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Lipid Oxidation in Muscle Foods: A Review

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ABSTRACT

Lipid oxidation is a major cause of deterioration of the quality of stored meat and meat products. This review deals with the mechanism and the methods used to follow lipid oxidation, as well as the interaction of lipids/lipid oxidation products with food components and their possible biological effects. Emphasis is given to the presentation of the anti- and pro-oxidant properties of various compounds and conditions; their relative importance in oxidative rancidity is discussed.

MECHANISM OF LIPID OXIDATION

A major cause of muscle food quality deterioration is lipid oxidation and the changes associated with it. Lipid oxidation is a rather complex process whereby unsaturated fatty acids reacting with molecular oxygen via a free radical chain mechanism, form fatty acyl hydroperoxides, generally called peroxides or primary products of the oxidation (Gray, 1978). The primary auto-oxidation is followed by a series of secondary reactions which lead to the degradation of the lipid and the development of oxidative rancidity. The problems associated with lipid oxidation have gained much interest as they relate to flavour deterioration, loss of nutritional value and safety, biological

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damage, ageing, functional property changes and environmental pollution (Frankel, 1984).

Lipid oxidation is initiated when a labile hydrogen atom is abstracted from a site on the fatty acyl chain, with the production of a free lipid radical which reacts rapidly with oxygen to form a peroxyradical. The peroxyradical abstracts a hydrogen from another hydrocarbon chain yielding a hydroperoxide and a new free radical which can perpetuate the chain reaction (Pearson *et al.*, 1977; Enser, 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 (β-scission) of the fatty acid chain adjacent to the alkoxy radical produces low molecular weight volatile compounds, some of which have distinct aromas and can affect flavour properties at concentrations well below 1 ppm. These breakdown products causing rancidity, include complex mixtures of aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Frankel, 1984). Lipid hydroperoxides can also condense into dimers and polymers which may, in turn, oxidise and decompose into volatile breakdown products. 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.

The main unsaturated fatty acids comprising the lipids of animal tissues are oleic, linoleic, linolenic and arachidonic. Their autoxidation gives rise to a number of different hydroperoxides which, in conjunction with the many different decomposition pathways involved, lead to a large number of volatile compounds (Mottram, 1987).

INTERACTION WITH FOOD COMPONENTS

The nature and relative proportions of the compounds formed from lipid oxidation depend at least in part on the composition of the fat of the animal from which they are derived, which, in turn, may reflect a variety of factors including the nature of diet (Pearson *et al.*, 1977). Of course, other factors such as processing and storage conditions, type of ingredients and concentration of pro- or antioxidants, are very important in determining the rate of development and the possible deteriorative effects of lipid oxidation. The types of flavour developed from the volatile lipid oxidation compounds depend on a multitude of complex interactions, concentration ranges and the medium in which they are tasted (Frankel, 1984).

Although oxidation of lipids during storage is usually considered to produce off-flavours and rancidity, a notable exception is observed in dry-cured country hams and some fermented sausages, the desirable flavour of which 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 be a source of intermediates which react with other components to give important constituents of the desirable flavour of normal cooked meat (Enser, 1987).

Many of the reactions involved in the formation of volatile aroma compounds from lipid, follow the same basic pathways for both thermal and rancid oxidation and similar volatile products are formed. However, subtle differences in the precise mechanisms of oxidation under storage conditions and under thermal processing lead to mixtures of volatiles exhibiting both qualitative and quantitative differences (Mottram, 1987).

Cooked meats held in a refrigerator develop rancid odours and flavours which usually become apparent within 48 h at 4°C. These flavours are particularly noticeable after reheating the meat and are referred to as warmed-over flavour (WOF) (Tims & Watts, 1958). The rapid development of oxidised flavour in refrigerated cooked meats is in marked contrast to the slow onset of rancidity commonly encountered in raw meats, fatty tissues, rendered fat or lard, which is normally not apparent until they have been stored for weeks or months (Pearson *et al.*, 1977).

