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Review Article

Role of Lipid Per-Oxidation in Quality Aspects of Muscle Foods during Storage

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Abstract

Lipids are imperative component of meat and contribute to numerous enviable characteristics of meat and meat products. Lipids are contributing factor for flavor and aroma profiling along with improved tenderness and juiciness of meat. However, scientific findings established the fact that lipid oxidation is the primary process responsible for quality deterioration of meat during storage. The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage followed by nutrient loss, color change, formation of toxic compounds, and heath risk to consumer. The rate of lipid per-oxidation in meat depends upon the balance between endogenous and exogenous factors of the meat that too varies among meats from different animal species and muscles from the same animal. Application of natural/synthetic anti-oxidant source in the meat is beneficial to modify the oxidative stability of meat.

Keywords: Lipid Per-Oxidation; Antioxidant; Meat; Factor; Iron.

Introduction

Consumer concerns on the quality of meat and meat products have significantly improved during past decades. The most important attitudes in purchasing meat include appearance/color, texture such as tenderness and juiciness, flavor/taste, firmness, cohesiveness, functional properties for instance water-holding capacity, emulsifying ability, microbial quality and nutritive values (Toldra, 2007). Among these, however, three sensory properties viz. appearance/color, texture, and flavor are the main factors which consumers utilize to evaluate meat quality (Ponnampalam et al., 2001). Consumer's purchase decision for meat is more strongly affected by changes in appearance/color and texture than those in flavors because visual appearance is more decisive factor especially at the market (Min, 2006)

Lipid oxidation is one of the primary factors limiting the quality and acceptability of meats and other muscle foods (Zamora and Hidalgo, 2001). The precarious balance between pro-oxidative and anti-oxidative factors in muscle is destroyed during conversion to meat after slaughter, resulting in initiation and propagation of lipid per-oxidation, generating compounds that might be detrimental to human health. The susceptibility of meat to lipid per-oxidation depends upon endogenous and exogenous factors. It is supposed that meat with higher heme pigment content produce more hydrogen peroxide (H_2O_2) during oxymyoglobin auto-xidation which reacts with metmyoglobin to generate ferrylmyoglobin, eventually initiating lipid per-oxidation. Therefore, catalase activity can be an important determining factor for lipid per-oxidation in addition to various iron catalysts along with differences in fat content, fatty acid composition,

endogenous antioxidants such as carnosine and related dipeptides. Antioxidant enzymes may also play important roles in maintaining oxidative stability of meat. Primary and secondary products of lipid oxidation are extremely reactive with other components of meat, changing their physical and chemical properties, imparting a specific flavour and aroma. Oxidised proteins take on a yellowish red through brown hue. Furthermore, toxic substances (such as biogenic amines) are formed as a result of interactions between meat components, e.g. protein-lipid or protein-protein combinations as well as transverse bonds in protein structures. On one hand it is essential to imbue flavours and aroma characteristic for ripening products, on the other hand excessive amounts or unwanted transformations may cause the meat product to become a risk to health of consumers.

Process of Lipid Per-Oxidation

Lipid per-oxidation, particularly the phospholipids in membranes of muscle cells, is a free radical-driven process of fatty acid oxidation producing fatty acid peroxides accelerated by presence of main oxidation factors i.e. oxygen and light (Fellenberg and Speisky, 2006). It occurs in three steps: initiation, propagation and termination. Initiation involves removing a hydrogen atom, most commonly located between two double bond molecules, from a free polyunsaturated fatty acid or fatty acid of a phospholipid molecule (Morrissey et al. 1998) transforming it into an alkyl free radical with a group of conjugated bonds (Fellenberg and Speisky 2006). Initiation is stimulated the presence of hydroxyl, alkoxyl, peroxide and alkyl radicals, as well as nitrogen oxide and dioxide, ozone, and sulphur dioxide (Hêœ and Korczak 2007 a, Fellenberg and Speisky 2006). The propagation step leads to oxygen molecules binding to the alkyl free radical, resulting in the formation of peroxide free radicals which stimulate the initiation of subsequent polyunsaturated fatty acids, eventually leading to the formation of lipid peroxide. This chain reaction stops with the production of a non-radical species (termination step). Products of the termination step are lipid dimers, oxo- or hydroxy fatty acids, and other modified and damaged lipid molecules. Further degradation of PUFA residues produces molecules with a dozen or so carbon atoms, including malondialdehyde (MDA), 4-hydroxyalkenals, 2-alkenals, hepta 2, 4-dienal, 4-hydroxynonenal (4-HNE), 4-hydroxy-*trans*-nonenal, and hydroxyoctanal.

