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Biochemistry of lipid and myoglobin oxidation in postmortem muscle and processed meat products – effects on rancidity

Graham R. Trout
Food Science and Nutrition
Program, Griffith University, Brisbane,
Logan Campus, Queensland. 4131
AUSTRALIA

SUMMARY

Although many of the biochemical processes that affect myoglobin oxidation in post-mortem muscle have been identified the relative importance and integration of these different process is still unclear. However, as discussed below, it is possible to utilize some of our knowledge of these processes to minimize the rate of myoglobin oxidation in post-mortem muscle and hence reduce the potential for premature discolouration of the meat during refrigerated display.

In most frozen processed meat products, lipid oxidation and the development of rancid odours can be prevented by the use of nitrite or traditional antioxidants. However, where animal production practices have increased the level of highly oxidizable PUFAs, the supplementation of the animals diets with Vitamin E can reduce the incidence of lipid oxidation in processed meat products made from the meat from these animals. Vitamin E is not effective as an antioxidant, in cases where the increased level of PUFAs is the result of dietary fish oils particularly those high levels of highly oxidizable fatty acids (C22:5 and C22:6). In this situation the recommended approach is to eliminate fish oils in the animals diets at least in the six weeks prior to slaughter.

KEYWORDS

Meat products. Lipid oxidation, rancidity, frozen meat.

INTRODUCTION

Oxidation of myoglobin and lipids during refrigerated and frozen storage greatly reduces the colour and flavor acceptability of fresh meat and processed meat products. Intrinsic properties of the muscle and the nutritional background of the animals have a profound effect on the extent of oxidation of myoglobin and lipids in the muscles post-mortem. These intrinsic differences in properties of the muscles result in extreme variation in the extent of myoglobin and lipid oxidation (and hence colour and flavour deterioration) that occurs during storage and display of postmortem muscle or meat. Although a number of external factors such as temperature (O'Keefe and Hood, 1982) and gaseous atmosphere (Giddings, 1974) have a large effects on both forms of oxidation, these factors are now fairly well understood and easily controllable.

From a consumer point of view, colour is one of the major factors taken into consideration when purchasing fresh meat, since consumers judge the quality of meat by its colour. Once fresh meat is cut, it progressively discolors during subsequent refrigerated display, and becomes increasingly unacceptable. The colour changes from the initial bright cherry red to greenish-brown as the result of spontaneous autoxidation (i.e. oxidation without assistance from exogenous agents) of oxy-myoglobin to metmyoglobin. Pre-mature discolouration of meat during storage and refrigerated retail display results in a significant economic loss to the meat industry. US research indicates that approximately 15% of beef sold through supermarkets is discounted by up to 20% because of pre-mature discolouration even though the meat is

wholesome (Smith et al., 2001). This results in an overall loss of 3% of retail sales of meat or approximately a $US 1billion per year.

The rate at which myoglobin oxidizes (and hence the rate at which meat discolours) during refrigerated display is extremely variable (Hood, 1980) and depends on both the display conditions and the intrinsic properties of the meat. Although the effect of display conditions is well understood, the effects of the intrinsic properties of the meat are poorly understood.

Display conditions that have greatest effect on discoloration rate are the oxygen concentration in which the meat is displayed (Giddings, 1974), the display temperature (Hood, 1980), the light intensity during display (Kropf, 1980; Greer and Jeremiah, 1981) and the rate of bacterial growth (Faustman and Cassens, 1990).

The intrinsic properties of muscle have a large effect on the post-mortem display life of the meat. It is well established that there are large differences in discoloration rate of meat from different muscles, from different species and from animals from different nutritional backgrounds. For example, within the same species there is a 1-3 fold difference in discolouration rate between different muscles with the **psoas major** having low oxidative stability (discolours rapidly), **biceps femoris** intermediate oxidative stability and **longissimus dorsi** high oxidative stability (discolours slowly) (Hood, 1980; Renerre, 1990; Gutzke, 1997). Similarly there is a 3-4 fold difference in discolouration rate between the same muscles from different domestic animal species (porcine>bovine=ovine>cervine) (Gutzke et al., 1997). There are also reported differences in discoloration rate of meat from grain fed cattle compared to grass or pasture feed cattle (Yang et al., 2002).

