NATURAL BUTYLATED HYDROXYTOLUENE (BHT): A REVIEW

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Abstract. The 2,6-bis (1.1-dimethylethyl)-4-methylphenol (BHT), also known as butylhydroxytoluene, is a lipophilic (fat soluble) organic compound that is primarily used as an antioxidant food additive as well as in cosmetics, pharmaceuticals, jet fuels, rubber, petroleum products, and electrical transformer oil. BHT is prepared by the reaction of p-cresol (4- methylphenol) with isobutylene (2-methylpropene) catalyzed by sulfuric acid. Recently scientists have found out that BHT has carcinogenic effects on human foods and has been banned for such uses. A naturally occurring BHT was identified in the halophyte plant Mesembryanthemum crystallinum. It was also demonstrated that four freshwater phytoplankton, including a green alga (Botryococcus braunii) and three cyanobacteria [Cylindrospermopsis raciborskii, Microcystis aeruginosa and Oscillatoria sp.] were capable of producing BHT. The fact that BHT can be produced naturally in plant leaves or in Alga is very interesting and must be considered and explored for others species. The present paper was prepared to show the difference between natural and synthetic antioxidant and to focus research on the replacement of synthetic antioxidant by natural ones produced in nature like natural BHT.

Keywords: *antioxidant compounds, BHT, synthetic antioxidant replacement, halophyte plants, phytoplankton*

Introduction

Antioxidant chemistry has developed rapidly. In addition to natural antioxidants present in natural substances, scientists have developed a number of synthetic antioxidants, which have either replaced or supplemented natural antioxidants. Ironically antioxidants are not always effective or safe for a long period as they themselves are oxidized and their utility diminishes with time. Nevertheless antioxidants have effective's uses for a reasonable time. For many decade Butylated hydroxytoluene was the most prominent antioxidant having uses in diversified disciplines of food products, polymers, fats and oils. BHT was considered very safe for both human food and animals feeds. Recently scientists have found out that BHT has carcinogenic effects on human foods and has been banned for such uses. However, in many developing countries BHT is still used in food products because of its easy availability and relative cost advantages. Traditionally BHT has been used in petroleum industry in lubricating oils, as fuel additive, in petrochemicals, in vegetable oils. BHT which was not any longer recommended for human consumption was substituted by other antioxidants such as Butylated hydroxyl anisole (BHA) and tert-btuyl-hydroquinone (TBHQ) which was considered safe for prevention of oxidation for human food products.

BHT is prepared by the reaction of p-cresol (4- methylphenol) with isobutylene (2-methylpropene) catalyzed by sulfuric acid (Fiege et al., 2000). A naturally occurring BHT was identified in the halophyte plant Mesembryanthemum crystallinum (Bouftira et al., 2007). The species Mesembryanthemum crystallinum (family: Aizoaceae, order: Caryophyllales), also termed the common ice plant, has emerged as a model organism in plant molecular physiology (Adams et al., 1998). The plant's rise to relative fame started with the serendipitous discovery of a stress inducible switch from C3 photosynthesis to Crassulacean acid metabolism (CAM) (Cushman and Bohnert, 1999; Winter and von Willert, 1972). A second major area of interest centres on the plant's extreme stress tolerance, particularly tolerance to high salinity. During normal metabolism, plants generate reactive oxygen species (ROS), including superoxide radical (O2•−), hydrogen peroxide (H2O2), hydroxyl radical (HO•), and singlet oxygen (O2) (Qiu-Fang et al., 2005). ROS are overproduced in plants under stress, including drought and desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolet radiation, air pollutants, such as ozone and SO2, mechanical stress, nutrient deprivation, pathogen attack and high light stress (Allen, 1995). To mitigate the oxidative damage initiated by ROS, plants have developed a complex antioxidative system (de Azevedo Neto et al., 2006). In the halophyte plant Mesembryanthemum crystallinum, high photosynthetic active radiation and ultraviolet irradiance induce the accumulation of flavonoids (Ibdah et al., 2002). Abiotic stress particularly salinity induces BHT accumulation in M. crystallinum leaves (Bouftira et al., 2007).

Both algae and cyanobacteria are considered to be commercially potential sources of antioxidants. Although most photosynthetic organisms are exposed to a combination of light and high oxygen concentrations, which leads to the formation of free radicals and other strong oxidizing agents, they seldom suffer from serious photodynamic and DNA damage in vivo, suggesting that their cells contain protective antioxidative mechanisms and compounds (Park et al., 2005; Sigaud-Kutner et al., 2005; Sinha et al., 2003). A green alga (Botryococcus braunii Ku¨tz.) and three cyanobacteria [Cylindrospermopsis raciborskii (Wołłosz.), Microcystis aeruginosa (Ku¨tz.) and Oscillatoria sp.] were capable of producing this compound. Hexane extracts from all the studied species exhibited various degrees of antioxidative properties when they were tested with the b-carotene-linoleate (b-CL) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay. The highest antioxidant activity was observed in the crude extracts of M. aeruginosa and B. braunii, which displayed a similar activity to synthetic BHT. Gas chromatography/mass spectroscopy (GC-MS) analysis of the purified fractions revealed that the active compound was identical to synthetic BHT. Culturing under various irradiances gave rise to different magnitudes of BHT production in cyanobacterial cells (Babu and Wu, 2008).

