Review Paper:

**Peroxide value in foods**

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**Abstract**

Foods that contain lipids or cooking oil can decrease quality due to the oxidation process that produces primary products peroxide which is hydroperoxide. The presence of peroxide content in food containing lipids or oils can be analyzed so that the peroxide numbers can be obtained which can be used as a determinant of oxidation status. Peroxide value is an indicator. Peroxide value can be obtained with iodometric titration, colorimetric method and chromatography method. It depends on the nature matrix in food samples.

**Keywords:** Lipids, Oils, Lipid oxidation, Peroxide value, Hydroperoxide.

**Introduction**

Lipids obtained from plants better known as oil, have a liquid form and can be used for cooking. Various kinds of oils obtained from plants are coconut oil, palm oil, olive oil, soybean oil, peanut oil, corn oil, flower oil sun and sesame oil. These edible oils are susceptible to photo-oxidation and auto-oxidation during manufacturing and storage processes.

Oxidation can change the taste and aroma of food, reduce or decompose nutritional quality in food and can produce toxic compound to the body. Oxidation in oil can be influenced by several factors i.e. degree of unsaturation, temperature, oxygen, light, water, oil making process, the presence of antioxidants and the presence of metals (e.g. Cu, Fe). Repeated use of oil which often occurs in roadside sellers and other food vendors to reduce sales capital has an impact on the deterioration in the quality of the oil used. Repeated heating in cooking oil can increase the tendency for peroxidation lipids in oil. The oil that is used repeatedly usually will change color to darker and will be discarded if oil smells bad.

Lipid oxidation in food is a complex chain reaction which produces the main product, namely peroxide, especially hydroperoxide. It is an intermediate compound in the lipid oxidation process which can be further oxidized to produce secondary oxidation products, for example aldehydes, ketones, epoxides, hydroxy compounds, oligomers and polymers. Most of the products have a negative impact to the body, so it is necessary to be controlled.

The presence of peroxide can be one of the benchmarks regarding status or quality of food so that its existence can be analyzed with variety methods. Lipid peroxidation in food, either in unprocessed or in processed food is the main cause of damage, decreased stability and change in food taste so that it will have a bad impact on the quality and storage of these food products. Peroxide value is a measurement that is often used to know the unwanted degradation reactions of food. In addition, peroxide value can also be used on samples biology where the reaction is present at the initiation of tumors and others degenerative diseases, changes in cell membrane structure and modification of DNA and protein.

Lipid peroxidation in food can be prevented by the presence of antioxidants which works with a variety of mechanisms for example by controlling substrate which plays role in oxidation (lipids and oxygen), controls prooxidants and free radical inactivation. Antioxidants can be found naturally inside plants so that they are safe and function as food additives, plant extracts which contain high phenolic component can be used as a food stabilizer. Antioxidant can prevent lipid peroxidation in food and provides protection against oxidative damage in the membrane that can damage the functioning of the biological system. Many diseases are caused due to the lipid peroxidation and lipid oxidation, for example atherosclerosis and aging processes, so it is very important to prevent lipid peroxidation and to determine the analytical method to determine peroxidation value.

**Mechanism of Lipid Oxidation**

There are 3 different ways in which oxidation occurs in lipids to produce different oxidation products. The mechanism is free radical mechanism or also known as autoxidation, photooxidation and a process related to lipoxygenase activity.

**Autoxidation:** Autoxidation is a spontaneous reaction between oxygen molecules and a lipid leads to oxidative damage. Autoxidation occurs because of a mechanism free radical chain that takes place in 3 stages, namely the initiation stage, stage propagation and termination stage.

The initiation stage takes place when the hydrogen atom apart from the methyl group produces an alkyl radical (R*). Propagation stage is the stage where the formation of peroxy radicals (ROO*) is due oxygen that will react with the unsaturated fatty acids that produces hydroperoxide (ROOH). At the termination stage there will be formation of non-radical products because there is an interaction between the alkyl radical and the peroxy radical ROOR and oxygen.

