

REVISIÓN

Synthetic and natural antioxidants: food quality protectors

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RESUMEN

Antioxidantes sintéticos y naturales: protectores de la calidad de los alimentos.

La oxidación de los componentes lipídicos de un alimento, conocida como rancidez oxidativa, es una de las reacciones que deteriora y afecta en forma más importante la calidad de un producto. La rancidez oxidativa es iniciada por radicales libres del oxígeno o por el ataque del oxígeno molecular a radicales libres pre-formados en los ácidos grasos poliinsaturados que forman las grasas y aceites. La oxidación puede ser prevenida o retrasada por los antioxidantes, sustancias orgánicas de origen sintético o natural que actúan como atrapadores de los radicales libres del oxígeno involucrados en la oxidación de los ácidos grasos. Los antioxidantes sintéticos son los más populares y ampliamente utilizados, sin embargo existe preocupación respecto a la seguridad de estos para la salud tanto humana como animal. Esta situación ha estimulado la investigación sobre sustancias de origen natural con actividad antioxidante. Pocas de estas sustancias naturales han probado ser efectivas como antioxidantes cuando se les compara con los productos sintéticos en las mismas condiciones experimentales. Este trabajo resume las principales características de los antioxidantes sintéticos más importantes. También discute las características de cuatro antioxidantes naturales, compara las ventajas y desventajas del uso de antioxidantes naturales en relación a los sintéticos y visualiza el futuro de los productos naturales con actividad antioxidante.

PALABRAS-CLAVE: *Alimento – Antioxidante natural – Antioxidante sintético – Protector de la calidad – Rancidez oxidativa – Revisión (artículo).*

SUMMARY

Synthetic and natural antioxidants: food quality protectors.

Oxidation of food lipid components, known as oxidative rancidity, is one of the major deteriorative and quality-affecting reactions. Oxidative rancidity is initiated by oxygen free-radicals or by the reaction of molecular oxygen with pre-formed organic free-radicals from polyunsaturated fatty acids composing fats and oils. Oxidation may be prevented or delayed by antioxidants, these substances being organic molecules of either synthetic or natural origin which can scavenge the oxygen free-radicals involved in fatty acid oxidation. Synthetic antioxidants are the most popular and widely used antioxidants, however concerns about it safe to both human and animal health is encouraging research on substances from natural origin showing antioxidant properties. Few natural antioxidants have been proved to be

effective when compared to synthetic products in the same experimental conditions. This work summarizes the main characteristics of the most important synthetic antioxidants, also discuss the principal characteristics of four natural antioxidants, comparing the advantages and disadvantages of using natural products compared to synthetic ones, and sight the future for natural products with antioxidant activity.

KEY-WORDS: *Food – Natural antioxidant – Oxidative rancidity – Quality protector – Review (paper) – Synthetic antioxidant.*

1. INTRODUCTION

Lipid oxidation is one of the major causes of food deterioration. The development of this process, generally referred as oxidative rancidity, is often the decisive factor determining the useful storage life of food products, even when their fat content is as low as 0.5% or 1%. Oxidative rancidity produces undesirable changes in color, flavor, aroma, and other quality factors of a food, and causes the rancid taste and odor that develops in unprotected lipids (fats and oils). The nutritional value of a product is impaired and even toxicity may be induced. The texture may also change as a result of side reactions between proteins and the product of fat oxidation. In short, oxidative deterioration of lipids may be considered as a spoilage factor affecting all the aspects of food acceptability. Much research has been conducted to understand better the mechanism of oxidative rancidity of polyunsaturated lipids, and the effect of decomposition products of lipid oxidation on the development of rancidity on foods.

Antioxidants are organic molecules of either synthetic or natural origin which can avoid or delay the development or the progress of oxidative rancidity. Much research has been done on antioxidants. Synthetic antioxidants have been a matter of concern during the last two decades because some deleterious and potentially dangerous effects have been observed for some of them. Natural antioxidants arise as an alternative for substitute the questioned synthetic antioxidants. However, there are

also many drawbacks to natural antioxidants when compared to the synthetic products.

2. OXIDATIVE RANCIDITY: MECHANISMS AND PRODUCTS

The classical mechanism for oxidative rancidity is via oxygen free-radical attack, and the initial substrates for oxidation are polyunsaturated fatty acids composing fats and oils (Labuza, 1971). The high the polyunsaturation of a lipid, the high its susceptibility for oxidative rancidity development. It has been proposed that the number of pentadienic units present in the structure of a polyunsaturated fatty acid, correlates with its susceptibility for develop oxidation (Nawar and Ultin, 1988). A pentadienic unit is defined as a structure formed by five carbon atoms bearing two double bonds separated by a methylene group.

