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ORIGINAL CONTRIBUTION

Measurement of Degree of Rancidity: An Approach for Food Quality Evaluation

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ABSTRACT

Rancidity or development of repugnant flavors has been recognized as a major problem during storage of fat rich food products. Changes in lipid molecules usually result in the destruction of valuable nutrients with generation of various toxic compounds. Lipid oxidation can occur during extraction and refining of oil as well as during processing and storage of food products. The reaction results in the formation of lipid hydro-peroxides which rapidly break down into secondary products having strong off-flavor. Therefore, appropriate analytical tools are necessary for periodical monitoring of rancidity in order to predict food quality. Due to complexity of chemistry involved in the mechanism, the possibility of single universal test for proper evaluation of oxidative status of food lipids is challenging. A large number of methodologies have been developed and implemented for determination of both primary and secondary oxidation products including sensory analysis; chemical analysis by peroxide value, thiobarbituric acid reactive assay or various chromatographic techniques and instrumental methods utilizing fluorescence emission, Raman spectroscopy, infrared spectroscopy or nuclear magnetic resonance etc. Stability of food materials towards oxidation during storage is also an important aspect of quality evaluation determined by various accelerated tests.

KEYWORDS— oxidation, off-flavor, hydro-peroxides, analytical tools, stability

1. INTRODUCTION

Lipids play a major role in food system due to their contribution to palatability, satiety, nutrition, and textural quality. Therefore, lipid quality is significant to the consumers and may be directly associated with many health problems. Oxidation of unsaturated fatty acids in food lipids is of great concern for the food processors as it produces compounds that degrade wholesomeness of foods, develop unpleasant off-flavor, alter textural properties, adversely affect the nutritional value and ultimately lead to food spoilage. Oxidation of oil destroys essential fatty acids and produces toxic compounds and oxidized polymers. Many of the volatile lipid oxidation products are detectable by humans at the parts per million and even parts per billion thresholds. The development of off flavors in lipid is usually denoted by a general term 'rancidity'. However, the qualitative nature of rancid flavors varies significantly from product to product and even in the same food item. The rancidity may be developed in food items via different pathways: radical mechanism (known as autoxidation), singlet oxygen mediated mechanism (known as photooxidation) and also the enzymatic hydrolysis catalyzed by lipoxigenases. Susceptibility of lipids to oxidation increases with the degree of unsaturation of fatty acids due to increasingly lower bond dissociation energies of methylene interrupted carbons (1). Polyunsaturated fatty acids (PUFAs) are the essential fatty acids and generally considered as healthier for consumers. As per the latest Dietary Guidelines of WHO, EFSA and U.S. Dept. of Health and Human Services (2, 3, 4) the manufacturers reduce solid fat content of foods by simply substituting PUFAs for saturated fats resulting in increased

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oxidation and decreased food acceptability and shelf life.

Hydrolytic rancidity results from the release of free fatty acids from lipids. Since only the short chain fatty acids have unpleasant odors, the problem is typically encountered in milk and dairy products. Foods that are prone to oxidative rancidity include edible fats and oils used for frying, fat rich processed foods, fried foods like potato chips, roasted nuts, dried soups, broths and seasonings, dried meat, frozen fish and fish oil, dried milk, cakes, chewing gum, concentrated vitamin preparations, flavorings and essential oils. Several research works was carried out to evaluate lipid oxidation in various food samples. According to a research work the lipid oxidation in meat samples is dependent on haemoglobin content of the muscle (5). The oxidative status of various food samples like vegetable oils (6), olive oil (7), fish sausages (8) and milk samples in presence of catechins and ascorbic acid (9) were determined using various methods.

Evaluation of degree of rancidity is a challenging task because different compounds are formed in the food products depending on the composition, time, extent of oxidation and mechanism involved. Some products like volatile aldehydes are formed during later stages of reaction which can be detected only after a lot of oxidation has occurred while others like conjugated dienes are formed in large amounts early in the reaction but breakdown easily to immeasurable levels later in the process. Therefore, choosing just one parameter to analyze the oxidative status is rather difficult and hence, it is more convenient to combine different methods. With the continuous improvements of sensitivity and resolution in analytical instrumentation the detection of whole series of new compounds is made possible, and interpretations regarding flavor become more complex. This review will briefly describe the methods to determine both primary and secondary lipid oxidation products in foods by traditional titrimetric, spectroscopic and chromatographic techniques as well as latest instrumental methods with their characteristics, advantages and limitations. The procedures for evaluating the oxidative stability of lipids in food materials during storage are also reviewed hereunder.

