EVALUATION OF OXIDATIVE PROCESSES IN LAMB MEAT

ZHODNOCENÍ OXIDAČNÍCH PROCESŮ V JEHNĚČÍM MASE

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ABSTRACT

The effects of different fat sources (soya oil, linseed oil and fish oil) and the effect of time on feed on oxidative processes in lamb meat during storage were evaluated from the view of colour, protein and lipid oxidation. Animals fed linseed oil and soya oil had higher a*-values compared to fish oil. The a*-values decreased over time of storage for all dietary groups. The lowest proportion of MetMb was observed in meat from animals fed linseed oil. TBARS-value was the lowest in animals fed soya oil. It increased with time of storage and decreased with time on feed. The highest carbonyl content was in samples fed fish oil, the lowest in animals fed linseed oil. Carbonyl content decreased with time of storage and also with time on feed.

Keywords: lamb meat, oxidation, soya oil, linseed oil, fish oil

ABSTRAKT

Byl zkoumán účinek různých typů olejů (sójový, lněný a rybí) a účinek délky krmení na průběh oxidačních procesů (oxidace barvy, tuků a bílkovin) v jehněčím mase. Maso zvířat do jejichž krmiva byl přidán lněný nebo sójový olej mělo vyšší hodnotu a* ve srovnání s masem zvířat do jejichž krmiva byl přidán rybí tuk. U všech skupin zvířat hodnota a* s dobou skladování klesala. U zvířat krmených lněným olejem byl zjištěn nejnižší obsah MetMb. Hodnota TBARS byla nejnižší u zvířat krmených sójovým olejem. Vzrůstala s délkou skladování a klesala s délkou krmení. Nejvyšší obsah karbonylů byl ve vzorcích ze zvířat krmených rybím tukem, nejnižší ze zvířat krmených lněným olejem. Obsah karbonylů klesal s délkou skladování a také s délkou krmení.

INTRODUCTION

Meat, and particularly red meat, plays a very important role in the diet. It is a very good source of high quality protein, essential minerals and trace elements, haem iron and a range of B vitamins. However recently, consumers prefer convenience foods. The research is consequently focused on the identification of novel, safe, and quality enhancing antioxidants like dietary vitamin E in meat, through the use of natural oils, rich in PUFA, in the animal diets and through the interest in the contribution and the impact of natural ingredients (e.g. grass (silage), grains). At the point of consumers, appearance, texture and flavour are the most important factors of

meat quality (Liu et al., 1995), whereas at the retail level, colour and colour stability are important. Consumers equate an attractive bright red colour with a long shelf-life and good eating quality, and various approaches have been used to meet this expectation (Hood and Mead, 1993). Discoloration in retail meats during display conditions may occur as a combined function of muscle pigment oxidation and lipid oxidation in membrane phospholipids (Sherbeck et al., 1995). In general, muscle foods are susceptible to oxidative activity of their lipid, protein, pigment, vitamin and carbohydrate composition (Kanner, 1994). Lipid oxidation causes the development of rancidity and "warmed-over flavours". It is a major cause of deterioration of muscle foods and can directly affect many quality characteristics such as flavour, colour, texture, nutritive value and safety of the food (Buckley et al., 1995). Proteins in muscle cells are also targets for radicals *in vivo*, but the study of protein oxidation is a relatively new area. The dietary regime of the animals is one of the important factors affecting meat colour and quality. The type of diet fed to meat-producing animals can influence the PUFA composition of phospholipids (Larick and Turner, 1989) and thereby meat flavour and lipid oxidation of meat. It is also well accepted that increasing the degree of polyunsaturation accelerates oxidative processes. As lipid oxidation is primary initiated in the membrane phospholipids, the use of vitamin E, as a dietary lipophilic antioxidant, has been recommended to protect muscle components against oxidative damage caused by free radicals. Oxidative damage to body cells and molecules has been impact in a wide variety of diseases. The combination of vitamin E supplementation and the type of diets on ruminant meat quality is an area of research that needs to be addressed, particularly in relation to sheep meat, which has received little or no attention.

MATERIALS AND METHODS

1. Experimental set-up

40 weaned lambs were bought from an organic farmer and slaughtered at the Department of Animal Production (Ghent University, Melle). 4 male animals were used as a control group. The other 36 female lambs were devided in 3 main groups, depending on the oil included in the diet. They were fed a concentrate/roughage (50/50 on dry matter) mixture. The concentrates were cereal based supplemented with 4% of oil: soya oil, linseed oil or fish oil. Roughage consisted of hay originated from intensive ryegrass or from organic pastures. Animals were fattened indoors and slaughtered after 1 and 3 months on the diet. After slaughtering carcasses were cooled at 2-3°C. Twenty four hours post mortem, sub-samples were taken from the *longissimus thoracis* for the different analysis.

