Towards models for the prediction of beef meat quality during cooking

Kondjoyan, Alain [a*], Kohler, Achim [b,c], Realini, Carolina Eva [d]., Portanguen, Stéphane [a], Kowalski, Ryszard [e], Clerjon, Sylvie [a], Gatellier, Philippe [a], Chevolleau, Sylvie[f], Bonny, Jean-Marie [a], Debrauwer, Laurent [f].

[a]INRA, UR370 QuaPA, F-63122 Saint-Genès Champanelle, France

[b]Department of Mathematical Sciences and Technology (IMT), Norwegian University of Life Sciences, PO Box 5003, 1432 Aas, Norway

[c]Nofima AS, Osloveien 1, 1430 Ås, Norway

[d]IRTA Finca Camps i Armet, Monells (Girona), E-17121 Spain

[e]Institute of Meat Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland

[f]INRA, UMR1331 Toxalim, Research Center in Food Toxicology, BP 93173, F-31027 Toulouse Cedex 3, France

Abstract

Heating of beef muscles modifies the water content, the micronutrient content and the colour of beef meat. Juice expelling and loss of water soluble micronutrients were predicted by combined transfer-kinetics models. Kinetics modeling and crust formation are needed to progress toward a reliable prediction of HAAs formation. HAAs formation in uniformly heated beef meat slices was compared with the values issued from the kinetic models developed in literature in liquid systems. The models of literature were adapted to meat slices but the parameters values were different from those determined in liquid systems. Results in meat slices were confronted to the HAAs formation at the surface of bigger meat pieces subjected to air roasting conditions. The transposition of the results from the meat slices towards the bigger meat pieces was not direct because the formation of HAAs was affected by the thickening of the crust and the migration of precursors.

Highlights:

Prediction of cooking losses and vitamins content in cooked meat; Kinetics of the formation of HAAs during roasting; Mitigation of HAAs formation during grilling and roasting.

* Corresponding author: alain.kondjoyan@clermont.inra.fr; Phone: +33(0)473624492; Fax: +33(0)473624089

Keywords:
Meat quality; Nutritional properties; Heterocyclic Aromatic Amines; Cooking process; Mathematical modeling.

List of abbreviations

Muscle types

IS: *Infraspinatus;*
LT: *Longissimus thoracis*;
MA: *Masseter*
SM: *Semimembranosus;*
ST: *Semitendinosus*.

Heterocyclic aromatic amines (HAAs)

IQ: 2-amino-3-methyl-3*H* -imidazo [4,5-*f*]quinoline;
MeIQ: 2-amino-3,4-dimethyl-3*H* -imidazo[4,5-*f*]quinoline;
IQx :2-amino-3-methyl-3*H* -imidazo[4,5-*f*] quinoxaline;
MeIQx :2-amino-3,8-dimethyl-3*H* -imidazo[4,5-*f*] quinoxaline;
DiMeIQx :2-amino-3,4,8-trimethyl-3*H* -imidazo[4,5-*f*]quinoxaline;
PhIP : 2-amino-1-methyl-6-phenyl-imidazo-[4,5-*b*]pyridine.

Other abbreviations:

DM: Dry Matter;
FTIR: Fourier Transform InfraRed;
LC-APCI-MS/MS: Liquid Chromatography –Atmospheric Pressure Chemical Ionization tandem Mass Spectrometry;
$MgSO_4$: Magnesium Sulfate;
MRI: Magnetic Resonance Imaging;
MW: Microwave;
NaCl: Sodium Chloride;
NMR: Nuclear Magnetic Resonance;
SE: Standard Error;
STPP: Phosphates Sodium triPolyphosphate;
W: Watt.

Introduction

Today, most meat and meat-based products are cooked before being eaten. The cooking process not only destroys pathogenic or spoilage microorganisms but develops also sensorial properties which are specific of the cooked product. Cooking has an important effect on the nutritional properties of the meat product and at the same time on its possible toxicity. This paper deals with: the juiciness, the nutriments content, the colour of cooked meat and the

formation of Heterocyclic, Aromatic Amines (HAAs) during grilling and roasting. Discussion on the prediction of meat tenderness which would have required mechanical modeling is beyond the scope of this study. Colour and juiciness are with tenderness the main sensorial properties of beef meat. Juiciness is related with the variation of the water content in the meat during cooking which also determined the cooking yield which is a critical factor for industry. Meat is rich in bioavailable micronutrients (vitamins B, iron, zinc, selenium). A lot of these micronutrients, as the B vitamins, are water-soluble, and are expelled with meat juice during cooking. Some of the B vitamins are also temperature-sensitive as thiamin (B1), pyridoxin (B6) and cobalamin (B12), while other as niacin (B3) are known to be more heat-resistant. Despites its importance for the quality of beef meat, vitamin B12 is seldom studied due to difficulties in its quantification, Szterk (2012a). In this paper only the B3 and B6 vitamins have been studied to validate a combined-modeling approach. It will be possible to extend afterwards this approach to other vitamins as the B12.

This paper overviews the work performed during the ProsafeBeef project to improve the quality of the cooked beef meat as it is ingested by consumers. To reach this objective it is necessary to know how the variations in the process conditions and in the quality of the raw meat will affect the quality of the cooked meat. In practice consumer habits, types of heating equipment and raw meat quality vary a lot. Moreover, quality is most often analyzed averagely while its evolution is local and depends on the complex thermal and water gradients generated in the meat during heating. This can explain why the results of literature are sometimes contradictory, and often difficult to transpose from one case to another. This leads scientists and engineers to repeat experiments as soon as the type of meat, the size of the meat cut, the type of equipment, or the cooking conditions are changed. Combined transfer to quality modeling is appropriate to respond to this situation.

This paper describes how the combined modeling approach was used to progress in the project. Text is separated into three parts: (1) the analysis of the evolution of meat water content and colour during cooking linked to protein denaturation and contraction, (2) An example of how the combined models can be used to predict the cooking loss and the B vitamin content in cooked beef meat, and (3) the analysis of the formation of heterocyclic aromatic amines during the roasting and the grilling of beef meat. At the beginning of each part the literature is shortly reviewed to analyze the basic phenomena which are involved in the development of the studied quality. When possible, the results obtained at lab-scale are confronted with what can be observed in household equipment. Combined transfer to quality models requires the knowledge of the time-evolution of the target quality at a given

temperature and at given water content. Thus, quality kinetics were measured in slices of meat uniformly heated. These kinetics have been combined to a transfer model to predict the evolution of the weight loss and the B vitamin content during the roasting of SM muscle by air convection. The new results and the need for future research are discussed in the paper.

1) Protein denaturation and contraction, links with meat juiciness and colour

Basic knowledge gained at lab-scale will be compared to what can be observed in a controlled microwave equipment. Then, the mathematical relations issued from the experiments on the uniformly heated slice are presented and discussed.

Basic knowledge on protein denaturation and effect on water binding capacity and colour

Denaturation of muscle proteins which is linked to the organoleptic qualities (tenderness, juiciness and colour) of cooked meat has been studied for a long time. Myosin is known to denatured at about 54 and 58°C, whereas actin, actomyosin complex and titin are denatured at around 80°C and the transition temperature of sarcoplasmic proteins is about 65-67°C (Tornberg, 2005). Collagen contraction occurs between 58°C to 65°C. Protein structural changes in muscle tissue due to thermal changes have been studied using FTIR microspectroscopy which is a versatile spatially resolved technique. (Kirschner, Ofstad, Skarpeid, Høst & Kohler, 2004; Bertram, Böcker, Ofstad & Andersen, 2006; Astruc et al., 2012). Increasing in meat temperature, leads to an increase in β-sheet and a decrease in α-helical structures, which is more pronounced for the intracellular proteins than for the connective tissue and is practically independent of the fibre type (Kirschner et al., 2004; Astruc et al., 2012). Salting can also affect the protein structure which is important to consider when marinated products have to be cooked (Böcker, Ofstad, Bertram, Egelandsdal & Kohler, 2006; Böcker, Kohler, Aursand & Ofstad, 2008; Carton, Böcker, Ofstad, Sørheim & Kohler, 2009). Meat salting is known to increase the water holding capacity of the meat. However, sodium is detrimental for human health and thus it can be interesting to replace sodium by other salts. During the ProSafeBeef project, investigations have been focussed on the analysis of the effect of different salt types on protein structures by FTIR microscopy and Raman microscopy (Perisic, Afseth, Ofstad & Kohler, 2011; Perisic, Afseth, Ofstad, Hassani & Kohler, 2013). Clear differences in protein structures could be detected for the different salt mixtures. The samples that were treated with mixtures containing $MgSO_4$ hydrated

earlier with increasing salt concentration. An increased hydration of the proteins in meat tissue was related to a partial unfolding of the proteins and thereby to their destabilization. This unfolding of the protein may, at moderate salt concentrations led, to an increase of hydration, since large parts of the proteins were accessible and thus able to bind to water molecules. A further increase of the salt concentration led to a further destabilization of the proteins and consequently to their denaturation. These last results are important to reduce the salt content in cooked meat products.