The interactions of lipid hydroperoxides and their secondary products with proteins or amino acids have a considerable impact on flavour stability during processing, cooking and storage. The form of these interactions is determined by the point at which proteins enter the lipid oxidation reaction chain, depending on whether radicals or secondary products predominate. Factors such as pH, temperature and water activity also play an important role (Hall, 1987). Non-covalent complexing of desirable volatile compounds to proteins may increase their stability during storage. Radical induced cross-linking or scission of the proteins may be responsible for most nutritional losses; hydroperoxide radicals are very reactive with sulphur and amine, functional groups of amino acids, whereas aldehydes and epoxides, secondary products of lipid oxidation, also react with thiols from cysteine (Gardner, 1979). Non-enzymic browning reactions (Maillard) also give rise to loss in nutritional value as well as to organoleptic changes. Carbonyls, such as aldehydes and dialdehydes forming Schiff bases with amines, lose their effect on rancid odour; however, high molecular weight brown materials known as melanoidins, resulting from polymerisation of Schiff bases, are unstable and produce new volatiles which affect flavour characteristics, especially during cooking and processing (Frankel, 1984). Recent work has shown that the interaction of lipid oxidation products in the thermally-

induced reaction between sugars and amino acids, is very important for the development of desirable meaty flavours in cooked meat (Mottram & Edwards, 1983; Whitfield *et al.*, 1987). Muscle phospholipids may be the essential lipids in these reactions (Mottram, 1987).

Protein solubility, emulsification and water-binding capacity associated with texture and rheological properties, also appear to be affected by the interaction between lipid oxidation products and proteins (Hall, 1987).

HEALTH IMPLICATIONS OF LIPID OXIDATION

Although the organoleptic aspects of lipid oxidation were considered, until recently, to be the most important to both producer and consumer, great attention has now been given to health risks that lipid oxidation might impose. Lipid hydroperoxides and their decomposition products may cause damage to proteins, membranes and biological components, thus affecting vital cell functions (Frankel, 1984). Products of lipid oxidation have been indicted as chemical toxicants and are believed to lead to deteriorative processes in man, including ageing (Tappel & Dillard, 1981). Lipid peroxides and oxidised cholesterol may be involved in tumour promotion and in atherosclerosis, whereas malonaldehyde, a secondary product of lipid oxidation, has been implicated as a catalyst in the formation of *N*-nitrosamines and also as causing mutagenesis (Pearson *et al.*, 1983; Jurdi-Haldeman, 1987; Sanders, 1987). At present, however, the significance of such compounds for human health is unknown (Pearson *et al.*, 1983; Balogun *et al.*, 1984).

MEASUREMENT OF THE EXTENT OF LIPID OXIDATION

Lipid oxidation in muscle foods may be followed by a variety of methods, measuring either primary or secondary changes. The suitability of each of these methods depends on the type of product and the way it has been processed and stored (Coxon, 1987), as well as the degree of correlation of the method with sensory analysis (Igene *et al.*, 1979).

The methodology for measuring the extent of oxidative deterioration of lipids in muscle foods has been reviewed recently (Melton, 1983; Coxon, 1987). Of the various methods reported, the TBA test (2-thiobarbituric acid test) appears to be the most widely used (Melton, 1983). The method is based on the spectrophotometric determination of the extracted malonaldehyde and can be performed (a) directly on the food product, involving extraction of the coloured complex or (b) on an extract of the food or (c) on a portion of

a steam distillate of the food (Tarladgis *et al.*, 1960). The method involving the steam distillate is the most popular for measuring TBA in muscle foods (Rhee, 1978). Modifications of the method, according to the type of product analysed have been proposed (Zipser & Watts, 1962; Rhee, 1978; Siu & Draper, 1978). TBA measurements have been frequently found to give useful correlation with sensory scores, in looking at the development of WOF in cooked meats (Poste *et al.*, 1986). The appearance of malonaldehyde in frozen poultry meat has also been followed by HPLC (MacNeil *et al.*, 1987).

Methods involving measurement of other secondary changes, e.g. formation of carbonyls, hydrocarbons and fluorescent products, are also used to follow lipid oxidation (Melton, 1983; Kamarei & Karel, 1984).

Methods measuring primary changes such as oxygen uptake, loss of polyunsaturated fatty acids and formation of hydroperoxides (Peroxide Value), are generally more suited to measuring low levels of oxidation in uncooked products stored at low temperature (Coxon, 1987). The oxygen absorption method may be particularly useful in following the mechanism of lipid oxidation in muscle tissue homogenates (Silberstein & Lillard, 1978). Determination of peroxides may not be useful as a measure of lipid oxidation in muscle foods during prolonged storage, especially if the muscle is ground (Melton, 1983).

