Combined effect of meat composition and heating parameters on the physicochemical state of proteins

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Combined effect of meat composition and heating parameters on the physicochemical state of proteins

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Abstract

During meat cooking, proteins undergo some oxidations and conformation changes which can induce a loss in the nutritional value of products. The objective of this study was to determine the effect of heat treatments on the physicochemical state of proteins (oxidation and thermal denaturation). A great number of experiments were performed on mimetic models. Two mimetic models were used: a basic model, composed of an aqueous suspension of myofibrillar proteins, and a complex model, in which oxidants were added in physiological concentrations. Heat treatments were applied on the two models at 45°C, 60°C, 75°C and 90°C during 5, 10, 30, 60 and 120 min. Then, the results were compared to those obtained on a pork meat model (M. Longissimus dorsi), cooked in the same conditions. In these three models, myofibrillar proteins were the target of the treatments. Protein oxidation was assessed by the carbonyl groups and thermal denaturation was evaluated by the measurement of protein surface hydrophobicity. The mimetic models showed that carbonyls can not be produced under the thermal process alone; oxidants are required for their formation. A synergic effect of the oxidants and heat treatments was noticed on protein oxidation. Carbonyl production was considerably higher in the complex mimetic model than in meat. On the contrary, changes in protein hydrophobicity were dominated by the thermal process and hydrophobicity values were higher in meat than in the mimetic model.

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Keywords: heat treatment; meat; myofibrillar proteins; protein oxidation; thermal denaturation;

1. Introduction

Meat and meat products are an important source of proteins for human. These proteins are well balanced in amino acids and contain all the essential amino acids that humans can not synthesize. In developed countries, the needs in amino acids are generally properly provided by the diet but, some

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segments of the population such as the elderly, the children and seriously ill persons often present some nutritional deficiencies in amino acid due to a low consumption of meat or to a decreased assimilation of proteins. For these populations it is of the highest importance to provide proteins with a well preserved amino acid composition and with a high digestibility. Meat is almost always cooked before consumption. Cooking is used to inactive pathogenic microorganisms and to develop sensorial qualities. During meat cooking, the free oxygenated radical production, in which iron in reaction with peroxides plays a key role [1], and the decrease of the antioxidant defence systems lead to the accumulation of oxidative damages on amino acids [2] [3]. This oxidation leads to a decreased nutritional value of meat, especially when it affects essential amino acids. In addition to these oxidative modifications, thermal treatments can also alter the secondary and tertiary structure of proteins, inducing changes in the physical properties of proteins such as solubility, hydrophobicity and formation of protein aggregates [4] [5]. To enter the bloodstream, proteins must be broken down into amino acids or small peptides by proteases within the digestive tract and pass through the small intestine wall. Preliminary in vitro studies have demonstrated that changes in proteins physicochemical state (oxidation, denaturation, and aggregation) can decrease their digestibility [6] [7] [8] with a negative impact on the nutritional value of meat products. Besides poorer amino acids bioavailability, a reduced protein digestion rate could have a deleterious effect on human health by the formation of fermented products which can increase the risk of colon cancer.

Therefore, due to its importance with regard to the nutritional and health values of meat and in a context of an increasing demand of more elaborated foodstuffs, studies are needed to better understand the mechanisms involved in protein changes induced by cooking and to assess how much the related kinetics are affected by products initial characteristics and process conditions.

This study is leaning on the European DREAM project (Design and development of REAlistic food Models with well-characterised micro- and macro-structure and composition) which has for goals the development of realistic, physical and mathematical food models to be used as tools to better understand and assess the impact of processing techniques on food qualities. Our particular objective was to determine changes in the proteins state (oxidation and thermal denaturation) induced by meat cooking with the ultimate goal to create a mathematical tool predictive of these changes. At first, a great number of experiments were carried out on mimetic model systems. The basic model was composed of an aqueous suspension of myofibrillar proteins (which represent 60% of the whole meat proteins). This model was then complexified by addition of the main muscle oxidative compounds. In this way, confusing effect due to uncontrolled biological variability of meat tissues can be avoided and the incidence of various compounds can be assessed independently. Then, the results obtained with these mimetic models were compared to those obtained with a selected pork meat tissue with well characterized composition and structure and cooked in the same conditions than the mimetic models.