However, even under controlled conditions within the same muscle and species, there are frequent occurrences of unexplained incidences of rapid discolouration of meat during refrigerated display. Examples of this include complete discolouration of meat within 1-2 hours of cutting, unexplained rapid discolouration of vacuum packaged meat and reduced display life of long-term vacuum packaged meat (Renerre et. al., 1996, Gutzke, 1997).

Flavour is also an important attribute of meat and meat products. Lipid oxidation is the primary cause of off-flavour development during frozen storage of meat and processed meat. The end-products of lipid oxidation produce undesirable rancid odours and flavours in the products. Lipid oxidation does not usually cause rancidity and off-flavour development in fresh meat since it will usually discolour or develop off-odours due to bacterial spoilage before rancid odours develop. The propensity for rancidity development is also related to intrinsic properties of lipids within the muscle. It is well established that pork-based products are much more susceptible to rancidity development than other types of meat products because they contain highly unsaturated fatty acids. (Buckley et al., 1989).

This paper will discuss the biochemistry of myoglobin and lipid oxidation in fresh and frozen meat and processed meat products and discuss approaches for prevention of both forms of oxidation.

MYOGLOBIN OXIDATION

Endogenous Mechanisms Controlling Myoglobin Oxidation In Muscle – Differences Between Muscles

No convincing comprehensive theory has been developed to explain the intrinsic difference in myoglobin oxidation rate, and hence the differences in discolouration rate, seen between muscles from different origins. However, the biochemical properties of the muscles that affect myoglobin oxidation rate are reasonably well understood and outlined below. This summary also includes how each of these biochemical properties of muscle can be controlled to minimize myglobin oxidation.

Myoglobin properties.

One explanation for this difference in discoloration rate of different muscles is that the myoglobin from different muscles and different species oxidizes at different rates. For example, research on myoglobin from different marine animals has shown that there are differences in oxidation rate of the myoglobins from different species (Livingston et al., 1986). Similarly, more recent research on myoglobin from different bovine muscles also indicates that there are differences in oxidation rate of myoglobins from the different muscle types (Foucat et al., 1994).

These differences in oxidation rate of myoglobin from different muscles and different species are not surprising since it is well established that there are structural and chemical differences between myoglobin from different sources (Brown and Dolev, 1963; Brown and Mebine, 1969; Livingston and Brown, 1981; Livingston et al., 1986;). For example, differences in crystallographic, immunological and electrophoretic behavior among various myoglobins from different mammalian species have been known for many years (Kendrew et al., 1954; Perkoff and Tyler, 1958). Moreover, differences in amino acid composition of myoglobins from different species has also been well established (Boardman et al., 1956; Kendrew 1959).

Although there are a number of differences between myoglobins from different species, most of the properties of myoglobin are conserved. For instance, early x-ray analyses on myoglobin from different species indicated that tertiary structures for different myoglobins are essentially the same (Kendrew et al., 1954; Scouloudi 1959). Also, the functional properties of tuna myoglobins, including a high affinity for oxygen, are similar to each other and to that of mammalian myoglobins (Rossi-Fanelli and Antonini 1958; Rossi-Fanelli and Antonini 1960; Matsuura et al., 1963).

However, recent research by Gutzke and Trout (2002) has shown that there are only minimal differences in oxidation rate in myoglobin from different terrestrial mammal species (porcine, bovine, ovine and cervine) and that the differences observed do not explain the difference in oxidative stability of the different muscles (Trout and Gutzke, 1995). Hence difference in oxidation rate of the different myoglobins does not appear to explain the differences in discolouration rate between muscles from different origings.

A possible explanation for difference in discoloration rates of muscles from different species may be due to differences in concentration of myoglobin in the muscles not the differences in oxidation rate of constituent myoglobins. Muscles from species with high myoglobin levels (eg cervine and bovine) discolour more rapidly than muscles from species with low myoglobin levels (porcine). Muscles with high myoglobin level generate larger amounts of ROS (reactive oxygen species) during normal autoxidation of myoglobin which in turn initiates lipid oxidation and further accelerates myoglobin oxidation.

Reducing compounds and reducing enzymes. Another explanation for this intrinsic difference in oxidative stability between muscles is related to the reducing activity in the muscles. This includes variation in concentration or activity of reducing compounds or reducing enzyme or enzyme systems such as NADPH, NADH (Faustman & Cassens, 1991); ascorbate (Deneke et al., 1978); non-protein sulphydryls (Faustman & Cassens, 1991); Metmyoglobin reductase (Faustman & Cassens, 1990); and mitochondrial reduction (Ouali, et al., 1988). The role of these reducing systems **in vivo** is to reduce any metmyoglobin formed so as to maintain the myoglobin in its reduced form so that it can bind oxygen (Haggler et al., 1979).

