LIPID STABILITY IN MEAT AND MEAT PRODUCTS

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ABSTRACT

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and meat products. Oxidative damage to lipids occurs in the living animal because of an imbalance between the production of reactive oxygen species and the animal's defence mechanisms. This may be brought about by a high intake of oxidized lipids or polyunsaturated fatty acids, or a low intake of nutrients involved in the antioxidant defence system. Damage to lipids may be accentuated in the immediate post-slaughter period and, in particular, during handling, processing, storage and cooking. In recent years, pressure to reduce artificial additive use in foods has led to attempts to increase meat stability by dietary strategies. These include supplementation of animal diets with vitamin E, ascorbic acid, or carotenoids, or withdrawal of trace mineral supplements. Dietary vitamin E supplementation reduces lipid and myoglobin oxidation, and, in certain situations, drip losses in meats. However, vitamin C supplementation appears to have little, if any, beneficial effects on meat stability. The effect of feeding higher levels of carotenoids on meat stability requires further study. Some studies have demonstrated that reducing the iron and copper content of feeds improves meat stability. Post-slaughter carnosine addition may be an effective means of improving lipid stability in processed meats, perhaps in combination with dietary vitamin E supplementation.

INTRODUCTION

Meat has traditionally held a special place in the diet because of its appealing flavour and texture and its high nutritional value. However, in recent years the meat industry has come under increasing scrutiny because of concerns such as those relating to saturated fat, cholesterol and heart disease, food safety, animal welfare, and even environmental protection. In general, there is now a greater demand than ever by consumers for foods perceived as natural, fresh-tasting, healthy and more nutritious, and this is reflected in the results of a recent pan-EU survey in which the five most important factors influencing consumer food choice were "quality/freshness", "price", "taste", "trying to eat healthy" and "family preferences" (Lennarnäs et al., 1997). One of the main factors limiting the quality and acceptability of meat and meat products is lipid oxidation. This process leads to discolouration, drip losses, off-odour and off-flavour development, and the production of potentially toxic compounds (Morrissey et al., 1994a; Gray et al., 1996). The low oxidative stability of meat and precooked and restructured meat products is a problem for all those involved in the meat production chain, including the primary producers, processors, distributors, and retailers, and understanding and controlling the processes which lead to lipid oxidation is a major challenge for meat scientists. This review will focus on three critical phases of lipid oxidation. In phase one, we consider the production of reactive oxygen species and antioxidant defence mechanisms in the living animal. Phase two of oxidative damage occurs in the immediate post-slaughter period, while phase three occurs during handling, processing, storage and cooking. Here, the mechanisms are probably the same as those occurring in stressed tissues in vivo. Dietary factors capable of influencing lipid oxidation in meats and meat products will be highlighted.

REACTIVE OXYGEN SPECIES

Under normal physiological conditions, animal cells are continuously challenged by stressors arising from both internal and external sources. The most important of these are reduced derivatives of oxygen called reactive oxygen species (ROS). These include free radicals having one or more unpaired electrons which can exist independently for a brief period (Kehrer & Smith, 1994). Examples are hydroxyl radical ($HO^{\cdot}$) (the most potent oxidant encountered in biological systems), superoxide anion radical ($O_2^{\cdot-}$), and oxygen-centred radicals of organic compounds (peroxyl, $ROO^{\cdot}$ and alkoxyl, $RO^{\cdot}$). Other ROS include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and hydroperoxide and epoxide metabolites of endogenous lipids. These are not free radicals but contain chemically reactive oxygen-containing functional groups (Kehrer, 1993).

ROS can either be produced accidentally or deliberately (Kehrer, 1993; Halliwell et al, 1995). During normal aerobic metabolism, mitochondria consume molecular oxygen and reduce it sequentially to produce H_2O. During this process, $O^{2-\cdot}$, H^2O^2 and $HO^{\cdot}$ are produced accidentally at a low rate. Peroxisomal enzymes and cytochrome P_{450} mixed-function oxidases also produce ROS accidentally. On the other hand, phagocytes generate $O_2^{-\cdot}$, H_2O_2, and HOCl deliberately and use them to inactivate bacteria or viruses. ROS can oxidize lipids, proteins, nucleic acids, and other macro-molecules leading to cell death and tissue injury. Although frequently occurring as a late event accompanying rather than causing cell death (Halliwell & Chirico, 1993), lipid oxidation is probably still the most widely used measure of oxidative stress in living animals.