Natural antioxidant

Natural antioxidants are those antioxidants that are found in natural sources, such as fruits, vegetables, and meats. There are several common natural antioxidants which are found in everyday foods, the most common of which being Vitamin C (ascorbic acid), Vitamin E (tocopherols), Vitamin A (carotenoids), various polyphenols including flavonoids, and anthocyanins (a type of flavonoid), Lycopene (a type of carotenoid), and Coenzyme Q 10, also known as Ubiquitin, which is a type of protein (*Figure 1*).

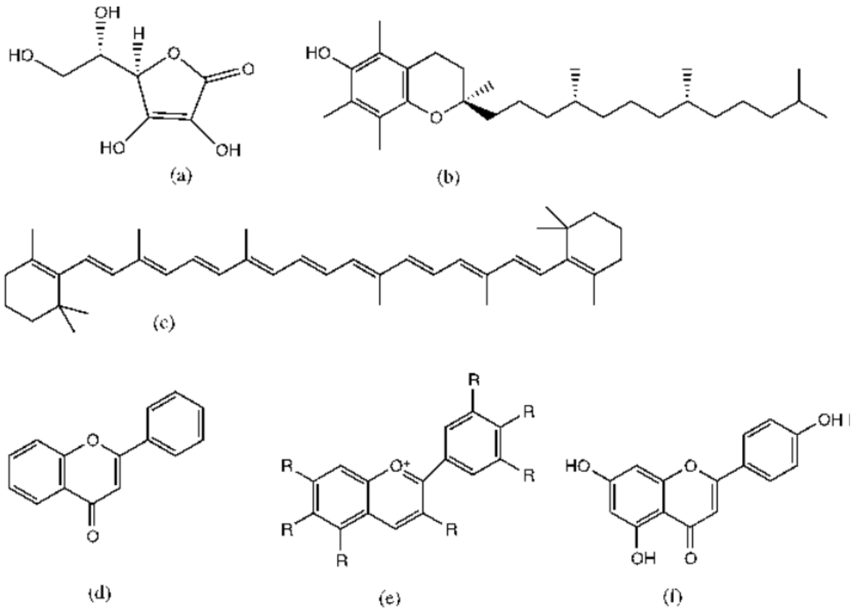


Figure 1. *Chemical structures of natural antioxidants. This figure shows the chemical structure of the following antioxidants (a) Ascorbic Acid, (b) Vitamin E, (c) Beta-Carotene, (d) Flavonoid, (e) Anthocyanin, and (f) Polyphenol.*

Types of natural antioxidants

Vitamin C, also known as ascorbic acid is one of the most prevalent natural antioxidants in most everyday diets. It is a polar, water soluble antioxidant and is found most commonly in fruit, particularly in citrus fruits such as oranges and lemons (McGhie and Walton, 2007). Additionally, it is found in some vegetables, such as tomatoes (Urquiaga and Leighton, 2000). It has been observed in laboratory trials that in lower doses (less than 30 mg), absorption of dietary Vitamin C is fairly high (approximately 50%). Vitamin E, part of a family of antioxidants known as tocopherols is a non-polar, fat soluble antioxidant. It is commonly found in several types of produce, such as cereal grains, broccoli, and Brussels sprouts (Croft, 1998). It can also be found in more lipid-rich source such as cooking oil like olive oil, sunflower oil, or safflower oil, and nuts like almonds and hazelnuts (Urquiaga and Leighton, 2000). In general, bodily absorption of Vitamin E is thought to be rather inefficient, with the body absorbing only 20-40% of dietary intake. β-carotene (which can be thought of interchangeably with Vitamin A) is a fat soluble antioxidant member of the carotenoid family. Vitamin A is mostly found in vegetables such as kale, carrots, sweet potatoes, apricots, papayas (Urquiaga and Leighton, 2000). Carotenoid bioavailability is considerably very low, approximately 10-30%, and just as with other antioxidants, absorption levels decrease as dose increases

