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A Review of the Physiological Implications of Antioxidants in Food

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A Review of the Physiological Implications of Antioxidants in Food

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Abstract

Antioxidants have been known to prevent oxidation reactions, inhibiting conditions such as cancer and heart disease. The purpose of this IQP is to explore natural and synthetic antioxidants used as preservatives. It was found that both the concentration and the absorption mechanism of natural antioxidants are important in obtaining the maximum beneficial effect. Additionally, synthetic antioxidants used as preservatives have shown evidence of toxicity in animal models, though more research needs to be done to fully understand their physiological effects.

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Table of Contents

Abstract **2**

Acknowledgements **3**

Table of Contents **4**

Table of Figures **5**

List of Tables **6**

1. Objectives **7**

2. Introduction **8**

3. Methodology **10**

Abstract **14**
1. Introduction **15**
2. Oxidation of Foods **18**
2.1 Antioxidants 20
2.2 Antioxidants as Preservatives 20
2.3 Synthetic and Natural Antioxidants 21
3. Natural Antioxidants **23**
3.1 Types of Natural Antioxidants 24
3.2 Chemical Pathways for Natural Antioxidants 29
3.3 Biological and Cellular Effects of Natural Antioxidants 32
3.4 Health Benefits and Risks 33
4. Synthetic Antioxidants **37**
4.1 Types of Synthetic Antioxidants 39
4.2 Chemical Action of Synthetic Antioxidants 42
4.3 Biological Effects of Synthetic Antioxidants 45
4.4 Health Risks and Benefits 47

5. Public Awareness of Antioxidants used as Preservatives **51**
5.1 Survey Results 53
6. Conclusions **57**
Appendices **62**
Appendix A: Survey 62
References **64**

Conclusions **69**

Appendices **71**
Appendix A: Letter Sent to Various Journals **71**
Appendix B: Letter Sent to Nutritionists **72**

Table of Figures

Figure 1: Some popular food and drink brands which advertise the beneficial effects of natural antioxidants 16

Figure 2: Initiation reaction (1), propagation reactions (2, 3), and termination reactions (4, 5, 6) 19

Figure 3: Chemical structures of natural antioxidants.. 23

Figure 4: Oxidation of Vitamin C via the donation of an electron from a hydroxyl group and the generation of a double bond. 29

Figure 5: Oxidation of ß-carotene via the donation of an electron from a double bond. 31

Figure 6: Various types of synthetic antioxidants. 37

Figure 7: Chemical structure of synthetic antioxidants. 38

Figure 8: Boiling points of synthetic antioxidants. 41

Figure 9: Antioxidant mechanism of a radical terminator via the donation of an electron from a phenolic hydroxyl group. 43

Figure 10: Resonance stabilization of a phenolic antioxidant. 44

Figure 11: Oxidation of BHA via donation of an electron from a phenolic hydroxyl group. 44

Figure 12: Generation of a phenoxy radical, with the intramolecular hydrogen bond shown. 45

Figure 13: Responses of various groups regarding Question 6 in the survey. 54

Figure 14: Responses of various groups regarding Question 7 in the survey. 55

List of Tables

Table 1: List of the most common natural antioxidants used as food preservatives, and their typical sources. 24

Table 2: List of natural antioxidants and techniques to maximize absorption. 28

Table 3: Additional data on structure, solubility, melting point, potential daily intake (PDI), and countries in which each synthetic antioxidant is banned. 42

Table 4: Typical synthetic antioxidants used as preservatives, their legal limits in foods, and their common sources. 48

Table 5: Major foods consumed in the USA and the typical natural antioxidant content in each category. 60

1. Objectives

The major objectives of this review were:

1. To understand the process of oxidation in foods, find various definitions for the term "antioxidant" and elucidate the necessity for the addition of antioxidants to common food products

2. To identify the most common natural and synthetic antioxidants present in common food items and discuss their sources, health benefits and risks, antioxidant mechanisms, and cellular and physiological effects

3. To understand government regulations placed on natural and synthetic antioxidants, and learn general guidelines currently established for their consumption

4. To begin to understand the general public's awareness of antioxidants, the difference between natural and synthetic antioxidants, and the potential health risks associated with consuming synthetic antioxidants.

2. Introduction

Oxidation, a nearly ubiquitous chemical reaction that involves the transfer of electrons from one compound to another, has long been known to have negative effects, especially in physiological contexts. Many diseases such as cancer and heart disease can be attributed to the oxidation of molecules such as DNA or lipids, both of which are necessary for proper life function. Similarly, oxidation reactions can occur in common food items, often as a result of prolonged exposure to the atmosphere. These oxidation reactions can lead to browning, rancidity, and the development of unpleasant flavors.

To combat oxidation in both foods and physiological contexts, compounds known as antioxidants can be utilized. Antioxidants provide electron density to compounds likely to undergo oxidation, thus preventing them from losing electrons. While there are several different types of available antioxidants, they can be grouped into two major categories: natural and synthetic. Natural antioxidants are those that can be harvested directly from organic sources such as fruits, vegetables, grains, and meat. Synthetic antioxidants are those created in laboratories, generally for use in the preservation of foods.

Natural antioxidants are often advertised as key supplements by vitamin and health food manufacturers, but consumers must remain aware of the sources from which they consume these natural antioxidants, as well as the concentrations in which these compounds are consumed. These factors can be important in understanding how much of the consumed antioxidant is actually absorbed into the body. Conversely, food manufacturers generally do not prominently mention their use of synthetic antioxidants, which are cheaper than natural antioxidants, to preserve packaged foods. However, it is very important for consumers to be aware of which synthetic antioxidants they consume and their concentrations, as some commonly used synthetic antioxidants have been shown to have toxic effects in high concentrations.

The purpose of this IQP is to elucidate the natural and synthetic antioxidants commonly found in foods, and to understand their chemical modes of action and their physiological effects. Additionally, this study aims to gain insight into the general public's awareness of antioxidants and their actions, their understanding of synthetic antioxidants and their potential toxic effect, and their level of concern regarding these food additives.

3. Methodology

The main goal of this IQP was to understand the physical and chemical actions of natural and synthetic antioxidants as preservatives. In addition, public perceptions and awareness about antioxidants in foods was analyzed. This research was used to develop a paper to be published in a peer-reviewed journal, which was submitted to the *Journal of Young Investigators* in December 2010. It is currently being reviewed.

1. Journal Search

Before beginning to write the paper, many journals were browsed and researched in order to choose an appropriate target journal to which the paper would be submitted. Journals were searched for impact factor, types of articles (review, original research, etc.), and target audience. The list of journals considered was as follows:

- Appetite
- Journal of Nutrition Education and Behavior
- Journal of Food Products Marketing
- Trends in Food Science and Technology
- Health Promotion International
- American Journal of Public Health
- Journal of Nutrition
- Science News

- Nutrition Research Newsletter
- Journal of Young Investigators

Emails were sent to all the above journals to determine their level of interest in the chosen research topic. A copy of this email can be found in Appendix A. Finally, the *Journal of Young Investigators* was chosen due to its interdisciplinary focus, and frequent publication of review articles.

2. Literature Review

In order to gain background information regarding oxidation, synthetic antioxidants, and natural antioxidants, an extensive review of the existing literature on these topics was conducted. Scientific search engines (the most predominantly used of which were ScienceDirect and Google Scholar) were used to retrieve peer-reviewed papers, which provided information about antioxidants, their cellular and physiological effects, and their health benefits and risks. This information was collected, analyzed and presented in the body of the review.

3. Survey

In order to gain insight in to the attitudes of the general public regarding the use of antioxidants as preservatives in commonly consumed foods, two very similar surveys were created using Google Documents. Both surveys aimed to gauge the respondent's awareness of antioxidants used as preservatives, his or her knowledge about the differences between natural and synthetic antioxidants, and his or her concern about the possible health effects of consuming synthetic antioxidants. The first survey was disseminated among the WPI community (students, faculty, and staff). The second survey was targeted towards the general public, and was advertised mainly via word of mouth. The surveys were then compared against each other and analyzed via the chi-squared method of analysis in order to identify any differences in attitudes and awareness regarding antioxidants used as preservatives between the two groups. A sample survey is included in the paper submitted to the journal.

4. <u>Contacting Nutritionists</u>

A few nutritionists from various institutions (such as the University of Massachusetts at Amherst, Northeastern University, and the Massachusetts College of Pharmacy and Health Sciences) were contacted in order to get their expert opinions regarding antioxidants. A sample of the e-mail sent can be found in Appendix B.

The results of this IQP are presented in a paper submitted to the *Journal of Young Investigators.* This paper is presented in the following sections.

4. Paper Submitted to the *Journal of Young Investigators*

A Review of the Physiological Implications of Antioxidants in Food

Abstract

The use of antioxidants as dietary supplements has long been known to prevent harmful oxidation reactions in physiological systems. In particular, antioxidants have recently gained considerable attention, due to their role in preventing conditions such as cancer and heart disease. However, both natural and synthetic antioxidants have long served as preservatives, used to prevent oxidation reactions, which lead to browning, and rancidity in foods. The purpose of this review is to explore various types of natural and synthetic antioxidants used as preservatives, their chemical and biological actions, and their associated health risks and benefits. It was found that while natural antioxidants can indeed have many positive physiological effects such as the prevention of DNA oxidation, the sources from which they are consumed must also be carefully considered to maximize absorption. Additionally, while synthetic antioxidants are harmless in small concentrations, studies in animal models have shown evidence of their toxicity in higher concentrations. A survey of the general public indicates a general lack of knowledge about antioxidants. An organized effort to educate individuals about foods rich in natural antioxidants and the ability to recognize the major synthetic antioxidants on food labels would be highly beneficial.

