Lipid Oxidation In Foods

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ABSTRACT: This review discusses the basic chemical reactions that affect food flavor quality. Although there are many reactions that can lead to the deterioration of quality in foods, this review focuses on lipid oxidation and how it adversely affects flavor principles. It also presents technological advances for studying the basic mechanism of lipid oxidation, for measuring its intensity, and for retaining food quality. The food commodities that provide the subject matter for this review include vegetable oils, legumes, cereal grains, eggs, beef, lamb, poultry, seafoods, and catfish. The methodologies for assessing food quality form a multidisciplinary approach that includes primarily instrumental analysis by direct gas chromatography, chemical analysis by the TBA test, and sensory analysis by quantitative descriptive determinations. The author hopes that the information presented in this review is applicable to food commodities not discussed.

KEY WORDS: quality, lipid functionality, oxidation, rancidity, mechanism, TBA test, gas chromatography, descriptive analysis, sensory, antioxidants, shelf-life, storage, vegetable oils, peanuts, rice, muscle foods, beef, meat flavor deterioration, warmed-over flavor, poultry, catfish, lamb, tenderization, seafood, eggs.

I. INTRODUCTION

Quality can be defined as a degree of excellence. For scientists, however, this definition may be too simple with respect to foods. Also, some quality traits that pertain to a particular food may not necessarily be applicable to another. In 1962, the American Association for the Advancement of Science (AAAS) sponsored a symposium on Food Quality. This symposium
described milk quality as “milk that meets all the regulatory requirements for safety, cleanliness, and composition, and has a desirable flavor.” Pearson
described meat quality in broad terms that include overall nutritive value, edibility, wholesomeness, and freedom from disease. Speaking of meat quality, Hammond simplified the definition by stating that “quality was that which the public likes best.”

Other scientists have also presented their definitions of food quality. For example, Kramer divided food quality into three main categories: appearance factors (i.e., eye appeal, size, shape, wholeness, color, flowability), kinesthetic factors (i.e., hand and mouth feel of firmness, softness, juiciness, chewiness), and flavor factors (i.e., odor, taste, off-flavor). Later, Potter described quality as the composite of characteristics that have significance and make for acceptability. He further stated that we select foods for consumption by utilizing all our physical senses, including sight, touch, smell, taste, and even hearing (such as hearing the snap of a potato chip or the crackle of a breakfast cereal). Collectively, the views of the speakers from the AAAS symposium seemed to fit the definition that was later used by Molnar: “quality is a concept that is based on a number of product attributes that basically determine their level of suitability to a concrete and predetermined use.” He then discussed the calculation of an overall quality index model that can apply to foods and food products.
Martin, when reporting on fish quality, claimed to have searched for years before reaching a definition of quality. He ultimately defined quality as a combination of such characteristics as wholesomeness, integrity, and freshness. Martin defined "wholesomeness" as a quality of a food fit to eat, clean and uncontaminated, and packed and stored in a sanitary environment. This quality would ensure that the product is not contaminated with any bacteria and/or other microorganisms, leading to spoilage. He defined "integrity" as a product's being what it is supposed to be according to the supplier's claims. This would relate to net weight, count, ingredients, and so on. He defined "freshness" as a quality of appearance, taste, and texture.

Martin was addressing the quality of seafoods. However, these definitions, when integrated with other physical, chemical, and aesthetic properties, can apply to many other food commodities. So, in a broader sense, quality can entail a variety of properties discerned by the ultimate user of the product, and because this is not a static concept, the definition will change as our concepts of quality change. In general, quality can include sensory properties, safety factors, and nutritional value. However, as discussed by Lawless, it can also include product consistency and product reliability.

The information presented herein will discuss one particular aspect of food quality, the "flavor factor" as it relates to freshness, taste, aroma, and odor. This article will also review basic chemical reactions that affect the food quality flavor factor. Although there are many reactions that can lead to the deterioration of quality in foods, this article will focus on lipid oxidation and how it adversely affects flavor principles. It will also present technological advances for studing the basic mechanism of lipid oxidation, for measuring its intensity, and for retaining food quality.

One additional point should be noted. The literature is replete with reports on the effects of lipid oxidation in all types of foods and food products. These reports were presented by scientists from many different research facilities, representing industry, academia, and government institutions. It would be impractical and beyond the scope of this review to summarize those reports. Therefore, the food commodities that provide the subject matter for this review include vegetable oils, legumes, cereal grains, eggs, beef, lamb, poultry, seafoods, and catfish. The methodologies for assessing food quality focus essentially on direct gas chromatography, the TBA test, and quantitative descriptive analysis. The author hopes that the information presented in this review will be applicable to food commodities not discussed.

II. FUNCTIONALITY OF FOOD LIPIDS

Before a discussion of lipid oxidation and its effects on the flavor factor of food quality, perhaps a few words should be said about the beneficial attributes of lipids. Lipids are heterogeneous compounds, components of living systems, insoluble in water but soluble in non-polar solvents, such as hydrocarbons. Included in this class of compounds are the oils or fats of our diet and also phospholipids, which are associated with cell membranes, where they effect compartmentalization of cellular function through bilayer structures. Lipid structures may be esters of long chain fatty acids, steroids, or terpenes. Unlike proteins and carbohydrates, they do not contribute structural strength to plant and animal tissues, but rather act as depot storage forms of energy metabolites in adipose tissue. Lipids are primarily a source of fuel for living organisms, both animal and plant. As an ingested nutritious fuel, they contain 9 kcal/g, or a little over twice the caloric content of proteins and carbohydrates.

