Could eating food containing lipid autoxidized products formed during preparation and storage be harmful to your health? Can anything be done to prevent or minimize lipid peroxidation within your body?

The answer to both questions is potentially yes—potentially because researchers do not know the extent of oxidation nor the quantity of oxidation products needed to produce a harmful effect.

Lipid oxidation is a normal biological process by which we obtain energy from fat. Deleterious lipid oxidation occurring in foods generally is referred to as autoxidation; the corresponding deleterious lipid oxidation occurring in the body generally is called peroxidation.

Various aspects of lipid oxidation have been studied for many years. How to identify lipid oxidation products in foods and in biological systems, however, continues to be investigated. What the results mean and the mechanisms involved also are unresolved.

There is tremendous interest in the topic, according to David Mills, chairperson for the AOCS Health and Inform Nutrition Division: "Oxidation, particularly with its possible health and nutrition implications, is probably one of the most important areas of current lipid research. There are weighty problems, such as chronic diseases and the process of aging, that have been found to be influenced by lipid oxidation."

Oxidized foods
Many believe that oxidized foods do not present a real risk because people will throw out food that smells or tastes rancid without consuming it.

Kathleen Warner, research food technologist at the U.S. Department of Agriculture's National Center for Agricultural Utilization Research, however, wonders if people could be consuming foods that may be slightly oxidized.

"Based on our sensory research, untrained testers are less sensitive to rancidity than trained sensory panelists. Therefore, consumers may be eating oxidized food. Certainly when rancidity is noticeable, people avoid consuming the product. Just think how much food we may eat that's slightly or moderately oxidized but we eat it anyway. I'd like researchers to answer how much we can eat before we should be concerned," Warner said, adding, "I don't believe anyone has set levels of when these oxidation products become harmful."

Because the body has many protective mechanisms, it is questionable whether moderately oxidized products are harmful in the body when eaten, according to Edwin N. Frankel of the Department of Food Science and Technology, University of California at Davis. For example, fish oil capsules may increase the levels of highly unsaturated fatty acids circulating in the body. Some authorities recommend taking additional vitamin E...
LIPID OXIDATION

(continued from page 800)

along with these fish oil health supplements, but a definite need remains to be established.

The food industry is interested primarily for economic reasons in how to prevent and measure oxidation, according to David B. Min, professor at Ohio State University: “Flavor changes in food are due mainly to lipid oxidation. Off-flavors mean products will be less acceptable to consumers.”

Lipid oxidation also means a loss of essential fatty acids, and highly oxidized foods may have a negative effect on health. “The question is how we can minimize lipid oxidation,” Min said, noting this needs to include information about the role of the various antioxidants in preventing oxidation.

In a talk given at a Lipidforum seminar in October 1992 in Denmark, Hans Lingnert of SIK (The Swedish Institute for Food Research) noted that oxidative processes are complex in nature and affect many aspects of food quality. “Flavor deterioration is one important effect, but impaired color and texture are also common consequences of lipid oxidation, as are losses in nutritional value due, for instance, to vitamin reactions,” he said.

The main targets of oxygen attack are unsaturated fatty acids, but other compounds such as sterols, carotenoids and aroma compounds also may react. “In addition to the undesirable sensory deterioration of autoxidized foods, questions have been raised about the safety of oxidation products in food. Mutagenic effects are reported for cholesterol oxidation products, but the possible actions of hydroperoxides, epoxides and certain carbonyl compounds also are being debated,” Lingnert said.

According to Lingnert, the reaction between oxygen and lipids does not take place spontaneously. “It needs to be initiated either by the formation of free radicals from the lipids or by the formation of active oxygen species that are able to react directly with the lipid molecule.” The main prooxidative factors in foods are enzymes, metals and metal compounds, light and heat. Protection against lipid oxidation should be taken as early as possible, Lingnert noted. Oxidation can occur in raw materials and during processing. One way to limit oxidation is to limit the oxygen and light available during food processing and packaging. “Inactivation of enzymes is crucial to the oxidative stability of many food products,” Lingnert added. Low-temperature storage is another way. A third is the use of antioxidants.

“Despite considerable progress in the understanding of lipid oxidation in foods during the last 20 years, our knowledge is still far from complete. Lipid oxidation remains a major problem in the food industry and an important cause of quality deterioration,” Lingnert said.