1. Introduction

Of the many commonly occurring natural chemical reactions, reduction and oxidation are two of the most important. Oxidation reactions, in which electrons from a given compound are lost, and reduction reactions, in which a given compound gains electrons, are coupled. That is, oxidation cannot occur without reduction occurring simultaneously, and vice versa. Oxidation can occur in many contexts. For instance, it is an important physiological reaction without which several cellular functions such as respiration would not be possible. Additionally, oxidation can occur in foods and is one of the most common causes for food to lose flavor or texture, as it can lead to browning and rancidity. Thus, it has been the goal of many food manufacturers to add compounds, which prevent or retard oxidation in order to improve the shelf life of packaged foods.[2]

Antioxidants are generally defined as compounds that prevent oxidation. They vary greatly in size, composition and molecular weight. While some are small and have low molecular weights, others are enormous in size and can even be macromolecules such as proteins. Antioxidants can have several uses, both in physiological systems and in human-made applications. As the loss of electron density among some vital physiological compounds can lead to improper cellular function, the body produces, utilizes, and regulates several types of antioxidants to ensure that physiological processes are not interrupted due to oxidation. However, the presence of antioxidants is not limited to physiological processes; antioxidant compounds can be found in food sources. The two major types of antioxidants found in food sources are natural antioxidants and synthetic antioxidants. While natural antioxidants are compounds found in foods consumed without much processing such as fruits and vegetables, synthetic antioxidants are compounds produced artificially and added to processed or pre-packaged food to prevent rancidity, browning, or loss of fresh taste or texture. [2]

As natural antioxidants have been shown to have significant benefits in preventing cancer and heart disease, many food advertisers have taken note and begun publicizing this fact. Antioxidant content in foods has achieved prominence on many food labels, ranging from fruit juices to chocolate products. (Figure1)

Figure 1: Some popular food and drink brands which advertise the beneficial effects of natural antioxidants

However, though synthetic antioxidants are often present in those same food items, manufacturers and advertisers generally never discuss them. Thus, consumers are often unaware of their existence. Therefore, as synthetic antioxidants are major food ingredients, they need to be studied further in order to elucidate for consumers their role in processed and packaged foods. The purpose of this review is to examine natural and synthetic antioxidants and their role in food preservation. Additionally, their metabolic pathways, cellular and physiological effects, and health benefits and risks are discussed. Finally public attitudes and awareness of antioxidants in food items is examined.

2. Oxidation of Foods

Most foods are made up of several compounds that can easily undergo oxidation. While all compounds possess the ability to undergo oxidation, lipids (such as fats, oils, and waxes) in general have the greatest tendency to lose electrons. Auto-oxidation of lipids in food triggered by exposure to light, heat, ionizing radiation, metal ions or metallo-protein catalysts can have a deteriorating effect on the food color, flavor, texture, quality, wholesomeness and safety[80]. For instance, oxidation of lipids leading to the destruction of Essential Fatty Acids (EFAs) or fat soluble vitamins can lead to important quality changes in the food and oxidation of cholesterol can affect the safety levels of the food. Also, the Malliard type reactions between the reducing substances originating from lipids can lead to color changes, and formation of volatile odoriferous compounds and hydroxyacids can affect the food's aroma and taste [80].

Fats contained in food are chemically composed of triglycerides and oxidation leading to the rancidity of foods occurs at the unsaturated sites of the triglycerides. The reaction causing the oxidation of these unsaturated sites is an auto-catalyzed radical chain reaction consisting of three steps- initiation, propagation and termination (Figure 2).

$$RH \longrightarrow R^{\bullet} + H^{\bullet} \quad (1)$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \quad (2)$$

$$ROO^{\bullet} + RH \longrightarrow R^{\bullet} + ROOH \quad (3)$$

$$R^{\bullet} + R^{\bullet} \longrightarrow RR \quad (4)$$

$$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR \quad (5)$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOOOR \quad (6)$$

Figure 2: Initiation reaction (1), propagation reactions (2, 3), and termination reactions (4, 5, 6)

Since the oxidation products further the reaction by themselves acting as initiators for a new chain reaction, the rate of this process increases with time. During the initiation step lipid free radicals are formed through abstraction of hydrogen, which in turn react with oxygen in its excited state to form lipid peroxy radicals during the propagation step as shown in reactions 1-3. The lipid peroxy radicals are very reactive, and can thus participate in various radical-mediated reactions following the propagation step and finally during the termination step lead to formation of the primary products of auto-oxidation, hydroperoxides as shown in reactions 4-6. These hydroperoxides then decompose to form products such as alcohols, aldehydes, ketones, hydrocarbons, acids and epoxides that result in rancid flavors. Additionally, hydroperoxides are able to form possibly toxic polymerization products which cause color changes, or lead to insolubilization of proteins causing functionality and texture changes[80,81].

2.1 Antioxidants

The main function of antioxidants is to prevent oxidation in various contexts. It has been known for some time that antioxidants play a very important biological role in the body by protecting against oxidative damage (particularly oxidative damage to DNA), thus preventing cardiovascular, neurological and carcinogenic diseases and delaying chronic health problems like cataracts[20, 23] The Food and Drug Administration (FDA) defines antioxidants only as dietary supplements to be taken in addition to normal food consumption in an effort to prevent these diseases.[1] However, antioxidants do not only serve as dietary supplements; they can also be added to packaged foods as a form of preservative. Antioxidants used in foods are generally added in order to maintain freshness and prevent browning or rancidity, and are particularly important in foods containing large quantities of fats or oils, which are the most vulnerable to oxidative rancidity. Thus, the addition of antioxidants to packaged food items greatly extends their shelf lives, and keeps flavors and aromas fresh for as long as possible.

2.2 Antioxidants as Preservatives

The addition of antioxidants to the food items as preservatives can be during many different stages of food production[22,33,44.] Generally, the preservative action of antioxidants is more effective if they are added early in the manufacturing process, as antioxidants cannot reverse the oxidation of food products[44]. While the most practical application of antioxidants as food preservatives is their addition to the fats and oils used in food production[23], antioxidants are used in other food products for preservation, such as vegetables and vegetable products; fruits and fruit products; cereals and bakery products; milk and milk products like cheese; meat, fish and their products; spices and other dry foods like sugar, honey, beverages, and chewing gum[20].

In addition to varying the stages at which antioxidants are added to foods, the form in which the antioxidant is added can also differ. For instance, sometimes the antioxidant might be a food grade solvent that is sprayed onto food items such as nuts and cereals[23]. Besides the direct addition to food items, the antioxidants can be used to preserve food by preventing the degradation of food packaging during processing and storage. Thus, antioxidants can be added to packaging materials like paper, polyethylene, plastic and paperboard preventing the oxidation of the material itself, or allowing the added antioxidants to migrate into the packaged food inside and prevent oxidation there[20,23,44]. For addition in any form, the effectiveness of antioxidant activity depends on the complete dissolution and proper dispersion throughout the finished product[20].

2.3 Synthetic and Natural Antioxidants

Antioxidants to be used as preservatives can be both naturally derived as well as chemically synthesized. However, natural and synthetic antioxidants differ in their performance levels. The performance level of antioxidants (their effectiveness as preservatives) can be measured by the number of peroxides formed in lipids over time and also by observing their carry-through properties i.e. the ability of the antioxidant to provide stability under different processing conditions like heat (such as frying or baking), varying solubility, etc. Both synthetic and natural antioxidants function as preservatives by donating electron density to fats, thus preventing their breakdown. Synthetic antioxidants have been shown to have higher performance levels than the natural ones, since the natural antioxidants show a greater reluctance when donating hydrogen atoms when preventing oxidation. Synthetic antioxidants, which are generally priced at approximately $10-20 per pound, are less expensive than the natural ones, as the derivation processes of the natural antioxidants from the various natural sources add to their cost.

Natural and synthetic antioxidants also differ in their fortification values. Generally, natural antioxidants are known to have higher additional beneficial health effects such as their ability to prevent diseases like cancer and heard disease as compared to the synthetic ones. The type of antioxidant used depends greatly on the type of food being preserved and overall price considerations. For instance, preservation of foods with high rancidity levels is better achieved by using synthetic antioxidants since these have higher performance levels, while natural antioxidants with lower performance levels can suffice for hydrogenated oils with lower rancidity levels[23].

