Review Article

Update on the Methods for Monitoring UFA Oxidation in Food Products

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**Abbreviations: UFA**, Unsaturated-fatty-acid, **ROS**, reactive oxygen species, **OSI**, Oil Stability Index, **OBM**, oxygen bomb method, **PV**, peroxide value, **CD**, conjugated diene, **CT**, conjugated triene, **DB**, double bond, ***p*-AnV**, *p*-anisidine value, **TBARS**, thiobarbituric acid reactive substance, **TBA**, 2-thiobarbituric acid

Abstract

Unsaturated fatty acids (UFAs) as well as natural food ingredients containing them are being added to food products more often. Unfortunately, UFAs are susceptible to oxidative degradation, and can therefore reduce the stability of a food matrix. The assessment of oxidation and stability of lipids has never been standardized, and has long included a variety of approaches. These techniques can differ greatly in the reactions/compounds being assessed, and therefore the eventual conclusions may be affected dramatically by selection of methodology. The aim of this review is to provide an update on the methods of historic and current use for the assessment of lipid stability in food products. Accelerated storage tests, assessment methods of lipid oxidation, and rapid indicators of stability are discussed in the contexts of their modern prominences, uses, and concerns.

**1. Introduction**

Unsaturated fatty acids (UFAs) as well as natural food components containing significant quantities of UFAs (*e.g.* whole grains) are being added to food products more often. Unfortunately, UFAs are unstable compounds and their decomposition can negatively impact food quality as well as the nutrition and health status of consumers. UFAs such as linoleic (C18:2, ω6) and α-linolenic (C18:3, ω3) acids are prominent in vegetable oils, whole grains, nuts and seeds, and are essential UFAs for human growth and development. Oxidation of such UFAs can result in the reduced uptake of these important nutrients as well as the release of ‘rancid’ volatile compounds that can render foods undesirable to consumers and lead to an increase in food waste.

A fairly sophisticated understanding of many mechanisms of UFA oxidation has been known for many decades; however, the prediction and mitigation of these reactions within systems continues to be an ongoing challenge. There exist a large number of confounding variables that can affect UFA oxidation in bulk oil form, and even more so in food systems. Many contributing factors to UFA oxidation have been cited including: fatty acid composition (degree of unsaturation), presence of free fatty acids (FFAs), and the concentrations of pro-oxidants and antioxidants (natural or added), to name a few [1, 2]. The interaction of these factors, as well as matrix effects, is still not well understood on a quantitative level [3]. Even when examining a single factor within controlled systems, the effect of the factor may vary greatly according to its concentration and the system it is within. As an example, higher antioxidant concentrations in bulk oils have been shown to not always directly correlate with a higher oxidative stability of the lipids containing them [4]. In the case of emulsions, studies on UFA oxidation must also take into account the heterogeneous physical properties inherent to the lipids analyzed and the role of these micro-environments upon their oxidative stability [5], among other concerns. This inherent complexity to UFA oxidation renders the means by which to accurately monitor UFA degradation, and the strategies developed for their stabilization, indeed a challenging exercise.

Another complicating factor in the understanding of oxidative stability is the wide array of means by which oxidation and stability are assessed and defined. The various methods and assays in these determinations can differ greatly – not only in methodology, but also in the compounds and reactions being assessed. Thus, it is highly possible the results and conclusions of a study may be just as thoroughly affected by method-selection as by the true stabilities of the assessed samples. It is therefore important to choose methods and techniques that are compatible with both the product and the objectives of the study. For example, studies focused on the sensorial shelf-life of a particular product will typically require different assessments of stability than those focused exclusively on the nature of degradation reactions within laboratory emulsions.

In the continued absence of reliable means to accurately predict oxidative stability according to composition, the most dependable techniques to ascertain product stability continue to involve straining the product, monitoring the evolution of its oxidative state, and effectively summarizing the output. The most historically classical methods to monitor current oxidative state (peroxide value, conjugated dienes and trienes, *p-*anisidine, and 2-thiobarbituric acid reactive substances) have been in use for more than six decades. These assays are not comprehensive measures of oxidation, but are considered still to be of merit for their simplicity, minimal equipment requirements, and documented associations with sensory scores. Their use also allows for easy comparison to the wealth of published studies that have acquired data by comparable means. The employment of these assays continues to be common in modern science.

Advances in machinery and methodologies have given rise to additional methods to determine oxidation and oxidative stability – including those using chromatography, spectroscopy, or acceleration chambers for rapid assessment.

The aim of this review is to provide an update on the methods that are of considerable current use for the monitoring of UFA decomposition in food products. The classical assays are reviewed in some detail, and the evolution of their respective uses over time is examined. The emergence of more novel approaches is also discussed. The design of accelerated storage tests, which are required for the successful implementation of many oxidation studies, will also be evaluated herein to put some current challenges in perspective. Rapid measures of stability are also reviewed – including discussion of their modern presence in scientific literature, and their established benefits and concerns. A brief review of the most predominant mechanisms of UFA oxidation has been included to put the methods of assessment into context.

**2. Mechanisms and outcomes of UFA oxidation**

The term lipid oxidation is a general classification for chemical interactions occurring between reactive oxygen species (ROS) and unsaturated fatty acyl groups, and can include a large variety of specific chemical reactions and products [6]. The process of lipid oxidation in refined edible fats and oils is generally viewed as consisting of the two subcategories of autoxidation and photooxidation [1]. The distinction between autoxidation and photooxidation lies in the dissimilarity of the environmental variables required for their occurrence, as well as the different possible electron orbital states of molecular oxygen that are present in the two different reaction mechanisms [7]. These different orbital states of molecular oxygen are defined as triplet oxygen (3O2) and singlet oxygen (1O2), respectively.