A number of products formed are believed to be or shown to be cytotoxic, carcinogenic or atherogenic, according to industry consultant Gerhard Maerker, who, however, added, “At the U.S. Department of Agriculture’s Eastern Regional Research Center (ERRC), we have done work demonstrating that carcinogenic products formed—the epoxides of cholesterol—do not survive exposure to simulated gastric juice. So one would not expect them to survive ingestion in the human stomach. However, the product formed from these epoxides is shown to be cytotoxic.”

Other work at ERRC has demonstrated that irradiation of food containing cholesterol generates certain cholesterol oxidation products which exist normally but are not known to be toxic. Thus, these compounds potentially could be used as a marker to indicate past irradiation of foods. “The issue of finding a marker for irradiation is not trivial,” Maerker, said, explaining, “People have looked for such compounds for the past 35 years. This indicates the changes to foods when irradiated are very minute.”

Deep-fat frying
People like the taste of some oxidized lipids, according to Robert G. Ackman, Research Professor at the Canadian Institute of Fisheries Technology, Department of Food Science and Technology, Technical University of Nova Scotia, who noted that desirable flavors often are oxidation products or a result of the oxidation process.

Fried snack products are a good example of this, according to Edward G. Perkins, professor of food chemistry and nutritional science at the University of Illinois. “American consumers like a little hexanal, which is primarily an oxidation product,” he said.

Warner noted that USDA researchers have found that the desirable fried food flavor typical of potato chips is related to the oxidation of linoleic acid and the development of breakdown products such as 2,4-decadienal.

Lipid oxidation occurs very rapidly in deep-fat frying because of the high temperature, Min said, adding that 30% of the frying fat is oxidized after 72 hours. Studies have shown that rats fed highly oxidized frying fat experienced decreased growth and increased liver weight and fatty tissue deposits.

Citing studies at the University of Illinois in which rats were fed fat from commercial deep-frying operations, Perkins confirmed similar results. “There are indeed nutritional and toxicological effects in rats,” he said, pointing to reduced growth, a change in liver enzyme levels and enlarged livers. Previous studies showed that when foods were fried in these fats, the foods absorbed some of the frying oil, and the absorbed fat contained the same concentration of oxidation products as the bulk of the frying medium.

“When the fat gets bad enough to cause excess smoking and color development, it is thrown out. Generally speaking, I think food operators throw it away before it is harmful,”

INFORM, Vol. 4, no. 7 (July 1993)
Perkins said, adding, "I think we've identified the lipid oxidation products from heated fats, and most of the higher molecular weight products formed from heating fats are probably excreted, based on rat studies."

Of more concern, he said, may be compounds such as cyclic monomers of fatty acids which are formed both during processing of the fats and in deep frying. "I think the primary concern should be the cyclic fatty acids in the fats and their health effects," he said. "These have already been shown to cause toxicological effects at levels as low as 0.1% of the diet in rat studies."

"What is needed is to have someone fund studies to synthesize large quantities of lipid oxidation products and do definitive studies with rats. It also would be interesting to do a study of the effects of exposure of persons who operate the fryers all day and may inhale volatile fat oxidation products. No one yet has done a classical chronic toxicity test with some of these heated fat compounds. You can't buy them. You have to generate them in a laboratory so you are working with only small amounts."

At the University of Illinois, researchers are studying these effects in tissue cultures. J.-L. Sebedio's laboratory at INRA in Dijon, France, is the only other group in the world studying the nutritional and toxicological effects of cyclic fatty acids, according to Perkins.

David Firestone of the U.S. Food and Drug Administration, meanwhile, believes there needs to be more regulation of fats and oils used for deep-fat frying. In his talk at the 1993 AOCS Annual Meeting and Exposition, Firestone noted that although many studies have shown that improper use of frying fats causes fat degradation and reduced quality of the fried food, there are no general worldwide regulations for the control of frying fats.

**Biological systems**

There is evidence that, in the body, lipid oxidation can cause inflammation and play a role in such diseases as arthritis, atherosclerosis, heart disease and breast and colon cancer.

Free radicals of polyunsaturated fatty acids can form in living systems, and oxygen can react with the radicals to form peroxyl radicals and hydroperoxides. These compounds can chain react to cause membrane damage. For instance, in cardiovascular disease, arterial clogging apparently begins when low-density lipoprotein (LDL) is oxidized in a reaction induced by free radicals.