Although both the enzymic and non-enzymic reducing ability have been demonstrated **in vitro**, no source of the reducing agent required for either type of reduction has been identified in post-mortem muscle. This does not, however, minimise the potential role of these reducing systems in controlling myoglobin oxidation in post-mortem muscle; it may illustrate our lack of understanding of these reducing systems.

Changes in the activity of these reducing enzymes may also explain the rapid discolouration rate during refrigerated display of long-term vacuum-packed meat. This may be caused by denaturation of these enzymes during processing. This may result from proteolytic break down of the reducing enzymes during long term vacuum packaged storage of the meat as the result of rapid postmortem pH fall in combination with slow cooling which produces conditions similar to PSE (Pale Soft and Exudative) and can occur in both bovine and porcine muscle. The net effect of this denaturation is unexpectedly rapid discolouration of the meat immediately after slicing. Hence, the most effective approach to reducing this type of discoloration is to eliminate conditions that produce PSE type conditions in muscles and to limit the length of vacuum packaged storage.

Anti- and pro-oxidants. A third explanation for the difference in discolouration rate between muscles is that different muscles contain different levels of anti-oxidative and pro-oxidative compounds that control the oxidation rate of myoglobin in the muscle. There are many anti- and pro-oxidative compounds in muscle with the potential for affecting myoglobin oxidation. These include anti-oxidants such as ascorbate (Decker and Crum, 1991) carotenoids, and tocopherols (Yang, et al., 1992) and metal chelating antioxidants carnosine/anserine (Gutzke, 1997), ceruloplasmin (Decker et al., 1993), metallothionein (Waalkes and Goering, 1990), transferrin (Moser et al., 1993), and uric acid (Smith, & Nunn, 1984). Muscles also contain trace levels of pro-oxidant metals such as copper, iron (Trout and Gutzke, 1996). Also having a similar role are enzymes that inhibit production of ROS or destroy ROS once produced such as superoxide dismutase (Adachi, et al., 1992); catalase (Renerre et al., 1996); glutathione peroxidase (Nakano et al., 1992) and glutathione (Griffith, 1985). By so doing, these enzymes prevent ROS from inducing and accelerating myoglobin oxidation.

Determining the relative importance of different antioxidants in controlling myoglobin oxidation in post-mortem muscle is difficult since many antioxidants produce much greater effects when they interact with other antioxidants. For example, ascorbate plays an important role in the antioxidant effects of alpha tocopherol; it acts to reduce and maintain the activity of any alpha tochoperol that has been oxidised as a result of its antioxidant role (Faustman et al., 1986). Similarly, ascorbate, carnosine and free copper interact strongly with each other to produce antioxidant effect and in some cases pro-oxidant effects which are several orders of magnitude higher than that of either individual component (Gutzke, 1997). Although many of these interactions have been well described **in vitro**, it is still unclear what effect these interactions have **in vivo** or in post-mortem muscle.

There has been one successful approach to using antioxidants to reduce the discolouration rate of post-mortem muscle. This approach is the to feed dietary Vitamin E to animals during production. It has been well demonstrated that supplementation with Vitamin E can reduce discoloration rate and hence increase the display life of beef by up to 20% (Faustman et al., 1986; Faustman et al., 1989 Smith et al., 2001). However, it has been shown more recently that this benefit is mainly observed in feed-lot cattle that are primarily deficient in Vitamin E and has little effect on pasture feed cattle (Yang et al., 2002). This research does highlight the practical role of antioxidants in discoloration rate and hence myoglobin oxidation in postmortem muscle.

In spite of our lack of full understanding of how these inhibitors of ROS operate in postmortem muscle, our limited knowledge in this area may help explain the cause

of variations in discolouration rate between muscles. And it also may provide strategies for increasing the oxidative stability of muscle. For example, the cervine muscle shown to be an oxidatively unstable muscle in research from my laboratory (Gutzke, 1997) was from animals raised in a region that is deficient in both copper and selenium (Booth et al., 1989). These trace metals are required for production of superoxide dismutase and glutathione peroxidase, respectively, two antioxidant enzymes in muscle. Hence, our research indicates that if this role is verified, supplementation of animal feeds with dietary copper and selenium may have beneficial effects on oxidative stability of the muscles where these animals are raised in areas where these deficiencies occur.