3. Natural Antioxidants

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 3)

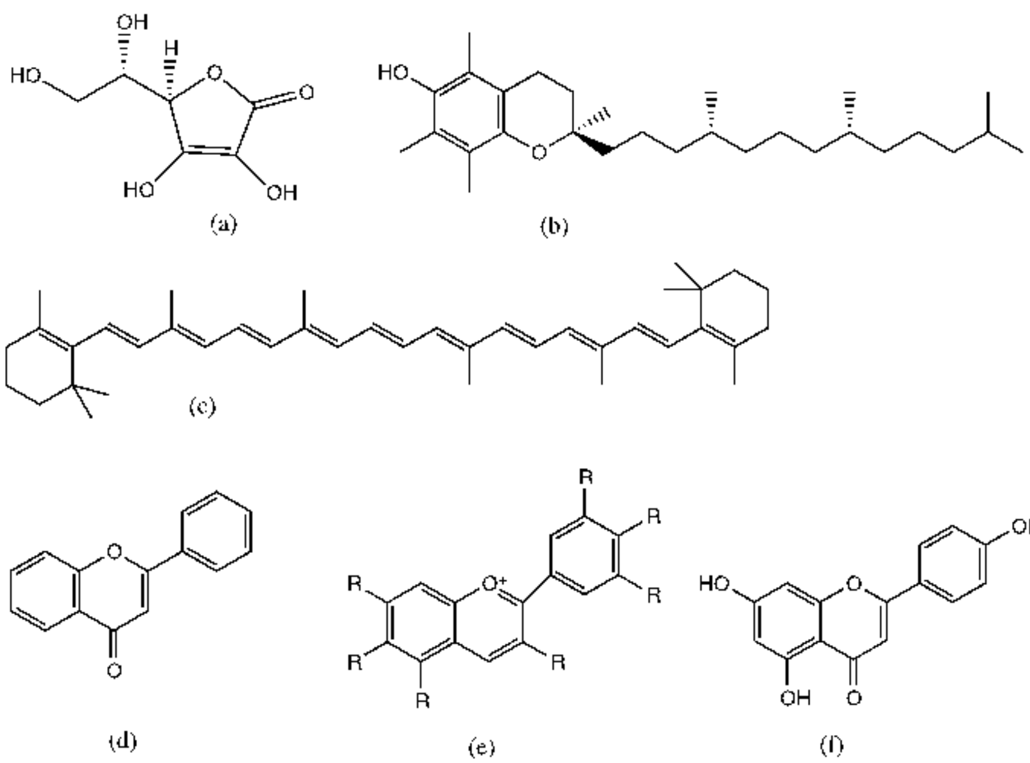

Figure 3: Chemical structures of natural antioxidants. This figure shows the chemical structure of the following antioxidants: (a) Ascorbic Acid, (b) Vitamin E, (c) Beta-Carotene, (d) Flavonoid, (e) Anthocyanin, (f) Polyphenol.

As shown in Table 1, each natural antioxidant is available in vastly different sources and each must be consumed in a different manner in order to maximize its absorption.

Table 1: List of the most common natural antioxidants used as food preservatives, and their typical sources.

Compound Name	**Natural source**
Vitamin C (ascorbic acid)	Most fruits (particularly citrus fruits) [16], some vegetables, tomatoes[19]
Vitamin E/Tocopherols	Cereal grains, broccoli, Brussels sprouts, cauliflower[18], cooking oils (olive, sunflower, safflower), almonds, hazelnuts[19]
Beta-Carotene	Vegetables such as kale, red paprika, spinach, parsley, and tomatoes [18], carrots, sweet potatoes, apricots, papayas[19]
Flavinoids (a type of polyphenol)	Potatoes, tomatoes, lettuce, onions, wheat, dark chocolate, Concord grapes, red wine, black tea[19]
Anthocyanins (a type of flavonoid)	High content in red wines, some in whiskey, sake[17]
Various polyphenols (in figure: apigenin)	Teas (mainly green, some rooibos) [16], as well as many red/purple hued fruits or vegetables, such as Concord grapes, red cabbage, blueberries, blackberries, açai berries, etc. [17]
Lycopene	Tomatoes, papaya, watermelon, pink grapefruit, guava, the skin of red grapes[19]
CoQ10 (an antioxidant enzyme)	Wheat bran, fish, organ meats (eg. Chicken liver) [19]

3.1 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.[16] Additionally, it is found in some vegetables, such as tomatoes.[19] 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%). However, the higher the dose, the lower the percent absorption. Thus, it is commonly recommended that in order to optimize Vitamin C absorption, smaller doses should be consumed several times throughout the day, instead of consuming one large supplement.[51]

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.[18] It can also be found in more lipid-rich sources such as cooking oils like olive oil, sunflower oil, or safflower oil, and nuts like almonds and hazelnuts. [19] In general, bodily absorption of Vitamin E is thought to be rather inefficient, with the body absorbing only 20-40% of dietary intake. As with Vitamin C, it has been seen that the absorption levels of tocopherols are decreased as consumption levels increase. It has also been seen that when taken in conjunction with dietary fats, tocopherols have a higher bioavailability. [51]

ß-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, and other orange-hued produce items. [19] Carotenoid bioavailability is considerably very low, approximately 10-30%, and just as with other antioxidants, absorption levels decrease as dose increases. Because of the low absorption levels of Vitamin A, excess is generally released from the body via solid waste. Additionally, it should be noted that when Vitamin A is taken with high levels of dietary fiber, absorption decreases significantly. [51] Lycopene, another fat-soluble antioxidant, is also a member of the Carotenoid family, but has one major difference: it cannot be converted into Vitamin A, as all other carotenoids can. Lycopene is found in tomatoes and tomato products and interestingly, it has been found that lycopene levels in processed tomato products (such as tomato paste, pasta sauce, ketchup, etc.) are far greater than those in tomato fruits. [19] As with other carotenoids, lycopene is best absorbed in the presence of dietary fats, and hindered by the presence of dietary fiber. [51]

Polyphenols are another major class of natural antioxidants. They are most commonly found in products such as teas, particularly green tea and rooibos (red) tea[16], in addition to dark fruits such as Concord grapes.[17] 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.[55]

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.[19] 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%). [53] One type of flavonoid of particular interest are anthocyanins, which are typically found in dark fruit (such as blueberries, blackberries, Concord grapes, açai berries) and red wine.[17] While anthocyanin absorption is very low, it has been shown that the bioavailability of anthocyanins in darker fruits is much greater than that of lighter fruits (such as strawberries). [54]

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 (eg. chicken liver), as well as in high-protein plant sources such as wheat bran.[19] The bioavailability of CoQ10 from meat sources and poultry has been seen to be approximately 60%. [5]

Table 2: List of natural antioxidants and techniques to maximize absorption.

Compound Name	How to optimize consumption
Vitamin C (ascorbic acid)	~Consume fruit, particularly citrus fruit ~Instead of taking one large supplement a day, consume moderate quantities of citrus throughout the day to increase absorption
Vitamin E/Tocopherols	~Eat whole grain foods such as wheat bread, and consume lots of green vegetables ~Eat tocopherol-rich foods with some sort of unsaturated fat (such as olive oil)
Beta-Carotene	~Consume fruits and vegetables with a reddish-orange color (such as carrots or papaya) ~Avoid consuming food with high beta-carotene levels in conjunction with high fiber foods (eg. Shredded Wheat) as this decreases absorption
Flavinoids (a type of polyphenol)	~Consume foods such as potatoes, onions, black tea, grapes ~Flavinoids are better absorbed when the molecule is not attached to any sugar molecules (so, flavinoids obtained from onions would be better absorbed than those obtained from grapes)
Anthocyanins (a type of polyphenol)	~Eat fruits such as blueberries, blackberries, Concord grapes, as well as dark chocolate ~Though other foods such as strawberries and raspberries contain anthocyanins, the anthocyanins from darker-hued foods are absorbed better.
Various polyphenols	~Consume foods such as teas, dark berries, Concord grape juice, etc. ~Consumption guidelines from anthocyanins and flavinoids apply to most polyphenols.
Lycopene	~Consume tomato products, or red fruits (such as tomatoes or papaya) ~It is much easier to absorb lycopene from tomato products (such as tomato sauce of ketchup) than from raw tomatoes ~Consuming tomatoes with dietary fat (eg. Cooking oils) increases absorption of lycopene
CoQ10 (an antioxidant enzyme)	~Consume whole wheat bread; meats ~Organ meats contain more CoQ10 than do muscle-derived meats

3.2 Chemical Pathways for Natural Antioxidants

After absorption, all antioxidants undergo certain chemical reactions in order to protect other compounds from oxidation**.** While some natural antioxidants, such as Coenzyme q10 have complicated enzymatic mechanisms to prevent oxidation in other molecules, most other natural antioxidants have areas of high electron density within themselves in order to prevent other molecules from maintaining radicals (that is, having unpaired electrons) for extended periods of time. Natural antioxidants donate electrons from two major electron-rich sources: hydroxyl groups and double bonds. After donating electrons, natural antioxidants undergo additional chemical reactions in order to facilitate their breakdown. While many compounds are recycled by physiological systems in order to re-use them for their antioxidant capacities, most natural antioxidants can also be broken down in to metabolites for excretion.

The first major method that several antioxidants use in order to prevent oxidation in other compounds is to donate electrons from their hydroxyl (-OH) groups. (Figure 4)

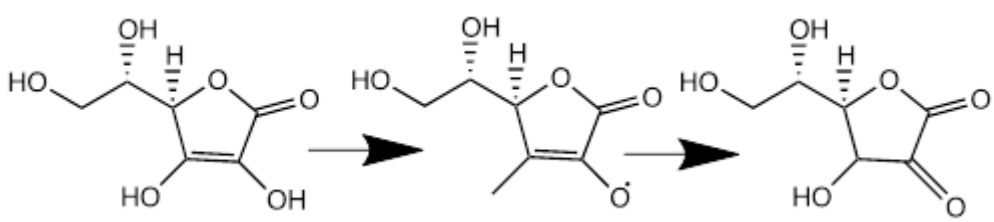

Figure 4: Oxidation of Vitamin C via the donation of an electron from a hydroxyl group and the generation of a double bond.

