PS2.03 Feeding and protein oxidation of meat constituents 347.00

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Abstract— **One of the factors affecting meat quality is oxidation, comprising colour changes, lipid and protein oxidation. Oxidation of meat proteins does not only affect the nutritional and sensorial but also the technological value of meat. The impact of dietary and management strategies on meat protein oxidation and the correlation with other oxidation parameters has been compiled in this paper. Feeding higher amounts of (poly)unsaturated fatty acids does not seem to affect protein oxidation. In most studies a protective effect of dietary α-tocopherol on protein oxidation was observed, evaluated by the content of carbonyls or thiols. Although the carbonyl content was not significantly correlated with the α-tocopherol content in meat in a compilation of data from 12 studies, a highly significant correlation was found between the content of thiols and α-tocopherol (r = 0.580). No correlation was found between the thiol and carbonyl content. Across studies, a significant correlation was also found between the content of carbonyls and the colour a^* value and TBARS (respectively r = 0.401 and r = 0.314) and between the content of thiols and the colour a^* value and TBARS (respectively r = -0.447 and r = -0.444). The effect of protein oxidation on the activity of endogenous antioxidative enzymes, or conversely the role of these enzymes in protecting against protein oxidation has not been well studied and deserves more attention**.

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***Index Terms*— meat, protein oxidation, antioxidative enzymes.**

I. INTRODUCTION

One of the factors detrimental to the sensorial, nutritional and functional quality of meats and meat products is oxidative processes [1], resulting in colour changes, increased insolubility of proteins, off-flavour development and production of oxidized compounds. Although oxidation is generally considered negative, it should be kept in mind that a limited degree of oxidation also contributes to the typical flavour of meat and meat products [2]. In the past, the main focus of studying oxidative processes in meat was on discolouration and lipid oxidation, but it is now well recognized that also protein oxidation influences meat quality, due to protein fragmentation or aggregation, the formation of amino acid derivatives, changes in protein solubility and hydrophobicity. These changes will affect the functional properties of the proteins [3,4]. Protein oxidation undergoes similar processes (initiation, propagation and termination) as those involved in lipid oxidation [5]. Formation of carbonyl groups, a decrease in free thiol groups and aromatic hydroxylation are the main known chemical modifications of amino acids during oxidation [3].

The oxidative stability of meat depends upon its balance between present antioxidants and prooxidants. In muscle tissue the main prooxidants are free or haem bound iron and copper, as well as unsaturated fatty acids. In recent years, interest has grown, from a nutritional point, in increasing the polyunsaturated fatty acid content in meat by including unsaturated oils and fats in the animals' diet. As more unsaturated fatty acid enriched products are more prone towards oxidation, more attention should also be paid to the antioxidant supplementation in the animals' diet and to practices for safeguarding oxidative stability. Supplementation with high doses of α-tocopherol is now well established and its effects on delaying colour and lipid oxidative deterioration in muscle is well-known, however, the impact on limiting protein oxidation is not clear yet. Recently, several studies have been conducted using natural antioxidants or plant extracts in the feed aiming at improving oxidation parameters. The impact of these new antioxidant supplements on protein oxidation is

equally not known at this time. In addition, the oxidative stability of meat is not only dependent on the supply of exogenous antioxidants but also on endogenous enzymatic and non-enzymatic antioxidant compounds. The relationship between the endogenous antioxidant enzymes and protein oxidation has also poorly been studied until now.

The present review will focus on and limit to (mainly refereed) studies that have reported on the effects of dietary constituents on protein oxidation of muscle foods. Relationships between protein oxidation and lipid and colour oxidation will also be addressed, as well as scarce information on the relationship between endogenous antioxidant enzymes and protein oxidation.

II. EFFECT OF DIETARY CONSTITUENTS ON PROTEIN OXIDATION IN MUSCLES

An overview of studies reporting measurements on protein oxidation as affected by dietary factors is given in Table 1. Increasing the dietary polyunsaturated fatty acid content of meat is achieved by including unsaturated oils in the diet or by using high pasture diets. In general the effect of oil source on protein oxidation of fresh meat is not clear. In turkey muscle after 9 days storage, 6% inclusion of soybean oil increased the amount of carbonyls compared to rapeseed or tallow as dietary fat source (3.28, 1.79 and 1.57 nmol DNPH/mg protein for soybean oil, tallow and rapeseed fed groups respectively) [6]. This dietary oil effect was not observed by measuring the thiol content, demonstrating the importance of the methodology that is used to assess oxidation. In other studies on pork no effect of the dietary oil source on protein oxidation was found [7,8,9]. Petron et al. [10] detected differences in protein oxidation in lamb meat from animals grazed on different pastures, with a higher degree of protein oxidation in meat from lambs that had been grazing on botanically diverse pastures compared to leguminosa rich or intensive ryegrass pastures (respectively 1.12, 0.98 and 0.98 nmol DNPH/mg proteins after 8 days cooled storage). However, a possible confounding effect of a much lower growth rate of the animals on the botanically diverse pastures on protein oxidation measurements could not be excluded. On the other hand, in a recent study of Savary-Auzeloux et al. [11] no effect of an impaired growth of lambs on protein oxidation was found.