Re-initiation of lipid peroxidation may be influenced by the presence of transition metal ions – haem (ferryl radical (Fe^{4+}, Fe^{2+} or Fe^{3+}) (Bartosz, 2006) and non-haem iron, glucose or glycosylated peptides. Heme metal ions break lipid peroxides into free radical products, Fe^{2+} ions reacting faster than Fe^{3+} ions (Carlsen et al., 2005). Non-haem iron may accelerate lipid oxidation processes, especially in acidic environments (4.5 pH found in raw/ ripening meat products) (Carlsen et al., 2005). Hernández et al. (1999) noted increased levels of peroxides in raw pork loin as compared to cured and dried product due to reduction in the peroxide number and concurrent increase in the TBA value as a result of transformation of primary products of lipid degradation into secondary products like MDA. However, MDA is just one of many products of lipid degradation and may be subject to further transformations.

Although oxygen is essential for life, excess oxygen causes toxicity by the increased production of Reactive Oxygen Species (ROS) that are normally produced in just enough amounts under normal physiological conditions to be dealt by the natural defence systems in body. Excess ROS is reduced by way of one-electron reduction processes which further produces short-lived but highly reactive oxygen products such as hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroperoxyl radical ($HO_2^{\bullet}$), and iron-oxygen complexes (ferryl and perferryl radical), all of which may directly or indirectly participate in lipid per-oxidation processes in meat and meat product.

Superoxide anion radical ($O_2^{\bullet}$) is an intermediate in various biochemical reactions in body. It is poorly reactive in aqueous solution whereas highly reactive in hydrophobic environments. Physiologically, it could be generated in muscle tissues by various components of electron transport chain in mitochondria such as NADPH-dependent dehydrogenase and ubiquinone which may leak electrons onto O_2. Hydroperoxyl radical ($HO_2^{\bullet}$), the protonated form of $O_2^{\bullet}$ is more reactive than O_2 itself.

Factors Affecting Lipid Per-Oxidation

Lipid per-oxidation in muscle food is determined by both intrinsic and extrinsic factors for example the presence of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, oxygen consumption reaction and the fatty acid profile (Andreo et al., 2003). Metals, besides iron (copper, especially) supplied by water, processing equipment and spices, can also encourage oxidation. The susceptibility of meat to lipid per-oxidation

varies among meat from different animal species as well as muscles from the same animal.

Endogenous Factors

Comprise of total lipid content, its fatty acid composition, types and amount of iron present, reducing compounds (e.g., ascorbic acid), natural antioxidants (carnosine, anserine and á-tocopherol, â-carotene, Coenzyme Q), and antioxidant enzymes (Glutathione peroxidase, catalase, superoxide dismutase etc). Concentration of heme pigment and catalase activity determines the rate of lipid per-oxidation in raw meat. Meat with higher heme pigment content supposedly produces more hydrogen peroxide (H_2O_2) during oxymyoglobin autoxidation than that with less heme pigments which further reacts with metmyoglobin to generate ferrylmyoglobin and initiate lipid per-oxidation. Reducing compounds such as ascorbic acid plays a critical role in the progress of lipid per-oxidation by serving as an electron donor in free radical-mediated oxidative processes. Lipoxygenase activities are found in various mammalian tissues and can be an important determinant for the oxidative susceptibility of muscle tissues.

