Session 4.II

Biochemistry and functional properties of meats

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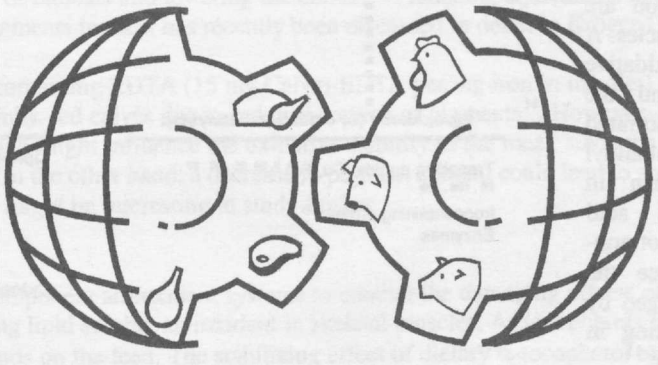

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Oxidation, shelf-life and stability of meat and meat products

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Introduction

Oxidation of meat and meat products results in changes in colour, flavour, odour and formation of potentially toxic products. Fresh meat is normally fairly stable against oxidation. However, freezing, thawing, as well as mincing increase the oxidative stress due to damage of cellular structure[1]. Light exposure during storage as well as irradiation will promote the formation of free radicals increasing oxidative processes in the meat, the extent of which, however, depends on the inherent antioxidative capacity[1] and furthermore on pre-slaughter physiological conditions[2].

Oxidative changes in processed meat are influenced by a larger number of factors than in fresh meat. Heat treatment may denaturate antioxidative enzymes as catalase, superoxide dismutase and gluthation peroxidase and promote release of catalytic active iron or transform meat pigments into prooxidative forms, resulting in meat which is even more vulnerable to oxidative changes[3]. Curing of meat on the other hand results in meat products which are surprisingly stable against lipid oxidation[4]. Other additives used in meat processing might either promote or inhibit oxidative changes. Thus, controlling oxidation in meat products requires different strategies, where critical control points in the entire production chain are taken into consideration as recently suggested and described in Skibsted *et al.*[5].

This review focuses on four main areas: i) *Intrinsic factors* influencing oxidative balance in raw meat ii) *pre-slaughter physiological conditions* of importance for oxidation iii) *processing* with focus on antioxidative additives and finally iv) *packaging* and *storage conditions*. Mainly more recent results (literature published 1998-2000 or unpublished) will be included in the review, and directions for future research for a better understanding of oxidative processes in meat and meat products will be outlined.

Intrinsic factors influencing oxidative balance in raw meat

The oxidative stability of skeletal muscle is dependent on the composition, concentrations, and reactivity of (i) oxidation substrates, (ii) oxidation catalysts (prooxidants), and (iii) antioxidants[6], see Figure 1.

Oxidation substrates

Lipolysis and lipid oxidation are the main causes of lipid degradation. The most important substrates in lipolysis and lipid oxidation are the phospholipids (PL)[7]. The content of PL and its composition are related to the metabolic type of muscles. A higher content of PL is found in oxidative muscles (1.0% versus 0.5%) and the proportion of the most unsaturated phospholipids cardiolipin and phosphatidyl ethanolamin (PE), is higher than in glycolytic muscles[7]. The fatty acid composition of muscle triglycerides of non-ruminants and to a lesser degree the membrane lipids (PL) can be changed by altering dietary fat sources, resulting in increased lipid and protein oxidation[8-11].