Hydroxyl groups are a good source of electron density, as oxygen, a highly electronegative element, holds many electrons per atom. While several types of antioxidants follow this pathway to prevent oxidation, two of the most prominent of these are vitamin C and polyphenols. Vitamin C, or ascorbic acid, generally acts as an antioxidant by donating hydrogen atoms from its own hydroxyl groups in order to quench reactive radical species and generating double bonds in place of the lost hydrogens to make up for he lost electron density. However, once this occurs, the Vitamin C molecule itself is oxidized, and so it is reduced back into a useable form of the Vitamin C molecule by a variety of enzymes, including glutathione or thioredoxin reductase.[42] Like vitamin C, all polyphenols (including anthocyanins and all other flavonoid molecules) also rely on the donation of hydrogen atoms from their multiple hydroxyl groups which protrude from their central ring structure in order to carry out their antioxidant functions. [47] While the specific mechanism of the metabolism of polyphenols has not been elucidated, it is thought that polyphenol metabolism is enzyme mediated and occurs in the gut. [49] In particular, it is thought that flavonoid metabolism is aided by the microflora found in the guts of most organisms.[48]

The other strategy with which antioxidants prevent oxidation is to use double bonds to donate electron density. (Figure 5)

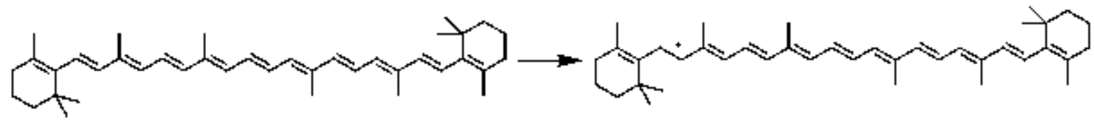

Figure 5: Oxidation of ß-carotene via the donation of an electron from a double bond.

As the electrons in double bonds are less tightly held to the molecule, they are more easily available for donation. Generally, antioxidants that use this strategy are non-polar, and contain hydrocarbon chains of moderate length. Three prominent natural antioxidants that utilize this method are ß-carotene, lycopene, and vitamin A. ß-carotene and lycopene have very similar mechanisms of antioxidant activity, as both have similar chemical structures and fall into the carotenoid family of molecules. As both have many double bonds, they are able to lend some of this electron density to other molecules that need to be reduced in order to avoid forming radical species. From here, the oxidized ß-carotene is metabolized into Vitamin A[45] , and this vitamin A is sometimes metabolized into retinol[46]. Lycopene, however, cannot be converted into vitamin A, and thus the oxidized form of lycopene is immediately reduced back into a re-usable form of the molecule. Additionally, it has been found that lycopene has a greater antioxidant capacity than ß-carotene. [51] Vitamin A or other tocopherols, also donate their own electron density to other molecules in order to prevent them from forming radicals. However, instead of generating double bonds in order to stabilize themselves, tocopherols are converted into tocopheroxy radicals. These radicals can either be reduced back into useable tocopherols (generally by another antioxidant, such as Vitamin C or Coq10)[51], or metabolized for excretion. However, in most cases, physiological systems prefer to reduce and re-use tocopherols as antioxidants instead of excreting them as metabolites. [44]

3.3 Biological and Cellular Effects of Natural Antioxidants

Many natural antioxidants have been much touted for their positive effects on health, especially applications that prevent cancer and heart disease. While each antioxidant works in a different manner, the act of preventing or reversing oxidation, which is a natural occurrence in many physiological processes, is hypothesized to have significant effects in the prevention of many diseases.

Though Vitamin C is one of the most commonly consumed natural antioxidants, there has been no definitive cellular mechanism that has been found to elucidate its effects on cancer and heart disease. But, it is thought to have an impact on these diseases. [51]

Tocopherols, in particular vitamin E, have been observed to have a significant effect on the oxidation of lipids. When vitamin E is consumed, it is passed through the liver and generally delivered to the Low Density Lipoproteins (LDL, commonly known as “bad cholesterol”). From there, it is generally transferred to adipose (fat) tissue, where it prevents oxidation. [51] Similarly, carotenoids, Vitamin A in particular, are also passed through the liver and deposited in adipose tissue, where they inhibit the oxidation of fats. Additionally, it is thought that carotenoids may have some regulatory function in cells, but no conclusive evidence has been found to support this hypothesis. [52]

While the function of most carotenoids in cellular regulation has not been elucidated, Lycopene has been shown to have a definitive role in preventing the cellular process that leads to cancer. In particular, Lycopene has been observed to bind to IGF-I, one of the main growth factors that leads cells to become cancerous. By binding to IGF-I, Lycopene inhibits its ability to communicate with cells by binding to its membrane surface proteins. This lack of communication prevents or slows down the cell cycle, thus reducing the risk for cancer.

All polyphenols (including anthocyanins and flavonoids) have shown evidence of preventing the oxidation of LDL molecules. When LDL is oxidized it is converted into plaques that build up in the interior walls of arteries, increasing the risk for heart disease. By preventing the oxidation of these LDL molecules, polyphenols prevent the buildup of plaque.[52] Additionally, some flavonoids have been found to prevent oxidation of enzymes, thus preserving their proper function.[53]

3.4 Health Benefits and Risks

Due to the power of natural antioxidants to prevent the generation of free radicals, it has been found that they are particularly useful in preventing certain diseases. However, though it is apparent that natural antioxidants have many positive effects on health, it should also be taken into consideration that they could also have harmful effects if taken in excess. While natural antioxidants must be consumed in very large doses in order to produce overdose effects, the possibility of overdose remains a realistic one.

The health benefits of consuming natural antioxidants have been long touted, and recently, manufacturers have taken advantage of the natural antioxidants' abilities to prevent cancer and heart disease. Although those positive health effects are the best advertised, each natural antioxidant also has potential negative side effects. Vitamin C, perhaps the most popular and commonly consumed natural antioxidant, has many positive health effects, besides helping to prevent the common cold[24]. Vitamin C has also been found in studies to prevent the oxidation of DNA, thus leading to certain cancers and cardiovascular disease[22]. In recent years, polyphenols have been some of the most touted antioxidants available from natural sources. Flavonoids, in particular anthocyanins, have been greatly promoted for their positive effects on heart health. Polyphenols have been seen to prevent the buildup of plaque in artery walls a condition known as arthrosclerosis)[32], and possibly prevent oxidative DNA damage, which is thought to be one of the factors which leads to cancer. [26]

Vitamin A and ß-carotene, just like many other antioxidants, have been found to prevent carcinogenic diseases. In particular, Vitamin A has been observed to be particularly useful in the prevention of lung cancer, though the reason for this specificity in disease has yet to be determined.[23] Because tocopherols and Vitamin E generally act as lipid antioxidants, their positive health effects generally come in the form of preventing cardiovascular disease by decreasing the amount of plaque buildup in the blood vessels. Additionally, they have been found to increase the amount of HDL (High Density Lipoprotein, commonly known as "good cholesterol") in the blood, thus also preventing heart disease.[22]

Though overdose on natural antioxidants is a very rare occurrence, it is a definite possibility. When natural antioxidants are taken in greater than certain concentrations, they can produce significant, sometimes even deadly, physiological effects. For example, while an overdose of Vitamin C is fairly difficult to achieve, as the concentration ingested in order to produce overdose-like effects is approximately 3000 mg/day, exceeding this dose can lead to some negative physiological effects. Consuming a dose larger than 3000 mg/day for several days can lead to illnesses such as kidney stones, and an increased need for oxygen. Also, as with any acid, increased consumption of Vitamin C can lead to excess uric acid excretion and erosion of dental enamel. [20] Additionally, all polyphenols have exhibited pro-oxidant behavior (that is, they cause other compounds to become oxidized instead of reduced) [42]. Furthermore, some polyphenols have been shown to interfere with the metabolism of certain medicines when taken in excess.[40]

Tocopherols, Vitamin E in particular have been studied somewhat extensively for their adverse effects, as tocopherol overdoses are one of the more severe forms of dietary supplement overdoses. In doses of 1600-3200 mg/day for extended periods of time, Vitamin A overdoses can lead to symptoms such as fatigue, breast soreness, gastrointestinal stress, vascular inflammation, and thyroid problems. [22] While the specific concentrations that lead to overdose in Vitamin A seem to vary vastly on a case-by-case basis, it has been found that in very high doses for extended periods of time, excess Vitamin A consumption can lead to hypercalacemia,. This condition can be a very serious disease, leading to nausea, dementia, renal failure, and sometimes even death. [41]

Coenzyme q10, also known as Ubiquinone, has also been found to have several positive health effects when ingested as a dietary supplement. It has been seen to alleviate hypertension, prevent ischemic heart disease, and promote congestive heart failure.[31] However, as with all other antioxidants, Coq10 has negative side effects if taken in excess. It may cause severe hemorrhages if taken in very large quantities, and just as with polyphenols, it could act as a pro-oxidant in high concentrations. [38]

4. Synthetic 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[2]. These antioxidants fall into two major categories depending on their mode of action- Primary antioxidants and Secondary antioxidants. The primary antioxidants, which prevent the formation of free radicals during oxidation, can further include three major categories (Figure 6)- Free Radical terminators, Oxygen scavengers, and Chelating agents[80,79,57].