2. MECHANISM OF LIPID DEGRADATION

2.1 Autoxidation

Autoxidation, the spontaneous reaction of atmospheric oxygen with lipids, is the most common process leading to deterioration of food lipids. Oxidation of unsaturated fatty acids proceeds through a free radical chain reaction mechanism classified into three distinct steps of initiation or formation of free radicals in presence of transition metal ions like copper and iron, propagation or free radical chain reaction and termination through formation of non radical compounds (1, 10, 11). A simplified schematic representation of the autoxidation mechanism is given below (12):

Initiation: $RH \longrightarrow R^{\bullet} + H^{\bullet}$

Propagation: $R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$

Termination: $R^{\bullet} + R^{\bullet} \longrightarrow RR$

$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$

Hydroperoxides (ROOH) are the main primary oxidation products of unsaturated lipids (RH), accumulating during the initiation and propagation step of the oxidation process. The

hydroperoxide content gradually increases with progression of lipid oxidation. After the maximum amount of hydroperoxide has been formed in the food, a drop in hydroperoxides level is noticed due to decomposition of hydroperoxides into a variety of secondary oxidation products (Fig. 1). The hydroperoxide breakdown starts with the loss of a hydroxy radical ($OH^{\bullet}$) to form a lipid alkoxy radical ($RO^{\bullet}$) which rearranges to produce various volatile and non volatile rancid-smelling secondary oxidation products like aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, epoxides, hydroxy compounds, oligomers and polymers (13).

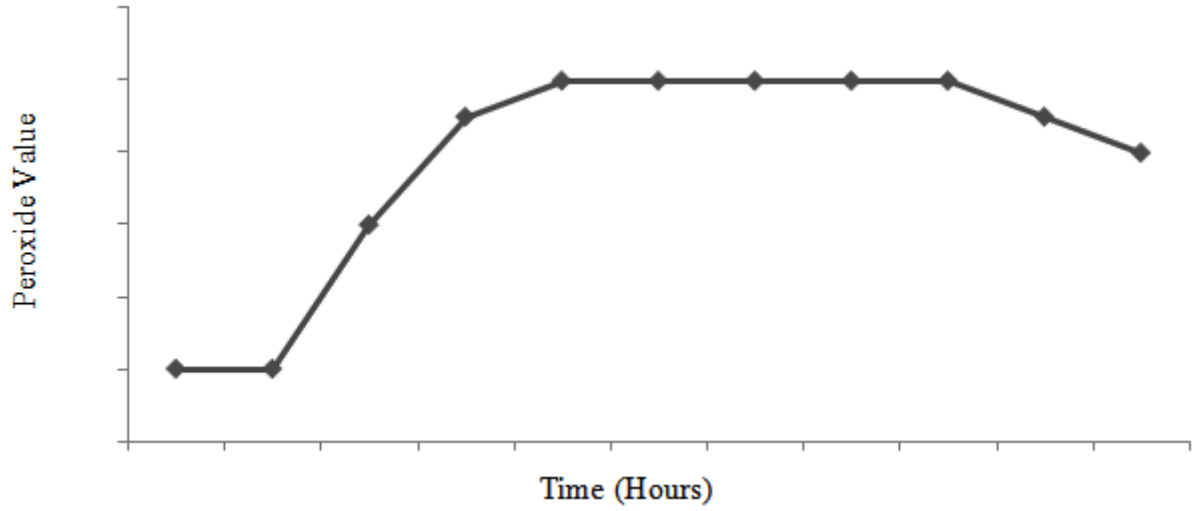

Figure 1: Peroxide formation and decomposition as a function of time

2.2 Photooxidation

Photooxidation is the stoichiometric oxidation of unsaturated fatty acids by singlet oxygen ($^1O_2$) to produce allylic hydroperoxides via addition of oxygen at either end of the double bond (14). The singlet oxygen is produced by excitation of triplet molecular oxygen ($^3O_2$), under exposure to light in presence of photosensitizers like riboflavin, chlorophyll, myoglobin, erythrosine, and heavy metal ions (15, 16).

