Review Article

Oxidation of Lipids in Foods

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Abstract | Lipid oxidation is a major cause of deterioration in the quality of food and food products. Oxidation can occur in both triglycerides and phospholipids of food because lipids are divided into two main classes; polar lipids (phospholipids) and neutral lipids (triglycerides). Lipid oxidation has been long been recognized as a major problem in the storage of fatty acids in foods. Oxidation occurs by several molecular mechanisms such as generation of reactive oxygen precursors and free radicals. Oxidation affects many interactions among food constituents, leading to both desirable and undesirable products. Food lipids are the foods components that are most susceptible to oxidation, therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing, storage, distribution and final preparation of foods. Lipid oxidation products are omnipresent in foods, although much variation exists in their kind and levels present. Although levels of these compounds are generally low, the problem of lipid oxidation severely compromises the quality of some food products and limits the shelf-life of others. Oxidative changes can cause rancidity such as off flavours, loss of colour, altered nutrient value, and may produce toxic compounds, which can be detrimental to the health of consumers. Antioxidants and chelating agents are the most helpful inhibitors of lipid oxidation.

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Lipids are triacylglycerol and formed when three fatty acids esterifies with a molecule of alcohol or glycerol (Ubhayasekera, 2009). Unsaturated fatty acids mainly consist of omega-9, omega-6 and omega-3 series are highly susceptible to oxidation (Galano et al., 2015). Rate of oxidation depends on degree of unsaturation and increases with increase in double bond of fatty acids. When oxygen reacts with unsaturated lipids, a wide variety of oxidation products are produced by lipid peroxidation (Adrian et al., 2015). Lipid oxidation products have mutagenic, carcinogenic and cytotoxic properties and considered to be risk factor for human health (Julia et al., 2015). These metabolites cause severe health problems, such as growth of tumor cells through lipid peroxidation as hydroxides of fatty acids are cytotoxic. Oxidation of long chain fatty acids causes neuromyopathic disease both in infant and adults (Olpin, 2005). Oxidation in marine lipids causes loss of nutritive value and development of unpleasant flavour (Juan et al., 2015). Lipid oxidation reduces the shelf life of many complex food products and nutritive value of food by limiting the content of essential polyunsaturated fatty acids (Böttcher et al., 2015). A fishy malodour associated with DHA and EPA oxidation products is the result of odour synergy effects. When two lipid oxidation products – heptanal and (E, Z)-3, 5-octadien-2-one – are smelled together, a fishy malodour is perceived (Marsili and Laskonis, 2014).

Highly unsaturated fatty acids are present in marine lipids, when exposed to atmospheric oxygen their oxidation starts (Barden et al., 2015). But fish oil capsules are less susceptible to oxidation as compared to non-encapsulated oil (Juan et al., 2015). Free radical mechanism of autoxidation relating an attack of oxygen at the allylic position with the formation of unsaturated hydro-peroxides. These primary oxidation products further decompose into secondary oxidation products and make marine lipid rancid. Fish oil quality can be determined by solid phase micro extraction method. This is an accurate and reproducible method for the determination of volatile and semi volatile compounds form during oxidation (Bruheim, 2009). Hydro-peroxides isomers may also be formed through the process of photo-oxidation and enzymatic oxidation mechanisms in addition to autoxidation (Uchida, 2015). Hydro-peroxides initiate new chain reaction after reacting with free radicals. Since isomer of hydro-peroxides such as cis and trans have different activities in biological system and food, there is a need to evaluate importance of lipid oxidation activity (Porter, 2013). These factors highlight the significance of understanding the mechanism of lipid oxidation and to classify the factors which manipulate the oxidation reaction pathways.

This review elucidate the different types of lipids oxidation which is one of the most important issues related to food quality in term of shelf life of food containing lipids and nutritional values of lipids and lipids soluble vitamins and the health effect such as oxidative stress relates to chronic disease, mainly inflammation and cardiovascular disease.

