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**A Problem That Stinks**

Oxidation-induced rancidity in fats and oils has been a problem since ancient times. For hundreds of years, various substances have been added to foods for the purpose of delaying the onset of such rancidity. However, only in recent years have the mechanisms of oxidation and the effects of added substances been made clear. The discovery of outstanding antioxidant properties in butylated hydroxyanisole (BHA) in the 1940's marked the beginning of chemical antioxidant technology.8) Although this has brought solutions to the problems of food spoilage, it has also raised some concerns over the possible negative effects of widespread chemical use in food processing. Yet, people have seldom considered the potential of natural antioxidants as a practical solution to prevent rancidity. Already, honey and rosemary extracts have shown promising antioxidant qualities after preliminary tests conducted at Clemson University and the University of Illinois.

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**The Process of Oxidation**

The oxidation of fatty substances occurs in three stages: initiation, propagation, and termination. During initiation, fatty molecules (e.g. fat or oil molecules) split into a fatty free radical ("free radical") and a hydrogen ion. (A free radical is defined as any atom or group of atoms with an unpaired electron and is therefore highly reactive.) Fatty free radicals can catalyze further free radical formation because each free radical must steal an electron from another molecule to stabilize itself. However, this stabilization effort leaves another substance without an electron, and this sets off a chain of free-radical reactions. (� indicates a free radical)

RH -----------> R� + H

(fat molecule) (fatty free radical) (hydrogen ion)

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Elements such as moisture, heat, light, air, and metallic elements (e.g. iron, copper, platinum, and other multi-valent elements) can promote oxidation, and are therefore called "prooxidants." In propagation, previously formed free radicals can combine with (atmospheric) oxygen to form peroxide free radicals which, in turn, react with the substrate (the molecules in which the free radical acts) to form more fatty free radicals and hydroperoxides (a peroxide with an additional hydrogen atom).

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R� + O2 ------------> ROO�

(peroxide free radical)

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ROO� + RH -------------> ROOH + R�

(hydroperoxide)

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It is during this stage that the decomposition of hydroperoxides leads to the odor and flavor characteristics associated with rancid food fats and oils.

ROOH -----------> RO� + �OH

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�OH + RH -----------> R� + H2O

(thus, the cycle repeats itself)

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Termination, or the ending of the oxidation chain reaction, occurs with the deactivation of the free radical. This can be achieved in several ways; for example, the substrate may be entirely used up or the free radical may be destroyed. 8)

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**How Antioxidants Work**

Antioxidants delay rancidity by binding to oxygen molecules and therefore making them unavailable to free radicals. They also function by reacting with initiating and propagating free radicals and forming non-reactive substances that lay dormant within the substrate. However, it must be kept in mind that antioxidants can only delay, and not stop, oxidation. The remarkable characteristic of antioxidants is that they have optimum effectiveness in low concentrations in oil. This can be attributed to the fact that when great quantities of antioxidants are added to an oil, they act as prooxidants.

Antioxidants can be classified into four groups: primary, synergistic, secondary, and miscellaneous. Primary antioxidants function by terminating the free-radical chain reaction. They donate hydrogen or electrons to free radicals and then convert them into more stable products. They may also function in reactions with lipid radicals, forming lipid-antioxidant complexes. Most primary antioxidants are chemical antioxidants, such as phenols (e.g. nordihydroguairetic acid), 'hindered' phenols (e.g. BHA and BHT), and other miscellaneous primary antioxidants (e.g. ethoxyquin). Phenols are benzine rings, compounds that contain hydroxyl groups and that are highly reactive.

Synergistic antioxidants are broadly classified as oxygen scavengers and chelators. Synergists may act as hydrogen donors to free radicals, which regenerate the primary antioxidants. Synergists may also provide an acidic medium that improves the stability of primary antioxidants. Oxygen scavengers include ascorbic acid (vitamin C), ascorbyl palmitate, sulfites, and erythorbates. These react with free oxygen and remove it from a closed system. Chelators, such as citric acid, phosphates, and tartaric acid, are not true antioxidants but are highly effective as synergists with both primary antioxidants and oxygen scavengers. They react with prooxidant metals like iron and which raises the activation energy of initiation reactions.

Secondary, or preventive, antioxidants include esters (the product of a reaction between an alcohol and an acid to form a solid that is soluble in oil), which function by decomposing the lipid peroxides into stable end products. Ascorbic acid can be reacted with alcohol so that it forms a salt, or ester.