FACTORS PROMOTING LIPID OXIDATION

Effect of metals, haem compounds and salt

Lipid oxidation is enhanced by metals such as iron, cobalt and copper, which facilitate the transfer of electrons leading to increased rates of free radical formation (Ingold, 1962). The most common way that metal ions enter food is via the water used and in some instances via salt and spices (Taylor, 1987).

The form of the metal is as important as the amount of metal present (Taylor, 1987). Ferrous iron has been shown to have greater pro-oxidant activity than ferric iron in cooked uncured meats (Pearson *et al.*, 1977). Low levels of ascorbic acid may increase the efficiency of iron as a catalyst for lipid oxidation, presumably by regenerating the active ferrous form (Sato & Hegarty, 1971). It has also been reported that the relative effectiveness of metals in absorbing oxygen, when added to fish homogenate, is in the following decreasing order: iron (II), copper (II) and iron (III) (Mizushima *et al.*, 1977). At low water contents, the catalytic effectiveness of metallic catalysts is lowered through hydration and in some cases through the formation of insoluble hydroxides; as the moisture content increases, water promotes oxidation through its solvent activity (Labuza *et al.*, 1971).

Lipid oxidation may also be accelerated by a variety of haem compounds (Pearson *et al.*, 1977). However, uncertainty still surrounds the exact nature of the haematin complexes exhibiting catalytic activity.

Many workers have tried to assess the relative importance of haemoprotein and non-haem iron as catalysts of lipid oxidation in various animal tissues. Both non-haem iron and haemoproteins can function as prooxidants when in contact with purified lipids. The situation in muscle, however, is much more complex. Yong and Karel (1978) found inorganic iron and copper to be strong catalysts of mackerel meat lipid oxidation, whereas Khayat and Schwall (1983) postulated that haem iron is the major catalyst of lipid oxidation in mullet fish. The oxidative rancidity in cooked meats has been attributed to both haem and non-haem iron (Liu & Watts, 1970; Roozen, 1987). On the other hand, Sato and Hegarty (1971), presented evidence that non-haem iron and ascorbic acid catalyse lipid oxidation in cooked meat. Igene *et al.* (1979) further reported that the increased rate of lipid oxidation in cooked meat is due to the release of non-haem iron during cooking. Haem pigments may be more active lipid oxidation catalysts with iron in the ferric state, whereas non-haem iron appears to be more active in the ferrous state (Greene & Price, 1975). Recent work by Verma *et al.* (1985), Tichivangana and Morrisey (1985) and Ledward (1987), revealed that, in raw meat and model emulsions, ferric haematin pigments are powerful catalysts of lipid oxidation, whereas in heated meats the system is more complex and inorganic iron may play a more important role.

Haem proteins so far have been considered catalysers of the propagation step and not truly initiators of lipid peroxidation, since all commercially available unsaturated fatty acids used in model system studies contain traces of preformed hydroperoxides. According to Harel and Kanner (1985) active species, formed by the interaction of hydrogen peroxide with metmyoglobin or methaemoglobin, could be described as true initiators of lipid peroxidation. Furthermore, Rhee *et al.* (1984) have suggested, that microsomal oxidase systems coupled with iron (III) or iron (II) may also be initiators of lipid oxidation.

High concentrations of haem compounds have been reported to inhibit lipid oxidation (Hirano & Olcott, 1971). According to Nakamura and Nishida (1971) the association of fatty acids with haemoglobin is responsible for the observed dependence of lipid oxidation on haemoglobin concentration. Furthermore, Lee *et al.* (1975) have suggested that the extent of lipid oxidation occurring in muscle may be influenced by the ratio of haemoprotein to unsaturated fatty acids. At low linoleate to haematin ratios, inhibition of lipid oxidation occurs. However, as Ledward (1987) points out, this antioxidant effect of haematin compounds can only be seen at concentrations that are unrealistically high for meat products.

Sodium chloride accelerates oxidation of the triglycerides, although the mechanism of salt catalysis is not completely known (Love & Pearson, 1971). It has been reported that sodium chloride induces rancidity in freezer-stored, cooked, cured meat (Zipser *et al.*, 1964), in cured pork (Ellis *et al.*, 1968) and in raw and cooked beef, both during cooking and subsequent storage (Chen *et al.*, 1984). The effect of sodium chloride on fat oxidation depends on the level of free moisture in the system (Pearson, 1977; Roozen, 1987). According to Love and Pearson (1971), the oxidative effect of sodium chloride may be attributed to the action of the reactive chloride ion on lipids, or to a modification of the haem proteins catalysing lipid oxidation.