Oxidative rancidity of unsaturated lipids has been well reported and, unless mediated by other oxidants or enzyme systems, proceeds through a free radical-

chain mechanism involving initiation, propagation and termination steps. The free radical chain mechanism has been generally accepted as the only process involved in oxidative rancidity. The initiation step has been and still remains the subject of much research and general uncertainty (Gray and Monahan, 1992). The direct reaction of polyunsaturated fatty acids with molecular oxygen is thermodynamically unfavorable. However, the spin restriction that prohibits the interaction of ground state oxygen with a polyunsaturated fatty acid can be overcome by a number of initiating mechanisms, including singlet oxygen, partially reduced or activated oxygen species such as hydrogen peroxide, the superoxide free radical or the hydroxyl free radical; active oxygen iron complexes, and iron mediated homolytic cleavage of the hydroperoxides, which generate organic free radicals at the polyunsaturated fatty acid molecule (Gunstone, 1984). Figure 1 shows the sequential reduction of molecular oxygen leading the formation of the superoxide free radical, hydrogen peroxide, and the hydroxyl free radical (Namiki, 1990).

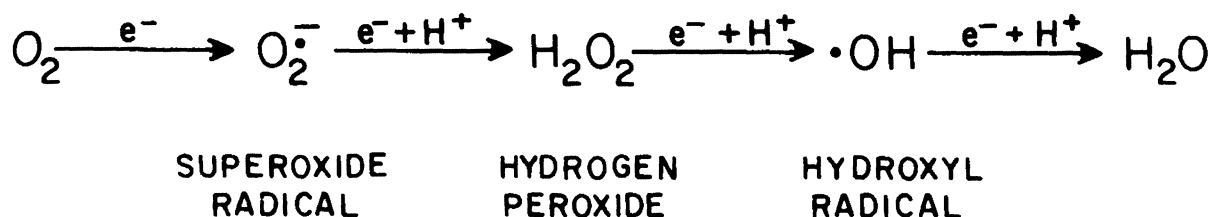


Figure 1
Formation of oxygen free radicals

The resulting fatty acid free radical reacts with molecular oxygen to form peroxy radicals. This is the propagation step. In this process the peroxy radicals react with more polyunsaturated fatty acid molecules to form fatty acid hydroperoxides, which are the fundamental primary products of oxidative rancidity. Decomposition of fatty acid hydroperoxides constitutes a very complicated process and produces a multitude of materials that may have biological effects and cause flavor deterioration in fat-containing foods. This decomposition proceeds by homolytic cleavage of the fatty acid hydroperoxides to form alkoxy radicals. These radicals undergo carbon-carbon cleavage to form breakdown products including aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones. Fatty acid hydroperoxides can react again with molecular oxygen to form secondary products such as epoxyhydroperoxides, ketohydroperoxides, dihydroperoxides, cyclic peroxides and bicyclic

endoperoxides (Janero, 1990). These secondary products can in turn decompose like monohydroperoxides to form volatile breakdown products. Lipid hydroperoxides can also condense into dimers and polymers that can break down and produce volatile material generating the typical «rancid odor» of the oxidized material (Frankel, 1984). The formation of such diversity of organic products constitutes the termination step.

Finally, fatty acid hydroperoxides and some of their bifunctional breakdown products can interact with proteins, membranes and enzymes. These reactions with biological components are of most concern to biochemists and cellular physiologists because they can affect vital cell functions and structures (Logani and Davies, 1980; Kubow, 1993). The development of fat oxidative rancidity in complex food systems is also greatly affected by the interaction of proteins and aminoacids with the oxidation products (Halliwell, 1994). Complex high-molecular-weight interaction

products are formed during processing and cooking of foods, and their further degradation into volatile compounds is a very complex chemical process not well understood (Kubow, 1992). Figure 2 shows a simplified scheme of one of the mechanisms proposed for the initiation, and of the various further steps of the oxidative rancidity of a polyunsaturated fatty acid (Valenzuela, 1991).

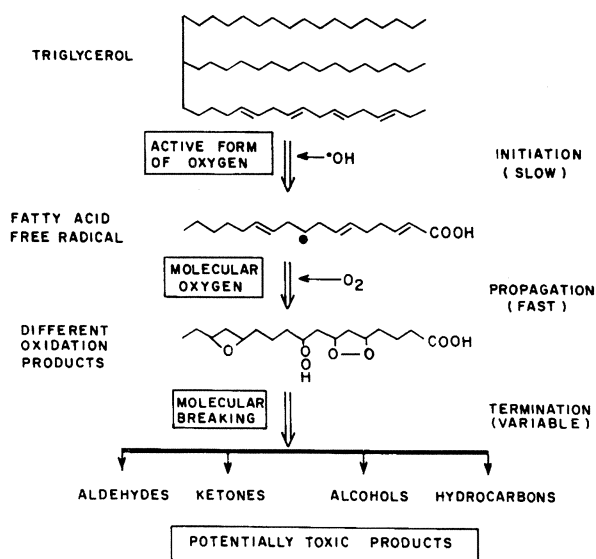


Figure 2
Steps of oxidative rancidity

3. SYNTHETIC AND NATURAL ANTIOXIDANTS: QUALITY PROTECTORS

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

Antioxidants may be classified as synthetic or natural. The synthetic antioxidants have been generally considered as relatively «safe» and are widely applied in a number of manufactured products including pharmaceuticals, cosmetics, human foods and animal feeds. The most popular are those derived

