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# EVALUATION OF MEAT AND MEAT PRODUCT OXIDATION AND OFF-FLAVOR FORMATION: MANAGING OXIDATIVE CHANGES

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## Abstract

One of the primary issues with processed foods during heat treatment and freezing storage is fat oxidation, which causes significant changes in fats due to their interaction with reactive oxygen species (ROS). This interaction leads to the creation of various aldehydes that have a high affinity for large molecules, such as proteins, leading to the formation of final products of advanced oxidation processes that contribute to food spoilage. Co-oxidation can also result in extensive damage. Another problem affecting the quality and nutritional value of meat products is protein oxidation, which can occur during storage via freezing and thawing or as a result of heat treatment. Heat treatment can cause physical and chemical changes, such as the loss of some essential amino acids and the transformation of certain amino acids into carbonyl compounds via various mechanisms. Protein oxidation is indicated by the accumulation of these carbonyl compounds, and the heat treatment can lead to the denaturation of myoglobin, which is responsible for the brown color of cooked meat and is influenced by several factors. Active protein aggregates can interact with the oxidation products of polyunsaturated fatty acids and with carbohydrate glycation or glycoxidation to produce Maillard products. It is critical to understand the oxidative changes that occur in fats and proteins in food, particularly in meat products, since these components are among the primary constituents of food.

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## Introduction

Meat and meat products (i. e., burgers, nuggets, shawarma meat) are highly valued for their nutritional and sensory properties making them popular among consumers. However, they are also particularly vulnerable to spoilage due to their chemical composition. To preserve them, several meat preservation methods are used in addition to freezing and heat treatment, such as chilling, smoking, canning, dehydration, and irradiation, which are widely used methods that help maintain the quality of meat and meat products during storage [1]. The quality of frozen meat products depends on such factors as the growth of microorganisms, physical and chemical changes, and storage temperature, which should be at or below -18 °C as indicated, for example, in Council Directive 89/108/EEC for quick-frozen foodstuffs [2]. Other factors that can affect the quality of meat and meat products include the length of the storage period, the duration of the thawing process, and the type of packaging material used. These factors also play a key role in the formation of ice crystals [3]. The size and distribution of ice crystals within the cells and spaces of frozen meat can vary depending on the rate, at which the meat is frozen.

During the freezing process, many interactions can occur between the components of meat, which can alter their properties. For example, the oxidation of fats can lead to the formation of various byproducts, such as peroxides and aldehydes, including malondialdehyde (MDA), as documented in studies by [4,5,6]. These fat oxidation products can make proteins in meat more vulnerable to oxidation during cryogenic storage and heat treatment. Protein oxidation (P-OX) is a complex chemical-physical reaction that depends on various factors, such as the starting conditions of the reaction and the sensitive targets of the reaction, and involves several modifications to the peptide backbone and amino acid side chains, which are very sensitive to oxidative stress. Chemical changes associated with protein oxidation include the loss of sulfhydryl groups, loss of tryptophan fluorescence, and gain of carbonyl derivatives, as well as the formation of intra- and intermolecular cross-links [7]. The DNPH technique is commonly used to estimate the total amount of protein carbonyls in meat as a way of assessing P-OX.

The practice of heat treatment in meat and meat products has been around for a long time and has evolved into modern technologies. This process is necessary to make raw

meat suitable for consumption by eliminating pathogens and enhancing its sensory qualities. When meat is heated, the protein myoglobin undergoes a change that results in the appearance of a brown color, which varies based on the level of maturity. Although consumers often view the change in meat color from red to brown as an indication of maturity and product aesthetics, it can also be a health concern. The final color of a meat product can also be affected by browning, which can induce oxidative changes in fats that produce harmful compounds with metabolic effects on human health. This article aims to identify these oxidative changes in meat and meat products during freezing and heat treatment, and to explain how these changes are linked to color changes and the basic mechanisms that affect the final color of meat and meat products. The article provides a brief overview of freezing and heat treatment and their role in protein oxidation.

### **Objects and methods**

The sources of information were the following scientific databases: ScienceDirect, PubMed, Scopus, ResearchGate, and Google Scholar (accessed 03/25/2023). The search strategy included the following keywords: cryopreservation, oxidation of proteins, carbonyls, Mailard products, oxidation of myoglobin, cross-links. The following acceptance criteria for research characterization were considered: meat and meat products, oxidative reactions, analytical methods to determine lipid and protein oxidation, original research. The parameters of the publications were as follows: publication from 1988 until 2023 (86 references were selected for this review); language: English. Exclusion criteria: no access to the full-text articles. Based on the review, the authors compiled information on meat and meat products, preservation of meat, oxidative reactions, formation of crosslinking, and the analytical methods to determine lipid and protein oxidation in meat and meat products.

### **Meat and meat products**

Meat consumption, sourced mainly from herbivorous animals including cattle, buffaloes, goats, sheep, camels, and horses, is widely favored by individuals in both developed and developing countries. Meat is a very nutritious food that is widely used as a primary source of animal protein by people around the globe. Nevertheless, the American Heart Association [8] advises restricting daily meat intake to a maximum of six ounces. Meat is an essential element of human diet, and its chemical makeup might differ based on characteristics such type, breed, sex, age, animal feed, and tissue quality [9]. Meat and meat products provide vital nutrients, including premium proteins, lipids, and minerals. These goods are available in different forms, including cured meats, patties, nuggets, and meatballs. Processed meat products are food items that have been modified through methods including smoking, salting, and curing to improve their quality and increase their

shelf life. The composition of meat products exhibits variations in terms of amount, type, and manufacturing procedures, as well as disparities in shape and size. However, the main ingredient is beef, which can have a fat content of up to 30%. The high fat content enhances the flavor, taste, juiciness, and emulsion of the product.

### **Burgers**

A burger is a type of minced meat product that is flavored with spices and condiments, shaped to achieve, as a rule, a flat, disc-like shape, and cooked either by frying or baking. The name of a burger is linked to a meat type used, such as pork, beef, or chicken. Various proportions of fat are used in burger production. For example, according to the U. S. Code of Federal Regulations, hamburgers should not contain more than 30% of fat [10]. Fats are important for the sensory properties of the burger. To make a burger, meat must first be minced effectively to create a tender texture, then salted and mechanically mixed to bind the components before cooking [11].