Colour change due to temperature increase is initially due to myoglobin denaturation, shifting from deep red to pink and then on to a greyish colour before finishing in a light brown. It is recognized that these changes occur near 60°C, between 60 and 70°C, and between 70-80°C, respectively (Lawrie, 1985). Beyond the 85°C threshold, Maillard molecules begin to form along with the melanoid pigments which are associated with the grilled-meat colour.

Confrontation of previous knowledge with weight losses and colour evolutions measured during microwave cooking

Microwave cooking has been chosen as an example because it is a cooking method widely used at domestic scale. Moreover, there are limited published data about the quality of beef cuts as affected by rapid heating methods including microwave (Tang, Lyng, Cronin & Durand, 2006).Traditional cooking methods (such as convection, contact, immersion, and infrared radiation) lead to heterogeneities between the product surface and its center. Microwave cooking/reheating is known to lead to more complex patterns of heterogeneity, related to either the geometric shape of the product (overheated corners and angles of parallelepipeds, or in cylindrical products, overheating of the product center) or to its composition (Ryynanen & Ohlsson, 1996). Work was dedicated during the ProSafeBeef project to microwave cooking to evaluate the effect of the difference sources of variations encountered in practice on the quality of cooked beef meat (Perez-Juan, Kondjoyan, Picouet & Realini, 2012). To discuss the results in the light of previous basic knowledge the heterogeneity of thermal treatment and its duration were determined using six or eight optical probes inserted in the roast. The dimensions of the roastbeef and its the position in the microwave were also perfectly controlled to ensure repeatable gradients of temperatures in the sample. Meat issued from different muscles (*Semitendinosus* and *Semimembranosus*) coming from animal of different ages (Friesian yearling heifers and mature cows) were cooked using combinations of microwave power (182 W and 654 W power) and final temperature (60 and 80°C). The gradient of temperature due to microwave heating was

mainly along the vertical cross-section of the sample. Underdone areas were observed at the roast surface being more evident in the central section while the edges were overcooked or almost burnt. Maximum temperature depended on the targeted temperature of each treatment, and therefore, was higher for roasts heated to a final temperature of 80ºC compared with 60ºC (Table 1). Most of the observed results agree with what is known from literature at a laboratory scale or what can be obtained using other heating sources than microwave. For example, microwave power affected treatment duration but neither the cooking loss, nor the product temperature. While cooking loss and colour variation were affected by the final meat temperature. A significant increase of lightness was observed in the SM when cooked to 80ºC compared with 60ºC (Table 2). However, some of the colour differences due to animal age were still evident after cooking. Moreover, other observations were less clear for example about the effect of animal age, which should have had no influence on the final meat temperature, which was not observed. This was partly due to the difficulties in controlling the microwave cooking under practical conditions. The initial difficulty to position the fiber sensors in raw meat and movement of these sensors during cooking can for example lead to artificially greater or smaller temperature. This is especially important when this temperature is used to control the treatment-time because heating can be stopped earlier/later which will lead to a lower/greater cooking loss than expected. This effect was clearly observed during some experiments in the SM muscle.

This study illustrates the difficulty to use basic literature knowledge issued from laboratory experiments to interpret what can be actually observed at a household scale. Despite the attempt of specialists of thermal science and of meat science to control the MW cooking of calibrated pieces of beef meat some non-expected results were observed. This was mainly due to the fact that quality was analysed too averagely while its evolution was locally dependent on the complex thermal gradient generated in the meat. Thus, at household scale it is often difficult to know if the observed effects come from a variation of the quality of the raw beef meat or if they come from a difference in the functioning of equipments. Combined transfer to quality modelling approach is developed in the following to predict weight losses and nutrient contents during the convection cooking of beef meat roasts. Convection cooking has been chosen since thermal gradients are quite easy to model in such a situation. However, combined transfer-quality modelling requires the knowledge of the time-evolution of the target quality at a given temperature which is studied in the next paragraph.

Representation of the time evolution of the water holding capacity of the meat and of its colour using mathematical functions

Effect of temperature on water de-bounding due to protein denaturation is generally taken into account in models by a mathematical function which describes the effect of temperature on the water holding capacity of the meat. Experiments are usually performed by immerging thin slices of meat in water bath at given temperature and waiting for equilibrium (no more weight loss). The results are often presented as the evolution of the equilibrium water content with temperature using a sigmoid function (Van der Sman, 2007, Goni and Salvadori, 2010). The difference between the initial and the equilibrium water contents is used to determine the loss of juice. It is considered that the value of the equilibrium water content is not dependent on the dimension of the sample and that the sigmoid shape of the function is verified whatever the type of muscle. This has been validated during the ProsafeBeef project using pieces of beef muscles of different dimensions and types (Fig. 1, Kondjoyan, Oillic, Portanguen & Gros, 2013). Effect of dimension which exists between thin meat slices of meat and 10mm-side-cubes becomes negligible when the dimension of the cube increases. Except for specific muscles as *Masseter*, the evolution of the equilibrium water content with temperature keeps a sigmoid shape. However, equilibrium values can be different from one type of muscle to another (Kondjoyan et al., 2013).

The knowledge of temperature thresholds is not enough to predict the evolution of colour which results from the kinetics of the previously mentioned chemical reactions. Thus, experimentations have been conducted during the ProSafeBeef project in order to model the effect of time and temperature on the kinetics of meat colour. Samples were cut in slices to ensure a uniform heating of the meat. The evolution of the three colour parameters in the CIELAB system (D65-10°-L*a*b*-d/8 SCE) were measured during steam heating at three temperatures: 66, 98 and 205°C. These colour parameters were normalized relatively to their initial value measured on the raw meat. At 66°C or 98°C, L*/ L*0 increases to a maximum during the first 30 to 60 seconds of the treatment and then stabilizes. At 205°C, L*/ L*0 increases during the first 10 s of the treatment and then decreases sharply toward a minimum value (Fig. 2). The variations of a*/a*0 are opposite to those of L*/ L*0.

Visually, the change of the colour parameters corresponds to the whitening, the browning and the darkening of the sample in the course of the cooking treatment. For the 66 and 98°C temperatures, sample whitened and then does not change colour until the end of the treatment where spots of brown were noticed. On the contrary, for the 205°C treatment the whitening is

limited to the first 10 s of the treatment, afterwards sample gets brown and darkens very quickly. A kinetics model based on two successive first order chemical reactions was developed to take into account the evolutions described previously (Portanguen, Lebert & Kondjoyan, 2009).

2) Modeling the cooking yield and micronutrients content in cooked beef meat

2.1) Modeling the mass transfer and the cooking yield during cooking and cooling

This discussion concerns water transfer in pieces of whole beef meat (grounded meat not considered here) during their cooking and following cooling. The temperature increase in the beef meat pieces leads to water debinding from the myofibrillar proteins and water migration under pressure in channels of different dimensions formed by the contraction of the complex muscle structure (Laroche, 1978; Lepetit, 2007; Lepetit, Grajales, & Favier, 2000). Van der Sman (2007) has modeled water transport in meat pieces during cooking by using the Flory–Rehner theory and the Darcy law. Feyissa, Gernaey, and Adler-Nissen (2013) have extended this work by inserting the mechanical forces to model the effect of protein contraction on the water transport inside roast meat. These works assumes that juice circulates in a uniform porous material which does not vary during heating. This is disputable because contraction of muscle structure leads to a network of interconnected channels of different sizes. The parameters introduced in these models are also difficult to determine for whole beef meat. Feyissa et al. (2013) reported data for ground meat and emphasized the need for more quantitative knowledge of the effect of temperature on meat permeability and meat elastic modulus. Another approach, which combines heat transfer and chemical kinetics, has been used in literature to model the cooking of whole beef meat (Goni & Salvadori, 2010). This approach is simpler than the previous one and can be used for the multi-objective optimization of beef roasting (Goni & Salvadori, 2012). A limit of literature is that all previous models have been validated directly on a few pieces of meat of given size and shape derived from one type of muscle and subjected to air cooking conditions. This falls short, since a real determination of model performance requires a wide range of sample sizes and muscle types. Moreover, oven-cooking in dry air is not the best situation for a first test of model performance, since: (i) it is a complex situation where uncertainties on heat transfer "are mixed" with the uncertainties due to the mass transfer phenomena which drive to crust

formation, and (ii) air-cooking makes it difficult to effectively separate the water loss by evaporation from the water loss by protein denaturation–contraction.

Confronted with this literature knowledge a two sides approach was followed during the Prosafebeef project. On one side the simplest modeling approach of literature was improved and extensively validated to be able to predict, in a first step, the weight loss (Kondjoyan et al., 2013) and, in a second step, the loss of micronutrients associated with the juice migration. On the other side, an experimental method was developed to visualize the contraction of the connective tissue and of the muscle fibers during heating and to map out the water movement in the beef meat due to this contraction (Bouhrara et al, 2011).

