorbic acid; however, natural antioxidants are often, at least partially, lost during processing or storage, thus exogenous antioxidants are intentionally added to products or their precursors participate in the formation of antioxidants during processing. Although there are many of compounds that have been proposed to inhibit oxidative deterioration processes, only a few can be used in food products (10). Antioxidants for use in food processing must be inexpensive, nontoxic, effective at low concentrations (0.001–0.02%), capable of surviving processing (carry-through), stable in the finished products, and devoid of undesirable color, flavor, and odor effects. In general, the selection of antioxidants depends on products, compatibility, and regulatory guidelines (11). In this chapter, the properties and applications of antioxidants in foods as well as their regulatory status are discussed.

2. SYNTHETIC ANTIOXIDANTS

Although the use of antioxidants dates back to ancient times when herbs and spices were used in food preservation, modern antioxidant technology is only about 60 years old. Since free radicals were found to be responsible for lipid oxidation, hundreds of natural and synthetic compounds have been evaluated for their efficacy as radical scavengers or for their other inhibitory effects. Among them, only four synthetic antioxidants are widely used in foods; namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) (7, 12). Scientists are attempting to develop novel synthetic antioxidants aimed at retarding the effects of free-radical-induced damage in various food products as well as in the human body cells (13). Synthetic antioxidants used in the food industry can be added as direct additives or indirectly through diffusion from packaging material (6).

All antioxidants have points of strengths and weaknesses. Therefore, certain points, such as thermal stability, effective concentration, and synergism, should be taken into consideration when selecting antioxidants for use in particular foods. Regulatory status is another factor that cannot be ignored, especially for some antioxidants that have been reported to show potential adverse health effects. Synthetic antioxidants have been tested for safety and approval for use in food at low concentrations on the basis of complex toxicity studies (10). Allowable limits for use of antioxidants vary greatly from country to country, and depend on the food product under consideration (11).

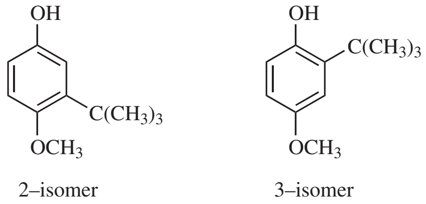


Figure 1. *Chemical structures of BHA molecules.*

2.1. BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene)

Phenolic and polyphenolic compounds are the most active dietary antioxidants (14). The structural variation of phenolic antioxidants directly influences their physical properties, resulting in differences in their antioxidant activity. BHA and BHT are examples of phenols, in which the aromatic ring contains alkyl groups (hindered phenols), which are extremely effective as antioxidants (11).

Chemically, BHA is a mixture of two isomers (2-*tertiary*-butyl-4-hydroxyanisole and 3-*tertiary*-butyl-4-hydroxyanisole) (Figure 1). The 3-isomer is generally considered to be a better antioxidant, and accounts for 90% of the commercial BHA (12). BHA is a white, waxy solid that is sold in the form of flake or tablet. It is a highly fat-soluble monophenolic antioxidant that is extensively used in bulk oils as well as oil-in-water emulsions (10–12). It is effective in animal fats and relatively ineffective in vegetable oils. Demonstrating considerable effectiveness in controlling the oxidation of short-chain fatty acids, BHA is frequently used for the preservation of coconut and palm kernel oil in cereal and confectionery products (10). BHA is good in baking because of its stability to heat and its mild alkaline conditions, although its application in frying is limited due to its volatility (11). However, it can be added to packaging materials to provide protection to food products inside the package through volatilization (12). BHA is particularly useful in protecting the odor and flavor of essential oils (10). Furthermore, BHA has been reported to possess antimicrobial activity (15–17) and is known to act synergistically with other antioxidants such as BHT.

BHT (3,5-di-*tert*-butyl-4-hydroxytoluene) (Figure 2) is a white crystalline solid with properties similar to BHA (12). It is appropriate for thermal treatment but not as stable as BHA (11). Being able to regenerate BHA, BHT is commonly used in

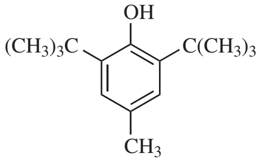


Figure 2. *Chemical structure of BHT.*

combination with BHA to provide greater antioxidant activity (18). BHT does not have an optimum concentration; usually, BHA/BHT mixtures are added to foods at levels of up to 0.02% (10, 11). Both BHA and BHT have a slight phenolic odor, and may impart undesirable odor in foods when used at high temperature for an extended period of time (10, 11).

Although synthetic antioxidants have widely been used in the food industry, there are some arguments about their safety (19). The use of BHA and BHT in foods has been decreased due to their potential action as promoters of carcinogenesis (20). In addition to the carcinogenicity of BHA in the forestomach of rodents, BHA and BHT have been reported to be cytotoxic (21–23). Furthermore, a suggestion has been made that BHT be withdrawn from use in all foods because of its possible adverse effects on the kidney and liver as well as lung tissues of rat (24, 25). However, some scientists have noted that the metabolism of BHT in rat and man are too widely different to allow a proper hazard assessment of BHT in humans (26). It is generally considered that permitted food antioxidants, such as BHA and BHT, have a considerable safety margin; for instance, the dose for enhancement of carcinogenesis is at least 1500-fold greater than that in human exposure (27, 28). Meanwhile, BHA and BHT have been reported by some researchers to pose no cancer hazard to humans and, on the contrary, have health benefits related to their anticarcinogenic and antimutagenic properties as well as inhibition of cholesteral oxidation (29–32).