The formation of singlet oxygen from triplet oxygen can occur due to chemical processes, enzymatic actions, gaseous discharges, and the decomposition of hydroperoxides [8]. In food systems, the majority of singlet oxygen is formed as a result of the interaction of light, photosensitizers, and triplet oxygen [9]. The reaction of unsaturated fatty acids with singlet oxygen is autocatalytic and can proceed at a rapid rate. **Figure 1** depicts a comparison of the relative reaction rates of UFAs with both triplet and singlet oxygen. As shown in **Figure 1;** reaction rates with singlet oxygen are often faster [5, 10]. This fact, however, is counterbalanced in the food industry by packaging, storage conditions, and also the nature of oil production. For foodstuffs that incorporate refined edible oil, the refinement process typically removes compounds capable of acting as photosensitizers. For all food products, the exposure of sensitive lipids to ultraviolet/visible electromagnetic radiation can be retarded by proper packaging materials and storage. This results in a minimization of the likelihood for photosensitized oxidation being a contributing factor in the oxidative degradation of commercially-refined edible fats and oils [11, 12].

Autoxidation does not depend on the formation of singlet oxygen, and instead begins with the conversion of a fatty acid or acylglycerol into a radical state *via* the removal of a hydrogen atom. Autoxidation therefore is of significantly greater concern to the food industry [1]. This initial transition of fatty acid into alky radical is known as “initiation”, and represents the first step in the sequence of reactions of autoxidation. This sequence is generally characterized as follows [13, 14]:

|  |  |
| --- | --- |
| **Initiation** | The formation of free-radical species. |
| **Propagation** | The chain reactions involving the free-radical species. |
| **Termination** | The formation of non-radical products. |

The chemical reactions of these stages can then be summarized as follows (R represents a lipid alkyl chain) [1]:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Initiation** | RH | 🡪 | R• + H• | (1) |
| **Propagation** | R• + 3O2 | 🡪 | ROO• | (2) |
|  | ROO• + R′H | 🡪 | ROOH + R′• | (3) |
|  | RO• + R′OOH | 🡪 | ROO• + ROH | (4) |
|  | RO• + R′H | 🡪 | ROH + R′• | (5) |
| **Termination** | ROO• + R′• | 🡪 | ROOR′ | (6) |
|  | R• + R′• | 🡪 | RR′ | (7) |
|  | RO• + R′O• | 🡪 | ROOR′ | (8) |
|  | RO• + R• | 🡪 | ROR′ | (9) |

This initial conversion of singlet state lipid RH into the free radical R• in reaction (1) is fundamental to the occurrence of autoxidation, and its mechanism is still not fully understood [7]. This reaction can theoretically occur in an autocatalytic manner, but much work has been done to determine factors capable of either triggering or promoting the degradation reaction [6, 15]. Throughout a great number of studies, initiation has been associated with the action of heat, light, certain enzymatic reactions, acidity, ozone, nitrogen dioxide, pressure, and others [16, 17, 18, 19, 20, 21]. The presence of metal catalysts has received much attention as a possible enacting agent of initiation of autoxidation, which may occur *via* the mechanism RH + M3+ $\rightarrow $ R• + H+ + M2+ [7].

There has also been a longstanding attribution of the initiation reaction (1) to the presence of hydroperoxides, which are therefore at once both a primary product of oxidation as well as a compound often considered centrally responsible for radical generation [18]. The role of hydroperoxides as positively affecting initiation rates has been supported by studies, and it provides a possible explanation for the exponential increases of initiation rate that is often observed over time [3, 22]. There does exist, however, a minority opinion which challenges the belief that hydroperoxides are centrally responsible for autocatalytic radical generation [23, 24]. Morita and Tokita [18] investigated the question experimentally and concluded that the main-product hydroperoxide has little autocatalytic radical-generating activity at room or body temperature.

As a result of the initiation reaction (1), alkyl radicals are formed on the unsaturated lipid molecule. The free-radical electron present on the alkyl group then delocalizes over the double bond system and results in a double bond shift. In the case of polyunsaturated fatty acids (PUFAs), this shift can also cause the formation of conjugated double bonds (meaning the double bonds are not interrupted by a methylene moiety). An example of this process occurring on linoleic acid is depicted in **Figure 2**; note the different end locations of the electrons that can result from the described delocalization. The relative occurrences of radical electron location resulting from the autoxidation of oleic acid, linoleic acid, and linolenic acid are shown in **Table 1**.

Following free-radical mediated initiation (Eq. 1) of autoxidation, the oxidation pathways and mechanisms of subsequent reactions are well characterized in the literature. A radical propagation reaction (2) follows in which triplet oxygen reacts with the alkyl group generated in intiation to form a highly reactive peroxy radical (ROO•). This peroxy radical then in turn abstracts a hydrogen atom from another unsaturated fatty acid and produces a hydroperoxide and a new alkyl radical as shown in reaction (3) [3]. The formation of a hydroperoxide from the peroxy radical is illustrated in **Figure 3**. These hydroperoxides are classified as “initial” or “primary” oxidation products and often serve as a crucial indicator of the current oxidative state of oils and fats. The accumulation of hydroperoxides over time can also be monitored in order to determine the oxidative stability of lipids. It is important to note that hydroperoxides are not sensorially active. It is only once they undergo decomposition into smaller aromatic compounds and associated fragments that they are perceived and thus can compromise product quality.