from phenolic structures or those having a phenolic configuration within their molecular structure. The presence of conjugated ring structures and hydroxyl groups allows phenolic compounds to actively scavenge and stabilize free radicals (Shahidi et al., 1992). The presence of carbonylic and carboxylic groups in numerous phenolic compounds can also result in the inhibition of oxidative rancidity by metal-chelation (Hudson and Lewis, 1983). Among the phenolic-derived structures the gallic acid esters, the butylated hydroxyanisole (BHA), the butylated hydroxytoluene (BHT), and the tertiary butylhydroxyquinone (TBHQ), identified as «primary antioxidants» are the most widely used food synthetic antioxidants.

4. GALLIC ACID ESTERS

Early in the 1940s, good antioxidant properties of several alkyl esters of gallic acid were reported and some of them were authorized for food use in a number of countries. Based in the trihydroxyl structures of the gallic acid (3, 4, 5-trihydroxybenzoic acid), they would be expected to have relatively high antioxidant potency in oils. Use of the higher gallates (octyl and dodecyl gallates), serves to overcome solubility difficulties that the butyl and propyl esters present (high water and low oil solubility). However, the strong tendency of all gallate esters to complex with traces of iron to give a blue-dark discoloration serves to minimize the use of these relatively potent antioxidants in vegetable oils. In addition, the gallates are heat sensitive, especially under alkaline conditions, and are lost from cooking oils rather rapidly at high temperatures, especially those encountered in baking and deep frying of foods. Figure 3 shows the structure of the propyl ester of gallic acid.

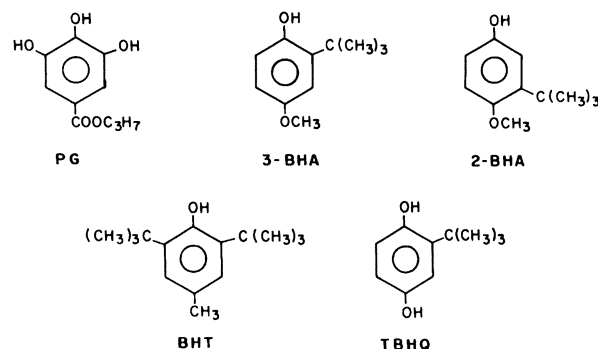


Figure 3
Synthetic antioxidants

5. BUTYLATED HYDROXYANISOLE (BHA)

Butylated hydroxyanisole is commercially available from 1948, when was first approved for food use in the USA, and now found widespread use in food fats and oils in many countries. It is a mixture of 2- and 3-isomers of tertiarybutyl-4-methoxy phenol. Because of the tertiarybutyl group ortho- or meta- to the hydroxyl group, BHA is referred as a hindered phenol. This steric hindrance is believed responsible for the relative ineffectiveness of BHA in vegetable oils because the tertiarybutyl group interferes with the antioxidant activity of the phenolic structure. However, this same steric hindrance also serves to protect the active hydroxyl group under some conditions and is probably responsible for the carry-through effect of BHA in fats and oils used in baked or fried foods. BHA is a white, waxy solid highly soluble in oil and insoluble in water. Although it is not a particularly effective antioxidant in vegetable oil, BHA is commonly used in combination with other primary antioxidants (such as gallates) in order to take advantage of the synergistic effects observed when certain phenolic antioxidants are used together and also to benefit from the carry-through protection which BHA may afford. BHA has a strong phenolic odor which becomes particularly noticeable when an oil treated with it is subjected to high temperatures, such as in baking or frying operations. Figure 3 shows the chemical structure of the BHA isomers.

6. BUTYLATED HYDROXYTOLUENE (BHT)

BHT, 2,6-ditertiarybutyl-4-methyl phenol is another hindered phenol which is in wide commercial use as an oxidation inhibitor in petroleum and rubber products for a number of years. In 1954 this compound was also approved for use in food oils in USA on the basis of favorable toxicity and potency test results showing it to be safe and effective antioxidant for fats and oils. As a food antioxidant, it has become known commonly as BHT (butylated hydroxytoluene), and, as might be expected from their similar molecular structures, BHT and BHA are similar in performance, including their relative weakness as antioxidants in vegetable oils. BHT is a white crystalline solid with good oil solubility and insoluble in water. It provides relatively low antioxidant effectiveness in vegetable oils, but like BHA, is often used in combination with other primary antioxidants to take advantage of the carry-through protection it may afford in baked and fried foods. Figure 3 shows the chemical structure of BHT.