### **Nuggets**

Consumers readily accept restructured breaded products, such as nuggets, as a substitute for chicken meat. Chicken nuggets are made from chicken meat trimmings, skin, water, phosphate, and salt. The nugget-making process involves using small and irregular pieces of meat, then binding them together with a binder to form a larger size. To meet the increasing demand for high-quality and long-lasting nuggets, many manufacturers add additional ingredients, such as phosphate. Although the use of phosphate is still uncertain in terms of health benefits, it is added to produce a chewy and durable product [12]. The ability to bind meat particles with other ingredients is a critical factor in determining the success of a product. Finally, chicken nuggets are coated in batter and breadcrumbs, deep-fried or baked, and then quickly frozen.

### **Shawarma meat**

Nowadays, shawarma meat is widely recognized as one of the most favored item, although it is not an indigenous product in many countries. In Sudan, for example, shawarma made its first appearance in the 1980s, originating from the Turkish region [13]. The term “shawarma” refers to thin slices of lamb, chicken, turkey, beef, veal, or a combination of meats seasoned with sauce, mixed with fat and spices, and prepared by arranging strips of fat and seasoned meat on a vertical skewer that rotates. The exterior of meat is roasted while the interior remains mostly uncooked. For serving, thin shavings are cut from the meat block, while the remaining portion of meat is kept warm on the rotating skewer [14].

### **Preservation of meat**

Meat is vulnerable to microbial and oxidative spoilage, which makes it one of the most perishable food

items. Raw meat can harbor harmful bacteria such as *Salmonella*, *Campylobacter*, and *Escherichia coli*, and meat products derived from pork, turkey, and broiler are known to be a significant source of foodborne illnesses such as salmonellosis [15]. This has made meat preservation important for transporting meat over long distances without deterioration of its texture, color, or nutritional value, especially with the development and growth of supermarkets [16]. Preservation methods primarily aim to prevent microbial spoilage, but other preservation techniques are also used to minimize other types of deterioration such as color and oxidative changes [17]. At present, traditional preservation methods such as drying, smoking, brining, fermentation, refrigeration, and canning as well as newer methods such as high hydrostatic pressure (HHP), modified atmosphere packaging (MAP), active packaging (AP), natural antimicrobial compounds, and biopreservation have been used [18]. The goal of preservation methods is to prevent microbial spoilage as well as to minimize oxidation and enzymatic spoilage. Current meat preservation techniques fall into three broad categories: controlling temperature, controlling water activity, and using chemical or bio-preservatives. These preservation techniques can be used in combination to reduce the rate of spoilage [15,16].

### *Chilling*

Proper chilling of meat is crucial for maintaining hygiene, safety, shelf life, appearance, and eating quality [17,19]. Chilling meat with air is effective in reducing the temperature of the surface and preventing bacterial growth. Decreasing temperature and increasing air velocity can shorten the chilling time, but cooling the deeper tissue of the carcass is challenging. Natural-convection air chilling is slower and less controlled, while forced-convection air chilling with fans is more efficient. Quick chilling improves product yield by reducing surface evaporation and bacterial growth. However, ultra-rapid chilling of pre-rigor meat can cause cold-shortening and toughening [16,17]. Spray-chilling helps oxygenate the surface myoglobin, maintain brightness, and prevent weight loss without increasing metmyoglobin [17].

### *Freezing*

Freezing meat on a large scale began in Britain around 1880 with the arrival of frozen beef and mutton from Australia [18]. The benefits of freezing meat below the freezing point include extending its useful storage life and discouraging microbial and chemical changes. Quick freezing creates tiny ice crystals within meat cells, reducing drippings when thawed. The freezing rate depends on a size of meat, its thermal properties, a temperature of the refrigerating environment, and a method of refrigeration, as well as a type of wrapping material used for smaller cuts. To prevent quality changes in frozen meat, a temperature of  $-55^{\circ}\text{C}$  has been recommended as ideal

storage conditions, where few deteriorative changes occur during storage due to minimal enzyme reactions, oxidative rancidity, and ice recrystallization [17]. Cryogenic freezing is faster than conventional air freezing because of the large temperature differences between the cryogen and the meat product, resulting in a high rate of surface heat transfer caused by boiling the cryogen. Cryogenic freezing can be achieved with a cryogen tank and suitable spray equipment, without the need for mechanical refrigeration equipment. However, the process may cause some distortion to the shape of the product, which could affect its commercial application. Additionally, the cost of cryogenic liquid is relatively high, which may limit its commercial use [17].

### *Curing*

Various methods of curing meat are practiced, including dry cure, pickle cure, injection cure, and direct cure, using ingredients such as sodium chloride, sodium nitrate, sodium nitrite, and sugar. Preservation of meat through heavy salting has been a long-standing practice, and sodium chloride has been historically used in food preservation due to the lack of refrigeration facilities [16]. Later, the use of common salt and sodium nitrate resulted in improved products. Sodium chloride inhibits microbial growth by increasing osmotic pressure and decreasing water activity. Concentrations as low as 2% can inhibit some bacteria, and a concentration of 20% can inhibit many food spoilage yeasts. However, some microorganisms from the *Bacillus* and *Micrococcus* genera can tolerate high concentrations of salt. The current limit for nitrite in meat products is 156 ppm in the US and 200 ppm in Canada, as the use of nitrite as a food additive can form carcinogenic nitrosamines with prolonged exposure. However, there is no epidemiological evidence to support the relationship between nitrate consumption and a specific cancer or cancer risk [16,19,20].

### *Smoking*

Smoking meat has historically been used to preserve it for extended periods of time. Smoke contains a variety of substances resulting from the breakdown of wood, such as organic acids, aldehydes, ketones, and phenols, among others. Preservation of meat through smoking is also accomplished by surface dehydration, reduction of pH of the meat surface, and the antioxidant properties of smoke components. Curing and smoking of meat are closely linked, with smoke generated in specialized smokehouses where sawdust, hardwood, or both are burned at around  $300^{\circ}\text{C}$ . The combustion process generates numerous organic compounds and their condensation products, with aldehydes and phenols accounting for 50% of smoke components and contributing significantly to the color of smoked meat products. Phenols are the primary bactericidal compounds [16].