Karen Schaich, assistant professor in the Food Science Department at Rutgers University, noted that recommendations to increase the proportion of unsaturated fats and decrease total fat intake in the diet may have been made prematurely from observations that the material found in plaques contained cholesterol oxides and saturated fats.

"The belief was that atherosclerosis was caused by cholesterol and saturated fats and thus these should be decreased in the diet. However, we now know that neither cholesterol nor saturated fats per se causes atherosclerosis. Instead, the original lesions are thought to arise through oxidative events in the blood vessel walls. When lesions are formed, they put out chemical markers that are like antennas that attract mast cells. This action triggers a series of events, such as deposition of cholesterol. What we missed was that the initial trigger was oxidation," Schaich said. "By increasing PUFA (polyunsaturated fatty acid) consumption, we may be aggravating our susceptibility to cardiovascular disease. We've been treating the symptom, not the disease."

According to an article in the April 1993 issue of Food Technology, the late John E. Kinsella and co-authors E.N. Frankel, B. German and J. Kanner noted that each cell is exposed to numerous free radical attacks. Unless controlled, these agents can damage tissue. The peroxidation of tissue lipids potentially can disrupt membrane-related functions, alter platelet functions, modify macrophage functions, alter the arachidonic acid cascade, cause protein polymerization, promote atherogenesis by modifying LDL and cause DNA mutation. However, the body has an array of protective mechanisms to control peroxidation and to quench, eliminate or inactivate free radical generators. These include enzymes (superoxide dismutase, catalase, glutathione peroxidase), antioxidants and complementary agents such as ascorbic acid, ß-carotene and retinoids. Natural antioxidants, primarily provided by diet, include vitamins E and C.

In the March/April 1990 issue of Food & Nutrition News, Paul B. Addis, professor in the Department of Food Science and Nutrition, Universi-
ty of Minnesota, St. Paul, cited evidence that lipid oxidation products may accelerate all three phases of chronic heart disease: initiation, progression and termination. "Evidence exists, strong in some cases, that dietary lipid oxidation products are involved in arterial injury, atherosclerotic plaque formation and thrombosis/spasm and therefore may be more deleterious than was once assumed," he noted.

Lipid oxidation products involved may include cholesterol oxides, fatty acid hydroperoxides, malonaldehyde and other secondary breakdown products, and may be obtained from the diet or formed in vivo. Addis noted that cholesterol oxides possess antioxidant properties although pure cholesterol does not. There also have been studies indicating that consuming rancid oils directly accelerates foam cell formation, the hallmark of atherogenesis.

"In the context of cholesterol oxidation, it appears that much of the toxicity of oxidized lipoproteins can be attributed to the cholesterol oxide content," according to Alex Sevanian of the University of Southern California's Institute of Toxicology, School of Pharmacy. Sevanian and H. Hodis and colleagues have done rabbit studies that show cholesterol oxide content goes up in hypercholesterolemia but can be reversed by the antioxidant probucol.

Addis' studies on cholesterol oxide absorption indicate that cholesterol oxidation products found in the diet eventually end up in plasma lipoproteins. "The main sources of cholesterol oxidation products in the human diet are probably not fresh, freshly cooked or cured meat products and fresh dairy products, but rather French fried potatoes (if fried in animal fat), powdered eggs and other dehydrated foods," Addis wrote, adding, "Much further quantitative work is needed to identify foods which supply lipid oxidation products to the diet of humans. Research is needed to eliminate lipid oxidation products from frying oils, dehydrated foods and powdered eggs."

Future research, he concluded, should emphasize protecting lipids from oxidation—"an attainable goal at reasonable cost"—rather than attempting to remove cholesterol from foods—"a very expensive process."

Ironically, however, some researchers have found that lipid oxidation products might help to destroy cancer cells in the body, according to Mills, professor and chairperson of the Department of Health Studies at the University of Waterloo, who this month becomes the Bureau Chief of the Scientific Lab Division of the Department of Health for New Mexico.

Because cancer tumors have very low tolerance to lipid oxidation products, the latter might be used for therapeutic purposes, Mills said, citing studies by Michel Bégin showing PUFAs are capable of killing cancer cells but not healthy cells in vitro. In articles published in Anticancer Research (Vol. 6, pp. 291–296, 1986) and the Journal of the National Cancer Institute (Vol. 77, pp. 1053–1062, 1986), Bégin related this phenomenon to the oxidation products of PUFAs.