Significance of Iron

Iron is the most abundant transitional metal in biological systems, present in various oxidation states (Fe^{2+} and Fe^{3+} most dominant), reduction potential, and electron spin configuration. It may serve in multifunctional roles as a protein cofactor, catalysis of lipid per-oxidation in the model systems with various meat species such as beef and fish. Rhee and Ziprin (2001) showed that the levels of lipid per-oxidation depended on animal species and muscle type in raw meat, and beef was more susceptible to lipid per-oxidation than pork and chicken muscle. Hydrogen peroxide (H_2O_2) and ascorbate present in the cytosol of muscle cells can release free iron from heme pigments and ferritin, respectively, which can catalyze lipid per-oxidation in meat and meat products (Boyer et al., 1987).

The decomposition of lipid hydroperoxides involves further free radical mechanisms and the formation of non-radical products. Homolysis of lipid hydroperoxides to hydroxy and alkoxy radicals, followed by cleavage of the fatty acid chain adjacent to the alkoxy radical produces low molecular weight volatile compounds (complex mixtures of aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones) imparting distinct aromas and flavor at as low concentrations as <1ppm (Frankel, 1984). Further oxidation may occur in the original peroxides or in the unsaturated aldehydes, which then undergo further degradation to form epoxides, cyclic peroxides and bicyclic endoperoxides (Enser, 1987). These secondary oxidation products can also break down to form volatile materials and dialdehydes contributing to flavour deterioration.

Auto oxidation of main unsaturated fatty acids in lipids of animal tissues (oleic, linoleic, linolenic and arachidonic) produces different hydroperoxides during storage, producing off-flavors and rancidity, a notable exception observed in dry cured country hams and some fermented sausages, where the pleasing flavor does not occur until hydrolysis of some of the fat and a certain degree of oxidation has taken place during ripening (Pearson et al., 1977). On the other hand, lipid oxidation during cooking may form key constituents needed to generate desirable flavor of normal cooked meat (Enser, 1987).

Exogenous Factor

The exogenous factors consist of oxygen, heating, addition of salts, temperature abuse during handling and distribution, prolonged storage, etc.

Light and Oxygen

Numerous kinds of light like blue-purple fluorescent (and ultraviolet), appear to photo activate meat pigments and elevate oxygen to a high-energy state increasing their abilities to commence oxidation (Jadhav et al., 1996). Oxygen is a fundamental part of the reaction. The interior of whole muscle cuts contains very little oxygen; however mechanical manipulation like grinding, chopping, mechanical deboning, chunking and forming, mixing and tumbling may mix large amounts of air into the product, promoting oxidation and Warmed Over Flavour (WOF) if the product is precooked and stored.

Temperature

High temperature causes the release of oxygen and free iron as well as the reduction of activation energy for lipid per-oxidation and production of free radicals from hydroperoxides which stimulates autoxidation process and off flavor production (Kanner, 1994). Heating disrupts muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments. Free iron is readily converted into its oxidized ($Fe3^{+}$) form, the conversion is essential for generation of free radicals from meat fats (Love, 1987). Oxidation of lipids also occurs

during postmortem storage of muscle tissue. Lynch et al. (2001) demonstrated that lipid oxidation occurred progressively in stored ground beef at 4°C and produced a variety of aldehydes. Fatty fish undergo rapid lipid oxidation during iced storage due to the high content of polyunsaturated fatty acids (Chaijan et al., 2006). Lipid oxidation rates enhance with temperature and time.

Pro-Oxidants

Furthermore, NaCl is capable to catalyze lipid oxidation in muscle tissue. Alternatively, the Na^+ may replace iron from a cellular complex via an ion exchange reaction (Kanner and Kinsella, 1983). The displaced iron may then contribute in the instigation of lipid oxidation.