Miscellaneous antioxidants such as flavonoids (metabolites in plants that occur in almost all plant parts) and related compounds and amino acids function as both primary antioxidants and synergists. Compounds that fall under this category include spice extracts, vitamin A, vitamin E, and tea extracts. 4)

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**Saturated and Unsaturated Oils**

There are two categories of fats and oils: saturated and unsaturated. Unsaturated oils are less stable because they have more double bonds between carbons in their fatty acid complexes, and therefore are weaker and more reactive. 1) Unsaturated fats and oils include monounsaturated fatty acids, which have a single pair of hydrogens missing. Monounsaturated fats, such as olive oil, are the best for the body. Polyunsaturated fats, which have more than one pair of hydrogens missing, are found mostly in seed oils. Saturated fats have single bonds between carbons, and a pair of hydrogen atoms around each carbon, making them nonreactive and stable. Saturated fats in the bloodstream have been found to raise the level of cholesterol and polyunsaturated fats tend to lower it. Saturated fats generally are solid at room temperature; polyunsaturated fats are liquid. 6) In addition, saturated fats can accumulate in arteries and lead to arteriosclerosis (hardening of the arteries) and heart attacks.

During the oil refining process, hydrogen is pumped into the oil so that the hydrogen breaks the double bonds of the fatty acid chains and forms single bonds, therefore making an unsaturated fat more similar in structure to saturated fats. This hydrogenation of oil stabilizes it. Because the oil structure is altered to become more similar to that of saturated fats, it may build up in blood vessels and cause heart problems. Hydrogenation is currently a very widespread commercial processing method.1)

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**The Advantages and Disadvantages of Natural Antioxidants**

Natural antioxidants, such as those derived from herbs, vegetables, and other plants, have many advantages over chemical antioxidants (and processing procedures) currently used. However, they may also have disadvantages. 4)

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| Advantages | Disadvantages |
| Readily accepted by consumer, as considered to be safe and not a "chemical" | Usually more expensive if purified and less efficient if not purified |
| No safety tests required by legislation if a component of food is "generally recognized as safe" (GRAS) | Properties of different preparations vary if not purified. |
| Readily available, could be easily incorporated into home-cooking and use. | May impart color, aftertaste, or off-flavor to the product\* |
| Works best in small amounts, therefore, relatively inexpensive | � |

\*However, natural antioxidants can be deodorized and decolorized.

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**Methods Used for Testing Antioxidant Effectiveness**

Currently, there are several methods through which oxidation can be accelerated and oxidative stability can be analyzed.

Standard Accelerated Stability Tests

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| Test | Conditions | Characteristics |
| Ambient storage | Room temperature, atmospheric pressure | Too slow |
| Light | Room temperature, atmospheric pressure | Different mechanism |
| Metal catalysts | Room temperature, atmospheric pressure | More decomposition |
| Weight-gain method | 30-80 oC, atmospheric pressure | Endpoint questionable |
| Schaal oven | 60-70 oC, atmospheric pressure | Fewest problems |
| Oxygen uptake | 80-100oC, atmospheric pressure | Different mechanism |
| Active oxygen (AOM) | 98 oC, air bubbling | Different mechanism, possibly dangerous in non-industrial settings |

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�Analytical Methods to Measure Oxidative Stability of Fats and Oils

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| **Chemical methods** | **Chromatographic methods** |
| Peroxide value | Gas chromatography |
| Thiobarbituric acid test | Headspace gas analysis |
| Kreis test | Measurement of oxygen absorption |
| Carbonyl value | Dissolved oxygen meter |
| **Spectrophotometric methods** | Warburg's manometer |
| UV absorption | Weighting method |
| IR spectrometry | **Sensory Methods** |
| ESR spectrometry | Flavor and odor evaluations |

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Chemical methods are used to measure peroxides, hydroperoxides, free fatty acids, and decomposition products. The Peroxide Value (PV) test is the most common measure of oxidative rancidity. The oxidizing sample is reacted with a saturated aqueous solution of potassium iodide. It is then titrated with sodium thiosulfate, to measure the iodine liberated by the peroxides. The Kreis test is a colorimetric method which measures changes in color with a spectrophotometer. The carbonyl value test is a measure of the carbonyl compounds formed during oxidation. Oxygen uptake can be measured with a Warburg manometer. Storage tests conducted under normal conditions provide the most realistic determination of stability, but these tests are very time-consuming. The Schaal Oven Test method involves storing the oil under controlled conditions and evaluating it periodically for organoleptic (odor) changes. It is often used for evaluating fats, oils, baked goods, and food-packaging materials. The samples are placed in an oven at approximately 62.8oC, and the odors are evaluated at appropriate intervals. The samples are aired every day, to further accelerate oxidation. Sensory evaluations provide the most useful information related to consumer acceptance products. This method is highly sensitive, but is dependent on the quality of the training the smell or taste panel has received. 4)