Polyphenols are another major class of natural antioxidants. They are most commonly found in products such as teas, particularly green tea and rooibos (red) tea16, in addition to dark fruits such as Concord grapes (Carr et al., 2000). Bioavailability of polyphenols is approximately 15-20% of consumption, with absorption increasing when the consumed polyphenols do not have sugar molecules attached to them. Therefore, polyphenols from tea sources have superior absorptions to those from fruit sources, due to the relatively high sugar content of fruits (Quintavalla and Vicini, 2002). Among polyphenols, flavonoids are one of the most common sub-categories found naturally in foods. Flavonoids have been seen to have particularly high concentrations in foods such as dark chocolate, potatoes, lettuce, wheat, red wine, and black tea (Urquiaga and Leighton, 2000). Flavonoids have been seen to have much greater bioavailability when consumed from sources containing low quantities of sugar (approximately 50%) than when consuming flavonoids from sources with higher quantities of sugar, such as fruits (approximately 15%).53One type of flavonoid of particular interest are anthocyanins, which are typically found in dark fruit (such as blueberries, blackberries, Concord grapes, acai berries) and red wine (Carr et al., 2000). The final common type of natural antioxidant found in foods is the protein CoenzymeQ 10 (CoQ10), also known as Ubiquitin. Coq10 is a small, soluble protein used by animals as a natural antioxidant. Thus, it is found in substantial quantities in food sources such as fish and other meats, particularly organ meats (e.g. chicken liver), as well as in high protein plant sources such as wheat bran (Urquiaga and Leighton, 2000). The bioavailability of CoQ10 from meat sources and poultry has been seen to be approximately 60%.

Synthethic antioxidants

Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation (Shahidi et al., 1992). These antioxidants fall into two major categories depending on their mode of action. The primary antioxidants, which prevent the formation of free radicals during oxidation, can further include three major categories. Free Radical terminators, Oxygen scavengers, and Chelating agents (Venkatesh and Sood, 2011) (*Figure 2*). The radical terminators constitute the bulk of the synthetic antioxidants used as preservatives in food and these antioxidants prevent lipid oxidation by terminating the free radical chains. The important examples of radical terminators include Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary butyl-Hydroquinone (TBHQ), and gallates such as propyl gallate (PG), dodecyl gallate (DG) and octyl gallate (OG). The examples of oxygen scavengers which function as reducing agents, include sulphites, glucose oxidase and ascorbyl palmitate. The chelating agents prevent oxidation of lipids by binding the lipid oxidation catalysts such as heavy metals (iron, copper, etc). They do so by either precipitating the metal or by occupying all its coordination sites. Examples of such agents include Polyphosphatases and Ethylene diaminetetraacetic Acid (EDTA). Secondary antioxidants function by breaking down hydroperoxides formed during lipid oxidation into stable end products. Thiodipropionic acid and Dilauryl theodipropionate are examples of secondary antioxidants (Venkatesh and Sood, 2011) (*Figure 3*).

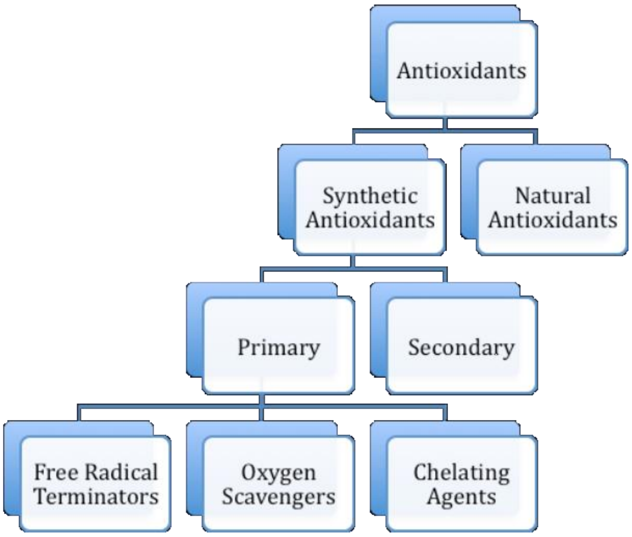


Figure 2. *Various types of synthetic antioxidants.*
Source: Venkatesh and Sood (2011).