"Healthy cells have a protective mechanism. However, if the cells already have been altered, you may be able to use the toxicity of the lipid oxidation products to your advantage to kill the cancer cells," Mills said.

The role of antioxidants

Antioxidants in the diet are seen as a way to minimize lipid oxidation. Researchers, for instance, are studying the role β-carotene and vitamins E and C might play in inhibiting LDL-oxidation.

"Vitamin E is seen by some as a 'cure-all,' although it is not that simple," noted Ackman, who cited a trend toward more use of natural antioxidants because consumers are perceived as more willing to accept "natural" vs. synthetic additives.

Antioxidants, in fact, have been shown to provide protective mechanisms. Primary antioxidants such as vitamin E donate hydrogen to inactivate initial free radical peroxy or hydroxy radicals. Thus, dietary vitamin E helps offer protection, as does vitamin C which spares and may regenerate vitamin E. Carotenoids also have been shown to be effective free radical quenchers in membranes, according to Frankel and colleagues at the University of California at Davis.

"We can show beneficial effects of antioxidants which are far greater than the possible adverse effects. Consuming fruits and vegetables and red wine—beverages containing fruits and vegetables—is a very healthy way of counteracting the effects of lipid oxidation," Frankel said.

Frankel and colleagues, for instance, have shown that the nonalco-
holic phenolic components of red wine may reduce the oxidation of human LDL. Their study found that in in vitro studies red wine’s phenolic substances effectively inhibited copper-catalyzed oxidation of LDL (Lancet, Feb. 20, 1993).

At Ohio State University, Min and researchers continue to look at β-carotene’s role in food and body systems. Thomas Webb at Ohio State University, for instance, has a National Cancer Institute grant to study the effects of β-carotene on mammary cancer in rats. Min, meanwhile, is studying the possible health benefits of β-carotene and soluble fiber.

In fact, many researchers throughout the world are looking at phytochemicals from plants to possibly ameliorate the effects of lipid peroxidative reactions. Research efforts on “designer food” products include possibly mixing compounds found in fruits, vegetables and other plant sources to perform as effective free radical quenchers and protective antioxidants in foods [INFORM 4:344 (1993)].

An article in The Wall Street Journal of April 13, 1993, said that studies indicating possible benefits of antioxidants have boosted their sales, particularly of vitamin E. “Vitamin E’s growing popularity comes as more studies suggest that antioxidants lower risk of various diseases by blocking cell damage from highly reactive oxygen-containing chemicals called free radicals,” the article noted.

The article explained that the argument for taking vitamin E supplements in addition to eating vitamin-rich foods is stronger than that for most other vitamins because the main dietary sources are vegetable oils, nuts and other fatty foods. With consumers told to cut back on fat intake, it is difficult to get large quantities of vitamin E in the diet. According to the Vitamin E Research & Information Service, a not-for-profit organization established by Henkel KGaA, one would have to drink two quarts of vegetable oil or eat more than five pounds of wheat germ to obtain 400 international units (IUs) of vitamin E, the amount in a typical supplement capsule. When used as antioxidants in foods, vitamins C and E are used only in small, pharmacologic amounts.

The U.S. recommended daily allowance (RDA) for vitamin E is 30 IUs. However, studies indicate higher doses may be needed to achieve disease-prevention benefits.

Ishwarlal Jialal and Scott Grundy at The University of Texas Southwestern Medical Center, for instance, have shown that megadoses of vitamin E may slow the development of atherosclerosis. The results of their study, published in the June 1992 issue of the Journal of Lipid Research, noted that the oxidation rate of LDL was reduced by half in volunteers given daily doses of 800 IUs of α-tocopherol (vitamin E).

However, Jialal and Grundy stopped short of recommending megadoses of either vitamin C or E without clinical studies to establish benefits. In fact, researchers generally agree on the need for further research on the role of antioxidants in the protection against oxidation of the PUFAs of LDL. Recent work by M. Stamford of Brigham and Women’s Hospital, for instance, suggests that vitamin E supplementation above 100 IUs a day serves no useful purpose.

Addis personally eats plenty of fruits and vegetables and takes some amounts of supplemental antioxidants, especially if traveling. “I am convinced there should be more use of antioxidants in foods,” Addis said. Benefits include retarding lipid oxidation in the food, so consumers are less exposed to secondary lipid oxidation products. Also, they help maintain vitamin levels in the foods; vitamins not spent in the foods to extend shelf life will be absorbed in the body.