Lipids in foods, as described by Kinsella, serve several important functions. For example, food lipids contribute to food quality by providing organoleptic character notes that make particular foods desirable. These properties include flavor, aroma, color, texture, and mouthfeel. Food lipids also impart nutritious value by providing a source of metabolic energy, fat-soluble vitamins (e.g., A, D, E and K) and essential fatty acids (e.g., linoleic, linolenic, and arachidonic). When oxidized, lipids lead to primary and secondary products that can adversely affect these functions. Therein lies the need for protecting the biological and chemical integrity of lipids.
III. MECHANISM OF LIPID OXIDATION

Over the past decades, there have been many comprehensive reviews written on the mechanism for the oxidation of lipids, including several that have appeared recently. This review will not describe all of these papers but will highlight some of the basic mechanisms that have been proposed recently.

How are fats oxidized? To answer that question, one may say that there are many catalytic systems that oxidize lipids. Among these are light, temperature, enzymes, metals, metalloproteins, and microorganisms. Many of these reactions involve some type of free radical and/or oxygen species, such as singlet oxygen. The substrate for these reactions is generally unsaturated fatty acids. When oxidized, they can form an autocatalytic process, that is, the oxidative products so formed can further catalyze the reaction, which causes the rate to increase with time.

In 1987, Simic and Taylor presented a very simple, yet striking, explanation of autoxidation, that merits repeating here. They stated that “autoxidation” was a misleading expression because autoxidation refers to self-oxidation, which they interpreted as oxidation without assistance from exogenous agents. They further said that this term was accurate only if oxygen were considered to be an integral part of the system. They finally concluded that oxygen-mediated oxidation in food systems is commonly referred to as autoxidation and generally proceeds through free-radical reactions comprising initiation, chain propagation, and termination, as shown in equations 1 to 3. The generally accepted free-radical pathway for the oxidation of lipid molecules, RH, proceeds through three stages:

\[
\begin{align*}
\text{Initiation:} & \quad \text{RH} + \text{initiator} \rightarrow \cdot R \\
& \quad \text{RO}_2\text{H} \rightarrow \text{RO}_2\cdot \\
\text{Propagation:} & \quad \cdot R + \text{O}_2 \rightarrow \text{RO}_2\cdot \\
& \quad \text{RO}_2\cdot + \text{RH} \rightarrow \text{RO}_2\text{H} + \cdot R \\
\text{Termination:} & \quad \cdot R + \cdot R \rightarrow \cdot R - \cdot R \\
& \quad \text{RO}_2\cdot + \cdot R \rightarrow \text{RO}_2\text{R}
\end{align*}
\]

In the initiation step, Equation 1, the free radical \( \cdot R \) is formed from an unsaturated lipid molecule at an allylic methylene group, RH, or a lipohydroperoxide, \( \text{RO}_2\cdot \), by the action of an initiator. In the propagation step, 2, the free radical \( \cdot R \) can then react with oxygen to form a peroxy radical \( \text{RO}_2\cdot \), which can further react with another lipid molecule to generate a hydroperoxide, \( \text{RO}_2\text{H} \), and another lipid radical, R. In the termination stage, Equation 3, two radicals react to give products that do not sustain the propagation phase. Termination also occurs when antioxidants or free radical scavengers react with free-radicals generated during propagation.

For a number of years, the initial reaction was thought to proceed via the decomposition of a lipid hydroperoxide or by the direct reaction between an olefin and an oxygen, that is, by one direct bimolecular attack of oxygen on a fatty acid molecule:

\[
\text{RH} + \text{O}_2 \rightarrow \cdot R + \text{HOO}.
\]

However, because the activation energy of reaction, Equation 4, is reported to range from 30 to 45 kcal/mol, Uri concluded that, on the basis of the thermodynamics of the reaction, it would be unlikely that this reaction would play a significant role in the initial reaction for autoxidation. Subsequently, Heaton and Uri postulated that the initial molecular oxygen reaction requires metal interaction as shown in Equation 5, where \( n \) equals the oxidation state of the metal and \( n + 1 \) signifies a loss of 1 electron. They also proposed that metal catalysis is involved in the decomposition of hydroperoxides.

\[
\text{M}^n + \text{O}_2 \rightarrow \text{M}^{(n+1)+} + \text{O}_2^-
\]

Most scientists agree that autoxidation in fats proceeds via chain reactions involving peroxy radicals, \( \text{RO}_2\cdot \). However, the exact origin of the initial free radical is still unknown. According to Simic and Taylor, it can be either a fatty acid radical, \( \text{HL} \cdot \), or it can be a completely different radical, \( \cdot R \). Even though the generation of this initial radical is still controversial, what is known about the initiation of autoxidation reactions is that it can be activated by certain factors, such as temperature, singlet oxygen, photosensitizers, physiological reduction of oxygen to superoxide radical, or radiation. These initiators trigger
autoxidation reactions by oxidizing the substrate, which could also include amino acids or some vitamins, adding to a double bond or sulfur atom, or by abstracting a hydrogen from a polyunsaturated fatty acid (PUFA).