The most likely decomposition pathway of hydroperoxides is that of homolytic cleavage between the two bound oxygen atoms (ROOH 🡪 RO• + •OH), resulting in an alkoxyl radical and a hydroxyl radical [7]. This alkoxyl radical is then cleaved *via* *β*-scission of a carbon-carbon bond that can occur on either side of the bound oxygen. The potential products of these reactions (known as “secondary” products) are numerous, and their relative formations are dependent upon the initial reactants, additional reacting compounds, and the specific atomic locations of the steps of decomposition and cleavage [3, 25]. **Figure 4** illustrates the decomposition of a hydroperoxide molecule. **Table 2** lists the many potential decomposition products resulting from the *β*-scission reaction outlined in **Figure 4**.

A large proportion of these secondary products will be volatile and capable of contributing off-flavors to oils and fats [7], and the accumulation of these volatile compounds often serves as a limiting factor in the shelf-life of lipids [1]. In assessing the potential impact of lipid oxidation products upon sensory quality, it is often useful to note both the perceived flavor associated with the end products as well as their sensory threshold values. It is important to recognize that the most abundant oxidation products of a given lipid will not necessarily be that which are of the greatest concern in regards to lipid quality. There can exist great differences in the partitioning of different volatile lipid decomposition products into the headspace of a food product, as well as differences in the relative tendency of those compounds to be perceived by the consumer. Further, different compounds can impart different off-odors and flavors that can affect the lipid’s perceived quality. Sensory threshold values for many common end-products of lipid oxidation are summarized in **Table 3,** but one should note that these threshold values can vary depending on the matrices and competing volatiles of a food substance [26]. Some common sensory descriptors attributed to oxidized lipids, and their characteristic volatiles, are given in **Table 4** [27]. Due to their frequently high concentrations and their relatively low threshold values, aliphatic carbonyl compounds are generally considered to have the greatest influence on oxidized oil flavor [1].

**3. Storage testing to monitor UFA oxidation**

Oxidative stability is not an intrinsic trait of food products, but rather, it is the sum total of the effects of all relevant factors intrinsic and extrinsic to that product throughout its life-cycle [3]. Thus, evaluation of the oxidative stability of UFAs in foods requires the measurement of UFA degradation over time. Methods designed to determine UFA oxidation within a timeframe may do so according to two very different approaches. One approach is that of a rapid test, which involves exposing oil or fat samples containing UFAs to extreme conditions for short periods of time and monitoring their degradation (*e.g.* Rancimat test or oxygen bomb method, *vida infra*). These rapid tests will be discussed in more detail later in this review. The other approach is that of an oven storage test, during which a lipid sample is placed in a highly controlled environment and UFA oxidation is monitored over time with a variety of physicochemical methods (please see **Table 5** for a non-exhaustive list). Given the costs associated with the storage of large volumes of food products and ingredients and the time needed to run such tests (*e.g.* if a food product is being examined for long shelf-life of ≥ 2 yr), accelerated storage tests are often employed to reduce resource input [28]. This acceleration of oxidation is typically attained by controlled storage within incubators of temperatures greater than room-temperature.

Storage testing may involve the holding of intact products, or may include the extraction of the lipids from the product prior to storage. The advantage of the former may be greater relevance to real-world degradation rates of the product’s quality, while the latter allows for greater control of the conditions for the lipid-content of interest. Whether the extraction of lipids occurs prior to storage testing or after, the method and thoroughness of extraction may significantly affect the eventual indications of oxidation. For instance, if using direct extraction, the retention of water within the oil sample may not only directly affect oxidation rates, but the reduced proportional mass content of lipid in a sample can greatly skew results when not accounted for.

Many solvent-based extraction procedures exist for lipids including crude extraction *via* a Soxhlet apparatus (*i.e.* normally hexanes and petroleum ether), the Folch method (chloroform / methanol) [29], and the Bligh and Dyer method (chloroform / methanol) [30]. In order to reduce the amount of toxic solvents (*e.g.* chloroform) used as well as to accelerate and improve the extraction process, new high-pressure liquid extractions have been developed [31]. The extraction method chosen is often dependent on the physicochemistry of the food being analyzed (solid, liquid, emulsion, etc.) and can include initial sample preparation steps. For example, in the case of emulsions like mayonnaise, sample preparation can include modification of the pH and ionic strength of the sample followed by centrifugation and / or liquid-liquid extraction, coupled with subsequent evaporation of solvents [32]. In addition to being tedious, such sample preparatory procedures can also result in low extraction yields / recoveries as well as result in damage to the UFAs being extracted due to exposure to heat, air and water (*vide infra*).

Although storage tests are often carried out at ambient temperature (~22-23 °C) in the food industry, they can be also be employed at higher temperatures (*e.g.* 25-80 °C) to reduce analyses times; often termed an ‘oven storage test’ (Cg 5-97 from AOCS) [33]. Heating oils has been shown to result in an increase in the kinetics of UFA oxidation [34]. The term “accelerated” suggests that the increase in oxidation is merely an enhancement of oxidative rate, rather than an instigation of different oxidative reactions and processes than those observed at ambient temperature. To temperatures as high as 60 °C, the effect of acceleration is usually considered to be directly attributable to the input of sufficient activation energy to support the initiation of UFAs radical formation [35]. However, concerns of heat altering the mechanisms and results of oxidative reactions have motivated ongoing research for less destructive means to accelerate oxidation. In 2014, Van Durme *et al.* [36] concluded that temperatures of 70 °C produced different degradation products than room-temperature, and proposed an innovative technique of non-thermal plasma technology as a less destructive method of accelerating oxidations. Heat has also been shown to potentially degrade antioxidants as well as result in the production of pro-oxidant compounds - effects which may further alter oxidative stability in manners not compatible with degradations that would occur at lower temperatures [37, 38]. If using temperature to accelerate reactions, the choice of temperature should consider both practical considerations (*e.g.* the length of time to be dedicated to the study) and also the expected stability of the samples.