Despite positive and negative reports of these synthetic antioxidants on human health, their use is subject to regulation, in the United States, under the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA); in Canada, the Food and Drug Regulations (National Health and Welfare); in Europe, the European Economic Community (EEC); and in Japan, the Food Sanitation Law. Many other countries have adopted regulations similar to those used in the United States, with significant differences existing both in the antioxidants approved and in their application and level of usage (10, 11). According to the existing food additive regulations published by the FDA, BHA and BHT are lawful for use individually or in combination at a maximum level of 0.02%, or 200 ppm, based on the lipid content of food products, as specified by the Code of Federal Regulations (CFR) (6, 7, 12). Although BHA and BHT are effective at low concentrations, they become pro-oxidant at high levels in foods (11, 33). As specified in 21CFR, 172.100, and 172.115, limitations for BHA and BHT, alone or in combination for specific products, are as follows: 10 ppm in potato granules; 50 ppm in dehydrated potato shreds, dry breakfast cereals, potato flakes, and sweet potato flakes; and 200 ppm in emulsion stabilizers for shortenings (11). BHA and BHT are not allowed in fish products (5). The summery of regulations, applications, and properties of BHA and BHT are shown in Tables 1 and 2.

The daily dietary intakes of BHA and BHT have been estimated in many countries. The daily intakes of BHA and BHT in Japan in 1998 were 0.119 and 0.109 mg/d/person, which reflect 0.5% and 0.7% of the acceptable daily intake (ADI), respectively (35). The estimates of theoretical maximum daily intake (TMDI) of BHA and BHT in Brazil published in 2001 were in the range of

TABLE 1. Properties, Applications, and Regulations of BHA.

NAME:	**Butylated hydroxyanisole (BHA)**	
CATEGORY:	Antioxidant	
FOOD USE:	Bakery products/Meat products/Spices/Cereals/Dehydrated mashed potatoes/Beverage mixes/Dessert mixes/Nuts/Vitamins/Yeast/Vegetable oils/Animal fats/Processed cheeses/Margarine/Essential oils/Chewing gum base	
SYNONYMS:	Mixture of two isomers: 3-*tertiary* butyl-4-hydroxyanisole and 2-*tertiary* butyl-4-hydroxyanisole/(1,1-dimethylethyl)-4 methoxyphenol/E320/ Antracine 12/Embanox/Nipantiox/Sustane BHA/ Sustane 1-F/Tenox 4B/ Tenox 5B	
FORMULA:	$(CH_3)_3CC_6H_3OCH_3OH$	
MOLECULAR MASS:	180.25	
PROPERTIES AND APPEARANCE:	White waxy flakes or tablets	
MELTING RANGE IN °C:	48–55	
FLASH POINT IN °C:	130	
PURITY %:	Not less than 98.5 of 2-isomer and not less than 85 of 3-isomer	
SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:		
in water:	**at 20°C**	Insoluble
in vegetable oil:	**at 25°C**	30% cottonseed oil 40% coconut, corn, peanut oils 50% soybean oil
in ethanol solution:	**100% at 25°C**	$>$25%
in propylene glycol:	**at 20°C**	70%
FUNCTION IN FOODS:	Antioxidant preservative by terminating free radicals formed during autoxidation of unsaturated lipids. It also possesses antimicrobial activity as a phenolic compound.	
ALTERNATIVES:	BHT; PG; TBHQ	
SYNERGISTS:	BHT; propyl gallate; methionine; lecithin; thiodipropionic acid; citric acid; phosphoric acid	
FOOD SAFETY ISSUES:	This antioxidant has not been subjected to great criticism of safety. However, suspected for tumor formation in animals with forestomach.	
LEGISLATION:	**USA:** Maximum usage level approved for general use; FDA 0.02% and USDA 0.01% of weight of fat. Special applications include: Chewing gum base: 0.01% by weight of chewing gum base Active dry yeast or dry material Emulsion stabilizer: 0.02% by weight of emulsion, shortenings, stabilizer Potato flakes, sweet potato flakes: 0.005% by weight, dry breakfast cereal, of food material, packaging material Potato granules: 0.001% by weight of potato granules Dry mixes for beverages: 0.009% of material and desserts Beverages and desserts, prepared from dry mixes: 0.0002% Dry diced glazed fruits: 0.0032% Flavor substances: 0.5% of essential oil content **U.K. and EUROPE:** approved **CANADA:** approved **AUSTRALIA/PACIFIC RIM and JAPAN:** approved	

Adapted from (34).

TABLE 2. Properties, Applications, and Regulations of BHT.