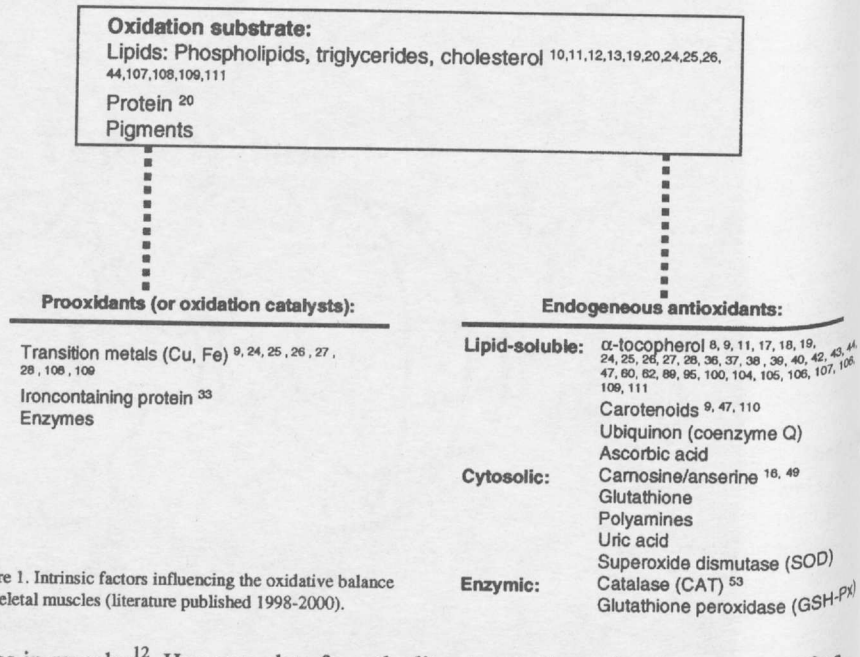

Figure 1. Intrinsic factors influencing the oxidative balance in skeletal muscles (literature published 1998-2000).

Lipolysis occurs due to activity of lipases and phospholipases. Very little is known about *post mortem* activity of the lipolytic enzymes in muscles[12]. However, data from the literature indicate that the amount of free fatty acids (FFA) increases during storage[13]. Oxidative muscles from chicken and rabbit contain more FFA than glycolytic muscles[7]. The latter finding is in agreement with Hernández *et al.*[12], who found higher lipolytic activity in oxidative muscles from pigs than in glycolytic muscles. However, Alasnier *et al.*[13] explained the higher content of FFA in oxidative muscles by a high triacylglycerol

content of oxidative muscles as compared to glycolytic muscles. In fact the rate of phospholipid hydrolysis was the same in all muscles, while that of triacylglycerol hydrolysis was slightly higher in glycolytic muscles than in oxidative ones[13].

It is generally presumed that lipolysis promotes lipid oxidation[14], although recent studies have shown that lipolysis in dry cured ham not always promotes lipid oxidation[15]. The mechanism by which lipolysis might promote oxidation is unknown, although several hypotheses have been put forward. Muscle cell contain enzymes (lipoxygenases, cycloxygenases) which oxidize free fatty acids and, in addition, low level of hydrolysis of phospholipids will result in disordering of the cell membran, which might give better opportunities for prooxidative components such as oxygen radicals and iron to penetrate the membrane[7].

Several studies[16-18] have pointed out that oxidative muscles oxidize faster than glycolytic ones. Whether this might be explained by a higher content of lipids, higher phospholipid content, higher proportion of the most unsaturated classes of phospholipids (cardiolipin and PE), higher content of FFA and/or higher content of heme iron remains to be established.

Further investigations are also required to understand how lipolysis is regulated, its relationship to lipid oxidation and the relative contribution of both pro- and antioxidant substances in oxidation processes[7]. Furthermore, it should be taken into account that muscles are non-homogenous with several compartments where enzymic reactions and non-enzymic reactions do not occur at the same rate. Additionally, prooxidants are generally found in the cytosol, while the lipids are in the hydrophobic environment[7].

Most of the studies on oxidative changes in meat have been focused on the lipid fraction, affecting development of off-flavours. Protein oxidation might affect the functionality of muscles, and several recent studies have shown that protein oxidation occurs widely in meat[11,19]. In a recently study[20] it was concluded that protein fibres promoted lipid oxidation by increasing the interaction of the catalysts (low molecular iron) with membrane lipids. Further understanding of the contribution of proteins to oxidation and the interaction between lipid oxidation and protein oxidation could be useful.

Prooxidants

'Free' transitions metals like iron and copper are prooxidants in meat systems due to their ability to catalyze breakdown of lipid hydroperoxides, possibly by a redox cycle driven by reducing compounds as in the Fenton reaction, as discussed by Kanner[21]. In fresh meat, however, the free metal ions are likely to be of minor importance for lipid oxidation compared to the effect of iron contained in heme proteins[22]. It is widely accepted that both types of processes contribute to lipid oxidation in meat, with different impact depending on the actual product. Heat treatment of meat appears to increase the level of free iron[21], but high-pressure treatment up to 8000 atm. was not found to increase the level of non-heme iron in chicken breast[23].

Copper (II) sulphate is often added to pig diet as a growth promoter. Copper is known to be a prooxidant in model systems, however dietary copper (0.035-0.175 g kg^{-1} feed) was found to have no prooxidative effect in several recent studies involving pigs[24-28], probably owing to the fact that increased dietary level of copper did not increase deposition of copper in muscle tissue[29]. Likewise, no decrease in lipid oxidation was seen when broilers were deprived of iron and copper during the last three weeks before slaughter[9].

