TOWARDS THE PREDICTION OF OXIDATION IN MEAT INDUSTRY

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Abstract – Oxidations which appear during meat storage and processes can result in impairment of technological, sensorial and nutritional qualities of products. So, it would be very interesting for industrials to be able to predict meat susceptibility to oxidation. For that purpose, different studies were conducted in our laboratory to investigate the relationships between the early *post-mortem* sarcoplasmic proteome, which contains the majority of enzymes involved in the oxidative process, and oxidations generated during meat refrigerated storage and cooking. Proteomic analyses were performed by coupling 2D electrophoresis and mass spectrometry. Protein and lipid oxidation were estimated in pork meat by the measurement of carbonyl groups and TBARS after a refrigerated storage of 4 days and a subsequent cooking at 100°C. Meat color was estimated by L* values measured 36 h post-mortem. Significant correlations ($p<0.05$) were observed between oxidation and color parameters and spots of the 2D electrophoresis showing the involvement of some of sarcoplasmic proteins in the muscle oxidative stress. The interest for industrials of the meat sector for an early selection of carcasses on the basis of oxidation susceptibility is discussed.

Key Words –color, oxidation, proteomics

I. INTRODUCTION

In *post-mortem* muscle tissue, free oxygenated radicals are produced which can promote lipid and protein oxidation. These radicals can be issued from a complex cascade of chemical reactions. The pH decline, observed during the first 24 hours following slaughter, leads to the release of iron from ferritin. This free iron can react with hydrogen peroxide to give hydroxyl radicals (OH°). Myoglobin oxidation generates hydroperoxide (HO_2°) and superoxide ($O_2^{\circ -}$) radicals. These oxidative processes are known to be the major cause of quality deterioration during meat refrigerated storage, affecting sensorial qualities like colour, flavour and tenderness. Technological properties like protein solubility, gel forming ability, emulsification properties, and water binding capacity are also impacted by oxidation. In modern society meat is almost always cooked prior consumption. By increasing the free radical production while the antioxidant protection is altered, cooking greatly accelerates oxidations in meat. Polyunsaturated fatty acid and essential amino acid oxidation affect negatively the nutritional value of meat. Therefore, in an attempt to minimize quality impairment and to preserve the nutritional value of meat during processes, predictive methods on susceptibility of products to oxidation are of first importance in meat industry. The proteomics (2-D electrophoresis in combination with mass spectrometry) had already been used with success to correlate muscle proteome with different meat quality traits like tenderness [1] and drip loss [2]. We present here a synthesis of our work performed during the last decade on the identification of proteomic markers related to color degradation [3] and oxidation of proteins [4] and lipids [5] in pork during ageing and cooking. The potential use of these markers, in industry, to develop rapid and reliable tools predicting meat oxidation is discussed.

II. MATERIALS AND METHODS

Protein and lipid oxidation was evaluated on pig M. *longissimus lumborum* after 4 days of refrigerated storage on air and at 4°C, and after cooking of the 4 days aged samples. Samples were heated at 100°C during 30 minutes. Protein oxidation was evaluated by the measurement of protein carbonyl groups with 2,4 dinitrophenylhydrazine (DNPH) according to Oliver *et al.* [6]. Lipid oxidation was measured by the ThioBarbituric Acid Reactive Substances (TBARS) method according to Lynch and Frei [7]. For color evaluation L*

values (luminance) were measured 36 h *post-mortem* on pig M. *semimembranosous* with a Minolta CR 300 chromameter. Proteomic analysis was performed on samples taken on the carcass exactly 30 min after exsanguination. For 2D electrophoresis, samples, prepared in a buffer containing 7M Urea, 2M Thiourea, 2% (w/v) CHAPS, 0.4% (v/v) carrier ampholyte and bromophenol blue, were loaded onto immobilized pH-gradient strips (pH 4-7) and isoelectric focusing was performed. In the second dimension, proteins were resolved on 12 % SDS-PAGE gels. After Blue Coomassie staining, gel images were acquired using a GS-800 imaging densitometer (BioRad). Protein quantity in a spot was evaluated by the spot volume, expressed by spot density x spot surface. After trypsin digestion spots of interest were analysed by mass spectrometry. Identification of specific markers was based on statistical analysis. In our studies on protein and lipid oxidation, a protein was considered as a marker when its quantity was correlated with the oxidation parameter at the level of $p < 0.05$. In color study the strategy used was different. Two groups of pigs were selected on the basis of extreme L* values of meat. The resulting set of spot quantities was submitted to a one-way analysis of variance (ANOVA) and a protein was consider as a marker when it was differentially expressed (on the basis of $p<0.05$ in ANOVA) between dark and light groups.