NAME:	**Butylated hydroxytoluene (BHT)**	
CATEGORY:	Antioxidant	
FOOD USE:	Breakfast cereals/Baked goods/Potato chips/Vegetable oils/Snack foods/Butter/Margarine/Frozen seafoods/Chewing gum base	
SYNONYMS:	2,6-*bis* (1,1-dimethylethyl)-4-methylphenol/2,6-di-*tert*-butyl-*p*-cresol/2,6-di-*tert*-butyl-4-methylphenol/E321/Antracine 8/Ionol CP/Dalpac/Impruvol/Vianol/Tenox BHT/Tenox 8/Sustane BHT	
FORMULA:	$[(CH_3)_3C]_2C_6H_2CH_3OH$	
MOLECULAR MASS:	220.34	
PROPERTIES AND APPEARANCE:	White granular crystals with slight odor	
MELTING RANGE IN °C:	69–72	
FLASH POINT IN °C:	118	
PURITY %:	Not less than 99	
SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:		
in water:	**at 20°C**	Insoluble
in vegetable oil:	**at 25 °C**	30% cottonseed, coconut, corn, peanut and soybean oils
in ethanol solution:	**100% at 25°C**	25%
in propylene glycol:	**at 20°C**	Insoluble
FUNCTION IN FOODS:	Antioxidant preservative; prevents oxidative rancidity development in oil-containing foods by terminating free radicals formed during autoxidation of unsaturated lipids. It possesses antimicrobial activity as a phenolic compound.	
ALTERNATIVES:	BHA; PG; TBHQ	
SYNERGISTS:	BHA	
FOOD SAFETY ISSUES:	This antioxidant has not been subjected to great criticism over safety.	
LEGISLATION:	**USA:** Maximum usage level approved for general use; FDA 0.02% and USDA 0.01% of weight of fat. Special applications include: Enriched rice: 0.0033% Nonalcoholic beverages, frozen raw breaded shrimp, mixed nuts and margarine: 0.02% based on oil content Dry sausage: 0.003% Fresh pork sausage, brown-and-serve sausage, pre-grilled beef patties, pizza toppings, meatballs, dried meats: 0.01% Rendered animal fat or combination with vegetable fat, poultry fat or various poultry procucts: 0.01% Dry breakfast cereals: 0.005% Emulsion stabilized for shortening: 0.02% Potato granules: 0.001% Potato flakes, sweet potato flakes, dehydrated potato shreds: 0.005% **U.K. and EUROPE:** approved **CANADA:** approved **AUSTRALIA/PACIFIC RIM and JAPAN:** approved	

Adapted from (34).

0.09–0.15 and 0.05–0.10 mg/kg body weight, respectively, indicating that it is unlikely to exceed the ADI (0.5 and 0.3 mg/kg body weight) (36). In the Netherlands, the mean intake of BHA and BHT in 2000 was 105 and 351 μg/day (37). In Italy, the likelihood of exceeding the ADI for BHA was very low. However, the TMDI of BHT was above the ADI. The three food categories, ‘‘pastry, cake, biscuits,’’ ‘‘chewing gums,’’ and ‘‘vegetable oils and margarine,’’ were the major sources of BHT and contributed 74% of the TMDI (38).

2.2. TBHQ (*tert*-Butylhydroquinone) and Gallates

TBHQ (Figure 3) is a beige powder or is a white-to-tan crystal that is used frequently in frying applications with highly unsaturated vegetable oils. Its solubility in different solvents declines in the order of alcohol > fats > water. As a diphenolic antioxidant, TBHQ is more effective in vegetable oils than BHA and BHT. It is stable to heat and is regarded as the most effective antioxidant in preventing the oxidation of frying oils and an alternative or supplement to oil hydrogenation for increasing oxidative stability (10, 11). TBHQ shows excellent synergism with other antioxidants such as citric acid. A ternary mixture containing TBHQ, monoacylglycerol citrate (MGC), and ascorbyl palmitate (AP) exhibited the highest thermal stability and provides optimum protection for oil during high-temperature processing (39). TBHQ mixed with BHA and BHT can increase the smoke point of fats and oils (40).

Three esters of gallic acid are approved for use in foods, namely propyl gallate (PG), octyl gallate, and dodecyl gallate (Figure 4). PG is a white crystalline powder that is slightly soluble in both water and fat, whereas the higher octyl and dedecyl gallate are practically insoluble in water but dissolve easily in fats and oils (10).

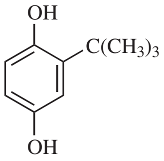
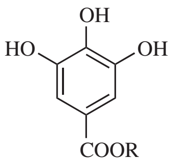


Figure 3. *Chemical structure of TBHQ.*



R = C_3H_7	Propyl Gallate
= C_8H_{17}	Octyl Gallate
= $C_{12}H_{25}$	Dodecyl Gallate

Figure 4. *Chemical structures of different alkyl gallates.*

Therefore, PG is widely used in foods where lipid-soluble antioxidants such as BHA, BHT, and TBHQ are not suitable. PG is inappropriate for frying due to its poor stability at high temperatures. It decomposes at its melting point of 148°C (11, 12). Gallates can form undesirable, dark-colored complexes with iron and copper; thus, they are sold as a mixture with metal chelators such as EDTA. Gallates also act synergistically with other antioxidants (11, 12).

As synthetic antioxidants, the safety of TBHQ and gallates has been questioned and, similar to BHA and BHT, both drawbacks and benefits of TBHQ and gallates have been reported. According to the literature, TBHQ exhibited a nontypical mode of cell death and proved cytotoxic toward human monocytic leukaemia cells (41). It caused apoptosis and significantly promoted DNA damage (42–44). PG suppressed humoral immunity (45). The coadministration of TBHQ or PG with sodium nitrite promoted forestomach carcinogenesis (46). Meanwhile, TBHQ and PG have been reported to be beneficial to human health. TBHQ was reported to be an effective inhibitor of cholesterol oxidation (32, 47), and PG provided inhibition of foodborne pathogens (48). Anticarcinogenic and antimutagenic activities of TBHQ and PG have also been reported (29, 49).

