PS2.02 Protein oxidation in meat during chill storage in high-oxygen atmospheres 52.00

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***Abstract*—Modified atmosphere packaging with a high level of oxygen (70-80% O_2 and 20-30% CO_2) is increasingly used for retail packaging of fresh meat. The packaging method is advantageous as it preserves the bright red color of fresh meat and reduces microbiological spoilage concurrently, but the high concentration of oxygen in the packaging atmosphere holds the risk for increased oxidation of both lipids and proteins. Protein oxidation of meat is associated with texture deterioration. Two considerable aspects of protein oxidation of meat are i) inhibition of calpains and hereby reduced tenderization early *post-mortem* and ii) oxidation of structural proteins to form cross-linked polymers resulting in a strengthening of the myofibrillar structure.**

The aim of the present work was to investigate how storage in high-oxygen atmosphere affected meat tenderness due to oxidation and inactivation of calpains and/or oxidation of structural proteins and cross-linking. Comparative studies were performed for meat stored without oxygen. Furthermore, the protective effect of two selected antioxidant systems on meat proteins was tested. Tenderness was determined by sensory analysis, protein oxidation by determination of protein carbonyls and free proteins thiols, calpain activity by casein zymography, and oxidative protein cross-linking was determined by SDS-page and subsequent MS analysis.

Storage in high-oxygen atmosphere caused decreased meat tenderness and reduced content of free protein thiols compared to packaging without oxygen, showing that meat proteins are in fact oxidized when stored in high-oxygen atmospheres. The calpain activity was not found to be affected by packaging atmosphere. Myosin was found to cross-link in meat stored only in high-oxygen atmosphere, and these results indicate that the reduced tenderness observed in high-oxygen atmosphere packaged meat is caused by oxidative modification of structural proteins, most likely by formation of myosin cross-links, while inactivation of μ-calpain does not seem to be of major importance. The selected antioxidant systems proved not to inhibit protein oxidation in meat stored in high-oxygen atmosphere, while they effectively inhibited lipid oxidation.

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***Index Terms*—protein oxidation, high-oxygen atmosphere packaging, protein cross-linking, meat tenderness, antioxidant protection.**

I. INTRODUCTION

Lipid and pigment oxidation are recognized as the most important causes of quality deterioration of both fresh and processed meat during storage [1]. Protein oxidation in meat is only sparsely understood and described contrary to lipid oxidation. The importance of protein oxidation for quality deterioration has only been partially investigated and the coupling between protein oxidation and lipid oxidation is elucidated to an even lesser extent. However, protein oxidation has been investigated extensively in relation to aging and pathology and it is well-known that proteins are modified in human tissue under oxidative stress [2].

Retail modified atmosphere packaging with high levels of oxygen is widely used for storage of fresh meat. Packaging in high-oxygen atmospheres with 70-80% O_2/20-30% CO_2 has proven effective to reduce microbial growth in meat and protect the red meat colour for both beef and pork [3]. The presence of oxygen causes oxidation of meat lipids and has been found to cause production of unacceptable rancid off-flavours during storage [3]. However, the extent of protein oxidation in fresh meat stored in high-oxygen atmospheres and the consequences for meat quality have only recently received attention.

Reaction of radicals with proteins and peptides in the presence of oxygen gives rise to alterations of both the backbone and of the amino acid side chains. These oxidative changes include cleavage of peptide bonds, modification of amino acid side chains, and formation of covalent intermolecular cross-linked protein derivatives. The nature of protein oxidation products formed is highly dependent on how oxidation is initiated. In general, the more reactive the formed radicals are, the less selective reactions are initiated [2]. Some of the most general amino acid modifications

are the formation of protein carbonyl groups and protein hydroperoxides, while cross-linking has mostly been described as formation of disulfide and dityrosine. Oxidation of proteins may cause changes in protein hydrophobicity, solubility and conformation, inactivation of proteolytic enzymes, and altered susceptibility of protein substrates to proteolytic enzymes [2]. Hence, it is important to know the impact of protein oxidation on meat quality as increased oxidation due to storage in high-oxygen atmosphere holds the risk for reduced protein functionality and potentially meat quality deterioration.