III. RESULTS AND DISCUSSION

III.1. Identification of markers of protein oxidation:

Protein oxidation after the 4 days storage was correlated with 30 identified proteins, and protein oxidation in cooked meat was correlated to 19 identified proteins. Figure 1 shows a representative 2D gel electrophoresis of the sarcoplamic proteins with the different identified spots and in table 1 are listed some of the identified proteins, displaying good correlations ($p<0.05$) with carbonyls and whose implication in oxidative process can be clearly established.

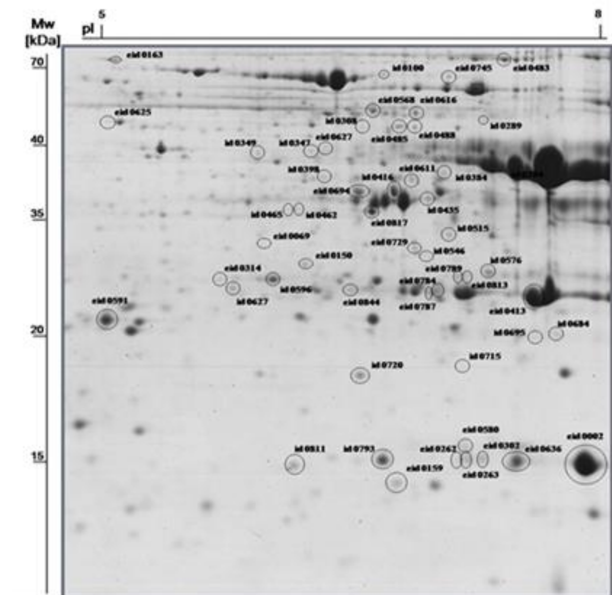

Figure 1: 2-D electrophoresis gel of *Longissimus lumborum* muscle sarcoplasmic proteins. Circles indicate proteins that were correlated to protein oxidation during storage or cooking.

Table 1 : Correlation (r) of protein quantity with protein oxidation during storage and cooking.

Protein name	After 4 days storage	After 100°C cooking
Myoglobin	-0.505	
Selenium-binding protein	-0.44	
Mitochondrial SOD	-0.412	-0.42
Annexin A4	0.368	
S-formylglutathione hydrolase	-0.365	
Heat shock protein 70 kDa	-0.315	
Serotransferrin	-0.304	-0.305
Heat shock protein beta-1	0.304	
Heat shock protein beta-6		-0.397
Peroxiredoxin 6		-0.358

Among these proteins many metal proteins were found. Myoglobin was the best marker of protein oxidation in aged meat. In literature, myoglobin has been described as oxidant or antioxidant depending on conditions. Here, a negative correlation was obtained with carbonyl content. An explanation is that, during storage, most of the myoglobin was oxidized into metmyoglobin, which is very reactive towards hydrogen peroxide. Through reaction with

peroxides the metmyoglobin is transformed into perferryl-myoglobin, whose oxidant activity is lower than the activity of radicals which could be produced by the reaction of peroxides with free metals. So, by neutralizing peroxides, metmyoglobin could reinforce the antioxidant activity of other peroxidases like catalase and glutathione peroxidase. The two following proteins in table 1, selenium binding protein and mitochondrial superoxyde dismutase, also displayed a negative correlation with carbonyls thus indicating their implication in the muscle antioxidant protection. Indeed, selenium is the cofactor of the glutathione peroxidase (GPx), an enzyme involved in the degradation of the hydroperoxides produced during the lipid peroxidation, and superoxide-dismutase neutralizes superoxide radicals. S-formylglutathione hydrolase was also negatively correlated with carbonyls. Actually, this enzyme is not an antioxidant enzyme, but it may play an important role in the production of glutathione which is also a cofactor of GPx. The negative correlations observed between carbonyls and proteins involved in the selenium and glutathione supplies strongly suggest that GPx is involved in the detoxification of reactive oxygen species during meat storage, even though it was not in the 30 identified proteins. HSP β-1 and Annexin A4 are positively correlated to carbonyls. Neufer and Benjamin [8] have reported an over-expression of chaperone proteins in response to oxidative stress and according to Rhee et al. [9] annexins can be assimilated to stress proteins induced by heat or oxidative stress.