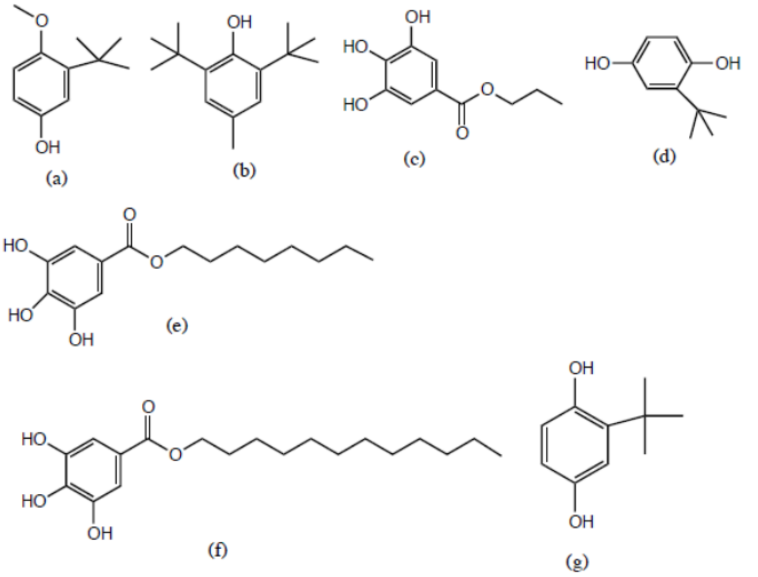


Figure 3. *Chemical Structure of synthetic antioxidants: (a)BHT, (b)BHA, (c)PG, (d)OG, (e)DG, (f)EDTA, and (g)TBHQ.*
Source: Venkatesh and Sood (2011).

Types of synthethic antioxidants

BHT and BHA are the most prevalent synthetic antioxidants in food as reported by the National Research Council Food Additive Committee. Chemically, BHT and BHA are monohydric phenols with commercial Butyl hydroxyanisole (BHA) consisting of two isomers 3-tertiary butyl 4-hydroxyanisole and 2-tertiary butyl 4-hydroxyanisole in the ratio of 9:1. BHA is available commercially as white waxy flakes, while BHT as a white crystalline solid, with both being extremely soluble in fats but not in water due to their phenolic structures with bulky hydrocarbon side chains. Both of these additives has good carry through properties, which determine their ability to withstand various processing steps such as baking and frying and maintain their functionality (Devlieghere et al., 2004). BHA has slightly higher stability and thus is more effective especially in protecting the flavor and color of foods. Moreover, BHA is more effective in preserving foods containing animal fats with short chain fatty acids, such as those found in coconut and palm kernel oils used in confectionary products. Since, BHT and BHA are fairly volatile with their boiling points around 265 0C and 268 0C respectively, they are also used in the food packaging materials either by direct addition in the waxed inner linings or indirectly as emulsions, from which they migrate into the food. BHT and BHA have been shown to have synergistic effects when used in combination especially in nuts and nut products (Venkatesh and Sood, 2011).

Tertiary butyl hydroquinone (TBHQ) is another important synthetic antioxidant most often used in the preservation of food items containing frying oils. Chemically, TBHQ is a diphenol and is commercially available as beige colored solid, which is 40 soluble in fats just like BHT and BHA due to similar structural features. With its boiling point range of about 271.3-311.3 0C, it is less volatile as compared to BHT and BHA (Naidu, 2000). TBHQ is an effective supplement to the expensive process of liquid oil hydrogenation to provide increased oxidative stability and color improvement, and provides good carry through properties to the fried product. In addition to its use as a singular additive, TBHQ has been shown to have good synergistic effects when used in combination with BHT and/or BHA. Its stabilizing effects on the food lipids can also be enhanced when used in combination with chelating agents like citric acid, in substances such as vegetable oils, animal fats and particularly shortenings. But, the use of TBHQ with Propyl gallate (PG) is prohibited (Venkatesh and Sood, 2011). Propyl gallate (PG) is the most important and widely used antioxidant among all the gallates. Chemically, it is again a phenol and is prepared commercially by treatment of gallic acid with propyl alcohol and further distillation to get rid of the excess alcohol. PG is available as a white crystalline solid, which is sparingly soluble in water due to the presence of a number of hydroxyl groups in its structure. With a boiling point range of 161.3-201.3 0C, it is the most volatile among BHT, BHA and TBHQ 57. PG is particularly effective in stabilizing vegetable oils and animal fats in products such as meats, spices and snacks; but it is less effective as compared to TBHQ in preserving vegetable oils. Its use in chewing gum bases is also considered important. PG has been shown to have good synergistic effects with BHA and/or BHA. In addition it is always used along with a chelating agent like citric acid, since in its absence PG chelates metal ions like iron forming an aesthetically unappealing blue-black complex (Venkatesh and Sood, 2011).

Another important synthetic antioxidant Ethylenediaminetetraacetic acid (EDTA) falls under the category of chelating agents. It forms complexes with pro-oxidative metal ions like copper and iron, through an unshared pair of electrons in its molecular structure and thus lowers the amount of soluble reactive metal (Venkatesh and Sood, 2011).