Existing kinetic models of Goni & Salvadori (2012) was improved by introducing an explicit determination of the effect of sample thickness on mass transfer (Kondjoyan et al., 2013). Performance of the new model to predict weight loss was evaluated on a SM muscle using a wide range of samples which size varied from thin steaks to big muscle cuts (Oillic et al. 2011). Different air/steam conditions were applied to analyze the transition from the wet to the dry air situation and sets of experiments were performed on other muscles than the SM to extend the application of the model (Kondjoyan et al., 2013). Some predicted and measured weight losses are compared in figure 3 to illustrate the results. The predicted cooking losses agreed with the measurements on all the meat samples regardless of their dimensions and of the time-temperature conditions. During cooking by air convection water evaporation at the meat surface can be a further cause of weight loss. However, measurements and simulations led to the conclusion that during the study of Kondjoyan et al. (2013) most of the evaporation came from the juice already expelled by the denaturation and the contraction of proteins. Globally the simple model which combined heat transfer and a kinetic approach was enough accurate to predict the average weight loss under very different cooking conditions. However, it does not take into account neither the coupling between the mechanical phenomena, which induce the meat contraction, and juice flow, nor the effect of the juice flow and of the meat contraction on the temperature variations close to the meat surface.

The new method developed to visualize the contraction of the muscle structure and its effect on juice flow was based on magnetic resonance imaging (MRI). MRI was used because it is a noninvasive, nondestructive tool that can be used to characterize properties and structures both locally and dynamically (Bouhrara et al, 2011; Bouhrara, Clerjon, Damez, Kondjoyan &

Bonny, 2012). A novel device was designed to heat the sample in an NMR imager. Rapid MRI methods were developed both to contrast the connective tissue and the muscle fibers in the images and to map water during heating. The contrasted images were used to quantify deformation fields during heating (Bouhrara, Lehallier, Clerjon, Damez & Bonny, 2012). Finally, global models were developed which link the water content and the deformation to temperature (Fig. 4). The contraction of myofibrillar and collagen networks was observed at 38°C, and water began to migrate toward the interfascicular space at 38°C. A strong deformation occurred between 54 and 70°C. Variation of the interfascicular space and matrix contraction led to complex juice flow patterns within the muscle.

The previous MRI method is able to analyze in 3 dimensions the anisotropic deformation of the sample and the formation of the channels of various sizes through which the juice will be expelled outside the meat. This method will be very helpful for the design and the validation of models such as those of Feyissa et al., (2013) but which will be more realistic, because they will take into account the non-uniformity and time-variation of the porosity of the meat and the anisotropy of the mechanical deformations.

2.2) *Prediction of the loss of micronutrients during cooking*

Literature has shown that the loss of water soluble vitamins during cooking was often close to the measured weight loss. This is the case for Lombardi-Boccia, Lanzi & Aguzzi (2005), who have analyzed the content of B vitamins in raw and cooked beef cuts issued from 5 types of muscles (sirloin, fillet, roast beef, topside and thick flank) and find 36 % to 42 % of niacin loss depending on the type of muscle. Leskova et al. (2006) have reviewed the retention of most of the vitamins during heat treatments and its prediction by mathematical models. They stress on the effect of the cooking method and of the cooking conditions on the loss of vitamins. For example the retention of vitamin B6, which was 6.5 % during meat frying, ranged from 43 to 71 % during roasting and broiling. Similarly, the retention of niacin varied from 45 to 90 % depending on the culinary treatment. Leskova et al. (2006) finally mentioned the lack of kinetic models to predict the loss of vitamins and they insisted on the necessity for the models to take into account the effect of the type of vitamin, of the cooking method, and more generally of all the process conditions on the vitamins losses.

Thus, a modeling approach was developed during the ProSafeBeef project to predict the concentration of vitamin B3 and B6 according to the size of the meat cut and to the heating conditions. Vitamin losses by juice expelling was predicted using the weight loss model of

Kondjoyan et al. (2013) while the thermal degradation kinetics of these two vitamins were measured in juice and in thin slices of meat under controlled conditions. Validation experiments were performed on meat cut of different sizes. Results prove that the concentration in vitamin B6 decreased faster in the juice than in the meat cuts probably because of the difference in the degradation due to light. Vitamin B3 was almost only present in nicotinamide form, nicotinic acid being in very small proportion (from 1 to 5 µg/g DM). On the contrary, to vitamin B6, no thermal denaturation of nicotinamide content was measured in the meat cubes heated in water-bath, even after 15 hours at 90°C. This was coherent with literature which asserts that vitamin B3 is particularly heat-resistant. Thus, the loss of vitamin B3 was predicted afterwards directly from the calculated quantity of expelled juice while the thermal degradation of the vitamin B6 was added to the quantity of B6 expelled in the juice to determine the total loss of this vitamin (Fig. 5). The model was validated during the oven-roasting of meat cuts of different size heated under different conditions. Values predicted by the model were consistent with experimental values. The average of the algebraic difference between the predicted and the measured values ranged from -2.2 to 11.6 % of the experimental value. Then, the validated model was used to predict the loss of vitamin B3 and B6 under different heating conditions. During grilling or pan frying of steaks, the loss in vitamin B was only due to juice expelling and was ranging from 4 to 23 % depending on the degree of doneness (cooking time). During roasting, the loss of vitamin B3 in beef meat was in between 25 and 32 % mainly depending on the final core temperature of the meat (50-70°C), while it was in between 30 and 41 % during simmering. The additional loss fraction of vitamin B6 due to thermal denaturation was between 5 and 10% during roasting and simmering and reached 30% during very long boiling/steam treatments. This study illustrates how heat mass transfer models can associated to denaturation models to predict the nutritional quality of cooked beef meat.

3) Formation of Heterocyclic Amines during the roasting and the grilling of beef meat

The HAAs are usually formed as end-products of the Maillard reaction at moderate temperatures (150-200°C) and products of pyrolysis *via* radicalar mechanisms for high temperatures (>300°C) (Messner & Murkovic, 2004). Beef meat contains creatinine and tryptophan which can lead to the formation of HAAs once the product temperature goes over the 90-100°C threshold (Skog, Johansson & Jagerstad, 1998). According to Polak, Dosler,

Zlender & Gasperlin (2009) the increase of creatinine and free aminoacids during meat ageing favors the formation of HAAs. This difference in the content of precursors is also put forward by Sterk, Roszko, Malek, Kurek, Zbiec,& Waszkiewicz-Robak (2012b) to interpret the difference of HAAs formation between the *Psoas Majors* and the *Gluteus Medius*. In their study, storage temperature has also an effect on HAAs formation. However, the variations due to the muscle type or to storage were much smaller that the differences due to the cooking method. The rate of HAAs formation increases with temperature, reaching very high rates between 150 and 200°C, which are the temperatures commonly found when grilling or roasting meat. HAAs formation tends to be promoted by low water activity but slowed by marination (Pais, Salmon, Knize & Felton, 1999; Sinha, Knize & Felton, 1997). Some literature results have concluded that increasing the content in lipids decreases the formation of HAAs (Hwang & Ngadi, 2002) while lipid oxidation promotes their formation as recently shown for PhIP (Zamora, Alcon & Hidalgo, 2012). Thus, antioxidants like vitamin E have been used to prevent HAAs formation (Balogh, Gray, Gomaa & Booren, 2000). Wine, garlic, rosemary or other ingredients in the marinade could also have a similar effect as vitamin E (Busquets, Puignou, Galceran, & Skog, 2006; Gibis, 2007). Phenolic compounds in the frying oil (Persson, Graziani, Ferracane, Fogliano, & Skog, 2003) or lipid oxidation compounds (Randel et al., 2007) appear to be linked to lower quantities of HAAs. HAAs mainly form at the product surface, in the “crust”. The quantity produced is directly dependent on the cooking process and on the cooking equipment. However, integrating these elements remains a complex task due, in particular, to the difficulty in measuring the temperature at the product surface (Knize, Cunningham, Avila, Jones, Griffin, & Felton, 1994; Knize et al., 1995; Murkovic & Pfannhauser, 2000). Epidemiological studies have widely reported an indirect link between the quantity of HAAs produced and the cooking stage, which is itself assessed through the colour of the cooked meat (Sinha et al., 1998; Sinha *et al.*, 1999; Rohrmann & Becker, 2001, 2002; Rohrmann, Zoller, Hermann, & Linseisen, 2007; Aaslyng, Duedahl-Olesen, Jensen, & Meinert, 2013). Finally, it can be concluded from literature that although, cooked meat and meat juices are significant sources of HAAs, it remains difficult to reliably estimate the consumer exposure depending on his practice, on the type of meat and on the type of equipment (Skog, 2002; Murkovic, 2004). Combined transfer-reaction modeling can be a great help in dealing with the variety of conditions encountered in practice. Such, an approach has already been followed by Tran, Salmon, Knize and Colvin (2002) to simulate the formation of HAAs during pan frying of beef patties. However, the mass transfer (no juice migration) and the formation of the crust at the surface of the patties were not erally

modeled. Gradient of temperature in the meat patties was calculated using a conduction model and assuming either that heat capacity of the meat was constant or variable depending on the local temperature value. The simulated temperature agreed with the measurement at the center of the patties. This was not the case at 6 mm from the surface where the differences of temperature between the simulated and the measured values were greater than 10°C at the end of cooking. Differences were probably even greater in the crust area which thickness during pan frying is usually less than 2 mm. A first order kinetic model and an Arrhenius relation were used to predict the formation of HAAs, using the same activation energy value for all the HAAs. A more sophisticated modeling of the heat-mass transfer during frying of beef patties has been developed recently Sprague and Colvin (2011). A diffusion model was used to predict the mass transfer (lipid and water) while the temperatures were simulated using a mixture-enthalpy formulation to account for the liquid and the vapor state of water. The coherence between the predicted and the measured quantity of HAAs in the simulations of Tran et al. (2002) and Sprague & Colvin (2011) proves the interest of the transfer-reaction approach. However, the transfer and reaction models have not been validated enough to ensure reliable predictions on HAAs formation. The objective of the work performed during the Prosafebeef project was to progress on the kinetic modeling of HAAs formation in beef meat and to analyze experimentally this formation during roasting, in relation with the development of the crust at the surface of beef meat pieces. These are two key points to predict reliably HAAs formation by transfer-reaction models. In a first step, experiments and kinetic modeling were performed on uniformly heated slices of meat. The results were compared to the kinetics model developed in liquid systems (Arvidson, van Boekel, Skog, Solyakov, & Jägerstad, 1999; Murkovic, 2004). In a second step, the results obtained on meat slices were confronted to what occurred at the surface of bigger meat pieces. Experiments on meat slices and bigger meat pieces were also used to validate different mitigation strategies to decrease the formation of HAAs.