Oxidation of proteins in processed meat products tends to result in reduced water-holding capacity and texture-forming ability [4]. For fresh meat, it has been shown that protein oxidation induced by irradiation, which creates a highly oxidative environment, leads to reduced tenderness [5], but the fundamental mechanisms behind this change in protein functionality has not been clarified. Storage of meat in high-oxygen atmospheres has been found to reduce tenderness in a number of studies [6-8]. In addition, the changed functionality of myofibrillar proteins caused by oxidation may affect water binding capacity and hereby reduce juiciness of the meat [9].

The presence of elevated levels of molecular oxygen (or triplet state oxygen) in packaging atmospheres holds the risk for increased oxidation of both structural proteins and meat enzymes. The most abundant meat protein, myosin, is highly susceptible to oxidation and is known to polymerize through intermolecular cross-linking in model systems [10]. Additionally, myosin is known to cross-link during aging of meat [11], a reaction which may be increased by oxidation and influence meat quality negatively. The reaction mechanism of myosin oxidation has only recently been described in details [12]. Additionally, it is hypothesized that calpain activity is affected by high levels of oxygen in the packaging atmosphere, as oxidative stress may reversibly inactivate calpain due to the reactive cysteine residue at the active site [13]. As μ-calpain is believed to play a major role during *post-mortem* tenderisation, inhibition of μ-calpain is expected to be a reasonable explanation of decreased tenderness in high-oxygen atmosphere packaged meat.

Antioxidants are widely used to protect meat products from oxidation and the protective effect of various compounds such as tocopherols and different plant phenolics towards lipid oxidation in meat products is generally accepted [14] but the reports on antioxidative protection of proteins are ambiguous.

II. MATERIALS AND METHODS

The experiments have been described in [6] and [15] unless otherwise stated. An additional storage experiment was performed to further elucidate the effect of high-oxygen atmosphere packaging on calpain activity. The storage experiment was performed similarly to the experiment described in [6] with the following modifications: the number of animals was 4 and storage time was 0, 1, 2, 8, and 14 days *post-mortem*. Beef (2 animals) was also included in the study and was packed on day 2 *post-mortem*, and stored similarly as the pork samples.

III. RESULTS AND DISCUSSION

Storage of pork *longissimus dorsi* (LD) in high-oxygen atmosphere resulted in significantly less tender meat evaluated by sensory analysis already after 4 days of storage compared to storage without the presence of oxygen. The difference in tenderness between the two packaging atmospheres was found to increase further with storage time [6].

Determination of free protein thiol groups showed that increased protein oxidation had occurred in meat stored in high-oxygen atmospheres compared to storage without oxygen [6]. In this study [6] it was not possible to detect any μ-calpain activity on day 4 post-mortem, possibly because of calpain autolysis, and an additional storage experiment was therefore conducted to determine the extent of μ-calpain inactivation due to storage in high-oxygen atmosphere. Samples were analyzed for μ-calpain activity after 1 and 2 days of storage (day 2 and 3 *post-mortem*), but no significant difference of packaging atmosphere was observed (Table 1).

Table 1 μ-Calpain activity in pork and beef LD stored in high-oxygen atmosphere (+) and without oxygen (-) for up to 2 days. Pork was packed on day 1 *post mortem* and beef on day 2 *post mortem*.

Species	O_2	Storage day		
		0	1	2
Pork	+	1.16 ± 0.84	0.26 ± 0.24	0.05 ± 0.05
	-		0.22 ± 0.22	0.10 ± 0.12
Beef	+	1.02 ± 0.27	0.34 ± 0.08	0.22 ± 0.07
	-		0.29 ± 0.20	0.30 ± 0.40

These results indicate that μ-calpain is not inactivated during storage of meat in high-oxygen atmospheres. The results were similar for m-calpain (data not shown).

The effect of high-oxygen atmosphere packaging on the structural proteins of meat was

studied by extracting myofibrillar proteins from pork LD after storage and analyzing the protein fraction by SDS-page. In the gel a high-molecular weight protein band above 500 kDa appeared only in the samples from high-oxygen atmosphere storage [6]. The high-molecular weight band was identified as myosin heavy chain (MHC) by MS analysis showing that MHC forms intermolecular cross-links in the presence of oxygen. By running the samples reduced and non-reduced the cross-linking type was identified as disulfide bonding as the cross-linked MHC (CL MHC) band did not appear when the samples were reduced.