### Canning

The process of canning meat helps maintain its sensory properties, including its appearance, flavor, and texture, to a significant degree. Additionally, canned meat products can last for at least two years at room temperature. Canning involves various stages, such as meat preparation, precooking, filling, exhausting, seaming, thermal processing, cooling, and storage [16].

### Dehydration

This process of preserving meat involves removing water from a meat concentrate, which makes water-soluble nutrients inaccessible to microorganisms. In ancient times, people used sun drying of meat chunks for preservation, but rehydrating such meat was challenging. Mechanical drying involves passing hot air with controlled humidity, but it also poses difficulties for rehydration. However, freeze drying is a satisfactory process for dehydration and preservation due to better reconstitution properties, nutritive quality, and acceptability of meat products. This process involves removing water from food by sublimation from a frozen state to a vapor state by keeping it under vacuum and giving it low heat treatment. Freeze drying of meat is carried out in three steps: pre-freezing, primary drying, and secondary drying. Meat is first frozen at  $-40^{\circ}\text{C}$ , and then it is dried under vacuum for 9–12 hours at low temperature in plate exchangers at 1–1.5 mm pressure of Hg [20]. During primary drying, free and immobilized water of meat, which constitutes about 90–95% of total moisture, is removed. Secondary drying is done at high temperature to remove the remaining 4–8% bound water. After freeze-drying, meat products are packed under vacuum and have excellent storage stability. This process is commonly used for preparing dehydrated meat soup mixtures [16].

### Irradiation

Irradiation, also referred to as “cold sterilization,” involves the propagation of energy through materials. Electromagnetic waves are emitted in a continuous form, which can ionize molecules in their path. These waves can break down the DNA molecules of microorganisms and ionize the water inside them, thereby destroying them. It is worth noting that irradiation destroys microbes in food without significantly raising the temperature of the food. Gamma radiation only affects food during irradiation and has no impact once the source is removed. Consequently, it is extensively employed for food preservation. While UV radiation mostly kills bacteria, it lacks sufficient penetrating power and is only utilized for surface sterilization of meat [16,21].

### Heat treatment

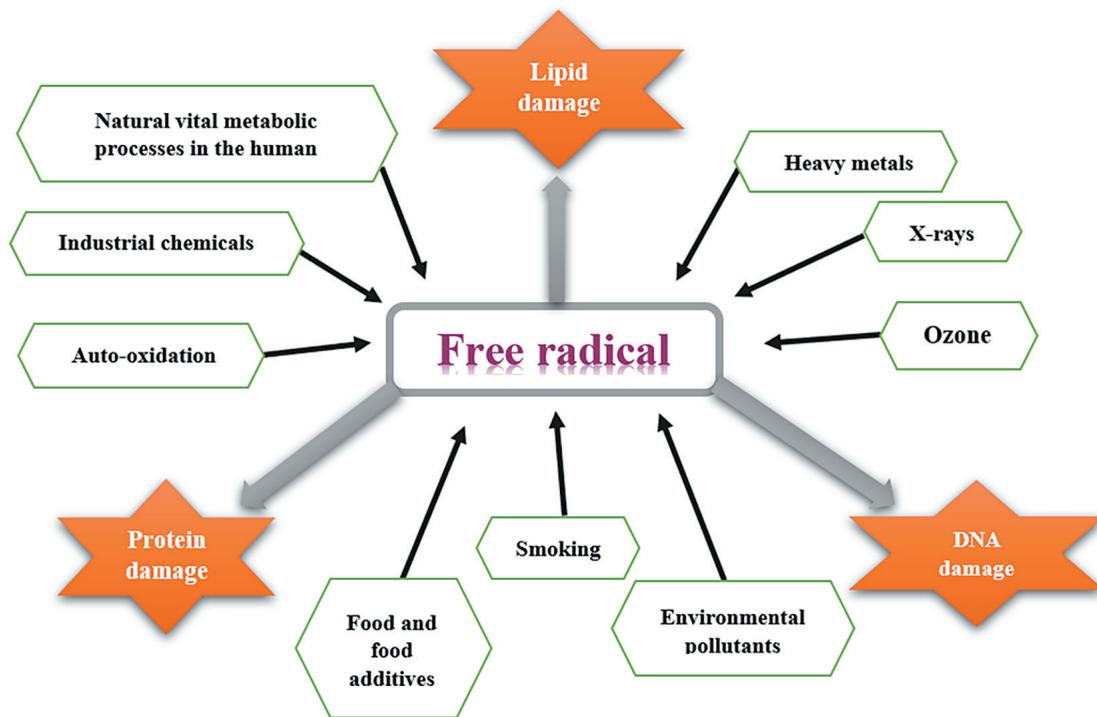
The previous studies mentioned in Table 1 show that when meat products are subjected to heat treatment, their chemical composition is affected. This means that cooking reduces the amount of moisture in a meat product, and increases the levels of fat and protein.

### Oxidative reactions

An imbalance between the generation of reactive oxygen species (ROS) and the capacity of biological systems to remove them leads to oxidative stress. It is widely recognized that elevated levels of ROS can cause direct harm to proteins, lipids, and nucleic acids. Among the ROS that have a considerable impact on lipids, the hydroxyl radical ( $\cdot\text{OH}$ ) and the hydroperoxyl radical ( $\text{HO}_2$ ) are the two most commonly occurring ones. The hydroxyl radical ( $\cdot\text{OH}$ ) is highly mobile and soluble in water, which makes it the most reactive ROS. As demonstrated in Figure 1, it can attack biomolecules [30].