Oxygen is an unusual diatomic molecule in which the spins of the two valence electrons of lowest energy are unpaired in the most stable ground state. Ground state oxygen is a triplet. Singlet molecular oxygen is produced when triplet oxygen absorbs sufficient energy to shift one of the unpaired ground state electrons to a higher energy level. Once in this excited, unstable energy state, singlet oxygen must release its excess energy by reacting directly with electron-rich double bonds, such as are found in unsaturated fatty acids and other singlet state compounds. It should be noted, however, that oxidation reactions can occur in the presence of either ground state oxygen or singlet oxygen. Nonetheless, singlet oxygen has been shown to react with linoleic acid at least 1450 times faster than triplet oxygen. Over the years, many comprehensive reviews have been written on the chemical and biological properties of singlet oxygen.

Any reaction that prevents propagation of peroxidation or removes free radicals from the system plays a key role in the termination mechanism. Thus, antioxidants are a very effective class of compounds that are able to inhibit chain peroxidation reactions. According to the type and concentration of antioxidant used in foods, autoxidation can be either inhibited or retarded. A chain-breaking antioxidant can interfere with either the initiation step or the propagation step as described below:

$$\text{RO}_2^- + A^- \rightarrow \text{nonradical product}$$  \hspace{1cm} (6)

$$\text{RO}_2^- + \text{AH} \rightarrow \text{RO}_2\text{H} + A^-$$ \hspace{1cm} (7)

IV. MEASUREMENT OF LIPID OXIDATION

Foods that contain polyunsaturated fatty acids are highly susceptible to lipid oxidation. The greater the degree of unsaturation of the fatty acid, the more susceptible is the food to oxidation because allylic sites flanking double bonds permit stabilization of free radicals through delocalization of the unpaired electron. Lipid oxidation leads to the formation of hydroperoxides, which are very unstable and decompose to form secondary reaction products that adversely affect food flavor quality. These secondary reaction products may include hundreds of compounds, such as aldehydes, ketones, alcohols, acids, or hydrocarbons. In many cases, the compounds generated are associated with the term oxidative rancidity and are described as having objectionable off-odors and off-flavors.

The determination of the extent of oxidation in foods and food products is of major importance in the control of quality and flavor characteristics. Many analytical methods for the measurement of lipid oxidation in foods have been reported in the literature. Many of these were more than adequately presented in reviews. On a subject such as this, there tends to be some overlap in the lengthy treatments, but some differences appeared among the comprehensive reviews. For example, the reviews by Lundberg and by Gray, presented chemical and physical methods for measuring flavor quality of fats and oils. They discussed in detail the peroxide value (PV) method, the 2-thiobarbituric acid (TBA) test, the determination of total and volatile carbonyl compounds, the Kreis test (a rancidity index), the ultraviolet method for determining conjugated dienes, the use of fluorescence to detect lipid oxidation damage in biological tissues, and the measurement of fat stability by the active-oxygen method. Chan and Coxon discussed briefly many of the same methods, but in less detail. Their review included discussions on the PV method, the TBA method, and the use of thin-layer chromatography for determining lipid hydroperoxides. They also emphasized the detection and measurement of lipid hydroperoxides by utilizing infrared spectral properties of the hydroperoxides and their ability to absorb in the ultraviolet region. They also discussed the use of nuclear magnetic resonance spectra and mass spectrometry for determining the structural properties of peroxides.

Rossell presented yet another review on the measurement of rancidity by methods including PV values, the TBA test, the Kreis Test, determin-
nation of oxirane oxygen in epoxidized products, the anisidine value method for determining degree of oxidation, and a method for determining the total oxidation value (or ToTox Value) from the PV determination. He also gave several methods that can be used to measure the resistance of a fat to oxidative rancidity, including the Schaal oven test, the Sylvester test, the Swift test (or Active Oxygen Method, AOM), and the use of the Rancimat apparatus. Further, he described instrumental methods for measuring rancidity. These included the UV determination at 232 and at 268 nm and their correlations with oxidation of fatty acids, the use of infrared spectroscopy, chemiluminescence, bioluminescence, fluorimetry, polarography, and determination of oxidation by physical measurements of the dielectric constant. Rossell also discussed the use of liquid column chromatography to determine concentrations of thermally labile peroxides, hydroperoxides, and volatile and nonvolatile secondary oxidation products.

Recently, Berner \(^{44}\) reviewed the American Oil Chemists’ Society’s “Official and Tentative Methods” for monitoring the processing and quality of fats and oils. He discussed alternative methods being investigated to replace hazardous chemicals used in traditional methods. This review included references for determining residual hexane (by head space and GLC methodology), free fatty acid content (by titration with alcoholic KOH), iodine values (by use of carbon tetrachloride, cyclohexane), peroxide values (chloroform, isooctane), AOM (conventional method and the Rancimat), fatty acid profiles (by methyl esters/GLC) and trans-fatty acid content (by GLC).

Although occasionally our laboratory has used several of these analytical methods to evaluate food quality, the present report describes the principal methods that we use most often to assess food quality. These methods include chemical (TBA), instrumental (volatile components), and sensory technologies, which we refer to collectively as a multidisciplinary approach for the assessment of food quality. Because all foods contain lipids, and the oxidative mechanisms are generally similar, it is hoped that this article will illustrate our approach to assessing food quality and will provide information that is applicable to the assessment of food quality over a wide range of commodities.