(continued on page 808)
LIPID OXIDATION

(continued from page 807)

“...It is important to have a variety of antioxidants in your diet, from such sources as whole grain cereals, fruits and vegetables,” Addis said.

Agreeing with Addis, Schaich believes the official recommendation for vitamin E intake will be increased. “Our dietary recommendations for antioxidants are probably too low,” Schaich said.

Measuring lipid oxidation

Another crucial aspect of lipid oxidation concerns methodology.

“The biggest confusion is caused by unreliable methods,” according to Frankel. “The effect of lipid oxidation in biological systems is confused because researchers are using such tests as TBA (thiobarbituric acid assay, used to measure the decay products of lipid hydroperoxides). The major drawback is it measures many things besides lipid oxidation and thus the impact of liquid oxidation may be exaggerated.”

Agreeing, Mills noted that measuring for the many various compounds is tricky. “Current methods aren’t adequate, and there is a lot of confusion over what is really being measured because the methods are so nonspecific,” Mills said. “I think there is a lot of energy wasted trying to measure these compounds. In fact, I believe this will slow down the interpretation.”

Several sessions at the 1993 AOCS Annual Meeting and Exposition held in April focused on methods for measuring the oxidative status of lipids and lipid stability. Research continues on various methods, including front-face ratiometric fluorometry, potentiometry, high-performance liquid chromatography, spectrophotometry, nuclear magnetic resonance spectroscopy, headspace gas chromatography of volatile oxidation products, chemiluminescence assays and conductometric determination. The latter has resulted in a conductometric determination method which the Japan Oil Chemists’ Society has adopted as an official method for testing autoxidation of fats and oils, as an alternate to the active oxygen method (AOM).

To evaluate oil stability, different measuring methods are used after a sample is oxidized under standardized conditions to a given endpoint. Frankel is critical of the use of AOM in this application because the mechanism of oxidation is different at the high temperature used, vs. at lower temperatures. “The mechanism of oxidation changes drastically at 100°C and above, and the results of the AOM test do not predict well the future stability or quality of oils under normal storage conditions,” he said, adding, “Similarly, in the evaluation of antioxidants, the AOM test, like other high-temperature stability tests, gives unreliable and misleading results that do not translate well to actual antioxidant performance at normal storage conditions.”

Meanwhile, lipid peroxidation in biological tissues usually is investigated by measuring major peroxidation products, lipid hydroperoxides and conjugated dienes and minor products such as malonaldehyde, hexanal, fluorescent carbonyl-amine products and volatile hydrocarbons. Most studies for lipid oxidation in biological systems have been done in vitro.

However, there are two kinds of in vivo studies, according to Frankel. One is to give the animal oxidative stress and to measure the toxicological effect after the animal is killed. The second analyzes exhaled breath to measure volatile hydrocarbons such as pentane and ethane that form when unsaturated lipid hydroperoxides decompose. Al Tappel described the latter—a gas chromatographic method—in the book Lipid Peroxides in Biology and Medicine, published in 1982.

Frankel believes researchers need to accept more valid methods for analyzing lipid oxidation. “We are dealing with an extremely complicated series of reactions. We don’t know which events are significant. Unless we do, we have a long way to go before we can approach preventive medicine,” he said.

Frankel and colleagues have developed a static headspace gas chromatographic method to determine human LDL oxidation. “This simple, rapid and sensitive method for oxidative susceptibility plays a useful role in analyzing prooxidant/antioxidant status of biological samples,” Frankel and co-workers wrote in the December 1992 issue of Lipids.

Because this method measures specific volatile aldehydes, it not only provides information on the degree of hydroperoxide decomposition but also can distinguish between oxidation products of n-6 PUFA (pentane, hexanal) and n-3 PUFA (propanal).

Lipid oxidation “is a very, very complex reaction,” Frankel said, noting that hundreds of compounds may be formed. “To sort them all out is a monumental task.”

Schaich also sees the need for better methodology. “When one looks for damage in a cell, it is very difficult to differentiate between oxygen radicals and lipid oxidation. A few people have tried to measure lipid oxidation concurrently with a loss of cell function and they haven’t always found a correlation. One of the reasons is that the TBA test is used extensively as a measure of lipid oxidation products in biological systems. TBA measures a downstream product in small quantities from only some lipids and thus is a very limited test. The TBA test is not going to show the early free radicals from lipids. Most other tests require the extraction of lipid from the tissues. These are tedious and time-consuming,” she said.