2. Colour and colour stability measurements

After 1 hour blooming, samples were overwrapped in O_2-permeable film and stored at 4°C under continous light (900 lux). Colour measurements were done on 1, 3, 6 and 9 days post mortem. Colour was measured using a HunterLab Miniscan apparatus and colour was expressed

using the CIELAB-coordinates. In addition, reflections at certain wavelenght as an indication for colour stability were measured using the same apparatus. Using the method fo Krzywicki (1979) the relative contents of myoglobin, metmyoglobin and oxymyoglobin can be calculated from the reflection values.

3. Lipid stability

Lipid oxidation was determined using the TBARS-method (the 2-thiobarbituric acid method). The meat samples were minced and 10 g of meat were weighed in a plastic tube. Then 40 ml destilled water and 1 ml BHT-solution (1.5 g 2,6-di-tertiar-butyle-4-methylphenol was dissolved in 100 ml absolute ethanol) were added. The meat was then homogenised, the homogenate was then poured into a Markham destillation apparatus. Some drops (4 to 5) antifoam solution was added. The plastic tube was rinced with 30 ml destilled water and poured in the destillation apparatus. Then 3 ml 4M HCl was added. Destillation was carried oud till 100 ml of the destilate was collected in a 100 ml flask. A standardcurve was made using dilutions (between 0 and 20 nmol/5 ml) of a standard solution of 14.4 µl 1,1,3,3-tetramethoxypropane dissolved in 100 ml destilled water. In glass tubes, 5 ml of the destillate were brought, together with 1 ml TBARS-reagens (dissolve 865 mg 2-thiobarbituric acid in 100 ml glacial acetic acid). The tubes were placed in a boiling waterbath for 35 min and then cooled under running tap water. The absorbance was meaured at 532 nm. Using the obtained standardcurve, the amount of lipid oxidation products, expressed as µg malondialdehyde/g fresh meat, could be calculated.

4. Protein oxidation

Protein oxidation was measured by an estimation of carbonyl groups using the method of Mercier et al. (1998) with slight modifications. 1 gram of frozen muscle was ground in 10 ml of sodium phosphate buffer 100mM (pH 7). Two equal aliquots of 150 µl were taken away from the muscle extract. Proteins were precipitated in both aliquots with 3 ml of 10% trichloroacetic acid (TCA) and centrifuged. One pellet was treated with 1.5 ml of 2N HCL and the other with an equal volume of 0.2% (w/v) 2,4-dinitrophenyl hydrazine (DNPH) in 2N HCL. Both samples were incubated for 1 h at room temperature. The samples were reprecipitated with 10% TCA and centrifuged. The pellets were washed three times with 1.5 ml of ethanol:ethyl acetate (1:1, v/v). Proteins were finally dissolved in 3 ml of 6M guanidine-HCL in 20mM sodium phosphate buffer (pH 6.5). Protein concentration was calculated at 280 nm in the HCL control using Bovine serum albumine (BSA) in 6M guanidine as standard. Carbonyl content was measured on the treated sample by measuring DNPH incorporated on the basis of an absorption of 21.0 mM^{-1} cm^{-1}. The results were expressed as nanomoles of DNPH fixed per miligram of protein.