**Table 1. Influence of heat treatment (cooking) on the chemical composition of meat and meat products**

Product	Chemical composition, %					References
	Moisture	Protein	Fat	Ash	carbohydrate	
Beef (lean)	75.0	22.30	1.80	1.2		
Fried beef (lean)	58.4	30.40	9.20	—		[22]
Raw beef burger	45.50	12.28	15.75	2.00		
Cooked beef burger	32.74	14.55	24.00	1.94		[23]
Raw chicken burgers	—	58.3	22.8	3.58		
Cooked chicken burgers (oven)	—	54.7	20.1	2.94		[24]
Patty burger before cooked	61.97	23.21	10.45	4.06	0.31	
Patty burger after cooked	64.95	22.12	8.86	3.86	0.17	[25]
Raw chicken burger		59.41	31.60	6.18		
Cooked chicken burger		63.53	30.39	6.01		[26]
Raw beef burgers	70.50	17.14	10.05	2.06		
Cooked beef burgers	57.81	25.54	10.15	2.29		[27]
Raw shawarma meat	69.24	23.0	3.34	2.84		
Cooked shawarma meat	55.72	28.0	10.0	3.67		[28]
Raw beef burger	59.60	14.29				
After frozen storage (3 month) and cooking	48.80	19.53				[29]



**Figure 1.** Sources and harmful effects of free radicals

#### Lipid oxidation in meat

Lipids are a vital element in all types of meat and meat-based products, as they provide desirable properties such as aroma, flavor, tenderness, and juiciness. However, the process of lipid oxidation is the primary factor responsible for quality deterioration and reduced shelf life. This natural and inevitable process affects various aspects of meat, including color, texture, nutritional value, taste, and aroma. It causes rancidity, leading to off-flavors and unpleasant taste, which often result in consumer rejection [31]. Lipid oxidation also causes the breakdown of fatty tissues in meat, ultimately affecting its commercial value [32]. Lipids are highly susceptible to oxidative reactions, which occur due to various factors such as the type of lipid structure, degree of unsaturation in fatty acids, exposure to light and heat, and presence of molecular oxygen, pro-oxidant, and antioxidant components. These reactions are quite complex and can significantly impact the oxidative stability of lipids [31].

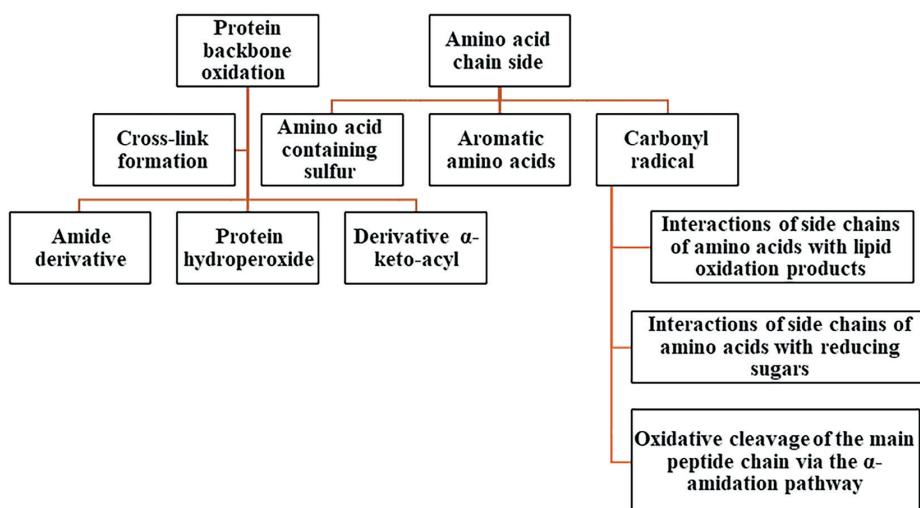
Muscle tissue contains natural components such as iron, myoglobin, hydrogen peroxide, and ascorbic acid that can cause lipid oxidation. They act as catalysts or promote the formation of reactive oxygen species, which can damage lipids. Oxidative reactions can also be triggered by physical factors such as radiation and light. There are three primary ways lipids undergo oxidation in biological systems: photo-oxidation, enzymatic oxidation, and autoxidation. Photo-oxidation occurs when radiant energy, such as ultraviolet radiation, interacts with sensitizers like myoglobin, leading to radical reactions that form hydroperoxides different from those produced without light and sensitizers [33]. Autoxidation is a complex chemical process that involves self-propagating radical reactions and depends

on such factors as temperature, pH, metal ions, and free radicals. Enzymatic oxidation is catalyzed by lipoxygenase, which oxidizes fatty acids, adding oxygen to the hydrocarbon chain. This process forms peroxides and hydroperoxides with conjugated double bonds that can undergo various degenerative reactions, forming multiple products.

#### Protein backbone chain oxidation

The peptide main chain and the amino acid side chains are susceptible to oxidative damage by reactive oxygen species (ROS). When metal ions and hydroxyl radicals are present, hydrogen atoms are removed from the  $\alpha$ -carbon in proteins, creating an  $\alpha$ -carbon radical and transforming the protein into a radical. This radical can become a peroxy radical (POO) in the presence of oxygen, which can then transform into an alkyl peroxide (POOH) by removing a hydrogen atom from an unstable molecule. Additional reactions with ROS and metal ions such as iron and copper can result in the formation of alkoxy and hydroxyl derivatives. When  $O_2$  is available, the radicals centered around the carbon atom turn into a peroxy radical  $RO_2\cdot$ , and alkyl peroxy reacts with  $H_2O_2$  to form hydroperoxides. Cross-links within or between protein molecules can form under anaerobic conditions due to radical interactions [3,34,35]. Figure 2 shows the oxidative modification of protein backbone and amino acid side chains.

Moreover, the process of protein oxidation by ROS can cause the breaking of peptide bonds. This occurs when alkoxy radicals and alkyl peroxide derivatives undergo cleavage through either the  $\alpha$ -amidation or di-amide pathways. Both pathways result in the formation of an amide derivative at the N-terminal end of the protein via the  $\alpha$ -amidation pathway and the derivative  $\alpha$ -keto-acyl at the C-terminal end of the protein via the di-amide pathway [36].