In accordance with regulations concerning the use of antioxidants in foods, TBHQ is permitted for food use by the FDA and the USDA at less than 0.02% and 0.01%, respectively. At levels higher than 0.02%, TBHQ may exert a pro-oxidant effect. The CFR specifies the maximum addition of TBHQ as 0.02% (200 ppm) (6, 12). However, the combination of TBHQ and PG is illegal (12). In Japan and European coutries, addition of TBHQ in foods is not allowed (7, 11), although its use in Canada is quite recent and dates back to 1999. PG is the only gallate permitted in foods in the United States and Canada, but the use of higher alkyl gallates is approved in several European countries (10). Gallates have optimum concentrations for antioxidant activity and may act as pro-oxidants when used at high levels (10). Tables 3 and 4 show detailed information on the regulatory status of TBHQ and PG in various applications, respectively. With respect to daily intake, according to investigations in Brazil and Italy, the estimate of TMDI for TBHQ and gallates was very low and unlikely to exceed the ADI (36, 38).

2.3. Erythorbic Acid and Ascorbyl Palmitate

Erythorbic acid (or D-ascorbic acid) (Figure 5) is a white or slightly yellow crystalline powder that is often used in fruits and cured meats to enhance curing action and to stabilize the color of food products (34). Unlike its isomer L-ascorbic acid, erythorbic acid is not a natural constituent of foods and has minimal Vitamin C activity (11).

Ascorbyl palmitate (Figure 6), a synthetic derivative of ascorbic acid, is a white powder with a soapy taste and citrus-like odor (34). It has better lipid-solubility compared with that of ascorbic acid and its salts, and is often used in combination with α-tocopherol in lipid-containing foods (11). Ascorbyl palmitate prevents oxidative rancidity by quenching singlet oxygen, among other modes of action (34).

TABLE 3. Properties, Applications, and Regulations of TBHQ.

NAME:	***Tert*-butylhydroquinone (TBHQ)**
CATEGORY:	Antioxidant
FOOD USE:	Dry cereals/Edible fats/Margarine/Pizza toppings/Potato chips/Poultry/Dried meats/Sausages/Beef patties/Vegetable oils
SYNONYMS:	2-(1,1-dimethylethyl)-1,4-benzenediol/mono-*t*-butyl hydroquinone/ Sustane TBHQ/Tenox TBHQ
FORMULA:	$(CH_3)_3CC_6H_3(OH)_2$
MOLECULAR MASS:	295
PROPERTIES AND APPEARANCE:	White to tan color solid crystals, having a characteristic odor
MELTING RANGE IN °C:	126.5–128.5
FLASH POINT IN °C:	171
PURITY %:	99
SOLUBILITY % AT VARIOUS TEMPERATURE /pH COMBINATIONS:	
in water:	**at 20°C** $<1\%$ **at 100°C** 5%
in vegetable oil:	**at 20°C** 10% in corn, cottonseed, and soybean oils
in ethanol solution:	**100%** 25%
in propylene glycol:	**at 20°C** 30%
FUNCTION IN FOODS:	Prevents oxidative rancidity developmentin foods by terminating free radicals formation
ALTERNATIVES:	BHA; BHT
SYNERGISTS:	BHA; citric acid
FOOD SAFETY ISSUES:	Has shown mutagenicity *in vivo*; therefore, some countries consider that TBHQ does not meet current standards of toxicity testing.
LEGISLATION:	**USA:** Not allowed to use in combination with PG. For general usage, FDA-0.02%, USDA-0.01%, based on lipid content of food. Special food use: Nonalcoholic beverages Margarine, mixed nuts: 0.02% alone or in combination based on lipid content Dried meats Fresh pork or beef sausages Pre-grilled beef patties Pizza toppings Meatballs: 0.01% based on weight of finished product Rendered animal fats **EUROPE, U.K., NORWAY, DENMARK, SWEDEN, SWITZERLAND:** not allowed for food use **CANADA:** allowed for food use **AUSTRALIA/PACIFIC RIM:** **AUSTRALIA, NEW ZEALAND:** allowed for food use **JAPAN:** not allowed for food use

Adapted from (34).

TABLE 4. Properties, Applications, and Regulations of PG.

NAME:	**Propyl gallate (PG)**
CATEGORY:	Antioxidant
FOOD USE:	Chewing gum base/Nonalcoholic beverages/Margarine/ Mixed nuts/Fresh or dry sausages/Pre-grilled beef patties/Rendered animal fat/Pizza toppings and meatbal
SYNONYMS:	*n*-propyl-3,4,5-trihydroxybenzoate/3,4,5-trihydroxybenzoic acid/Gallic acid, propyl ester/E310/Nipa 49/ Nipagallin P/Tenox PG/Sustane PG
FORMULA:	$(HO)_3C_6H_2COOCH_2CH_2CH_3$
MOLECULAR MASS:	212.20
PROPERTIES AND APPEARANCE:	White crystalline powder with slight odor
MELTING RANGE IN °C:	146–150
FLASH POINT IN °C:	187
PURITY %:	Not less than 98 and not more than 102.5 on the dried basis
SOLUBILITY % AT VARIOUS TEMPERATURE/pH COMBINATIONS:	
in water:	**at 20°C** $<1\%$
in vegetable oil:	**at 20°C** 1% in cottonseed oil 2% in soybean oil insoluble in corn oil
in ethanol solution:	**100% at 25°C** $>$**60%**
FUNCTION IN FOODS:	Prevents oxidation rancidity development in lipid-containing foods by terminating free radicals formation during autoxidation of unsaturated lipids.
ALTERNATIVES:	BHA; BHT; TBHQ; octyl gallate; dodecyl gallate
SYNERGISTS:	BHA; BHT
FOOD SAFETY ISSUES:	Not subjected to great criticism over safety.
LEGISLATION:	**USA:** not allowed to use in combination with TBHQ. For general usage, FDA- 0.02% and USDA – 0.01% alone or in combination with BHT or BHA by weight of lipid proportion of food. Special applications include: Chewing gum base: 0.1% Nonalcoholic beverages: 0.1% Margarine: 0.1% Mixed nuts: 0.02% based on oil content French beef or pork sausages Brown-and-serve sausages Pre-grilled beef patties Pizza toppings and meatballs: for all these 0.01% based on weight of finished product Rendered animal fats or combination of such fats with vegetable fat: 0.01% based on lipid content **U.K.:** approved **EUROPE:** listed **CANADA:** approved **AUSTRALIA/PACIFIC RIM and JAPAN**: approved