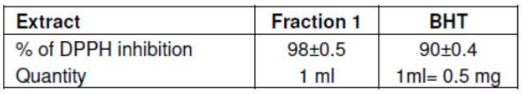
Biological effects of synthethic antioxidants

Both Butylated hydroxyanisole (BHA) and Butylated hydroxytouluene (BHT) influence cellular metabolism through induction of various drug metabolizing enzymes such as cytochrome P450, cytochrome b, NADH reductase, glucose-6-phosphate dehydrogenase. BHA also has been shown to cause increase in organ weight like hypertrophy of liver61, thyroid, adrenals, lungs and proliferation of endoplasmic reticulum in liver cells, whereas BHT has been shown to cause cell proliferation like alveolar cells and liver cells in addition to the proliferation of endoplasmic reticulum in liver cells (Gould, 1995). BHT may also cause increase in macromolecular synthesis in liver, lung cells & decrease in kidney cells and cell growth inhibition in kidney cells. Again, both BHA and BHT might cause induction of chromosomal and sperm abnormalities, cause inhibition of bacterial growth & toxin formation, lipid peroxidation in biological membranes and platelet aggregation, interfere with leukemia cell differentiation, prostaglandin synthesis and with immune response, enhance microsomal H2O2 formation, cause decrease in smooth muscle and heart muscle contractility and can have an influence on lipid metabolism and dietary levels of vitamin E61. In addition, BHA might cause membrane labialization, cell lysis, alteration of lipid composition in liver, serum and platelets and might cause lesions in for estomach. On the other hand, BHT may cause inhibition of repair DNA synthesis in human lymphocytes, of drug cooxygenation during prostaglandin synthesis and of metabolic cooperation between V79 cells, might interfere with intracellular cGMP concentration and with prostaglandin synthesis, might cause shortening of life cycle in human leucocytes, membrane labialization leading to cell lysis and alteration of lipid composition in liver, serum & platelets.

Moreover, BHT could also have antiviral activity, anti-inflammatory action and might play a role in the protection of sperm membrane from cold shock. BHT plays an important role in vitamin K antagonism leading to a decrease in clotting factors80. Propyl Gallate (PG), the most important gallate used as antioxidant preservative, may induce drug metabolizing enzymes like cytochrome b, NADH reductase, epoxide hydrolase, might inhibit mitosis in human cancer cells, lipid peroxidation in biological membranes and bacterial growth & toxin formation, may interfere with leukemia cell differentiation, with immune response and with prostaglandin synthesis, may enhance microsomal H2O2 formation and decrease smooth muscle and heart muscle contractility. Furthermore, PG has been shown to have local anesthetic action and anti-inflammatory action (Venkatesh and Sood, 2011).

Identification of naturally occuring BHT from purple leaves of the halophyte plant Mesembryanthemum crystallinum

The potential antiradical activity of extract from purple leaves of the halophyte plant M. crystallinum was investigated. Semi-preparative HPLC was used to obtain different fractions of the plant extract at 540 nm; the six peaks were tested for their antiradical activity (*Figure 4*). The results showed a very high antioxidant level in fraction 1 (1 ml=98±0.5 % of DPPH inhibition). Further, it was important to identify the molecule present in fraction 1 which seemed to be responsible for the significant anti-oxidative activity observed (Bouftira et al., 2007). The antioxidant molecule was identified as the 2, 6-bis (1.1-dimethylethyl)-4-methylphenol (BHT) using GC/MS (*Figure 5* and *Figure 6*). Synonyms: 2,6-Di-tert-Butyl-4-Methylphenol; BHT; 2,6-Di-tert-Butyl-1-Hydroxy-4 Methylbenzene; 3,5-Di-tert-Butyl-4-Hydroxytoluene; 4-Hydroxy-3,5-Di-tert-Butyltoluene; Dibutylated Hydroxytoluene; 4- Methyl-2, 6-di-t-butyl-phenol. Molecular Weight: 220.18 g/mol. Molecular Formula: C15H24O. Melting Point: 71°C. Boiling Point: 265°C. It was insoluble in water and in propylene glycol, but was freely soluble in alcohol (Bouftira et al., 2007). Another study on M. crystallinum (Ibtissem et al., 2010) showed that BHT synthesis is dependent on plant growth stage. The results showed that M. crystallinum extract at seeds stage has the highest BHT concentration. At this period, the plant leaves are purple resulting from tolerance to stress of environment. In the seeds stage, the plant is exposed to solar radiation. The light stress can attribute in part to stimulate the plant for the synthesis of BHT. The results of this study showed also that the method of extraction influences the yield of BHT. The method using hexane and acetonitrile is better than that using methanol 80% for BHT extraction (Ibtissem et al., 2010).