Myoglobin is quantitatively the most important iron-containing protein in meat. Other iron-containing proteins are hemoglobin, cytochromes, ferritin, and enzymes such as catalase and peroxidase. The processes of lipid and pigment oxidation are tightly linked, but the mechanisms are still not fully understood. Tappel[30] suggested that myoglobin promotes lipid oxidation by catalytic breakdown of lipid hydroperoxides. Another possible mechanism is the pseudoperoxidase reaction of myoglobin, whereby myoglobin is activated by hydrogen peroxide and other hydroperoxides to form hypervalent species. Hypervalent myoglobin species have been found to abstract hydrogen atoms from unsaturated lipids[31,32] and are known to oxidize a number of compounds present in meat, thereby causing formation of radicals and lowering the content of reducing equivalents in the tissue. The mechanism of radical formation by hypervalent iron pigments in meat has recently been discussed in detail in Kröger-Ohlsen *et al.*[33].

Feeding calves with grain diets containing EDTA (15 mg $CaNa_2$-EDTA per mg iron in the diet) produces veal with the same desired pale colour as that produced by milk-fed calves due to reduced content of pigments[34]. However, meat from EDTA-fed calves showed a significant decrease in pH, which might influence the oxidative stability of the meat, since the susceptibility to oxidation generally increases with decreasing pH[35]. On the other hand, a decrease in pigment content could lead to an increase in oxidative stability. Such an effect of low pigment content might be interesting to study further.

Antioxidants

Skeletal muscles contain multicomponent antioxidant systems to combat the damaging effects of prooxidants. α-tocopherol (vitamin E) is the major naturally occurring lipid soluble antioxidant in skeletal muscles. As tocopherols can not be synthesized in animals the content in skeletal muscles depends on the feed. The stabilizing effect of dietary α-tocopherol on lipids in meat and meat products is well documented and was reviewed by Morrissey *et al.*[1]. As it appears from Figure 1 α-tocopherol is still an active research area. Among the most prospective results published recently is that it now seems to be documented that vitamin E is of importance for the colour stablity not only in beef[17,18,36-39] but also in fresh pork during extended storage[24,40]. The exact mechanism by which lipid-soluble α-tocopherol maintains oxymyoglobin in the oxygenated form in meat is, however, unknown[36,41], and deserves further attention. Another factor discussed widely is whether α-tocopherol affects water-holding capacity of meat. From recently published papers it seems evident that α-tocopherol may have a positive effect on water holding capacity in pork and in pork products[8,42], in beef[17], in rabbit[43] and in broiler[44]. Here too, the exact mechanism is not known. However, Ashgar *et al.*[45] and Monahan *et al.*[46] suggested that α-tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membranal

phospholipids during storage and this could inhibit the passage of sarcoplasmic fluid through the muscle cell membranes. Besides the positive effect on drip loss, Castellini *et al.*[43] also found that a higher level of α-tocopherol significantly reduced shear value of rabbit meat. The latter may partly be explained by an effect on proteinases (cathepsin activity), as found by Sárraga & García-Regueiro[9] for broilers, although reduced protein oxidation as found by Sárraga & García-Regueiro[9] and Gatellier *et al.*[11] may also be an important factor. Further studies are necessary in order to better understand what role vitamin E might play in controlling proteolytic activity of enzymes[9] and how it affects protein oxidation and the implications for meat quality.

Another group of lipid-soluble, endogenous antioxidants are the carotenoids, which also are obtained from the diet. Morrissey *et al.*[1] concluded that further studies are necessary to understand more fully the effects of carotenoids on lipid oxidation, and what specific role, if any, they might play in enhancing the quality of meat and meat products. Recently, Ruiz *et al.*[47] reported that the effects of dietary β-carotene on vitamin E content and lipid oxidation in raw, cooked and chill-stored broiler meat depended on its concentration in the feed. At 15 ppm β-carotene acted as an antioxidant, whereas a prooxidant effect was seen when the supplementation level was 50 ppm, probably due to a decrease in vitamin E content. However, in a study by Sárraga & García-Regueiro[9] a low antioxidant effect was seen in broiler muscles with a supplementation level of 50 ppm, showing that the situation today is not clear cut regarding the effect of dietary carotenoids as also concluded in a recent review by Mortensen & Skibsted[48]. One important mechanism of carotenoids is their ability to inactivate singlet oxygen[6], whereas their role as radical scavengers still is being discussed. Singlet oxygen is not a major prooxidant in dark stored meat and meat product, although it could be formed in thermal reactions. However, it might be interesting to study the effect of dietary carotenoids in light exposed meat and meat products, where singlet oxygen may be a significant prooxidant at least at the surface of the product.