7. TERTIARY BUTYLHYDROXYQUINONE (TBHQ)

Increased use of highly oxidation-sensitive polyunsaturated vegetable oils in food processing and in the human diet gave rise to the need for more potent antioxidants (and possibly ones less troublesome to use than already available compounds such as the gallates). Tertiary butylhydroxyquinone (TBHQ) was identified by extensive laboratory testing as a potential answer to this need. TBHQ was approved for food use in USA in 1972. Since then, the use of this compound to stabilize vegetable oils has been approved in a number of other countries. The response of vegetable oils to treatment with TBHQ generally is found to be greater than with any of the other approved primary antioxidants. Also, the laboratory tests and results of commercial experience indicate that the greater antioxidant performance of TBHQ in polyunsaturated oils may likely be achieved without certain problems (color and solubility, for instance) encountered with the use of other antioxidants. TBHQ is a white crystalline solid with moderate oil solubility and very low water solubility. Figure 3 shows the chemical structure of TBHQ.

In spite of the widespread use of the antioxidants described above and of other synthetic antioxidants of minor importance such as nordihydro guaiaretic acid, 2, 4, 5-trihydroxy butyrophenone (THBP), thiodipropionic acid (TDPA), dilauryl thiodipropionate (DLTDP), and 1, 2-dihydro-6-ethoxy-2, 2, 4-trimethylquinoline (ethoxyquin), which is approved for animal feed only (Dzanis, 1991), during the past two decades, both consumers and legislation officers have become suspicious about synthetic antioxidants. The «safe» status for most of the synthetic antioxidants is now a matter of concern (Ito et al., 1986) because the potential toxicity of some of them in biological models has been described (Anonymous, 1986; Thompson and Moldéus, 1988; Federal Register, 1990). BHA and BHT are now included in the class 3 status of the Food, Drug and Cosmetic Act of the Food and Drug Administration (USA) (Winter, 1994). This means that these antioxidants, as nineteen other substances, are requiring that certain safety test be undertaken within a specific time. Although no definitive conclusions may be drawn from the experimental research about synthetic antioxidants, and because other researchers claim positive effects for synthetic antioxidants in different experimental conditions (Xiu, et al., 1994; Mahajan et al., 1994; Kashfi, 1994), the public have been aware about questions for synthetic products. In this context natural products appear as more healthy and safe than synthetic antioxidants. The majority of natural antioxidants, as the synthetic ones, are also phenolic or phenolic-derived compounds.

The empirical use of natural compounds as antioxidants is very old. The popularity of smoking and spicing in the home for the preservation of meat, fish,

cheese and other fat-rich foods, may be due, at least partly, to the recognition of the rancidity retarding effect of these treatments. A number of natural products, extracted mainly from vegetables have been claimed for antioxidant properties. Products extracted from sesame oil (sesamol), from the seeds of rice (orizanol), from different spices such as: sage, mace, black pepper, oregano, allspice, and a number of other natural substances have been demonstrated effectiveness as antioxidants when assayed in different experimental models (Namiki, 1990). Specific type of molecules such as carotenoids, phenolic flavonoids, tannins, porphyrin-related substances, and Maillard reaction products, also show antioxidant properties (Decker, 1995). However, only a few of them have been proved as effective in retarding or inhibiting oxidative rancidity of oils, fats or more complex products. The most important proved natural antioxidants are the tocopherols, some flavonoids, the extracts from rosemary, and boldine a new recently described natural antioxidant.

8. TOCOPHEROL ISOMERS (VITAMIN E) AS ANTIOXIDANTS

Among the natural antioxidants, tocopherols deserve special attention. Tocopherols occurs as minor constituents in all vegetable oils, and are the best known and most widely used antioxidants. The alpha, beta, gamma and delta isomers of tocopherol differ in the degree of methylation of the dihydrochromanol ring. The natural tocopherol isomers (also named tocots) mixture with the tocotrienol isomers (analogs to tocopherol but having an unsaturated side chain) are collectively named Vitamin E. Certain vegetable oils, particularly palm oil (Qureshi et al., 1991) and rice

bran oil (Rogers et al., 1993) are rich sources of tocotrienols which have weak vitamin E activity but act as antioxidants and provide stability against oxidation. Vitamin E is the most important dietary component contributing to antioxidant defenses in tissues (Bieri, 1984). Tocopherols work as antioxidants by donating the hydrogen of the hydroxyl group (see figure) to a fatty acid free-radical. The resulting tocopherol radical is very stable and does not continue the chain reaction of producing new fatty radicals. The stability of the tocopherol radical is due to its aromatic structure and its ethereal oxygen. The one paired electron is distributed around the aromatic ring, to the ethereal oxygen, and on the side chain.

