POLYPHENOLS AS POWERFUL NARURAL ANTIOXIDANTS

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Abstract: Antioxidants occupy a special place in food industry as food additives, used against food deterioration. Antioxidants include natural and synthetic preservatives, but a lot of people do not know the difference between these two types of food additives. Meanwhile, synthetic antioxidants are much more dangerous than natural additives. So we should study and develop the proprieties of the natural antioxidants. Powerful natural antioxidants are plant pigments – polyphenols, the major class of which is represented by flavonoids. Polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress, such as cancer, osteoporosis, diabetes mellitus etc. Numerous factors affect the polyphenol content of plants, such as environment, storage, methods of culinary preparation etc. Flavonoids (red pigments) changes their color in different mediums. This fact helped us to implement a qualitative reaction for the presence of flavonoids in red cabbage.

Keywords: *antioxidant, polyphenols, preservatives, flavonoids, pigments*

1. Antioxidants, as food additives

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules.

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent – antioxidant. Oxidation reactions can produce free radicals (atoms, molecules, or ions with unpaired electrons on an open shell configuration, that cause radicals to be highly chemically reactive). In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [1].

Antioxidants (class of food additives indicated in the range from E300 to E324) are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors [2]. Consequently, packaging of fresh fruits and vegetables contains ~8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food [3]. These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) [4].

Some of the synthetic food preservatives are well known to a modern consumer as E320, E321 etc., because we meet it every day on the packages of food products. But most people don't know what Sodium Bisulfite, Benzoyl Peroxide, TBHQ, BHA, or BHT are, along with hundreds of other chemicals that are added to food. The public is unaware of why certain chemicals are used and what kind of effects they have on human bodies. The questionnaire[3] showed that 51% of the people do not read ingredients of the foods that they eat, and that they regularly consume products that contain BHT, BHA etc. In addition, it was shown that 74% of the people are not aware of what BHT and BHA are.

[3] The questionnaire was conducted among students and profesors of the Technical University of Moldova

Meanwhile, the synthetic chemicals BHA and BHT are very dangerous and threatening. The HACSG (Hyperactive Children's Support Group) recommends to avoid them. These additives are not permitted in foods for infants or young children. [5]

The toxicology of the synthetic food preservatives BHA and BHT was compared with the proprieties of the naturally occurring vitamin E (alpha-tocopherol). In high dosages all three compounds induce in animals impairment of blood clotting, which can be explained by an antagonism with vitamin K. Specific toxic effects to the lung have only been observed with BHT. The other described toxic effects of BHA and BHT are less characteristic and often occur only after high dosage and long-term treatment. However, BHA induces in animals tumours of the forestomach, which are dose dependent, whereas BHT induces liver tumours in long-term experiments. Because there is no indication of genotoxicity BHA and BHT, all published findings agree with the fact that BHA and BHT are tumour promoters. In contrast to BHA and BHT, vitamin E is not carcinogenic. On the other hand, all three antioxidants have also anticarcinogenic properties. The intake of the necessary high doses as for these effects are, however, contraindicated with BHA and BHT because of their carcinogenic effects. The present overview concludes that the concentrations of BHA and BHT nowadays used in food, drugs and cosmetics are probably harmless. In addition, vitamin E can also be used in higher doses without the occurrence of adverse effects [6].

Thus natural food preservatives are much safer than synthetic chemicals. The questionnaire showed that 81% of the people would like to consume more expensive, but natural and safe food products.

2. Polyphenols, as powerful natural antioxidants

Polyphenols – plant pigments, which are powerful natural antioxidants. They comprise a wide variety of molecules that have a polyphenol structure (i.e. several hydroxyl groups on aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are: favonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (Table 1) [7].

Table 1. *The main edible sources of polyphenols*

The class of polyphenols	**The subclass of polyphenols**	**The main edible sources of polyphenols**
Flavonoids	*Flavonols*	onions (up to 1.2 g/kg fresh wt), curly kale, leeks, broccoli, and blueberries; tea and red wine
	Flavones	parsley, celery, mandarin
	Flavanones	oranges, lemons, tomatoes, mint
	Isoflavones	leguminous plants: soya, soybeans (140-1530 mg/kg fresh wt) and soy milk (12-130 mg/L)
	Anthocyanins	red wine (up to 350 mg /L), cereals, vegetables (cabbage, beans, onions, radishes), cherry, strawberry, black currant, blackberry
	Flavanols	Catechins: apricots (250 mg/kg fresh wt), cherry (250 mg/kg fresh wt), green tea (up to 800 mg/L), chocolate (up to 600 mg/L), red wine (up to 300 mg/L).
Phenolic acids		tea, blackberry, raspberry, olive oil, coffee, kiwi, blueberries
Phenolic alcohols		olive oil, white and red wine, beer
Stilbenes		grapes, berries and peanuts, red wine, grape juice
Lignans		linseed oil

As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress. Experimental studies, in fact, strongly support a role of polyphenols in the prevention of cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative disease [8].

3. Flavonoids, as the major group of the polyphenols

Flavonoids consist of two benzene rings joined by a linear three-carbon chain. The central three-carbon chain may form a closed pyran ring with one of the benzene rings.

Flavonoids are themselves divided into 6 subclasses, depending on the oxidation state of the central pyran ring: flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols (catechins and proanthocyanidins) (Table 1) [9].