Ubiquinone or coenzym Q is another lipid-soluble endogenous antioxidant. Very little is known about the role of ubiquinone as an antioxidant in muscles but their presence in mitochondria suggests that they might be of importance for the oxidative stability of red meat[6].

The cytosolic endogenous antioxidants encompass ascorbic acid, carnosine/anserine, glutathione, polyamines and uric acid. It appears from Figure 1 that the focus of recent research has been on carnosine/anserine. Carnosine (N-β-alanyl-L-histidine) is an endogenous dipeptide with antioxidative properties and a pH-buffering effect. The content of carnosine is high in glycolytic muscle (>300 mg/100 g) and low in oxidative muscle (<200 mg/100 g), as determined for pork[49]. Dietary treatment of pigs with β-alanine alone or together with histidine has been reported to have little effect on muscle levels of carnosine and to cause no change in the oxidative stability of the meat[16]. No research papers have appeared on the effect of dietary administration of ascorbic acid since this subject was reviewed[1]. Dietary ascorbic acid was concluded to have a very minor if any effect on the oxidative stability of muscles[1]. According to Decker & Xu[6], the literature is scarce about the antioxidant role of polyamines and uric acid.
Several researchers[50-52] have reported activities of the antioxidative enzymes super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in skeletal muscles. Recently Pradhan *et al.*[53] investigated the stability of catalase and its potential role in lipid oxidation in ground beef muscle. It was concluded that catalase would be stable during storage and distribution and contribute significantly to the antioxidative prosesses in raw meat products.

Pre-slaughter conditions affecting oxidative stability

Pre-slaughter treatment such as stress is known to affect the quality of the meat. Recently, Gasperlin *et. al.*[54] studied the colour differences between fresh normal beef (pH_u ~5.6) and fresh DFD (dark, firm and dry) beef (pH_u >6.6). As expected normal beef was more pale and bloomed to a higher extent than DFD beef. The pH_u in the meat is determined by the physiological conditions in the live animals at the time of stunning[55,56]. However, very little knowledge is available concerning the relationship between pre-slaughter physiological conditions and oxidative changes in meat and meat products. The metabolic conditions at the time of stunning are a result of an interaction between genetic factors and the environmental stress imposed on the animals in connection with pre-slaughter handling[57], but even animals of the same genotype may behave differently when exposed to stress[58]. To overcome these problems experiments were performed in which the pre-physiological conditions of the pig muscles were standardized by eprinephrine injection and treadmill exercise[57], and the treatment resulted in variations in energy metabolites (glycogen, lactate, creatine phosphate, ATP) and in pH_u. Chops from meat with high glycogen level (27-32 mmole/kg meat) at the time of stunning and the low pH_u (pH_u = 5.7) had a much higher L*-value (were more pale) than chops from meat with low glycogen level (3-9 mmole/kg meat) and high pH_u (pH_u = 5.9-6.2). Furthermore, chops with the lowest pH_u bloomed to a higher degree, as also found by Gasperlin *et al.*[54]. However, the colour stability during six days of retail storage was inferior for chops with the low pH_u, and the low pH_u resulted in a higher degree of lipid oxidation[2]. A threshold value of approximately 5.8 was identified. Above this value the effect of pH_u on the oxidative stability was found to be of minor importance. The differences found in oxidative stability of unprocessed pork with different pH_u were even more pronounced, if the meat was freezer-stored prior to retail storage (unpublished results). Notably, the effect of pH_u on colour stability and lipid oxidation was also realized in nitrite-cured products, although not as pronounced as in the unprocessed meat (unpublished results). The results of our experiments show that the effect of pH_u on colour stability and lipid oxidation in fresh and processed pork is pronounced, and these novel findings are now being studied in more details.