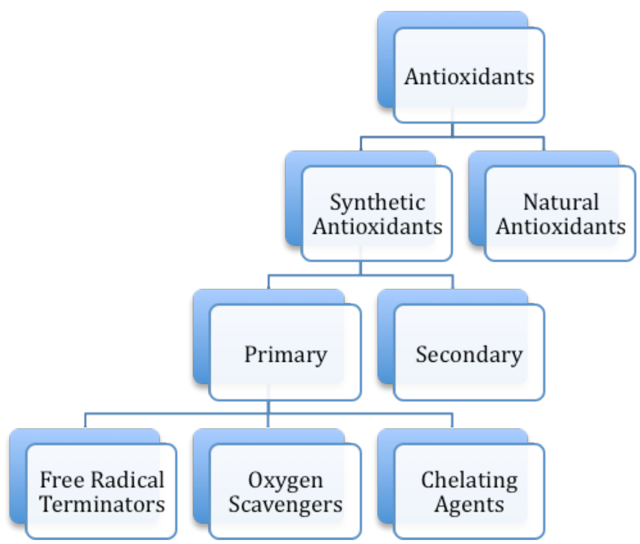

Figure 6: Various types of synthetic antioxidants.

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[82]. 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[80,81]. (Figure 7)

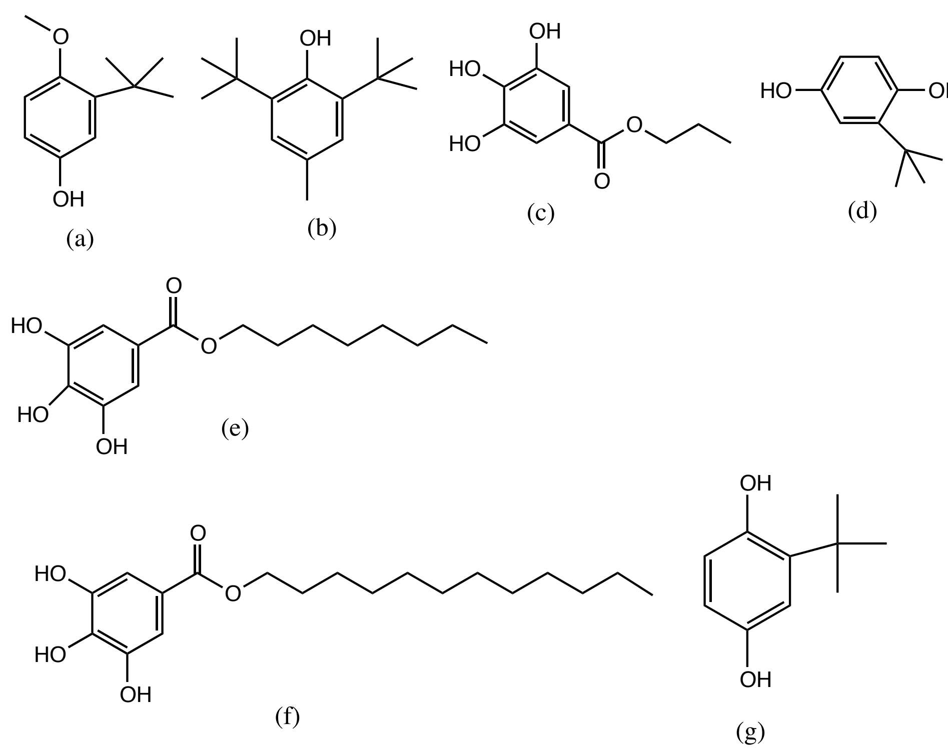

Figure 7: Chemical structure of synthetic antioxidants. a)BHT (b)BHA (c)PG (d)OG (e)DG (f)EDTA (g)TBHQ

4.1 Types of Synthetic Antioxidants

BHT and BHA are the most prevalent synthetic antioxidants in food as reported by the National Research Council Food Additive Committee[81]. 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 (Figure 7). Both of these additives have good carry through properties, which determine their ability to withstand various processing steps such as baking and frying and maintain their functionality[60]. 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 ^{0}C and 268 ^{0}C 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[80,81,57,58].

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 soluble in fats just like BHT and BHA due to similar structural features. With its boiling point range of about 271.3-311.3 ^{0}C, it is less volatile as compared to BHT and BHA [57]. 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[80,81].

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 (Figure 7). With a boiling point range of 161.3-201.3 ^{0}C, 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[80,81].

Octyl gallate (OG) and dodecyl gallate (DG) are the other two gallates used as antioxidants in food. Both OG and DG are white to creamy white crystalline, odorless solids[64,65]. OG is a diphenol like TBHQ, with similarities in their sizes and structures, while DG has more hydroxyl groups and a long hydrocarbon side chain in its ring structure (Figure 7). The increased solubility of DG in water due to the presence of a number of hydroxyl groups is counterbalanced by the decrease in solubility due to the long hydrocarbon side chain. As a result, both OG and DG are insoluble in water like TBHQ, BHT and BHA[57]. With their boiling points ranging between 442.9-522.9 ^{0}C and 476.7-566.7 ^{0}C respectively, OG and DG are the least volatile among all the gallates[57] (Figure 8).

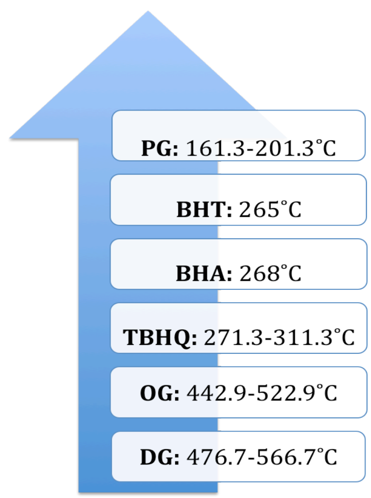

Figure 8: Boiling points of synthetic antioxidants.

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[81, 82]. It is mostly used in processed fruits and vegetables, soft drinks, salad dressings, margarine and canned sheelfish[58]. However, the use of EDTA in combination with certain natural antioxidants is not advisable. For example, citric acid hampers the antioxidant capability of EDTA, since EDTA and citric acid combination forms catalytically active iron chelates that enhance oxidation reactions[82]. The various properties of the important synthetic antioxidants discussed in this section are summarized in Table 3 .

Table 3: Additional data on structure, solubility, melting point, potential daily intake (PDI), and countries in which each synthetic antioxidant is banned.

Compound Name	Chemical Structure/ Family	Morphology/Solubility	Thermal Degradation beyond melting temp	Amount consumed	Countries Banned In
BHA (butylated hydroxyanisole)	Monohydric Phenol[2] Radical scavenger[34]	White waxy flakes soluble in fat, insoluble in water[2,3]	Steam Volatile[2,3] Melting point: 50-52 ^{0}C [2,33]	PDI: 5.6mg/kg/capita[51]	Japan [52]
BHT (butylated hydroxytoluane)	Monohydric Phenol[2] Radical scavenger[34]	White crystalline compound soluble in fat, insoluble in water[2,3]	Steam Volatile[2,3] Melting point: 69-70 ^{0}C [2,33]	PDI: 2mg/kg/capita[51,41]	None[43]
PG (Propyl Gallate)	Phenol[2] Radical scavenger[34]	White crystalline powder sparingly soluble in water[2,3]	Melting point: 146-148 ^{0}C [2,3,33]	PDI: 1.4-3.9mg/kg/capita[51,41]	None[43]
TBHQ (tertiary butylhydroquinone)	Diphenolic[2] Radical scavenger[34]	Beige colored powder soluble in fats[2,3]	Melting point: 126-128 ^{0}C [2]	PDI: 14mg/capita[44]	Canada, Japan, European countries [52,53,40,2]
OG (Octyl gallate))	Diphenolic[33] Radical Scavenger[34]	White to creamy white crystalline solid, insoluble in water[33]	Melting point: 91-92 ^{0}C [33]		None[43]
DG (dodecyl gallate)	Phenol[33] Radical Scavenger[34]	White to creamy white crystalline solid, insoluble in water[33]	Melting pont: 94-96 ^{0}C [33]		None[43]
EDTA (Ethylenediaminetetraa cetic acid)	Metal chelator[34]				None[43]

4.2 Chemical Action of Synthetic Antioxidants

Radical terminators, the major class of synthetic antioxidants, fall under a group of chemical compounds called phenols. Their specific mechanism of action involves transfer of the hydrogen of the phenolic hydroxyl group to a lipid free radical in order to form stable free radicals, which do not initiate or propagate the oxidation chain reactions. (Figure 9)

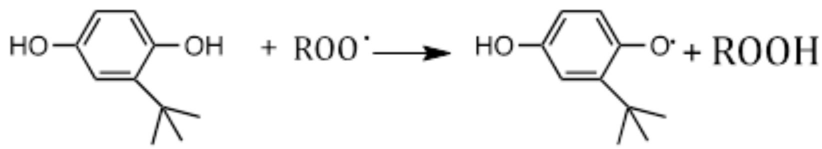

Figure 9: Antioxidant mechanism of a radical terminator via the donation of an electron from a phenolic hydroxyl group.

These reactions involving the donation of hydrogen to the lipid free radicals are highly exothermic and compete with the chain propagation reactions during the lipid auto-oxidation[80,81].