Interrelationship between Lipid and Myoglobin Oxidations in Muscle Foods

Heme pigments, particularly myoglobin, catalyze the lipid oxidation in meat. Myoglobin and other heme compounds in red meats function as pro-oxidants in muscle tissue. It has been usually believed that lipid oxidation in meat is non enzymatic process and is catalyzed by myoglobin which gets oxidized ensuing production of pro-oxidants, specifically metmyoglobin and H_2O_2 (Chan et al., 1997). It has been proposed that production of superoxide, hydroperoxyl radical ($HOO^{\bullet}$), and H_2O_2, during oxidation of oxymyoglobin to metmyoglobin, induce the development of red-brown color by changing the oxidation state of the iron in myoglobin. Additionally, H_2O_2 can react with metmyoglobin to form a pro-oxidative ferrylmyoglobin radical (Baron and Andersen, 2002).

These reactive products of autoxidation of oxymyoglobin, can cause damage to muscle lipids through oxidation. Metmyoglobin is an effective pro-oxidant at acidic pH and in the presence of hydroperoxides, but undergoes a rapid neutralization at physiological pH and in the presence of lipids, due to formation of the noncatalytic heme pigment. However, further denaturation of the heme proteins in a high lipophilic environment may result in heme release or further exposure of the heme group to the surrounding lipids, thereby inducing lipid per-oxidation (Baron and Andersen, 2002). Metmyoglobin acts as a pro-oxidant in raw fish more effectively than in raw turkey, chicken, pork, beef and lamb. Lipid to heme protein ratio is a significant factor affecting the pro-oxidative activity of heme proteins reaction.

Ferrylmyoglobin in Lipid Oxidation

Interaction between metmyoglobin and H_2O_2 is an intricate mechanism, resulting in the generation of two discrete hypervalent myoglobin species, perferrylmyoglobin and ferrylmyoglobin (Baron and Andersen, 2002). H_2O_2 activation of metmyoglobin is an essential step in the conversion of metmyoglobin to a pro-oxidant (Kanner and Harel, 1985).

Oxidized Lipid/Protein Reactions

The interaction between oxidized lipids, proteins and constituting amino acids may cause either the arrangement of physical complexes between the oxidized lipids and the protein or the formation of various types of covalent bonds, besides the production of protein radicals (Gardner, 1979). Protein polymerizes with peroxy free radicals through hydrophobic association and/or hydrogen bonds during non-enzymatic browning. The strength of these lipid-protein complexes can be defined by a series of extraction steps, and complexes may be broken with urea/ sodium dodecyl sulfate (Kanner and Karel, 1976). "Secondary product" is a generic term used to describe a mixture of aldehydes, epoxides, ketones, and other products obtained from the decomposition of lipid hydroperoxides. Although most of the peroxidized lipids usually can be removed from protein by methods that disrupt hydrogen bonds, the lipid resistive to these methods can be separated only after chemical treatment and it is likely to be bound mainly by covalent bonds.

Warmed Over Flavor

WOF begins with oxidation of meat fats (Willemot et al., 1985). Meat and meat products containing more polyunsaturated fat (PUFA) are more prone to get oxidize and develop WOF. PUFA's are mainly located in the cell membrane as phospholipids, are more readily oxidized than saturated fatty acids by losing additional hydrogen atoms from the carbon adjacent to the points of unsaturation. Hydrogen abstraction from these points forms lipid (fat) free radicals which are extremely reactive and tend to take up oxygen (oxidize) very quickly, breaking apart into smaller molecules, such as pentanal, hexanal, and 2,4-decadienal, responsible for the off-odors and flavors as warmed over (Vega and Brewer, 1994). These products are extremely volatile, traceable in very low concentrations (parts per billion), fat-soluble and may partition into the melted fat phase where they are retained until the fat is reheated. Because of the relative higher PUFA content, the oxidation and WOF follows the rate of order as: fish > poultry > pork >

beef > lamb. Re-heating (cooking) is one of the principal causes of WOF development. It causes proteins to coagulate so that they lose their functional capabilities i.e. enzymes no longer assist with reactions, fibrous proteins can no longer hold onto water so they shrink, and the globin-bound heme fractions of hemoglobin and myoglobin can no longer hold onto iron. High temperature also causes the release of oxygen and free iron as well as the production of free radicals. The globin-bound heme group usually holds onto and protects from coming into contact with oxidizable substances. Conversion of free iron from its reduced (Fe^{2+}) state into its oxidized (Fe^{3+}) form assists in generation of free radicals from meat fats (Love, 1987). There is still substantial debate as to the importance of heme-bound iron and free iron in the role of lipid oxidation.