Processing factors influencing oxidative stability

The various processes used for manufacturing of different type of meat product result in great variation in flavour, appearance, and microbial and chemical stability. These differences highly affect various quality parameters of the meat products during storage and retail display. For instance warmed-over-flavour is mainly related to pre-cooked meat products intended for re-heating. In contrast, rancidity is rarely observed in pickled-cured (salt, nitrite/nitrate and ascorbat) meat products (with low fat content), due to the antioxidative action of nitrite. Newer investigations on oxidative stability of meat products have mostly been related to the effect of antioxidants or antioxidative ingredients either supplemented via the diet (endogenous antioxidants) or added during processing (exogenous antioxidants). Furthermore, considerable efforts have been directed at clarifying or understanding how the oxidative stability and thus the product quality of traditional, dry-fermented pork products is maintained during a lengthy maturation period and afterwards during non-refrigerated storage.

Antioxidative additives for processed foods

In the review by Chizzolini *et al.*[59] dealing with factors influencing the oxidative stability of processed meat products, it was concluded that the effect of natural antioxidants present in the muscles from the diet or added during manufacturing had not been thoroughly studied. As it appears from Table 1 the use of natural antioxidants have been an active research area since then. It appears that studies aiming at examining the effect of *post mortem* addition of α-tocopherol on oxidative changes have been performed on cooked turkey[60], cured sausages[61], roast pork[62] and beef patties[63]. In all studies a positive effect has been obtained on lipid oxidation and/or on colour stability. However, *post mortem* addition of α-tocopherol is less effective than dietary α-tocopheryl acetate supplementation, as both Higgins *et al.*[60] and Kerry *et al.*[62] concluded that 'endogenous meat' was more stable to lipid oxidation, although the α-tocopherol levels were higher in 'exogenous meat' than in 'endogenous meat'.

Numerous phenolic antioxidants from a variety of plant extracts have been shown to inhibit lipid oxidation in a range of meat products[64-71]. The phenolics responsible for the antioxidative activity are known for rosemary and comprise carnosol, rosmanol, rosmaridiphenol, carnosoic acid, and rosmaric acid[6]. Knowledge about the antioxidative components in the remaining plant extracts is insufficient and deserves further attention. It is difficult to predict the ability of different plant phenolics to inhibit lipid oxidation in a complex system such as meat products. The different solubility of various phenolic compounds leads to differences in their antioxidative activity, since the highest effect will be obtained if the antioxidative phenolics are partitioned in the lipid fraction where lipid oxidation is most prevalent[72]. Knowledge about the antioxidative components in the plant extracts combined with knowledge about these structure-function relationships is critical in understanding and predicting the antioxidative potential of the plant phenolics in meat products[6].

Table 1. Effects of natural antioxidants or antioxidative ingredients added post slaughter on oxidative processes in meat products (literature published 1998-2000)

Antioxidants/ antioxidative ingredients	Product	Storage conditions[1)]	Effect on[2)]			Ref.
			Colour stab.	Lipid oxi.	Chol. oxi.	
α-tocopherol	Cooked turkey patties	Aerob.,4°C, 9d	+	+	-	60
	Cured pork/chick. sausages	4°C, 8w	+	+	-	61
	Cooked beef patties	Vac.,5°C, 30d	-	+	-	63
	Pork roast	Aerob/Vac.,4°C, 8d/8w	-	+	-	62
Sod. lactate + glu-del.lactone	Cooked, cured pork emul.	MAP, 5 °C, 4w	+	+	-	80
Sodium lactate	Cooked pork patties	Aerob.,-18°C, 14w	+	+	-	79
Sodium polyphosphat	Cooked pork patties	Aerob.,-18°C, 14w	+	+	-	79
Ascorbyl palmitate	Cooked beef patties	Vac.,5°C,30d	-	+	-	63
β-caroten	Cooked beef patties	Vac.,5°C,30d	-	+	-	63
Borage seeds	Meat model	Aerob., 4°C, 7d	-	+	-	70
Marjoram,ginger,carry, clove	Minced chicken	Alu., -18°C, 6mo.	-	+	-	71
thyme,sage, nutmeg,caraway,	Minced pork	or 4°C, 7d	-	+	-	71
peppermint, cinnamon, basil	Cooked pork		-	+	-	71
Rosemary	Heat-sterilised pork	Aerob, 20°C, 8-9w	-	+	-	64
	Cooked chicken	Aerob., 4°C, 4d	-	+	-	65
Sesamol, quercetin, rutin,	Pork patties	Aerob.4°C, 7d	+	+	-	68
rosemary oleoresin	Cooked pork patties	Aerob.4°C, 7d	-	+[3)]	-	68
Cherry tissue	Beef patties	Aerob., 4°C, 9d	-	+	+	67
	Cooked beef patties	Aerob., 4°C, 4d	-	+	+	67
Pepper	Cooked, ground pork	Vac., 4°C, 8d	-	+	-	66
Dittany	Cooked turkey homogenate	Aerob, 37°C, 90min.	-	+	-	69
Carnosine	Cooked chicken patties	Aerob., 4°C, 7d	-	+	-[73], +[74]	73,74
	Chicken patties	Aerob., 4°C, 10d	-	+	-	73