LIPID OXIDATION IN VIVO

The first step in lipid oxidation is the removal of a hydrogen from a methylene carbon in a fatty acid (RH). This becomes easier as the number of double bonds in the fatty acid increases, which is why polyunsaturated fatty acids are particularly susceptible to oxidation (Halliwell & Chirico, 1993). The initiation step can be catalysed by $HO^{\cdot}$ or by certain iron-oxygen complexes (e.g. ferryl or perferryl radicals).

$$RH + HO^{\cdot} \longrightarrow R\bullet + H_2O \qquad \text{(reaction 1)}$$

The fatty acyl radical ($R^{\cdot}$) reacts rapidly with O_2 to form a peroxyl radical ($ROO^{\cdot}$):

$$R + O2 \longrightarrow ROO^{\cdot} \qquad \text{(reaction 2)}$$

The rate-constant (k_1) for this reaction is $3 \times 10^8\ M^{-1}s^{-1}$ (Buettner, 1993).

Because $ROO^{\cdot}$ is more highly oxidized than the fatty acyl radical or the fatty acid itself, it will preferentially oxidize other unsaturated fatty acids and propagate the chain reaction:

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot} \qquad \text{(reaction 3)}$$

The rate-constant (k_2) for this step is relatively low ($10^1\ M^{-1}s^{-1}$) (Buettner, 1993).

Lipid hydroperoxides (ROOH) formed in the propagation reaction are both products of oxidation and substrates for further reaction with Fe^{2+} and Cu^{+} to yield $ROO^{\cdot}$ and alkoxyl radicals ($RO^{\cdot}$) (Morrissey et al., 1994b). Fe^{2+} reductively cleaves ROOH (reaction 3) as follows:

$$Fe^{2+} + ROOH \xrightarrow{Fast} Fe^{3+} + RO^{\cdot} + OH^{-} \qquad \text{(reaction 4)}$$

and Fe^{2+} can be regenerated as follows:

$$Fe^{3+} + ROOH \xrightarrow{Slow} ROO^{\cdot} + Fe^{2+} + H^{+} \qquad \text{(reaction 5)}$$

$O2^{-\cdot}$ also reduces ferric iron to ferrous and cupric copper to cuprous in vivo, allowing a redox cycle in which the transition metal ion is used several times:

$$O_2^{-\cdot} + Fe^{3+} \longrightarrow Fe^{2+} + O_2$$

$$O_2^{-\cdot} + Cu^{2+} \longrightarrow Cu^{+} + O_2$$

Other strong reductants such as ascorbic acid and paraquat also reduce Fe^{3+} to Fe^{2+} (Buettner & Jurkiewicz, 1996).

Both $ROO^{\cdot}$ and $RO^{\cdot}$ can initiate further reactions (e.g. reaction 3 and the following):

$$RO^{\cdot} + RH \longrightarrow ROH + R^{\cdot} \qquad \text{(reaction 6)}$$

The $RO^{\cdot}$ can also undergo β–scission and degrade to alkyl radicals ($R'CH2\bullet$) and a range of aldehydes (R''CHO) depending on the particular hydroperoxide present (Morrissey et al., 1994b):

$$RO^{\cdot} \longrightarrow R'CH_2^{\cdot} + R''CHO \qquad \text{(reaction 7)}$$

$RCH2^{\cdot}$ can initiate further chain reactions resulting in the formation of ethane and pentane, while the aldehydes, including hexanal, malondialdehyde and 4-hydroxynonenal, can react readily with ε-amino groups of proteins to yield Maillard-type complexes.

ANTIOXIDANT DEFENCE SYSTEM

Animals have evolved several mechanisms which limit inappropriate exposure to ROS (Yu, 1994). Enzymes including superoxide dismutase, catalase and glutathione peroxidase work together to convert $O_2^{\cdot-}$ through H_2O_2 to H_2O, thereby minimising the production of $HO^{\cdot}$. Storage and transport proteins (e.g. transferrin, lactoferrin, haptoglobin, caeruloplasmin, metallothionein (Thurnham, 1990) and carnosine (Chan & Decker, 1994) sequester transition metals in forms which cannot catalyse the conversion of $O_2^{\cdot-}$ and H_2O_2 to the more damaging $HO^{\cdot}$ (Halliwell et al., 1995). Retinol also makes an important contribution by maintaining tissue integrity and limiting the release of highly catalytic free iron.

Another important protective mechanism involves so-called chain-breaking antioxidants. Much of this work has been carried out on vitamins E, C and β-carotene, but lutein and other carotenoids as well as ubiquinol-10, thiols and uric acid are also capable of interrupting free-radical chain reactions (Stocker et al., 1991). Vitamin E (as α-tocopherol) (TOH) is the most important of these compounds in plasma lipids because it is present in concentrations at least 15-times higher than any of the others (Burton et al., 1983). It is also an indispensable component of cell membranes. When it encounters a peroxyl radical ($ROO^{\cdot}$) TOH donates a hydrogen from its chromanol phenolic group to form a hydroperoxide and a tocopheroxyl radical ($TO^{\cdot}$) (Packer, 1993):

$$TOH + ROO^{\cdot} \longrightarrow ROOH + TO^{\cdot} \qquad \text{(reaction 8)}$$