3.1) *Experiments on meat slices*

Two set of experiments were performed on meat slices. The first set aimed at determining the effect of process conditions (time-temperature, relative humidity) on HAAs formation in meat tissues issued from different muscles. The second set of experiments aimed at studying the effect of marination on HAAs formation.

HAAs formation in non-marinated beef meat

During the first set of experiments jets were used to heat 1-2 mm meat slices of lean *Longissimus thoracis* and *Semimembranosus* muscles (Kondjoyan et al., 2010 a, b). These muscles were aged and stored under the same conditions. The jets were either, superheated steam jet, or hot air jet to be able to vary the water activity of the meat. The temperature was considered as rapidly uniform in the meat and the whole slice as being as a formed crust. Experiments were restricted to 20 minutes because afterwards the slice was "bursting". HAAs content was measured by LC-APCI-MS/MS according to a method specially adapted for beef meat (Kondjoyan et al., 2010 a, b). Analysis of the results led to the conclusion that four HAAs namely IQx, 4,8-DiMeIQx, MeIQx and PhIP were mainly formed during the heat treatments and that their concentration followed regular kinetic patterns. After only 10min of treatment the formation of HAAs was plateauing or followed by degradation. HAAs formation increased significantly between 170 and 200°C. Results depended on the jet conditions. The results were compared to the ones obtained in literature in liquid systems (Arvidson, van Boekel, Skog, Solyakov, & Jägerstad, 1999; Murkovic, 2004). Under superheated steam jet conditions, the amounts of IQx and 4,8-DiMeIQx formed in LT slices were 3 to 4-fold smaller than those reported in literature for meat juices, while quantities of MeIQx and PhIP remained comparable. Under hot-air jet conditions the amount of HAAs formed in the SM muscle was clearly lower than that formed in the LT muscle as soon as the heat treatment was longer than 300s. In this study, the content of the two muscles in creatine and in amino acids and other precursors was similar except for phenylalanine and glycogen which have to be hydrolysed before affecting HAAs formation. The difference in the content of amino acids and sugar between the two raw muscles was very small and thus could not explain the difference between the two muscles. Thus, the difference between the two muscles was attributed to variations in the water migration and content. The extreme dehydration obtained with the hot-air jets slowed the formation of IQx, MeIQx and, particularly, 4,8-DiMeIQx compared with superheated steam treatments. The reverse effect was observed for PhIP concentrations which increased 1.4 to 5.5-fold. These original results obtained on meat slices confirm what was observed in juice system i.e. there is a temperature threshold of 150°C above which the formation of HAAs is really boosted. They also highlight the importance of the nature of the muscle tissue, and of the water activity variation on HAAs formation. The first-order kinetic model used in literature to describe the results obtained in liquid systems was adapted to predict the results on meat slices, taking into account the time-temperature variation in the slice over the course of the experiment. The parameters of the

kinetic model were different from one HAA to another and also different from the values obtained in liquid systems.

HAAs formation in marinated beef meat

The second set of experiments, which aimed at studying the effect of marination on HAAs formation, was performed on meat slices 3 mm in thickness and 60 mm in diameter (10 g) cut from Roastbeef muscles issued from three young bulls of Holstein breed. These meat slices were grilled on a hot plate at 220ºC for 10 min. (5 min per side, turned over every 1 min). Marination is often proposed in literature as a mean way to decrease the formation of HAAs in grilled and roasted beef meat. This HAAs decrease is generally attributed to the antioxidant effect of plant extracts placed in the marinade. However, other compounds such as NaCl or phosphates can affect HAAs formation by modifying the transfer of juice from the center of the product to its surface.

The objective of this set of experiments was to determine how the combined effect of a modification of juice migration and of the addition of an antioxidant compounds can decrease the formation of HAAs. Rosemary extracts were chosen for their well-known antioxidant activity. NaCl and polyphosphate were examined because they are used in main of the delicatessen products and are known to affect the water holding capacity of the meat. They can also affect iron, or oxygen concentration or solubility, in meat with some contradictory effects on lipid and protein oxidation. In literature, the effect of NaCl on oxidation is still much debated. For many authors, NaCl may act as pro-oxidant in meat products (Kanner, Harrel, & Jaffe, 1991; Sarraga, Carreras, & Garcia Regueiro, 2002). Nevertheless, in some conditions, inhibition of oxidation by NaCl has also been reported. For example, Rhee, Smith & Terrell (1983) reported that NaCl activated lipid oxidation at low concentration but inhibited at concentration greater than 2 % in ground pork. In dry-cured pork loins, Sarraga et al. (2002) have also observed an antioxidant effect at 3 %. Other authors have demonstrated that polyphosphate can inhibit myoglobin and lipid oxidation during meat storage (Allen, & Cornforth, 2006; Lee, Hendricks, & Conforth, 1998). At the pH of meat, polyphosphates have multiple negative charges which can bind cations and contribute to its antioxidant properties in meat. Thus, NaCl and Phosphate at higher concentrations than 3 % should both increase the water concentration of the meat pieces and increase the antioxidant reactions. The four HAAs (2-amino-3-methylimidazo [4,5-f]-quinoline (IQ) regularly formed in greatest quantity at these temperatures, 2-amino-3,4-dimethylimidazo[4,5-f]-quinoline (MeIQ), 2-amino-3,4,8-trimethylimidazo[4,5-]quinoxaline (DiMeIQx) and 2-amino-1-

methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were quantified in the meat extract. Extraction and quantification of HAAs were performed using the same procedures as the one used in the previous set of experiments (Kondjoyan et al., 2010 a, b).

The largest concentrations of HAAs were found in the non-marinated control steaks. Rosemary extracts decreased the formation of MeIQ and PhIP but increased the concentration of IQ and DiMeIQx. Addition of Sodium, Chlorite and Tripolyphosphate led to an important decrease of the concentration of IQ, DiMeIQx and PhIP but had no effect on the formation of MeIQ (Table 4). These results can explain some contradictions of literature when different antioxidative extracts are added to marinade to decrease the formation of HAAs. If these marinades already contain NaCl and Sodium Polyphosphate, the assumed active effect of the extracts can be biased by the presence of the salts. It is also reasonable to think that the formation of HAAs in delicatessen issued from pork meat can be mitigated by the presence of salts. However, it is not recommended to increase the NaCl content in marinated beef products because sodium is known to have detrimental effects on human health. The kinetics of the oxidation reactions and, the effect of antioxidants on the rate of the reactions are not known in meat. Moreover, it has been shown during the project that salt present in the marinade can modify the mass transfer. Thus the situation in marinated product is very complex and the following of the work was focused on crust formation at the surface of non-marinated beef meat.

3.2) *Crust development and HAAs formation at the surface of small roast beefs*

Previous experiments on meat slices aim at limiting the temperature and the water gradients to better quantify the effect of process conditions or of meat composition on the formation of HAAs. However, in practice, crust is only a thin area close to the surface of the meat which thickened during grilling and roasting. The HAAs content in the whole meat piece depends on the gradients of temperature and of water content in the thickening crust and of the relative importance of this crust area with respects to the non-crusted area. Thus, it was important to analyze the crust development and its effect on HAAs formation. This was performed during the project at the surface of a 5 cm thick cylindrical piece of meat subjected during up to 90 min to a jet at 210°C which mimics the cooking by air convection of a roast beef in a fan-assisted oven. Analysis of the thermal exchanges in the crust area requires the measurement of surface and under surface temperatures. This is not easy when meat is cooked in an oven due to problems of accessibility and to probe movements generated by the heat shrinkage of the meat sample. Thus, experiments were performed using: (1) an open jet system which

enables the IR measurement of surface temperature, and (2) a specific device which partially compensates heat shrinkage and thermocouple movements. Results prove that during these experiments, no plateauing and no degradation of the quantity of HAAs were observed even after 90 min at 210°C. This was the contrary to what had been observed in meat slices where the formation of HAAs was plateauing or followed by their degradation after only 10 min of treatment at 170-210°C (Kondjoyan et al., 2010 a, b). This degradation had also been observed in liquid systems when HAAs were heated in test tubes at 200°C (Arvidsson et al., 1999). This contradiction can be due to the fact that the crust area, where the temperature was higher than 200°C, only represented a small portion of the area where HAAs were forming (temperature higher than 150°C). Thus, the small degradation which occurred in the 200°C area of the crust was overwhelmed by the HAAs formation in the other parts of the crust. Another cause can be related to the variation of the precursors content in the crust. When thin slices of meat are subjected to air flows, precursors are quickly consumed which can explain why in meat slices HAAs degradation occurred just after ten minutes of treatment. On the contrary, in roasts the juice which comes from the core to the surface brings precursors which can be used for further formation of HAAs.

