# A KINETIC MODEL TO SIMULATE THE EFFECT OF COOKING TIME-TEMPERATURE ON THE GASTRIC DIGESTION OF MEAT

Kondjoyan, A., Daudin J.D., Portanguen, S., Aubry, L., and Santé-Lhoutellier, V.

UR370 Qualité des Produits Animaux, INRA, 63122 Saint-Genès-Champanelle, France

Abstract - A kinetic model was developed to predict the effect of cooking time and temperature on the digestibility of myofibrillar proteins. The predictions were confronted to the measurement of the *in vitro* digestibility of myofibrillar proteins coming from either slices of beef meat heated in water bath or from a piece of meat roasted in a domestic oven. The model was able to simulate the *in vitro* measurements for the meat pieces of different sizes cooked under different conditions. Simulation calculations can help to investigate the relative effects of cooking and residence time in the stomach.

Keywords— Model, Cooking, Beef, Digestibility.

# I. INTRODUCTION

Animal proteins are part of the daily diet in most countries. Beef is also sought by many consumers for its sensorial quality. Meat is a major source of proteins that provide all the essential amino acids. The digestion rate of proteins is important for amino acids assimilation. This is a key problem especially for elderly people, who are prone to sarcopenia [1]. Moreover, undigested proteins entering the colon are suspected of favoring carcinogenesis [2]. Gastric digestion by pepsin, which takes place before the trypsin/chymotrypsin reactions, affects protein digestibility. The incidence of various process parameters on the in vitro gastric digestibility of myofibrillar proteins has been studied in a previous study [3]. Results have proved that aging and mincing have a little effect on the digestibility of proteins, whereas digestibility was affected by salting, and above all by heating temperatures [3, 4]. Present work deals with the development of a kinetic model to predict the effect of cooking time and temperature on the digestibility of myofibrillar proteins. The predictions have been confronted to the measurement of the *in vitro* digestibility of the myofibrillar proteins coming either from slices of beef meat heated in water bath or from a piece of meat roasted in a domestic oven.

# **II. MATERIALS AND METHODS**

# A. Meat samples and heat treatments

Two sets of experiments were performed; the first one on slices and the second one on a piece of meat cooked under domestic conditions. All the muscles were excised from two years old Charolais cows, vacuum packaged, aged 14 days at 4°C and cut in large pieces which were frozen at -18°C until use.

During the first set of experiments 2 mm thick slices of meat (*Infraspinatus* or *Semitendinosus* muscles from one animal), were immersed in a bioreactor to fix the initial pH and NaCl content of the meat. Then, they were vacuum-packed, heated in water bath at 60, 70 or 90 °C, during a given time, and cooled rapidly in melted-ice. Then, samples were frozen and stored at -80 °C until proteolysis measurement. Each condition was repeated 4 times. The experimental conditions used in this paper as references come from the *Infraspinatus* muscle, salted at 0.7% and marinated at pH 5.5. This corresponds to marinating in a solution with the same ionic strength and same pH as aged beef meat.

The second set of experiments was performed using  $110 \ge 60 \ge 60$  mm parallelepiped pieces of beef meat cut from frozen *Semimembranosus* muscles. Holes were drilled in the frozen sample to introduce 2

thermocouples along the longest symmetry axis: one at core and one 5 mm below the surface. The sample was then thawed in a bag by immersion in water bath at 6 °C. When the two thermocouples indicated 6 °C the sample was removed from the bag and introduced into a fan equipped air oven which was pre-heated at 250 °C. The roast was removed from the oven when the core temperature reached 60 °C. It was immersed in melted-ice until the core temperature be less than 40 °C. Then the thermocouples were removed and the crust at the surface was eliminated. Two samples of 10 g of meat were taken for proteolysis measurements: one at the surface of the roast and one at core. These samples were stored at -80 °C until proteolysis measurement. The experiment was repeated three times.

# B. In vitro digestibility

The *in vitro* digestibility was measured on the different replicates for each treatment condition using the protocol described by Bax et al. [4]. The myofibrillar proteins were extracted from the samples: grinding and a series of washing steps with salts solution and phosphate buffer at pH 6. Proteins were digested by porcine gastric pepsin (10 U/ mg proteins) in conditions of pH (glycine buffer, 1.8) and temperature (37 °C) that simulated stomach digestion during 4 h. Digestion was stopped by addition of 15 % trichloroacetic acid at times indicated in figure 1. After centrifugation (10 min, 4000 g) the amount of hydrolysed peptides (PM< 15 kDa) in the soluble fraction was measured by absorbance (OD) at 280 nm.