The rate-constant (k_3) for this reaction is $8 \times 10^4\ M^{-1}.s^{-1}$ (Buettner, 1993) which is nearly 10^4 times faster than the propagation reaction. This means that TOH can scavenge $ROO^{\cdot}$ about 10^4 times faster than they can react with RH, so that only relatively small amounts of TOH need be present for it to be an effective antioxidant. It is also believed that $TO^{\cdot}$ can be reduced back to TOH by other intracellular reductants such as ascorbate, glutathione and dihydrolipoate (Packer & Kagan, 1993), although this has not yet been rigorously proven (Halliwell et al., 1995).

Ascorbate is considered the most important antioxidant in extracellular fluids (Sies et al., 1992). It efficiently scavenges H_2O_2, $^{\cdot}OCl$, $O_2^{\cdot-}$, $HO^{\cdot}$, and $ROO^{\cdot}$ (Sies et al., 1992) and is reactive enough to effectively intercept oxidants in the aqueous phase before they can attack and cause detectable oxidative damage to lipids. It may also restore vitamin E by re-reducing the tocopheroxyl radical to its native state. However, ascorbate can also reduce Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{+}, thereby increasing the prooxidant activity of these metals and generating $O_2^{\cdot-}$, H_2O_2, and $^{\cdot}HO$ (Buettner & Jurkiewicz, 1996). Thus, it can serve as both a prooxidant and an antioxidant. In general, ascorbate tends to be a prooxidant at low concentrations and an antioxidant at high concentrations. However, the exact concentration at which this crossover effect takes place depends on the catalytic metal ion concentration (Buettner & Jurkiewicz, 1996).

The antioxidant properties of carotenoids have been attributed to their extended system of conjugated double bonds (Stahl & Sies, 1996). The radical quenching and antioxidant activity of carotenoids have been extensively reviewed in the literature (Krinsky, 1989; Sies & Stahl, 1995). Some specific issues will be discussed later.

The production of ROS and the animal's antioxidant defences are approximately balanced *in vivo*. However, it is easy to tip the prooxidant-antioxidant balance in favour of the ROS and create a situation of oxidative stress which may cause tissue damage. Examples relating to diet include a high intake of PUFA or highly oxidised fats, or an inadequate intake of nutrients that contribute to the defence system. Dietary factors which contribute to the antioxidant defence system and which may affect the optimal balance are listed in Table 1.

Table 1: Dietary factors contributing to antioxidant defences in vivo (Strain, 1993).

Vitamin A	Iron	Carotenoids
Vitamin C	Copper	Flavanoids and related
Vitamin E	Zinc	compounds
Riboflavin	Selenium	Phytic acid
Niacin	Manganese	
Folate	Magnesium	
Vitamin B12		

LIPID OXIDATION DURING CONVERSION OF MUSCLE TO MEAT

Phase two of lipid oxidation is likely to occur immediately pre-slaughter and certainly during the early post-slaughter phase (Morrissey et al., 1994a). The biochemical changes that accompany the conversion of muscle to meat give rise to conditions where oxidation in the highly unsaturated phospholipid fraction in subcellular membranes is no longer tightly controlled and the balance between prooxidative factors and antioxidative capacity favours oxidation. It is highly unlikely that the defensive mechanisms available to the cell in the live animal still function in the post-slaughter period because of the quantitative changes in several metabolites and physical properties (Table 2) (Morrissey et al., 1994a). The rate and extent of oxidation in phase two are also likely to depend on the extent of tissue damage in the live animal. Post-slaughter events such as early postmortem pH drop, carcass temperature and tenderising techniques such as electrical stimulation are also likely to disrupt cellular compartmentalization and release catalytic metal ions (Morrissey et al., 1994a).

Table 2: Post-slaughter changes which predispose muscle foods to oxidation

- Stunning and bleeding, circulation of blood ceases
- Anaerobic metabolism - lactic acid accumulates, pH declines to approximately 5.5
- Circulation of nutrients rapidly ceases
- Preventative antioxidant enzyme system - superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase - unlikely to function
- Acute phase proteins which scavenge iron - caeruloplasmin, transferrin, haptoglobin - unlikely to be activated
- Sarcoplasmic reticulum loses its Ca-accumulating ability
- Ca-dependent proteinases degrade muscle proteins
- Some destruction of cell compartmentalization
- Low-molecular-weight chelatable iron released
- Iron-catalysed chain reactions
- Membranal lipid oxidation initiated

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The third phase and in many cases the most significant phase of lipid oxidation occurs during handling, processing, storage and cooking. During these processes, iron is released from high molecular weight sources (e.g. haemoglobin, myoglobin, ferritin, haemosiderin) and made available to low molecular weight compounds such as amino acids, nucleotides and phosphates with which it is believed to form chelates (Decker et al, 1993). These chelates are thought to be responsible for the catalysis of lipid oxidation in biological tissues (Halliwell & Gutteridge, 1986).Some high molecular weight iron sources such as haemoglobin, myoglobin and ferritin can also directly catalyse lipid oxidation (Apte & Morrissey, 1987; Decker et al., 1993; Monahan et al., 1993). However, the relative contributions of the different forms of iron have not been clearly defined (Gray et al., 1996).