2.3 Hydrolytic Reactions

In this reaction the fatty acids are released from triglyceride molecules as free fatty acids (17). These free fatty acids produce off flavour, reduce oxidative stability, cause foaming, reduce smoke point and ultimately render the food unacceptable. This hydrolysis reaction requires either high temperature, e.g., deep fat frying with water present or lipase enzyme activity. The reaction mechanism is represented below:

$$\begin{array}{l} CH_2COOR_1 \\ CHCOOR_2 \\ CH_2COOR_3 \end{array} \xrightarrow[\text{Lipase}]{\text{Hydrolysis}} \begin{array}{l} CH_2COOH \\ CHCOOH \\ CH_2COOH \end{array} + R_1COOH + R_2COOH + R_3COOH$$

3. METHODS FOR DETERMINATION OF DEGREE OF RANCIDITY

Lipid oxidation is a complex process involving numerous reactions resulting in various sensory, chemical and physical changes in food. Therefore, analysis of lipid oxidation products is difficult because of their complex nature, instability, presence of large quantities of interfering substances in foods, natural antioxidants and sometimes lack of specific and adequate analytical methods. Since oxidative decomposition is of major significance with respect to both the acceptability and nutritional quality of food products, many methods have been developed for evaluation of the oxidative status of food lipids. However, a single test is not sufficient to measure all oxidative products at the same time, at all stages of the oxidative process and for all foods. Therefore, combination of tests is needed to represent degree of rancidity in foods. Keeping these considerations in mind following commonly used methods is reviewed.

3.1 Organoleptic Tests

The value of any objective chemical or physical method is judged primarily on how well it correlates with results from sensory evaluation. Today the food manufacturers rely much on the trained food tasters for product quality control. Organoleptic studies play an important role in the assessment of flavor, taste and aroma of foods where the terms like rancid, oxidized, metallic, cardboard, grassy, fishy, unclean etc. are used to indicate the characteristic off-flavor note. The complete, detailed and accurate descriptive representation of the sensory profile of a product involves the detection and description of both qualitative and quantitative sensory aspects of the product by trained taste panels using highly specific universally accepted scales.

3.2 Chemical Tests

3.2.1 Acid Value: Acid value represents the degree of hydrolytic rancidity in foods and is the measure of free fatty acids present in a sample of fat or oil. It is defined as the number of milligrams of alkali required to neutralize the free fatty acids present in 1 gm of fat. Acid value is determined by direct titration of the sample in alcoholic medium against standard alkali solution using phenolphthalein indicator (18) and calculated using the formula:

$$\text{Acid Value} = \frac{56.1 \times V \times N}{W}$$

Where, V is the titer value, N is normality of alkali solution and W is the weight of sample taken.

3.2.2 Peroxide Value: Peroxide Value measures the total amount of hydroperoxide and peroxide oxygen content of lipid containing food materials. The peroxide value is applicable for determination of rancidity at the early stages of oxidation. During the course of oxidation, peroxide values reach a peak and then decline which is the function of temperature and presence of other food components. As per Codex Alimentarius guidelines, a level of 10 meq/ kg of peroxides are acceptable in fats and oils for edible purpose.

a) Iodometric Titration Method: In this method the hydroperoxides and peroxides oxidize aqueous potassium iodide solution to liberate iodine which is then titrated with standard sodium thiosulfate solution using starch indicator (19). The reactions and stoichiometries for this method are:

$$ROOH + 2KI + H^+ \longrightarrow I_2 + ROH + K_2O$$

$$ROOR + 2KI + H_2O \longrightarrow I_2 + 2ROH + K_2O$$

$$I_2\,(\text{purple}) + 2Na_2S_2O3 \longrightarrow Na_2S_4O_6 + 2NaI\,(\text{colorless})$$

Where ROOH is a lipid hydroperoxide and ROOR is lipid peroxide. The peroxide value is then calculated as milli-equivalents of oxygen per kilogram of sample using the following formula:

$$\text{Peroxide Value} = \frac{(V - B) \times N \times 100}{W}$$

Where, V is the volume of sodium thiosulphate required for sample, B is the volume of sodium thiosulphate required for blank; N is the normality of sodium thiosulphate solution and W is the weight of the sample taken.