Flavonols have a double bond between C2 and C3, with a hydroxyl group in the C3-position. They represent the most ubiquitous flavonoids in foods, with quercetin as the more representative compound. It is important to note that flavonols biosynthesis is stimulated by light, so they accumulate in the outer and aerial tissue of fruits. Interestingly, differences in concentration can exist among fruits on the same tree and even between different sides of a single piece of fruit, depending on exposure to sunlight [10].

Flavones have a double bond between C2 and C3, and are the less common flavonoids.

Flavanones are characterized by the presence of a saturated three-carbon chain and an oxygen atom in the C4. The solid parts of citrus fruit, in particular the white spongy portion (albedo) and the membranes separating the segments, have a very high flavanone content; this is the reason way the whole fruit may contain up to 5 times as much as a glass of orange juice [11].

Isoflavones have structural similarities to estrogens – hydroxyl groups in the C7 and C4 positions. They can bind to estrogen receptors and are classified thus as phytoestrogens. Isoflavones are sensitive to heat and are often hydrolyzed to glycosides during industrial processing and storage [12].

Anthocyanins are water-soluble pigments, responsible for most of the red, blue, and purple colours of fruits, vegetables, flowers, and other plant tissues or products. They occur primarily as glycosides of their respective aglycones form, called anthocyanidins. Food contents are generally proportional to colour intensity and reach values up to 2-4 g/kg fresh wt in blackcurrants or blackberries; the contents increase as the fruit ripens. Anthocyanins are found mainly in the skin, except for some red fruits (cherries and strawberries) in which they also occur in the flesh [13].

Flavanols contain a saturated three-carbon chain with a hydroxyl group in the C3. They exist in both the monomer and the polymer form (catechins and proanthocyanidins respectively). Unlike other classes of flavonoids, flavanols are not glycosylated in foods. Proanthocyanidins are dimers, oligomers, and polymers of cate chins. They are responsible for the astringent character of fruit (grapes, apples, berries, etc.) and beverages (wine, cider, tea, beer etc) and for the bitterness of chocolate. It is important to note that this astringency changes over the course of maturation and often disappears when the fruit reaches ripeness [14].

4. Factors, that play a key role in determining the polyphenol content

Numerous factors, such as ripeness at the time of harvest, environmental factors, and storage, may affect the polyphenol content of plants.

Environmental factors, such as climatic (sun exposure, rainfall) or agronomic (different type of culture, fruit yield per tree, etc.) play a key role in determining the polyphenol content. In particular, the exposure to light has a considerable effect on most flavonoids. The degree of ripeness differently affects the concentrations and proportions of the various polyphenols: generally phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase. Storage may also affect the content of polyphenols that are easily oxidized, leading to the formation of more or less polymerized substances, which alter particularly the colour and the organoleptic characteristics of fruits. Cold storage, in contrast, did not affect the content of polyphenols.

Polyphenol content of foods is also influenced by the methods of culinary preparation. Simple peeling of fruits and vegetables can significantly reduce polyphenol content, because these substances are often present in high concentrations in the outer parts. Cooking, also, have a remarkable effect: for example onions and tomatoes lose about 75% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying. Potatoes contain up to 190 mg chlorogenic acid/kg mainly in the skin [15].

5. Heating and pH changes, as the main method to determine the presence of flavonoids in a product

Polyphenols are not just some mysterious particles, contained in food products of plant origin, but most of them are represented by well-known pigments – flavonoids, that color fruits and vegetables in red.

The pigments in fruits and vegetables fall into three classes: chlorophyll, flavonoids and carotenoids. Chlorophyll is a green pigment found in many vegetables such as peas, broccoli and spinach. Flavonoids are the red pigments in red cabbage, red grapes, and red onions. Carotenoids are the yellow, orange, and red-orange pigments found in foods such as carrots, squash, and tomatoes. Heating, pH changes, and chemical reactions can alter the appearance of food by causing changes in the color [16].
An experiment with red cabbage was conducted to determine the presence of the flavonoids in it. It had three main steps, that included heating 150 ml of deionized water to 100ºC (10 minutes) with pieces of red cabbage in three different mediums – acid, normal and basic. The cabbage boiled in water became purple, while cabbage in acid became bright red, and cabbage in basic turned blue-green.

6. Conclusion

Antioxidants play an important role in modern society. They occupy a special place in food industry and are used as food additives to help guard against food deterioration. Antioxidants include natural preservatives such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic preservatives such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321). Synthetic additives are much more dangerous than natural antioxidants, and a very big problem is related with the fact that people do not know the difference between these types of preservatives. However, almost 82% are ready to pay more for safe and natural products.

Powerful natural antioxidants are plant pigments – polyphenols. Polyphenols are divided into several classes: phenolic acids, phenolic alcohols, stilbenes and lignans, but the major class is represented by favonoids. Polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress, such as cancer, osteoporosis, diabetes mellitus etc.

Numerous factors affect the polyphenol content of plants. They are ripeness at the time of harvest, environmental factors (climatic – sun exposure, rainfall; agronomic), storage, methods of culinary preparation, cooking. Flavonoids (red pigments) change their color in different mediums (pH=7 – purple, pH<7 – bright red, pH>7 – blue-green). This fact helped us to implement a qualitative reaction for the presence of flavonoids in red cabbage.

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