A. The TBA Method

As hydroperoxides, formed from the oxidation of lipids, begin to degrade into the myriad of secondary reaction products (e.g., alcohols, aldehydes, ketones, and hydrocarbons), one of the products formed during the oxidative process is malondialdehyde (MDA), a three-carbon dialdehyde with carbonyl groups at the C-1 and C-3 positions. For quantification of MDA, the TBA test is one of the most commonly used methods for determining rancidity in lipid-containing foods, particularly in animal foods; it is one of the three major analytical methods used extensively in our laboratory. Lipid oxidation values are reported as mg of malonaldehyde/kg (or ppm) of sample.

The TBA method was first described by Kohn and Liversedge,\(^ {45}\) who observed that aerobically oxidized brain tissue, when heated in an acid solution with 2-thiobarbituric acid, produced a brilliant red color with an absorption maximum at 532 nm. They theorized that the red color was produced by a carbonyl compound because the reaction did not occur when blocked by semicarbazide or phenylhydrazine. Many investigations of the TBA reaction concerning the formation of the complex followed their report. Bernheim et al.\(^ {46}\) reported that the red pigment consisted of a 3-carbon moiety containing 1 oxygen atom, either a ketone or an aldehyde, which was derived from the oxidation of fatty acids. These findings led to the proposal that the TBA reaction should be used to assess the oxidative products of unsaturated fatty acids.\(^ {47}\) Later, Patton and Kurtz\(^ {48}\) showed that freshly prepared \(\alpha\) or \(\beta\) unsaturated aldehydes did not give the TBA test but did so when oxidized or heated with Cu\(^ {2+}\). Subsequently, a conclusion was reached that the TBA reaction was caused by a low molecular weight compound, possibly MDA, which was present in oxidized milk fat. Owing to the instability of MDA, proper standards could not be run to prove these theories about the red compound until the newly available compound 1,1,3,3-tetraethoxypropane (TEP) was hydrolyzed under mild
acid conditions to yield MDA and ethanol. The MDA so formed could then react with TBA to give the typical red color.

Thus, TEP could be used as a standard for quantitative determinations of MDA in products subject to oxidation.

In addition to the reddish chromophore that absorbs at 532 nm in the TBA reaction, a second color, yellow orange, was found to absorb from 450 to 460 nm. This compound was reported to result from many different types of compounds, that is, α, β-unsaturated carbonyl compounds, carbohydrates, formic acid, furfurals, glyoxal, glycolic aldehyde, glyceraldehyde, dihydroxy acetone, epiphridin aldehyde, and glyceraldehydes. The absorbance at 452 nm was also reported to result from the reaction of the TBA reagent with aldehydes that were not oxidation products. However, it was later shown that beef tallow, which has no C18:3 fatty acid, would not produce high levels of MDA in storage, but would produce alkanals, which react with TBA to form the yellow complex that absorbs at 454 nm.

Over the years, several modifications of the TBA method have been made. The most popular modification of the TBA method for determining lipid oxidation in muscle foods was the distillation method of Tarladgis et al., who first concentrated the TBA-reactive compounds by codistillation from aqueous acid solution. This method, which is used in our laboratory, has been used by several different investigators to follow lipid oxidation in foods, in particular, freshly cooked and cooked/stored muscle foods, such as beef, pork, and poultry. Several other modifications of the TBA reaction have been reported. In addition to the spectrophotometric method for measuring the red chromophore, the TBA reaction has also been modified where it can be monitored instrumentally with high-performance liquid chromatography and gas chromatography. The TBA method has several limitations; for example, the data are not transferable from species to species. The data are reported to be grossly erratic when frozen samples are analyzed. TBA numbers do not always increase with storage of muscle foods, and the data are quite variable depending on the method of analysis. The distillation method is time-consuming and requires a multiple distillation apparatus that can limit the number of samples analyzed per day. Furthermore, the TBA test could yield erroneous results as substances other than MDA could react with the TBA reagent. For a recent comprehensive review on the TBA method for measuring lipid peroxidation in muscle tissue and for identification of those nonlipid materials that react with the TBA reagent, the reader should consult the review by Raharjo and Sofos.

B. Direct Gas Chromatography

Headspace vapors were analyzed in early attempts to employ gas chromatographic techniques. These procedures were used to detect fruit and vegetable aromas, and volatiles of food products. Such methods require special sample preparation and subsequent transfer of a vapor aliquot to a gas chromatograph and were later reported to lack sensitivity and reproducibility. Methods that involved extractions and distillations of the samples and reactions to form derivatives were also utilized to provide quantities of volatiles sufficient for instrumental detection and analysis. Vegetable oils were generally assessed for their quality and stability by peroxide values and the accelerated oxygen method, official procedures of the American Oil Chemists' Society. In many cases, these methods are complex, tedious, and time-consuming, and have potential for creating artifacts. Moser et al. reported sensory assessments of oils by flavor panels.

Some of the earliest work that correlated gas liquid chromatographic evaluations of vegetable oil with a lipid oxidation product, pentane, was reported by Scholz and Ptak. These investigators correlated pentane content with flavor scores based on a ranking system, but not on a point-to-point correspondence with the pentane concentrations because the average rancidity ratings varied widely within each group. They noted that correlations should be established independently for each type of vegetable oil.