Phenolic antioxidants such as BHA, BHT, TBHQ and PG all act as excellent antioxidants due to two main reasons. Firstly, their hydrogen bond dissociation energy is low making them efficient hydrogen or electron donors. Secondly, the phenoxy radicals generated after the donation of electrons to the lipid free radicals do not themselves generate other free radicals. They are relatively stable due to resonance stabilization by delocalization of electrons across the aromatic ring and the lack of suitable sites for attack by molecular oxygen. (Figure 10)

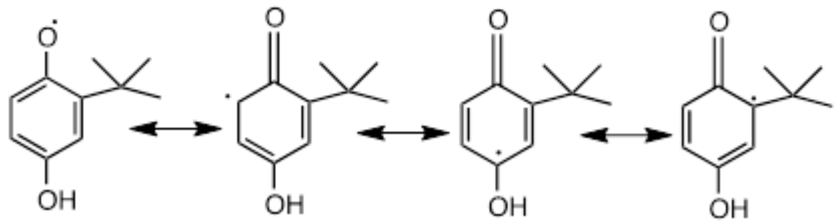

Figure 10: Resonance stabilization of a phenolic antioxidant.

Furthermore, the efficiency of these phenolic antioxidants varies depending on the absence or presence of certain other groups on the aromatic ring, mainly at the *ortho* and *para* positions. For example, the stability of the phenoxy radical is increased by the presence of bulky groups at the ortho position as in butylated hydroxyanisole (BHA), which is 2,6-di-tertiary-butyl,4-methoxyphenol. (Figure 11)

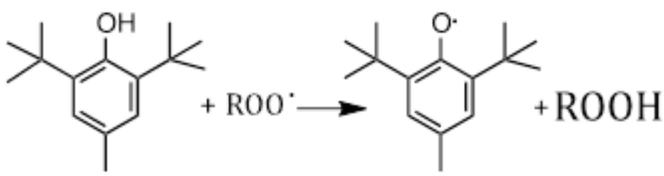

Figure 11: Oxidation of BHA via donation of an electron from a phenolic hydroxyl group.

The presence of bulky group introduces steric hindrance in the region of radicals, decreasing the rate of further propagation reactions. Another example, which illustrates the increase in antioxidant activity, is the presence of an extra hydroxyl group at the ortho or para position of the hydroxyl group of phenol. The stability of the phenoxy radical in this case is enhanced by the formation of an intra-molecular hydrogen bond [80,81]. (Figure 12)

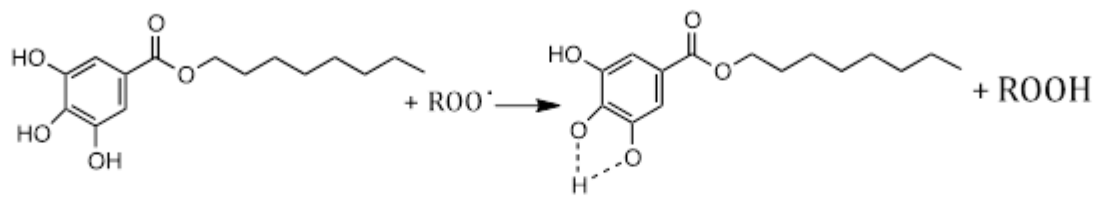

Figure 12: Generation of a phenoxy radical, with the intramolecular hydrogen bond shown.

Besides the structure, the antioxidant activity is also affected by other factors such as concentration. The phenolic antioxidants often lose their activity at higher concentrations and rather behave as pro-oxidants participating in the initiation reactions[69,57]. Finally, the added synthetic antioxidants are incapable of retarding the deterioration of already spoiled food items and therefore, must be added to food as early as possible[80,81]. This early addition can be achieved by adding the antioxidants in their commercial forms to the fats and oils used in food production, by spraying them as food grade solvents on the food items or by incorporating them in the packaging materials[57].

4.3 Biological Effects of Synthetic Antioxidants

The synthetic antioxidants used in food as preservatives can exert effects in biological systems similar to their effects when used as dietary supplements[69,57]. The synthetic antioxidants, specifically the phenolic antioxidants have several common biological effects on the molecular, cellular and organ levels. Their biological effects can be grouped into the following major categories- modulation of growth, macromolecule synthesis and differentiation, modulation of immune response, interference with oxygen activation and miscellaneous. Not all of these effects have been observed with each of the synthetic antioxidant used[83].

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 show to cause increase in organ weight like hypertrophy of liver[61], 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 [61]. 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 & 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 H_2O_2 formation, cause decrease in smooth muscle and heart muscle contractility and can have an influence on lipid metabolism and dietary levels of vitamin E[61, 83]. In addition, BHA might cause membrane labialization, cell lysis, alteration of lipid composition in liver, serum & platelets and might cause lesions in forestomach[80].

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 factors[80].

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 H_2O_2 formation and decrease smooth muscle and heart muscle contractility. Furthermore, PG has been shown to have local anesthetic action and anti-inflammatory action[83].

Besides the above-described direct biological actions of the synthetic antioxidants, a large number of interactions with physical and chemical noxae have been described. These interactions can be either harmful or beneficial and are most often directed to the same biological point[83].

4.4 Health Risks and Benefits

There are only a few synthetic antioxidants such as BHA, BHT, PG, and TBHQ currently permitted for usage in food as preservatives, because the toxic effects of their degradation products is still under study[80,69,57]. Even for the above-mentioned synthetic antioxidants, the Food and Drug Administration (FDA) requires that their presence be mentioned on food labels along with an explanation of their intended usage[80]. The permissible levels of synthetic antioxidant in food is decided based on the fat content in the recipient food item, and are usually limited to 0.02% total antioxidants[81]. The maximum limits of synthetic antioxidants in food are regulated by various legislating authorities such as European Union Directives and Regulations, the FDA in the United States, Food Standards Australia New Zealand for Australia and New Zealand, Joint FAO/WHO Expert committee on food additives[57]. Table 4 summarizes the permissible levels in food for the synthetic antioxidants.

Table 4: Typical synthetic antioxidants used as preservatives, their legal limits in foods, and their common sources.

Compound Name	**Limits in food**	**Found In**
BHA (butylated hydroxyanisole)	Less than 200 mg/kg [22,23]	Cereals, chewing gum, potato chips, vegetable oils, biscuits, cakes, pastries, sugar, honey, spices, meat products, milk products, etc [20,21]
BHT (butylated hydroxytoluane)	Less than 100 mg/kg [22,23]	Cereals, chewing gum, potato chips, oils, biscuits, cakes, pastries, beverages, sugar, honey, spices, meat products, milk products, etc [20,21]
PG (Propyl Gallate)	Less than 200 mg/kg [22,23]	Vegetable oil, meat products, potato sticks, chicken soup base, chewing gum, sugar, honey, spices, milk products, etc [20,21]
OG (Octyl gallate)	Less than 200 mg/kg [22]	oils and fats, cereals, snack foods, dairy produce, sugar, honey, meat products, etc [20,21]
DG (dodecyl gallate)	Less than 200 mg/kg [22]	oils and fats, cereals, snack foods, dairy produce, meat products, etc [20,21]
EDTA		Salad dressing, margarine, sandwich spreads, mayonnaise, processed fruits and vegetables, canned shellfish, soft drinks. **[20,21]**
TBHQ (tertiary butylhydroquinone)	Not allowed [22,23]	Milk, milk products like cheese, meat & meat products, chewing gum, fish & fish products, sea food, sugar, honey, spices, etc [20,21]

When used within the recommended levels, most synthetic antioxidants have been shown to be effective in preventing the lipid deterioration in food. In fact, at those low concentration levels in food, the synthetic antioxidants have been observed to add beneficial effects to our health especially anti-carcinogenic and anti-mutagenic effects[79]. They are known to prevent the metabolic activation of a pre-carcinogen by inhibition of the activating enzyme or by alteration of the metabolic pattern of a carcinogen via selective enzyme induction. Also, some of them may prevent the reaction between the ultimate carcinogen and DNA by direct interaction with carcinogenic species, by increased detoxification by antioxidant-inducible enzymes and by competition between the carcinogen and the antioxidant at the binding process[83].

Although, in general the antioxidants when used within the recommended levels are known to have beneficial effects on health while increasing the shelf life of food items, recent studies with rodents and monkeys have shown that these antioxidants might exert toxic side effects at higher concentrations[72,75,66]. The daily intake of antioxidants like BHT and BHA are estimated to be about 0.1mg/kg. At larger doses of about 500 mg/kg i.e. 5000 times the normal intake, both BHT and BHA have been shown to exert pathological, enzyme or lipid alterations or carcinogenic effects[61]. It has been suggested that their degradation products might exert toxic effects. For example, the formation of tert-Butylhydroquinone (tBHQ), one of the degradation products of BHA is known to exert carcinogenic effects by causing oxidative damage to DNA[67, 77].

Even though at current levels of intake the synthetic antioxidants seem to pose no reasonable threat to health, but long term chronic ingestion of these antioxidants may aid in modifying the acute toxicity of several carcinogenic and mutagenic chemicals and lead to chronic side effects. Moreover, the effects exerted by the antioxidants also depend on their pattern of metabolism and the excretion of the metabolic products. Both BHA and BHT are excreted mainly as glucuronide conjugates in urine with small amounts being excreted as ether sulphates or phenols and the rest in feces. But BHT has been observed to be excreted at a much lower rate than BHA, increasing the chance of its accumulation in the body[61]. Thus, considering all the factors like intake levels, metabolism patterns and excretion, further research needs to be conducted in order to establish the occurrence of their toxic effects on human health and also to better determine the efficient levels and combinations of the synthetic antioxidants in various food items.