Adapted from (34).

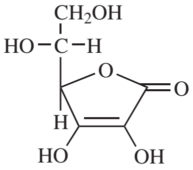


Figure 5. *Chemical structure of erythorbic acid.*

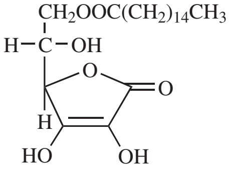


Figure 6. *Chemical structure of ascorbyl palmitate.*

More recently, replacement of the palmitate moiety with oleate has been proposed to increase solubility as well as antioxidant activity in oils (50).

Both erythorbic acid and ascorbyl palmitate have "Generally Recognized as Safe (GRAS)" status with the FDA (11). No restrictions on their usage levels are imposed except for the maximum addition of 0.02% for ascorbyl palmitate in margarine (34).

Table 5 shows the maximum levels permitted by the FDA for the four major synthetic antioxidants (BHA, BHT, PG, and TBHQ) in specific applications (51). The regulatory status for these antioxidants in the USA, Canada, and Europe is given in Table 6; Table 7 summarizes their status in other countries for which a listing could be found. In addition to the major synthetic antioxidants discussed above (BHA, BHT, TBHQ, gallates, erythorbic acid, and ascorbyl palmitate), several other

TABLE 5. Maximum Levels Permitted for Antioxidants in Specific Applications.

Food Type	Maximum Permitted Levels (ppm)			
	BHA	BHT	PG	TBHQ
Active dry yeast	1000	—	—	—
Beverages from dry mixes	2	—	—	—
Dehydrated potato shreds	50	50	—	—
Dried meant	100	100	100	100
Dry breakfast cereals	50	50	—	—
Dry diced fruits	32	—	—	—
Dry mixed for beverages and desserts	50	—	—	—
Dry sausage	30	30	30	30
Emulsion stabilizers for shortenings	200	200	—	—
Fresh sausage	100	100	100	100
Potato flakes	50	50	—	—
Poultry products	100	100	100	100

Adapted from (51).

TABLE 6. Regulatory Responsibility for Major Antioxidants.

	U.S. FDA	USDA	Canada (NHW)	Europe (EEC)
BHA	21 CFR 182.3169	9 CFR 318.7	Table XI, Part IV, B.1, 320	E320
BHT	21 CFR 182.3173	9 CFR 318.7	Table XI, Part IV, B.2, 321	E321
Gallates	21 CFR 184.1660	9 CFR 381.147	Table XI, Part IV, P.1, 324	E310-312
TBHQ	21 CFR 172.185	9 CFR 361.147	Table XI, Part IV, T.1A, 325	Not approved
Tocopherols	21 CFR 182.3890	9 CFR 318.147	Table XI, Part IV, T.2, 325	E306-309

Adapted from (10).

TABLE 7. Regulatory Approval Status of Major Antioxidants in Different Countries.

	Antioxidants			
Country	BHA	BHT	Gallates	TBHQ
Afghanistan	+	+	−	−
Argentina	+	+	−	+
Australia	+	+	+	+
Austria	+	−	−	−
Bahrain	+	+	+	+
Barbados	−	−	−	+
Belgium	+	+	−	−
Brazil	+	+	+	+
Chile	+	+	+	+
China	+	+	+	−
Columbia	+	+	+	+
Cyprus	+	+	−	−
Denmark	+	+	+	−
Ecuador	+	+	−	−
Finland	+	+	+	−
France	+	+	+	−
Germany	+	+	+	−
Gibraltar	+	+	−	−
Greece	−	−	+	−
Hong Kong	+	+	−	−
Hungary	+	+	−	−
Indonesia	+	−	−	−
Iran	+	+	+	+
Ireland	+	+	+	−
Israel	+	+	−	+
Italy	+	+	+	−
Jamaica	−	−	−	−
Japan	+	+	+	−
Kenya	+	−	+	−
Korea, South	+	+	+	+
Luxembourg	+	+	+	−
Malaysia	+	+	+	+
Malta	+	+	−	+
Mauritius	+	+	+	−
Mexico	+	+	+	+
Morocco	−	−	−	+

TABLE 7. (*Continued*)

Netherlands	+	+	+	−
New Zealand	+	+	+	+
Nigeria	+	+	−	−
Norway	+	+	+	−
Pakistan	+	+	−	−
Panama	+	+	+	+
Papua New Guinea	+	−	−	−
Peru	+	+	−	−
Philippines	+	+	+	+
Portugal	+	+	−	−
Saudi Arabia	+	+	+	+
Singapore	+	+	+	−
South Africa	+	+	+	+
Spain	+	−	−	−
Sweden	+	+	+	−
Switzerland	+	+	+	−
Taiwan	+	+	+	+
Thailand	+	+	+	+
Trinidad/Tobago	+	+	+	−
Turkey	−	−	+	+
United Kingdom	+	+	+	−
Uruguay	+	+	−	−
Venezuela	+	+	+	+
Zimbabwe	+	−	−	−

Adapted from (10).

synthetic antioxidants have been used less frequently in the food and feed industry. These include ethoxyquin, trihydroxybutyrophenone (THBP), and some secondary antioxidants such as thiodipropionic acid and dilauryl thiodipropionate (10, 11). Novel synthetic antioxidants have been created in order to obtain stronger antioxidant activity than that of traditional ones (52). However, the general consumer rejection of synthetic food additives has led to a decrease in their use and an increased interest in their replacement with natural ingredients.