The tenderness of pork LD slices stored without oxygen increased during the storage period from day 4 to day 14 *post-mortem* ($p = 0.0374$) indicating that development of tenderness still occurs after day 4 [6]. In contrast, tenderness of LD slices stored in high-oxygen atmosphere decreased further over time ($p = 0.0436$), which indicates that presence of oxygen not only inhibits development of tenderness but strengthens the myofibrillar structure as well, for example through protein cross-linking. However, the part of myosin heavy chain cross-linking after 14 days *post-mortem* appears to be quite small when comparing band intensities of MHC and CL MHC and whether cross-linking of myosin was the only cause for the observed tenderness decrease remains to be investigated. Nevertheless, these results indicate that protein oxidation causing cross-linking of myosin has a negative effect on meat tenderization.

Likewise, recent studies show that beef stored in high-oxygen atmospheres also becomes less tender compared to storage without the presence of oxygen evaluated by sensory assessment [7; 8] and by Warner Bratzler shear force although these findings were not statistically significant [16]. However, if beef is stored in vacuum packaging prior to packaging and storage in high-oxygen atmospheres the negative effect of oxygen on beef tenderness is limited to some extent [7; 16]. These observations might suggest that proteolytic enzyme activity early *post-mortem* plays a role in beef tenderisation during storage in high-oxygen atmospheres. In one of our storage experiments where beef steaks were packed in high-oxygen atmospheres and without oxygen on day 2 *post-mortem* no significant effect of packaging atmosphere was found on μ-calpain activity after 1 and 2 days of storage (day 3 and 4 *post-mortem*, respectively) (Table 1). However, only two cows were included in this experiment so the data material is rather limited, and it is possible that a higher number of animals will reveal measurable differences in μ-calpain activity as a consequence of packaging atmosphere. The authors are not familiar with other reports investigating μ-calpain activity early *post-mortem* after storage in high-oxygen atmospheres for a few days. It is also possible that other enzymes than calpain are involved in *post mortem* tenderisation and it can be speculated that these are also affected by oxidation.

When meat is stored in high-oxygen atmospheres, it takes several days before the oxygen pressure is equalized in the meat, even with a meat thickness of only 2 cm. Consequently, during the first days of storage, only the surface of the meat will most likely be affected by oxygen. Since μ-calpain activity is primarily restricted to the first days of storage according to the fast decline *post-mortem*, the time span for activity coincide with the time, where large areas in the middle of the meat is still not reached by oxygen and likely not oxidatively affected to the same extend as the surface. If any, the effect of oxidation on μ-calpain in the surface area of the meat will be diminished when transverse cut is used for determination of activity. Enzyme systems responsible for tenderisation at later times *post-mortem* may, if susceptible to oxidation, more likely be affected by storage in high-oxygen atmospheres due to a more pronounced oxygen consumption by the meat over time. In addition, enzymes involved in *post-mortem* proteolysis may be indirectly affected by oxidation as oxidative modification of proteins may make them less susceptible as substrates.

Antioxidative protection of meat proteins has until now only received little attention. α-Tocopherol and some plant phenolics have been shown to inhibit protein oxidation in meat but the results are dependent on animal species, muscle type, protein fraction, and antioxidant structure [5; 17; 18]. Generally, it seems that α-tocopherol protects proteins from oxidation while the reports on plant phenolics are ambiguous, but no positive effect of α-tocopherol on tenderness of beef [5] and pork [19] has been observed, while addition of plant phenolics to chicken has been reported to increase tenderness [20]. In our study two antioxidant systems (a commercially available rosemary extract and a mixture of ascorbate and citrate (1:1)) were tested in minced beef patties stored in high-oxygen atmospheres, but no protective effect of the two systems on the meat proteins was observed [15]. Further research into this area is therefore encouraged in order to find solutions to prevent protein oxidation in meat and meat products.

IV. CONCLUSION

Presence of oxygen in the packaging atmosphere affects meat tenderness negatively through oxidative cross-linking of myosin but it cannot be excluded that proteolytic enzymes also are affected either directly by inactivation or indirectly by loss of protein substrate susceptibility due to increased oxygen pressure. Further

research is needed to elucidate the ability of phenolic compounds to protect meat proteins from oxidation.

ACKNOWLEDGEMENT

The authors thank the LMC graduate school FOOD Denmark for supporting the work.