Storage tests, whether accelerated or ambient, can be conducted in both open and closed containers (Cg 5-97 from AOCS 1998) [33]. Given that atmospheric oxygen plays such a key role in UFA peroxidation, when oxygen is present only at low concentrations in the headspace of a product container it may serve as a limiting factor in the reaction. Andersson [39] and Kacyn *et al.* [40] showed that lipid oxidation rates cease to be dependent upon oxygen concentration and are more substrate dependent at oxygen concentrations of 4-10% in the headspace.

**4. Historically common methods to assess UFA oxidation in storage**

As discussed previously, the compounds resulting from the autoxidation of UFAs are often denoted as “primary” or “secondary” lipid oxidation products. While primary lipid oxidation products such as hydroperoxides are not sensorially active, secondary lipid oxidation products are often comprised of volatile compounds that can drastically affect the sensorial perception of foods. As such, the decision to monitor either primary products, secondary products, or both throughout storage can lead to very different results and conclusions. The proliferation of primary oxidation products can be considered the earliest sign of chemical degradation within a sample, while the proliferation of secondary oxidation products may reasonably be expected to show more relevance to perceptible reduction in product quality. Two of the most commonplace assays for analyzing UFA primary oxidation products are discussed below (peroxide value and conjugated dienes and trienes), followed by a discussion of two of the most historically common assays for secondary oxidation (*p*-anisidine and 2-thiobarbituric acid reactive substances test). See **Figure 5** for a comparison of the relative presence of these assays within literature since the 1930s. In a later section, the emergence of more modern and novel approaches will be discussed.

The historically prominent peroxide value (PV) test is a reverse titrimetric method and is one of the most common tests used to assess the primary oxidative status of high-UFA oils [27, 28] (Cd 8b-90 from AOCS) [33]. It depends upon the separation of I2 from KI in the presence of hydroperoxides, which produces a visible yellow hue as an indicator of hydroperoxides within the sample. An advantage of this assay is that the measured interaction is not upon only a subset of hydroperoxides, but should in fact serve as a measure of all hydroperoxides within the system. This can be particularly important when measuring the oxidation of samples rich in monounsaturated fatty acids, as these fatty acids will produce hydroperoxides but not the conjugated products measured in the conjugated acids assays (to be discussed subsequently). Another advantage is that the assay is fairly inexpensive, is relatively simple to conduct, and does not require the use of sophisticated machinery. One possible disadvantage of the assay is its reliance upon the experimenter’s visual evaluation of the sample’s hue – leading to a possible subjectivity and/or imprecision in the results. This possible imprecision can be mitigated somewhat by carefully lowering the concentration of the titrant.

Given its cost, ease of use, and historical ubiquity, the PV test has maintained its popularity as a quality assurance indicator in the food industry, and is often included in specification sheets provided by fat and oil suppliers. Such quality control situations provide a good example of cases in which measuring primary oxidation products can be preferable to measuring secondary ones. In these cases, evidence of chemical degradation is highly important to eventual product quality even if sensorially active products have not yet been produced in substantial quantities. When screened, fresh fully-refined oils and fats of high quality are expected to have a PV < 0.1 meq/kg; with a PV > 10 meq/kg suggesting advanced lipid oxidation has already taken place within the lipid sample [28]. Cold pressed lipids are special cases and often have higher acceptable PVs than typical refined vegetable oils when they arrive from the supplier (*e.g.* for fresh virgin olive oil PV ≤ 20, whereas a refined olive oil PV ≤ 10) [41].

Although not directly detecting volatile compounds, PVs still indicate quality deterioration and are therefore often a useful predictor of sensory and consumer scores. The strength of these correlations will vary according to food substance, competing flavors, and study designs. In 2014, a storage study upon oat-based biscuits found PVs to significantly correlate with sensory scores of aroma (r = 0.78), flavor (r = 0.75), and aftertaste (r = 0.72) [42]. In 2011, Zajdenwerg *et al.* [43] determined that in Brazil nuts, a PV of 18.8 meq/kg corresponded to rejection by consumers. A different study upon stored fish found that PVs showed greater correlations (r2 values ranging from 65.7% to 68.4%) with sensory detection of off-odors than did gas chromatographic assays directly detecting volatile compounds [44].

Given the fact that hydroperoxides can be formed and subsequently decompose over the course of UFA autoxidation (*i.e.* resulting in an inverted U-shape) [45], the relative presence or absence of peroxides in a sample at a given moment must be interpreted with care. As an illustrative example of the interpretation of oxidative stability data of fats and oils, please see **Figure 6** [46]. Taken at a single point in time, a relatively low PV could indicate either a maintained stability or it could also indicate that hydroperoxides have already formed and then subsequently degraded into secondary products. Repeated measurement of primary products at intervals compatible with expected rates of degradation is therefore necessary to ensure the data can be interpreted properly.

