Oxidative changes in lipids and proteins in beef during storage

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SUMMARY
The development of lipid and protein oxidative stability in beef m. Longissimus dorsi during storage was measured. The samples from the same individual animals were either chill stored for 6 days and then subsequently frozen for 90 days (non vacuum packaged) or vacuum aged for 14 days and stored at 4°C after opening the bags for maximum of 6 days. Lipid and protein oxidation during storage were measured respectively by quantification of thiobarbituric acid reactive substances (TBARS) and protein carbonyl groups. Time had significant impact on the TBARS formation during storage of the non vacuum packaged meat samples as they increased significantly at the end of the chilling period (P<0.05) and after freezing (P<0.001). Vacuum aging influenced the lipid oxidation in beef muscle during storage as it was more pronounced than in the non vacuum packaged meat. In the course of storage of both non vacuum and vacuum packaged samples the measured reflectance values significantly decreased (P<0.001) but remained higher in non vacuum when compared to vacuum packaged meat (P<0.001). Duration of the storage had significant impact on the formation of carbonyl substances as it increased over time in the vacuum packaged meat samples.

Keywords: beef, storage, TBARS, proteins

INTRODUCTION
Beef industry serves many markets and numerous demands are placed on the manufacture, distribution, sales and marketing of beef products to multiple segments of the food industry. To supply safe, high quality products to the customers, storage conditions and packaging are of great importance.

It is known that during storage the oxidative processes that occur in both lipid and protein fractions of meat are one of the major causes for changes in its quality parameters. Lipid oxidation is often responsible for quality loss via formation of rancid flavour (Asghar et al., 1988) and is affected by the duration and temperature of storage of meat (Sun et al., 2002, Smet et al., 2005, Tan and Chen, 2005), as well as the presence of oxygen. During chilled and frozen storage lipid oxidation is usually slow but does not stop since the reactive
species are soluble in the lipid fraction and stable at low temperatures (Zarzycky and Swiniarska, 1993). Protein oxidation produces different amino acid modifications leading to carbonyl formation and decreased sulfhydryl content (Martinaud et al., 1997; Xiong, 2000) that alter the water holding capacity and tenderness of meat (Rowe et al., 2004), as well as its nutritional value (Sante-Lhou tellier, 2008).

In order to reduce the negative impact of the oxidation on the meat quality beef retailers use vacuum packaging. On the other hand through vacuum packaging meat continues its maturation process in a safe manner, becomes tender, with a better taste and excellent colour.

Since the development of oxidation process in meat is important, related to its quality and consumers’ acceptance, the aim of this study is to examine the oxidative changes in lipids and proteins during storage in beef meat.

**MATERIAL AND METHODS**

The study was carried out with 6 animals (steers and heifers) of Limousin crossbreed at the age of 24 months and average body weight 418 kg. The animals were raised on pasture and slaughtered according the EU requirements. About 24 hours after slaughter *m. Longissimus dorsi* (m.LD) was removed from the left half of each carcass and sliced into 2 cm thick chops which were placed in a plastic tray, wrapped in an oxygen permeable film and stored at 4°C for a period of 6 days, after which the storage continued at -20 °C up to 90 days. The rest of m.LD muscles were vacuum-packaged and stored at 4°C for 14 days until required for analysis. After 14 days of storage under vacuum, the bags were opened and the meat was cut into 2 cm thick chops, wrapped and placed as described before and stored for a period of 6 days.

Lipid oxidation during the storage of the meat was measured by determination of the thiobarbituric acid reactive substances (TBARS) according to the method of Lynch and Frei (1993), modified by Mercier et al. (1998). Muscle samples (1 g) were homogenized in 10 ml KCl 0.15 M + BHT 0.1mM with Ultraturrax (1 min, medium speed). Samples of homogenate (0.5 ml) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 ml) and 2.8% (w/v) trichloroacetic acid (0.25 ml) in a boiling water bath for 10 min. After cooling at room temperature for 20 min, the pink chromogen was extracted with n-butanol (2 ml) and its absorbance measured at 535 nm against a blank of n-butanol. The results were expressed as milligram malondialdehyde (MDA) per kilogram of meat (TBA units).

Reflectance of the meat was measured on Spekol 11 as the sample was placed in a cuvette and the values of the reflectance were read at 525 nm.

Protein oxidation was determined for the myofibrillar proteins by the content of the carbonyl substances formed during storage as described by Oliver at al. (1987) and modified by Mercier et al., 1998. Carbonyl groups were detected by reactivity with 2,4 dinitrophenylhydrazine (DNPH) to form protein
hydrazones. The results were expressed as nanomoles of DNPH fixed per milligram of protein.

The statistical evaluation was carried out by JMP7, 2007. Student’s t-test was used to compare and evaluate the difference in analyzed parameters between the two types of storage of beef. To compare the changes in the quality traits during storage ANOVA was used, and in case of difference between the intervals, it was evaluated by Student t-test. The levels of significance are as follows: * -P<0.05; **- P<0.01; ***- P<0.001.