A few years later, Dupuy et al. developed an objective instrumental technique that was able to quantitate residual solvents in vegetable oils. They reported excellent correlations with flavor panels on a variety of food substances, including salad oils, peanut butters, and raw and roasted
peanuts.\textsuperscript{104} The method is referred to as the direct gas chromatography (DGC) method and is different from the static or dynamic headspace methods. Wampler et al.\textsuperscript{105} defined the static headspace technique as one that involves the injection of a small (1 to 3 ml) sample of the atmosphere around a warmed sample onto a GC; headspace gas is withdrawn with a gas-tight syringe, and injected directly into a GC inlet for analysis. They defined dynamic headspace as a concentration technique in which the sample is warmed and the atmosphere continuously purged with a carrier gas to remove the volatiles. The carrier gas is then passed through a trap to retain the organics, while the carrier itself is vented. There have been several variations of this method, but all involve some type of headspace gas enrichment procedure.

In the original DGC method of Dupuy et al.,\textsuperscript{101} as much as 1 g of vegetable oil was placed directly into a glass liner (8.5 × 0.84 cm) containing volatile-free silanized glass wool, and the tube was next inserted into the heated injection port of a GC; the glass liner was sealed with an injector septum nut. The volatiles were purged rapidly from the oil by action of a carrier gas and heat and were swept onto a packed GC column without contaminating the column with oil. The non-volatiles were retained in the glass liner. Elution took place without any apparent decomposition of the oil; trace amounts of volatiles were easily detected at the ppb level.\textsuperscript{102} This approach eliminated the need for special entrapments because the gas chromatographic column (packed, or more recently, capillary) acts both as the solid support/concentrating medium as well as, subsequently, the separation vehicle. The method requires no extractions, distillations, formation of derivatives, or need of special entrapment devices. Although the volatile profiles obtained by this method are similar to those obtained by earlier GC methods, the DGC method minimizes or eliminates errors and/or losses of volatiles through transfer or trapping inefficiencies. This innovation to direct inlet gas chromatography therefore permitted the elution of volatiles from food samples directly onto a GC column with efficiency so great that GC pattern recognition could be used to judge sample quality.

The original method of Dupuy utilized Porapak P as the adsorbent in the packed columns in the GC. In 1976, Williams and Wille\textsuperscript{106} modified the method by changing the column-packing material to Poly MPE-Tenax, because the bleed rate of the Tenax above 145°C did not increase as sharply as the Porapak adsorbent did. The Tenax packing was also stable to 275°C, which permitted efficient column reconditioning between GC runs. This modification improved the stability of baseline of the chromatogram and thus improved analysis precision and measurement of the peaks. Both of these packing materials were water tolerant.

A few years after the modification by Williams and Wille,\textsuperscript{106} another major modification was made. This was the invention by Legendre et al.\textsuperscript{107,108} of an external closed inlet device (ECID), which can be interfaced with practically any GC. The ECID has three major parts, an inlet assembly, a condenser assembly, and a six-port rotary valve. The glass inlet that contains the sample is placed in the injection port inlet between two perforated silicon septa in such a manner as to provide a seal on both sides. Carrier gas from the GC is forced to flow down and through the sample during the heating phase. Volatile components pass from the heated sample on through the condenser and through the six-port rotary valve to reach the GC column. The sodium sulfate-coated support matrix contained in the condenser removes moisture from the volatiles as they travel to the GC column. The eluted volatiles are then analyzed by GC or combined GC/mass spectrometry, which is interfaced with the ECID. The eluted volatiles are primarily lipid oxidation marker compounds such as the aldehydes (e.g., hexanal, pentanal, heptanal, nonanal, octanal, the decadienals), the ketones (e.g., 2,3-octanedione, 2-heptanone), and hydrocarbons (e.g., pentane). After completion of the purge cycle, the spent glass liner is removed, replaced with a blank liner, and a combination of heat to the condenser and purge gas flow regenerates the sodium sulfate desiccant.

The original ECID unit used packed columns; the DGC method was later upgraded by the use of capillary columns.\textsuperscript{109–111} In the Dupuy et al. modification\textsuperscript{109,111} (Figure 1), the condenser was eliminated. The moisture contained in a sample is generally controlled by sample size and volatile-free glass wool placed in the glass liner. In its applica-
tion with capillary GC, 300 mg of vegetable oil were added directly on top of a volatile-free glass wool plug secured into the glass liner, that was then inserted into the ECID. Volatiles were purged and concentrated at the entrance of the capillary column by cooling the GC oven with either solid or liquid carbon dioxide. (Liquid nitrogen can also be used.) Temperature programming of the column oven facilitated resolution of the volatile components. This technique has all the advantages of the packed column system,\textsuperscript{102,103} and is adaptable for analysis.
by the higher resolution capillary columns. Moreover, this method minimizes or eliminates the loss of low-molecular-weight volatile components and improves sensitivity. The method is also adaptable as a trap-and-purge method using an ECID equipped with two chambers, one chamber interfaced to the inlet port and the second interfaced to the exit port of the six-port rotary valve. The length of the tubing used to interface the two inlet ports is kept to a minimum length (about 4 cm). A 1 g sample can be placed in the first chamber and an adsorbent trap (e.g., Tenax-GC) can be placed in the second chamber to adsorb the volatiles as they are purged by heating the first chamber. After purging, the volatiles are thermally desorbed by heating the second chamber and then collected onto the head of the cold (-30°C) capillary column. This method also offers another advantage: it allows the analyst to store the volatiles-laden Tenax cartridge for later analysis. In our laboratory, we refer affectionately to this method as "the double whammy."