2. Material & Methods

2.1. Mimetic model making

The basic mimetic model was composed of myofibrillar proteins in suspension in a phosphate buffer with the same pH and ionic strength than muscle. Our objective was to determine kinetic laws of protein changes in pure fibre types and to extrapolate these laws to mixed fibre types. In this goal, myofibrillar proteins were extracted, one day post-mortem, from two pure fibre type muscles of rabbit; the Psoas major muscle, which is a 100% α-white fibre type muscle and the Conoideus muscle, which is a 100% β-red fibre type muscle. For practical reasons, only results on Psoas major muscle are presented here. Muscles were frozen at -80°C until myofibrillar proteins extractions. Myofibrillar proteins were prepared according to the method of Pietrzak et al., 1997 [9]. The final pellets of myofibrillar proteins were suspended in a 40 mM pH 6 phosphate buffer at the concentration of 25 mg/ml. The whole myofibrillar

protein extracts were delipidated by addition of 2 volumes of butanol/di-isopropyl ether (in a 40:60 v/v ratio) to 1 volume of extract.

2.2. Thermal treatments

Heat treatments were performed in polypropylene test tubes (inner diameter = 13 mm and thickness = 1 mm) with a digital temperature-controlled dry bath. Four heating temperatures (45°C, 60°C, 75°C and 90°C) were tested. To reach the target temperature in less than one minute, 1 ml of myofibrillar proteins suspension, preincubated at 20°C, was added to 4 ml of preheated buffer at a temperature (T1) higher than the target temperature (T2): T1 (°C) = [(T2 (°C) x 5) – 20] / 4. In these conditions the thermal treatment was divided in 3 periods: a rapid rise of sample temperature to its target value, a dwelling period while temperature was maintained at the target value, a cooling period at 1°C which stopped the heating treatment. Reactions were stopped after 5, 10, 30, 60 and 120 min. In case of heating at 90°C the calculated T1 (107°C) can not be reached so, the temperature of the preincubated buffer was restricted to 99°C. In this case a small lag phase of 2 min was observed before the temperature reached 90°C.

2.3. Addition of oxidants

The basic model was complexified by addition, just before heating, of a mixture of oxidants composed of hydrogen peroxide (2 mM), ascorbate (0.1 mM) and ferrous iron. Different iron concentrations, 0.05 mM, 0.2 mM and 0.6 mM, were tested to screen all kinds of lean meat tissues. These oxidants produce hydroxyl radicals (OH°), by the Fenton reaction, which are very reactive towards proteins. To stop the oxidative reactions after the heat treatments, the proteins from the complex model were immediately centrifuged (2000 g/10 min) at 1°C and the oxidants were eliminated by washing the pellets two times with 5 ml of the 40 mM pH 6 phosphate buffer. The final pellet was suspended in 5 ml of the phosphate buffer.

2.4. Pork meat model

To avoid confusing effect due to biological differences between muscles from different animals, the experiments on meat were carried out on only one pig muscle. In these conditions, only variability inherent to the extraction process and to the measurement methods existed. After one day, the muscle Longissimus dorsi (fibre type: 11% β-red, 16% α-red, 73% α-white) was removed from the carcass and was stored for 3 days at 4°C before freezing in vacuum plastic bags at -80°C until use. To have a good control of the time-temperature couple, thin slices of meat (1.5 mm) were enclosed in plastic bags and heated in a water bath in the same conditions than described with the mimetic model. Indeed, the heating time during which the temperature distribution was heterogeneous was about 30 seconds. Analyses were performed on myofibrillar proteins which were extracted from the heated samples and delipidated using the same protocols as described above.

2.5. Characterisation of protein physicochemical changes

Protein oxidation was evaluated by the measurement of carbonyl groups with appropriate probe 2,4 dinitrophenylhydrazine (DNPH) as described by Oliver et al., 1987 [10]. The results were expressed as nanomoles of DNPH fixed per milligram of protein. Protein surface hydrophobicity was measured with the hydrophobic probe, bromophenol blue (BPB) according to the method of Chelh et al., 2006 [11]. The results were expressed as the percentage of BPB bound to protein.

2.6. Statistical analysis

All values are reported as the mean ± SEM (standard error of mean) of 6 determinations. The effect of the heat treatments in the pork meat model was evaluated in a mixed model by a two way variance analysis (ANOVA): one fixed effect (temperature) and time as repeated variable. In the mimetic complex model, the combined effect of oxidants and heating parameters and their interactions were measured in a mixed model by a three way variance analysis: two fixed effects (oxidants and temperature) and time as repeated variable.

3. Results & Discussion

Figures 1 to 4 present the results at 45°C and 90°C; all the conclusions derived from these figures are in agreement with the measurements at 60°C and 75°C.