Extract	Fraction 1	BHT
% of DPPH inhibition	98±0.5	90±0.4
Quantity	1 ml	1ml= 0.5 mg

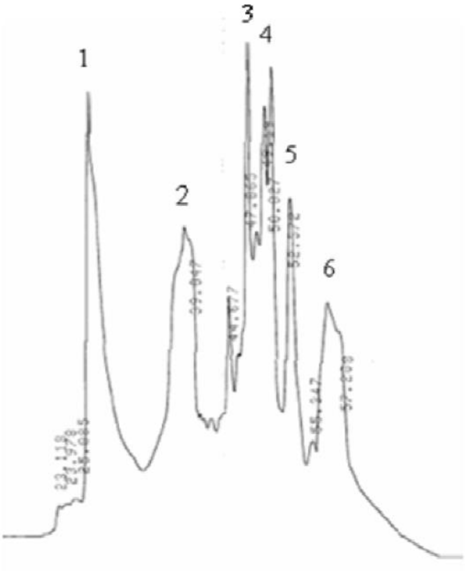


Figure 4. *HPLC chromatograms of methanol extract at 540 nm.*

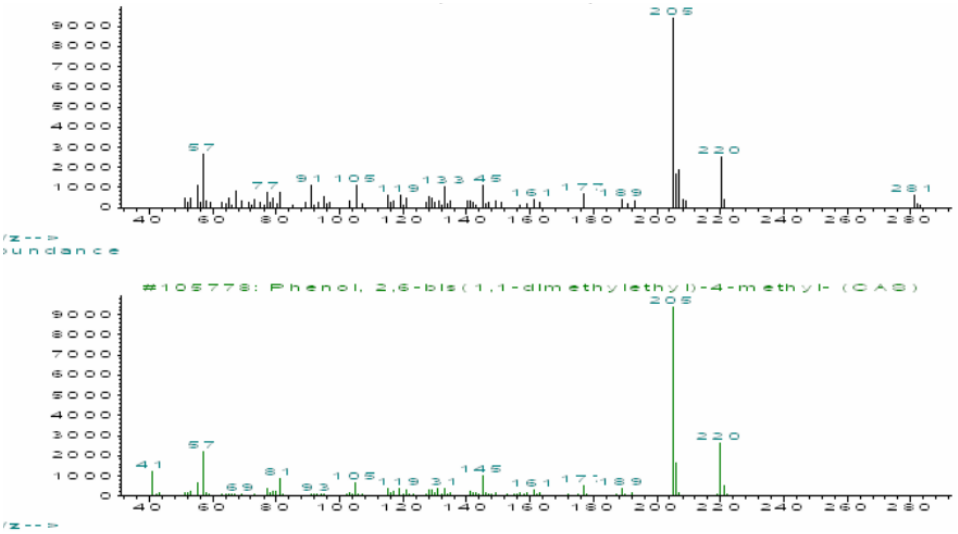


Figure 5. *Mass spectra corresponding to fraction 1. The molecule identified by GC/MS was the 2,6-bis (1.1- dimethylethyl)-4-methylphenol.*