### **II. MODELS DEVELOPMENT**

Pepsin is an endoprotease of the stomach, which cleaves peptide bonds after an aromatic amino acid (Trp, Phe or Tyr). Our primary model is based on the accessibility of the enzyme to these cleavage sites. Under different assumptions it can be demonstrated that the formation of the hydrolysed product (P) follows a first-order reaction and that the variation of the absorbance (OD) with the digestion time  $t_{OD}$  is:

$$OD \approx OD_{max} \left( 1 - \exp(k_{f} t_{OD}) \right)$$
 (1)

 $k_f$  is the rate constant of the reaction and  $OD_{max}$  the maximum absorbance measured when all the proteins have been digested with:

$$OD_{max} = \delta_{max} \frac{E_{T}^{pH}}{E_{T}^{pH} + K}$$
(2)

 $E_T^{pH}$  is the active form of pepsin at a given pH. K and  $\delta_{max}$  are unknown parameters which shall be determined from the results.

A secondary model is required to take into account the effect of the heat denaturation of meat protein on the digestion kinetics. It assumes that protein unfolding and aggregation modify the number of sites available for pepsin reaction. The irreversible thermal denaturing of myofibrillar proteins has been followed in the literature by measuring their surface hydrophobicity [5], and modeled using a first-order relationship of the type [6]:

$$X^{\text{th}} = \left(X^0 - X^{\text{end}}\right) \exp\left(-\alpha t_{\text{h}}\right) + X^{\text{end}} \qquad (3)$$

where  $X_0$  is the initial hydrophobicity, and  $X_{end}$  is the stable value obtained after a long heat treatment time,  $t_h$ . The time scale of heat denaturing is characterized by  $\alpha$ .

# **III. RESULTS**

# A. Determination of model parameters

Figure 1 presents the variation of  $OD_{max}$  with heating time for two heating temperatures, 60 and 90 °C. The  $OD_{max}$  value increased during the first 5–10 minutes of heating, and remained constant thereafter. This increase was faster and larger for the higher temperature. The change in k<sub>f</sub> due to the heating time and temperature is shown in Figure 2. It decreased during the first 5–10 min of heating, and stabilized thereafter. It can be concluded that the increase in heating temperature promotes an increase in the value of  $OD_{max}$ . but parallel, due to protein denaturing, a decrease in the value of  $k_f$ .

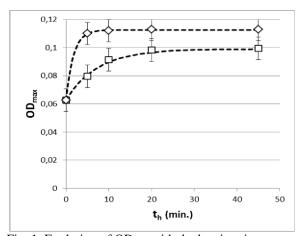


Fig. 1. Evolution of OD<sub>max</sub> with the heating time, t<sub>h</sub>, measured on samples subjected to heating temperatures:
60 °C, squares, and 90 °C, diamonds. Dotted lines represent the results calculated with the relation (3)

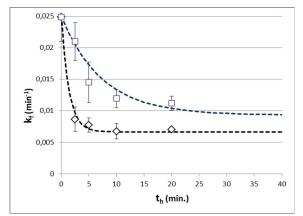


Fig. 2. Evolution of the constant rate of the reaction  $k_f$ , with the heating time,  $t_h$ . Symbols are the values determined from the measurements at two heating temperatures: 60 °C, squares, and 90 °C, diamonds. Dotted lines represent the results calculated from the relation (3)

### B. Effect of temperature on the digestion of meat slices

Temperature in the meat slice reached the water bath temperature after about 45 s of heating. The variation in digestibility due to an increase in the cooking

temperature was subjected to two opposing effects: (i) an increase in  $OD_{max}$  and (ii) a decrease in  $k_f$ . During our *in vitro* digestion tests the highest digestibility value was obtained at the end of the experiment which lasted 4 hours and under the highest heating time-temperatures (70-90 °C). However, simulation calculations, based on equation 1 with the previously estimated parameters  $OD_{max}$  and  $k_f$ , showed that digestibility depends on the digestion-time. For example a decrease in the digestion time from 4 to 2 hours led to a raw meat being more digestible than the meat heated at 70 °C (Fig. 3).