The limitations involving in this procedure are poor sensitivity and selectivity, possible addition of iodine into unsaturated bonds leading to low results, oxidation of iodide by dissolved oxygen and variations in reactivity of different peroxides.

b) Ferric Ion Complexes: This method is based on spectrophotometric measurement of the ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions. The resulting ferric ions are treated with either thiocyanate or xylenol orange to develop colored complex. Ferric thiocyanate is a red-violet complex that shows strong absorption at 500 – 510 nm whereas; xylenol orange forms a blue-purple complex with a maximum absorption at 550 – 600 nm (20). This colorimetric method of peroxide value determination is simple, rapid, reproducible, insensitive to ambient oxygen or light and more sensitive than the standard iodometric assay, and has been used to measure lipid oxidation in various food products including milk, fats and oils.

3.2.3 Determination of Conjugated Dienes/Trienes: Hydroperoxides formed during oxidation of polyunsaturated fatty acids are generally stabilized with the formation conjugated dienes and trienes through double-bond rearrangement. These relatively stable compounds can be measured quantitatively by spectrophotometric UV measurement at wavelength 235 nm and 270 nm respectively to assess oxidation level (13, 21, 22). This technique is simple and rapid which does not depend on any chemical reaction or color development and requires relatively small amounts of sample (11). The amount of conjugated diene or triene is determined by simply diluting about 0.1 gm sample in iso-octane and measuring the absorbance directly using spectrophotometer. The value based on the detected absorbance is expressed as µmol hydroperoxides /g sample.

It is a sensitive method used during early stages of the oxidation process. Limitations of the method are overlapping of secondary oxidation products in the same UV detection range, possibility of overestimation if conjugated double bonds are already present in original fatty acid, strong dependence on fatty acid composition of the sample, incompatibility of evaluating samples heated under conditions that decompose hydroperoxides etc.

3.2.4 Thiobarbituric Acid Reactive Substances (TBARS) Assay: This is the oldest and one of the most widely used methods for evaluating the extent of lipid oxidation. The method is based on the formation of a pink colored complex with strong absorbance at 532-535nm when secondary oxidation products from unsaturated fatty acids react with TBA. The major oxidation product that reacts with TBA is malonaldehyde (MDA), one mole of which reacts with 2 moles of TBA. The measurement procedure usually consists of homogenization and centrifugation of the sample with trichloroacetic acid followed by heating at high temperature of around 90-100ºC where the monoenolic form of MDA attack the active methylene groups of TBA to produce the colored complex chromophore (13). Other TBA-reacting substances are alkanals, 2- alkenals, 2, 4-alkdienals, ketones etc. The measurement of secondary oxidation products by the TBARS method is usually expressed as µmoles TBARS / g of sample. The potential limitations of TBRS assay are lack of sensitivity and specificity; variation in color development depending upon temperature, time of heating, pH, presence of antioxidants and metal ions; reaction of non

oxidation products like sucrose urea, pyridines, pyrimidines etc. with TBA to give higher values; poor results when malonaldehyde reacts with proteins in an oxidizing system etc. The TBA test is very useful for comparing samples of a single material at different stages of oxidation.

To overcome the shortcomings of conventional TBRS assay, more advanced chromatographic determinations have been developed with more accuracy, sensitivity and specificity. These methods involve formation of MDA-TBA complex, purification by gas chromatography or HPLC and subsequent detection by UV-Vis, mass spectroscopy or fluorometric detector. Sometimes reaction with 2, 4-dinitro-phenylhydrazine (DNPH) or penta fluoro phenyl hydrazine to convert into pyrazole and hydrazone derivatives with HPLC separation and spectrophotometric/fluorometric detection are also used (23, 24).

3.2.5 Kries Test: This was one of the first tests used commercially to evaluate oxidation of fats. The procedure involves measurement of a red color resulting from the reaction with phloroglucinol (Kries reagent). The test suffers from deficiencies like development of some color by fresh samples free of oxidized flavor upon reaction with the Kries reagent, and inconsistent results among different laboratories.

3.2.6 Anisidine value: It is the measure of secondary oxidation products which are non-volatile α-unsaturated aldehydes like 2-alkenals and 2, 4-alkadienals. The method is based on the reactivity of the aldehyde carbonyl bond on p-anisidine amine group in presence of acetic acid to produce yellow colored Schiff base with absorbance at 350 nm. The Anisidine value is expressed as 100 times the absorbance of a solution made of 1 gram fat in 100mL isooctane solvent and p-anisidine reagent (11, 25). The reaction does not use any strong acids or high temperature and therefore the chances of hydroperoxide decomposition are minimized.