The antioxidant activity of tocopherols depend very much on the food to which they are added, the concentration used, the availability of oxygen, and the presence of heavy metals and various synergists. At high concentrations and in the presence of iron or copper salts tocopherols form a hydroperoxy radical that is responsible for the prooxidant effect observed for the antioxidant in these conditions. The prooxidant effect of alpha tocopherol can often be observed at relatively low concentrations, an effect not observed with gamma and delta tocopherols. This leads to the conclusion that for antioxidant properties in foods the gamma and delta tocopherols should be used instead of alpha tocopherol. This means natural Vitamin E should be chosen in preference to the synthetic compound which contains mainly alpha isomer. Satisfactory antioxidant activity is usually achieved only when tocopherols are used in combination with synergists such as ascorbic acid, citric acid, some synthetic antioxidants, or various chelating agents (Yi et al., 1991). The antioxidant activity of tocopherols range in the order: delta isomer > gamma isomer > beta isomer > alpha isomer (the less active) (Pokorny, 1991). Table I shows the relative effectiveness of tocopherol isomers as antioxidants.

Table I
Antioxidant activity of tocopherols compared to biological activity

	Antioxidant activity	Biological activity*
d - Alpha Tocopherol	100	1.49
d,l - Alpha Tocopherol **	100	1.10
d - Gamma Tocopherol	200	0.15
d - Delta Tocopherol	400	0.05

* International units/mg

** Synthetic analog

Natural Vitamin E increases the stability of animal fats, vegetable oils and processed foods. Vegetable oil naturally contains Vitamin E, but it is lost during the purification process. Therefore, it is useful to add Vitamin E at the final stage of product processing. Animal fats contain little or no antioxidants such as Vitamin E. To obtain greater stability, therefore, it is also useful to add Vitamin E. Natural Vitamin E is very stable with respect to heat. It is very tolerant to heat, it evaporates only very slightly even at high temperatures and it has excellent carry-through effect. These characteristics make it particularly useful as an antioxidant for frying oils. In technology, the natural Vitamin E content of many oils is important for their stability. However, on equal weight basis, Vitamin E is

less effective than synthetic phenolic antioxidants, and much more expensive.

Synthetic dl-alpha tocopherol or its acetate derivative is frequently added to fats and oils blends, but the addition of natural tocopherols is preferred because the synthetic preparation contains biologically inactive stereoisomers. Tocopherol oxidation products also have some antioxidant activity, and the structurally related alpha, beta, gamma and delta tocotrienols, which are present in some vegetable oils, as palm oil or wheat germ oil, have antioxidant activities similar to those of the respective tocopherols. Figure 4 shows the chemical structure of the four tocopherol isomers.

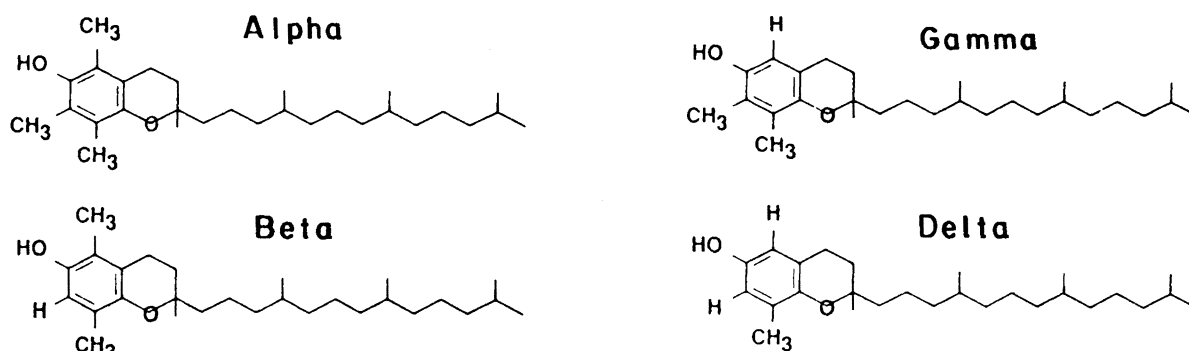


Figure 4
Tocopherol (Isomers)

9. FLAVONOIDS A SPECIAL CASE OF NATURAL ANTIOXIDANTS

Flavonoids constitute a large group of naturally occurring plant products that are widely distributed in the vegetable kingdom. All of them are structurally derived from the parent compound flavone (2-phenylchromone or 2-phenylbenzopyrone) and are characterized by two benzene rings joined by a C3 structure, which is condensed as a six-membered ring and changes with the nature of the flavonoid. Some of the carbons, at any of the three rings, are often hydroxylated and some of these hydroxyl groups may have transformed to methoxy groups (Havsteen, 1983). Flavonoids are ubiquitous in photosynthesizing cells, seeds, fruits and flowers. More than 500 different types of flavonoids are now known. Flavonoids, when incorporated into the alimentary chain may also be present in insects, molluscs, reptiles, and even mammals (Middleton, 1984). It has been estimated that the average daily Western diet may contain up to 1g of mixed flavonoids.