Effect of processing, storage and fat composition

Sato and Hegarty (1971) postulated that any process causing disruption of the muscle membrane system, such as grinding, cooking and deboning, results in exposure of the labile lipid components to oxygen, and thus accelerates development of oxidative rancidity. Destruction of the extremely well organised structure of living animal cells, will bring together lipids, oxidation catalysts and enzymes responsible for lipid oxidation (Hall, 1987). Pearson *et al.* (1977) suggested that chopping and emulsification are at least as likely to cause WOF as grinding or mincing of samples. MacNeil *et al.* (1973) and Dawson and Gartner (1983) attributed the high oxidative potential of mechanically deboned poultry to the extreme stress and aeration during the process and the compositional nature (bone marrow, haem and lipids) of the product; TBA values increase most rapidly with decreasing particle sizes, as the latter are related to greater cell disruption. On the other hand, comminuted beef has a storage life similar to that of intact pork, despite the differences in fatty acid composition (Enser, 1987).

The extent of lipid oxidation in cooked meat appears to be related to the intensity of heat treatment. In a survey of the malonaldehyde (MA) content of retail meats and fish (Siu & Draper, 1978), it was reported that 38% of all fresh meat samples tested had MA contents less than 1 μg/g whereas 60% were in the range 1–6 μg/g. Highest MA values were found among cooked chicken, cooked pork and cooked beef roasts. Keller and Kinsella (1973) observed increases in TBA values on cooking up to 70°C; further increases were observed when cooked samples were stored for 36 days at −18°C. Pearson *et al.* (1977), reported that meat heated at 70°C for 1 h developed rancidity rapidly. However, TBA values decreased when the cooking temperature was raised above 80°C. According to Huang and Greene (1978), meat subjected to high temperatures and/or long periods of heating developed lower TBA values, than did samples subjected to lower temperature for a shorter period of time. This phenomenon was postulated

to have resulted from antioxidant substances produced from browning reactions during the heating of meat. Earlier on, Sato *et al.* (1973) had demonstrated that reductic acid, maltol and products of the amino–sugar reaction were effective inhibitors of development of WOF in cooked ground beef. This is not irrelevant to the observation that precooked beef roasts prepared by low temperature cookery for food service establishments, are not as stable to oxidative rancidity as roasts cooked in a more conventional manner (Allen & Foegeding, 1981). Similarly, Einerson and Reineccius (1977) reported that retorted turkey meat was found to have significant resistance to development of WOF, as opposed to less severely cooked turkey. The active antioxidant material extracted from retorted turkey exhibited strong reducing properties similar to those of reductones (known intermediates of the browning reaction) and was thought to act as a primary antioxidant, interrupting the free radical mechanism (Einerson & Reineccius, 1978).

Tims and Watts (1958) noted that flavour deteriorated rapidly in cooked beef after only a few hours of refrigerated storage. Keller and Kinsella (1973) revealed marked increases in TBA numbers of frozen stored hamburgers and suggested that uncured cooked meats should not be stored for prolonged periods. Upon removal of cooked hamburger from frozen storage, discoloration and off-odours were perceptible. Keller and Kinsella (1973) also noticed a progressive increase of TBA values of raw hamburger during frozen storage at $-18°C$ and suggested that this temperature may not be optimum for prevention of lipid oxidation. Siu and Draper (1978) reported that fresh fish samples yielded lower MA levels than the frozen samples. The toughened texture, poor flavour and unappealing odour of poorly stored frozen seafood, has been attributed to the binding of oxidised unsaturated lipids to proteins, a process by which insoluble lipid–protein complexes are formed (Khayat & Schwall, 1983).