As hydroperoxides are formed in the early stages of UFA oxidation, often too are conjugated double bonds (DBs) [47]. In the case of oxidized UFAs with two conjugated DBs, these are referred to as conjugated dienes (CDs), whereas three conjugated DBs are considered conjugated trienes (CTs). Collectively, fatty acids containing these conjugated regions are commonly referred to as conjugated acids. The occurrence of such conjugated regions within a fatty acid will not universally occur in all hydroperoxides. Such regions cannot occur in oxidized monounsaturated fatty acids (MUFAs), and will occur only in a proportional subset of PUFAs. However, with the exception of samples known to be very rich in MUFAs, the assessment of CDs and CTs is typically considered to provide a meaningful representation of the occurrence of primary oxidative products within a system. CDs and CTs absorb UV radiation at 232 nm and 270 nm respectively [28], and are easily quantified using a simple bench-top spectrophotometer (see 2.505 from IUPAC) [48].

This type of test is rapid, requires no reagents, and can be helpful for monitoring UFA oxidation in its early stages. Among its advantages is the capability to discern between different conjugated products, and the high precision that can be attained with a spectrophotometer. Among its disadvantages is its inability to detect all hydroperoxides, and that the quantifications can be interfered with by other food components containing conjugated DBs. Carotenoids are a prominent example of such conjugated compounds, and their possible presence should be considered when either considering the use of this assay or interpreting its results [27, 49].

The measurement of CDs and CTs often has been shown to correlate well with measures of PV, and to therefore show similar strength of associations with sensory evaluations [50, 51]. In a 2011 study upon walnut oil, Martínez *et al.* [52] found strong correlations between CDs and sensory scores of oxidized (r = 0.77), oily film (r = 0.89), painty (r = 0.86), astringent (r = 0.87), and pungency (-0.72). In all cases, CTs showed similar correlations. Conjugated acids were not significant predictors of sweetness, nuttiness, or bitterness.

The *p-*anisidine value (*p-*AnV) test involves the measurement of the concentration of secondary UFA oxidation products within a lipid sample. The method relies upon the reaction between the *p-*anisidine reagent and aldehydes present in a decomposed lipid sample within acidic milieu and results in the production of a yellow-colored chromophore [53]. The occurrence of this yellow pigment is then quantified *via* spectrophotometry at a wavelength of 350 nm [56] (see Cd 18-90 from AOCS) [33].

The test is predominantly sensitive to unsaturated aldehydes, which are more likely to produce off-flavors than saturated aldehydes [55]. This gives the *p*-anisidine assay a particular relevance to food quality assessment in regards to sensory. Studies have shown the *p-*AnV of oils to have strong correlation with total volatile substances, as well as with sensory scores [55, 58]. An excellent study in 1999 examined the correlation of *p-*AnV within oils with both the chemical measure of volatile compounds, and the sensory detection of flavor notes. In this study, Tompkins & Perkins [57] found *p-*AnVs to correlate very well with measures of *trans*-2-octenal (r = 0.92), *trans-trans-*2,4-decadienal (r = 0.86), *trans*-2-hexenal (r = 0.81), and hexanal (r = 0.81). Interestingly, this study found that *p-*AnVs correlated very well with overall flavor intensity (r = 0.81), but did not correlate significantly (α = 0.05) with any of the individual flavor notes evaluated (fried food, burnt, acrid, fishy, doughy, hydrogenated, and waxy).

Despite its usefulness as a quality indicator, the *p-*anisidine reagent also presents toxicity concerns. The prominence of *p-*AnV in scientific literature has not kept pace with the assessment of secondary oxidation products by the 2-thiobarbituric acid reactive substance (TBARS) test.

Rising substantially in relevance since its first description in 1944 [58], the TBARS test has consistently been a very prominent assay for oxidation within the literature since the 1960s (refer to **Figure 5**). Like the *p-*AnV assay, this test relies upon a reaction between an introduced reagent and secondary lipid oxidation products present in an oxidized lipid sample (see Cd 19-90 from AOCS) [33]. The reagent, 2-thiobarbituric acid (or TBA), interacts with malondialdehyde and malondialdehyde-type products via a colorimetric reaction to form a pink complex that can be measured *via* spectrophotometer at an absorption maximum at 530-535 nm [49, 59]

Although popular, the TBARS test suffers from a lack of specificity and sensitivity [60]. Malondialdehyde is only a minor compound in oxidized oils rich in oleic [C18:1, ω9] and linoleic [C18:2, ω6] acids such as sunflower oil, and thus, is not a ‘universal’ oxidation marker [63]. Further, TBA can react with a variety of other compounds (*e.g.* proteins, carbohydrates, pigments, etc.) to give colored end products that also absorb visible light at 532 nm and can inflate results. Scientific study towards the diminishment of such unfavorable conflicts is ongoing, with a recent study finding that aqueous or diluted acid solutions of TBA exposed to temperature of 100 °C for less than one hour minimized interference from yellow pigments in meat [62]. The TBARS test is ubiquitous in the evaluation of meat, but in the case of bulk oils, the TBARS test continues to often be neglected in favor of the *p-*AnV test [27], due to the aforementioned limitations.

The correlations observed between TBARS results and those of sensory analysis vary according to individual food matrices, but those in literature frequently depict correlations of statistical significance but only moderate strength. A study upon chicken nuggets found TBARS to correlate only decently (r = 0.56) with sensory scores for warmed over flavors – a correlation weaker than that observed between hexanal and sensory (r = 0.68) [63]. Interestingly, TBARS and hexanal showed only a very weak correlation to one another (r = 0.14), illustrating the possible vulnerabilities of using “representative” markers as indicators of oxidation within a system. In 2013, Lorenzo *et al.* [64] found TBARS in sausage to be a significant predictor (α = 0.01) of rancid odor, rancid flavor, overall flavor, overall odor, and product acceptability, but in no cases did the magnitude of the correlation coefficient exceed 0.55.