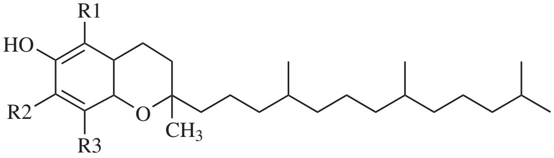
3. NATURAL ANTIOXIDANTS

Concerns about the safety of synthetic antioxidants have given rise to a large body of research on natural sources of antioxidants. Natural antioxidants allow food processors to produce stable products with “clean” labels of all-natural ingredients, as described by Reiche (11). They bring less rigorous burden-of-safety proof than that required for synthetic products (11). In addition to their antioxidant activity, some natural antioxidants, such as vitamins, minerals, and enzymes, are also regarded as nutrients due to their bioactivity. However, natural antioxidants may possess several drawbacks, including high usage levels, low antioxidant efficiency, undesirable flavor or odor, and possible loss during processing (11). The safety of natural antioxidants cannot be taken for granted because of their potential mutagenicity, carcinogenicity, teratogenicity, or other pathogenic activities (10). A case in point is nordihydroguariaretic acid (NDGA), which was removed from the GRAS list and is no longer of practical use (11, 19). NDGA is a natural constituent of creosote bush.

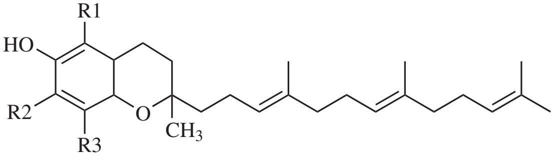
Ascorbic acid and tocopherols are the most important commercial natural antioxidants. In addition, many naturally occurring phenolic antioxidants have been identified in plant sources and vegetable extracts that may lend themselves for use in a variety of food applications (53). Recent research has focused on isolation and identification of effective antioxidants of natural origin (11).

3.1. Tocopherols and Tocotrienols

Tocopherols and tocotrienols, collectively known as tocols, are monophenolic and lipophilic compounds that are widely distributed in plant tissues (7). The main commercial source of natural tocopherols is the soybean oil. Tocotrienols, less common than tocopherols, are present in palm oil, rice bran oil, as well as cereals and legumes (11). Tocopherols and tocotrienols are classified into α-, β-, γ-, and δ-, depending on their chemical structures (Figure 7). In general, tocotrienols have a stronger antioxidant effect on lipid oxidation than tocopherols. The antioxidant activity of tocopherols is dependent on temperature and is in the order of δ- > γ- > β- > α-tocopherol (7). Tocopherols (mixed natural concentrate) are a golden



R1	R2	R3	
CH_3	CH_3	CH_3	α-tocopherol
CH_3	H	CH_3	β-tocopherol
H	CH_3	CH_3	γ-tocopherol
H	H	CH_3	δ-tocopherol



R1	R2	R3	
CH_3	CH_3	CH_3	α-tocotrienol
CH_3	H	CH_3	β-tocotrienol
H	CH_3	CH_3	γ-tocotrienol
H	H	CH_3	δ-tocotrienol

Figure 7. *Chemical structure of tocopherols and tocotrienols.*

brown colored, slightly viscous liquid with a characteristic odor. However, synthetic tocopherol (mixed α-, γ-, and δ-) is a yellow to brownish viscous oily and odorless liquid (34). Tocols are soluble in vegetable oils but insoluble in water. They function as a free radical terminator in autoxidation reactions, and they are often used in food products deficient in natural antioxidants, such as animal fats, waxes, and butterfat, among others (34, 54). Tocopherols act synergistically with ascorbic acid, citric acid, and phospholipids.

As natural antioxidants, tocopherols have GRAS status, and they are regarded as safe food additives. However, as noted earlier for other antioxidants, excessive addition of tocopherols may lead to pro-oxidant effects (6, 11). Furthermore, the hemorrhagic toxicity of a large dose of α-tocopherol has been reported (55). Therefore, use of tocopherols as antioxidants in foods is subject to regulations. The FDA (21CFR 182.3890) and the USDA (9CFR 318.147), Canada NHW (Table XI, Part IV, T.2, 325), and the EEC (E306-309) govern the regulations of tocopherols in foods in the United States, Canada, and European countries, respectively (10). In the United States, natural tocopherols are limited to 0.03%, i.e., 300 ppm in animal fats, and 0.02% in combination with BHA, BHT, and PG (9CFR 318.7). In the UK and some other European countries, their maximum addition is not to exceed 500 ppm (10, 34). With respect to ADI of α-tocopherol, it has been reported that an intake of 1000 mg/day is without risk, and 3200 mg/day is without any consistent risk (56). Actually, in the United States, the majority of men and women fail to meet the current recommendation for Vitamin E intake, according to a recent report on American diets (57).