Frankel, however, noted that his headspace gas chromatographic test measuring hexanal and other volatile oxidation products can be carried out directly with biological tissues and foods without extraction of lipids.

Lipid extraction allows researchers to look for conjugated dienes, peroxide values and carbonyl products. “By putting those all together, you can get a picture of the extent of lipid oxidation. But, because lipid oxidation is a dynamic process, if you pick any point, you won’t get any accurate pic-
tecture. For that, you have to do a finger-print of the whole process. If you do extracts and only have small amounts of tissue, you have to have many animals available to provide adequate amounts of tissues.” For these reasons, Schaich said, “The definitive studies required to clarify the role of lipid oxidation in pathological processes really haven’t been done.”

Currently working on chemiluminescence assays, Schaich hopes this methodology may eventually prove successful in determining lipid peroxidation. Her first goal is to try to develop an alternative method to the TBA test for food quality. A subsequent goal is to apply such methodology to determine lipid oxidation in living tissue.

What about plant sterols?
Although much research has been done on cholesterol oxides, not much is known about plant sterol oxidation, Perkins and others pointed out.

“As oil chemists, we should not just be concerned with lipid oxidation products but plant sterol oxidation in general,” according to Michael Eskin, professor in the Department of Foods and Nutrition at the University of Manitoba and chairperson of the AOCs Lipid Oxidation/Quality Division. “We need to look at the health implications of the consumption of lipid oxidized products including sterols.

“A few of the oxidized products from plant sterols have been recognized but they have not been characterized,” Eskin said, pointing to the need to remedy this. “I think we’ve only identified a small number of them. There hasn’t been much emphasis on plant sterols, so there is not much of a database.”

Maerker agreed that the mechanisms of plant sterol oxidation still need to be understood. “Frying oils contain substantial amounts of plant sterols. Chemically these compounds are very similar to cholesterol, but they exist in vegetable oils in much higher concentrations than cholesterol levels in meat and poultry,” Maerker said, noting that some oils are higher in plant sterols than others. “Plant sterols can range from 500 to 800 milligrams per 100 grams, where all meats and poultry contain approximately 75–85 milligrams of cholesterol per 100 grams. If a large amount of oxides of these plant sterols are formed, what is their effect?”

He added, “It is known that plant sterol content drops dramatically during frying operations. It is expected that the principal products formed are plant sterol compounds very similar chemically to the oxidation products of cholesterol. But essentially nothing is known about the health effects of plant sterol products. This is a very valid concern.”

Eskin agreed. “We don’t want to make things more difficult for industry, but we want to do whatever we can to minimize oxidized products and improve shelf life of products. If oxidation is occurring, we can take the appropriate measures to counteract it. We should minimize the damage.”

Analytical techniques have not yet been developed to measure plant sterol oxidation products, although techniques used for measuring cholesterol oxides possibly could be used. “However, there are no standards for plant sterol oxidation products as there are for cholesterol oxidation products,” Maerker pointed out.

Further research needed

Thus, improving methodology will continue to be a crucial research assignment in this field, both for detecting lipid oxidation in the food chain and in living tissue.

At the October 1992 Lipidforum lipid oxidation seminar in Denmark, Gunhild Hølmer of the Technical University of Denmark noted that more research is necessary to understand the complicated process of the digestive absorption of hydroperoxides and volatile and nonvolatile secondary oxidation products.

“Further research is needed on analytical procedures, the various lipid oxidation products and the question of absorption—which lipid oxidation products are absorbed in the bloodstream and which are not,” Addis said. “For example, although research shows cholesterol oxides are readily absorbed into the bloodstream, we don’t have enough information on the mechanism involved.

“The kinetics of absorption is very complicated,” Addis said, noting he hopes to do more research on this aspect.

In the meantime, however, Addis is convinced food companies and consumers can take steps to cut down on exposure to lipid oxidation. “I don’t think we have to do 30 more years of research before we tell people they shouldn’t consume oxidized products. It is prudent to tell people to use higher and broader levels of antioxidants now. Use them so we don’t have problems with lipid oxidation, or toss out the products before they are consumed.”