**5. Rapid methods to assess UFAs oxidative stability**

Unlike the assays described above that measure current oxidative state within a sample, there exist some techniques to rapidly strain and evaluate a sample. These produce rapid outputs meant to predict oxidative stability.

Appearing in the 1980s, Oil Stability Index (OSI; also often referred to as a “Rancimat Test”) quickly became a relatively prominent method for the rapid determination of stability (see **Figure 7**). The method involves the use of elevated temperatures (100-120 °C) and infused air to degrade UFAs and result in the release of volatile UFA oxidation products [65], which are then captured in water and result in a measured change in conductivity (#6886 from ISO) [66]. Although a popular method, there has been increased concern in recent literature that the usefulness of such tests is limited by the possibility that the high temperatures employed therein may alter the decomposition patterns of the lipid samples analyzed [28]. Another concern highlighted by Farhoosh [67] in the case of the OSI, is the potentiality of high variability in OSI results due to simple differences in the operational parameters employed in the assay. In 2013, García-Moreno *et al.* [68] found the trends of OSI behavior differed greatly with changes in temperature – a result the authors concluded to indicate the oxidative mechanisms within OSI are not equivalent to those occurring at room temperatures. In its favor, OSI results have in some cases demonstrated strong correlations with the shelf-stability of products containing UFAs as evaluated by sensory descriptive analysis [69].

The oxygen bomb method (OBM), like the OSI, is a rapid test for determination of the oxidative stability of lipids. This method was being used for stability studies as far back as the 1930s [70]. However, with the emergence of the OSI method, the OBM has been relegated to relative obscurity. Like the OSI, it runs the risk that the small changes in procedure can greatly affect the results obtained [59]. The OBM involves the measurement of the uptake of oxygen from a lipid sample containing UFAs while put under high pressure. It has been suggested that this method may show a better correlation with the results of sensory shelf-life tests than OSI, but inter-laboratory studies have recently demonstrated an unacceptable degree of variation in results obtained using the OBM [28] thereby limiting its perceived reproducibility and effectively removing the assay from quality assurance protocols in food industry.

In 2002, the use of Differential Scanning Calorimetry (DSC) was investigated as a possible alternative to OSI for the rapid determination of oxidative stability. In the study, Tan *et al.* [71] determined that the use of DSC with high temperatures (110-140 °C) and an oxygen-flow cell produced outputs with high correlations to that of OSI. The best correlation (r = 0.976) was observed between OSI at 110 °C and DSC at 110 °C. The authors conclude this may be a good, simple, non-toxic means to assess stability – and the assay has been used for this purpose in recent years (see Bryś *et al.* [72] and Naik *et al.* [73] for good modern examples).

**6. Modern approaches to assess UFA oxidative stability: Volatile compounds by chromatography**

In addition to the historically classical methods described above, there exist numerous more modern methods to evaluate oxidation. The use of many of these date back several decades, so the term “modern” is used only relative to the very longstanding history of the classical methods. Unlike the more historic methods, these methods rely on sophisticated machinery and their implementations continue to undergo modifications and optimizations. These methods can also differ from the historical ones in that the results typically target specific compounds or reactions, rather than producing the comprehensive/representative results of PV, CDs and CTs, TBARS, and *p-*AnV.

Among the most common of these methods (see **Figure 8**) is the measurement of the composition and concentration of specific volatile organic compounds (*e.g.* alkanes, aldehydes, and ketones) present in a challenged lipid sample by Gas Chromatography (GC). The use of GC to assess oxidation of food lipids became notably prominent in the 1990s, and has been a fairly common tool for assessing lipid oxidation in the years since. The technique oftenimplements theuse of GC with a Mass Spectrometry detector (GC-MS), but can also be accomplished with other detectors as well. This method is particularly useful because it can target specific volatile compounds so as to produce assessments that better parallel sensory descriptive analysis and similar measurements [28, 74]. Some specific compounds often measured *via* GC methods include hexanal, pentane, pentanal, propanal, and 2,4-decadienal. This assay requires careful consideration of the degradation compounds to be assessed – in their relevance both to sensory and also to the expected outcome compounds associated with the lipid substance of interest. The quantification of hexanal has become a common “benchmark” of oxidation with lipids, but in some sources its proliferation has been shown to in fact be an inferior indicator of degradation. For example, in 2014, Saraiva *et al.* [75] found 2 and 3-methylbutanal, 2 and 3-methylbutanol, 1-pentanol, 1-hexanol, 2,3-octanedione, 3,5-octanedione, octanal and nonanal to be better predictors that hexanal of spoilage in beef.

The decomposition of foods rich in ω6 (or n-6) fatty acids such as linoleic acid [C18:2, ω6] and arachidonic acid [C20:4, ω6] are often assessed by measuring hexanal accumulation over time [76, 77]. Foods rich in ω3 (or n-3) fatty acids such as *a*-linolenic acid [C18:3, ω3] as well as eicosapentaenoic acid [C20:5, ω3] and docosahexaenoic acid [C22:6, ω3] are often effectively assessed by measuring the accumulation of propanal [27, 39]. The decomposition of UFAs from fish or algal sources can result in the generation of compounds such as (*Z*)-1,5-octadiene-3-one, (*E,Z*)-2,4-heptadienal, 1-penten-3-ol and (*Z*)-4-heptenal [78, 79]. There currently exists no ‘universal marker’ compound, or reference set of marker compounds, to compare UFAs decomposition across lipid sources. The complex food matrix can also greatly affect sensorial observations of assessed volatile compounds; therefore making the derivation of meaningful correlations between GC outputs of different foodstuffs/sources a very challenging task.