Soon after the Legendre invention was patented and modified, the ECID was commercialized by Scientific Instruments Service, River Ridge, LA (see Figure 2). In the commercialized ECID model, the condenser was eliminated. The temperature and pressure flow controls were placed in one compartment and the external inlet and six-port valve in another.

Two other pretrapping procedures, in which beef samples (15 and 100 g) were placed in round-bottom flasks, have also been described. In the first instance, the sample was steam distilled and the volatiles were adsorbed onto a Tenax-GC trap. In the second instance, the sample was steam distilled at 60°C under vacuum (8 psi). Again, the volatiles were adsorbed onto a Tenax-GC trap. In both instances, the Tenax traps were placed in the ECID, and volatiles were thermally desorbed onto the cold capillary column. In addition to having the advantages of the methods described previously, these methods allowed samples with high moisture content to be analyzed without any problem of contaminating the column or blowing out the flame used in the detector. The Suzuki/Bailey method is also a direct sample analysis method, similar to the first purge-and-trap method described by Dupuy et al."

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**FIGURE 2.** Closed external inlet device, including control panel, rotary valve, and injection port. External dimensions are 31.5 cm x13 cm x18.6 cm.
Originally used to assess vegetable oil quality and correlate instrumental methods with organoleptic values, that is, sensory scores (a highly significant breakthrough), this methodology has been utilized during the past 2 decades to assess the quality of many different food types, such as soybean oil, raw and roasted peanuts, peanut butter, mayonnaise, cereal grains, soybean proteins, vegetable oils, eggs, coffee, southern pea, dried legumes, pecans, and molasses. The method has also been used to assess the quality of muscle foods by correlating chemical, instrumental, and sensory data, for example for beef, ham and pepperoni, chicken and turkey, bacon, meat loaf supplemented with cottonseed flour, lamb, seafoods, fish oils, and catfish. The method has also been used to study the oxidation of linoleic acid by the enzyme lipoyxigenase, and has nonfood uses such as identification of volatiles produced from fumigants, weeds, biological control agents, and Aspergillus flavus.

In 1988, Vercellotti et al. utilized the Dupuy capillary DGC technique to detect and identify volatiles removed at mild temperatures under vacuum from food and beverage products. Volatiles were stripped from the sample between 30 and 60°C and thermally adsorbed onto a Tenax-GC trap under a vacuum of 8 psi. Next, the moisture-free volatiles were desorbed directly onto a cold capillary GC column and resolution was accomplished by temperature programming the GC. This modification of the Dupuy method allowed the sample to be assessed with minimal decomposition and permitted the detection and identification of trace volatiles in foods and beverages down to the ppb level.

Judging from the vast differences in the commodities analyzed, the DGC method has over the years proven to be highly versatile in the assessment of food quality and in correlating the GC data to chemical (TBA) and sensory data. An authority on food assessment has honored the method by saying, “because of the broad application of this technique by Dupuy and co-workers, it has become the inspiration for much of the current evaluation of flavor volatiles of oils and oil-based products.”

Very recently, another variation of the DGC method was introduced. This method is referred to as direct thermal analysis (DTA), or short-path thermal desorption. It is similar to the DGC method except it does not have the six-port valve attached, the glass liner is replaced by a glass-lined stainless steel tube (GLT), and the manner used to interface with the GC is different (the short-path through which the sample passes). The principle of operation appears to be similar, nevertheless. A solid sample is inserted into the GLT unit; the tube containing the sample is purged with carrier gas, heated and then sparged into the GC injection port where the volatiles are cryotrapped onto the GC capillary column. The sparged components are then eluted from the GC. As with the Dupuy/Legendre DGC system, samples analyzed using the DTA system do not need to be extracted with solvents, nor do derivatives have to be made to identify the volatiles. One major difference that should be noted is that the DTA system uses much smaller samples, 1 to 5 mg of sample, compared with the Dupuy-Legendre system, which has used up to 500 mg of vegetable oil or up to 1.2 g of solid sample, such as beef. To analyze samples with high moisture content, the DGC method can use the ECID equipped with two chambers or with two chambers interfaced slightly differently, one chamber interfaced to the inlet port and a second chamber interfaced to the exit port of the six-port valve. The second chamber can hold a Tenax trap for adsorbing the eluted volatiles from the heated first chamber. A similar trapping system can be employed with the DTA system, but the amount of solid or semisolid sample analyzed by that method is reported to be limited by the amount of moisture present, usually less than 5%; samples can range from 1 to 500 mg. It should also be noted that the DTA unit is manufactured and sold by Scientific Instrument Services of Ringoes, NJ, and should not be confused with the ECID unit made by Scientific Instrument Services in Louisiana.

C. Descriptive Analysis

One of the most important attributes of a food is its flavor. Aside from those individuals who eat
particular foods for health, nutritional, or religious reasons, most people eat particular foods because they enjoy the tastes. If a particular food has an off-taste, it will probably be rejected as food in the future. Because of the many off-flavors (and tastes) that have been detected in foods and food products, sensory studies play an important role in the assessment of flavors, tastes, and aromas, both desirable and undesirable. The science of sensory evaluation has expanded far beyond the “come taste this product” benchtop panels of yesteryear. Food companies today rely heavily on the trained food taster for product quality control. Food scientists are always concerned with developing a lexicon that would characterize flavors perceived, as desirable and undesirable. According to Civelle and Dus, food scientists might be content with words to describe the apparent cause of an off-flavor, but sensory analysts are more interested in describing the observed flavor note. They stated that chemists studying rapid oxidation may use such terms as “rancid,” “oxidized,” or “warmed-over” to indicate the characteristic of the off-flavor notes, but for sensory investigators these words suggest the occurrence of definite chemical reactions or probable causes, while not providing an accurate description of the flavor perception itself.