For centuries, a number of different therapeutic and curative properties have been ascribed to flavonoids and many of them have been incorporated to the popular folk medicine. Flavonoids such as

quercetin (Beretz et al., 1982), taxifolin (Vladutiu et al., 1986) and silymarin (Valenzuela and Garrido, 1994) have been used as pharmacological principles, either as such, or mixed in several chemically complex preparations.

Among other properties, flavonoids exhibit high binding affinity to biological polymers and heavy metal ions, they catalyze electron transport reactions, and may be active in scavenging free radicals (Robak and Gryglewski, 1988). In view of these properties, their known liposolubility, and their hydroperoxide reducing properties, the possible antioxidant activity of flavonoids was investigated by our group. We demonstrated that some commercial flavonoids (such as rutin, morin and quercetin) as well as a group of flavonoids extracted and purified from Chilean native plants, show potent antioxidant activity when assayed against the temperature and metal-induced oxidation of fish oil (Nieto et al., 1993). Although the antioxidant capacity of some flavonoids has been demonstrated, both in *in vivo* and in *in vitro* models, their mechanism of action is still a matter of speculation. All flavonoids assayed as antioxidants have in common one or two hydroxyl groups (free or substituted) attached to the B ring of their structure (see figure 5), and their potency appears to be affected by the level of substitution and

the position of these groups in the ring. It has been proposed that flavonoids having free hydroxyl groups at the para position (3', 6') should exhibit a reduced antioxidative effect, whereas those containing hydroxyl groups at the ortho (3', 4') or at the metha position (3', 5') should be the most favored (Das and Pereira, 1990). The presence of a free or substituted hydroxyl group at the 3 position of ring C is also determinant for the antioxidative action of flavonoids (Nieto et al., 1993).

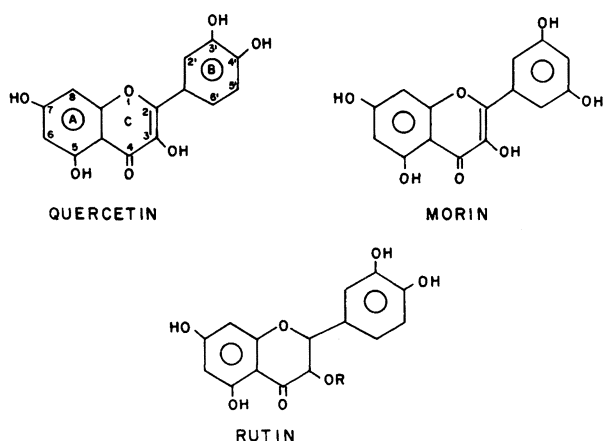


Figure 5
Some antioxidant flavonoids

Flavonoids have been defined as «high-level» antioxidants (Robak and Gruyglewski, 1988). That is, they act by scavenging those free radicals or excited forms of oxygen involved in the first steps of lipid oxidation, such as the singlet oxygen, the superoxide free radical or the hydroxyl free radical (Fraga et al., 1987). On the other hand, tocopherols defined as «low-level» antioxidants, act at the later steps of oxidation by stabilizing those free radical formed at the structure of the polyunsaturated fatty acid (methylene, alcohoxy or peroxy free radicals). These different sites of action in the oxidation chain may relate to the synergistic effect observed when flavonoids are assayed in mixture with tocopherols (mainly dl-alpha tocopherol) (Nieto et al., 1993). Additional antioxidative action of most flavonoids may be resulting from their metal-chelating properties. As a matter of fact, quercetin may form strong binding complexes, particularly with copper and iron (Thompson et al., 1976). Results indicate that some flavonoids could be used as natural antioxidants and might substitute for those synthetic antioxidants whose use has been questioned due to their potential undesirable secondary effects. However, further physicochemical and toxicological evaluations are required to assess the effectiveness and the future of flavonoids as antioxidants for fish oils or other oils rich in polyunsaturated fatty acids. Figure 5 shows the structure of some antioxidant flavonoids.