DIETARY FACTORS INFLUENCING LIPID STABILITY IN MEATS AND MEAT PRODUCTS

Fatty acid composition

Increasing the degree of unsaturation of muscle membranes reduces the oxidative stability of the muscle. The relative oxidation rates of fatty acids containing 1, 2, 3, 4, 5 or 6 double-bonds are 0.025, 1, 2, 4, 6 and 8, respectively (Horwitz, 1986). Monahan et al. (1992a) observed that pigs fed a diet containing 5% soyabean oil had a significantly higher linoleic/oleic acid ratio in the neutral and polar lipid fractions of skeletal muscle and in the total lipid fraction of adipose tissue than pigs fed a 5% tallow diet. Muscle from pigs fed the soyabean oil diet was significantly more susceptible to iron-

induced lipid oxidation than that from pigs fed the tallow diet. Genetic improvements in pigs in the UK over the past 20 years have led to a fall in the average fat content of pig carcasses and an increase in the level of unsaturation (Warkup, 1994). The ratio of PUFA to saturated fatty acids is now approximately 0.44, compared with values close to 0.10 and 0.05 in lamb and beef, respectively (Warkup, 1994). This softer fat is more susceptible to oxidative damage, and this may cause difficulties for the major retailers who are increasingly turning towards centralized butchery and modified athmosphere packaging, both of which lead to meats being exposed to higher levels of oxygen for a longer period of time prior to retail.

Dietary fat quality

Animal feeds sometimes contain fat blends based on spent commercial frying oil or on by-products such as distillation residues from edible oil refining. In general, consumption of oxidized fats and oils at realistic levels is not thought to be harmful to animals because the intake of lipid oxidation products is likely to be low. However, studies with pigs (Murphy et al., 1991; Monahan et al., 1992b, 1994) and poultry (Lin et al., 1989a; Sheehy et al. 1993a, 1994; Engberg et al., 1996; Galvin et al. 1997) suggest that caution should be exercised in the use of these types of fat sources for animal feeding. Through a series of balance studies, Engberg et al. (1996) showed that α–tocopherol retention in broilers was significantly reduced (by 4-10%) by including oxidized oil in the diet. This means that in addition to correcting for the tocopherol destroyed during oxidation of the oil, the α–tocopherol concentration of the diet must be increased even further to maintain a favourable antioxidant/prooxidant balance in the muscle membranes.

Dietary vitamin E supplementation and lipid stability.

Dietary supplementation with vitamin E, whether as natural RRR-tocopherol or synthetic all-rac-α-tocopheryl acetate, increases the concentration of α-tocopherol in muscle and reduces the susceptibility of the muscle to lipid oxidation. Supplementation of pig diets with α–tocopheryl acetate reduced lipid oxidation and improved colour stability of pork chops during simulated retail display (Monahan et al. 1992c). TBARS values were lower, and surface redness (Hunter 'a' values) were higher in pork chops from pigs given 100 or 200 mg α–tocopheryl acetate/kg diet, compared with chops from pigs fed 10 mg/kg diet after 2, 4, 6 and 8 days of refrigerated storage. Cannon et al. (1995) reported that providing supplemental vitamin E (100 mg/kg diet for 84 d) resulted in significantly lower TBARS and enhanced sensory properties in vacuum-packaged, precooked pork during refrigerated storage for periods of up to 56 days, compared with pork from pigs given an unsupplemented diet. Wen et al. (1997) observed that dietary supplementation of 30-35 kg pigs with 200 or 1000 mg α-tocopheryl acetate/kg diet for 4 weeks resulted in a progressive increase in the α–tocopherol content of whole muscle, mitochondria and microsomes, compared with corresponding values for pigs fed a control (30 mg/kg) diet. Concentrations of α–tocopherol in muscle, mitochondria and microsomes of pigs fed the 1000 mg/kg diet were 3.2-, 6.1- and 5.6-fold greater than those from the control group. The increase in α–tocopherol was associated with a progressive decrease in susceptibility of the whole muscle and subcellular membranes to iron-ascorbate induced lipid oxidation measured by conventional TBARS assay, and this was confirmed by first-derivative spectrophotometry.