Hydroperoxide is a primary product of oxidation that is easy to decompose through monomolecular or bimolecular reactions. Product decomposition such as peroxy and alkoxyl radicals are very reactive compounds and act as initiators of autoxidation. The hydroperoxide formed in the...
initial process of autoxidation is non-volatile and does not smell, but when decomposed, will form aromatic volatiles compounds that will smell bad and can be used as a sign of the food\(^1\).

**Photo-oxidation:** Another mechanism of oxidation occurs when there is a sensitizer and UV light thus undergoing oxidation via the photo-oxidation pathway. Photo-oxidation is alternative pathway to form hydroperoxides apart from radical mechanisms free. Excitation of unsaturated fatty acids or oxygen will occur in presence of light and sensitizer. Photo-oxidation occurs in 2 ways, namely the first way that occurs if there is an electron or hydrogen transfer between excited triplet sensitizers with substrate (PUFA / polyunsaturated fatty acids) to produce free radicals or radical ions and the second way is oxygen triplet (\(3O_2\)) which can be excited by light into a single oxygen (\(O_2\)) which will react with the double bonds of fatty acids to produce allylic hydroperoxide, this reaction causes the formation of trans configurations\(^10,11\).

**Lipoxigenase Activity:** The next mechanism of oxidation is based on lipoxigenase activity. Lipoxigenase is an enzyme that is very important for the formation of hydroperoxides during oil extraction. Lipoxigenase produces the same aroma that comes out with autoxidation. Lipoxigenase contains iron Fe (II) atoms that are oxidized to Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme will release hydrogen atoms from the fatty acid methyl group that is not saturated so that the iron will change back to Fe (II). The diene conjugated system is formed which then reacts with oxygen so that peroxy radicals and hydroperoxides are formed. The second type of enzyme will react with esterified substrate before the release of fatty acids by lipase forming ketodiene fatty acids\(^10\).

**Peroxide Value**

Peroxide value is an indicator that is often used to determine the initial oxidation of fats or oils that has occurred in hydroperoxide formation\(^12,13\). The range of oxidation rates for soybeans, sunflowers and canola based on peroxide value are 3-5 for mild oxidation, 10-12 for intermediate oxidation and 16-18 as high level of oxidation\(^14\). Determination of peroxide value is to determine the level of oxidation in foods which is the direct measurement of lipid peroxide and which is the main result of lipid oxidation. However, the disadvantage of this method\(^12\) is vulnerability interference from oxygen molecules.

Peroxide value is evidence of autoxidation from radical free reactions because autoxidation produces hydroperoxide which will react with component of food so that it makes food rancid\(^15,16\). Peroxide value in food can change because of heating at the beginning of cooking an increase in numbers peroxide at the maximum level due to the formation of hydroperoxide from unsaturated fatty acids is caused by lipid oxidation, then peroxide value will decrease because the hydroperoxide formed is unstable and will decompose with an increase in temperature resulting in secondary oxidation products which are volatile and non-volatile compounds. Fluctuation of oxidation numbers when cooking occurs due to peroxide decomposition becomes secondary oxidation products\(^1,16,17\).

Peroxide value is also influenced by packaging, when packaging using plastic bottles transparent and then it will be more exposed to light so that photooxidation is higher. Weather and climate conditions where the oil filtering process is carried out and the duration of storage can affect the quality of oil\(^1\).

The use of preservatives (UV-absorbers) such as Tinuvin 234 can protect oil from light exposure when using transparent packaging, the use of antioxidants can also increase the stability from oxidation and the use of chelating metals can reduce oxidation because metals are catalysts of oxidation\(^1,18\).

**Analytical Methods of Peroxide Value**

**Volumetric Method (Iodometric Titration):** Lipid hydroperoxide is a parameter that commonly used for the measurement of the quality of food, especially oil. Traditionally, the method used is iodometric titration by measuring the amount of hydroperoxide as a peroxide value\(^19\). This method is widely known because it is simple but requires a procedure for extracting lipids on food.

In acidic conditions, hydroperoxides and other peroxides react with iodide ions to produce iodine which is then titrated with a solution of sodium thiosulfate in the presence of starch as an indicator, this method has existed officially since 1965\(^20\). According to this method, the peroxide value considered to represent the amount of active oxygen (in meq) contained in 1 kg of lipid that can oxidize potassium iodide. Peroxide value is expressed as milliequivalent O\(_2\) kg\(^{-1}\) (meqO\(_2\)/kg\(^{19}\)).