10. ROSEMARY, A SPICE APPROVED AS NATURAL ANTIOXIDANT

The hexane extract of the leaves of the evergreen shrub rosemary (*Rosmarinus officinalis* L.) contains four effective antioxidants; carnosol, rosmanol, isorosmanol and rosmaridiphenol (Wu et al., 1982). These four compounds are odorless and tasteless diterpenelactones having an O-diphenolic group on the C ring, which is responsible for antioxidative activity. Among these, rosmaridiphenol and rosmanol show antioxidant activity stronger than BHA when assayed in lard as well as in a water-oil emulsion system. Rosemary is the only spice commercially available for use as an antioxidant in the United States and Europe (were rosemary products reportedly represent about 40 to 50% of the natural antioxidant market). One of its main potential uses is the suppression of «warmed over flavor» (Duxbury, 1988). However, because of their prime use as flavoring agents, rosemary extract products are not technically listed as natural preservatives or antioxidants.

Rosemary extracts have been used commercially in processed foods for over 30 years. It was not until Dr. S. Chang and colleagues of Rutgers University (USA) developed and patented a process to concentrate natural antioxidants from rosemary by solvent extraction and subsequent steam vacuum distillation of the extract. This procedure allows the obtention of an edible oil where rosemary extracts are available without imparting bitter flavour and rosemary odor to the finished product (Chang et al., 1984). Dr. Chang's work subsequently showed the efficacy of these deflavored extracts in retarding soybean oil reversion and in reducing hydroperoxide development in lard as well as in potato chips fried in the oils containing the spice extracts. Figure 6 shows the chemical structure of the four antioxidant active isomers of rosemary extract.

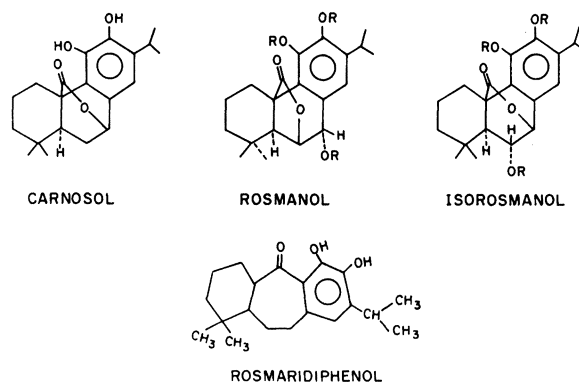


Figure 6
Isomers of rosemary extract

Kalsec Inc., which has the exclusive U.S. license to use Dr. Chang's patent, modified procedures to reduce color in its rosemary extract which is said to effectively retard oxidative rancidity, warmed-over flavors and color degradation in snack foods, mayonnaise, salad dressings, citrus oils, processed meats, poultry and seafoods. Drawbacks to using rosemary, however, include its high cost relative to the synthetic antioxidants and the larger quantities that often must be used to obtain the same effectiveness. For instance, marine oils, which are highly unsaturated, require addition of high level of rosemary extract. Rosemary extracts also must be used at higher levels than BHA or BHT to obtain the same amount of effectiveness in vegetable oils (Cuvelier et al., 1990). Even so, demand for rosemary extract antioxidants continues to grow (Fitch-Haumann, 1990).

11. BOLDINE: NEW PERSPECTIVES ON AN OLD NATURAL SUBSTANCE

Boldine is the most abundant alkaloid present in the bark and the leaves of boldo (*Peumus boldus* Mol.), a widely distributed native tree from the south region of Chile (Montes and Wilkomirsky, 1985). This substance was prepared in a pure state in 1922 in Merck's laboratory, and its structure was proved a few years later, by Spath and Tharrer (1933), to be (S)-2, 9-dihydroxy-1, 10-dimethoxy-aporphine. Mature boldo trees are usually between 6 and 12 m tall, although some individuals may attain heights of up to 20 m. Aqueous infusions of boldo leaves containing boldine have long been used by hundred of years by the natives of Chile for the treatment of gastric and hepatic diseases. The purported choleric, diuretic, sedative and digestive stimulant properties of boldo extracts and of boldine have been well described by the pharmacopoeias of natural medical products (Speisky and Cassels, 1994). As boldo only grows in abundance in Chile, this country has been for decades the sole original source of the leaves and bark, currently exporting about 1,000 tons of dried boldo leaves per annum, mainly to Argentina, Brazil, Italy, France, and Germany.

A few years ago, it was demonstrated that boldine, as pure form, extracted and purified mainly from the bark of boldo shows a potent antioxidant activity when assayed in both abiotic and biological systems. The aporphine shows a strong antioxidant activity when tested either against the spontaneous or the chemically-induced oxidation of biological systems undergoing peroxidative free radical-mediated damage (Speisky et al., 1991). In studies directed to elucidate the mechanism of the antioxidative action of boldine, it

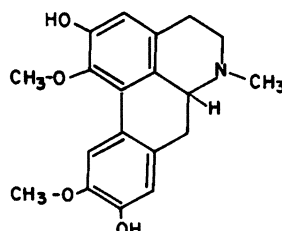
has been established that the boldine molecule acts as an efficient hydroxyl free radical scavenger (Cederbaum et al., 1992). The latter radicals, recognized as the most reactive oxygen species generated by biological systems, are trapped by boldine with even greater efficiency than that exhibited by dimethyl sulphoxide, a compound used experimentally as a paradigmatic hydroxyl free radical scavenger.