A factor which, until recently, has received little attention is the rate of uptake of dietary α–tocopherol by plasma and various tissues and the time required to achieve tissue saturation and optimal resistance to lipid oxidation. Morrissey et al. (1996) observed a relatively slow rate of α–tocopherol uptake in tissues of pigs fed a diet supplemented with 200 mg α–tocopheryl acetate/kg. α–Tocopherol levels increased with supplementation time up to d 91 in all tissues examined. In the case of plasma and muscle, an upward trend was observed between the results at d 91 and 126, but the values were not significantly different. α–Tocopherol concentrations of *longissimus dorsi* muscle increased at an average rate of 0.24 μg/g/week in the first 3 weeks and at a rate of 0.18 μg/g/week from then until the end of the trial. α–Tocopheryl acetate supplementation significantly decreased the susceptibility of muscle to lipid oxidation after 1 week, and a further significant reduction in TBARS numbers was observed each week up to d 35. Increasing supplementation beyond d 35 decreased TBARS even further and this decrease was significant between d 35 and 91, and also between d 91 and 126.

A significant protective effect of vitamin E supplementation against lipid oxidation (Lin et al., 1989a, b; Sheehy et al., 1993a, b, 1994; Jensen et al., 1995) and off-flavour development (Blum et al., 1992; O'Neill et al., 1995; De Winne &

Dirinck, 1996) has also been reported in poultry. Short-term feeding (for 5-12 d prior to slaughter) was effective in retarding lipid oxidation in raw whole breast muscle (Marusich et al., 1975). However, considering the relatively slow uptake of α–tocopherol by chicken muscle compared with other tissues (Sheehy et al., 1991) and the apparent requirement to lay down the vitamin in specific locations within the muscle membranes for optimum protection, some authors have questioned whether short-term supplementation would guarantee adequate stability in processed muscle. Morrissey et al. (1997) fed one group of broilers a basal diet containing 30 mg α–tocopheryl acetate/kg feed continuously up to slaughter at 6 weeks while other groups were given a supplemented diet containing 200 mg α–tocopheryl acetate/kg for 1, 2, 3, 4 or 5 weeks immediately prior to slaughter. A 4-week pre-slaughter supplementation period was required for muscle α–tocopherol levels to reach a plateau. The rate of α–tocopherol increase during this period was 3.8 μg/g/week in breast muscle and 4.0 μg/g/week in thigh muscle. Compared with values from broilers given the basal diet, supplementation for up to 5 weeks pre-slaughter significantly reduced the susceptibility of muscle homogenates to iron-ascorbate induced lipid oxidation (Morrissey et al., 1997). It also significantly improved the oxidative stability of ground muscle during refrigerated and frozen storage and protected against the prooxidant effect of salt (Brandon et al., 1993). Morrissey et al. (1997) recommended that broilers be given diets supplemented with 200 mg α–tocopheryl acetate/kg (some 20-times higher than the National Research Council (1994) requirement) for at least 4 weeks prior to slaughter in order to optimise muscle tocopherol concentrations and guarantee adequate stability against lipid oxidation. More recently, Galvin et al. (1998a) showed that supplementation with 400 mg α–tocopheryl acetate/kg feed over an 8-week period resulted in a further significant improvement in the storage stability of cooked chicken meat (monitored by TBARS) compared with samples from broilers given 100 or 200 mg/kg. Irradiation of the meat at 2.5 or 4.0 kGy did not significantly affect TBARS concentrations immediately after cooking compared with non-irradiated samples, but was associated with significantly higher TBARS concentrations in cooked meat after 5 days storage when the basal diet contained only 100 mg α–tocopheryl acetate/kg feed. This effect did not occur if the basal diet was supplemented with 200 or 400 mg α–tocopheryl acetate/kg.

Turkey tissues accumulate α–tocopherol much more slowly than chicken tissues, and a longer supplementation period is required to reach saturation. Wen et al. (1997) reported that in turkey poults fed a control diet containing 20 mg α–tocopheryl acetate/kg (approximately twice the NRC requirement), breast and thigh muscle α–tocopherol levels fell sharply between weeks 1 and 3 and remained low thereafter. Supplementing the diet with 300 or 600 mg α–tocopherol acetate/kg increased muscle α–tocopherol concentrations slowly up to week 13, at which time they reached a plateau. These dietary levels of α–tocopheryl acetate resulted in significantly lower TBARS concentrations in raw and cooked burgers during refrigerated and frozen storage (Wen et al., 1996) and improved the oxidative stability of muscle homogenates challenged with high amounts of catalytic Fe^{2+} (Wen et al., 1997). This suggests that turkey diets should contain at least 300 mg α–tocopheryl acetate/kg in order to ensure a high degree of oxidative stability in turkey meat.