The drawback of this method is the use of highly susceptible iodides against oxidation by air molecules and is accelerated by exposure to light. The other is that spontaneous hydroperoxide formation can occur via the higher calculation and there is no absorption of iodine by saturated fatty acids so the calculation is lower. In addition, anhydrous system is needed to avoid interference\(^8,21\).

**Colorimetric Method (Fox/ Ferrous Xylenol Orange):** Another simple method is to measure oxidation of ferrous (Fe\(^{2+}\)) to ferric ion (Fe\(^{3+}\)) by hydroperoxide with the orange xylene indicator. This method has high sensitivity and can be used in food\(^12\). This method is in acidic condition and uses orange xylene which can form complexes with ferric ions so that it can be detected by spectrophotometry because it provides maximum absorbance with peaks at 500 nm and 560 nm\(^8\).
The colorimetric method is carried out by dissolving oil samples in solvents such as propanol, then add FOX-containing reagents ammonium ferri (II) sulfate, sulfuric acid, methanol, BHT and xylene orange. Then it is incubated for 30 minutes at room temperature that can change the color intensity over time. Then centrifuge and the supernatant is measured with maximum absorbance for complex compounds colored (blue-purple) at 560 nm.

This FOX method can only detect peroxide concentrations at the small range and molar absorbptivity of the orange ferrile-xylene complex varies depending on how the dye is made or the indicator. However, the method can be said to be better when compared to the iodometry method. This method can use another indicator namely thiocyanate to produce the Fe (III) -thiocyanate complex, the use of thiocyanate relatively requires more solvents.

Iodide Oxidation Method: This method uses iodide which is then tested with spectrophotometry to determine the amount of hydroperoxide contained in the sample. Testing samples containing lipids are dissolved in the last acid solution mixed with iodide. Hydroperoxide in lipids contained in the sample will oxidize iodide to iodine. Excessive iodide will react with triiodide anions which can be detected using spectrophotometry wavelength at 350 nm. A catalyst can be added i.e. Fe (II) and then carried out in closed conditions to avoid the presence of oxygen in air that disrupts reaction rapid testing can reduce risk interference from reaction by products.

Chromatography Method: Determination of hydroperoxide content in food using chromatographic techniques produces accurate, reliable data and specific for the compound being analyzed so that the identification of compounds is also very good. Due to these advantages, this method is used more often to measure hydroperoxide compared to volumetric methods especially in terms of accuracy. However, this accuracy also depends on the analysis process conducted and sample preparation. The use of chromatography required skills, accuracy, maintaining optimal conditions for analysis, understanding ways process the data obtained after the analysis process so that the maximum results are obtained.

HPLC (High Performance Liquid Chromatography) is one of chromatographic techniques that can be used for the determination of hydroperoxides. This method is very sensitive and flexible in managing the condition or nature of the column and detector so that they can analyze compounds with differences in properties such as their volatility, molecular weight and polarity. However, this method usually requires a long preparation process and lipid extraction is needed from food.

This method can be used to determine the peroxide value of lipids that are given colored reagents i.e. ferrous xylenol orange uses a reaction with triphenylphosphine to form compounds that absorb light at 260 nm and sample through stages HPLC separation and UV detection so that the peroxidic value can be calculated. Stigmasterol can also be determined by its hydroperoxide content using a normal phase column and 2 types of detectors namely UV and fluorescence.

GC-MS (Gas Chromatography coupled to Mass Spectrometry) can also be used for the analysis of lipid hydroperoxide due to its thermolable properties. However, it is necessary to reduce lipid extraction and subsequent derivatization process that requires long time and complicated process.

Conclusion
Foods that contain lipids or cooking oil can undergo oxidation which can occur due to autooxidation, photooxidation or because of the activity of the lipoxygenase enzyme. The result of the oxidation is peroxide. The peroxide value of lipid can be determined by volumetric method using iodometric titration, the method colorimetric which is a ferrous xylene orange, chromatographic technique traditional or using HPLC or GC-MS.

References


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