Autoxidation

Autoxidation reaction leads to lipids breakdown and formation of wide range of oxidation products (Kubow, 1992). When lipid substrate (LH) exposed to heat, light or metal ions their hydrogen atom of double bond is extracted and free or alkyl radical ($L^{\cdot}$) is formed (Lee et al., 2004). These free radicals reacts with oxygen and peroxy radical ($LOO^{\cdot}$) are formed with subtracting hydrogen atom from another unsaturated fatty acids. It leads to the formation of primary oxidation products called hydro-peroxides (LOOH) by the mechanisms of initiation, propagation and termination (Julia et al., 2015). The length of propagation cycle is directly proportional to the degree of lipid unsaturation (Kubow, 1992). These primary oxidation products are not stable and further break down into carbonyl compounds such as aldehyde, ketones and alcohols (Tirosh et al., 2015).

- Initiation: $LH \rightarrow L^{\cdot} + H^{\cdot}$
- Propagation: $L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$
- $LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$
- Termination: $LOO^{\cdot} + LOO^{\cdot} \rightarrow$ non-radical product
- $LOO^{\cdot} + L^{\cdot} \rightarrow$ non-radical product
- $L^{\cdot} + L^{\cdot} \rightarrow$ non-radical product

Further reaction of hydro-peroxide (LOOH) with ferrous ion (Fe^{2+}) yield peroxyl radical ($LOO^{\bullet}$) and alkoxyl radicals ($LO^{\bullet}$). This reaction will be fast and Fe^{2+} will be regenerated but the regeneration of Fe^{2+} will be slow (Gutowski and Kowalczyk, 2013).

- Fast reaction: $Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\bullet} + OH^{--}$
- Slow reaction: $Fe^{3+} + LOOH \rightarrow LOO^{\bullet} + Fe^{2+} + H^{+}$

Alkyl radicals ($R'CH_2^{\bullet}$) and aldehydes (R" CHO) form due to degradation and β-scission of alkoxyl radical $LO^{\bullet}$. Free radicals are removed in termination cycle and two radicals combine to form a non-radical compound and finally the propagation cycle is terminated as shown in Figure 1 (Kubow, 1992).

Oxidation of Omega-3 Fatty Acids

Fish oil without having any antioxidant undergoes rapid autoxidation. Purified menhaden oil when exposed to air at ambient temperature deteriorates rapidly and peroxide value also increase 5 to 12 fold within two days (Umesha and Naidu, 2015). Normally during fish oil production from fish meal the Peroxide value (PV) ranged from 6-22 mEq O_2/kg. Different fatty acids have different rate of oxidation, where during oxidation PV increases and omega-3 fatty acids content decreases (Turner et al., 2006). Autoxidation of ethyle EPA and DHA is much faster as compared to ethyle linoleate (Lo) and ethyle linolenate (Ln) (Miyashita, 2014).

Oxidation in fish and fish oil occurs as a result of because of high concentration of polyunsaturated fatty acid exposure to air (Kubow, 1990). These fatty acids are more susceptible to radical initiated triple oxygen oxidation than monounsaturated fatty acids. As singlet oxygen can also react with electron rich double bonds of unsaturated molecules but the rate of triplet oxygen reaction increase as degree of unsaturation increase (Min and Boff, 2002). Volatile oxidation products formed in fish oil such as 1-penten-3-one,