Recently, cholesterol oxidation in foods has begun to attract attention because of the belief that cholesterol oxidation products (COPs) may be involved in atherogenesis (Kubow, 1993; Guardiola et al., 1996). Cholesterol oxidizes by a free radical mechnism involving the removal of a labile H from the molecule by peroxy or oxyradicals of polyunsaturated fatty acids (Smith, 1992). Oxidation of cholesterol can occur at C7, C20 and C25. In meats, processing conditions such as heating and long-term storage accelerate the formation of COPs (Paniangvait et al., 1995). Monahan et al. (1992b) reported that three COPs (5β,6β–epoxycholestan-3β–ol (-epoxide), cholest-5-ene-3β,7β–diol (7β–OH) and 7-oxycholest-5-en-3β–ol (7-keto)) were present in detectable amounts in cooked ground pork. After 2 days of storage at 4^0C, cooked pork from pigs fed supplemented diets (100 and 200 mg α–tocopheryl acetate/kg) had significantly lower levels of β–epoxide, 7β–OH, and 7-keto than pork from pigs fed the basal (10 mg/kg) diet. COPs represented 2.7% of the total cholesterol in refrigerated cooked pork from pigs fed the basal diet, compared with 1.6% in pigs given the 200 mg/kg diet. Engeseth et al. (1993) were unable to find a significant effect of vitamin E supplementation (500 mg/d) on COPs concentrations in veal immediately after cooking but COPs were reduced by 65% after 4 d of storage at 4^0C, compared with values from unsupplemented animals. Galvin et al. (1998b) observed significantly lower 25-OH concentrations after 12 days refrigerated storage of cooked ground breast and thigh muscle from broilers given 200 or 800 mg α–tocopheryl acetate/kg feed than from broilers given a basal diet containing 20 mg α–tocopheryl acetate/kg. In breast, total COPs

(the sum of 25-OH and 7-keto, the only COPs quantified) were on average 41 and 69% lower, respectively, for the 200 and 800 mg/kg groups, than those of the basal group. In thigh, total COPs were reduced by 50 and 72%, respectively, at these supplementation levels. Irradiation also increases COPs in beef, pork, veal and chicken and accelerates their formation during storage (Hwang & Maerker, 1993, Galvin et al. 1998a). Supplementation of broiler diets with 400 mg α–tocopheryl acetate/kg significantly reduced total COPs concentrations after 5 days refrigerated storage of irradiated, cooked breast and thigh meat compared with values for groups fed 100 or 200 mg α–tocopheryl acetate/kg (Galvin et al., 1998a).

The discoloration of beef from bright red to brown which occurs during retail display is a combined function of myoglobin oxidation and lipid oxidation. Several studies have demonstrated that dietary vitamin E supplementation is very effective in reducing the oxidation of lipids and myoglobin in fresh, ground, and frozen beef muscle, and these have been comprehensively reviewed by Liu et al. (1995, 1996) and Schaefer et al. (1995). Faustman et al. (1989) and Arnold et al (1993a) defined the relationship between muscle α–tocopherol concentration and metmyoglobin percentage and concluded that the target α–tocopherol level in fresh muscle for optimum protection against discolouration was in the region of 3-3.5 mg α–tocopherol/kg, depending on the muscle in question. Arnold et al. (1993b) stated that feeding cattle for a minimum of 44 d at a rate of 1300 IU (i.e. 1300 mg all-rac-α-tocopheryl acetate)/d was necessary to incorporate adequate α–tocopherol within *longissimus dorsi*. More recently, Liu et al. (1995) recommended a supplementation strategy of 500 IU of supplemental vitamin E per steer per day for 126 d in order to attain the desired levels in muscle.

Ascorbic Acid

Ascorbic acid has a number of important metabolic functions in living animals, one of which is believed to be the regeneration of α–tocopherol from the α–tocopheroxyl radical (Packer & Kagan, 1993). This suggests that supplementation of animal diets with ascorbic acid might enhance the protection afforded by α–tocopherol against lipid oxidation in meats after slaughter. However, relatively little is known about this subject because there is no dietary requirement for ascorbic acid in farm animals. Fletcher & Cason (1991) reported that ascorbic acid (973 mg/l) added to the drinking water of broilers for 24 hours prior to slaughter had no effect on live shrink, normal processing yield, breast meat yield, breast meat moisture content, or breast meat cooked texture. Santé & Lacourt (1992) gave groups of turkeys supplemental ascorbic acid (1000 mg/kg feed or 1000 mg/l drinking water) up to slaughter at 18 weeks of age and observed no significant effects on muscle pH over 48 hours, meat colour over 12 days or lipid oxidation over 7 days, when compared with values for unsupplemented controls. Likewise, studies in our laboratory have failed to demonstrate any enhancement of oxidative stability in broiler muscle by dietary ascorbic acid supplementation over and above that provided by α–tocopheryl acetate (unpublished data). In beef cattle, jugular infusion of sodium ascorbate (1.7 mol) 10 minutes before slaughter delayed oxymyoglobin oxidation and extended the colour display life of psoas, gluteus and longissimus muscles (Schaefer et al., 1995), but these authors concluded that dietary supplementation would probably not be as effective given the rapid rate at which ascorbic acid disappears from plasma. As already outlined, ascorbic acid has the ability to promote lipid oxidation by reducing transition metal ions, though careful sequestation of iron and copper normally means that its antioxidant properties predominate in vivo. However, the release of free or catalytic iron increases during the processing and storage of meat products (Kanner et al., 1988), and since free iron in the reduced state (Fe2+) is pivotal in the lipid oxidation process (Kanner et al., 1992), the potential benefit of dietary ascorbic acid supplementation as a means of improving the oxidative stability of meat systems is questionable.