The normal resistance of meat to the development of rancidity depends on the balance between the presence of antioxidants in the animal tissues and the level of unsaturation and the concentration of the fatty acids present (Enser, 1987). The most common antioxidant in animal tissue is vitamin E (tocopherol) which, however, is not all available to block oxidation because of the inhomogeneous nature of the animal tissue (Enser, 1987). Poultry meat is composed of relatively high levels of unsaturated fatty acids and low levels of natural tocopherols and thus poultry products are very susceptible to the development of off-flavours due to oxidative rancidity (Dawson & Gartner, 1983). According to Wilson *et al.* (1976) turkey meat, containing lower levels of natural tocopherol, is most susceptible to WOF development, followed closely by chicken, then by pork, beef and mutton. The use of mechanically deboned poultry meat enhances the tendency of poultry products to oxidise

(Moerck & Ball, 1974). However, the use of mechanically deboned beef in beef meat products did not result in flavour deterioration during storage, compared to control samples made of hand-boned beef, suggesting that lipid oxidation is not a problem as with chicken and fish; this was attributed to differences in the degree of unsaturation of fatty acids (Allen & Foegeding, 1981). The susceptibility of stored seafood products to autoxidation at low temperature may be related to the highly unsaturated long chain fatty acids present in these products (Siu & Draper, 1978). On the other hand, phospholipids have been shown to be the major contributors to development of rancidity in cooked meat because of their high unsaturated fatty acid content (Love & Pearson, 1971; Igene & Pearson, 1979). Wilson *et al.* (1976) demonstrated that phospholipids play a major role in development of WOF in all cooked meats except pork, where total lipid level seems to be the major contributor to WOF.

PREVENTION OF LIPID OXIDATION

With increased consumption of prepackaged raw meat and precooked convenience meat items, control of oxidation has become increasingly important. A great variety of substances and conditions may be considered as exerting antioxidant activities. According to Labuza (1971) they could be classified as follows:

(1) Free radical terminators (donating hydrogen to the free radical and thus stopping the chain reaction) such as phenolic compounds, e.g. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutyl hydroquinone (TBHQ) and tocopherol.
(2) Free radical preventors (controlling the production of free radicals during the ionization) such as metal complexing agents, e.g. ethylenediamine tetraacetic acid (EDTA), citric acid and phosphates.
(3) Environmental factors (such as redox compounds e.g. cysteine and ascorbic acid, and a_w regulators), physical conditions and packaging material.

Effect of free radical terminators

Single or combined antioxidant treatments, including BHA, BHT, propyl gallate, tocopherol and other antioxidants such as citric acid, ascorbic acid or phosphates may protect the product from oxidation.

Greene (1971) reported that a combination of propyl gallate or BHA and ascorbic acid is effective in retarding lipid and pigment oxidation in raw

ground beef for up to 8 days of refrigerator storage. The storage life of fresh salmon and trout is increased when treated with combinations of either BHA or TBHQ and EDTA or citric acid (Sweet, 1973). Injection of ducks with α-tocopherol or combinations of citric acid, polyphosphates, propyl gallate and BHA, prior to cooking, offers some protection against oxidation during cooking, storage and reheating (Klinger & Stadelman, 1975). The use of TBHQ as an approved antioxidant in certain meat and poultry products in the USA, is allowed at 0·02% in combination only with BHA and/or BHT, based on fat content (*Federal Register*, 1979). Using response surface methodology in experiments with cooked ground pork, Yun *et al.* (1987) presented evidence that TBHQ and BHA were the most effective antioxidants, among various compounds tested to find a suitable replacement to nitrite. Khayat & Schwall (1983) had earlier reported that the effectiveness of various antioxidants in inhibiting oxidation in mackerel skin lipids was of the following order: TBHQ > α-tocopherol > tempeh oil > BHA > BHT, at concentrations of 0·02% for all synthetic compounds and 0·1% and 5% for α-tocopherol and tempeh oil, respectively.

Experiments with raw and cooked beef have shown that α-tocopherol coated salt (2%) increased lipid oxidation, but only during storage of the cooked product; furthermore, Tenox 4 (BHA–citric acid–propylene glycol) coated salt and a mixture of BHA and BHT with salt (2%), have been reported to completely inhibit lipid oxidation in cooked meat (Chen *et al.*, 1984). Crackel *et al.* (1988) reported that TBHQ significantly reduced TBA numbers in raw and freshly cooked or in stored, cooked, restructured beef steaks. They also presented evidence that natural antioxidant formulations (containing mixed tocopherols, ascorbyl palmitate and citric acid) provided significant protection in freshly cooked meat and were as effective as TBHQ in retarding lipid oxidation.