“Even I cannot detect oxidized compounds at low levels. There may need to be concern at these low levels. People would be better off if they didn’t consume oxidized foods.”

Meanwhile, Eskin cautions that such risks should not be blown out of proportion. “Nothing in life is absolutely safe. It is unreasonable to expect no risk. Just think what would happen if we applied the same standards to driving a car? The problem is, when you improve your methods of detections to such a degree that you can detect a needle—or one-half or one-quarter of a needle—in a haystack, what you find could be way out of proportion to the actual risk. So, one has to be cautious concerning the actual significance of what has been found. If there is a risk, we need to determine what is an acceptable risk.”

It is evident lipid oxidation will remain a topic of interest to AOCs members. In fact, the Health and Nutrition Division and Lipid Oxidation/Quality Division already are gearing up to organize sessions on the health implications of lipid oxidation at future AOCs meetings.
Evaluation of oxidized lipids in foods

As is well known, flavor and aroma are two of the most important parts of human existence. Responses to flavor or aroma stimuli can be either positive or negative, and we are exposed to a wide variety of tastes and aromas each day. While our response to many foods and aromas is the result of lifelong conditioning, there are some aromas, e.g., vanilla, that have very broad appeal, and others, e.g., mercaptans, that are unpleasant to most humans.

When lipid-derived flavor components are studied, the problem of acceptance or rejection of individual flavor components remains. In addition, the level of the component in the food product must be considered. For example, mildly oxidized fatty acids are important to the perception of good beef flavor, yet too much of certain flavor components, e.g., hexanal, will ruin the overall flavor.

The early work concerning the flavor of oxidized lipids in foods was severely limited by the lack of development of instruments and procedures for studying flavor components. Derivatives, such as 2,4-dinitrophenylhydrazones, were valuable in the identification of individual compounds, but were not very useful for the quantitation necessary to decide whether a component was important to the overall flavor. There were other tests, such as the benzidine or anisidine tests which were reasonably quantitative, but their usefulness was limited primarily to the measurement of unpleasant aldehydes in fats and oils.

The TBA (thiobarbituric acid) reaction, on the other hand, has been used to measure lipid oxidation products in a variety of food systems. The outstanding advances in gas chromatography (GC), mass spectrometry (MS) and related computerized instrumentation have helped immensely in the understanding of both the qualitative and quantitative aspects of flavor. First the TBA reaction will be discussed then the use of GC and MS for evaluating flavor will be reviewed briefly.

This article is based on a presentation "Instrumental and sensory analysis of oxidized lipids in foods" by Glen A. Jacobson, a retired research fellow of Campbell Soup Company, and now a consultant from his home at 133 Windsor Ave., Haddonfield, New Jersey, during the AOCs Annual Meeting and Exposition held jointly with the Japan Oil Chemists' Society from April 25–29, 1993, in Anaheim, California.

TBA reaction, flavor studies

The TBA reaction has been known since 1916. In the 1940s, the TBA reaction was applied to biological systems such as liver tissue. Next came food systems, primarily because of the ability of TBA to detect lipid oxidation products at very low levels. Initially, many workers believed that the red chromogen that formed in the TBA test was the sole reaction product of malondialdehyde and TBA as shown in Scheme 1 (1).

As the TBA test was used in many laboratories for a variety of applications, it soon became apparent that there were many pitfalls to avoid. One of the first considerations was that TBA reacts with a number of aldehydes to give color in the yellow and orange region of the spectrum (450–510 nm) (Figure 1). It is inter-
LIPID OXIDATION

interesting to note that in the test shown, saturated aldehydes and monoenals gave yellow color, while the dienal TBA peak occurs in the 530 nm region, with appreciable absorption in the yellow (450) nm region. Also, some absorption occurred for the blank, which we found to result from traces of iron. When a source of ferric ion was added, about four times as much yellow color was developed during the TBA reaction with beef fat (Figure 2). It was later found that copper as well as iron affects the reaction.

A high degree of purity of TBA itself, glassware, acid, water and other entities used in the test is imperative. Several workers have shown that many aldehydes besides malondialdehyde will react with TBA to influence test results; some reactants, such as the dienals, form a red chromogen. In many cases, the level of the reaction products of these aldehydes is more related to off-flavors in foods than malondialdehyde itself. Reaction time and temperature also can be very important in rate of color development and the proportion of the chromogen developed. Temperatures from ambient to 100°C have been used.