**Various Studies Previously Done**

Spices and herbs provide not only good flavoring properties but also food-preserving properties. Early scientific studies done on antioxidant activity in spices indicated that a wide range of extracts have antioxidant properties. Later studies identified active components from various herbs and spices such as rosemary, sage, ginger, pepper, and cloves. Most of these compounds have been reported to be as effective as such chemicals as BHA and BHT. Several studies have been done to isolate odorless, tasteless compounds. Four odorless and tasteless compounds have been isolated from rosemary: rosmanol, carnosol, epirosmanol, and isorosmanol. Many antioxidant compounds have been identified in herbs and spices, but only with rosemary and sage have large-scale processing methods been developed to produce a purified product that is bland, odorless, and tasteless. Rosemary antioxidant is available as a fine powder that is soluble in fats and oils. Studies have recommended that 200-1000 mg be used per kilogram of the food product to be stabilized. In our experiment, we followed this recommendation, using .01g of antioxidant per 10 grams of oil. 4)

In 1996, scientists at Clemson University and the University of Illinois did studies on the antioxidant properties of honey. They mixed honey of different types into a batter used to make restructured turkey loaves. These loaves were then refrigerated, and after three days, the scientists tested for the oxidative stability of the various loaves. Their general findings were that honey did effectively resist oxidation and that darker honeys seemed to have more antioxidant properties than lighter honeys. 11) This apparent link with color and antioxidant capacities is strengthened by the fact that researchers at Tufts University have found that the general rule among leafy greens is that the more intense the green of the leaf, the more antioxidants. 2)

Researchers at Michigan State University worked together with two companies, Bil Mar Foods (a meat processing company) and Kalsec (a flavor/spice company) to produce turkey rolls with rosemary extracts added to some to compare with a few commonly used antioxidants. In addition, they added polyphosphates, which also help to prevent oxidation. The rosemary turkey roll compared well with the commonly used antioxidants. 5)

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**Substances Selected for Testing**

We selected eight substances to test on soybean oil for their antioxidant properties. We tried to select antioxidants representative of the four antioxidant categories. We chose soybean oil because it is an unsaturated fat and therefore highly reactive. (Soybean oil has more reaction sites than other salad oils, such as canola oil.) This helped accelerate our experiment. 1)

The natural antioxidants we chose were rosemary, garlic, ginger, green tea, and sesame seed oil. Rosemary was selected because it has already been used in some research, and we wanted to see how effective it was compared to other antioxidants that hadn't been widely tested before. Rosemary fits under the category of miscellaneous antioxidant. 4) Studies also conducted at Tufts University have shown that garlic is among the top five vegetables with antioxidant properties, so we expected garlic to be an effective antioxidant in oil. 2) Garlic, ginger, and green tea are miscellaneous antioxidants as well. Sesame seed oil has never been tested before, and we wanted to know its antioxidant potential. 4)

We also tested the tablet or capsule forms of vitamins A, C, and E. We chose these because we wanted to know whether we could identify if the antioxidant property is attributed to a specific substance in the plant or if the antioxidant potential lies within another component of the plant matter. These three vitamins were also chosen because they have been used in preliminary antioxidant tests. Vitamin E is a tocopherol, a kind of primary antioxidant. Vitamin C is a synergistic antioxidant. 4) However, Vitamin C may not be a very effective antioxidant because it is very sensitive to destruction. It can be destroyed by exposure to air (oxygen), light, heat, water, and time.

Iron sulfate was used as a negative control, a sample that is expected to perform the opposite of the expected result. Iron sulfate is a metallic element, and is therefore a prooxidant. It catalyzes oxidation.

To accelerate our experiment, we used the Schaal Oven Test, the simplest acceleration method. In our experiment, we set the oven at 65oC to further accelerate the oxidation, and we evaluated the odors by sensory evaluation daily.

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