Effect of free radical preventors

Experiments with cooked ground pork have demonstrated that sequestering agents such as catechol, EDTA, diethylenetriamine pentaacetic acid (DTPA), sodium pyrophosphate and, to a lesser extent, sodium tripolyphosphate, substantially lower fat oxidation and improve sensory quality of stored meat products (Shahidi *et al.*, 1986).

Addition of phosphates protects cooked meat from lipid oxidation. The mechanism by which phosphates prevent lipid oxidation appears to be related to their ability to sequester metals (Love & Pearson, 1974). Sato and Hegarty (1971) confirmed earlier results by Tims and Watts (1958) showing that pyro-, tripoly- and hexametaphosphate are important for preventing rancidity in cured meat products but orthophosphates are not. Enhanced antioxidant action is observed when sodium tripolyphosphate and lemon

juice concentrate are added in precooked frozen beef products (such as patties, steak and meat loaf) and soy-extended products (Haymon *et al.*, 1976). The stability toward oxidation of fresh broiler half-carcasses marinated in sodium pyrophosphate solutions (SPP), and subsequently subjected to freezing, cooking and re-freezing, was found to increase most by 6% SPP, followed by 3% SPP + 3% sodium chloride (Ang & Young, 1987).

In cooked meat, addition of 2% EDTA has been shown to effectively chelate the non-haem iron, released on cooking, and thus significantly reduce lipid oxidation (Igene *et al.*, 1979). Similar findings have been reported for cooked ground beef by Sato and Hegarty (1971), who have also demonstrated a surprising antioxidant activity by cupric salts ($CuCl_2$); this activity was attributed to the tendency of the free radicals to react with $CuCl_2$ in ground beef.

Effect of environmental factors, physical conditions and packaging material

Addition of reducing agents, e.g. ascorbic acid, isoascorbic acid and their salts, improves colour stability and product storage life. This activity is enhanced by addition of citric acid and its salts, which inhibit oxidation promoted by metals. These additives may be utilised in the preparation of salami, boiled sausage, ham, corned beef (Anon., 1971). Ascorbic acid at low levels (up to 250 ppm) catalyses the development of lipid oxidation, whereas at higher levels (500 ppm) it is considered to inhibit the reaction, possibly by upsetting the balance between ferrous and ferric iron, or by acting as oxygen scavenger (Sato & Hegarty, 1971; Benedict *et al.*, 1975; Igene *et al.*, 1985). Ascorbates, when used in fresh meat products (sausages or burgers) at a level around 200 ppm, will usually extend the shelf life before colour fading by 1 or 2 days (Ranken, 1987). However, at levels of 500–1000 ppm, in the presence of air, ascorbate decomposes to give hydrogen peroxide. When the concentration of peroxide rises above the capacity of the catalase and other meat enzyme systems to decompose it, destruction of myoglobin ensues, with bleaching of the meat colour and subsequent quality deteriorative changes.

Although the main use of sulphur dioxide in meat products is as an antimicrobial agent, it may also lead to better meat colour, reduction of oxidative spoilage and finally inhibition of lipolytic activity in stored meat (Wedzicha, 1987; Ranken, 1987). Sausages are the most common form of sulphited meat. The ability of sulphur dioxide to act as an antioxidant, is probably due to the ability of the sulphite ion to take part in redox reactions.

The most obvious precaution to take against oxidative deterioration is to remove the air. Wrapping raw meat in oxygen-impermeable film prevents metmyoglobin formation and lipid oxidation during storage, if sufficient reducing activity is present in meat; however, this is not always the case

(Pearson *et al.*, 1977). Ranken (1987), declared that vacuum packaging or controlled atmosphere packaging (carbon dioxide and nitrogen) of meat and meat products is a very satisfactory measure taken to prevent colour and rancidity problems. However, vacuum packaging of mechanically deboned turkey is comparable to packaging in nitrogen atmosphere but superior to packaging in carbon dioxide atmosphere (Dawson & Gartner, 1983). Nitrogen packaging and avoidance of elevated temperature, during frozen storage, have been reported as being effective in preventing rancidity of frozen fried chicken (Hanson *et al.*, 1959). Vacuum packaging improved sensory scores of salmon stored at $-18°C$ (Yu *et al.*, 1973), and of precooked and prefried chicken when compared to paper-wrapped or heat-sealed products (Arafa & Chen, 1976). Specially treated packaging material (BHA-impregnated coextruded polyethylene film) used for turkey patties was found to be partially effective in inhibiting lipid oxidation, adding a new dimension to future packaging and quality control procedures (Dawson & Gartner, 1983).