Measures to Reduce Oxidation

Antioxidants protect PUFA from oxidation by sacrificing themselves to the oxidation process; thereby extending the induction period (Jadhav et al., 1996). Primary antioxidants are free radical terminators that bind the oxidative radical. Their protective effect is mainly dependent on concentration, fat solubility and on the number of antioxidative sites on the molecule. Most antioxidants, including vitamin E, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG) are phenolic substances that may be naturally occurring or chemically synthesized. Their fat solubilites differ affecting their applicability in muscle food systems. BHA, BHT and TBHQ are effective at 100-200ppm, PG at 200-250ppm. The effectiveness of synthetic, phenolic antioxidants as compared to chelators, in the prevention of WOF is unclear; because being lipid soluble, they are difficult to distribute in the product. Oxidation, initiated or propagated by metal ions, can be effectively suppressed or delayed by chelating agents such as citric acid, ethylene diamine tetracetic acid (EDTA), and some phosphates which complex with metals (iron, copper) stabilizing them so they do not participate in the oxidation reaction. Ascorbic acid and its mirror image molecule, erythorbic acid, function as oxygen scavengers in fat-containing foods. They are added to cured meat products to prevent nitrosamine formation, but also serve to prevent lipid oxidation. Herbs and spices contribute a variety of antioxidant substances. Common spices with antioxidative properties include rosemary, marjoram, sage, thyme, mace, allspice, nutmeg, cinnamon and clove (Naveena et al., 2006). Certain herbs like lemon grass, mint, jimbu are having potent anti-oxidant properties. Rosemary is particularly effective because of a number of phenolic compounds including carnosic acid (odorless), rosmanol (odorless), rosmariquinone and rosmaridiphenol (Jadhav et al., 1996). Rosemary oleoresin (oil soluble) has been shown to be effective in turkey breakfast sausage and ground beef patties (St. Angelo et al., 1990). Physical means of delaying the onset of WOF include oxygen exclusion by the use of technologies such as vacuum tumbling and vacuum stuffing prior to cooking and vacuum packaging cooked products prior to storage. Because light can photosensitize meat pigments and oxygen, light exclusion may be a factor for some products. Pre-cooked products can be protected from oxidation by covering them with liquid or sauces prior to freezing.

Conclusion

Lipids are primarily responsible for desirable flavors and aromas in meat. However, lipid oxidation in most cases deteriorates the quality of meat and makes it unacceptable from aesthetic, nutritional and organoleptic points of view. Lipid per-oxidation may also lead to decrease shelf life, change in sensory characteristics, and sometimes may be detrimental due to formation of carcinogenic substances. Heat and light, catalysts, oxygen content, phospholipids, unsaturated fatty acids, condition of pre-slaughter, pH and processing conditions destroying muscle membranes etc. are the main factors accountable for lipid oxidation in muscle foods. The use of synthetic and natural antioxidants is done to prevent lipid oxidation, retard development of off-flavors, and improve color stability of meat and meat products. However, meat industry is demanding antioxidants from natural sources to replace synthetic antioxidants because of the negative health consequences and toxic effects. The application of hurdle technology like use of antioxidants with spices, irradiation etc. may also be helpful to retarded the lipid oxidation in meat. Appropriate packaging, super chilling and proper handling during storage and marketing may also have synergistic effect for augmenting the shelf life of meat products by retarding the lipid oxidation.

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