They may also be other as yet unrecognized antioxidants that have a large effect on discoloration rate. For example, it has been shown that **in vitro** the low molecular weight extracts from muscle have potent antioxidant effects on oxidation of purified myoglobin (Trout et al., 1996). The antioxidants present in these extracts have not be identified but do not appear to any of the antioxidants described above.

Oxygen consumption rate and oxygen penetration. A fourth possible explanation for the difference in oxidative stability of different muscles is related to the oxygen consumption rate of the muscle. Post mortem muscle actively consumes oxygen as a result of continuing activity of muscle mitochondria. It is well established that in post-mortem muscle there is considerable variation in oxygen consumption between different muscle types from the one species (O'Keefe and Hood, 1982) and different muscles from different species (Gutzke et al., 1997). During aerobic storage, post-mortem muscle with very high oxygen consumption rate will be prone to rapid myoglobin oxidation and hence discolouration. This occurs because the rate oxygen is consumed at the muscle surface is similar to the rate oxygen diffuses into the tissue and the net effect is a low surface oxygen concentration near the meat surface.

The depth of penetration of oxygen into the muscle is an indication of the oxygen consumption rate of the muscle that reflects the metabolic state of the muscle. The level of oxygen penetration is important to oxidative stability because myoglobin is unusual in that it oxidises most rapidly at low oxygen concentrations (1-2%) with the rate being 10-20 times higher than at atmospheric oxygen concentrations (Gidding, 1974). Hence, a low oxygen concentration near the muscle surface will promote myoglobin oxidation due to rapid production of ROS. This phenomenon is well known in the biomedical area where rapid oxidation during the ischaemic period (period when the heart is deprived on oxygen) in heart attacks where ROS produce massive tissue damage (Renerre et al., 1996 Gunther et al., 1999).

The practical aspect of this research is that it illustrates the importance of maintaining the activity of the enzymes and compounds responsible for maintaining oxygen consumption rate in muscle. It would indicate the possible adverse affect of rapid pH and temperature change on the enzymes responsible for oxygen consumption. If these were denatured early post-mortem it could affect the oxygen consumption rate and hence the discolouration rate.

Effect of lipid oxidation on myoglobin oxidation. The rate of myoglobin auto-oxidation is also affected by products of lipid oxidation. Interestingly, the oxidised myoglobin and myoglobin oxidation reaction products, can themselves catalyse lipid oxidation (Kanner et al., 1987). This occurs either directly or through the effects of the various radicals generated during myoglobin autoxidation such as ·O2-, hydroxy- and ferryl-radicals (Turner et al., 1991). Consequently, these two types of oxidation reactions are closely inter-related. (Decker and Crum, 1991)

Muscles that contain lipids that are very susceptible to oxidation, will produce high levels of oxidation products that will, in turn, accelerate myoglobin oxidation and increase discolouration rate. This effect has been recently demonstrated where it was shown that increased dietary levels of omega-3 fatty acids in lamb diets result in muscles with reduced oxidative stability (Ponnampalam, et al., 1997). Although this effect was clearly demonstrated, it was also shown that increased levels of dietary Vitamin E either supplemented or from pasture feeding, would reduce the detrimental of effect of highly unsaturated fatty acids on discolouration rate (Ponnampalam, et al., 1997; Yang et al., 2002).

LIPID OXIDATION

Lipid oxidation is the primary cause of the rancidity development during frozen storage of meat and meat products (Buckley et al., 1989). Lipid oxidation does not usually cause rancidity and off-flavour development in refrigerated fresh meat since it will usually discolour or develop off-odours due to bacterial spoilage before rancid odours develop. Skeletal muscle as a tissue is particularly susceptible to oxidative reactions since it contains high concentrations of pro-oxidants (transition metals, haem-containing proteins i.e. myoglobin, haemoglobin) and lipid membrane which contain higher percentages of polyunsaturated fatty acids (PUFAs) (Kanner, 1994; Sweeten **et al.** 1990). Furthermore when skeletal muscle is processed into meat products, the increased exposure to oxygen and destruction of muscle ultra-structure which allows intimate contact between membrane lipids and endogenous pro-oxidants in the presence of oxygen, greatly increases the potential for lipid oxidation.

