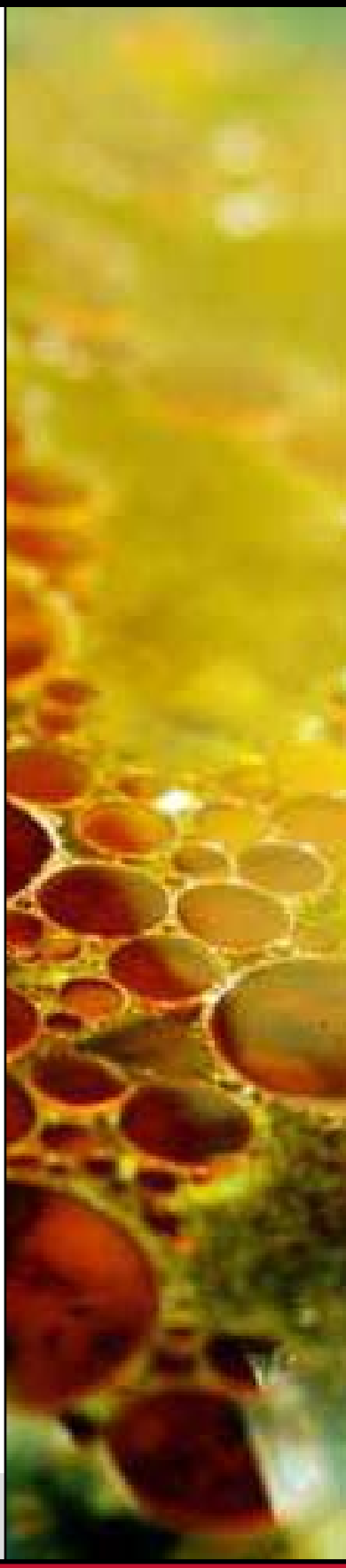
ChromaDex™

Tech Tip 0011

Fats and Oils



What is the best way to measure rancidity in my sample?

That is an often asked and simple question that, unfortunately, belies a simple answer. Many factors must be considered when selecting the most appropriate test or series of tests, and it is often difficult to predict which method will yield the most meaningful results.

What Causes Rancidity?

Fats and oils play an important role in the flavor, aroma, texture, and nutritional quality of foods, pet foods, and feeds. Fats and oils may be added during manufacturing or they may be inherent to the product or ingredient. The product may be pure oil or it may be part of a complex mixture with proteins, carbohydrates, minerals, and vitamins. The product may contain almost no fat or it may contain a considerable amount. Regardless of the source of fat, the amount of fat, or the product composition, predicting and monitoring fat and oil quality is an important component of developing and manufacturing high quality products.

As soon as a food, feed, or ingredient is manufactured, it begins to undergo a variety of chemical and physical changes. Oxidation of lipids is one common and frequently undesirable chemical change that may impact flavor, aroma, nutritional quality, and, in some cases, even the texture of a product. The chemicals produced from oxidation of lipids are responsible for rancid flavors and aromas. Vitamins and other nutrients may be partially or entirely destroyed by highly reactive intermediates in the lipid oxidation process. Oxidized fats can interact with proteins and carbohydrates causing changes in texture. Of course, not all lipid oxidation is undesirable. Enzymes, for example, promote oxidation of lipid membranes during ripening of fruit. For most products, though, predicting and understanding oxidation of lipids is necessary to minimize objectionable flavors and aromas arising from fat rancidity.

Two Types of Rancidity

Selecting an optimum test for lipid oxidation is difficult due to the complexity of the chemical processes involved. In fact, many of the oxidation pathways are not entirely understood. Two types of lipid oxidation cause the most concern. These are oxidative rancidity and hydrolytic rancidity.

Hydrolytic Rancidity:
Hydrolytic rancidity results in the formation of free fatty acids and soaps (salts of free fatty acids) and is caused by either the reaction of lipid and water in the presence of a catalyst or by the action of lipase enzymes. Low levels of free fatty acids are not necessarily objectionable, particularly if they are sixteen or eighteen carbon fatty acids as commonly found in soybeans, corn or animal fat. However, for other fats like coconut oil or butter fat, low levels of shorter carbon chain fatty acids may be quite objectionable.