5. Public Awareness of Antioxidants used as Preservatives

Antioxidants are almost ubiquitous in commonly consumed food products, as they are generally pre-existing compounds in the form of natural antioxidants, or are added during processing as synthetic antioxidants. As long as they are consumed in moderate concentrations, natural antioxidants have been proven to have many positive health effects, such as preventing plaque formation in the arteries and preventing other chronic conditions such as cancer and heart disease.[51-53] These beneficial properties have put natural antioxidants on the forefront of recent food advertising, and public levels of awareness concerning natural antioxidants and their positive effects have increased significantly.[84] However, while natural and synthetic antioxidants can both be used as food preservatives to prevent browning and rancidity and to extend shelf life, the Food and Drug Administration only defines antioxidants as dietary supplements.[1] Thus, antioxidants are almost always perceived in solely a positive light.

Synthetic antioxidants, like natural antioxidants, are present in several different types of foods, particularly those that are heavily processed. Unlike natural antioxidants, however, synthetic antioxidants have been shown to have potential toxic effects on the health of consumers.[61,80,83] Additionally, synthetic antioxidants are not advertised prominently on food labels and the number of studies done on public awareness of the synthetic antioxidants and their health effects is sparse, in direct contrast to the focus placed on the natural antioxidant content of food items. This lack of interest and inquest has led synthetic antioxidants to be the subject of few studies, particularly those assessing the public's awareness of their presence and toxic effects.

Considering recent increased awareness of beneficial natural antioxidants, as well as the lack of studies about the public's awareness of synthetic antioxidants and their potential toxic effects, an investigation was conducted. This study aimed to better understand public awareness of antioxidants, the differences between natural and synthetic antioxidants, and the potential toxic effects of synthetic antioxidants. To determine the level of awareness regarding antioxidants used as preservatives, two surveys (an example of which is shown in Appendix A), of two different populations were conducted: a) the general public, and b) the population of an academic institution (faculty, staff, etc.). Both surveys were similar, with each containing questions pertaining to the respondent's demographic information (age range, sex, occupation, etc.) as well as questions regarding the respondent's general knowledge of antioxidants, antioxidants used as preservatives, and studies carried out pertaining to the side effects of consuming synthetic antioxidants. The goal of the survey was to gradually introduce the topic of antioxidants to the respondents. Some questions were asked to assess how closely respondents examined food labels, their awareness about the existence of food additives, and finally their awareness and concern about natural and synthetic antioxidants as food additives. The respective sample size for each survey was 641 respondents (academic setting survey) and 128 respondents (general public survey).

5.1 Survey Results

Overall, the survey contained many questions regarding the respondents' awareness of food ingredients in general, their level of awareness concerning food additives, and their understanding of antioxidants in particular. Questions that indicated in particular the respondents' awareness, understanding, and concern towards antioxidants were analyzed using the Chi-squared method. The survey data showed that a significant number of people are aware of antioxidants and that they are present in foods. An examination of the p-value (0.003) shows that the data distribution has a significant correlation to the data. In this case, the p-value signifies that a significantly larger portion of the student population is aware of the definition of an antioxidant in comparison to the other occupation demographics tested.

Additionally, a significant majority of survey respondents are in fact unaware of the difference between the two types of antioxidants (Figure 13).

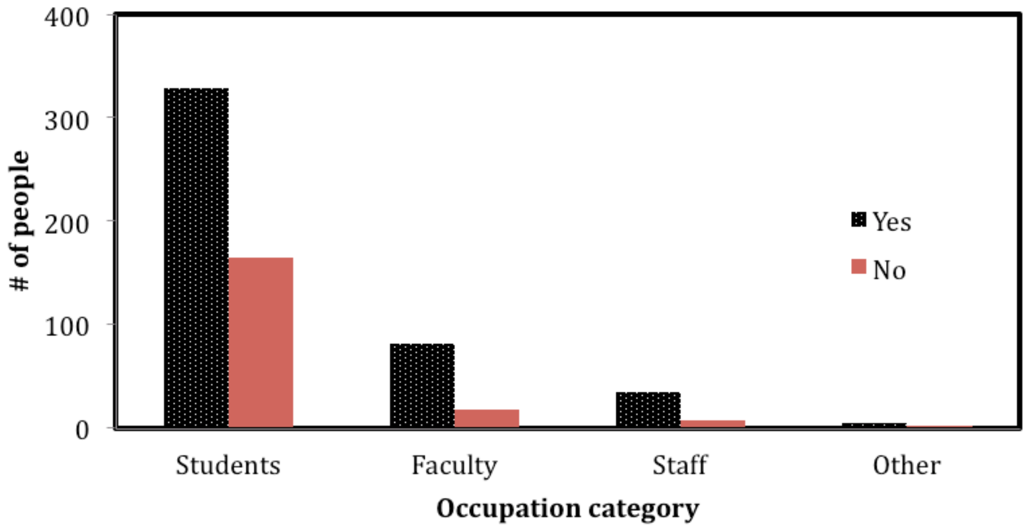

Figure 13: Responses of various groups regarding Question 6 in the survey.

However, the p-value (0.503) shows that the level of awareness does not differ greatly among the different groups tested i.e. all demographics are equally unaware of the difference between antioxidants used as preservatives and antioxidants used as dietary supplements. Similarly, it can be observed that the majority of respondents were unaware of the suspected toxic effects of synthetic antioxidants (Figure 14).

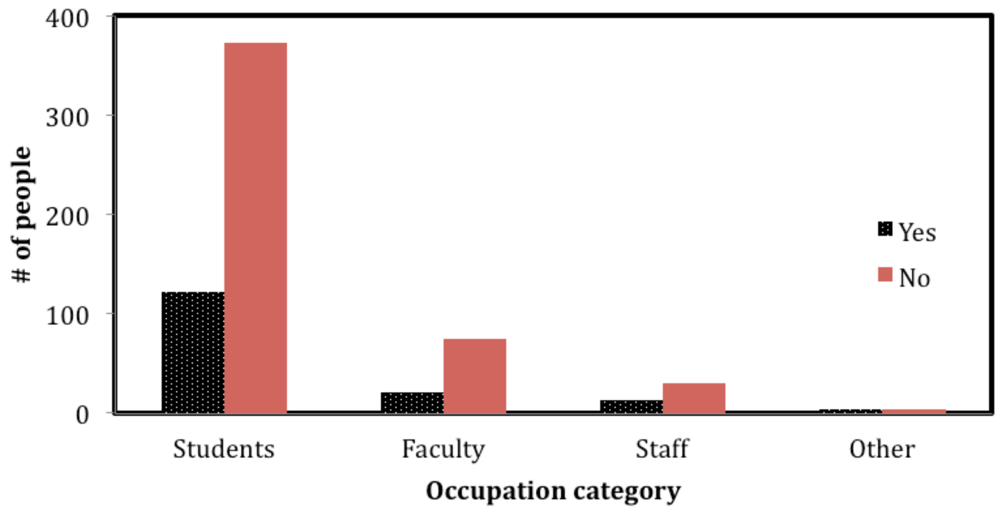

Figure 14: Responses of various groups regarding Question 7 in the survey.

Additionally, the p-value (0.927) showed that the level of unawareness was unrelated to the demographic of the respondent, and that all those surveyed were equally unaware of the possible toxic effects of synthetic antioxidants. While further studies must undoubtedly be undertaken in order to fully understand the awareness and attitudes of the public towards synthetic antioxidants, this survey provides at least one piece of evidence indicating that the public is unaware of the toxic effects of synthetic antioxidants. The results of this survey, in addition to the lack of literature concerning public awareness of synthetic antioxidants proves that this subject has not been explored to the necessary level, and much further study is required.

The chi-square p-values proved that there was little real statistical difference between the awareness levels of the two groups. Overall, it was concluded that while most of the population has indeed heard the term “antioxidant” used in terms of food, much of the public, regardless of occupation or education level, is unaware of the roles of antioxidants as preservatives in food. Additionally, it appears that a very small percent of the population is aware of the suspected toxic effects of synthetic antioxidants commonly used in food items. Research has shown that presence of certain synthetic antioxidants could hamper the functionality of the natural antioxidants in food. For example, studies have shown that EDTA reduces shelf life of products containing sorbates, since iron-EDTA complex enhances oxidation of sorbic acid[74]. In the context of such findings, it is particularly necessary for the general population to be aware of the negative effects and interactions between antioxidants. Thus, higher awareness would lead to consumers making informed choices about the foods they consume.

6. Conclusions

Antioxidants are compounds that are either present naturally or added to food items to prevent them from undergoing oxidation, which often leads to browning, rancidity, and a general lack of freshness. Two major types of antioxidants, natural and synthetic exist in foods. Natural antioxidants are found natively in fruits, vegetables, teas, grains and pulses, teas, and some types of meat. In addition, synthetic antioxidants are generally added to foods during the processing and packaging period in order to maintain the freshness of foods.

Natural antioxidants are generally found in fairly small doses in food sources such as fruits and vegetables. However, they are available in larger doses in processed food items that claim to be "fortified" with natural antioxidants such as vitamin C, as well as in most complete dietary supplements. These additional sources may be of little nutritive value to those who consume them, as studies have found that antioxidant absorption decreases when consumed in very high doses. Thus, consumption of antioxidant fortified foods which are high in sugar may not be beneficial at all (as antioxidants attached to sugars have low absorption levels), while foods with lower antioxidant concentrations but some fat content may prove more beneficial, as many antioxidants are fat-soluble. Additionally, although overdose on natural antioxidants is a rare occurrence, consuming these compounds in too high a concentration can lead to negative health effects such as increased oxidation and hypercalacemia.