To fully comprehend the assessment of food quality, there was a critical need, therefore to utilize a sophisticated sensory system, that produced data that could be statistically correlated to the chemical data determined on the same samples from foods and food products. To this end, Spectrum Descriptive Analysis (SDA), as defined by Civelle and associates, became the third major analytical method used to assess food quality in our laboratory. The principle of SDA is founded on a complete, detailed, and accurate descriptive representation of a product’s sensory characteristics and involves the detection and description of both qualitative and quantitative sensory aspects of a product by a trained panel utilizing a universal 15-point scale. A panel of 12 to 15 members to rate and rank intensity of the flavor descriptor being evaluated is selected on the basis of having “normal” ability to taste and smell. At the completion of an experiment, sensory chemical and instrumental data are statistically correlated using SAS for analysis of variance and principal components analysis (PCA) of factors. In this multidisciplinary approach, variables are experimental treatments and the sensory, chemical, and instrumental methodologies are attributes.

To sustain this sensory methodology, much thought and effort went into the building of our physical facility for sensory evaluation studies. It consists of a food preparation area, a row of 12 booths equipped with pass-through “breadbasket” windows and computers into which the panelist scores the data, a training room, and a set of offices for laboratory personnel. Inasmuch as the preparation of food samples generate odors that could interfere with flavor evaluation, the tasting booths and the preparation areas were separated. The air conditioning system in the evaluation room and the training room has a positive pressure compared with the surrounding rooms, to keep odors flowing out of the room. Vinyl floors instead of carpet were installed.

The evaluation and training rooms have adequate levels of illumination provided by fluorescent lighting. Special lighting systems are also used to mask the color of products. To reduce auditory and visual distractions, the booths are about 30 in wide with dividers extended about 18 in beyond the counter top. Counter tops are about 20 in deep and 30 in from the floor. The computer ballot system has the keyboard and monitor mounted above the pass-through window. The user-friendly software package allows the panelists to concentrate on the sample rather than the computer system. Panelists score the samples by touching the screens with light pens at the appropriate point on the electronic ballot. When testing is completed, samples are scored and the data are immediately available for discussion. Results are computed and the mean and standard deviations are determined for the panel for each property evaluated. The data are posted, and the results are discussed among the panelists. Emphasis is placed on deviations of the mean for each sample. The individual panelist is scored to determine his or her performance for that day.

Preparation areas contain convection ovens and electric grills and are constructed of easy-to-clean materials, such as stainless steel. (Plastic materials or porous materials are not suitable as
they may absorb odors and/or could harbor bacteria that can contaminate the sample.) Thermocouples are used to control internal temperatures of heated samples. Samples are placed in warming drawers to keep them warm for the few minutes between preparation and serving to the panelists. Cooking or reheating samples is staggered to minimize time in the warming drawers.

Descriptive flavor panelists, selected on the basis of their acuity for taste and smell, are oriented to the basic principles of descriptive flavor analysis by a trained panel leader. To develop the descriptors that are applied to the food product being evaluated, a range of samples that includes flavors and off-flavors similar to those of interest are presented to the panelists, who then describe the flavors perceived. The panel leader guides the discussion to eliminate redundant descriptors. The panelists then come to a consensus on the descriptors that eventually are placed on the ballot. This training procedure has been used in our research programs, including those on vegetable oils, peanuts, rice, meats, and catfish. The intensity reference scales of the spectrum method were used as panel calibration benchmarks in the sensory analysis.

V. ANTIOXIDANTS

Living tissues do not autoxidize because of the presence of antioxidants in tissue cells. When animals are slaughtered, the cells become filled with prooxidative compounds, peroxidized lipids, and oxygen radicals, because the cellular antioxidant capacity is no longer viable. In foods, including those made from muscle, there is a need to preserve the shelf life of the product until it has been consumed. In non-muscle foods, many of the naturally occurring antioxidants are destroyed during the processing of the raw product. Consequently, the manufacture of antioxidants for food use is a multibillion-dollar industry.

Antioxidants are compounds that in small quantities prevent or greatly retard the oxidation of substances that contain fats. Food antioxidants have been described as substances that function as free-radical inhibitors by interfering with the free-radical mechanism fundamental to antioxidation. The classical free-radical mechanism involving lipid oxidation depends on the production of free radicals, \( R^* \), from unsaturated lipid molecules, \( RH \), by their interaction with oxygen in the presence of a catalyst. This reaction is the initiation phase in which oxygen reacts with a carbon-carbon double bond (see Equation [4]). Sequentially, the lipid molecule becomes a free radical, which is then oxidized (Equation [2]). Free radicals are continually being made during the propagation phase, which proceeds until an event occurs that terminates the reaction (Equation [3]). Antioxidants react with free radicals, generated in the propagation phase to terminate the reaction (Equation [6]). Once reacted with a substrate molecule, antioxidant free radicals cannot initiate or propagate the oxidation reaction. It should be noted that substances containing many polyunsaturated fatty acids are more susceptible to oxidation than those that contain fewer. It is also equally important to note that antioxidants do not prevent oxidation in toto, but they do delay the cascade of free radicals in the oxidative process. The shelf life of the product will thus be increased.