RESULTS AND DISCUSSION

1. Colour and colour stability measurements

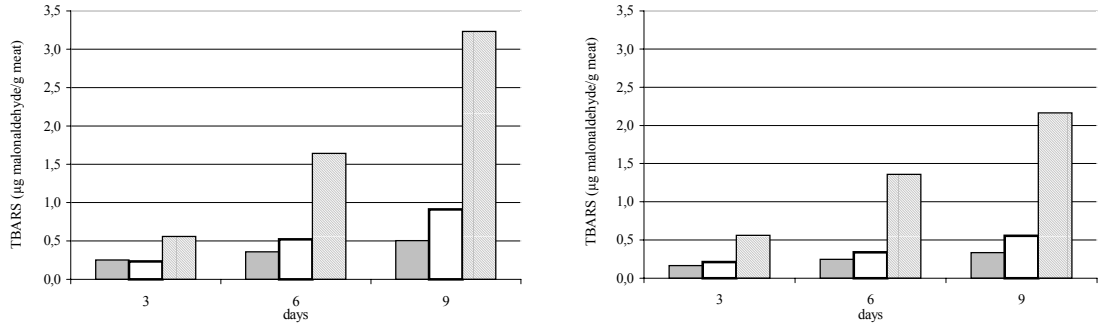
No significant effect of oil type was observed on the L* value but slaughtering time had a significant effect on the L* value ($P < 0.05$) at day 9. No significant effect of oil type on the a*-value was observed between groups, however certain trends were noticed. The a*-values decreased for all groups over time. The lowest values were almost always found in meat from animals fed fish oil after both 1 month and 3 months on the feed. These results correspond with other literature data. Mercier et al. (1998) carried out studies with turkey meat and they observed that the fat source had no influence on the L*-value. Santé and Lacourt (1994) found that L*-values for turkey meat did not change significantly during seven days of storage. Neither significant effects of fat source nor time on feed were observed on the proportion of MetMb. Also Mercier et al. (1998) did not note influences of fat source on MetMb proportions in turkey meat but O'Sullivan et al. (2002) observed, in studies carried out on beef, that there was a significant ($P < 0.05$) difference in the proportion of MetMb between the dietary groups.The content of MetMb in meat of the control group and of the groups fed different oils slaughtered after 1 month on the feed was very similar. Meat from the fish oil group fed 1 month had the highest proportion of MetMb. The highest proportion of MetMb in the samples of animals slaughtered after 3 months was observed in the meat of animals fed linseed oil, at 3, 6 and 9 days of storage. Overall, the proportion of MetMb increased over time of storage for all three dietary groups slaughtered after 1 month and 3 months on the feed. This observation corresponds with results from O'Sulivan et al. (2002). These authors reported a significant ($P < 0.001$) time on feed effect in the three dietary groups.

2. TBARS measurements

A significant effect ($P < 0.05$) of oil type on the TBARS-values between groups was observed at day 3, 6 and 9 of storage. No significant effect of time on feed on TBARS-values was noticed. A similar trend in TBARS-value during storage of the meat for the animals of the different feeding groups was observed when they were fed 1 or 3 month on the diets (Figure 1). In general, TBARS-value increased in all groups over storage time. Meat from animals fed fish oil had the highest TBARS-value. This fat source effect can be attributed to the polyunsaturated fatty acids (PUFA) content of the different diets. Fish oil contains high amounts of long-chain PUFA, C20:5 n-3 and C22:6 n-3, which are known to be very susceptible to oxidation. These results are in accordance with literature data. Mercier et al. (2001) and Mercier et al. (1998) showed a significant effect of fat source on lipid oxidation ($P < 0.001$) when turkeys were fed soya oil, rapeseed oil or tallow. Also other studies (Asghar et al., 1990; O'Sullivan et al., 2002) were reported where the rate of lipid oxidation was dependent on the type of the dietary oils.

Figure 1: Mean TBARS value after 3, 6, 9 days of storage of the *longissimus thoracis* of animals fed different oils, soya oil ■ , linseed oil □ , fish oil ▨ , during 1 month (a) and 3 months (b)

a) b)

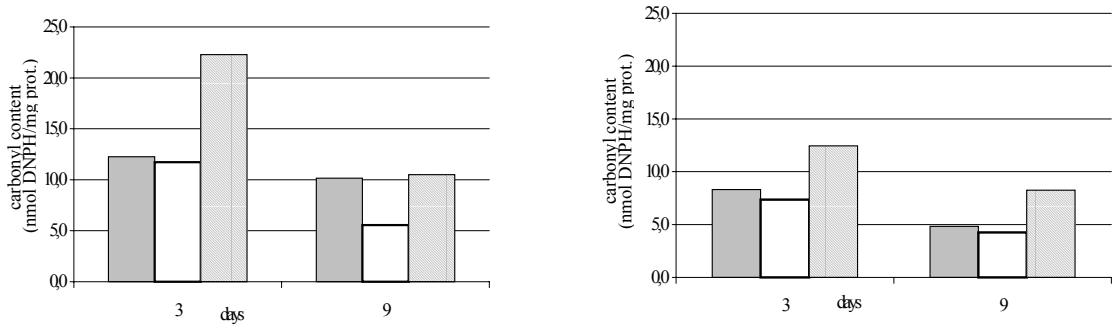


3. Protein oxidation

Significant effects ($P < 0.05$) of oil type and time on feed on the carbonyl content, as a measurement of protein oxidation, was observed when the *longissimus thoracis* was stored for 9 days (Figure 2). The carbonyl content in the control group increased slightly over time of storage. Compared to the control group, the carbonyl content decreased in all dietary groups over time on feed. The meat from the linseed oil fed animals during both 1 and 3 months showed the lowest carbonyl content at day 3 and day 9 of storage. The carbonyl content in meat from animals slaughtered after 3 months on the feed was generally lower compared to the carbonyl content in meat from animals fed only the diets during 1 month. These results do not completely correspond with those reported by Mercier et al. (2001) for turkey meat. These authors observed a significant effect ($P < 0.001$) of the fat source on the carbonyl content but the content increased over time of storage. Mercier et al. (1998) also noticed significant effects of oil types. Reznick et al. (1992) also found a reduction in carbonyl content during storage for rat muscle meat, however the diets were supplemented with vitamin E.