[1)]Aerob: Gaspermeable packaging material, Vac.: Vacuum, MAP: 80% N_2, 20% CO_2, [2)].+: Positive effect, +: No effect, -: Not analysed, [3)]Only sesamol

Carnosine is recognized as an endogenous water-soluble antioxidant, and its effect has recently been demonstrated in raw and cooked chicken thigh meat patties with and without salt, where added carnosine improved the oxidative stability as measured by MDA-TBARS and cholesterol oxidation products[73,74]. It has also been shown to improve colour stability and inhibit lipid oxidation (TBARS) in a ground beef patties model systems, although the effect in this study could have been caused by the buffering effect of carnosine rather than by a specific antioxidant action[75]. Notably, the antioxidative activity of carnosine needs to be re-evaluated after the discovery that commercial preparations of carnosine contain hydrazine in amounts that affect determination of lipid oxidation by TBARS and headspace analysis[76]. Recent data from our laboratory may also indicate that the antioxidative effect of carnosine has been overestimated due to the presence of hydrazine[77], but more experiments are needed to make a firm conclusion. Carnosine purified from contaminating hydrazine has been found to react with aldehydes such as those formed by lipid oxidation, and it has been suggested to act as a scavenger for lipid oxidation products in muscle and thereby minimize rancidity in muscle foods[78]. Carnosine-containing extracts of mechanically separated pork were found to inhibit lipid oxidation in model systems and in salted ground pork as measured by formation of lipid hydroperoxides and TBARS, but the antioxidative effect was too low for their use as a practical food additive[81].

Oxidative stability of dry-fermented pork products

It is intriguing how certain types of traditional meat products originating from Southern Europe maintain their wholesomeness during lengthy maturation periods and afterwards during non-refrigerated storage. It is evident that conditions such as high concentration of salt and low moisture content retard microbial spoilage in dry-cured meat products. However, it is still unknown whether these conditions also help to protect against oxidative changes in such products, e.g., dry-cured ham and dry-fermented sausage. With respect to dry-fermented sausage, the effect of microbial activity on lipid oxidation is still uncertain.

In *dry-fermented sausage*, flavour and oxidative status may be determined both by endogenous chemical changes in the meat batter, e.g., due to enzymatic action, and starter cultures added in the recipe. The starter culture is added to ensure a pH drop and flavour formation during maturation, but it seems from recent findings that such bacteria cultures also affect the oxidative stability of the final meat product[82]. Kenneally *et al.*[83] found free fatty acids in dry-fermented sausages to increase significantly during storage independently of the type of starter culture added. The oxidative status (TBARS) in sausages with one of the starter cultures added was lower in comparison to control sausages without any starter culture. Some starter cultures are believed to possess antioxidative capacity, which has been proven for strains of staphylococci and lactic acid bacteria when growing on media containing various fatty acids[84]. The antioxidative activity was improved for most strains when the medium was supplemented with manganese, suggesting a mechanism involving SOD activity. In another study the effect of incubation conditions on activity of nitrate reductase and catalase in several staphylococci strains was investigated, and it was found that certain strains had increased activity under anaerobic conditions and with nitrate present[85]. The latter could be correlated to the same strains effect on sausage aroma, when used as starter cultures in dry-fermented sausages.

In comparison to dry-fermented sausage the microbial action regarding flavour and colour development in *dry-cured ham* is most likely limited, although other opinions have been put forward. When dealing with dry-cured ham it is crucial to distinguish between variants with only salt added, e.g. Parma ham, and others with both nitrate/nitrite and salt added, e.g. Iberian ham. In general, extent of oxidation occurring in dry-cured ham during maturation and following storage is somehow controlled chemically, preventing development of off-flavours due to oxidative rancidity. For instance, the amount of hexanal, a marker of oxidative rancidity, was found to increase during drying and decline at the end of maturation in Iberian ham under different processing conditions[86]. When investigating lean and fat fractions of Parma ham from 12 to 24 months of ageing an actual decrease in TBARS was observed during storage[87]. The authors suggested that the low levels of oxidation observed, despite salt concentrations close to 6% and the absence of nitrate/nitrite, might be explained by the presence of natural antioxidants, e.g. carnosine and sulphide compounds.