Oxidative Rancidity:
Oxidative rancidity results from more complex lipid oxidation processes. The processes are generally considered to occur in three phases: an initiation or induction phase, a propagation phase, and a termination phase. In complex systems, the products of each of these phases will increase and decrease over time, making it difficult to quantitatively measure lipid oxidation. During the initiation phase, molecular oxygen combines with unsaturated fatty acids to produce hydroperoxides and free radicals, both of which are very

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reactive. For this phase to occur at any meaningful rate, some type of oxidative initiators must also be present, such as chemical oxidizers, transition metals (i.e., iron or copper), or enzymes (i.e., lipoxygenases). Heat and light also increase the rate of this and other phases of lipid oxidation. The reactive products of this initiation phase will, in turn, react with additional lipid molecules to form other reactive chemical species. The propagation of further oxidation by lipid oxidation products gives rise to the term "auto-oxidation" that is often used to refer to this process. In the final, termination phase of lipid oxidation, relatively unreactive compounds are formed including hydrocarbons, aldehydes, and ketones. A variety of tests for lipid oxidation have been developed since these mechanisms were first proposed in the mid-1940's. These tests measure either:

- products of hydrolytic rancidity,
- products of the initiation and propagation phases,
- products of the termination phase, or
- depletion of oxygen or substrate.

I Used the Same Formula, Why Did This Batch Go Rancid?

Lipid oxidation moves through each of these three phases as a product ages and a number of factors can influence the rate of lipid oxidation in a product. These include:

- the initial quality of the fat or oil used for manufacturing the product,
- conditions used to manufacture the product,
- storage conditions (heat, light, packaging),
- surface area exposed to atmospheric oxygen,
- presence of transition metals,
- concentration of active lipoxygenases,
- application of appropriate of synthetic or natural preservatives,
- presence of chemical oxidizers.

Early in the lipid oxidation process, peroxides and hydroperoxides are the predominate reaction products. These reaction products continue to increase until a) storage conditions change, b) one or more initiators is depleted, c) available oxygen is consumed, or d) the lipid substrate is exhausted. Increased peroxide and hydroperoxide concentrations will initiate a series of reactions that eventually lead to increasing concentrations of aldehydes, ketones, hydrocarbons, and other termination phase products. Because many compounds produced during the termination phase are volatile, their concentration in the product may also begin to decrease over time. The rate of decrease varies with storage conditions, packaging, and fat content. The consequence of all of these changing concentrations is that any attempt to evaluate the rancidity of a product will likely be taking aim at a moving target. Peroxide values could be low because minimal oxidation has occurred or because peroxide concentrations have begun to decrease. Low aldehyde concentrations may be the result of limited oxidation or the aldehydes may have volatilized. It is generally not possible to predict the best indicator of lipid oxidation and any attempt to characterize rancidity of a product will likely require multiple tests. Appropriate control samples (freshly manufactured or other non-rancid product) are also helpful when historical values are unavailable. And finally, while a variety of chemical tests can objectively quantify various lipid oxidation products, subjective sensory evaluations may be the key to understanding the data. Ultimately, correlation to sensory testing is the basis for determining which chemical tests are appropriate for measuring lipid oxidation in any product.

Lipid Oxidation Tests

Tests for lipid oxidation are either predictive tests or indicator tests. Predictive tests use accelerated conditions to measure the stability of a fat or finished product. These tests may be used to determine ingredient quality, measure effectiveness of preservatives, or estimate product shelf life. Indicator tests are intended to quantify product or ingredient rancidity. Some of the more commonly used tests are described briefly in the following paragraphs.

Predictive Tests

AOM (Active Oxygen Method):
This method predicts the stability of a fat by bubbling air through a solution of the fat using specific conditions of flow rate, temperature, and concentration. At intervals, peroxides and hydroperoxides produced by this treatment are determined by titration with iodine. The AOM value is defined as the number of hours required for the peroxide concentration to reach 100 meq/kg of fat. The more stable the fat, the longer it will take to reach that level. For products other than fats and oils, the fats must first be gently extracted with solvents. The method is very time consuming since a stable fat may require 48 hours or more before reaching the required peroxide concentration. While still used today, the AOM method is being supplanted by

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faster automated techniques. The method is included in compendia published by AOAC, AACC, and AOCS.