3.1. Carbonyls content

In proteins, carbonyl groups are formed by oxidation of the side chain of some amino acids [12]. Among them, basic amino acids (lysine, histidine, arginine) and threonine are essential amino acids for humans and their oxidation leads to an important decrease of the nutritionnal value of meat. Moreover, carbonyl groups can react with free amino groups of non oxidized amino acids of proteins to form amide bonds [7]. This reaction leads to protein aggregation with a negative impact on the protein digestibility.

Figure 1 shows the effect of heating time at 45°C on the carbonyl groups content in basic model, complex model and pork meat model. The initial level of carbonyl groups in the mimetic model was approximately 2 nmol per mg of proteins, a value close to those reported by Martinaud et al., 1997 [13] on bovine myofibrillar proteins and by Morzel et al., 2006 [14] on pig myofibrillar proteins. In the basic model, no increase in carbonyls was observed during the 120 min heating. In the complex model, ANOVA revealed significant effects ($p<0.001$) of heating time and iron content and their interactions on the carbonyls level. Carbonyls increased progressively with time and iron concentration up to a value closed to 8 nmol per mg of proteins. These results, obtained on the two mimetic models, demonstrated that heat alone is unable to catalyse the oxidation of amino acids and that to be formed carbonyls need oxidants. In pork meat model, the initial level of carbonyl was approximatively 1.5 nmol per mg of proteins. A slight, but significant ($p<0.001$), increase in carbonyl content was observed with increasing heating time and the value measured after 120 min heating was 30% higher than the initial value. This maximum value of protein oxidation was 4 times lower than the maximum observed in the complex model for iron concentrations of 0.2 and 0.6 mM which surround the value of 0.42 mM in total iron measured in this pork meat. The low protein oxidation observed in meat, when compared to the complex model, can be explained by the muscle antioxidant protection which was not taken into account in this preliminary version of the mimetic model. The enzymatic antioxidants (Superoxide dismutase, Catalase and Glutathion peroxydase) keep most of their activity up to 50-60°C [15] and some antioxidants like carnosine, anserine and vitamin E are more thermostable. Vitamin E can retain a great part of its activity up to 95°C [16]. In order to better reflect meat we plan to add antioxidants in the mimetic model.

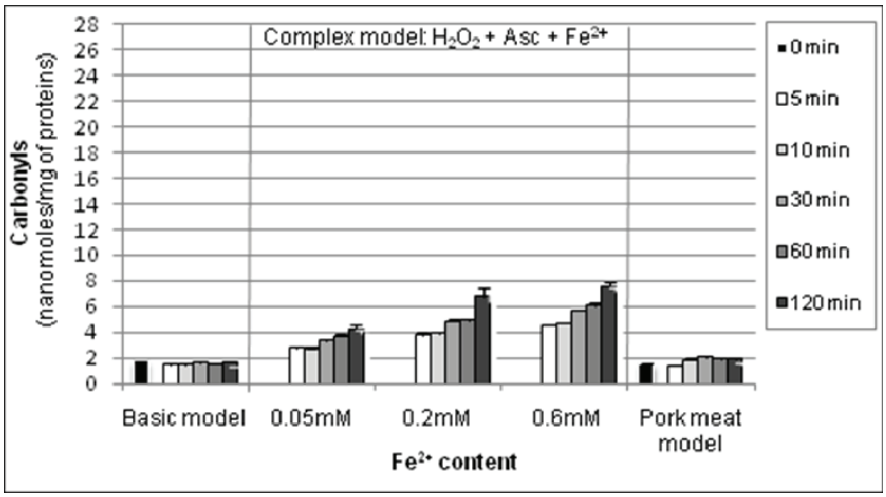

Fig. 1: Protein oxidations at 45°C evaluated by the formation of carbonyl groups

Figure 2 shows the effect of heating time at 90°C on the carbonyl groups content in the three models. In the basic model, ANOVA revealed a significant ($p<0.001$) effect of the heating time on carbonyl groups and a slight increase of 45% was observed between the unheated and the 120 min heated proteins. In the complex model the effects of heating time, iron content and their interactions were highly significant ($p<0.001$). The combination of heating and oxidants had a dramatic effect on amino acids and a ten-fold increase in carbonyls was observed in the highest oxidative conditions. For each iron concentration, the carbonyl production was considerably higher at 90°C than at 45°C. In the pork meat model, a significant ($p<0.001$) increase of carbonyls group was observed and a maximum of 3.5 nmol per mg of proteins, was reached at the end of cooking. These carbonyl values, observed in cooked pork meat, were of the same order than those observed by Gatellier et al., 2010 [3] in cooked bovine meat. As previously observed at 45°C, the level of carbonyls in pork meat was considerabely lower than that in the complex model. This difference can be attributed to the thermostable non enzymatic antioxidants which can limit the extent of protein oxidation during meat cooking.