**Figure 2.** Oxidative modification of protein backbone and amino acid side chains

#### Oxidation of amino acid side chains

The oxidative processes, such as oxidation of thiol groups in cysteine, hydroxyl in cyclic amino acids, and formation of carbonyl groups, affect the side chains of amino acids. These processes result in the loss of functional properties of proteins, ability of proteins to aggregate and loss of solubility, which affects their ability to carry water. Oxidation of proteins in the presence of ROS can convert histidine to oxohistidine derivatives and imidazolone derivatives, while leucine and valine are transformed into hydroxyderivatives. One of the most significant outcomes of the oxidation of side chains of amino acids is the formation of various products [36,37,38,39].

#### Loss of thiols

The sensitivity of amino acids to oxidation processes is determined by their chemical properties. Cysteine and methionine are two amino acids that possess sulfur atoms, which make them highly reactive even when the concentration of reactive oxygen species (ROS) is low. When the SH group of these amino acids is oxidized, a hydrogen atom is extracted, leading to the formation of thiyl radicals. These radicals can either react with other thiol groups to create disulfide bonds or interact with oxygen ( $O_2$ ) to produce thiyl peroxy radicals (RSOO). These thiyl peroxy radicals undergo a series of reactions, as described by [35,40].

#### Cyclic amino acid oxidation products

Amino acids containing hydroxyl groups, such as histidine, phenylalanine, tryptophan, and tyrosine, are sensitive and prone to oxidation. These amino acid groups are not easily oxidized by metal-catalyzed reactions but are preferred targets for free radicals, according to studies by [41,42]. Tyrosine is particularly vulnerable to oxidation compared to phenylalanine, both within the same protein chain and across different protein chains. When tyrosine is exposed to free radicals, ultraviolet radiation, beta rays, or lipid hydroperoxides, it can be transformed into dityrosine. This conversion occurs when a tyrosyl radical is formed by the loss of one electron from tyrosine, and two single ty-

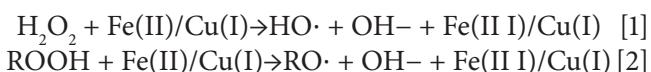
rosyl radicals can then combine to form dityrosine, which is a relatively stable molecule that is resistant to enzyme hydrolysis. Dityrosine can form cross-links both between and within proteins [42,43].

The rapid oxidation of the amino acid tryptophan is one of its distinguishing features. This is because tryptophan is among the amino acids that exhibit early oxidation. The oxidation of tryptophan results in several metabolites, such as -hydroxy-tryptophane when  $H_2O_2$  is present. Additionally, photo-oxidation of tryptophan produces formylkynurenine, kynurenine, and 3-hydroxykynurenine-3. The loss of tryptophan is an essential indicator of meat and meat product oxidation. This information is supported by research conducted by [35,36,41].

#### Protein carbonyl radical

Proteins are oxidized into carbonyl compounds through various pathways. The oxidation is irreversible and not carried out by enzymes. There are four main pathways for this reaction. The most common pathway involves the direct oxidation of certain amino acid side chains, such as lysine, threonine, arginine, and proline, which results in the formation of a carbonyl radical. Another pathway involves the interaction of amino acid side chains with reducing sugars, which contributes to the formation of carbonyl radicals. The third pathway involves the oxidative cleavage of the main chain of the peptide via the  $\alpha$ - amidation pathway [34,36]. Finally, certain amino acid side chains can interact with lipid oxidation products, which can also lead to carbonyl compound formation [34,36].

The way, in which certain amino acids are oxidized, directly depends on how metal ions interact with  $H_2O_2$  to create hydroxyl radicals, either  $\cdot OH$  or the highly reactive  $RO\cdot$  hydroxyl radical. These reactions occur near the metal binding site in the amino acids. In many biological systems, metals such as iron and copper act as either electron donors or acceptors. Metal-catalyzed oxidation (MCO) is thought to be the primary cause of oxidative damage because of the presence of these metals. This idea has been supported by research conducted by [36,44]



When  $\text{H}_2\text{O}_2$  and metals are present at high concentrations, almost all types of amino acids can be oxidized by resulting  $\text{HO}^\cdot$ . However, when the concentrations of iron or copper ions and  $\text{H}_2\text{O}_2$  are low, protein damage is likely to be limited. Metal binding sites on proteins are particularly sensitive to MCO catalysts, and oxidation occurs through a site-specific mechanism. This involves the binding of Fe (II) or Cu(I) to metal-binding sites on the protein, followed by a reaction with peroxide to form  $\text{OH}^\cdot$  and  $\text{RO}^\cdot$ , which are highly effective in reacting with amino acids. Research conducted by [36,45] has demonstrated this mechanism, as illustrated in Figure 3.

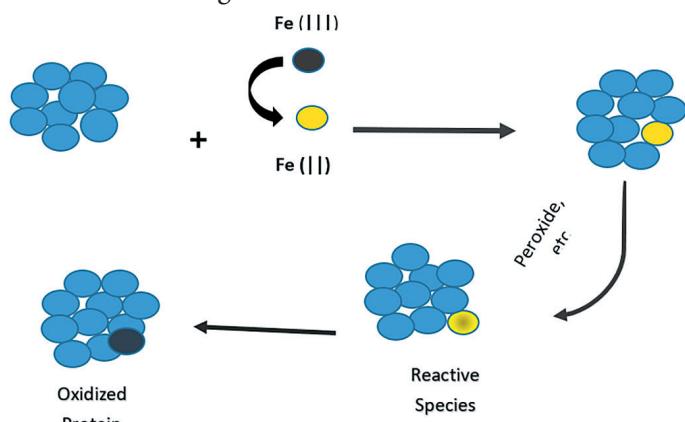


Figure 3. Metal-induced protein oxidation [36]

### Formation of crosslinking

ROS are produced in meat when it is exposed to oxygen and light during storage, and they react with the components of meat causing the oxidation of fats and proteins and the creation of radicals. There are various types of cross-links of meat proteins, including direct interaction between two carbon radicals, the formation of dityrosine from the interaction of two tyrosine radicals, and disulfide bonds formed from the reaction of radicals created from

the oxidation of cysteine. Cross-links are also formed from the reaction of the carbonyl radical of protein oxidation products with the  $\epsilon$ -amino group of lysine in the same or different protein, and the interactions of both aldehyde groups with malonaldehyde with two  $\epsilon$ -amino groups in different lysines in the same or different protein. Furthermore, junctions are created between the carbonyl radical of reducing sugars and their oxidation products with lysine or arginine in the same or different protein [36,46].