The addition of antioxidants to muscle foods during processing increases the stability of frozen meat when it is covered with a sauce or gravy (limiting contact with air) made with the fat (Lineweaver *et al.*, 1952). The inclusion of elevated levels of vitamin E in the diet of poultry and swine may also be helpful in reducing oxidation and off-flavours (Allen & Foegeding, 1981). The greater storage stability of chicken compared to turkey meat, has been attributed to the fact that turkey meat has lower levels of natural tocopherol than other poultry meat (Dawson & Gartner, 1983). Avoidance of substances like chlorine, pyruvic acid, ozone, metal ions, which are well known pro-oxidants, is essential in extending the shelf life of products (Ranken, 1987). The use of ion-exchangers and plastic piping to free the water used in meat products from metal contamination, would solve the problem of metal-induced lipid oxidation (Taylor, 1987). Furthermore, the use of salt treated to free it from transition metals, may be appropriate in some cases (Taylor, 1987).

The effect of temperature on oxidative deterioration has been described elsewhere. However, it is worth mentioning that temperature can affect lipid oxidation or fat stability in many ways. Refrigerated storage of mechanically deboned poultry delays or slows down the oxidation rate, and frozen storage further inhibits the reaction but does not stop it completely (Dawson & Gartner, 1983).

Effect of other substances as antioxidants

Reducing sugars may be included in meat product recipes, in the form of milk or whey products, improving the fresh meat colour through their

reducing properties and contributing also to the formation of Maillard-type colours, flavours and antioxidative substances on cooking (Sato *et al.*, 1973; Ranken, 1987).

Jurewicz and Salmonowicz (1971) reported that L-leucine and glycine had pro-oxidant activity on fish oil, whereas DL-valine, DL-methionine, DL-proline and L-cysteine had antioxidant activity.

Nitrite has been shown to eliminate WOF at a level of 220 ppm and to inhibit development of WOF at 50 ppm (Sato & Hegarty, 1971). Igene *et al.* (1979) reported that, in experiments with chicken white and dark meat, addition of nitrite was found to be significantly ($P < 0{\cdot}05$) more beneficial as a means of controlling oxidised flavour in cooked meat, than removal of haem pigments. Nitric oxide generated by nitrite in cured muscle foods, forms complexes with the iron present and, as shown in model system experiments, converts the activities of haem compounds from catalytic to antioxidative (MacDonald *et al.*, 1980; Kanner *et al.*, 1984). Igene *et al.* (1985) gave further evidence for the antioxidative effect of nitrite; they reported that the most important mechanism of this function is the formation of a strong complex with haem pigments. Interaction of nitrite directly with any liberated non-haem iron and to a lesser extent stabilisation of the unsaturated lipids within the membranes by nitrite, were also proposed to increase the shelf life of meat products. Morrissey and Tichivangana (1985), in experiments with pork, chicken and mackerel meat, reported that the antioxidative effect of nitrite was apparent even at 20 ppm and also that nitrite and nitrosylmyoglobin behaved synergistically toward the inhibition of lipid oxidation.

According to Kanner (1979), *S*-nitrosocysteine (RSNO) exhibited similar antioxidative properties in an aqueous linoleate system in the presence of myoglobin with other known antioxidants such as BHT; RSNO was shown to act not only as inhibitor of linoleic acid oxidation but as a hydroperoxide decomposer as well. The high inhibitory effect of added RSNO on lipid oxidation was demonstrated in experiments with ground, cooked turkey meat (1 mmol/kg meat) as well as with cooked, cured meat stored under anaerobic conditions; in this latter case, similar effects were obtained by 25 ppm of nitrite or the corresponding molecular concentration of RSNO, in colour development and inhibition of lipid oxidation (Kanner & Juven, 1980).

Antioxidative active polar lipids have been isolated from nitrite-treated laboratory-cooked ground pork and beef, during and after storage and also from commercially processed, nitrite-containing meats, including pepperoni, ham, frankfurters and bacon (Zubillaga & Maerker, 1987). This antioxidant activity of the polar lipids was found to be stable on storage and it was suggested that more than one antioxidant factor could be involved

and that at least one is associated with the acyl portion of the polar lipids. Zubillaga *et al.* (1984) had earlier reported that residual nitrite, carbon-nitroso and nitrogen-nitroso compounds, or products of the addition of nitrogen oxides to olefins, do not seem to account for the antioxidant activity observed.

