Incidence of various process parameters on in vitro protein digestibility of beef meat

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Incidence of various process parameters on *in vitro* protein digestion of beef meat

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Abstract—**Protein *in vitro* digestion was characterized by pepsin proteolysis of myofibrillar proteins extracted from processed beef samples using 2 descriptors of the kinetics: maximum value (ODmax) and half life time ($t_{1/2}$). An experimental fractional factorial design with 32 trials was used to investigate the effect of processes variables; it consists of 5 factors each taking 2 levels (muscle type, mincing, pH, NaCl content, cooking time) and 1 factor taking 4 levels (cooking temperature). The statistical analysis showed that (i) cooking temperature and time have a major effect, (ii) there is interaction between muscle type and NaCl content and (iii) mincing and pH have little effect. The evolutions of the 2 descriptors with cooking time were then analyzed with the 2 muscles at 2% NaCl content, 60 and 90°C. They show that the main changes occur in the first 10 minutes.**

Keywords— **Process, Beef, in vitro digestion.**

I. INTRODUCTION

There is an increasing consumer demand for processed beef meat: minced, marinated, pre-cooked… During processing biochemical and structural protein changes can decrease their digestion rate [1, 2, 3] which has been shown to be the main determinant in dietary protein assimilation [4, 5], especially for elderly people (sarcopenia). Moreover the amount of undigested protein entering into the colon is suspected to favour carcinogenesis [6]. So far if many studies focused on the effect of processing conditions on safety and sensory qualities of meat products little is known about their impact on nutritional quality.

Our aim was to assess the impact of the main processing variables that can affect protein digestion of beef meat.

II. MATERIALS AND METHODS

A. Meat samples and treatments

To get rid of biological variability and focus on processes effects all the experiments were performed on the muscles of one animal. They differed by their metabolic type and by their content of connective tissue (C): *Infraspinatus* (oxidative, C high) and *Semitendinosus* (glycolytic, C low).

The muscles were excised from a two years old Charolais heifer, vacuum packaged, aged 14 days at 4°C and cut in large pieces which were frozen at -18°C. Before treatments, one piece was thawed up to -2°C and either cut into thin slices (2 mm in thickness) or minced (2 mm diameter grid). In the latter case slabs of the same thickness than the slices were fabricated and placed on a support.

Two successive treatments were applied:

- Immersion (bioreactor Labfors, 3L) of 6 slices or slabs was performed during 20 h at 10°C so that the targeted meat pH and NaCl content were achieved (table 1). These values were checked (pH, Mettler InLab427- NaCl, Sherwood chloride analyser 926) on small samples (table2).
- Cooking: the samples were individually vacuum-packed. The bags were plunged into an agitated water bath to achieve the targeted (t,T) cooking condition (table 1) and cooled using an ice-water bath. Due to the low thickness of the samples heating and cooling times (less than 45 seconds) were negligible in comparison with cooking times.

Then the samples were frozen and stored at -80°C until proteolysis measurement.

B. Experimental design

As indicated in table 1, the experimental design consisted of 5 factors each taking 2 levels (muscle type, mincing, pH, NaCl content, cooking time) and 1 factor taking 4 levels (cooking temperature). To reduce the number of experiments a fractional factorial design with 32 trials instead of 128 was selected; each condition was repeated 4 times. All interactions at the order equal or higher than 3 were neglected. The simple effect of all the factors and most of the simple interactions between 2 factors were only aliased with interactions at the order equal or higher than 3. The statistical analysis was performed with the R software [7]

Table 1: List of the experimental design factors, abbreviations and levels.

Factor	Levels
Mincing (Mi)	Minced / Sliced
NaCl(Na)	0.7 / 2.0 % w :w
Muscle (M)	*Infraspinatus/ Semitendinosus*
pH (pH)	4.5 / 5.5
Time (t)	5 / 45 min
Cooking temperature (Tc)	60 / 70 / 80 / 90 °C

Additional experiments were carried out with the two muscles, pH equal to 5.5, salt content equal to 2 % and at the two extreme heating temperatures (60 and 90°C) to investigate the rate of change of the *in vitro* digestibility parameters with cooking time.

C. In vitro digestion

The *in vitro* pepsine digestion was measured on 4 samples for each treatment using the protocol described by Santé-Lhoutellier *et al.* [7, 8]:

- 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 IU/ mg protein) in conditions of pH (glycine buffer, 1.8) and temperature (37°C) that simulated stomach digestion during 4 h. Digestion was terminated by addition of 15 % trichloroacetic acid and cooling at times indicated in figure 1.
- After centrifugation (10 min, 4000 g) the amount of hydrolysed peptides (PM< 15 kDa) in the supernatant was measured by absorbance (OD) at 280 nm.

III. RESULTS

Table 2 shows that 4 distinct groups of samples were actually obtained after immersion.

Table2: Measured values after immersion.