However, in most cured processed meat products, the addition of nitrite and ascorbate greatly restrict the level of lipid oxidation that occurs (Trout and Dale, 1990). And as a general rule, most cured meat products are not susceptible to lipid oxidation and can be stored for extended periods of time (6-9 months) without detectable levels of lipid oxidation occur with concurrent production of

rancid odours. Even with uncured meat products, addition of antioxidants during manufacture will greatly reduce development of off odours ((Trout and Dale, 1990).

However, there are sporadic incidence in which during frozen storage pork-based products become particularly susceptible to oxidation and will produce rancid odours within 2-3 months frozen storage (Coxon, et al., 1986; Hertzman, et al., 1988; Trout et al., 1998). This appears to be due to their high PUFAs levels in pork muscle compared to similar lamb and beef muscle.

In pigs, the level of PUFA in the membranes can be increased by dietary means by incorporation of fish oils and or fishmeal and certain vegetable oils in the diet (Ponnompalam, et al 1997). Increased membrane PUFA levels can also result from the production of genetically leaner pigs since PUFA levels are higher in pigs with a higher muscle to fat ratio (Dunshea, 1994). Genetically leaner pigs accumulate a greater proportion of dietary PUFAs in the subcutaneous fat and membranes because they are more dependent upon dietary PUFAs due to their lower rates of **de novo** fatty acid synthesis.

Importantly, even small increases in PUFA levels in muscle can have substantial effects on rancidity development. Polyunsaturated fatty acids (PUFAs) are highly susceptible to oxidation due to the presence of labile double bonds. As the degree of unsaturation of fatty acids increases, susceptibility to oxidation, and hence rancidity, increases disproportionally. For example, the relative oxidation rates of C18 fatty acids at 25°C for Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2) Linolenic acid (C18:3) are 1: 100:1200:2500 (Shahidi, 1992). Moreover, the high level of incorporation of PUFAs into the phospholipid membranes further increases the potential for oxidation since the membranes themselves are highly susceptible to oxidation due to their high surface area and close proximity to pro-oxidant compounds in the cell.

Traditional approaches to reducing the oxidation of PUFA's in meat products are to incorporate antioxidants into the processed products during manufacture are not successful. For example, adding Vitamin E even at greatly elevated levels compared to those obtained through dietary means (Channon and Trout, 2002) and addition of traditional natural antioxidants such as rosemary extract, liquid and natural smoke, and milk powder (Coronado et al., 2002) have little beneficial effect on lipid oxidation in processed pork products.

The most effective approaches to reducing the oxidation of PUFA's and hence rancidity development in processed meat products, is to reduce the potential for the lipids to oxidize in the first place. This can be achieved through two approaches.

The first approach is to incorporate Vitamin E into the muscle via supplementation of the pigs' diets. This leads to a reduction in the development of oxidative rancidity in whole muscle and ground pork and reduced drip loss during thawing of pork muscles (Hertzman, 1988). Vitamin E is a potent biological antioxidant obtained only through the diet. The major function of Vitamin E, a lipid soluble compound in phospholipid membranes, is to act as a chain breaking anti-oxidant by inactivating free radicals in cell membranes. This effect occurs because dietary Vitamin E supplementation results in elevated concentrations of -tocopherol in cell membranes, especially in the mitochondria and microsomes, thus reducing the susceptibility of lipid oxidation in the membranes (Monahan, et al., 1991). The -tocopherol concentration required in porcine muscle for maximum stability to lipid oxidation is 7-10 mg/kg of fresh muscle which can be achieved by using 100-200 mg of Vitamin E/kg feed in the diet (Bruni, 1993).

The second approach is to minimize the level of highly oxidizable fatty acids in the diet. The PUFA's most susceptible to oxidation are fish oil fatty acids. Fish oils, either directly or through fishmeal, are incorporated in pig feeds to provide energy and for their Vitamin A and D levels. The level of fish oil fatty acids in pork fat is characterized by the level of C22:5 and C22:6 fatty acids (Hertzman et al., 1988). However, fish oils are characteristically high in unsaturated fatty acids and incorporation of them by pigs into their own fatty tissues can lead to a greater susceptibility of the fat from these animals to oxidize and produce rancid fish like off-odours (Coxon et al., 1986; Hertzman et al., 1988). Even low levels of these fatty acids (0.06% - 0.27% of C22:5 and C22:6) can result in rancid odours developing in frozen processed pork products within six weeks of frozen storage (Trout et al., 1998).

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