**7. Modern approaches to assess UFA oxidative stability: Spectroscopy methods**

Multiple spectroscopic techniques have been developed and refined for the purpose of detecting oxidation in foods. These include chemiluminescence spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, Infrared (IR) spectroscopy, and Electron Paramagnetic Resonance (EPR) spectroscopy. Each produces highly detailed outputs, and they are all capable of directly monitoring very minor changes within lipid systems. Frequently, these uses have emerged following the implementation of these techniques for the detection of oxidation within the field of biomedicine. Although established as useful for specific purposes, their implementation for the assessment of stability of food products remains fairly uncommon relative to the classical methods described earlier in this review (refer to **Figure 8** for a depiction of their relative presence in scientific literature in regards to oxidative stability within food science).

Chemiluminescence presents an extremely sensitive measure of electronically excited molecules (*e.g.* singlet oxygen), and can therefore detect very minor differences in oxidation occurrence in a sample [80]. Its main use is therefore in detecting minor changes in oxidative rates in response to controlled modification, such as the input of antioxidants [27]. A thorough investigation of the technique was conducted in 2013, in which Rusina [81] modeled the kinetic rates of many reactions in response to antioxidant input. The author concluded chemiluminescence to be the most rapid, sensitive, and accurate method to assess the content and quality of antioxidants in a system.

NMR is capable of detecting differences in hydrogen atoms within a system [82]. Although allowing for possibly highly specific interpretation, the noted outcome of lipid oxidation commonly detected by this assay is the gradually shifting concentrations of aliphatic and olefinic protons. As the fatty acids in a system degrade, the ratio of aliphatic protons to olefinic protons increases [27]. This change in ratio therefore serves as a useful representative indicator of oil oxidation, making NMR an effective alternative to standard tests such as PV, with which it has been shown to correlate well [83]. One of the main noted advantages of NMR over the classical titrimetric assay is in its reliance on highly precise machinery rather than upon titration.

IR spectroscopy is effective at detecting unusual functional groups and the occurrence of *trans* regions (relatively common among secondary oxidation products) [84]. Degradation of fatty acids into secondary products also results in a stretch of the C=O bond in the fatty acid - an effect observable in an IR spectra. In 2013, Tena *et al.* [85] implemented Fourier Transform mid-IR spectroscopy on virgin oil, and determined these C=O stretches to be the most useful parameter by which to differentiate the stabilities of the samples. The authors cited the advantages of this technique to be its rapidity and environmental-friendliness. Another benefit of this technique is that it can potentially simultaneously provide useful information regarding sample origin, authenticity, and other points of interest [86].

EPR spectroscopy differs from the other techniques discussed thus far, in that it assesses neither primary nor secondary oxidation products. Instead, it detects the radical intermediaries that occur very briefly (<10-3 s) during oxidation reactions [27]. So while traditional methods determine oxidation that has occurred prior to assessment, this technique measures the rate of oxidation occurring during assessment. The predominant utility of this technique to date has been in the assessment of antioxidant efficacy but it has also received attention as being a potentially powerful and versatile tool for assessing multiple indicators of lipid oxidation [87]. The implementation of spin label oximetry allows for an extremely sensitive measure of dissolved oxygen concentration – a technique that has compared favorably to TBARS in detecting early stages of lipid oxidation [87]. In a 2006 study upon olive oil, it was also determined that the use of EPR at accelerated temperature conditions (70 °C) was a viable alternative to the use of Rancimat as a rapid forecast of oxidative stability [88].

**8. Conclusions**

As reviewed above, a number of physicochemical methods have been developed to monitor UFA oxidation in food products. Despite advances in equipment, the most historically classical assays have remained common and relevant for many decades. These methods lack comprehensiveness, but have demonstrated consistent decent correlations to sensory analysis. More rapid methods and more modern methods have shown intriguing results, but still have not demonstrated enough advantages so as to replace the more traditional assays. Ongoing examinations of oxidation and its methods of analysis continue to show that the best choice of investigation strategy will require holistic consideration of the project objectives, the food components of study, and practical considerations (available time, accessible equipment, budget, etc.) Further research in the area of non-invasive analyses of UFA oxidation that are efficient, cost-effective, accurate, and can be utilized across a wide variety of investigations would be highly relevant and welcome.

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**Table 1** Free radical position on the fatty acid moiety following free-radical mediated autoxidation of various fatty acids [26]

|  |  |  |
| --- | --- | --- |
| **Fatty acid** | **Location of radical** | **Relative amount** |
| Oleic acid | C8C9C10C11 | ~27%~23%~23%~27% |
| Linoleic acid | C9C13 | ~50%~50% |
| Linolenic acid | C9C12C13C16 | ~32%~11%~12%~32% |

**Table 2.** Typical decomposition products of hydroperoxides following *β*-scission [3]

|  |  |  |
| --- | --- | --- |
| **Chain cleavage oxidation products** | **Di- and polymerization products:** | **Rearrangement products** |
| Alkanals, 2-Alkenals, Alkanes, 2,4-Alkadienals, Alkatrienals, α-Hydroxyaldehydes,Ketones, Malonaldehyde | Dimers and polymers linked ether, carbon, and/or peroxy bridges. | Hydroxy acids, Keto acids. |