### Impact of frozen storage and production techniques on the oxidation of meat proteins and meat-based food items

Various methods of storage and manufacturing have a significant impact on the physical and chemical properties of protein in food systems, particularly meat, resulting in protein oxidation. As a result, the quality of meat is affected. Numerous research studies have focused on the influence of frozen storage and heat treatment on protein oxidation, and these studies and their respective detection methods are listed in Table 2.

#### Myoglobin pigment oxidation

The hue of fresh meat and meat products is a crucial quality feature that signals their freshness, and it has a significant impact on how consumers perceive and accept them. Any alteration in color can be a crucial factor that influences whether consumers will buy meat or its products. According to [56,57], there is a strong connection between color preference and purchase decision.

The color of meat is determined by the pigment myoglobin (Mb), and is affected by the myoglobin chemical state and concentration in the muscles [58]. Myoglobin (Mb) consists of a protein part called globin and a non-protein part known as the heme ring. The stability of the color is influenced by both oxidation and reduction states. When oxygen is removed from meat or when it is not exposed to oxygen, deoxymyoglobin is formed, resulting

Table 2. Protein oxidation in various meat types and meat-based products

Product	Method of storage and heat treatment	Indicator	Methods	Sources
Chicken burgers	Frozen			[47]
Chicken thigh and breast meat	Frozen			[48]
Chicken burgers	Oven cooking and microwaving			[24]
Beef patties	Roasting			[49]
Beef patties	Frozen			[18]
Chicken patties	Boiling, grilling, roasting			[50]
Meatballs	Convection oven precooking, frozen storage and four reheating methods (boiling, pan-roasting, convection oven, microwave oven)			[51]
Beef patties	Frozen storage	Thiol content	DTNP	[18]
Russian sturgeon	Sous-vide cooking			[52]
Fish fillets	Boiling, microwaving, steaming, roasting, deep-frying			[53]
Beef and chicken patties	Frozen	Cross-linking proteins Schiff base	FS	[54]
Beef patties	Roasting			[49]
Chicken patties	Boiling, grilling, roasting	Cross-linking proteins Disulphide bond	4-DPS	[50]
Pork meat	Frozen			[55]

DNPH: 2,4-Dinitrophenylhydrazine, DTNP: 5,5-dithiobis (2-nitrobenzoate), FS: fluorescence intensity, 4-DPS: 4,4-dithiodipyridine.

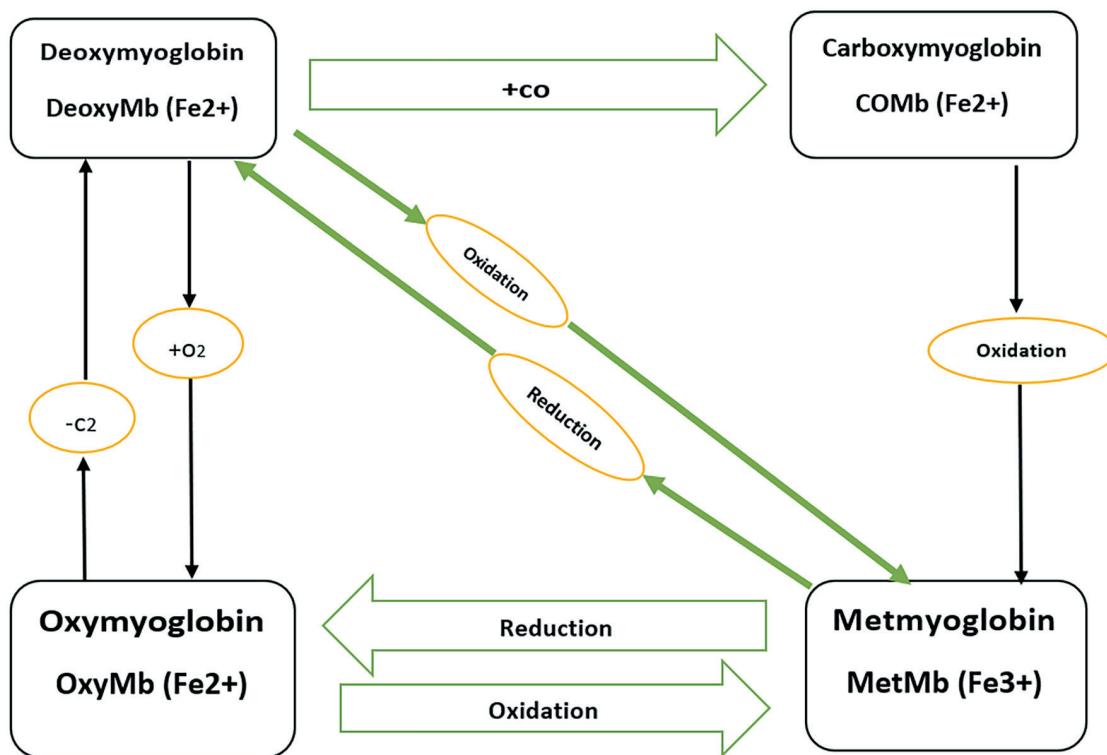


Figure 4. Transformations in the meat pigment

in a purple red color [58]. Exposure to oxygen causes the formation of oxymyoglobin, which gives meat a bright red color. However, excessive exposure to oxygen leads to the oxidation of ferrous to ferric state causing the pigment to change from oxymyoglobin to metmyoglobin, resulting in a brown color on the surface of meat (Figure 4). Metmyoglobin values greater than 40% can cause consumer rejection of meat and meat products. This is supported by various studies such as those conducted by [59,60].