A comparison of Spanish dry-cured and pickled-cured loins (both with nitrite added) found that TBARS values in the dry-cured product was about 20% of TBARS values present in the pickled-cured product[15]. The content of free fatty acids increased 10-fold and 6-fold for dry-cured and pickled-cured loins, respectively. Approximately, a 3-fold increase in free fatty acids was also seen in two batches of Iberian ham[86]. For dry-cured loins, mainly the phospholipid fraction was changed[15]. With higher concentrations of NaCl present, oxidation of membrane lipids catalyzed either enzymatic or by sacroplasmic protein showed an inverse relationship between increased NaCl concentration and degree of oxidation[88]. Under certain physico-chemical conditions, NaCl may have an antioxidative effect which goes against the generally recognized prooxidative effect of NaCl[74,89] when used as the only additive. A comparison of two processing technologies employing long and short salting time, respectively, showed no significant variation in lipid oxidation in the two kinds of dry-cured ham[90]. However, saturated free fatty acid increased most in dry-cured hams with high salt content (11.5%) during maturation, whereas dry-cured ham with low salt content (7.6%) obtained highest sensory scores for rancidity.

Recent studies in model systems have shown free fatty acids capable of inactivating the prooxidative effect of heme compounds on unsaturated lipids[91]. It may be speculated whether a similar mechanism prevails in dry-cured meat from Southern Europe in which extensive formation of free fatty acids take place during maturation. The remarkable colour stability of such products without nitrate/nitrite is another aspect that may be related to a possible antioxidative effect of free fatty acids. The few findings indicating an antioxidative effect of high NaCl concentrations and deserve further attention in relation to dry-cured meat products. Further elucidation of oxidative stability in dry-cured meat products may also prove useful in the manufacturing of Northern type of meat products. Especially the outstanding colour stability of Parma ham, where colour formation occurs without nitrate/nitrite, should be further investigated.

Effect of packaging and storage conditions on oxidative stability

Fresh meat

Research and development on modified atmosphere packaging (MAP) accelerated in the 1970`s and 1980`s. In recent years, however, packaging has not been the primary target for investigations, but has rather been included in investigations evaluating the effect of other parameters on oxidative stability[24,34,36,37,92-96].

Modified atmosphere packed meat is a complex and dynamic system. Packaging and storage parameters influencing the shelf-life of meat are, e.g., initial headspace atmosphere composition, headspace to product volumen ratio, gas transmission rate of the packaging material, gas absorption in the meat, storage temperature and exposure to light. All of these factors are acting and particularly interacting, resulting in quality changes of the meat during storage. Skibsted *et al.*[97] pointed out that conflicting results have been reported on whether increased O_2 concentration results in increased lipid oxidation. The picture is more clear today, as several papers have since appeared showing unambiguously that increased O_2 concentration results in increased lipid oxidation[36,37,94,95,98]. Thus increased knowledge is important to optimize the headspace gas composition considering both colour stability and lipid oxidation. Mathematical models may be of great help in understanding and describing changes in meat quality as a function of different combinations of storage and packaging parameters. By using mathematical models it was thus shown possible to reduce the O_2-level to approximately 40 % instead of the normally used 70-80 % without reducing the colour stability for fresh beef *Longissimus dorsi* and *Semimembranosus* muscles[112].

Mathematical models can be used to identify the most important factors affecting quality loss and for defining critical levels of different gasses and permeability characteristics of packaging materials and headspace to product volumen ratio, and thereby to form the basis for proposing the optimal atmosphere composition or best compromise. Models are needed to describe how the initial package microenvironment changes over time and how these changes influence product quality and shelf-life. More effort should be put into: 1) Modelling of dynamic headspace changes as a function of packaging and storage parameters (film permeability, initial gas composition, package geometry, product geometry, headspace to product volumen ratio, meat gas absorption etc.), and 2) Modelling of quality changes in the meat as a function of packaging and storage parameters (storage time, temperature, gas composition, light exposure etc.). Pfeiffer *et al.*[99] made computer simulations of quality deterioration processes mainly in dry products and combined the models with barrier properties for different packaging materials. The method provides a mean of determining the necessary barrier properties for a given shelf-life and can be a valuable supplement to many storage experiments. Predictive modelling seems promising for the development of product-specific packaging, taking into account the increasing demands for minimization of packaging, although at present there is a general lack of kinetic data for modelling of quality deterioration reactions in foods.