3.2. Ascorbic Acid and Ascorbate Salts

L-ascorbic acid (Vitamin C) and its salts (sodium ascorbate and calcium ascorbate) (Figure 8) are widespread in plant tissues or are produced synthetically in large quantities (11). Ascorbic acid is a white or slightly yellow crystalline powder that is extensively used to stabilize beverages, fruits, and vegetables. Its application in fats and oils, however, is limited because of the insolubility in lipids. It acts as an antioxidant with multiple functions, including quenching various forms of oxygen, reduction of free radicals, and regeneration of primary antioxidants (34). The effect of ascorbic acid on lipid stability in foods is mainly due to synergistic interactions with other antioxidant compounds (58). It shows excellent synergism with α-tocopherol, citric acid, BHA, BHT, and metal chelators. Ascorbic acid strongly inhibits the depletion of α-tocopherol by regenerating it (6, 59).

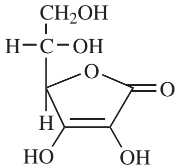


Figure 8. *Chemical structure of ascorbic acid.*

In addition to antioxidant activity, ascorbic acid also functions as Vitamin C, a flavorant, an acidulant, a color fixing, and a reducing agent in food products (11, 34). Moreover, it can diminish the generation of odor-active compounds in emulsions (60). However, the natural ascorbic acid in foods can be easily destroyed during processing as a result of susceptibility to heat, light, pH, oxygen, acrid smoke, and water activity; thus, it is often added to foods exogenously (61, 62).

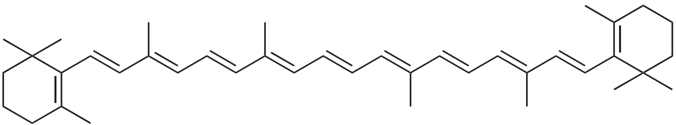
Ascorbic acid and ascorbate salts have GRAS status with no usage limits. According to the literature, Vitamin C is safe at supplementation levels of up to 600 mg/day, and higher levels of up to 2000 mg/day are without risk (56). The ascorbic acid and its salts carry GRAS status and with minimal associated organoleptic problems; thus, they are safe, stable, and good antioxidant candidates for use in foods (63). As natural or natural-identical products, they are highly recognized as antioxidant nutrients by consumers (11).

3.3. Carotenoids

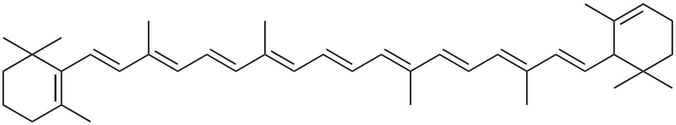
Carotenoids are yellow, orange, and red lipid-soluble pigments that occur widely in plants, fruits, and vegetables. They are 40-carbon isoprenoids with varying structures (Figure 9), and can be classified as carotenes and xanthophylls (11). Certain carotenoids are also referred to as pro-vitamins such as β-carotene, α-carotene, and β-cryptoxanthin. Carotenoids are antioxidant nutrients that act mainly as secondary antioxidants in foods by quenching singlet oxygen. They may also prevent oxidation by trapping free radicals in the absence of singlet oxygen (11). Carotenoids are a good synergist with tocopherols. β-Carotene, lutein, lycopene, and isozeaxanthin are typical carotenoids that effectively retard oxidation in foods. Astaxanthin has antioxidant activity that is ten times greater than that of β-caroten, lutein, zeaxanthin, and canthaxanthin, and is often used in fish products (64).

β-Carotene is a purple hexagonal prism or a red leaflet that is often used in fruit juices, cheese, dairy products, fats, and oils (34). It has poor solubility in most common solvents, and is highly reactive and unstable to heat, light, pH, oxygen, and the presence of metals, resulting in limited applications as a food antioxidant (11). In a high-oxygen concentration, β-carotene may exhibit a pro-oxidant, rather than an antioxidant effect in food products (61). Carotenoids are natural constituents of foods and have GRAS status. No limitation on their addition level has been stipulated.

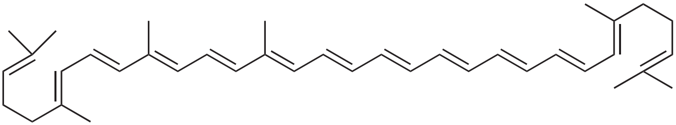
In addition to the three major classes of natural antioxidants (tocols, ascorbic acids, and carotenoids), several other natural substances have been identified that show antioxidant activity through different mechanisms; these include phospholipids, flavonoids, protein hydrolyzates, organic acids, sterols, Maillard reaction products, and enzymes. These are naturally occurring constituents of food and act as endogenous antioxidants that help prevent oxidation reactions. They are also regarded as a potential for replacement of synthetic antioxidants. A great deal of research has been conducted on evaluation of their antioxidant activity and methods of extraction. A variety of natural products can serve as sources of natural antioxidants, among which fruits and vegetables, spices and herbs, oilseeds, and animal