	Target	Mean	S.D.
pH	4.5	4.63	0.19
	5.5	5.45	0.11
NaCl % w/w	0.7	0.76	0.03
	2.0	2.0	0.07

A. Analysis of proteolysis kinetics

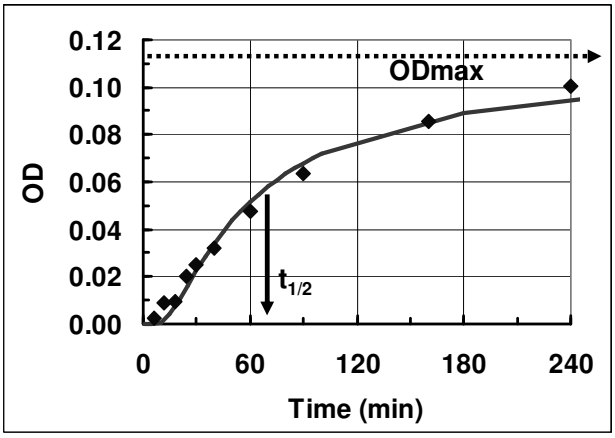

Figure 1: Fitting of a measured proteolysis kinetic (points) using equation 1 (curve).

$$OD = ODmax \cdot \exp(-B/t) \quad (1)$$

Where $B = Ln(2)\ t_{1/2}$

The increase in OD measured at 10 times during pepsin proteolysis was well fitted by equation 1. After a short lag time (10 to 30 min) the maximum proteolysis rate was reached and then decreased continuously with time. The shape of the curve is characterized by 2 parameters:

- ODmax corresponds to the maximal amount of hydrolysed peptides that would be obtained after an infinite time. It is equal to 0.113 in figure 1.
- $t_{1/2}$ is the time needed to reach half of OD max; mathematically, it gives the shape to the curve and is similar to the reverse of a global rate of proteolysis. It is equal to 71 min in figure 1.

The average values of the standard deviations for 4 repetitions were 0.011 and 4 min for ODmax (arbitrary unit) and $t_{1/2}$, respectively.

For all the treatments tested the values of both $t_{1/2}$ and ODmax were higher than those measured on the raw meat. This means that they induced a higher digestibility potential but an extended digestion time, especially cooking.

B. Statistical analysis

The statistical analysis was performed using the mean values of the 4 repetitions.

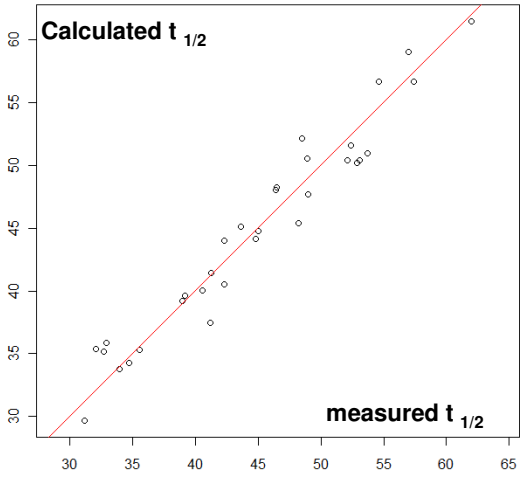

Figure 2: Prediction of $t_{1/2}$ using the linear regression for all the conditions of the experimental design.

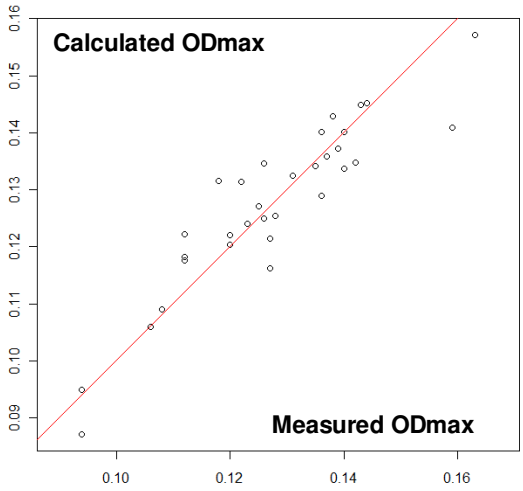

Figure 3: Prediction of ODmax using the linear regression for all the conditions of the experimental design.

Multiple linear regressions using all the factors and interactions between two factors were calculated: $t_{1/2}$ and ODmax were related to these variables with correlation coefficients equal to 0.95 and 0.87, respectively. Then forward stepwise multiple linear regressions were tested to decrease the number of terms in the equations while keeping the correlation coefficient values at a high level; 0.95 and 0.84 for $t_{1/2}$ and ODmax, respectively. Figures 2 and 3 show that the calculations well represent the data; in addition, the differences between the calculated and the measured values are homogeneously distributed over the studied range.