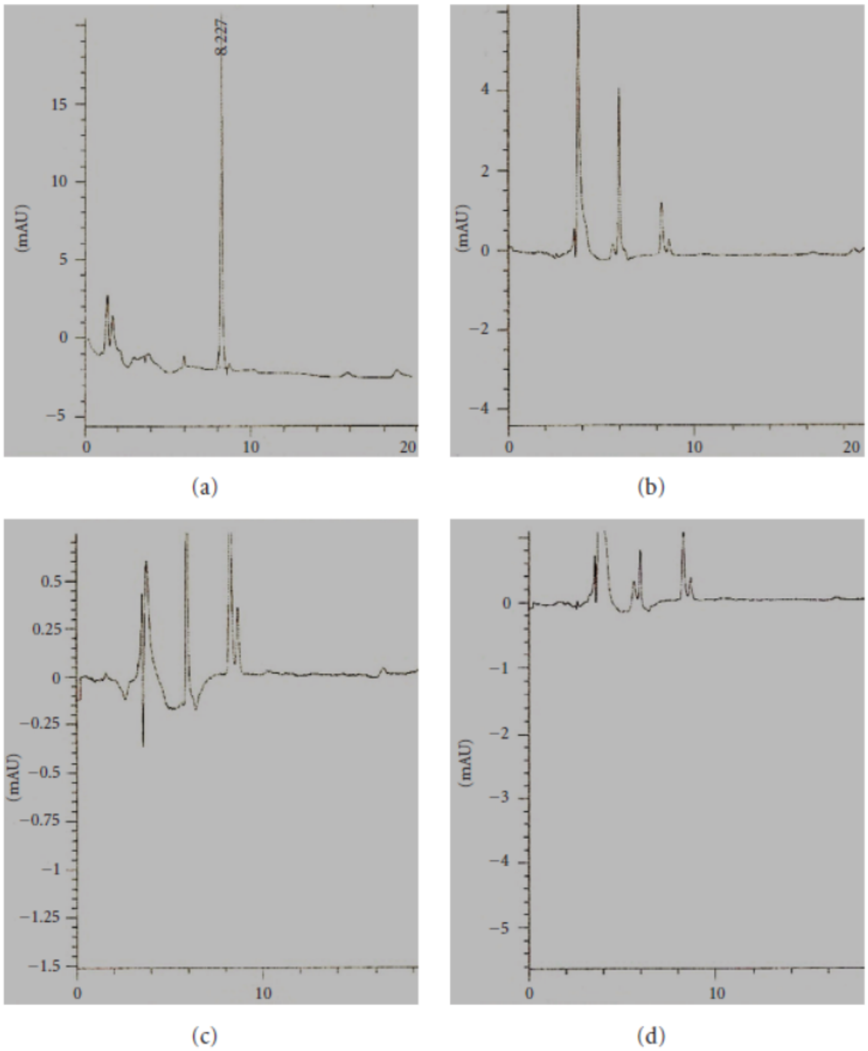


Figure 6. *BHT spectra: (a) standard (10mg/ml); (b) Seedlings extract; (c) Juvenile extract; and (d) Adult extract.*

Production of natural BHT by freshwater phytoplankton

The crude extracts of the four species were purified using the silica gel TLC plates with acetone:methanol:water (40:20:1 v/v/v) as the developing solvent. Six fractions were obtained for C. raciborskii (C1–C6), M. aeruginosa (M1–M6), and Oscillatoria sp. (O1–O6), and five were obtained for B. braunii (B1–B5). The gas chromatogram of the B5 fraction of B. braunii exhibited a distinct peak with a retention time identical to that of the synthetic BHT compound (*Figure 7* and *Figure 8*). The same result was obtained for M. aeruginosa (fraction M5), C. raciborskii (fraction C5), and Oscillatoria sp. (fraction O4) by GC-MS spectra. Three cyanobacteria were cultured under three irradiance conditions: 25, 75, and 150 µmol photons m-2 s-1, to study the relationship between the production of BHT and irradiance. The highest production of BHT was measured for those cultures growing under the highest irradiance, suggesting the dependence of the production of BHT on irradiance (Babu and Wu, 2008). Based on the GC-MS analysis, the antioxidant compound observed in the algal species is identical to that of a synthetic BHT, as it not only shared the same retention time in the total ion chromatogram but also had similar mass spectral data. The present study thus revealed that all the four algal species produce a natural BHT that exhibits antioxidant activity similar to that of synthetic BHT. Thus, these algal species have the potential to be used as an alternative commercial source for BHT production. Apparently, the amount of BHT produced by the cells is related to irradiance, rather than to growth rate or biomass. Thus, the production of BHT exhibits a light dependency and is considered to protect the cells from photooxidation (Babu and Wu, 2008).

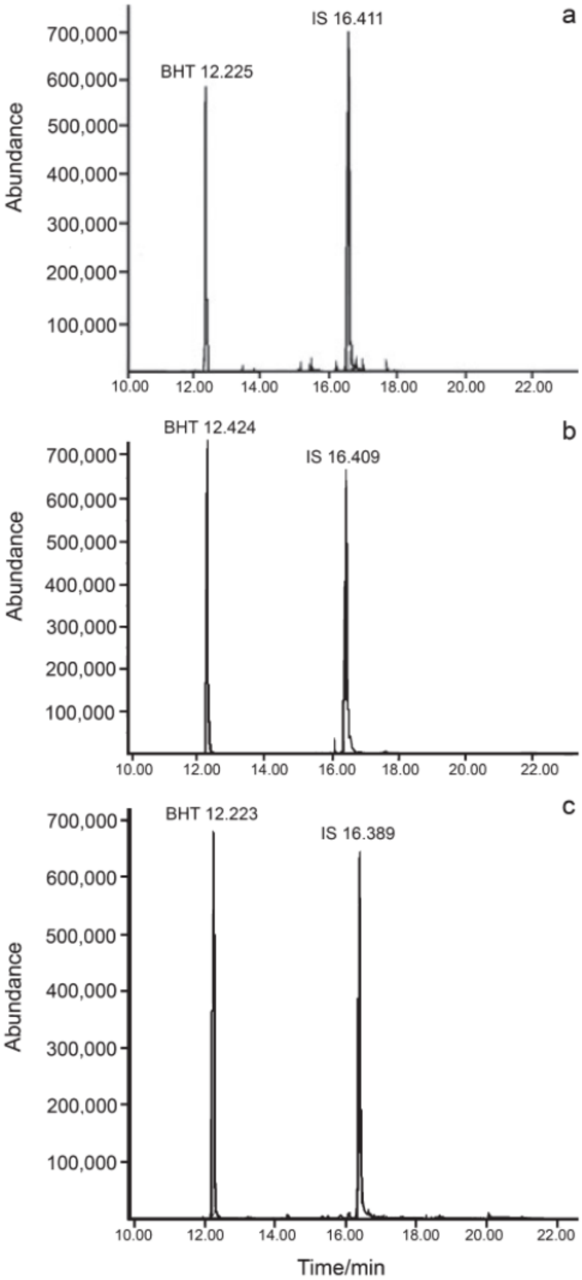


Figure 7. *Total ion chromatogram analysis of (a) the B5 fraction of Botryococcus braunii showing retention time (RT) (12.225) identical to synthetic BHT, (b) synthetic BHT showing RT of 12.242, and (c) the M5 fraction of Microcystis aeruginosa showing RT (12.223) identical to synthetic BHT. DM serves as the internal standard (IS). BHT, butylated hydroxytoluene; DM, dicyclopentenyloxyethyl methacrylate.*