Many types of compounds have been used as food grade antioxidants over the years. They include those that function as free-radical scavengers, oxygen absorbers, and chelators. Some of the more popular synthetic antioxidants used are phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), mono-tert-butyl-hydroquinone (TBHQ), and propyl gallate (PG). These compounds, referred to as "primary antioxidants," are very effective in terminating lipid oxidation. For example, BHA and BHT are fairly stable to heat and are often used in foods that are processed by heating. Antioxidants that are heat stable have the property referred to as "carry-through" or "carry-over." PG decomposes at 148°C, whereas TBHQ has poor carry-through properties in baking applications, but excellent carry-through properties in frying applications. These antioxidants function by interfering with the free radical mechanism. Some antioxidants, such as BHA and BHT, are used in various combinations with resulting synergistic effects, that is, the combined effect from two antioxidants is greater than the sum of the indi-
vidual effects obtained when used alone.\textsuperscript{175} BHA is also synergistic with PG. The combination of BHT and PG can result in negative synergism, that is, the shelf life of a product would be less than that anticipated from the sum of the effects of each when used alone.\textsuperscript{179}

The positive synergistic effect described above should not be confused with the synergism that occurs when a primary antioxidant is added with an acid chelator, such as citric acid. Acid chelators, or sequestrants, are known as “secondary antioxidants.”\textsuperscript{180} These include such compounds as citric, tartaric, oxalic, malic, and succinic acids and sodium tripolyphosphate and pyrophosphate, all of which are used as food-grade antioxidants. Another acid chelator, phytic acid (inositol hexaphosphate), is also reported to inhibit lipid oxidation.\textsuperscript{138,139,181}

When acid chelators, are combined with one of the phenolic-type antioxidants, the resulting synergism is referred to as “acid synergism.”\textsuperscript{175} In this capacity, the primary role of the chelator is to bind metals, or metalloproteins that promote oxidation, which in turn allows the primary antioxidant to perform its function and capture free radicals. In this manner, the acid synergistic mechanism functions differently from that of the combined BHA/BHT system.

Because of growing concern for the potential health hazard of synthetic antioxidants,\textsuperscript{182-184} there is renewed interest in the increased use of naturally occurring antioxidants. Because they occur in nature and in many cases are derived from plant sources, natural antioxidants are presumed to be safe. One of the naturally occurring antioxidants that is used widely by the food industry is ascorbic acid, which has a varied chemistry. Ascorbic acid and its esterified derivatives function as antioxidants by protecting double bonds and scavenging oxygen. As an oxygen scavenger, ascorbic acid acts by being preferentially oxidized over other oxidizable components in the food system because the 2- and 3-positions are unsubstituted. In this situation, oxygen is removed and converted into water at the expense of ascorbic acid, which is oxidized to form dehydroascorbic acid. In the presence of a metal ion, such as iron, ascorbic acid is able to scavenge oxygen faster due to a chelated intermediate. In this scheme, a reducing agent, such as reduced glutathione, is needed to convert the dehydroascorbic acid back to ascorbic acid.\textsuperscript{185}

Some important chemical characteristics of ascorbic acid include its redox properties, which enable it to function as a reducing agent and as a free-radical scavenger. In this role, ascorbic acid can donate a hydrogen atom for free-radical chain inhibition. Ascorbic acid can act as a synergist with tocopherol by converting oxidized tocopherol back to the reduced form. In these reactions, ascorbic acid can be regenerated by its action with reduced glutathione or NADH+.\textsuperscript{185}

Another function of ascorbic acid is to generate Fe(II) from Fe(III), which is part of the Haber-Weiss-Fenton reaction.\textsuperscript{20} In this reaction, peroxide is reduced to the hydroxyl radical, HO-, a very powerful oxidizing agent. Finally, it should be noted that ascorbic acid can chelate metals\textsuperscript{186} and can promote carbohydrate-amine browning reactions.\textsuperscript{187,188}

There has been much interest in using derivatives of ascorbic acid as antioxidants. One such compound is ascorbyl palmitate. In the structure of ascorbyl palmitate, the 2- and 3-positions are occupied by hydroxyl groups; the 6-position contains the fatty acid ester substituent. Other derivatives synthesized more recently are ascorbate-2-phosphate and ascorbate-2-triphosphate.\textsuperscript{189} Both of these compounds were reported to inhibit lipid oxidation as measured by chemical means in ground meat.\textsuperscript{138} Ascorbate-2-phosphate was also found to inhibit warmed-over flavor (WOF) as measured by sensory means in beef.\textsuperscript{138} The ascorbate-2-triphosphate was not tested as an inhibitor of WOF in this study, but one assumes that it, too, should prevent oxidation. Ascorbate-2-phosphate was later used to inhibit off-flavor development in cooked frozen turkeys.\textsuperscript{190} For comprehensive reviews on ascorbic acid, the reader is referred to Bauernfeind and Pinkert\textsuperscript{191} and Seib and Tolbert.\textsuperscript{192}

Other widely used natural antioxidants are the tocopherols, with \(\alpha\)-tocopherol being the most effective.\textsuperscript{193} Tocopherols, found in high quantity in vegetable oils and fresh, leafy plants, are particularly effective in stabilizing animal fats.\textsuperscript{194} They are also added to many other foods, and are very popular antioxidants. For example, tocopherols are estimated to represent 50% of the antioxidant