The most important synthetic antioxidants 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). Chemically, each compound is composed of phenol rings, and their specific mechanism of action involves the transfer of hydrogen from a phenolic hydroxyl group to a lipid free radical in order to form stable free radicals and terminate the oxidation chain reactions. Synthetic antioxidants have been shown to have both beneficial and harmful biological effects on the molecular, cellular and organ levels. As most synthetic antioxidants have been found to be carcinogenic in high doses, their maximum levels are recommended and controlled by various legislating authorities such as the FDA. The permissible levels of synthetic antioxidant in food is decided based on the fat content of the food item, and are usually limited to 0.02% of the total antioxidant content. Within the constraints of these levels, synthetic antioxidants are known to have beneficial physiological effects in addition to increasing the shelf life of food items. However, long-term ingestion of these antioxidants may still lead to chronic side effects. Thus, considering their metabolic patterns and excretion, efficient intake levels and combinations of synthetic antioxidants must still be determined.

While exploring the role of natural and artificial antioxidants in commonly consumed foods, it was found that the only definition the Food and Drug Administration provides of these compounds is as dietary supplements. Indeed, there is no mention of synthetic antioxidants' vast use as preservatives in packaged food. Upon further inspection, it was found that the only antioxidants mentioned prominently on food packaging were natural antioxidants (in particular, polyphenols) as beneficial supplements. Additionally, it was observed that very few studies have been conducted to assess the public awareness of antioxidants as food additives instead of as dietary supplements. This lack of public knowledge was concerning, as studies have shown that some antioxidants, particularly synthetic antioxidants could have negative effects on health.

While there is little conclusive research about antioxidant reaction mechanism and negative antioxidant effects on humans, animal models have shown that consumption of synthetic antioxidants could pose a health risk. While most normal, healthy individuals with resilient cardiovascular systems are not placed at a much significant risk of disease due to their consumption of synthetic antioxidant-rich foods, those individuals with pre-existing conditions (such as hardening of the arteries, heart disease, or cancer) should be aware of their synthetic antioxidant intake. While their effects have been shown to be subtle on most of the population, the health of the individuals with pre-existing conditions may worsen by consumption of these compounds.

It was generally shown through surveys that the public lacks sufficient knowledge about the potential benefits and perceived health risks associated with antioxidants. In order to maximize the beneficial health effects of natural antioxidants, consumers should be aware of a few simple guidelines. As seen in Table 2, while many fruits, vegetables, and other commercially available products may be high in natural antioxidants, absorption can be maximized by consuming dark fruits, processed tomato products (such as ketchup and pasta sauce), and dark chocolate. Additionally, absorption of natural antioxidants can be maximized if consumed in small quantities several times a day, instead of in large quantities once or twice a day.

Table 5: Major foods consumed in the USA and the typical natural antioxidant content in each category.

Name of Food	**Natural Antioxidants Found**
Pizza	Vitamin C (from tomatoes/tomato sauce), Beta-carotene (from tomatoes/tomato sauce), Lycopene (from tomatoes/tomato sauce) Vitamin E (from added oils and from crust)
Pasta	Vitamin E, Coenzyme Q 10
Potato Chips	Vitamin E (from cooking oil), Flavinoids (from potatoes)
Hot Dogs/Hamburgers	Vitamin E (from cooking oils and fats), Coenzyme Q 10 (from meats)
Bread	Coenzyme Q 10 (from wheat bran), Vitamin E (from wheat and cooking oils)

Similarly, consumers should be aware of a few important facts when making dietary decisions regarding synthetic antioxidants. Synthetic antioxidants are most prevalent in fatty, packaged foods such as potato chips, popcorn, and snack cakes, though almost packaged foods contain them. While not prominently advertised like their natural counterparts, synthetic antioxidants can indeed be found on food labels, listed along with all the other ingredients in the nutrition facts. However, generally their full names are not listed, so customers must learn to recognize acronyms such as BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene) in the list of ingredients. Additionally, consumers should evaluate their own health conditions and determine whether consuming synthetic antioxidants would pose them a significant health risk. In light of these findings, it can be concluded that the general public must be educated regarding antioxidants in food and their effects on health. This effort should complement ongoing clinical research on the effects and activity of antioxidants with the ultimate goal of acquiring conclusive evidence as to their suitability as dietary supplements.

Appendices

Appendix A: Survey

Typical survey administered to various groups in order to learn about public attitudes towards antioxidants.

1. How often do you read the list of ingredients when consuming packaged foods?
   - Always
   - Sometimes
   - Rarely
   - Never
2. How aware are you about the role of antioxidants as preservatives?
   - Greatly
   - Somewhat
   - Not at all
3. How do you think antioxidants affect food? Check all that apply
   - Prevent growth of organisms
   - Prevent fats from going rancid
   - Both
   - Not sure
   - Other
4. What foods do you think include antioxidants as preservatives?
   - Fats/Oils
   - Grains
   - Both groups
   - None
   - Other
5. In which forms do you think are antioxidants added to the food as preservatives? Check all that apply.
   - Just as an ingredient
   - In packaging
   - As solvents to be sprayed on foods
   - All of the above
6. Are you aware of the difference between antioxidants used as preservatives or antioxidants used as dietary supplements?
   - Yes

- No

7. Are you aware that some synthetic antioxidants are suspected to have toxic effects?
    - Yes
    - No
8. How concerned are you about the effects of synthetic antioxidants on your health?
    - Very much
    - Somewhat
    - Slightly
    - Not at all
9. Natural antioxidants have been shown to have a lower performance value and to be more expensive to extract as compared to the synthetic ones. Would you still pay a higher price for the 'All natural' tag?
    - Yes
    - No
10. Do you think you have ample accessible resources available to you if you want to further your knowledge on antioxidants?
    - Yes
    - No
    - I am not sure
11. What action would you like to see to educate the public about the prevalence and possible toxicity of synthetic antioxidants?
    - Legislature
    - Public health announcements
    - Integration into public primary education
    - Nothing

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Conclusions

The review of antioxidants indicates that while these compounds provide several beneficial health effects, they have potential toxic effects if not consumed in the proper fashion. Thus, consumers should take into consideration antioxidants' metabolic patterns and excretion, efficient intake levels and combinations of antioxidant compounds to maintain efficient absorption while minimizing negative health effects. The public must therefore examine their diets closely and make adjustments to increase natural antioxidant consumption. However, consumers should look beyond manufacturers' emphasis on the beneficial effects of antioxidants, and critically consider each compound's bioabsorption from different sources. Similarly, consumers should educate themselves about synthetic antioxidants, understand how to find them on food labels and analyze their own health conditions to determine the appropriate intake levels and combinations of these compounds.

Possibly as a direct result of their near-invisibility on food labels, a lack of awareness regarding synthetic antioxidants was seen in the survey of the general public. Overall, it was concluded that the entirety of the public must be educated regarding antioxidants in food, and their effects on health, either positive or negative. In order to further the awareness of the public about antioxidants in food and the health effects they may produce, public service announcements regarding these food additives could be disseminated, and information about synthetic antioxidants as well as natural antioxidants could be made prominent on food labels. However, care should be taken to attempt to spread the message in a manner which will be able to reach people of all demographics, as it was clearly seen in the data that members of all occupations were in need of greater information regarding antioxidants in food products.

Appendices

Appendix A: Letter Sent to Various Journals

To Whom it May Concern:

As part of our Interactive Qualifying Project (IQP) at Worcester Polytechnic Institute (WPI), we, Disha Sood (WPI class of 2012) and Rashmi Venkatesh (WPI class of 2011), are conducting an investigation into preservatives in food. At WPI, each student is required to complete an Interactive Qualifying Project, where students are challenged to address a current topic in science and technology which is also of social significance.

As part of our project, we plan on addressing the following issues: the biochemical aspect of preservative use in food, such as the major classes of food preservatives, the types of foods in which preservatives are found most often, and toxicology and carcinogenicity levels of various preservatives at different concentrations; levels of preservative consumption prevalence in different populations (varying geographic regions, socioeconomic levels, age groups, education levels, etc.); and finally, the level of public knowledge, as well as perception of, the use of preservatives in commonly consumed foods. As part of our research, we plan on speaking to several experts, as well as designing surveys to assess multiple populations.

We hope that you will be willing to review our research for possible publication in your journal. Thank you in advance.

Sincerely,

Rashmi Venkatesh and Disha Sood

Appendix B: Letter Sent to Nutritionists

Dear Dr.________,

I, Rashmi Venkatesh (Worcester Polytechnic Class of 2011), and my project partner Disha Sood (Worcester Polytechnic Institute Class of 2012), are currently conducting an investigation on the health effects and public awareness of synthetic antioxidants used as food preservatives. We are doing this research to complete a project, which is part of the Worcester Polytechnic Institute graduation requirements. We were hoping to conduct a short interview with you via e-mail in order to gain your expert perspective on this issue. If it is possible for you to help us, we will send you a few short questions about synthetic antioxidants, and would be grateful for your reply. Thank you for your time.

Sincerely,

Disha Sood

Biomedical Engineering

WPI Class of 2012

and

Rashmi Venkatesh

Biochemistry

WPI class of 2011