Carotenoids

Approximately 600 naturally occurring carotenoids have been characterised and about 40 are regularly consumed in the diet. Although many studies (especially *in vitro* and in cell culture and animal models) have highlighted the capability of carotenoids to act as antioxidants by reacting with peroxyl and alkoxyl radicals and quenching singlet oxygen, less information is available on whether or not they can increase the oxidative stability of meat and meat products when provided as dietary supplements to farm animals.

Woodall et al. (1996) investigated the effect of feeding chickens a control diet or diets containing β–carotene, zeaxanthin, canthaxanthin or α–tocopherol (100 mg/kg) on the susceptibility of liver and breast muscle homogenates to

cumene hydroperoxide/$FeSO_4$-induced lipid oxidation. Supplementation with α–tocopherol, β–carotene or zeaxanthin for 37 d reduced the susceptibility of liver to oxidation, but only α–tocopherol had a significant effect in muscle. Canthaxanthin supplementation did not influence susceptibility to oxidant stress in any tissue examined. Although the authors suggested that zeaxanthin may have significant antioxidant activity in tissues, further work is needed to substantiate this claim since only a small, non-significant protective effect was observed in muscle. Ruiz et al. (1997) reported that the effect of β–carotene supplementation on the oxidative stability of chicken leg meat varies depending on its concentration in the diet and on the type of fat present. When added to a diet containing 6% sunflower oil, β–carotene (15 mg/kg feed) significantly reduced TBARS concentrations in leg meat stored for 1 week at 4^0C and reduced the rate of iron-ascorbate induced lipid oxidation in leg muscle homogenates compared with controls fed a basal diet. However, in a second experiment, addition of β–carotene (50 mg/kg feed) to a diet containing 6% lard resulted in significant increases in TBARS and iron-ascorbate induced oxidation compared with controls. There is considerable *in vitro* evidence to suggest that other carotenoids, such as lutein, lycopene, β–cryptoxantin (Thurnham, 1994) and astaxanthin (Lawlor and O'Brien, 1995), are superior to β–carotene in quenching peroxyl radicals and are more efficient than α–tocopherol. Astaxanthin strongly suppressed malondialdehyde production and produced a distinct induction period superior to β–carotene and comparable with or superior to α–tocopherol using rat liver microsomal membrane systems (Palozza & Krinsky, 1992). Lawlor & O'Brien (1995) demonstrated that astaxanthin significantly protected chick embryo fibroblasts against paraquat-induced oxidative stress. However, further studies are necessary to understand more fully the effects of these carotenoids on lipid oxidation *in vivo*, and what specific role, if any, they might play in enhancing the quality of meat and meat products.

Withdrawal of Iron or Copper Supplement

Iron and copper are essential components of the antioxidant enzyme system, but they are also important catalysts of lipid oxidation. O'Neill et al. (1997a) demonstrated that removal of supplemental copper (8 mg/kg feed) from the diets of 6 week-old broilers 1 or more especially 2 weeks before slaughter significantly improved the oxidative stability of ground, cooked thigh muscle during refrigerated storage. This effect occurred even though muscle copper concentrations were not significantly affected by withdrawl of the copper supplement. Kanner et al. (1990) reported that removing the iron supplement (25 mg/kg) from turkey diets 5 weeks before slaughtering the birds did not change significantly the amount of heme- or total iron in dark or light muscle, but improved the oxidative stability of dark muscle by 50%. Conversely, however, the same group (Bartov and Kanner, 1996) were unable to demonstrate any adverse effects of high iron intake (dietary concentration 100-500 mg/kg as ferrous sulphate) on the oxidative stability of turkey thigh muscle stored at -18^0C.