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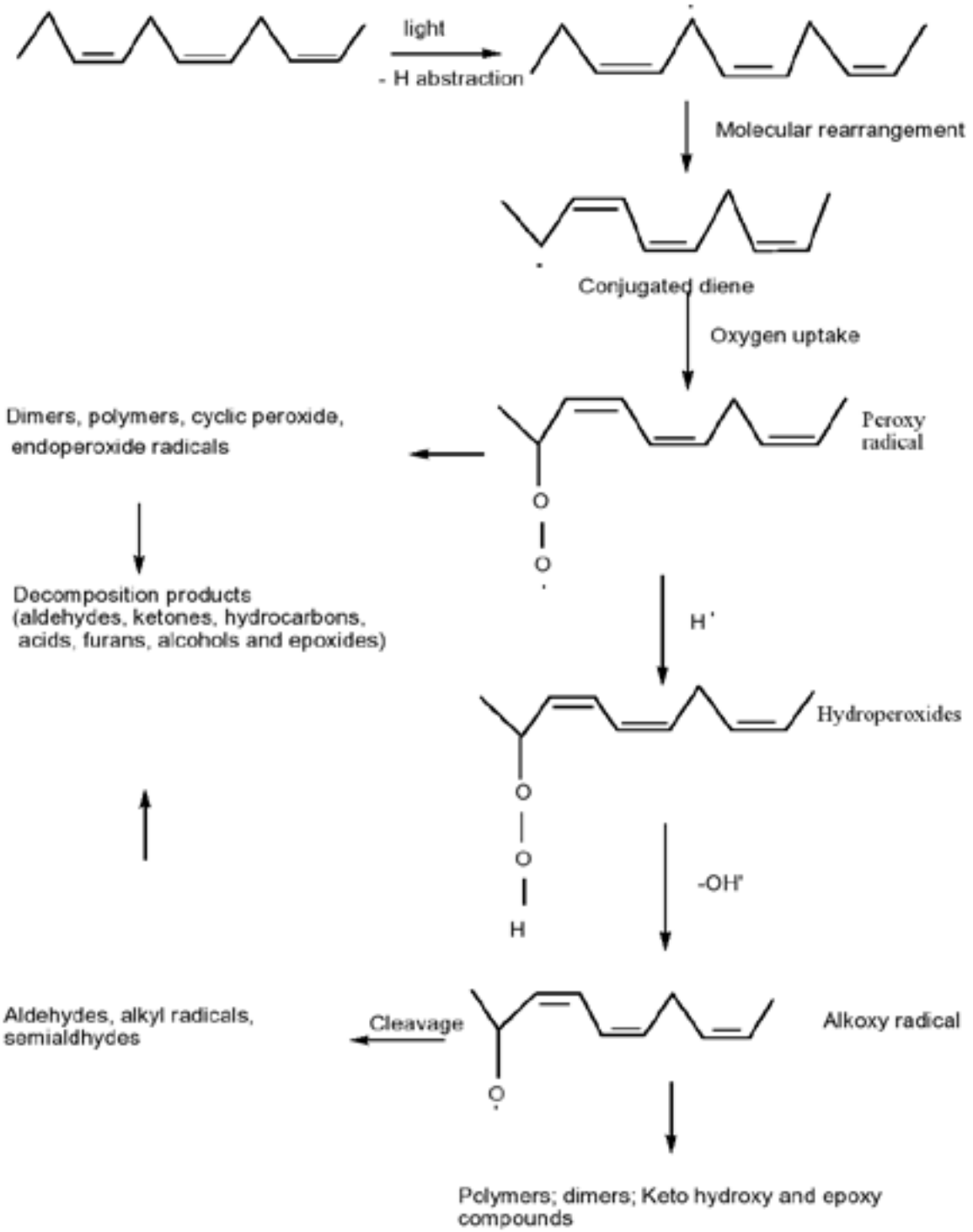


Figure 1: *Autoxidation of unsaturated lipids (Kubow, 1992)*

hexanal, 4-(Z)-heptenal, 2,4-(E,E)-heptadienal, nonanal and 2,6-(E,Z)-nonadienal are responsible for off-odors and are good indicators for the oxidation status in fish oil (Aidos et al., 2002).