**Table 3.** Sensory threshold values for some common volatile end-products from UFA oxidation [26, 89]

|  |  |
| --- | --- |
| **Volatile compounds** | **Threshold value (ppm)** |
| vinyl ketones | 0.00002-0.007 |
| *trans*, *cis*-alkadienals | 0.002-0.006 |
| isolated *cis*-alkenals | 0.0003-0.1 |
| isolated alkadienals | 0.002-0.3 |
| *trans*, *trans*-2,4-alkadienals | 0.04-0.3 |
| alkanals | 0.04-1.0 |
| 2-alkenals | 0.04-2.5 |
| 1-alkenes | 0.02-9 |
| vinyl alcohols | 0.5-3 |
| substituted furans | 2-27 |
| hydrocarbons | 90-2150 |

**Table 4.** Sensory descriptors for some common volatile end-products from UFA oxidation [25, 90]

|  |  |
| --- | --- |
| **Sensory descriptor** | **Dominant volatile compounds** |
| cardboard | *trans*,*trans*-2,6-nonadienal |
| cut-grass, grassy | hexanal, *trans*-2-hexenal, nona-2,6-dienal |
| deep-fried | *trans*,*trans*-2,4-decadienal |
| fishy | *trans*,*cis*,*trans*-2,4,7-decatrienal, oct-1-en-3-one |
| fresh oil, oily | aldehydes |
| mushroom | 1-octen-3-ol |
| painty | pent-2-enal, aldehydes |
| wood bug | *trans*-2-octenal |

**Table 5.** A non-exhaustive list of analytical methods for monitoring lipid oxidation [27, 91, 92]

|  |  |  |
| --- | --- | --- |
| **Stages of oxidation** | **Compounds/ Group of compounds** | **Analytical techniques** |
| Oxygen uptake | Molecular oxygen | Oxygen consumption test (drop of oxygen partial pressure) |
| Initial Stage | Free radicals | Electron paramagnetic resonance (EPR) |
| Primary oxidation products | Hydroperoxides | Peroxide value (PV) by iodometric titration, UV spectrometry using formation of ferric complexes, Fourier transform infrared spectroscopy (FTIR), liquid chromatography-mass spectrometry (LC-MS), reaction of hydroperoxides with luminol or isoluminol detected by chemiluminescence |
| Conjugated dienes and trienes | UV spectrometry (232-234 and 268 nm) |
| Secondary oxidation products | Thiobarbituric acid reactive substances (TBARS) such as malonaldehyde | TBA (2-Thiobarbituric acid) by UV-Visible spectrometry (530-532 nm) |
| Aldehydes such as alkenals | *p*-Anisidine value (absorption at 350 nm) |
| Carbonyls | Total carbonyls by spectrometry such as HPLC |
| Total volatile organic acids | Induction period by oil stability index (measurement of change in electrical conductivity) |
| Total volatile carbonyls and hydrocarbons | Head space-gas chromatography-mass spectrometry (HS-GC-MS) |
| Non-volatile polymers | Liquid chromatography-mass spectrometry (LC-MS) |
| Others | Formation of free fatty acids | Titration, electrical conductivity, liquid chromatography-mass spectrometry (LC-MS) |
| Loss of fatty acids | Quantitative loss of unsaturated fatty acids by gas chromatography (GC) |
| Measurement of onset and peak of exothermic reaction between oil and oxygen | Differential scanning calorimetry (DSC) |
| Both primary and secondary oxidation products | Nuclear magnetic resonance (NMR) spectroscopy |

**Figure Captions**

**Fig. 1.** Relative reaction rates of singlet and triplet oxygen with different fatty acids [10]

**Fig. 2.** Formation of conjugated radicals *via* the autoxidation of linoleic acid [1]

**Fig. 3.** Formation of a hydroperoxide resulting from the autoxidation of linoleic acid. This reaction proceeds following the formation of a radical product. [7]

**Fig. 4.** Potential pathways of the decomposition of hydroperoxides [7]

**Fig. 5.** Frequency of citation of historically common assays for the detection of lipid oxidation products. aDetermined according to queries within SciFinder® on October 3, 2014 (see https://scifinder.cas.org). Searches were made according to the terms “peroxide value”, “conjugated dienes”, “thiobarbituric”, and “*p-*Anisidine”. Results were filtered according to the keyword “oxidation”, and refined to the specified timeframes. The number of hits was divided by the number of years within the timeframe.

**Fig. 6.** Hydroperoxide accumulation, as demonstrated by the peroxide value test, in sunflower oil heated at 30, 47 and 67 °C over 100 days of storage [46]

**Fig. 7.** Frequency of citation of rapid assays for the estimation of oxidative stability. aDetermined according to queries within SciFinder® on October 3, 2014 (see https://scifinder.cas.org). Searches were made according to the terms “rancimat” or “oxidative stability index”, and “oxygen bomb”. Results were filtered according to the keyword “oxidation”, and refined to the specified timeframes. The number of hits was divided by the number of years within the timeframe.

**Fig. 8.** Frequency of citation of modern assays for the estimation of oxidative stability in food science. aDetermined according to queries within SciFinder® on October 3, 2014 (see https://scifinder.cas.org). Searches were made according to the terms exactly as listed. Results were filtered according to the keyword “lipid oxidation”, and also refined according to “edible or food or cooking or meat”. Results were refined to the specified timeframes and divided by the number of years within the timeframe.



**Figure 1.**



**Figure 2.**



**Figure 3.**



**Figure 4.**

**Figure 5.**

**Figure 6.**

**Figure 7.**

**Figure 8.**