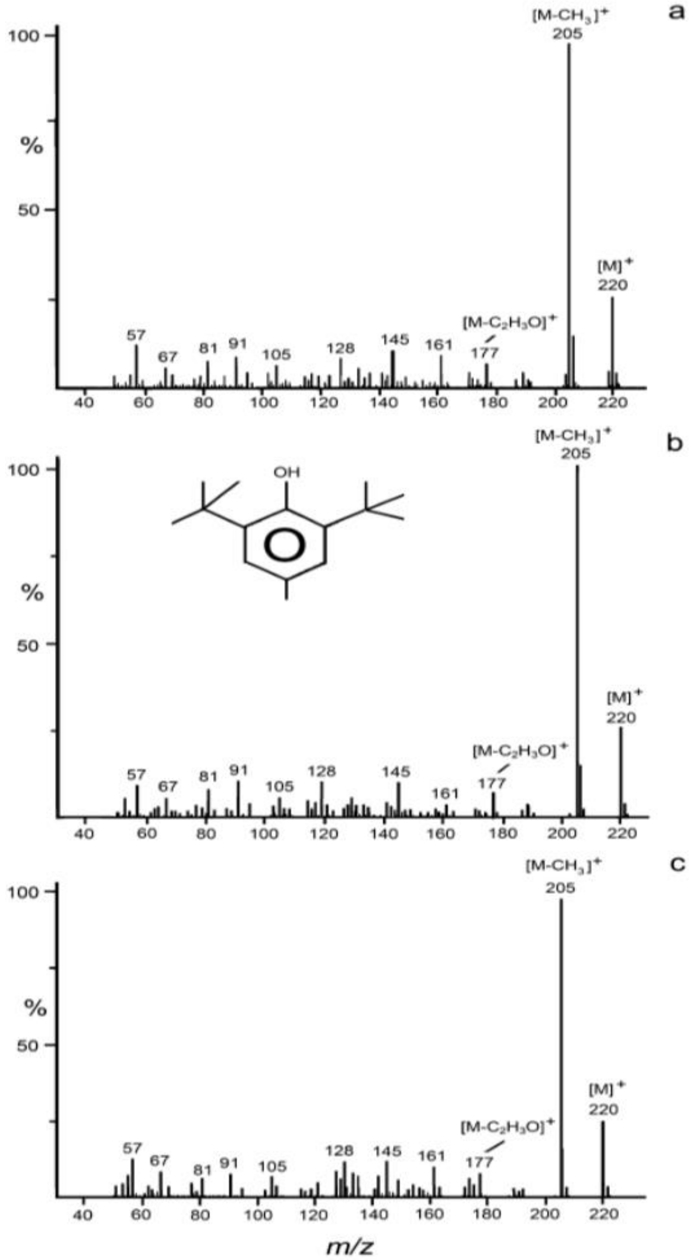


Figure 8. *Mass spectrum showing the fragmentation pattern of (a) the B5 fraction of Botryococcus braunii, (b) synthetic BHT, and (c) the M5 fraction of Microcystis aeruginosa. BHT, butylated hydroxytoluene.*
Source: Babu and Wu (2008).

Conclusion

Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. The 2,6-bis (1.1-dimethylethyl)-4-methylphenol (BHT), also known as butylhydroxytoluene, is a lipophilic (fat soluble) organic compound that is primarily used as an antioxidant food additive as well as in cosmetics, pharmaceuticals, jet fuels, rubber, petroleum products, and electrical transformer oil. Recently scientists have found out that BHT has carcinogenic effects on human foods and has been banned for such uses. BHT was found to naturally occur in Mesembryanthemum crystallinum leaves. The concentration of this phenol is dependent on the plant growth stage. In fact, the floraison stage has the highest BHT concentration. It was also demonstrated that four freshwater phytoplankton, including a green alga (Botryococcus braunii) and three cyanobacteria [Cylindrospermopsis raciborskii, Microcystis aeruginosa and Oscillatoria sp.] were capable of producing BHT. Culturing under various irradiances gave rise to different magnitudes of BHT production in cyanobacterial cells, showing that more BHT was produced in the cells irradiated with a higher light intensity, and its production was irradiance dependent. More research should be done on halophytes plants and algae to characterize and isolate the natural form of BHT to be an alternative use instead of its synthetic form.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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