Previous results illustrate that the transposition of the results obtained in thin slices to bigger meat pieces is not direct and that it will require both the modeling of the thickening crust and of the thermal gradient close to the surface, and probably also the modeling of the migration of the precursors with juice from the center of the meat piece towards its surface. An accurate modeling of these phenomena remains a challenge for the future modeling research. The second set of experiments on meat cylinder was also used to validate a mitigation strategy of HAAs formation based on the control of temperature at the surface of the meat. The idea was to subject the surface of the meat to a temperature less than 150°C to mitigate the formation of HAAs while promoting the formation of the crust as desired by many consumers which like the grilled or the roasted meat. Conditions were actually found where the meat product kept the traditional roasted aspect while being almost completely free of HAAs compared to beef meat as classically roasted in oven.

Conclusion

The variations of the water holding capacity of the beef meat, of its color, of the degradation of the B vitamins and of the formation of HAAs have been studied in slices of beef meat uniformly heated. Mathematical relations have been found to describe the quality kinetics.

Some of these relations were combined with a heat transfer model to predict the weight loss and the content of the B3 and B6 vitamins in pieces of SM muscle subjected to air/steam convection. The predictions of this combined model were validated experimentally on meat pieces of different sizes subjected to various air/steam conditions. This approach can be extended to other thermal treatments, to other beef muscles or to other micronutrients.

The use of combined transfer-reaction models to predict HAAs formation is more difficult. The transfers in the developing crust are complex and the routes of the reactions responsible for the formation of HAAs are not fully elucidated. However, ProsafeBeef work has led to some original results which can be used for the development of new models. For example the formation of HAAs seems to depend on the arrival of precursors which migrate with the juice towards the meat surface. Moreover, salts added with the marinade can affect both the oxidation reactions and the migration of juice.

The modelling approach, which combines heat transfer and chemical kinetics, was successful to predict the transfer of juice. Thanks to its simplicity it can be easily used for the multi-objective optimization of beef cooking. It can also lead, in the future, to lumped models usable by scientists and engineers which have no skill in numerical modeling. However, this approach has its limits. It is not linked with the anisotropic deformation of the beef meat sample and with the effect of meat contraction on juice expelling. These aspects are important to analyze the effect of cooking on the meat mechanical properties and tenderness. This is also important if local variations of the juice migration have to be taken into account to determine the formation of HAAs in the crust. More generally, the models existing in literature to predict the gradient of temperature in the crust at the surface of meat during grilling and roasting remain too simple and need to be improved. The MRI method developed during the project can be very helpful to design and to validate new transfer models. HAAs are not the only potential toxic compounds which form during grilling and roasting. Polycyclic Aromatic Hydrocarbures and products of lipid oxidation are other potentially toxic compounds which come from the same kind of precursors and reactions routes as flavour compounds. Thus, a more complete understanding of these reactions in meat is required to find how to promote flavour while mitigating the formation of the process-induced food toxicants.

631 **Acknowledgement**

632 This work was supported by a grant awarded as part of the ProSafeBeef project under the 6th
633 Framework Program of the European Union. The authors wish to thank S. Bruel, C. Touzet
634 and J. Molina for their efficient technical assistance in HAAs analyses.

635

References

Aaslyng, M.D., Duedahl-Olesen, L., Jensen, K. & Meinert, L. (2013). Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers. *Meat Science*, 93(1), 85-91.

Allen, K.E. & Cornforth, D.P. (2006). Myoglobin oxidation in a model system as affected by nonheme iron and iron chelating agents. *Journal of Agricultural and Food Chemistry*, 54, 10134-10140.

Arvidsson, P., van Boekel, M.A.J.S., Skog, K., Solyakov, A. & Jägerstad, M. (1999). Formation of heterocyclic amines in a meat juice model system. *Journal of Food Science*, 64(2), 216-221.

Astruc, T., Peyrin, F., Vénien, A., Labas, R., Abrantes, M., Dumas, P. & Jamme, F. (2012). *In situ* thermal denaturation of myofibre sub-type proteins studied by immunohistofluorescence and synchrotron radiation FT-IR microspectroscopy. *Food Chemistry*, 134, 1044-1051.

Balogh, Z., Gray, J.I., Gomaa, E.A. & Booren, A.M. (2000). Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. *Food and Chemical Toxicology*, 38(5), 395-401.

Bertram, H. C., Kohler, A., Böcker, U., Ofstad, R. & Andersen, H.J. (2006). Heat-induced changes in myofibrillar protein structures and myowater of two pork qualities. A Combined FTIR spectroscopy and low-field NMR relaxometry study. *Journal of Agricultural and Food Chemistry*, 54 (5) 1740-1746.

Böcker, U., Ofstad, R., Bertram, H.C., Andersen, H.J. & Kohler, A. (2006). Salt-induced changes in pork myofibrillar tissue investigated by FTIR microspectroscopy and light microscopy. *Journal of Agricultural and Food Chemistry*, 54 (18) 6733-6740.

Böcker, U., Kohler, A., Aursand, I.G., Veliyulin, E., Egelandsdal, B. & Ofstad, R. (2008). Effects of brine salting with regards to raw material variation of Atlantic salmon (*Salmo salar*) muscle investigated by FTIR microspectroscopy. *Journal of Agricultural and Food Chemistry*, 56, 5129-5137.

Bouhrara, M., Clerjon, S., Damez, J.-L., Kondjoyan, A. & Bonny J. M. (2011). Dynamic MRI and thermal simulation to interpret deformation and water transfer in meat during heating. *Journal of Agricultural and Food Chemistry*, 59 (4), 1229-1235.

669 Bouhrara, M., Clerjon, S., Damez, J.-L., Kondjoyan, A. & Bonny J. M. (2012). *In-situ*
670 imaging highlights local structural changes during heating: the case of meat. *Journal of*
671 *Agricultural and Food Chemistry*, 60 (18), 4678-4687.
672 Bouhrara, M., Lehallier, B., Clerjon, S., Damez, J. L. & Bonny J. M. (2012). Mapping of
673 muscle deformation during heating: *in situ* dynamic MRI and nonlinear registration.
674 *Magnetic Resonance Imaging*, 30 (3), 422-430.
675 Busquets, R., Puignou, L., Galceran, M.T. & Skog, K. (2006). Effect of red wine marinades
676 on the formation of heterocyclic amines in fried chicken breast. *Journal of Agricultural*
677 *and Food Chemistry*, 54, 8376-8384.
678 Carton, I., Böcker, U., Ofstad, R., Sørheim, O. & Kohler, A. (2009). Monitoring secondary
679 structural changes in salted and smoked salmon muscle myofiber proteins by FTIR
680 microspectroscopy. *Journal of Agricultural and Food Chemistry*, 57, 3563–3570.
681 Feyissa, A. H., Gernaey, K. V., & Adler-Nissen, J. (2013). 3D modelling of coupled mass
682 and heat transfer of a convection-oven roasting process. *Meat Science*, *93*(4), 810-820.
683 Gibis, M. (2007). Effect of oil marinades with garlic, onion, and lemon juice on the formation
684 of heterocyclic aromatic amines in fried beef patties. *Journal of Agricultural and Food*
685 *Chemistry*, 55, 10240-10247.
686 Goñi, S. M., & Salvadori, V. O. (2010). Prediction of cooking times and weight losses during
687 meat roasting. *Journal of Food Engineering*, 100 (1), 1-11.
688 Goñi, S.M. & Salvadori, V.O. (2012). Model-based multi-objective optimization of beef
689 roasting. *Journal of Food Engineering*, 111, 92-101.
690 Hwang, D.K. & Ngadi, M. (2002). Kinetics of heterocyclic amines formation in meat
691 emulsion at different fat contents. *Lebensmittel-Wissenschaft Und-Technologie-Food*
692 *Science and Technology*, 35, 600-606.
693 Kanner, J., Harel, S. & Jaffe, R. (1991). Lipid peroxidation of muscle food as affected by
694 NaCl. *Journal of Agricultural and Food Chemistry*, 39, 1017-1021.
695 Kirschner, C., Ofstad, R., Skarpeid, H.J., Høst, V. & Kohler, A. (2004). Monitoring of
696 denaturation processes in aged beef loin by Fourier transform infrared
697 microspectroscopy. *Journal of Agricultural and Food Chemistry*, 52 (12) 3920-3929.
698 Knize, M.G., Cunningham, P.L., Avila, J.R., Jones, A.L., Griffin, A. & Felton, J.S. (1994).
699 Formation of mutagenic activity from amino acids heated at cooking temperatures.
700 *Food and Chemical Toxicology*, 32, 55-60.

Knize, M.G., Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Levander, O.A., Cunningham, P.L. & Felton, J.S. (1995). Heterocyclic amine content in fast-food meat products. *Food and Chemical Toxicology*, 33(7), 545-551.