The main limitations of the method are low sensitivity, requirement of water free reagents due to incomplete reaction in presence of water, use of carbonyl free reagents to avoid interference with existing carbonyls in the sample, difficulties in interpretation of results if the aldehyde contains double bond etc.

3.2.7 TOTOX Value: For proper expression of lipid oxidation, a combined representation of primary and secondary oxidation products has been suggested by many researchers. TOTOX value measures both hydroperoxides and their breakdown products, and provides a better estimation of the oxidative status of fats and oils. TOTOX value i.e. total oxidation is equivalent to 2 × peroxide value + anisidine value. However, it has no scientific basis because it is a combination of two indicators with different dimensions. Since determination of anisidine value may not be always feasible, it can also be represented as 2PV + TBA (26).

3.2.8 Measurement of carbonyl Compounds: A wide range of carbonyl compounds having different functional groups: aldehydes, ketones, alcohols, short carboxylic acids and hydrocarbons are formed as secondary oxidation product during storage of food materials. Measurement of these compounds is important, since they contribute to the development of off-flavors. Methods for determining total carbonyl compounds are usually based on measurement of hydrazones that arise from reaction of aldehydes or ketones with 2, 4– dinitrophenylhydrazine. However, under the experimental conditions used for these tests, carbonyl compounds may be generated by decomposition of unstable intermediates, such as hydroperoxides, thus detracting from accuracy of the results. Attempts to minimize such interference have involved reduction of hydroperoxides to noncarbonyl compounds prior to determination of carbonyls, or conducting the reaction at a low temperature.

3.2.9 Oxirane Test: This method is a measure of epoxide content, which is based on the addition of hydrogen halides to the oxirane group. Epoxide content is determined by dissolving the sample in aqueous acetic acid in the presence of crystal violet, and titrating with hydro bromic

acid to a bluish green endpoint (27). The test suffers from the limitations like poor sensitivity, lack of specificity, and inappropriate result with some trans-epoxides. A colorimetric method based on the reaction of the oxirane group with picric acid is more sensitive than the oxirane test for measurement of epoxides.

3.3 Chromatographic Techniques

The main volatile compounds generated in the lipid oxidation process are various aldehydes, alcohols, ketones, furanones and lactones having characteristic off-flavor. Chromatographic techniques are more accurate, sensible and specific for a particular compound resulting in better identification of individual products of oxidation. This approach is based on the separation and quantitative measurement of specific fractions such as volatile, polar, or polymeric compounds or individual components typically produced during autoxidation. Various chromatographic techniques including liquid, thin-layer, size exclusion, high performance liquid chromatography (HPLC), direct gas chromatography (DGC), gas chromatography coupled to mass spectrometry (GC-MS), gas chromatography with flame ionization detector (GC-FID) etc. have been used to monitor lipid oxidation in food products.

3.4 Advanced Analytical Methods

The chemical methods are highly dependent on several experimental factors including personal skill, exposure to light and atmospheric oxygen as well as time-consuming. In order to overcome these limitations, direct spectroscopic analysis of samples based on magnetic resonance, fluorescence and vibrational spectroscopy, chemiluminescent properties etc. have been developed for analysis of both primary and secondary lipid oxidation products. These methods are advantageous as preliminary sample preparation is minimal or unnecessary, requirement of low amount of sample and accuracy of results. Table 1 summarizes various aspects of the advanced analytical techniques.

Table1: Summary of advanced analytical techniques for detection of lipid oxidation

Methodologies	Principle involved	Components	Applications
Chemiluminescence	Measurement of electromagnetic radiation emission generated from certain chemical reaction.	Hydroperoxides	Vegetable oils
Fluorescence spectroscopy	Free amino groups of proteins and aldehydes from lipid oxidation reacts to produce Schiff bases with characteristic fluorescence spectra	Aldehydes	Dairy products, meat, fish, oils
Infrared spectroscopy	Absorption of discrete energy levels from the IR region which are characteristic of atom-atom linkage of particular compound.	Hydroperoxides	Edible oils, horse mackerel patties, canned tomato juice
Raman spectroscopy	Detection of fundamental vibrational transitions by means of energy scattering originated from a UV, visible or IR laser.	TBRS particularly Malonaldehyde	Edible oils, meat and fish products
Nuclear Magnetic Resonance (NMR)	Hydrogen atoms in a strong magnetic field absorb energy in the radiofrequency range,	Hydroperoxides, carbonyl compounds and	Different oils