Processed meat products

Packaging is of utmost importance in preserving processed meat. For both pre-heated and cured meat products packaging should aim at excluding as much oxygen as possible from the product, and this is obtained either by vacuum or MAP. The shelf-life of these products is determined by a complex interaction between several factors: i) residual oxygen, ii) headspace to product volume ratio, iii) oxygen transmission rate (OTR) of packaging material and iv) illumination at the surface of the meat. Therefore, it should be stressed that such information always is provided in storage experiments dealing with packaging of meat and meat products.

For cured meat products discoloration of the primary pigment, nitrosylmyoglobin (MbFe(II)NO), is the most critical parameter pertaining to packaging and display. The pigment is very susceptible to photooxidation when exposed to light and oxygen. In fact, the ratio of pigment to oxygen is 1 for autoxidation of MbFe(II)NO, whereas the ratio exceeds 1 for photooxidation (Møller *et al.*, unpublished results). In practice, this means that more pigment will be converted to metmyoglobin (MbFe(III)) during photooxidation compared to autoxidation at equal quantities of residual oxygen. In a recent study performed by Houben & Gerris[100] sliced ham produced from meat with or without vitamin E supplementation discoloured only very little when packaged in either vacuum or modified atmosphere (60% CO_2/40% N_2) and stored at 7°C for 22 days. The latter might be due to very low residual oxygen in the packages. However, information on residual oxygen in the headspace was not provided in Houben & Gerris[100]. In contrast, packages of sliced, cured ham and sausages were found to fade quickly in redness[101,102]. In the study performed by Møller *et al.*[101] packages contained 0.5% residual oxygen. No colour fading was, however, observed for cured ham packaged with concentrations of residual oxygen below 0.1% during 28 days of illuminated (1000 lux) storage at chill temperature. This threshold value for residual oxygen will probably increase at lower headspace to product volume ratios or lower intensity of illumination. As mentioned for fresh meat, mathematical modelling will be a useful approach for predicting headspace changes as a function of packaging and storage parameters (film permeability, initial gas composition, package geometry, product geometry, meat gas absorption) and thus be used to obtain a residual oxygen level below the critical value during the entire storage period. The critical value of residual oxygen in the packages also depends strongly on intrinsic factors such as level of nitrite and vitamin E[103]. Thus, optimum oxidative stability can only be achieved by using a multifactorial approach, when both intrinsic and extrinsic factors are carefully considered.

Conclusions and perspectives

In order to improve the overall quality of the final product several strategies are recommended to provide sufficient resistance against oxidative deterioration. One strategy is to manipulate the diet with the purpose of increasing the concentrations of intrinsic antioxidants, decreasing the content of prooxidants and/or modifying the oxidative substrate (e.g. changing fatty acid composition). Another strategy is to try to obtain, if possible, a higher ultimate pH, but still sufficiently low to delay microbiological growth. The

third strategy is to improve the oxidative stability of processed meat by extensive addition of natural antioxidants or antioxidative ingredients. A final strategy is to improve packaging conditions, which are of utmost importance as packaging in several studies[94,96] have been shown to be the most important single factor in reducing oxidative changes.

Future research should be focused on several topics. The first one concerns more basic knowledge of the effect of intrinsic factors on the oxidative balance in meat. Two approaches are recommended: One is to use multivariate mathematical models incorporating the important parameters (lipid composition, lipolytic activity, vitamin E, heme iron etc.) in order to predict the relative importance of the various parameters on oxidative changes as introduced by Jakobsen & Bertelsen[98] and Hernández *et al.*[12]. The other is to carry out more basic mechanistic studies to establish the relationship between enzymatic (lipolytic and proteolytic) activity and oxidation and the relative contribution of both pro- and antioxidants in oxidative processes in both fresh meat during storage and in dry-cured meat products during processing and storage. The second topic concerns oxidative labile processed meat products where inclusion of natural antioxidants is an attractive approach for improving the oxidative stability. The focus of research should be to identify the minimum and critical levels of natural antioxidants, present in the muscle from the diet or added during manufacturing, in order to improve the quality of meat products. The third topic deals with optimisation of packaging conditions where predictive modelling seems promising for the development of product specific packaging, taking into account product quality as well as increasing demands for minimisation of packaging materials.

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