Kondjoyan, A., Chevolleau, S., Grève, E., Gatellier, P., Santé-Lhoutellier, V., Bruel, S., Touzet, C., Portanguen, S. & Debrauwer, L. (2010a). Formation of Heterocyclic Amines in slices of *Longissimus thoracis* beef muscle subjected to jets of superheated steam, *Food Chemistry*, 119(1), 19-26.

Kondjoyan, A., Chevolleau, S., Grève, E., Gatellier, P., Santé-Lhoutellier, V., Bruel, S., Touzet, C., Portanguen, S. & Debrauwer, L. (2010b). Modelling of the formation of heterocyclic amines in slices of *Longissimus thoracis* and *semimembranosus* beef muscles subjected to jets of hot air. *Food Chemistry*, 123(3), 659-668.

Kondjoyan, A., Oillic, S., Portanguen, S., & Gros, J. B. (2013). Combined heat transfer and kinetic models to predict cooking loss during heat treatment of beef meat. *Meat Science*, 95 (2), 336-344.

Laroche, M. (1978). Facteurs influencant les pertes d'eau pendant la cuisson de la viande. *Annales de technologie agricole*, 4, 849-871.

Lawrie, R.A. (1985). Eating quality of meat, *Meat Science*. 4th ed. Pergamon Press, Oxford. 169-173.

Lee, B.J., Hendricks, D.G. & Cornforth, D.P. (1998). Effect of sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on physicochemical characteristics of restructured beef. *Meat Science*, 50, 273-283.

Lepetit, J., Grajales, A. & Favier, R. (2000). Modelling the effect of sarcomere length on collagen thermal shortening in cooked meat: consequences on meat toughness. *Meat Science*, 54, 239-250.

Lepetit, J. (2007). A theoretical approach to the relationships between collagen content, collagen cross-links and meat tenderness. *Meat Science*, 76, 147-159.

Leskova, E., Kubikova, J., Kovacikova, A. Kosicka, M., Porubska, J. & Holcikova, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis*, 19, 252-276.

Lombardi-Boccia, G, Lanzi, S. & Aguzzi, A. (2005). Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *Journal of Food Composition and Analysis*, 18, 39-46.

734 Messner, C. & Murkovic, M. (2004). Evaluation of a new model system for studying the
735 formation of heterocyclic amines. *Journal of Chromatography B*, 802, 19–26.
736 Murkovic, M., & Pfannhauser, W. (2000). Analysis of the cancerogenic heterocyclic
737 aromatic amines in fried meat. *Fresenius Journal of Analytical Chemistry*, 366 (4), 375-
738 378.
739 Murkovic, M. (2004). Formation of heterocyclic aromatic amines in model systems. *Journal*
740 *of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*,
741 802(1), 3-10.
742 Oillic, S., Lemoine, E., Gros, J.B. & Kondjoyan, A. (2011). Kinetics analysis of cooking
743 losses from beef and other animal muscles heated in a water bath - Effect of sample
744 dimensions and prior freezing and ageing. *Meat Science*, 88 (3), 338-346.
745 Pais, P., Salmon, C.P., Knize, M.G. & Felton, J.S. (1999). Formation of
746 mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and
747 meat dripping. *Journal of Agricultural and Food Chemistry*, 47, 1098-1108.
748 Perez-juan, M., Kondjoyan, A., Picouet, P. & Realini, C. (2012). Effect of marination and
749 microwave heating on the quality of *Semimembranosus* and *Semitendinosus* muscles
750 from Friesian mature cows. *Meat science*, 92(2), 107-114.
751 Perisic, N., Afseth, N.K., Ofstad, R. & Kohler, A. (2011) Monitoring protein structural
752 changes and hydration in bovine meat tissue due to salt substitutes by Fourier
753 Transform Infrared (FTIR) microspectroscopy. *Journal of Agricultural and Food*
754 *Chemistry*,59, 52-61.
755 Perisic, N., Afseth, N.K., Ofstad, R., Hassani, S. & Kohler, A. (2013). Characterising protein,
756 salt and water interactions with combined vibrational spectroscopic technique. *Food*
757 *Chemistry*, 138, 679-686.
758 Persson, E., Graziani, G., Ferracane, R., Fogliano, V., & Skog, K. (2003). Influence of
759 antioxydants in virgin olive oil on the formation of heterocyclic amines in fried
760 beefburgers. *Food and Chemical Toxicology*, 41, 1587-1597.
761 Polak, T., Dosler, D., Zlender, B. & Gasperlin, L., (2009). Heterocyclic amines in aged and
762 thermally treated pork *Longissimus dorsi* muscle of normal and PSE quality. *LWT -*
763 *Food Science and Technology*, 42, 504-513.
764 Portanguen, S., Lebert, A. & Kondjoyan, A. (2009). Effect of animal diet and muscle type on
765 the evolution of the colour of cooked beef meat. *55th International Congress of Meat*
766 *Science and Technology*, Copenhagen, Denmark.

Randel, G., Balzer, M., Gruppe, S., Drusch, S., Kaina, B., Platt, K.L. & Schwarz, K. (2007). Degradation of heterocyclic aromatic amines in oil under storage and frying conditions and reduction of their mutagenic potential. *Food and Chemical Toxicology*, 45, 2245-2253.

Rhee, K.S, Smith, G.C. & Terrell, R.N. (1983). Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. *Journal of Food Production*, 46, 578-581.

Rohrmann, S. & Becker, N. (2001). Dietary intake of heterocyclic aromatic amines in Germany. *Ernahrungs-Umschau*, 48(11), 447-+.

Rohrmann, S. & Becker, N. (2002). Development of a short questionnaire to assess the dietary intake of heterocyclic aromatic amines. *Public Health Nutrition*, 5(5), 699-705.

Rohrmann, S., Zoller, D., Hermann, S. & Linseisen, J. (2007). Intake of heterocyclic aromatic amines from meat in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. *British Journal of Nutrition*, 98, 1112-1115.

Ryynanen, S. & Ohlsson, T. (1996). Microwave heating uniformity of ready meals as affected by placement, composition, and geometry. *Journal of Food Science*, 61(3), 620-624.

Sarraga, C., Carreras, I. & Garcia Regueiro, J.A. (2002). Influence of meat quality and NaCl percentage on glutathione peroxidase activity and values for acid-reactive substances of raw and dry-cured *Longissimus dorsi*. *Meat Science*, 62, 503-507.

Sinha,R., Knize,M.G. & Felton,J.S. (1997). Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. *Food and Chemical Toxicology*, 35, 433-441.

Sinha, R., Knize, M. G., Salmon, C.P., Brown, E.D., Rhodes, D., Felton, J.S., Levander, O.A. & Rothman, N. (1998). Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. *Food and Chemical Toxicology*, 36, 289-297.

Sinha, R. & Rothman, N. (1999). Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. *Cancer Letters*, 143, 189-194.

Skog, K.I., Johansson, M.A.E. & Jägerstad, M.I. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food and Chemical Toxicology*, 36, 879-896.

Skog, K.I. (2002). Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. *Food and Chemical Toxicology*, 40, 1197-1203.

Sprague, M. A., & Colvin, M. E. (2011). A mixture-enthalpy fixed-grid model for temperature evolution and heterocyclic-amine formation in a frying beef patty. *Food Research International,* 44 (3), 789-797.

Szterk, A, Roszko, M., Malek, K., Czerwonka, M, Waszkiewicz-Robak, B (2012a) Application of the SPE reversed phase HPLC/MS technique to determine vitamin B12 bio-active forms in beef. *Meat Science,* 91, *408-413.*

Szterk, A., Roszko, M., Malek, K., Kurek, M., Zbiec, M., & Waszkiewicz-Robak, B. (2012b). Profiles and concentrations of heterocyclic aromatic amines formed in beef during various heat treatments depend on the time of ripening and muscle type. *Meat Science*, 92 (4), 587-595.

Tang, X., Lyng, J., Cronin, D. & Durand, C. (2006). Radio frequency heating of beef rolls from *biceps femoris* muscle. *Meat Science*, 72, 467-474.

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. *Meat Science*, 70, 493-508.

Tran, N. L., Salmon, C. P., Knize, M. G., & Colvin, M. E. (2002). Experimental and simulation studies of heat flow and heterocyclic amine mutagen/carcinogen formation in pan-fried meat patties. *Food and Chemical Toxicology,* 40 (5), 673-684.

Van der Sman, R.G.M. (2007). Moisture transport during cooking of meat: An analysis based on Flory-Rehner theory. Meat Science, 76, 730-738.

Zamora, R., Alcón, E. & Hidalgo, F.J. (2012). Effect of lipid oxidation products on the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in model systems. *Food Chemistry,* 135(4), 2569-2574.

Figures legends

Figure 1: Variation of water content in the meat at equilibrium (longest cooking times), X_{eq}, at different water-bath temperatures. Black squares signify the value measured on ST muscle by Goni and Salvadori (2010). Other symbols represent the measurements reported by Oillic, Lemoine, Gros & Kondjoyan (2011) on IS, LT, MA, SM and ST muscles. The dashed line is the sigmoid function used by Goni and Salvadori (2010) to fit their experimental results.