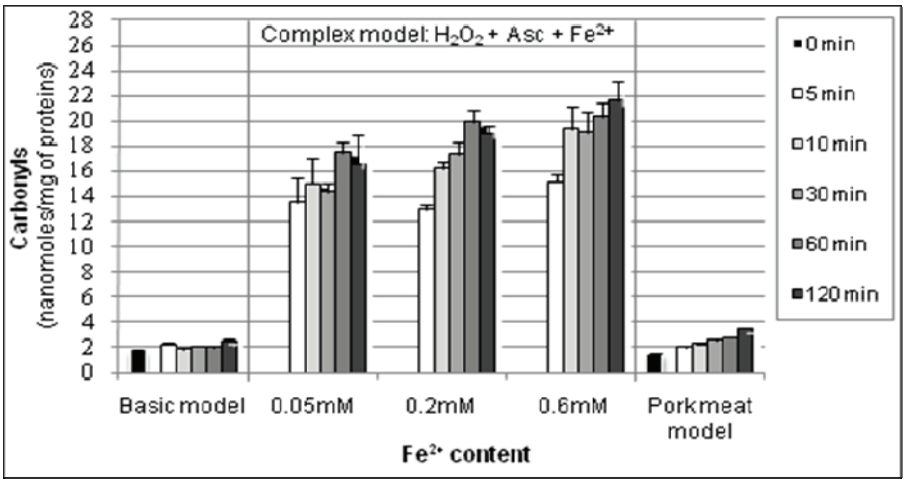

Fig. 2: Protein oxidations at 90°C evaluated by the formation of carbonyl groups

3.2. Protein surface hydrophobicity

Thermal treatments can impair the structure of meat proteins by breaking of hydrogen and electrostatic bonds. As a consequence of this thermal denaturation, an exposure to the protein surface of hydrophobic amino acids, which are mainly aromatic amino acids and long chain aliphatic amino acids, can occur [11] and favour a tendency of proteins to form aggregates.

Figure 3 shows the effect of heating time at 45°C on protein surface hydrophobicity in the three models. The initial level of hydrophobicity in the mimetic model was approximately 35% of BPB bound. In the basic model, a significant ($p<0.001$) increase in protein surface hydrophobicty was observed with increasing heating time. In the complex model, ANOVA revealed significant effects of heating time ($p<0.001$) and iron content ($p<0.001$) on surface hydrophobicity. But, when compared to the basic model, adding oxidants did not lead to a higher increase in surface hydrophobicity during heating. A slight decrease in protein surface hydrophobicity was even observed when the iron content increased from 0.05 to 0.2 mM. This can be explained by an oxidation of aromatic amino acids exposed to the protein surface. Indeed, free radicals can react with aromatic rings leading to hydroxylation products which are less hydrophobic than initial products. For example, the oxidation of tryptophan can induce the formation of hydroxytryptophan, phenylalanine can oxidize into tyrosine and tyrosine oxidation leads to the formation of dihydroxyphenylalanine or trihydroxyphenylalanine [2]. In raw pork meat the level of myofibrillar protein hydrophobicity was higher than in unheated mimetic model. This difference can be explained by the ageing period which was of 4 days in pork sample and only 24 hours in rabbit sample. During ageing some structural changes in proteins could already happen which change the protein hydrophobicity. Differences due to animal species or fibre type are also possible. In the pork meat model, ANOVA revealed a significant ($p<0.001$) increase of protein surface hydrophobicity with increasing cooking time. An increase of 25% was observed at the end of cooking which was of the same order than the increase observed in the mimetic model.