EPA and DHA are most polyunsaturated fatty acids (PUFA) in fish oil having five and six double bonds. These are susceptible to oxidation by three phases i.e. initiation, propagation and termination (Kerrihard, 2015). Free radicals and peroxides are produced during initiation phase when molecular oxygen reacts with unsaturated fatty acids. But in propagation phase some other reactive chemical products form from peroxides such dihydroperoxides because peroxides are unstable and have active methylene group, but in termination phase non-reactive secondary oxidation products such as hydrocarbon, aldehydes and ketones are formed and cause off-flavour (Kolanowski et al., 2007). In these PUFA peroxide value is not a good indicator of autoxidation because their hydro-peroxides are not stable and secondary oxidation products play important role in oxidative deterioration in these fatty

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acids ester (Miyashita, 2014).

Photo- Oxidation

Photo-oxidation produce aliphatic and aromatic oxidized compounds, such as ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxies, sulfoxides, sulfones, phenols, anhydrides, quinones and alcohols (Lee, 2003). In the presence of light photosensitizers convert triplet oxygen into singlet oxygen, which is highly reactive and non-radical molecule. It requires low energy to activate the reaction with food as compared to triplet oxygen, so temperature has little effect on the oxidation caused by singlet oxygen than triplet oxygen (Min and Boff, 2002). When oils are exposed to solar radiation, photo-oxidati on will occur in crude and refine oils (Lee, 2003). If wave length of solar light is less than 220 nm then unsaturated fatty acids cannot absorb light, but during photo oxidation sensitizer molecules like chlorophyll, porphyrin or dyes such as rose bengal absorb light energy and convert triplet state sensitiser to singlet state sensitiser. Fluorescent light is emitted by sensitiser molecules and to form triplet sensitiser, this triplet sensitiser react by two mechanism (Choe and Min, 2005). In first mechanism sensitiser react with lipid substrate to form complex mixture of free radical and singlet sensitiser then this mixture further react with triplet oxygen and give hydroperoxide similar in structure as form by autoxidation.

- ^{3}Sens + lipid substrate (LH) → Free radical (L$^\cdot$) + singlet sensitiser (SensH)
- Free radical (L$^\cdot$) + singlet sensitiser (SensH) $^3O_2$ → Hydroperoxide (LOOH)

In second mechanism triplet sensitiser also react with triplet oxygen and give singlet oxygen (Song et al., 2007). This singlet oxygen directly reacts with double bond of unsaturated lipids and produce hydroperoxides. These hydroperoxide are different from autoxidation products and in this case radical scavengers do not interfere because no involvement of free radicals (Galano et al., 2015; Kubow, 1992).

- ^{3}Sens + $^3O_2$ (triplet oxygen) → $^1O_2$ (singlet oxygen)
- $^1O_2$ (singlet oxygen) + LH → LOOH (Hydroperoxide)

Singlet oxygen produce peroxides by direct reacting with fatty acids without abstracting H atom and proceed chain reaction during lipid peroxidation process. Small amount of singlet oxygen also produce by colliding of peroxyl radicals, illumination of certain compounds in the presence of oxygen by absorbing light and entering to higher energy state (Rác et al., 2015).

Enzymatic Oxidation

Hydro-peroxide formed by enzymatic oxidation process are intermediates in the synthesis process of prostagladins and eicosanoids (Frey et al., 2006). Enzymatic mechanisms also catalysis the oxidation of lipids in foods by lipoxygenase and cytochromes P450 to produce hydro-peroxides. But oxidation of poly unsaturated fatty acids initiated by lipoxygenases via a free radical mechanisms to form specific hydro-peroxides (Kubow, 1992; Feussner and Wasternack, 2002). Lipoxygenase enzymes molecules contain one heme iron, 1, 4 –cis,cis-pentadiene system that desired in the fatty acid as a substrate for lipoxygenase. Ferric form of lipoxygenase enzyme oxidized 1,4 –cis,cis-pentadiene fatty acid moiety into pentadienyl radical, which react with molecular oxygen to form peroxyl radical and finally produce hydroperoxides (Gajera et al., 2015; Mandal et al., 2014). These hydro-peroxides are precursor of further transformation by enzymatic chemical reaction, because lipoxygenase reaction initiates the synthesis of a single fatty acid hydro-peroxide from substrate of fatty acids (Mandal et al., 2014).