Figure 2: Kinetics of L^*/ L^*_0 measured on beef samples subjected to superheated steam jets at different temperatures. A kinetics model based on two successive first order chemical reactions was developed. Colour measurement: CIELAB system (D65-10°-L*a*b*-d/8 SCE).

Figure 3: Cooking losses predicted by the combined heat transfer to kinetics model on SM meat cubes heated in the water bath (full lines) compared against measured losses (symbols □, Δ, ◊). The side length of the cube is (a) 10mm, (b) 30mm, (c) 50mm, and (d) 70mm, respectively. Symbols correspond to the different water bath temperatures.

Figure 4: Proton density versus average temperature (°C) (a). The proton density in the muscle decreases considerably with temperature, it is in part due to Curie's law and can also be explained by contraction that expels intramuscular water outside the muscle. Cumulative deformation (mm) *versus* temperature (°C) (b). Before 38°C, cumulative deformation is very slight and corresponds to the accumulation of the image registration error. The moderate strain between ~ 38 and ~ 54°C corresponds to myosin denaturing and the beginning of collagen denaturing. The acceleration of deformation after 60°C is due to the temperature effect on sarcoplasmic proteins and to the collagen inducing contraction of the connective network. Plateaus of deformations that occur from ~ 68°C can be explained by the end of one or several of these phenomena.

Figure 5: Evolution of the concentration of the B6 vitamin in meat samples. Squares are the values measured at 60°C, diamonds are those measured at 90°C; full lines are the values calculated by the model. Dotted lines represent the calculated quantities of the vitamin B6 expulsed in the juice during cooking at 60 and 90°C respectively.

Tables legends

Table 1: Effect of animal age, microwave power and temperature on maximum temperature (least squares means ± SE, ºC) for *Semitendinosus* beef roasts.

Table 2: Effect of animal age, microwave power and temperature on color lightness (L*), redness (a*) and yellowness (b*) for *Semimembranosus* beef roasts. Least squares means ± SE.

Table 3: The content of the studied aminoazarens in 3mm slice of beef meat (ng/g of lyophylized sample). The same letters by values indicate no statistically significant differences at the level of p=0.05. Data were analysed by Anova, range test at a significance level of P=0.05 (Statistica 8.0, StatSoft Inc., Tulsa, USA).