RESULTS AND DISCUSSION
A. Lipid oxidation

The development of lipid oxidation in non vacuum packaged beef meat is presented in Fig.1. Duration of the storage affected the overall TBARS formation of the meat (P<0.01). The amounts of malondialdehyde (MDA) increased during chilled storage with significant difference between the 1st and the 6th day (P<0.05). The subsequent freezing of the meat resulted in higher values of the TBARS on the 90th day of storage which were significantly different (P<0.001). The amounts of MDA formed during chilled storage were in the range 0.17-0.32 mg/kg meat and reached 0.64 mg/kg meat on the 90th day.

![Fig.1. Development of lipid oxidation during storage in non-vacuum packaged beef meat. Points, connected with different letters are statistically different](image)

Time had significant influence (P<0.01) on the development of oxidation in vacuum packaged beef similarly to the non vacuum packaged (Fig 2.). Significant difference was observed between the amounts of the TBARS
measured on 1\textsuperscript{st} and 2\textsuperscript{nd} (P<0.05) and 2\textsuperscript{nd} and 6\textsuperscript{th} days (P<0.01) due to the drop of the formation of MDA in the first interval of storage. Its content varied between 0.61-0.72 mg/kg meat.

![Graph](image)

Fig. 2 Development of lipid oxidation during storage in vacuum packaged beef meat. Points, connected with different letters are statistically different

![Graph](image)

Fig 3. Comparison in TBARS formation between non vacuum packaged and vacuum packaged beef meat; *** - P< 0.001

A comparison was made between the values of the TBARS formed in the common intervals of the two types of storage of beef meat samples (Fig.3). It was found that the type of storage had significant effect on the lipid oxidation
Unexpectedly vacuum packaged samples showed higher content of TBARS when subsequently chilled, compared to the non vacuum packaged meat as the difference was significant on the 1st and 6th day of storage (P<0.001).

In both non vacuum and vacuum packaged meat, the amounts of TBARS formed in the course of storage were far below the critical value of 3 mg/kg at which rancidity is detected (Wong et al., 1995). It could be supposed that the low intensity of oxidative processes was due to the raising mode of the studied animals which provided natural antioxidants, such as vitamin E, carotenoids, etc. (Yang et al., 2002). It was shown that pasture (Gatellier et al., 2005) increased significantly the content of vitamin E in bovine muscles and hence reduces the development of oxidation in meat.

Lipid oxidation involves changes in meat colour which is one of the important quality parameters. The reflectance measured at 525 nm was significantly influenced by the duration of the storage (P<0.001) in both non vacuum and vacuum packaged samples (Fig. 4 and Fig. 5). In the course of chilled storage of non vacuum aged meat samples the reflectance values decreased and were statistically different between the days of measurement (P<0.001) except 4th and 6th day and 2nd and 90th day where it was almost equal. A slight increase in the reflectance was observed at the 90th day but it remained lower than the values measured in the intervals of chilled storage (P<0.001). Reflectance in the vacuum aged samples decreased during storage but the difference was not significant.

Fig 4. Changes in color (R/525 nm) during storage of non vacuum packaged beef meat. Points, connected with different letters are statistically different.
The comparison made between the reflectance values in non vacuum and vacuum packaged meat samples at the same intervals (Fig. 6) showed higher values in the fresh meat. They differed significantly in the 1st and 2nd day of measurement (P<0.001). The difference in the reflectance values between the non vacuum and vacuum packaged beef could be explained with the lack of oxygen during the storage of the latter which leads to a purple brown colour and hence the lower reflectance.

**Fig. 5. Changes in colour (R/525nm) during storage of vacuum packaged beef meat**

**Fig. 6. Comparison in the changes of colour (R/525 nm) during oxidation between non vacuum and vacuum packaged beef meat; ***- P<0.001**
B. Protein oxidation

Carbonyl content was measured in vacuum packaged meat after opening the bags and on the 1\textsuperscript{st} and 6\textsuperscript{th} day of chilled storage (Fig.7). The total amount of carbonyl substances is increased in the samples overtime as the lack of significance was due to the variations in the animals.

Several proteins contain amino acids that are very susceptible to oxidation such as cysteine, histidine, methionine, lysine, and tryptophan (Xiong, 2000). Oxidative reactions involving the side chains of amino acids can lead to the formation of carbonyl groups as this conversion may ultimately result in a loss of catalytic activity and increased susceptibility to protein degradation (Stadtman, 1990) or protein aggregation and loss of solubility. Formation of carbonyl substances in meat can be caused by several oxidative treatments and has even been shown to occur in beef myofibrils during postmortem aging (Martinaud et al., 1997) which is in agreement with our results.

\textbf{CONCLUSIONS}

In the condition of observations carried out in this experiment the dynamics of lipid oxidative process and color changes show influence of time in both fresh and vacuum aged beef meat. Lipid oxidation had low intensity as the contents of TBARS in the course of storage remained below the threshold for detection of rancid odor, however it was higher in the matured meat samples.

Reflectance decreased during storage in non vacuum packaged meat as well as the vacuum packaged but remained lower in the latter.
Duration of the storage affected significantly the formation of carbonyl substances as it increased over time in the vacuum aged meat samples.

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