- Fat + Water $\xrightarrow{\text{Lipoxygenase / cytochromes P450}}$ Free fatty acids + Glycerol
- Free fatty acids $\xrightarrow{\text{oxidation}}$ Hydroperoxides $\xrightarrow{\text{degradation}}$ Aldehyde, Ketones

Fatty Acids Hydro-peroxides Isomerization

Isomeric monohydroperoxides formed from autoxidation of methyl linoleate are not stable and isomerizes further into geometrical hydro-peroxide cis-trans and trans-trans isomers (Morales et al., 2014). These hydro-peroxides go through rearrangement leading to their isomerization in which their OOH group is relocated and stereochemistry of double bond change by a free radical chain mechanism (Schneider, 2009). Trans, trans-conjugated dienes of hydro-peroxides are produce under thermodynamic control while trans, cis-conjugated dienes of hydro-peroxides are produce under kinetic control (Pratt et al., 2011). The ratio of trans, cis /trans, trans products form during the oxidation depend on temperature, concentration of fatty

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acids and solvent (Porter, 2013; Niki et al., 2005). Fatty acids hydro-peroxides are formed by lipoxygenases catalyze dioxygenation of polyunsaturated fatty acids. This reaction is based on initial stereospecific abstraction of a hydrogen atom from a bis-allylic methylene group followed by antarafacial attack by dioxygen at one of the terminal carbon atom of the pentadienyl radical (Oliw et al., 2006).

Formation of Hydro-peroxides of Fatty Acids

Hydro-peroxides, as primary oxidation products of lipid are formed through the mechanisms of autoxidation, photo-oxidation and enzymatic oxidation of lipids (Blair, 2001). Autoxidation is a reaction between oxygen and the unsaturated lipids to form a lipid hydro-peroxide, which then undergoes further reaction with or without the participation of other compounds. This reaction is enhanced by light and metal ions (Sun et al., 2011). Another pathway for the formation of hydro-peroxides is photoxidation, where hydro-peroxides are produced from unsaturated fatty acids with the reaction of singlet oxygen. During photoxidation mechanisms each double bond of fatty acid react with singlet oxygen and produces hydro-peroxide that causes flavour deterioration of food by monohydroperoxides hemolytic and heterolytic cleavage mechanisms (Medina-Meza et al., 2014). Enzyme such as lipoxygenase tightly controls the reaction with molecular oxygen that form mixed products and permit the release of free radicals (Mandal et al., 2014).

Decomposition of Hydro-peroxides

Long chain poly unsaturated fatty acids degrade in the presence of oxygen and metals ion that causes deterioration of sensory properties, like taste and odor due to formation of primary oxidation products by decomposition of hydro-peroxides (Jimenez-Alvarez et al., 2008). In the presence of metal catalysts hydro-peroxides decomposed thermally by involvement of hemolytic cleavage of O-O bond of hydro-peroxides to produce alkoxyl and hydroxyl free radicals, reaction of these radicals with lipid substrate propagate the chain reaction and produce hydrocarbons, alcohols and hydro-peroxides (Kubow, 1992). These hydro-peroxides decompose in to α., β-unsaturated aldehydes like 9, 12-dioxo-10(E)-dodecenoic acid, 4-oxo-2(E)-nonenal, 4, 5-epoxy-2(E)-decenal and 4-hydroxy-2(E)-nonenal (Williams et al., 2007). Decomposition of hydroperoxides of cyclohexene, linoleic, linolenic acids and their methyl esters produce carbonyls. Decomposition of methyl ester hydro-peroxides are slow than of free fatty acids (Elisia and Kitts, 2011) and produce wide range of carbonyl compounds such as hydrocarbons and furans that contributes to flavour deterioration of foods (Csallany et al., 2015). Lipids hydro-peroxides reduce into dimmers and polymers that can breakdown and produce volatile products such as malonaldehyde. These malonaldehyde as bifunctional breakdown products can interact with amino group of enzymes, proteins and DNA to cause cellular damage (Lu et al., 2014).