Carnosine

Carnosine (β–analyl-L-histidine) is a naturally occurring skeletal muscle dipeptide which has been suggested to act both as a buffering agent and an antioxidant. The antioxidant mechanism of carnosine appears to be a combination of its ability to act as a chelator and a free radical scavenger (Chan & Decker, 1994). The hydrophilic nature of carnosine is of biological significance because it may provide protection in the cystolic environment where many lipid oxidation catalysts and free radicals are found (Chan & Decker, 1994). The metal-chelating capability of carnosine appears to be metal ion specific (Decker et al., 1992). Nuclear magnetic resonance studies found that carnosine is capable of chelating cuprous and cupric ions, but not ferrous or ferric ions. The ability of carnosine to inhibit Fe-catalyzed lipid oxidation without chelation, as well as its ability to inhibit nonmetallic oxidation catalysts such as peroxyl radicals suggests that carnosine is capable of scavenging free radicals (Decker, 1995). Carnosine inhibits lipid oxidation catalyzed by iron, haem pigments, singlet oxygen and lipoxygenase (Decker & Faraji, 1990) and scavenges hydroxyl and peroxyl radicals (Chan et al., 1994). Chan et al. (1994) fed rats a diet containing 0.0875% carnosine (a concentration equivalent to that in a diet containing 20% beef) and failed to observe any increase in skeletal muscle carnosine concentration. However, the addition of carnosine during processing (typically at levels of 0.5-1.5%) inhibited lipid oxidation in uncooked ground turkey (Calvert & Decker, 1992), salted and unsalted cooked pork (Decker & Crum, 1993) and cooked minced chicken

(O'Neill et al., 1997b). COPs formation in chicken muscle during cooking and refrigerated storage and the prooxidant effect of salt on cholesterol oxidation were also significantly reduced by carnosine addition (O'Neill et al., 1997b). These authors suggested that a combination of dietary α–tocopheryl acetate supplementation and post-slaughter carnosine addition may be a very effective means of improving lipid stability in processed meats.

CONCLUSIONS

Lipid stability in meat and meat products is influenced by many factors, including species, muscle type, the amount and type of fat in the diet, the nutritional status of the animal at slaughter, the presence or absence of disease or infection, and, increasingly, the type of processing to which the meat is subjected (mincing, addition of salt, irradiation, refrigeration, freezing and cooking). There is now considerable evidence that dietary vitamin E supplementation reduces lipid oxidation as well as myoglobin oxidation, and, in certain situations, drip losses in meats. For broilers, studies in our laboratory indicate that vitamin E supplementation at a level of about 200 mg α–tocopheryl acetate/kg diet is very effective in stabilising meat lipids, though higher levels may be needed for stabilizing irradiated meats during storage. Turkey diets should probably be supplemented with higher concentrations (at least 300 mg α–tocopheryl acetate/kg) because of the slower uptake and deposition of α–tocopherol in turkeys. For beef cattle, Liu et al. (1995) recommended supplementing the diet with 500 mg α–tocopheryl acetate/day for 126 days prior to slaughter. For pig production, the Meat and Livestock Commission in the UK is encouraging producers to include vitamin E at a minimum level of 100 mg α–tocopheryl acetate/kg diet (Warkup, 1994).

The situation is less clear-cut regarding other components of the diet. Dietary vitamin C supplementation appears to have little, if any, beneficial effects on meat stability. Reducing the iron and copper content of feeds may improve meat stability but further work on the partitioning of transition metals within the muscle cells is necessary to explain why the improvements which have been noted up to now have occurred in the absence of any measurable changes in muscle iron or copper concentrations. The practical effects of feeding higher levels of carotenoids to animals also require further study, including their potentially negative effects on α–tocopherol status. Carnosine may be an effective means of improving lipid stability in processed meats, perhaps by post-slaughter addition in combination with dietary α–tocopheryl acetate supplementation. Other dietary components beginning to attract attention include α–lipoic acid and its reduced derivative dihydrolipoate. These seem to have several different kinds of antioxidant properties (e.g. metal chelating, radical scavenging, ascorbate- and tocopherol-regenerating ability) in specific model systems (Packer et al., 1995). Indeed, the redox couple has been described a 'universal antioxidant' (Kagan et al., 1992). However, like all antioxidants, they can also be made to act as pro-oxidants under certain conditions, and were they to be supplemented in the diet it is unclear at this stage whether either would be effective in stabilizing meats and meat products, which are much more complex than model systems.

In addition to producing demonstrable effects against lipid oxidation, other issues must also be addressed when supplementing animal diets with nutrients (or non-nutrients) for the purpose of improving meat quality. These include cost, the bioavailability of the component to the animal, and its likely intakes by consumers. The focus of research should be on identifying the minimum or critical levels of these components needed in animal diets and in the muscles in order to bring about measurable improvements in quality in meats and meat products. These levels and balances will probably be best established by utilizing physical and chemical laboratory techniques in conjunction with analytical and consumer sensory evaluation.

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