Spectroscopy	depending on their molecular structure	dienes	
Electron Paramagnetic Resonance (EPR) Spectroscopy	Measurement of the changes in energy levels of unpaired electrons when they are subjected to magnetic fields, typically from microwave radiation	Free radicals	Low moisture foods, cheese, milk powder

With the improvements in sensor technologies and software, the concept of electronic nose has gained popularity for assessment of sensory quality of oils and fat-containing foods. These devices consist of an array of electrochemical sensors of diverse specificity and equipped with an identification tool for differentiating simple or complex flavors (28, 29).

4. ACCELERATED STABILITY TESTS

To determine oxidative stability or resistance of lipids during processing and storage, the food samples are subjected to accelerated stability tests to initiate the changes that undergo in stored food. In these methods the food products are subjected to harsh conditions of temperature, light, UV radiation, pressure and oxygen to determine its stability during storage. Such testing parameters are highly relevant as the fats and oils are often subjected to such conditions during processing, food manufacture or domestic use. The results of these accelerated tests are usually expressed as an 'induction time' which is the time necessary to attain the critical point of oxidation either by sensorial change or a sudden acceleration of the oxidative process. The most popular methods are Schaal oven test, active oxygen method, Rancimat, oxygen uptake, ASTM oxygen bomb, use of light or metal catalysts etc. ion of the shelf-life (11). The main drawbacks of such stability tests are different mechanisms of oxidation at high temperature, rapid decomposition of hydroperoxides, side reactions like polymerization and cleavages, oxygen concentration and the end point of measurement is questionable (29).

4.1 Schaal Oven Test

In this method, the food sample is stored at about 60 - 70°C at atmospheric pressure and periodically examined for development of rancidity either by organoleptically or by measuring the peroxide value (11). This test can best correlate the oxidative changes with evaluation of the shelf-life.

4.2 Active Oxygen Method (AOM)

The most widely used test for estimating the oxidative stability of food materials during storage is the active oxygen method. The food sample is maintained at 98°C with continuous incorporation of air bubbles through it at constant rate. The time required to obtain a specific peroxide value is then measured to predict the stability of food towards oxidation.

4.3 Rancimat Method

The rancimat method is based on automatic determination of the volatile degradation products by measuring the change in electrical conductivity of the sample. Dry air is bubbled through hot oil at about 100°C to accelerate oxidation process. The volatile components formed are trapped in deionized water and increase in conductivity is measured. The graph obtained by plotting conductivity against time shows the progress of oxidation. The induction time is determined graphically after completion of the experiment as the time required to reach an end point of oxidation corresponding to either a level of detectable rancidity or a sudden change in the rate of oxidation (30).

Advantages of the Rancimat technique are continuous process, labor saving, and no requirement of supervision during experiment. The oxidative stability of commercially available infant formula in Tehran, Iran was determined using rancimat method at temperatures of 60, 80, 90, 100, 110, 120 and 130°C and the shelf life of the commercial infant formula studied was predicted to be approximately 18 months avoiding the time-consuming long-term studies (30).

4.4 Oxygen Absorption

The food sample is placed in a closed chamber and the amount of oxygen absorbed is determined to measure the stability. This is done by either measuring the time to produce a specific pressure decline, or by the time to absorb a pre established quantity of oxygen under specific oxidizing conditions. This test is very useful in the studies of antioxidant activity.

5. CONCLUSION

Different types and amounts of reaction products are formed by lipid oxidation depending upon innumerable factors. Even a single fatty acid may break down to form over a dozen significant products and complex food system may produce many hundreds. Therefore, it is really problematic to develop proper analytical tool as there is no single product to measure and losses of starting material before the product is obviously rancid. A wide range of tests from simple organoleptic evaluation to complex chemical and instrumental methods are routinely used for measuring the degree of rancidity in foods. However, there is no uniform and standard method for detecting all components responsible for rancidity in all food systems. Therefore, it is necessary to select simple, rapid and precise method for any particular application. The diversity and abundance of methods used to monitor lipid oxidation signify the complexity and significance of the matter and confirm the fact that multiple methods should be applied to get the maximum possible information regarding rancidity in foods.

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