Early alteration of beef colour packaged in a modified atmosphere: investigation of indicators involved in the phenomenon appearance

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Abstract— **Beef aging under vacuum before packaging under modified atmosphere with high oxygen content frequently leads to early colour alteration and occurrence of brown areas on meat surface, 3 to 5 days before the end of shelf life.**

The aim of this work was to identify the intrinsic meat parameters involved in this recurring issue. Experiments were carried out on *Longissimus dorsi* muscles of Charolais heifers aged under vacuum for 13 days. The muscles were then sliced, packaged in a modified atmosphere (70% O_2 and 30% CO_2) and stored at 4°C until the alteration occurs. Samples of the meat surface were taken from i) the damaged brown areas and ii) the intact red areas. Each sample was stored at -80 °C before analysis. Different biochemical indicators linked to the protein and lipid oxidation have been investigated.

Carbonyl and TBARS levels are significantly higher for the damaged areas than for the intact areas of the same slice (p=0.0128 and p=0.0010 respectively). Meat alteration is also related to a slight but significant acidification (p=0.0006). No difference was found concerning the total antioxidant status. Thus, early alteration of beef colour when packaged under high oxygen content modified atmosphere seems to be mainly related to oxidation phenomena and not to microbial growth as it was sometimes suggested. According to other experiments, antioxidant addition reduces myoglobin oxidation and improves colour stability.

Keywords— **colour, meat, oxidation, MAP.**

I. INTRODUCTION

As well as tenderness and flavour, colour of meat is a very important criterion for purchase. The colour of fresh meat and meat products depends on the concentration of meat pigments (the myoglobin) at the surface. Apart from the amount of pigment, other factors, such as the chemical state of pigment, are involved in the meat colour. Indeed, the oxidation state of iron atom located in the protein heme is responsible for meat colour. The pigment can be in several forms: the reduced form (red-purple colour), the oxygenated form (bright red) which is considered attractive by the consumer and the oxidized form called metmyoglobin (brown). When the pigment on the meat surface contains about 20% of metmyoglobin, sales decrease by a factor of 2 [1].

Since the 60s, a lot of works has been achieved on the colour of meat and showed that many factors can regulate meat colour stability [2] [3], specially myoglobin autoxidation [4]. Nevertheless, this mechanism leads to a gradual discoloration of meat during the storage and no study has yet dealt with the swift change in colour observed in case-ready beef under modified atmosphere packaging. This increasingly used packaging method leads to the occurrence of brown areas at the surface of meat.

This problem, mainly occurring when meat is aged under vacuum compared with meat aged on carcass, has not yet been elucidated. It constitutes an important economic problem due to more unsold products. While some professional of meat sector are focusing microbial growth issue, some others highlight biochemical origins.

The aim of this work was to identify the intrinsic meat parameters involved in the mechanism of early beef meat alteration during storage under modified atmosphere. This work is part of a more general study on the beef colour optimization.

II. MATERIALS AND METHODS

A. Raw materials

Experiments were carried out on *Longissimus dorsi* (LD) were removed 24h post-mortem from Charolais heifers carcasses (n=4) and stored under vacuum for

13 days aging. The top of the muscles were removed and the muscles were cut into slices packaged in a modified atmosphere (70%O_2 - 30% CO_2) and stored at 4°C until the alteration occurs.

B. Meat Treatments

Sampling: The early alteration occurrence was visually followed up on packaged meat each day during the storage. On each slice, samples of the meat surface were taken from i) the damaged areas and ii) the intact areas. Each sample was ground into a fine homogenous powder in liquid N_2 and it was stored at -80°C until its analysis.

Carbonyl: This is the most often used marker to evaluate protein oxidation level. The increase of carbonyls is quantified by the reaction with 2.4-dinitrophenylhydrazine (DNPH) [5]. A yellow colour complex is generated; it is detectable by spectrophotometry at 370 nm.

TBARS: Lipid peroxidation was measured using the thiobarbituric assay (TBARS) described by Lynch and Frei [6] and modified by Mercier et al.[7]. The content of TBARS may be expressed as malondialdehyde or MDA, which is one of the aldehydes generated during the final stage of peroxidation.