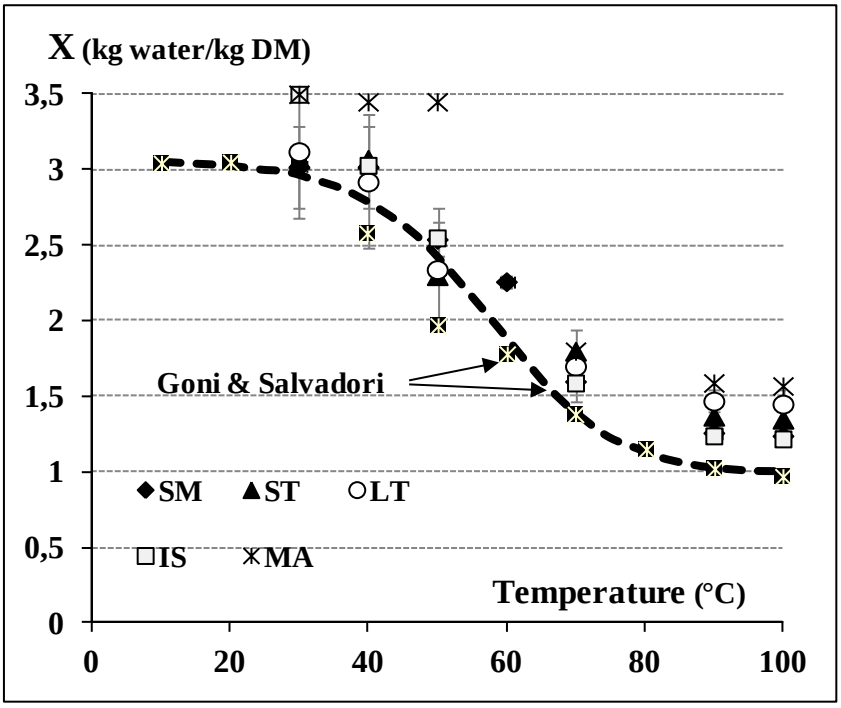


Figure 1

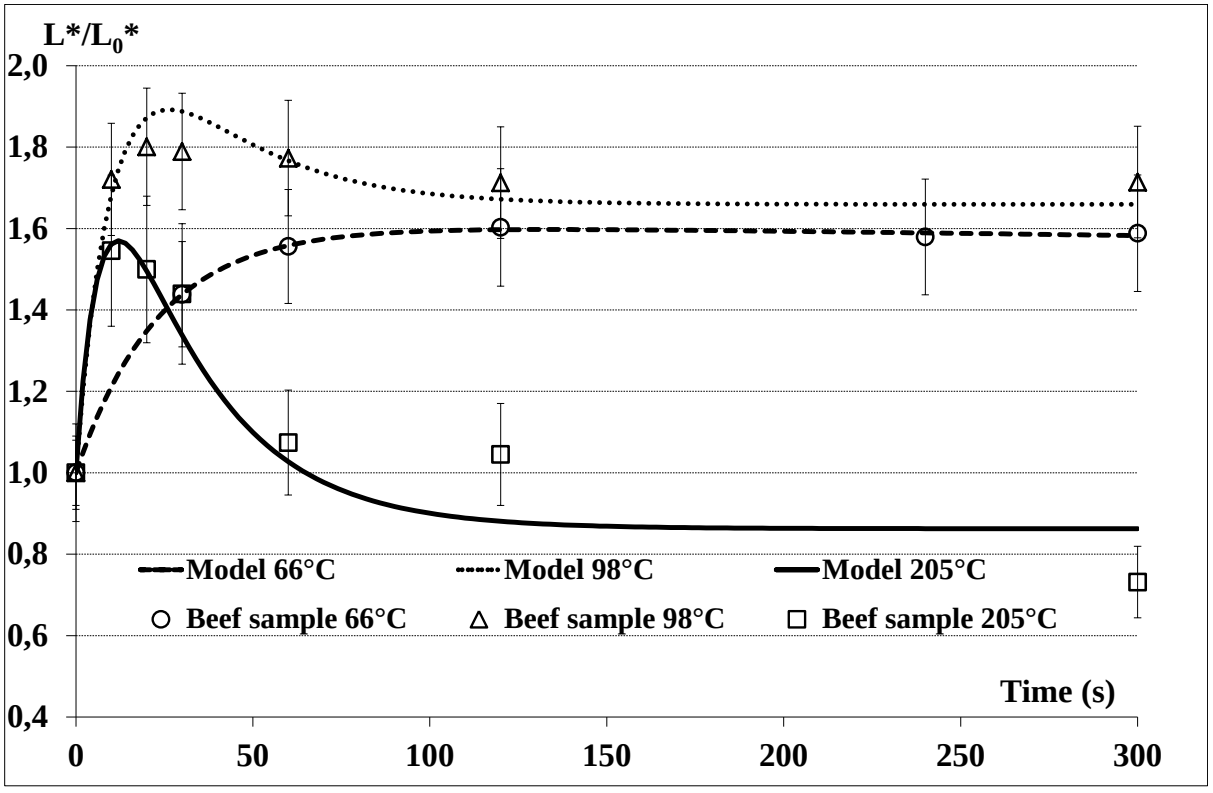


Figure 2

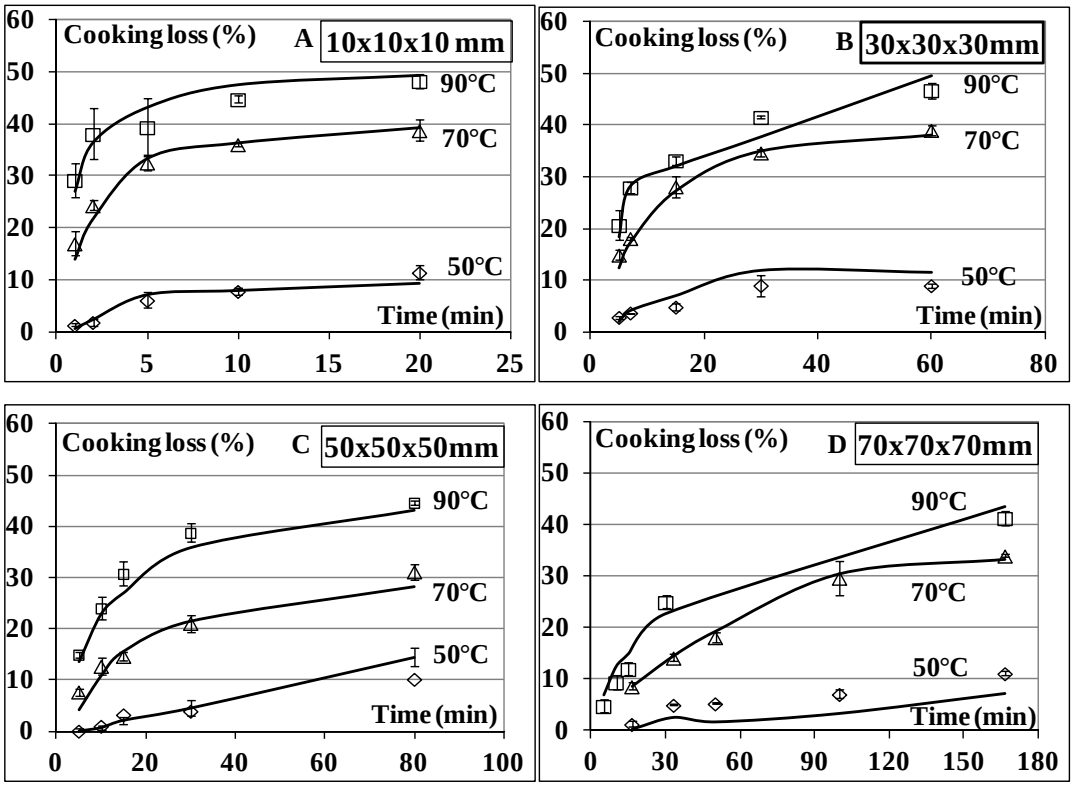

Cooking loss (%)
A 10x10x10 mm
90°C
70°C
50°C
Time (min)
0 5 10 15 20 25
Cooking loss (%)
B 30x30x30mm
90°C
70°C
50°C
Time (min)
0 20 40 60 80
Cooking loss (%)
C 50x50x50mm
90°C
70°C
50°C
Time (min)
0 20 40 60 80 100
Cooking loss (%)
D 70x70x70mm
90°C
70°C
50°C
Time (min)
0 30 60 90 120 150 180

Figure 3

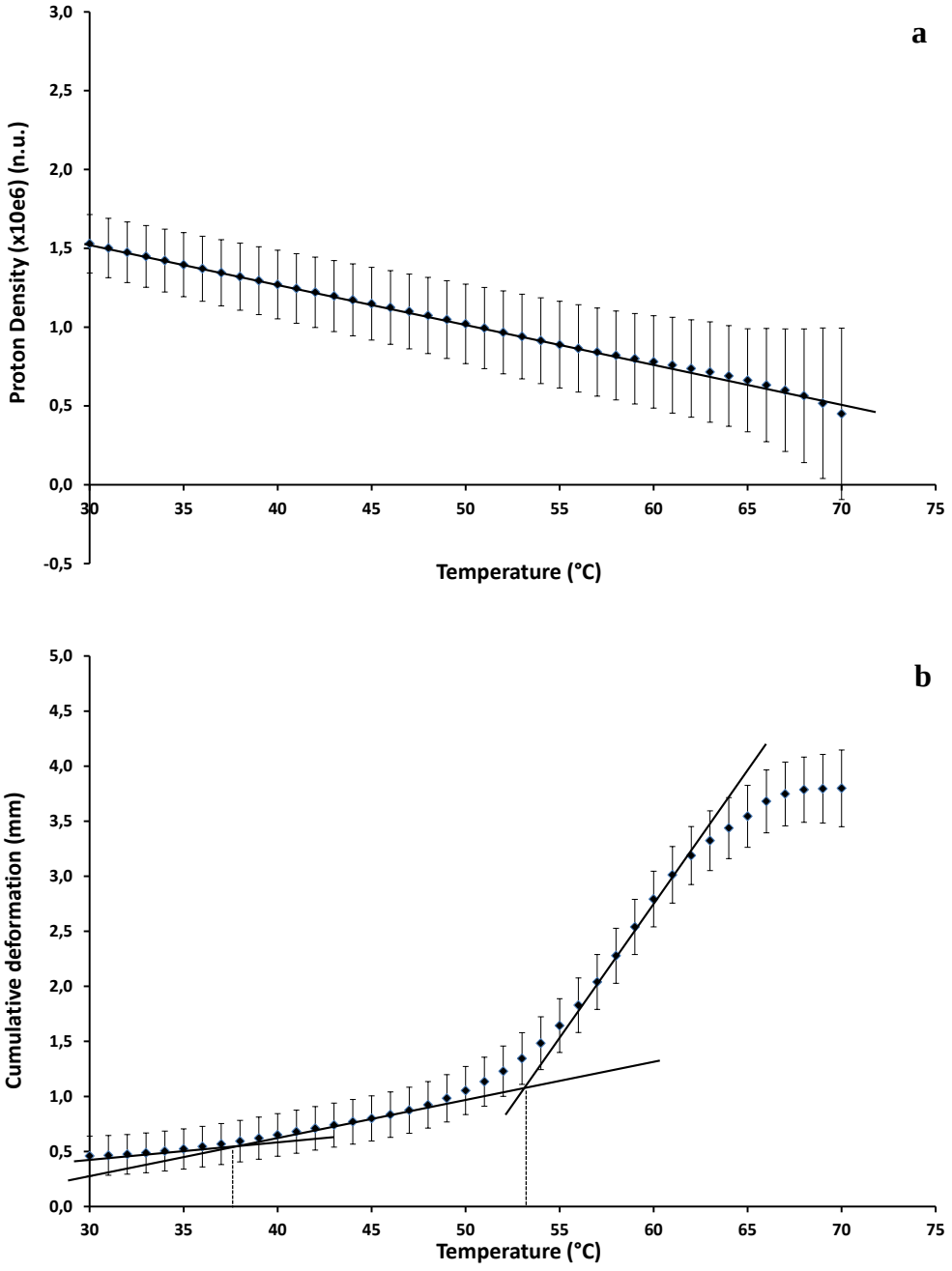
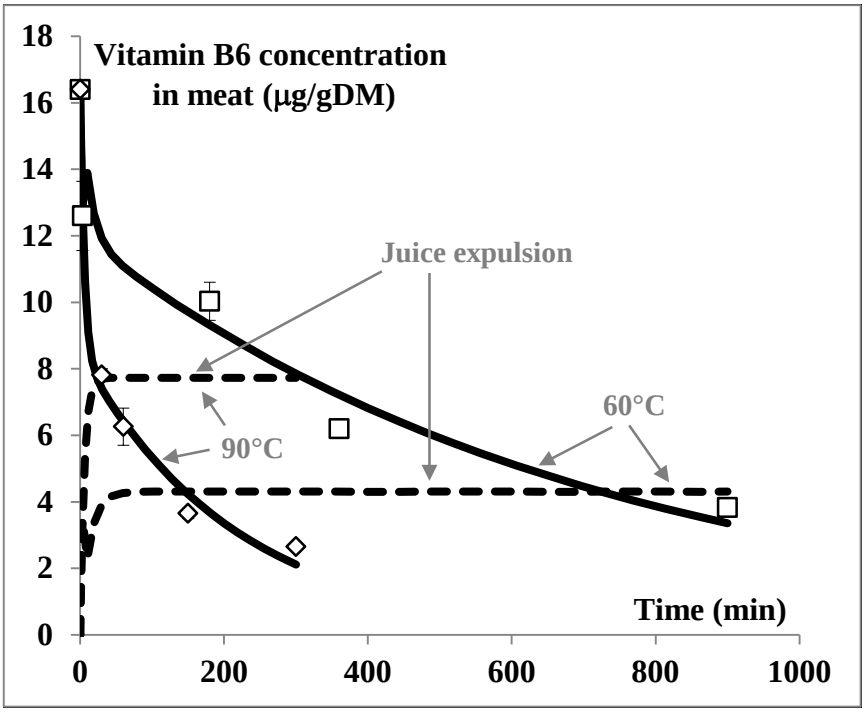

a
Proton Density (x10e6) (n.u.)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
30
35
40
45
50
55
60
65
70
75
Temperature (°C)
b
Cumulative deformation (mm)
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
30
35
40
45
50
55
60
65
70
75
Temperature (°C)

Figure 4

928

929



930

931 **Figure 5**

932

933

934

935

936

937

938

939

940

		Maximum temperature (°C)
Age	Heifers	76.9[b]±1.3
	Cows	82.1[a]±1.3
Power (W)	Temp (°C)	
182	60	71.8[c]±1.9
182	80	84.8[a]±1.9
654	60	77.7[b]±1.9
654	80	83.6[a]±1.8

[a,b,c] Within a column, main effect, and interaction effect, least squares means with different letters differ (P<0.05). Power*Temperature P<0.05.

Table 1

		L*	a*	b*
Age	Heifers	55.29[a]±0.40	7.71[b]±0.27	12.62[a]±0.19
	Cows	46.20[b]±0.40	10.18[a]±0.27	10.98[b]±0.19
Temp (°C)	60	49.70[b]±0.37	10.22[a]±0.25	11.98±0.18
	80	52.09[a]±0.37	7.66[b]±0.25	11.62±0.18

[a,b] Within a column, main effect, least squares means with different letters differ (P<0.05).

Table 2

Variant of steaks	IQ	MeIQ	DIMeIQx	PhIP
Control sample (non-marinated steaks)	0,020±0,005[b]	0,011±0,005$_b$	1,785±0,189[b]	124,819±19,646[c]
STPP 6%, NaCl 6%	0,001±0,001[a]	0,023±0,001[b]	0,086±0,020[a]	22,367±1,111[a]
Rosemary extract 0,35%, NaCl 6%	0,039±0,013[c]	0,006±0,001[a]	2,183±0,143[c]	80,406±13,632[b]

Table 3