In beef, Insani et al. [12] observed a higher protein oxidation after 1 and 9 days of storage for grain fed compared to pastured cattle. Protein oxidation increased during time of storage, independent of the dietary treatment. However, this effect of concentrate feeding versus pasturing was not confirmed by Santé-Lhoutellier et al. [13] in lamb meat. However, when the protein fraction was separated in a myofibrillar and sarcoplasmic fraction, an increase of 13 and 31% in the carbonyl content in the myofibrillar fraction during storage for 7 days was observed for the pasture and concentrate fed animals respectively [13]. As the major differences between the pasture and concentrate fed lambs were the intramuscular α-tocopherol content (6.42 and 1.61 µg/g in pasture and concentrate fed animals respectively) and PUFA content (13.9 and 7.82 g/100g FAME respectively), some protective effects of α-tocopherol on protein carbonyl formation was suggested. Indeed, in this study [13] a negative correlation was observed between the carbonyls and α-tocopherol content. Also other feeding trials suggested a protective effect of α-tocopherol on carbonyl formation, e.g. in turkey [6] and in beef [14]. Across 12 studies found so far, including 5 different meat species, the relationship between α-tocopherol and carbonyl content in muscle is not significant ($r = 0.080$; $P > 0.05$). In contrast, the amount of thiol groups was found to be significantly correlated with the α-tocopherol content ($r = 0.580$; $P < 0.05$).

There is an increasing interest for incorporating natural antioxidants in the animal's diet instead of synthetic ones. In two studies with pigs [8,9] rosemary was added to the diet, either or not combined with α-tocopheryl acetate. No additional effect of rosemary on protein oxidation during storage of fresh meat compared with only α-tocopheryl acetate supplementation was measured. Also Smet et al. [15] supplemented different natural antioxidant extract in the diet of broilers, but no effect on the thiol content during storage was measured. Further investigation on the mechanism of antioxidant activities of these plant extracts, and their impact on protein oxidation in vivo and post mortem is needed to evaluate their effectiveness in animal feeding.

In most of these studies, protein oxidation was followed in time during cooled storage to simulate retail display and assess effects on shelf-life. In general it can be concluded that the effect of storage time on fresh meat protein oxidation (measured by the carbonyls or the thiols content) was very small. These results suggest that only limited protein oxidation occurs during time of storage, or that both these

methods (thiol and carbonyl content) are not sensitive enough to detect differences in protein oxidation in time.

III. RELATIONSHIP BETWEEN PROTEIN, LIPID AND COLOUR OXIDATION IN FRESH MUSCLE

There are numerous studies that have looked at the relationship between pigment and lipid oxidation, however, the relationship between these phenomena and meat protein oxidation is far less studied. Santé-Lhoutellier et al. [13] found that protein oxidation occurred more rapidly than lipid oxidation in lamb muscle. In a study of Insani et al. [12], *Psoas major* muscle from pastured steers showed a lower lipid and protein oxidation compared with grain fed steers. This difference was already notable at 1 day post mortem, mainly due to a higher content of α-tocopherol (2.06 and 0.79 μg/g for pastured and concentrate fed animals respectively), and a higher amount of β-carotene (0.74 and 0.17 μg/g respectively). During storage lipid oxidation occurred, in both dietary groups to the same extent (in both groups a doubling of lipid oxidation values after 9 days of storage was found), mainly due to consumption of α-tocopherol. Also protein oxidation occurred during storage, and was different between the two feeding groups, with a lower amount of carbonyls in the pasture fed animals after 9 days of storage [12]. The relationship between α-tocopherol and carbonyl content was only seen in individual studies comparing pasture and concentrate diets [12, 13]. In studies investigating the effect of dietary tocopheryl supplementation on meat oxidation parameters [6,8,9,14], the relationship between carbonyl content and α-tocopherol content was not clear.

Compiling all available data in reviewed papers, measuring both protein and lipid and/or colour oxidation and/or α-tocopherol content, some significant correlations were observed. The carbonyl content is significantly positively correlated with the a^* value and the TBARS value ($r = 0.401$ and 0.314 respectively; $P < 0.05$), while the free thiol content is significantly negatively correlated with the a* value, the % metmyoglobin and the TBARS value ($r = -0.447$, -0.653 and -0.444 respectively; $P < 0.05$). From these data across studies, no relationship between the carbonyl content and thiol content, both measurements of protein oxidation, was obvious. This only confirms that both methods are measuring other amino acids.