As after storage, a negative correlation was observed between some proteins involved in the antioxidant protection (mitochondrial superoxide dismutase and peroxiredoxin 6) and the carbonyl level in cooked meat, thus demonstrating that these enzymes can limit protein damages during cooking. Peroxiredoxins can act with catalase to remove H_2O_2 produced during the oxidative process in muscle [10]. This result is surprising because most of enzymes generally lose their biological activity at temperatures higher than 70°C. In the present case, we can hypothesize that the antioxidant protection took place during the first six minutes of cooking, while the meat temperature was below 70°C. Contrary to the over-expression of HSP β-1 observed in aged meat, HSP β-6 was negatively correlated with carbonyls in cooked meat. From table 1, we can note that superoxide dismutase and serotransferrin are good markers of oxidation both during storage and cooking.

Other proteins were found to be significantly correlated to carbonyl levels in aged or cooked meat (results not shown), but did not participate directly to the pro- or anti-oxidant status of meat. Therefore their implication in the mechanisms of protein oxidation cannot be clearly established and they were not discussed in this paper.

III.2. Identification of markers of lipid oxidation:

In the second study 26 identified proteins were correlated to lipid oxidation after 4 days storage and 12 identified proteins were correlated to lipid oxidation after cooking. Redoxins (peroxiredoxin 4 and 6 and their mitochondria-specific electron supplier thioredoxin) were identified as good markers of lipid oxidation but displaying positive or negative correlations with TBARS. These contradictory results can be explained by the ambiguous role of redoxins in the oxidative process. The main function of redoxin is to catalyze the decomposition of hydrogen peroxide into water and oxygen, but it has been demonstrated that these peroxidases can also catalyze the production of hydroxyl radicals from hydrogen peroxide. Annexins A4 and A5 were correlated with lipid oxidation either in aged or cooked meat. We must notice that all these correlations were negative thus indicating a protective effect of annexins against lipid oxidation in meat. Peroxiredoxin 6 ($r = 0.382$ in aged meat and 0.323 in cooked meat) and annexin A4 ($r = -0.385$ in aged meat and -0.293 in cooked meat) are the only markers of lipid oxidation in both aged and cooked meat. Two fatty acid-binding proteins were positively correlated with lipid oxidation: the adipocyte fatty acid-binding protein in aged meat and the fatty acid-binding protein-5 in cooked meat. By modulating the level of intramuscular lipids, fatty acid-binding proteins can indirectly influence the level of TBARS produced during meat process.

III.3. Identification of markers of meat color:

Two groups (light and dark) of pigs were selected, based on meat L* values measured 36 h postmortem. Twenty-two proteins or fragments were differentially ($p < 0.05$) expressed between the two groups. Muscles leading to darker meat had a more oxidative metabolism, indicated by more abundant mitochondrial enzymes of the respiratory chain, hemoglobin, and chaperone or regulator proteins (HSP27, RB-crystallin, and glucose-regulated protein 58kDa). Conversely, enzymes of glycolysis were overexpressed in the lighter group. Such samples were also characterized by higher levels of glutathione S-transferase, and higher levels of cyclophilin D. This protein pattern is likely to have severe implications on postmortem metabolism, namely, acceleration of ATP depletion and pH fall and subsequent enhanced protein denaturation, well-known to induce discoloration.

III.4 Potential application in meat industry:

With the help of these markers the prediction of meat oxidation in industry can be now envisaged. Applying combined proteomic and immunologic approaches should provide reliable and rapid methods (for example in the form of ELISA tests) which could be used in the meat industry for an early selection of carcasses based on susceptibility to oxidation. Meat, detected as oxidative resistant, should be sorted preferentially for the commercialization as fresh products, while more oxidative meat should be devoted to processed products or canning. These immunochemical methods are rapid, non-invasive and not expensive. A miniaturization of these methods under the form of micro-system tools, easy to use for non-trained personnel in industry, can be achieved. Such immunochemical based microsystem tools still exist in food industry to determine genetically modified organisms, and allergens in products.

IV. CONCLUSION

These studies identified many proteins as potential markers of oxidation or color degradation in meat. Based on these results, the development of specific tools of prediction of oxidation could constitute an important axe of research in meat technology in the future.

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