Boldine also inhibits the metal or temperature-induced oxidative rancidity of fish oil, their antioxidative action being three to four times greater than those observed for dl-alpha tocopherol and conventional synthetic antioxidants such as BHA, BHT or mixtures of these antioxidants (Valenzuela et al., 1991). In addition, boldine shows a potent synergistic action when mixed with the flavonoid quercetin. Boldine is also effective for stabilizing highly polyunsaturated n-3 fatty concentrates (up to 80% concentration) (Valenzuela et al., 1995). Although the oil stabilizing activity of boldine resembles that of the natural flavonoid quercetin, the demonstration that the latter compound may be mutagenic *in vitro* (Namiki, 1990) is likely to curtail its technological development as a food antioxidant.

Recent studies addressing structure-activity relationships for benzyloquinolides suggest that all the known boldo alkaloids are likely to exhibit at least some antioxidative activity (Cassels et al., 1995). Interestingly, in the case of aporphine alkaloids, the presence of phenolic groups is clearly not essential for these molecules to display their activity. In the case of boldine, while O-methylation of the two phenol functions to afford glaucine (a boldine-derivative which shows anticough action) was not associated with a loss of potency, subsequent N-methylation led to a derivative which was shown to be virtually devoid of antioxidative activity. Thus, the available evidence suggests that in the case of aporphine alkaloids, in addition to the expected contribution of phenolic functions to their radical-scavenging ability, the hydrogen atom bonded to the benzylic carbon next to the basic nitrogen atom may be a key to the antioxidative activity displayed by the non-phenolic boldo alkaloids (Cassels et al., 1995).

Boldine is considered a non-toxic, non-mutagenic, and non-genotoxic substance (Speisky et al., 1995). However, although the effectiveness of boldine as an antioxidant and its relative innocuousness encourage the pursuit of more research directed to explore its future as an antioxidative food additive, additional toxicological research linked to its uses in food technology will be required. Figure 7 shows the chemical structure of boldine and the alternative sites for electron delocalization in the boldine free radicals.

A: CHEMICAL STRUCTURE OF BOLDINE



B: ALTERNATIVE SITES FOR ELECTRON DELOCALIZATION

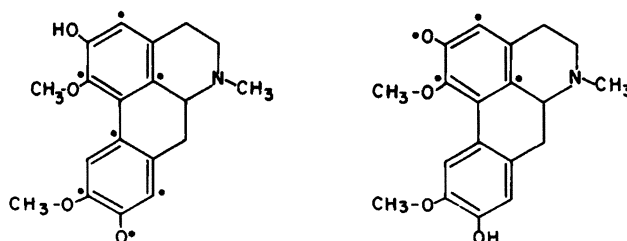


Figure 7

12. CONCLUDING REMARKS

Natural food additives, including antioxidants, are generally preferred by consumers, and more easily may gain legislative approval than synthetic additives, although most antioxidant are polysubstituted phenolic compounds, whether natural or synthetic. Natural food components do not possess high acute toxicity, as highly toxic substances would have been eliminated from traditional use as foods. However, the fact that a substance is natural and commonly found in a food is not guarantee that it is enterely nontoxic. It may have low toxicity, or be carcinogenic or mutagenic (Ames, 1983). Synthetic antioxidants are tested for such effects, but many natural food components have not yet tested.

The advantages and disadvantages of synthetic and natural antioxidants are summarized in table II. No rational scientific and technical argument can be given for preferences for natural antioxidants. They are more acceptable to consumers mainly on emotional grounds. While it is important for manufacturers to meet the requirements of consumers, it is imperative that the safety of additives that are not «generally recognized as safe» (GRAS) be tested before use. Perhaps the safest approach is to avoid the use of both synthetic and natural antioxidants by appropriate packaging and storage methods or by avoiding the use of ingredients that are readily oxidized. However, the benefits of using

antioxidants outweigh the risks. Without antioxidants in foods, oxidation products are created, causing a greater risk to health than the possible hazardous effects of antioxidants. Therefore, antioxidants may be considered as «food quality protectors», but if they are of natural origin we must consider them as «healthy food quality protectors».

Table II
Advantages and disadvantages of natural antioxidants, as compared with synthetic antioxidants

Synthetic antioxidants	Natural antioxidants
Inexpensive	Expensive
Wikely applied	Use restricted to some products
Medium to high antioxidant activity	Wide ranging antioxidant activity
Increasing safety concern	Perceived as innocuous substances
Use banned for some of them	Increasing use and expanding applications
Low water-solubility	Broad range of solubilities
Decreasing interest	Increasing interest

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