The total antioxidant status (TAS): it is a method to assess the balance between levels of antioxidants (endogenous and exogenous) and substances which are related to oxidation (polyunsaturated fatty acids, proteins ...). It represents the capacity of tissues to avoid oxidation reactions. The assay method applied to beef is adapted from the one proposed by Miller and al. [8].

The pH: The pH of sample was measured chemically in order to fully determine the value level of each zone.

C. Statistical Analysis

An analysis of variance (ANOVA) was performed to highlight significant effects between the damaged areas and the intact areas. A P-value lower to 0.05 was considered to be significant and a P-value lower to 0.1 was considered as a trend. As the alteration of colour was not necessarily apparent at the same time on different slices; this analysis was completed by a paired t-test allowing the treatment comparison between altered zones and unaltered, slice by slice. The statistical analysis focused on each biochemical criterion.

III. RESULTS

According to the statistical analysis performed, damaged areas show significant differences compared to the intact areas sampled from the same slice.

Regarding carbonyl measurement, the level is significantly higher in the damaged areas ($p = 0.0128$) (fig.1). This effect is confirmed by the probability obtained by a paired t-test ($p=0.0185$). The increase of carbonyl level is respectively by 31, 13, 28 and 14 % for animal 1, 2, 3 and 4.

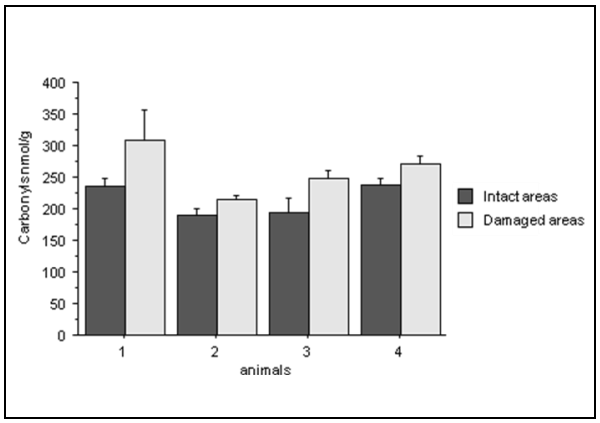

Fig. 1: carbonyl level (nmol/g) according the sampling zone: damaged or intact areas, for each animal

Concerning the TBARS values, the damaged areas present a significant higher level compared to intact areas ($p = 0.0010$) confirmed by paired t-test ($p = 0.0005$). The TBARS values are increased respectively by 144, 48, 34 and 66% for animal 1, 2, 3 and 4 (fig.2).

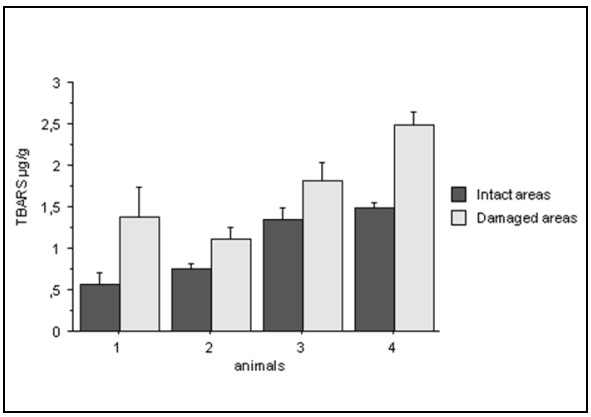

Fig. 2: TBARS values (µg/g) according the sampling zone: damaged or intact areas, for each animal

Regarding pH, a significant acidification is observed on damaged areas compared to the intact areas (p=0.0006 by ANOVA and p=0.0003 by paired t-test). This acidification is very slight but it is systematic since pH of animal 1, 2, 3 and 4 have decreased respectively by 0.09, 0.13, 0;07 and 0;08 unity (fig.3).

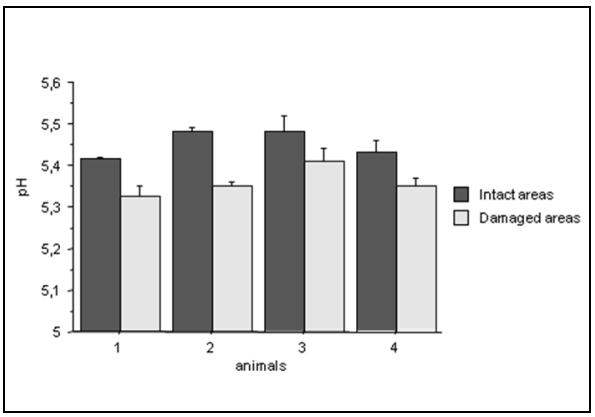

Fig. 3: pH values (µg/g) according the sampling zone: damaged or intact areas, for each animal

Regarding the analysis of antioxidant status, the results of Variance analysis are not significant (p=0,15) but paired t-test gives a probability of 0.0764.