Figure 2: Mean carbonyl content at day 3 and 9 of storage in meat of animals fed different types of oil, soya oil■ , linseed oil □, fish oil ▨ , and slaughtered after 1 month (a) or 3 months (b)

a) b)



CONCLUSIONS

No significant effect of oil type on the a*-value was observed between groups. The a*-values decreased for all dietary groups over time. It indicated loss of red colour and it corresponded to the increased %MetMb. Meat from group fed fish oil had almost always the lowest a*-value during storage. TBARS-value increased in all dietary groups over time of storage and decreased over feeding time. A significant effect of oil type on the TBARS-values was noticed between groups at day 3, 6 and 9 of storage but no significant effect of time on feed on TBARS was observed. Meat from the group fed fish oil had much higher levels of TBARS compared to other dietary groups. Fish oil is the most susceptible to lipid oxidation, compared to soya oil and linseed oil, because of its high amounts of long-chain PUFA, C20:5 n-3 and C22:6 n-3. The carbonyl content decreased in all dietary groups over time on feed and also over time of storage. The lowest carbonyl content was in the group fed linseed oil, followed by soya oil. Meat from animals fed fish oil had the highest carbonyl content. It indicates that fish oil induced a more pronounced oxidation of proteins compared to soya oil and linseed oil. These results do not completely agree with results from literature and therefore it would be suitable to confirm our results by other method to measure protein oxidation, e.g. thiol oxidation measurement. The results of this study indicate, that linseed oil and soya oil are more optimal dietary fat sources, compared to fish oil, concerning the meat stability against colour, lipid and protein oxidation. However, as it is known that fish oil is very good source of high quality PUFAs, it would be interested to combine fish oil supplementation with dietary vitamin E supply, as it is known that vitamin E is effective in inhibiting lipid oxidation.

REFERENCES

Asghar, A., Lin, C.F., Grey, D.J., Buckley, D.J., Booren, A.M., Flegal, C.J. (1990). Effect of lipid composition and α-tocopherol acetate enhances beef quality in Holstein and beef breed steers. *J. Food Sci.*, 55:46-50.

Buckley, D.J., Morissey, P.A., Gray, J.I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci.,* 73:3122-3130.

Hood, D.E., Mead, G.C. (1993). Modified atmosphere storage of fresh meat and poultry. In: *Principles and Applications of Modified Atmosphere Packaging of Food*, Parry, R.T. (ed.), London:

Kanner, J. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Sci*., 36:169-189.

Krzywicki, K. (1979). Assessment of relative content of myoglobine, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Sci*., 3:1-10.

Larick, D.K.,Turner, B.E. (1989). Influence of finishing diet on the phospholipid composition and fatty acid profile of individual phospholipids in lean muscle of beef cattle. *J. Anim. Sci.*, 67:2282-2293.

Liu, Q., Lanari, M.C., Schaefer, D.M. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *J. Anim. Sci*., 73:3131-3140.

Mercier, Y., Gatellier, P., Viau, M., Remignon, H., Renerre, M. (1998). Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.*, 48:301-318.

Mercier, Y., Gatellier, A., Vincent, M., Renerre, M. (2001). Lipid and protein in microsomal fraction from turkeys: influence of dietary fat and vitamin E supplementation. *Meat Sci*., 58:125-134.

O'Sullivan, A., O'Sullivan, K., Galvin, K., Moloney, A.P., Troy, D.J., Kerry, J.P. (2002). Grass silage versus maize silage effects on retail packaged beef quality. *J. Anim. Sci*., 80:1556-1563.

Reznick, A.Z., Witt, E., Matsumoto, M., Packer, L. (1992). Vitamin E inhibits protein oxidation in skletal muscle of resting and exercised rats. *Biochemical Biophysical Research Communications*, 189:801-806.

Santé, V., Lacourt, A. (1994). The effect of dietary α-tocopherol supplementation and antioxidant spraying on colour stability and lipid oxidation of turkey meat. *J. Sci. Food Agric*., 665:503-507.

Sherbeck, J.A., Wulf, D.M., Morgan, J.B., Tatum, J.D., Smith, G.C., Williams, S.N. (1995). Dietary supplementation of vitamin E to feedlot cattle affects retail display properties. *J. Food. Sci*., 60:250-252.