The aldehydes that result from the breakdown of lipids are highly active and can easily bond with myoglobin in the sarcoplasm, making it more prone to oxidation. When meat products have a higher fat content, they tend to have a browner color. During meat processing, the redness decreases significantly, and the oxymyoglobin content decreases, while the MetMb content and lipid oxidation increase. Ferric ions can also aid in lipid oxidation. The information in Figure 5 is based on the studies conducted by Wang et al. [61] and Zhu et al. [62].

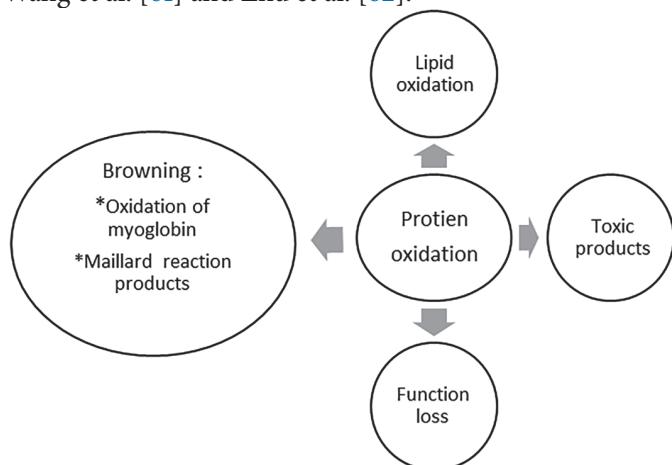


Figure 5. Effect of fat oxidation on proteins

The color of cooked meat at the end is influenced by how the myoglobin pigment reacts to heat treatment. This is determined by the protein's fundamental structure, how it interacts with other biomolecules, and self-oxidation, all of which impact the thermal stability of the pigment and its resistance to denaturation. These findings are depicted in Figure 6 [63].

The final color of cooked meat is influenced by heat treatments, which cause denaturation of myoglobin. It is crucial to consider the early development of the cooked meat color, as pathogenic microorganisms may still be present at this stage, posing a risk to consumers [64]. This early brown color can occur if myoglobin is in a particular chemical state before cooking. The delayed development

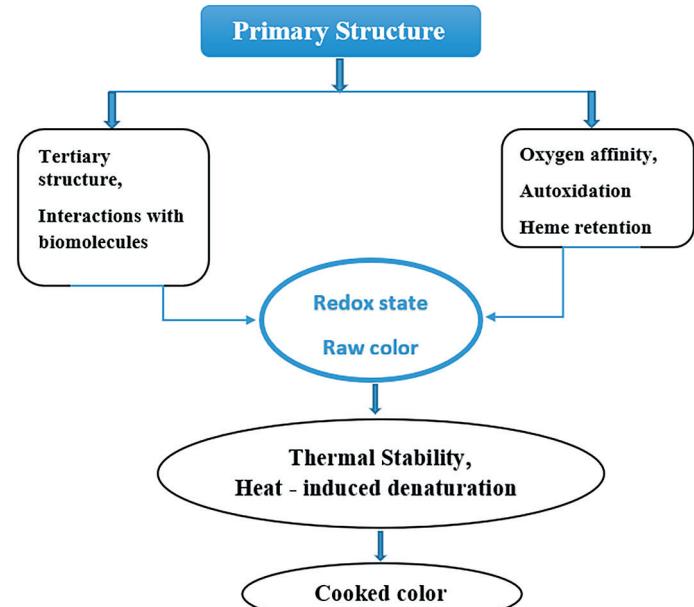


Figure 6. Crucial factors impacting the color of cooked meat

of color requires more time to reach the desired color and also depends on the myoglobin's chemical state in raw meat. Myoglobin's thermal stability varies based on its form, with metmyoglobin being the least stable and most likely to cause early browning. Oxymyoglobin and de-oxygenated myoglobin are both more heat-resistant than metmyoglobin. The pH, muscle source and type, product components, packaging, and storage all play a role in the internal color of cooked meat, which is a crucial factor in consumer acceptance and can also serve as an indicator of doneness [65].

#### *Maillard Reaction Products (MRPs)*

For centuries, people have used the Maillard reaction to enhance the taste and appearance of foods by heating, frying, roasting, and grilling them. This reaction also produces compounds that give food an appealing flavor and possess the antioxidant activity. However, it can also create harmful compounds in certain food products. The Maillard reaction is influenced by various factors such as a type of reactants, duration and temperature of heat treatment, pH, and water activity. Foods contain oxygen, minerals, and carbonyl compounds from fats that can act as a source of carbonyl for interaction with proteins, leading to the formation of Maillard products and a dark brown color [66, 67]. This reaction is defined as the interaction between the amine group of amino acids, peptides, and proteins with the carbonyl group found in reducing sugars, oxidized lipids, and vitamin C according to studies by [68] and other researchers such as [66,67,69,70].

The Maillard reaction products impact the processes of fat oxidation, with some of these products hindering and others promoting the oxidation processes. They interact with certain intermediate compounds and generate distinct compounds that would not typically form in the absence of fat. For instance, the development of a savory taste in cooked meat relies on the presence of fat. Both pathways involve co-polymerization mechanisms and generate comparable products in high-fat foods like meat and phospholipids. Many Maillard products stem from fat oxidation processes, which include the production of methylglyoxal (MG), glyoxal, and Amadori products [71,72]. The Maillard reactions that occur in meat products can be influenced by a type of heat treatment used depending on a method of heat transfer. The formation of Maillard reaction products (MRPs) is less efficient when using an oven and circulating air for heat (roasting), compared to using a hot surface (barbecue) or frying where heat is transferred through conduction. This information is based on research conducted by [73,74].