IV. DISCUSSION

The higher carbonyls and TBARS levels on damaged areas suggest the early alteration of beef colour in a modified atmosphere packaged is related to proteins and lipids oxidation. The oxidation of meat pigments leads to brown metmyoglobine development. According to several studies, the myoglobine oxidation and the membrane lipids peroxydation are two related processes [10], [11], [2].

Thus, the early alteration of beef color is induced by a lipid oxidation. The fatty acids oxidation leads to the production of free radicals able to oxidize proteins and especially the myoglobin.

Moreover, a slight acidification as observed on damaged areas is known to lead to an increase of myoglobin autoxidation rate and thereby to provide the browning occurrence on the meat surface [9]. This decrease of pH could be due to the lactic acid produced by lactic bacteria during vacuum ageing or it could be due to lipolysis.

In addition, iron is sequestered by a protein: the ferritin, at physiological pH. It is released during the meat ageing as free iron because of the low pH. The bound iron is not reactive while the free iron is a major source of oxidation. A lower pH can cause catalytic iron release and leads to more oxidation.

An imbalance between the pro-and antioxidants elements in meat would trigger this oxidation resulting in early brown areas occurrence on the surface of meat slices.

Modulating the pro-and antioxidant balance via animal feed could be a potentially interesting mean to improve the meat resistance to oxidation and thus prevent the early alteration of meat colour [12].

V. CONCLUSIONS

The early colour alteration of beef aged under vacuum and packaged under a modified atmosphere with high oxygen content is related to myoglobin oxidation. This reaction is probably induced and maintained by lipid peroxidation. However, without a full knowledge of the chemical composition (vitamin E, myoglobin, free iron, PUFAs, etc.), it is difficult to understand the origin of the phenomenon occurrence. Improving the antioxidant status of meat via animal feed could be a potentially interesting mean to increase the resistance to oxidation and prevent the early alteration of meat colour.

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REFERENCES

[1]. Renerre M, Mazuel J.P. (1985) Relations entre methodes de mesures instrumentales et sensorielles de la couleur de la viande. Sciences des Aliments. 5, pp 541-557

[2]. Faustman, C., Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: A review. Journal of Muscle Foods, 1, pp 217−243.

[3]. Renerre M. (1990) Review: factors involved in the discoloration of beef meat. International Journal of Food Science and Technology. 25, pp 613-630

[4]. Renerre M, Labas R (1987). Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Sciences, 19, pp151-165

[5]. Levine R. L., Garland D, Olivier C.N., Amici A., Climent I, Lenza A.G., Ahn B.W., Shaltiel S., Stadtman E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. Method Enzymol., 186, pp 464-478

[6]. Lynch S. M., Frei B., (1993). Mechanisms of copper- and iron-dependent oxidative modification of human low density lipoprotein. Journal of lipids Research, 34(10), pp 1745-1753.

[7]. 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 Science, 48, pp301-318

[8]. Miller N.J., Rice-Evans C., Davies M.J., Gopinathan V., Milner A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science, 84, pp 407-412.

[9]. Renerre M., Anton M., Gatellier P.(1992) autoxidation of purified myoglobin from two bovine muscles. Meat Science, 32, pp 331-342

[10]. Anton M, Salgues C., Gatellier P., Renerre M., (1993) Etudes des relations oxydatives entre les lipides membranaires et la myoglobine in vitro. Science des Aliments, 13, pp261-274.

[11]. Faustmann C., Cassens R.G. (1991). The effect of cattle breed and muscle type on discoloration and various biochemical parameters in fresh beef. Journal of Animal Science. 69, pp184-193

[12]. Gobert M., Gruffat D., Habeanu M., Parafita E., Bauchart D., Durand D. (2010) Plant extracts combined with vitamin E in PUFA-rich diets of cull cows protect processed beef against lipid oxidation. *Meat Science*, 85, 676-683