Free Radical Reactions in Decomposition of Hydroperoxides

Formation of peroxyl or alkoxyl radical is the initial step during decomposition of hydroperoxides through free radicals. This reaction is reversible for peroxyl radicals and irreversible for alkoxyl reaction (Yin et al., 2011).

- $LOOH \rightarrow LOO^{\cdot} + H^{\cdot}$
- $LOOH \rightarrow LO^{\cdot} + O\,H$

It relatively difficult to generate peroxyl and alkoxy radicals from hydro-peroxides. Rate of radical reaction is initiated by heat, light and catalyst species. The energy required to dissociate a LOO-H bond is 90 kcal/mol while for LO-OH bond is 44 kcal/mol (Yin et al., 2011). Hydrogen ion abstraction occurs with another radical then peroxyl radicals formed from hydroperoxides as shown is the below reaction:

- $LOOH + L^{\cdot} \rightarrow LOO + LH$

Bimolecular reaction decomposes hydroperoxides by forming peroxyl and aloxyl radical (Wardhani et al., 2013; Sun et al., 2011).

- $2\,LOOH \rightarrow LO^{\cdot} + LOO^{\cdot} + H_2O$

Trace metals play important role in the decomposition of hydro-peroxides, whereas activity of various metal ions also affected by various variables like pH, complexity of their ligands and solvents. Decomposition of hydro-peroxides through metals ion is high in speed than those of autocatalytic reactions (Waraho et al., 2011; Thanonkaew et al., 2006). Higher oxidation process by metal ions produce peroxyl radicals while with lower oxidation process metal ions produce alkoxy radicals (Min and Ahn, 2005; Yin et al., 2011).

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- $LOOH + M^{(n+1)+} \rightarrow LOO^{\cdot} + H^{+} + M^{n+}$ (peroxyl radicals)
- $LOOH + M^{n+} \rightarrow LO^{\cdot} + OH^{-} + M^{(n+1)+}$ (alkoxy radicals)

Conclusion

Lipid oxidation is one major cause of deterioration of the quality of stored food. This review deals with the mechanisms of lipid oxidation, as well as the interaction of lipid oxidation products with other food components. In order to prevent lipid oxidation in food the mechanisms of lipid oxidation should be comprehensively understood and in particular the control of free radical formation is very important because free radical is able to initiate free radical chain reactions. Iron and copper ions play very important role in lipid peroxidation and act as free radical which can directly and indirectly catalyze the initiation of lipid peroxidation by extracting hydrogen from lipid molecule. General chemistry of oxidation of lipids suggests that further efforts should be directed to a detailed understanding of the effects of this process in specific foods and food products. Codex Alimentarius provided some guidance that accepts levels of about 10 meq/kg of peroxides in fats and oils for edible purpose, based on establishment of acceptable limits of oxidation criteria of wholesomeness, nutritional and safety values. Possible targets for future research are:

i) Elucidation of the initiation step of oxidation in specific foods and possibilities and mechanisms of inhibition.
ii) Standardization and development of set of analytical procedures for evaluating the level of lipid oxidation in foods and completely decay the level of oxidation and isolation of oxidized lipids in individual foods under conditions that protect from breakdown of the unstable and formation of new products.
iii) Study of methods to identify the oxidation products from lipids, lipid extraction and analysis of the synergistic effects of per-oxidizing lipids and adjoining food constituents.
iv) Investigation of the content of antioxidant such as tocopherol, butylated hydroxytoluene and oxidized products from antioxidants such as tocopherol quinone or free radical.

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Authors' Contribution

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