The Maillard reaction is a complex chemical reaction that occurs between amino acids and reducing sugars, leading to the formation of a range of intermediate and end products that contribute to the flavor, aroma, and color of cooked and processed foods. The reaction occurs in three main stages:

- Early stage: In the first stage, a reducing sugar and an amino acid form a Schiff base, which undergoes a rearrangement to form an N-substituted glycosylamine. This compound can then undergo further rearrangement to form a variety of intermediate compounds, including 1-amino-1-deoxy-ketoses and 2-amino-2-deoxy-aldooses, which are key intermediates in the reaction.
- Intermediate stage: In the second stage, several key reactions occur, including the formation of diketosamines, which can decompose to form monofructosamines and non-nitrogenous carbonyl compounds. These compounds can undergo enolization, a process that leads to the formation of compounds responsible for flavor and color. Another important reaction in this stage is the Strecker degradation, which leads to the formation of a range of compounds that contribute to flavor.
- Final stage: In the final stage, some of the products formed in the previous stages can condense to form brown pigments and polymers, which are known as melanoidins. These compounds contribute to the brown color of cooked and processed foods and are also responsible for some of the unique flavors associated with these products. Overall, the Maillard reaction is a complex process that involves a series of chemical reactions occurring between reducing sugars and amino acids. The reaction results in the formation of a wide range of intermediate and end products, many of which contribute to the flavor, aroma, and color of cooked and processed foods [66,75]. Figure 7 shows the mechanism of the Maillard reaction.

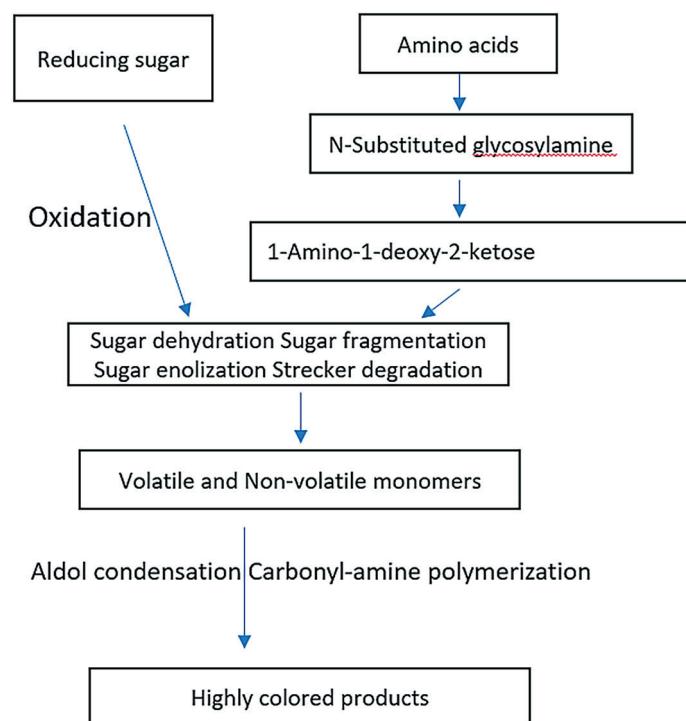


Figure 7. The mechanism of Maillard interactions

## The analytical methods to determine lipid and protein oxidation in meat and meat products

Analyzing lipid and protein oxidation in meat and meat products requires various analytical methods to assess the extent and progression of oxidation. Here are some common analytical methods used for this purpose:

- Peroxide value (PV) for lipid oxidation: PV measures the primary oxidation products in fats and oils, including meat lipids. It quantifies the concentration of peroxides, which are formed during the initial stages of lipid oxidation. Iodometric titration is a widely used method for determining PV [76].
- Thiobarbituric acid reactive substances (TBARS) assay for lipid oxidation: TBARS measures secondary lipid oxidation products such as malondialdehyde (MDA), a marker for oxidative rancidity. It involves reacting MDA with thiobarbituric acid to form a colored complex, which is measured spectrophotometrically [77].
- Anisidine value (AV) for lipid oxidation: AV assesses the quality of meat lipids by measuring the concentration of secondary oxidation products such as aldehydes and ketones. It is particularly useful for evaluating fish products. AV is determined by reacting the sample with anisidine reagent and measuring the absorbance at specific wavelengths [78].
- Free fatty acid (FFA) content for lipid oxidation: FFA content is indicative of hydrolytic rancidity and can be measured through acid or enzymatic hydrolysis of lipids followed by titration or colorimetric methods [79].
- Total oxidation (TOTOX) value for lipid oxidation: The TOTOX value combines PV and AV measurements to provide a more comprehensive assessment of lipid oxidation [78].
- Carbonyl content for protein oxidation: Carbonyl content in proteins is determined using the 2,4-dinitrophenylhydrazine (DNPH) assay. It quantifies the oxidative damage to amino acid side chains, particularly to lysine and arginine residues [80].
- Sulphydryl (-SH) group assay for protein oxidation: Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid)) can be used to measure the -SH groups in proteins, which are susceptible to oxidation [81].

- Electrophoresis for protein oxidation: Gel electrophoresis techniques, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), can reveal changes in protein profiles due to oxidation [82].
- Mass spectrometry for lipid and protein oxidation products: Mass spectrometry techniques, including liquid chromatography-mass spectrometry (LC-MS), are used to identify specific lipid and protein oxidation products and assess their abundance [83].
- Fourier transform infrared spectroscopy (FTIR): FTIR can analyze changes in the chemical structure of lipids and proteins in meat samples due to oxidation, providing information on functional groups and molecular changes [84].
- Electronic nose and electronic tongue: These instrumental methods can detect volatile compounds and taste profiles associated with oxidation, helping to assess meat quality [85].
- Sensory evaluation: Trained sensory panels or consumer panels can provide valuable information on the flavor, odor, and overall quality of meat products affected by lipid and protein oxidation [86].

The choice of method(s) depends on the specific research or quality control objectives, as well as a type of a meat product being analyzed. Often, a combination of these methods is used to obtain a comprehensive understanding of lipid and protein oxidation in meat and meat products [78].

## Conclusion

The evaluation of meat and meat product oxidation is an important consideration for ensuring product quality and safety. The oxidation of lipids and proteins can result in the formation of off-flavors and undesirable odors, which can reduce consumer acceptance and ultimately result in economic losses for producers. However, the management of oxidative changes can be achieved through the use of various strategies, including the addition of antioxidants, the use of modified atmosphere packaging, and the control of processing and storage conditions. These approaches can help to minimize the formation of off-flavors and extend the shelf-life of meat and meat products. Overall, managing oxidative changes is crucial for maintaining the quality and safety of meat and meat products and ensuring their acceptability to consumers.

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