Effect of natural antioxidants

Recently there has been growing concern about the possible health risks from the use of additives in foods. Imaida *et al.* (1983) reported that BHA and BHT may promote carcinogenesis in rats. In experiments with ground beef patties made with 5·7% or 10% fat, BHT was reported to increase mutagen formation whereas propyl gallate, BHA and Tenox 4 were found to considerably reduce mutagenicity development (Chen *et al.*, 1986). As a result, the development of natural antioxidants has been pursued with renewed vigour.

Spices have received particular attention as sources of antioxidants. MacNeil *et al.* (1973) reported that a rosemary spice extract was found to be quite effective in maintaining the flavour stability of mechanically deboned chicken. Experiments, where the oxidative stability of pork meat-balls during refrigerated storage was studied, have shown that rosemary and sage can exert strong antioxidant activity, whereas marjoram exerts pro-oxidant activity (Korczak *et al.*, 1988). However, it was also reported that no antioxidant activity of sage or rosemary was observed when these herbs were added as part of compounded seasoning preparations. The antioxidant activity of pepper was investigated and confirmed by Milbourne (1987), who concluded that although it was the ethanol-soluble fraction that was exhibiting the antioxidant properties, piperine (the main component) does not seem to be the only ingredient of the fraction that can exert antioxidant properties. Soaking beef cuts in lime or green pepper extracts for 24 h at 4°C had no antioxidant effect on the lipids during subsequent frozen storage at −10°C in polyethylene bags (Dessouki *et al.*, 1980). However, it was also stated that black pepper, chlortetracycline (CTC) and propolis (a resinous substance found in beehives), proved to be efficient inhibitors of oxidation, and that neither CTC nor propolis affected meat flavour. Onion juice (20%) was more effective than garlic juice (4·8%) in reducing rancidity development in lamb, in both refrigerated and frozen cooked samples (Jurdi-Haleman *et al.*, 1987). Antioxidant activity of 10 spices (allspice, black pepper, cardamon, cinnamon, clove, coriander, cumin, ginger, nutmeg and rosepetals) commonly used in the formulation of a fermented meat sausage (Pastourma) were evaluated for their antioxidative properties and clove, followed by rosepetals and allspice, were found to exhibit the highest antioxidant index when used in a dry form (Al-Jalay *et al.*, 1987).

Pratt (1972) has shown that extracts of soybeans and soy products can retard oxidation in slices of cooked beef. The shelf life of fresh, frozen and precooked pork patties, determined by TBA value, was improved by the inclusion of ginger extract from ginger rhizome (Lee *et al.*, 1986). In experiments with model systems and fresh beef homogenates, Rhee *et al.* (1981) showed that methanolic extracts of glandless cottonseed protein ingredients exerted uniquely and consistently higher antioxidant activity than those of peanut and soy protein ingredients. Total phenolic content was also markedly higher for the cottonseed protein extracts. Recently Gordon (1987) reported that flavonoids from cottonseed, peanuts, tempeh, grapeseed and hydroxycinnamic acid esters including esters of caffeic acid and ferulic acid have been shown to be active antioxidants. Flavonoids possess a phenolic structure, which is a structural feature shared by α-tocopherol, BHA and BHT.

The limitation of phenolic antioxidants is that they can become ineffective during prolonged heating at elevated temperatures, which occur during the deep fat frying of foods (Gordon, 1987). Recent work (Gordon, 1987) has shown that the naturally occurring sterol, Δ^5-avenasterol, although it has no antioxidant activity at 100°C or at room temperature, retards the oxidative deterioration of edible oils during prolonged heating at 180°C.

Although a great deal of research has been carried out on the factors affecting oxidative stability of meat and meat products, uncertainty still surrounds the mechanism of the antioxidative action of reducing sugars, amino acids, active polar lipids and natural antioxidants under the various processing conditions. Further investigation is required on the possible synergistic effect between the above-mentioned antioxidants in meat products, which eventually may lead to a gradual reduction or even elimination of the various chemical antioxidants.

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