IV. EFFECT OF DIETARY CONSTITUENTS ON THE ACTIVITY OF THE ANTIOXIDATIVE ENZYMES AND ITS RELATION WITH PROTEIN OXIDATION

The main endogenous enzymes involved in the antioxidative balance of muscle tissue are superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px). These enzymes have several cofactors (Cu, Zn, Mn, Fe or Se) [16] Although they are known to be important in controlling oxidative processes *in vivo*, their contribution to protecting meat against oxidative processes post mortem is less clear. To our knowledge, only studies on beef and lamb meat are available in which both protein oxidation and endogenous antioxidant enzyme activities were measured simultaneously. In a study of Insani et al. [12] comparing pasture and grain fed steers, the Cat activity was not different while the SOD activity tended to be higher in beef from pastured animals compared to beef from concentrate fed ones. In contrast, the GSH-Px activity was higher in beef from concentrate fed steers in this study. Also in this study the protein oxidation was higher for concentrate fed animals. This diet effect could not be confirmed in a study on lamb meat [13], where no effect was observed on either enzyme activities nor on protein oxidation. However in lamb meat, Petron et al. [10] found an effect of the type of pasture on the GSH-Px activity, with higher activities in the leguminosa rich pasture fed lambs compared to animals fed intensive ryegrass or botanically diverse pastures. The difference in GSH-Px activity was probably due to the higher Se content in the leguminosa rich pasture. However, the content of other trace elements that are cofactors for SOD or Cat was also higher in the leguminosa rich pasture compared to the other pasture types (e.g. Zn 1.5, Fe 1.25 and Cu 2 fold higher). In this study a significant correlation was observed between GSH-Px activity and the thiol content, while the Cat activity was significantly correlated with TBARS and carbonyl content. A significant correlation between thiol groups and GSH-Px activity could be due to the content of glutathione, an important non-protein thiol.

In summary, several studies have reported differences in antioxidant enzyme activities in meat from pastured versus concentrate fed animals. The underlying factors have not been elucidated, but differences in trace element content might be involved and deserve further attention.

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TABLE 1. OVERVIEW OF THE RANGE IN AVERAGE TREATMENT VALUES FOR OXIDATIVE MEASUREMENTS IN DIFFERENT STUDIES

Reference	(Dietary) factor studied	Number of animals (per treatment)	Days of display	a*	% MMb	TBARS (µg MDA/g meat)	Carbonyl (nmol DNPH/mg protein)	Thiol (nmol SH/mg protein)	α-tocopherol (µg/g)
Lamb									
Petron et al. [10]	Different pastures	7	0 – 8	11.3 – 15.4	20 – 41.8	0.31 – 0.68	0.65 – 1.42	48.6 – 70.7	1.09 – 1.72
Santé-Lhoutellier et al. [13]	Pasture vs concentrate	8	0 - 7	6.8 – 16.1		0.38 – 2.67	2.5 – 3.93		1.61 – 6.42
Savary-Auzeloux et al. [11]	Restricted feeding	4	0				0.78 – 1.11		
Beef									
Insani et al. [12]	Pasture vs. grain diet	5	1 - 9	9.6 – 15.3	23.5 – 72.1	0.2 – 1.2	0.66 – 1.91		0.34 – 2.06
Pork									
Haak et al. [8]	Oil source + antioxidant treatment	3	2 - 8	5.63 – 9.79	19.2 – 39.5	0.14 – 0.65	1.41 – 5.50	60.8 – 80.4	0.61 – 0.91
Haak et al. [9]	Antioxidant treatment	8	2 - 8			0.20 – 1.19	1.51 – 2.82	65.5 - 102	1.25 – 2.67
Lund et al. [7]		7	0			0.09	0.81 – 0.93	70.0 – 72.5	
Nurnberg et al. [17]	genotype	9 – 17	0 - 1			0.07 – 0.11	0.33 – 0.47		1.10 – 1.65
Ramirez et al. [18]	genotype	10	0 - 10	8.9 – 18.3		0.05 – 0.57	2.12 – 9.53		
Ventanas et al. [19]	Diet / genotype	11 – 13	0				1.03 – 1.56		1.38 – 2.11
Turkey									
Mercier et al. [6]	Oil / a-tocopherol	24	9	1.91 – 9.50		0.3 – 1.44	1.10 - 3.41	85.2 - 119	3.46 – 9.80
Poultry									
Smet et al. [14]	Antioxidant treatment	9	3 - 10			0.07 - 0.65		63.8 – 89.3	1.34 – 7.64
Vaithiyanathan et al. [20]		15	0 - 28			0.55 – 1.92		26.35 - 75	