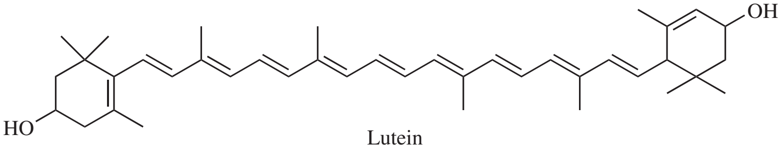
β-Carotene



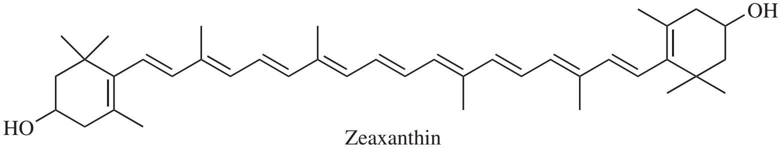
α-Carotene



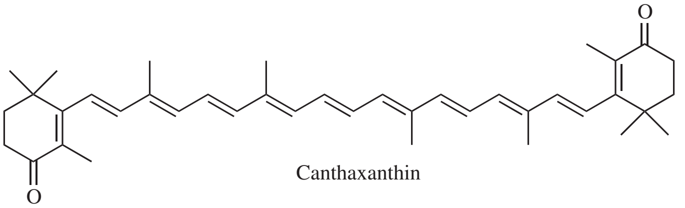
Lycopene



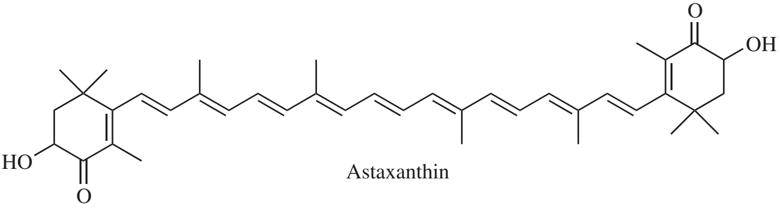
Lutein



Zeaxanthin



Canthaxanthin



Astaxanthin

__Figure 9.__ Chemical structures of carotenoids.

and microbial products have been considered (64, 65). Grapes, berry fruits, and citrus are rich sources of antioxidants (65). Among vegetables, garlic, broccoli, mushroom, and pulses have been shown to possess antioxidant effects (65–67); spinach powder has been reported to be capable of improving lipid stability in deep-fat-fried products (68). Aside from fruits and vegetables, several studies have confirmed that many spice and herb extracts show strong antioxidant activity, such as rosemary, sage, oregano, cinnamon, thyme, green tea, and evening primrose extract (65, 69–73). Flaxseed, sunflower, soybean, cottonseed, rapeseed, and sesame seed typify the sources of antioxidants from oilseeds (64, 74, 75). More recently, Shahidi et al. (76) have reported the antioxidant activity of de-fatted Niger seed extract. Animal products can serve as good sources of natural antioxidants, such as protein hydrolyzates (peptides and amino acids), carotenoids, chitosan, and enzymes (64, 77, 78). Furthermore, microbial fermentation is becoming a promising method for producing natural antioxidants (64).

These antioxidants are all-natural ingredients of foods and have GRAS status. However, because of the ability of some natural antioxidants to exhibit pro-oxidant activity, caution should be exercised when adding them to food systems (79). Furthermore, the safety of natural compounds with antioxidant activity should be established.

4. CONCLUSIONS

In foods that may undergo oxidation, antioxidants, endogenous or exogenous, function as an inhibitor to oxidation reactions through various mechanisms. Nevertheless, natural antioxidants are deficient in some foods and can easily deteriorate during processing or in storage, necessitating the use of synthetic antioxidants. However, most synthetic antioxidants are effective at low concentrations, and the addition of higher levels may lead to a pro-oxidant effect. Additionally, large doses of synthetic antioxidants have been reported to impart safety problems. Therefore, caution must be taken when selecting and adding antioxidants in food systems.

TABLE 8. Antioxidants Conventionally Permitted in Foods.

- ascorbic acid, sodium, calcium salts
- ascorbyl palmitate and stearate
- anoxomer
- butylated hydroxyanisole (BHA)
- butylated hydroxytoluene (BHT)
- *tert*-butylhydroquinone (TBHQ)
- citric acid, stearyl, and isopropyl esters
- erythorbic acid and sodium salt
- ethoxyquin
- ethylenediaminetraacetic acid (EDTA) and calcium disodium salt
- glycine
- gum guaiac
- lecithin
- ionox-100
- polyphosphates
- propyl, octyl, and dodecyl gallates
- tartaric acid
- trihydroxybutyrophenone
- tocopherols
- thiodipropionic acid, dilauryl and distearyl esters

Adapted from (64).

TABLE 9. ADI of Some Antioxidants Permitted in Foods.

Antioxidant	ADI (mg/kg body weight)
propyl gallate	0–2.5
BHA	0–0.5
BHT	0–0.125
TBHQ	0–0.2
tocopherols	0.15–2.0
gum guaiac	0–2.5
ethoxyquin	0–0.06
phosphates	0–70.0
EDTA	2.5
tartaric acid	0–30
citric acid	not limited
lecithin	not limited
ascorbic acid	not limited
sulfites (as sulfur dioxide)	0–0.7
ascorbyl palmitate or ascorbyl stearate (or the sum of both)	0–1.25

Adapted from (64).

Meanwhile, the safety of natural antioxidants should not be taken for granted as antioxidants from natural sources are attracting more and more attention. Adherence to regulatory guidelines remains a necessity.

The most common antioxidants permitted for use in foods in most countries are shown in Table 8. Table 9 presents the ADI of some antioxidants allocated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The food producer has full responsibility for the choice of suitable antioxidants according to the corresponding guidelines governed by regulatory laws of the individual country or the international bodies that declare their safety (64).

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