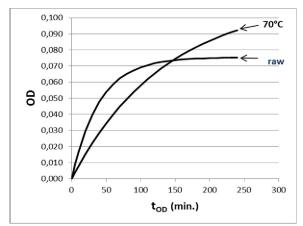


Fig. 3. Comparison of the simulated *in vitro* digestibility of myofibrillar proteins issued from raw meat with those coming from meat slices heated at 70 °C during 40 min

### C. Digestibility of roasted meat

Strong gradients of temperature were observed within the pieces of meat that were roasted in the oven (Fig. 4). Temperature 5 mm under the surface of the meat rose to reach the boiling temperature during the heating period and decreased rapidly during the ice cooling period. On the contrary the temperature at the center of the meat went on increasing for a while during the cooling period and then decreased slowly. Similar temperature measurements have been observed during the cooking of meat roasts [7]. In this case the effect of heating on digestibility should be different at the core and at the surface of the meat piece.

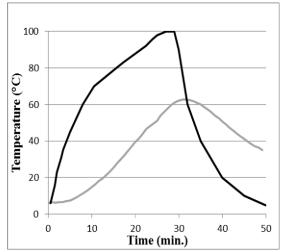


Fig. 4. Temperatures measured during oven cooking; black line at 5 mm from the surface, grey line at the centre of the parallelepiped

Digestibility values predicted by our calculations using the parameters values that corresponded to the temperatures in figure 4 are compared to the OD measured on the samples taken at the same locations in Figure 5. In this case the digestibility at the core of the roast is higher than at the surface due to the decrease in  $k_f$  with temperature which predominates on the increase in  $OD_{max}$  with temperature. Predictions issued from the model agree with the *in vitro* measurements.

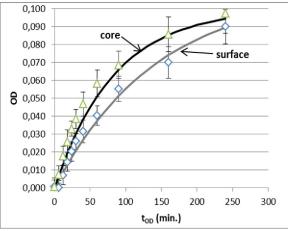


Fig. 5. Comparison of the *in vitro* digestibility of myofibrillar proteins at two locations of a piece of meat when roasted in the oven. Points are the digestibility measurements and lines are the values predicted by the model

# **IV. CONCLUSION**

Mathematical model was developed to predict the *in vitro* digestibility of myofibrillar proteins by pepsin. The model is able to simulate the *in vitro* results for meat pieces of different sizes cooked under different conditions. The effect of heating on digestibility depends on the digestion-time which *in vivo* can be linked to the residence time in the stomach. Model equations can also takes into account the variation of pepsin activity with pH and the effect of enzyme concentration on digestibility (not detailed here). This can help to analyse *in vivo* digestion experiments where pepsin concentration and pH in the stomach are sources of variability.

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# REFERENCES

- Dangin, M., Guillet, C., Garcia-Rodenas, C., Gachon, P., Bouteloup, C., Reiffers-Magnani, K., Fauquant, J., Ballèvre, O. & Beaufrère, B. (2003). The rate of protein digestion affects protein gain differently during aging in humans. Journal of Physiology 549: 635-44.
- Le Marchand, L., Donlon, T., Seifried, A. & Wilkens, L. R. (2002). Red Meat Intake, CYP2E1 Genetic Polymorphisms, and Colorectal Cancer Risk. Cancer Epidemiology, Biomarkers & Prevention 11: 1019-24.
- Hassoun, A., Santé-Lhoutellier, V., Lebert, A., Kondjoyan, A. & Daudin, J. D. (2011). Incidence of various process parameters on *in vitro* protein digestibility of beef meat. In proceedings 57<sup>th</sup> ICoMST, p140-141, 7-12 August 2011, Ghent, Belgium.
- 4.Bax, M. L., Aubry, L., Ferreira, C., Daudin, J. D., Gatellier, P., Rémond, D. & Santé-Lhoutellier, V. (2012). Cooking conditions affect *in vitro* meat protein digestion. Journal of Agricultural and Food Chemistry 60: 2569-2576.
- 5. Chelh, I., Gatellier, P. & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. Meat Science 74(4): 681-683.
- 6. Promeyrat, A., Daudin, J. D. & Gatellier, P. (2013). Kinetics of protein physicochemical changes induced by heating in meat using mimetic models: (1) relative effects of heat and oxidants. Food Chemistry 138(1): 581-589.
- 7 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: 336–344.