REFERENCES

1. Kanner J., Oxidative Processes in Meat and Meat-Products - Quality Implications, Meat Science 36 (1994) 169-189.

2. Davies M.J., Dean R.T., Radical-mediated protein oxidation, Oxford Science Publications, Oxford, 2003.

3. Asensio M.A., Ordonez J.A., Sanz B., Effect of carbon dioxide and oxygen enriched atmosphere on the shelf-life of refrigerated pork packed in plastic bags, Journal of Food Protection 51 (1988) 356-364.

4. Xiong Y.L., Protein oxidation and implications for muscle food quality, in: Decker E, Faustman C. (Eds.), Antioxidants in Muscle Foods, John Wiley & Sons, Chichester, 2000, pp. 85-111.

5. Rowe L.J., Maddock K.R., Lonergan S.M., Huff-Lonergan E., Influence of Early Postmortem Protein Oxidation on Beef Quality, Journal of Animal Science 82 (2004) 785-793.

6. Lund M.N., Lametsch R., Hviid M.S., Jensen O.N., Skibsted L.H., High-Oxygen Packaging Atmosphere Influences Protein Oxidation and Tenderness of Porcine longissimus dorsi during Chill Storage, Meat Science 77 (2007) 295-303.

7. Clausen I., Jakobsen M., Ertbjerg P., Madsen N.T., Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef, Packaging Technology and Science (2008).

8. Zakrys P.I., O'Sullivan M.G., Allen P., Kerry J.P., Consumer acceptability and physicochemical characteristics of modified atmosphere packed beef steaks, Meat Science 81 (2009) 720-725.

9. Huff-Lonergan E., Lonergan S.M., Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes, Meat Science 71 (2005) 194-204.

10. Decker E.A., Xiong Y.L., Calvert J.T., Crum A.D., Blanchard S.P., Chemical, Physical, and Functional-Properties of Oxidized Turkey White Muscle Myofibrillar Proteins, Journal of Agricultural and Food Chemistry 41 (1993) 186-189.

11. Martinaud A., Mercier Y., Marinova P., Tassy C., Gatellier P., Renerre M., Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems, Journal of Agricultural and Food Chemistry 45 (1997) 2481-2487.

12. Lund M.N., Luxford C., Skibsted L.H., Davies M.J., Oxidation of myosin by heme proteins generates myosin radicals and protein cross-links, Biochemical Journal 410 (2008) 565-574.

13. Rowe L.J., Maddock K.R., Lonergan S.M., Huff-Lonergan E., Oxidative Environments Decrease Tenderization of Beef Steaks through Inactivation of milli-calpain, Journal of Animal Science 82 (2004) 3254-3266.

14. Andersen M.L., Lauridsen R.K., Skibsted L.H., Optimising the Use of Phenolic Compounds in Foods, in: Johnson I., Williamson G. (Eds.), Phytochemical Functional Foods, Woodhead Publishing Limited, Abington Hall, Abington, Cambridge, UK, 2003, pp. 315-346.

15. Lund M.N., Hviid M.S., Skibsted L.H., The Combined Effect of Antioxidants and Modified Atmosphere Packaging on Protein and Lipid Oxidation in Beef Patties during Chill Storage, Meat Science 76 (2007) 226-233.

16. Zakrys P.I., Hogan S.A., O'Sullivan M.G., Allen P., Kerry J.P., Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere, Meat Science 79 (2008) 648-655.

17. Vuorela S., Salminen H., Makela M., Kivikari R., Karonen M., Heinonen M., Effect of plant phenolics on protein and lipid oxidation in cooked pork meat patties, Journal of Agricultural and Food Chemistry 53 (2005) 8492-8497.

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

19. Lahucky R., Bahelka I., Kuechenmeister U., Vasickova K., Nuernberg K., Ender K., Nuernberg G., Effects of dietary supplementation of vitamins D_3 and E on quality characteristics of pigs and *longissimus* muscle antioxidative capacity, Meat Science 77 (2007) 264-268.

20. Rababah T., Hettiarachchy N., Horax R., Eswaranandam S., Mauromoustakos A., Dickson J., Niebuhr S., Effect of electron beam irradiation and storage at 5C on thiobarbituric acid reactive substances and carbonyl content in chicken breast meat infused with antioxidants and selected plant extracts, Journal of Agricultural and Food Chemistry 52 (2004) 8236-8241.