From the correlation coefficients between both $t_{1/2}$ and ODmax and the factors, on the one hand, and from the level of statistical significance related to each parameter of the final regression equations, on the other hand, it was concluded that:

- Cooking temperature has the major impact. Its effect is not linear and differs for $t_{1/2}$ and ODmax. $t_{1/2}$ increases slightly from 60 to 70°C and strongly between 80 and 90°C. ODmax increases from 60 to 80°C and is almost constant above 80°C.
- There is an interaction between muscle type and NaCl content but no definitive conclusion could be drawn since $t_{1/2}$ and ODmax changes were of the order of magnitude of the standard errors of the repetitions.
- The effects of either decreasing pH by acidic marinating or grinding prior to cooking can be neglected in comparison to the other factors.
- The effect of cooking time varies with the other factors and needs further investigation.

C. Kinetics of $t_{1/2}$ and ODmax

Figure 4 and 5 present the changes of $t_{1/2}$ and ODmax with the cooking time, respectively.

For both muscles the increase in $t_{1/2}$ is sharp during the first minutes of heating and eases for longer times; it seems that cooking times longer than 45 min may lead to further increase. This suggests that the longer the cooking the lower the digestion rate. The increase in cooking temperature from 60 to 90°C has a greater effect on the *Infraspinatus* which has mainly oxidative fibres than on the *Semitendinosus* which has mostly glycolytic fibres.

ODmax increases slightly (20 %) and similarly for the 2 muscles. Due to the great standard deviation on the measurements it was not possible to highlight a difference between the 2 cooking temperatures.

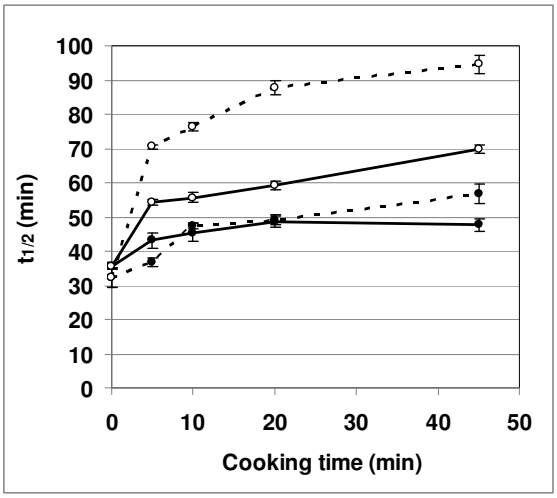

Figure 4: Evolution of $t_{1/2}$ with cooking time (mean ± standard error on mean) at 60°C (black points) and 90°C (white points) for *Infraspinatus* (dotted line) and *Semitendinosus* (full line).

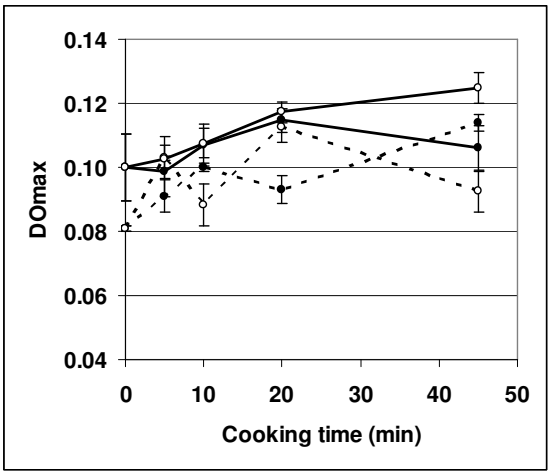

Figure 5: Evolution of ODmax (mean ± standard error on mean) with cooking time at 60°C (black points) and 90°C (white points) for *Infraspinatus* (dotted line) and *Semitendinosus* (full line).

The fact that both $t_{1/2}$ and ODmax vary mainly during the first minutes of cooking coincided with the results of Oillic et al. [9] on meat cooking loss kinetics in the same temperature range: they were also very steep at the beginning of cooking due to protein denaturation. This phenomenon probably modifies pepsin accessibility to myofibrillar proteins.

IV. CONCLUSIONS

Processes have an effect on pepsin proteolysis. The major impact is the (t,T) couple applied during cooking. Grinding and acidic marinating have negligible incidence. Complementary trials at 70 and 80°C and at 0.7 and 1.4 % NaCl contents are in progress to clarify the respective role of muscle type and NaCl content in interaction with cooking temperature.

While the myofibrillar protein digestibility potential increases slightly with cooking the mean pepsin digestion rate decreases markedly. *In vitro* trials with pancreatic proteases and *in vivo* tests are needed to derive definitive conclusions.

ACKNOWLEDGMENT

This work was supported by a grant awarded as part of the PRONUTRIAL project under the French ANR-ALIA program (ANR-09-ALIA-008-02). The authors gratefully acknowledge S. Portanguen and L. Aubry for their technical assistance.

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