OSI (Oxidative Stability Index):
The method is similar in principle to the AOM method, but it is faster and more automated. Air is passed through a sample held at constant temperature. After the air passes through the sample, it is bubbled through a reservoir of deionized water. Volatile acids produced by lipid oxidation are dissolved in the water increasing its conductivity. Conductivity of the water is monitored continuously and the OSI value is defined as the hours required for the rate of conductivity change to reach a predetermined value. Multiple samples can be tested simultaneously and software controls instrument parameters and data collection. The method has been collaboratively studied and accepted by AOCS.

Iodine Number:
While not a specific measure of fat stability, iodine number measures can indicate the potential of a fat to be oxidized. The method measures the reaction of iodine with double bonds of unsaturated fatty acids. Fats with a greater number of double bonds provide more sites for oxidation. Because other factors can influence fat stability, iodine number is not useful by itself for predicting fat stability.

Oxygen Bomb Test:
This method is used to predict stability and evaluate antioxidant systems in fats and finished products. Oxygen uptake of the sample is measured in a closed system. The rate at which oxygen is consumed indicates the oxidative stability of the tested product. An advantage of this technique is its ability to measure stability of the complete product without prior extraction of the fat. Because other components of a product, like transition metals or chemical oxidants, can promote oxidation, extracted fat may not be a suitable predictor of product stability.

Oxidation Indicator Tests

Peroxide value:
One of the most widely used tests for oxidative rancidity, peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with iodide ion. Peroxide values are not static and care must be taken in handling and testing samples. It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations.

TBA test:
Saturated aldehydes, 2-enals, and 2-dienals, produced in the termination phase of lipid oxidation, can be detected by reaction with 2-thiobarbituric acid. The reaction produces a red color which can be measured using a spectrophotometer. While originally developed to detect malonaldehyde, TBA has been shown to react with other aldehydes, as well as possible interfering substances like phenols in smoke flavors. As with peroxide value, a low TBA value is not an absolute indicator of fat quality. Aldehydes may have not yet formed or volatile aldehydes may have been lost during processing and storage.

Anisidine value:
When hydroperoxides break down, they produce volatile aldehydes like hexanal, leaving behind a non-volatile portion of the fatty acid that remains a part of the glyceride molecule. This non-volatile reaction product can be measured by reaction with anisidine. High anisidine values may be an indication that a fat has been oxidized even when TBA and other aldehyde tests give low results because volatile aldehydes may incidentally or intentionally be removed during processing. Anisidine value is defined as 100 times the absorbance (at 350 nm) of a solution resulting from reaction of 1 g of fat in 100 mL of solvent.

Hexanal value:
Hexanal, produced during the termination phase of lipid oxidation, can be measured by gas chromatographic analysis of the headspace over a sample. Methods vary, but generally a portion of sample is moderately heated in a sealed septum bottle. A gas syringe is used to withdraw a small portion of the headspace over the sample. The headspace sample is then injected onto a GC column to separate hexanal from other volatile components. Hexanal concentrations can vary widely depending on a number of factors including sample history, fat content, and fat composition. Generally, data is needed on a variety of samples of the same product to establish a correlation between hexanal concentration and product quality. Once that correlation is established, hexanal measurement can be a rapid and useful tool for lipid oxidation measurement.

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Headspace Profile:
Using techniques similar to those used for hexanal analysis, it is possible to measure the total volatile profile of the headspace over a product. Lipid oxidation produces a variety of volatile compounds including hydrocarbons, aldehydes, enals, dienals, ketones, and organic acids. As oxidation increases, the total of these volatiles tends to increase and can be measured by injecting a portion of the headspace into a gas chromatograph. Volatile flavorings may interfere and correlations of headspace values to samples of known quality are important.

Free Fatty Acids (FFA):
Free acids in a fat (or fat extracted from a sample) can be determined by titration. The FFA value is then expressed as % of a fatty acid common to the product being tested. Frequently, values are expressed as % oleic acid for tallows or soybean oils. For coconut oils or other oils that contain high levels of shorter chain fatty acids, FFA may be expressed as % lauric acid. FFA is an indication of hydrolytic rancidity, but other lipid oxidation processes can also produce acids. It may also be useful to know the composition of the free fatty acids present in a sample to identify their source and understand the cause of their formation. Extracts of samples can be analyzed for free fatty acid profiles when this information is required.

Many other methods have been used for assessing fat and oil quality, some very successfully in specific industries. Because of the complexity of lipid oxidation, no single method can suffice. Method selection and data interpretation require careful collaboration between the food scientist and the laboratory.

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