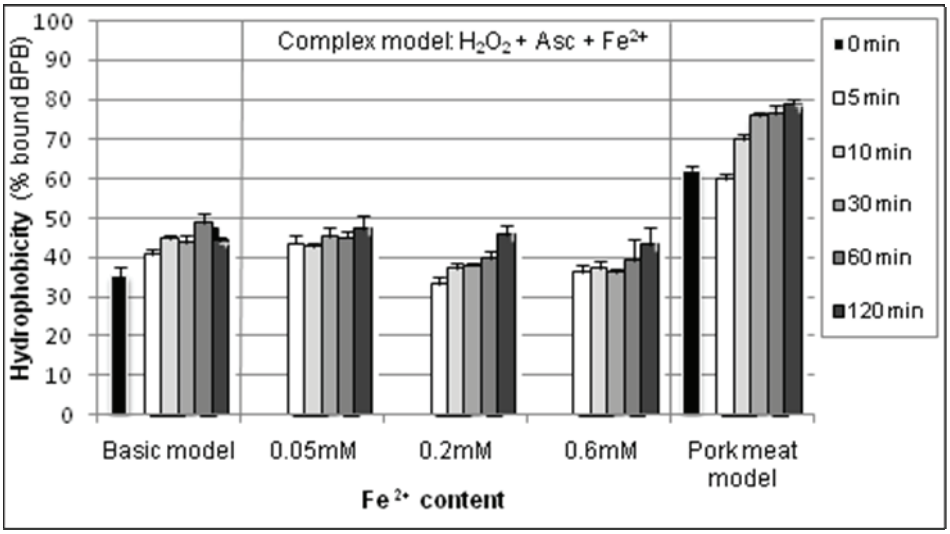

Fig. 3: Protein thermal denaturation at 45°C measured by change in surface hydrophobicity

Figure 4 shows the effect of heating time at 90°C on protein surface hydrophobicity in the three models. In the basic model, an important and rapid increase of 110% of hydrophibicty was observed. After 5 min of heating, the maximum value of 75% BPB bound to protein was already reached. Significant effect of iron concentration ($p<0.001$) was observed, in the complex model. As previously

observed at 45°C, change in protein surface hydrophobicity was mainly under the dependence of the thermal denaturation and oxidants poorly affected this change. A slight decrease was also observed with increasing iron concentration. ANOVA revealed a significant effect of heating time ($p<0.001$) for the three models. However, it is clear that the major changes occurred during the first 5 min. For example, in the pork meat model the maximum value of 94% BPB bound to protein was reached from 5 min and no further increase was observed with increasing cooking time.

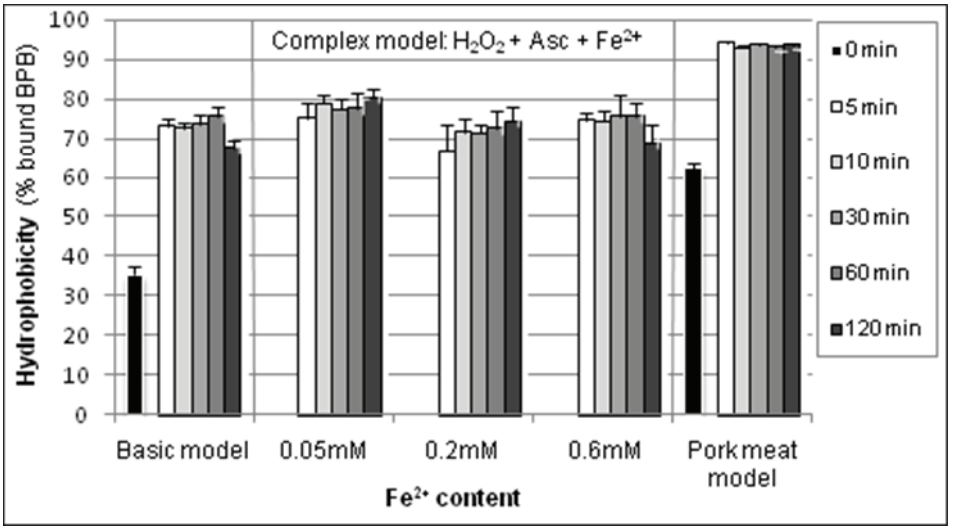

Fig. 4: Protein thermal denaturation at 90°C measured by change in surface hydrophobicity

4. Conclusion

The main advantage of using the mimetic models was to permit to assess the relative contributions of the chemical and thermal effects on the protein physicochemical changes: (i) synergic effect of oxidants and heat treatments on protein oxidations and (ii) negligeable incidence of oxidants and fast denaturation of proteins due to heating. To better reflect meat, the mimetic model must be complexified by adding antioxidants and other biological compounds like lipids and sugars which are known to interact with proteins under heating. Our ultimate goal is to predict through a mathematical tool the effects of heat treatments on the nutritional value of meat products. Thus, to build up this tool our effort will be firstly focussed on the modelling of the kinetics of protein oxidation and denaturation both in the mimetic models and in the meat. In a second time, kinetic models will be combined to heat-mass transfer models to predict protein evolutions under realistic cooking conditions in large pieces of meat.

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