Applying the TBA test to foods often involves a very complex system. Meat products are a good example of this. Several sources of malondialdehyde could be present and can affect the level of red color developed. Some non-lipid sources of malondialdehyde are nucleic acids/nucleotides, sugars, bile pigments and protein.

Despite numerous pitfalls of the TBA test, many laboratories use it, and good correlations can be developed between the TBA test and flavor scores. One of the ways to minimize artifacts is to steam distill the volatiles from the food sample and react the distillate with TBA. This procedure is widely used, but is cumbersome and slow if several samples must be analyzed. Recently Pikul and co-workers (2) modified Wite’s aqueous extraction method and applied it to chicken and other meats. The modified method data correlated very well with that of the

Figure 1. A comparison of the absorption spectra of TBA reaction products

Figure 2. The effect of ferric ion on the development of yellow color in the reaction of TBA with beef fat

INFORM, Vol. 4, no. 7 (July 1993)
LIPID OXIDATION

standard distillation procedure. The extraction procedure significantly reduces the analysis time, which makes the test more attractive for routine use. It also appears to be amenable to partially automated analysis.

The TBA test has been applied to many food products and ingredients, including dairy products, fats and oils, bakery products, and particularly meat, fish and poultry. The data shown in Figure 3 obtained with cracker meal are typical for bakery products and highlight how formulation and processing effects can be followed by the TBA reaction. When baked powdered cracker meal was added back to the unbaked ingredients at an 18% level, the TBA value increased dramatically. When a high baking temperature (320°F) was used, the cracker meal was borderline in flavor acceptability after the first six or seven weeks. An acceptable flavor score was found in these samples when the TBA number was below 1.5. Although routine testing of this type of ingredient is still needed for quality assurance, the TBA test could help offset the subjectivity of flavor scoring with limited or untrained personnel. The TBA reaction was used to show the relative oxidation rates of different parts of fish as they were held frozen (3). The data shown in Figure 4 definitively reflect the relative susceptibility to oxidation of different parts of frozen fish tissue.

GC, MS evaluation
Several years ago, St. Angelo and co-workers (4) at the U.S. Department of Agriculture's Southern Regional Research Center demonstrated the importance of hexanal to the warmed-over flavor of cooked beef. More recently, Hwang and co-workers (5), working with frozen cooked beef, also found that the presence of hexanal decreased the meaty flavor note and increased the warmed-over, cardboard and oxidized flavor notes. The correlation (continued on page 816)

Figure 3. TBA/flavor evaluation of cracker flour flavor stability

Figure 4. The reaction rates of lipid oxidation in terms of change of TBA molar value for autumn mackerel stored at -15 and -30°C
The correlation coefficient between hexanal level and degree of oxidation of linoleic acid was found to be 0.99. Young and Hovis (10) also used a rapid GC headspace technique for measuring the off flavor in raw and roasted peanuts. The peaks identified in the headspace chromatograph derived from heating ground peanuts in sealed vials for 30 minutes (Figure 10) reflect flavor defects used to grade peanuts. Threshold levels also were developed for the chromatographic peaks through use of trained panels and established descriptors. For example, the musty flavor of peak 14 (N-methyl pyrrole) was found to be detectable by most trained panelists at about one-third full scale. The ubiquitous hexanal, peak 16, was described as “beany” in peanuts.

(continued on page 818)

Figure 6. Comparison of peroxide values and total GC volatiles of vegetable oils during storage at 60°C

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Figure 7. Volatiles from beef fat aged at 55°C
It has been found that many foods contain a number of volatile components which impact total flavor. Using a stream splitter at the detector to sort volatiles by their aromas, "thresholds" in terms of chromatographic response can be estimated. In this way, better understanding can be developed of which peaks and which chromatograms reflect the right response and balance of pleasant and unpleasant volatiles.

Presented here have been a number of examples of how to solve problems brought on by lipid oxidation. The TBA reaction is relatively simple, has some potential for automation and correlates with flavor scores in many foods. GC and MS help to determine which flavor components are responsible for a good or poor flavor, and offer guidance in what to do to achieve the desired flavor.

References
Figure 10. Headspace chromatogram by the minimum analysis time system, showing the peak/flavor relationships of a raw peanut sample. Descriptive terms were derived using a trained flavor panel. Average threshold levels are approximately full-scale for musty aftertaste, one-half scale for fruity and one-third scale for musty flavors.