Food Chemistry 172 (2015) 265–271

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Modelling of pepsin digestibility of myofibrillar proteins and of variations due to heating

Alain Kondjoyan *, Jean-Dominique Daudin, Véronique Santé-Lhoutellier

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

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 9 July 2014
Accepted 26 August 2014
Available online 6 September 2014

Keywords:
Model
Digestion
Enzyme
Kinetics
Gastric
Heating

ABSTRACT

Digestibility of myofibrillar proteins by pepsin was determined by *in vitro* trials and mathematical modelling. A primary model was developed to predict *in vitro* digestion kinetics, and a secondary model based on the mechanisms of protein denaturation was then added to take into account the effect of meat heating. Model predictions agreed with measurements in the pH and pepsin concentration ranges 1.8–3.8 and 6–50 U mg^{-1} respectively. The utility of the model is illustrated by a simple example where meat is assumed to be heated homogeneously, and myofibrillar proteins to be directly in contact with pepsin. The combined effects of heating time, temperature, enzyme concentration and pH modified the digestibility value, which also depends on residence time in the stomach.

1. Introduction

Meat is a major source of proteins that provides all the essential amino acids. However, nowadays meat is more often processed to meet an increasing demand for minced, marinated or precooked foods. The digestion rate of proteins is known to be the main determinant in their assimilation in the diet, especially for elderly people, who are prone to sarcopenia (Dangin et al., 2003; Mosoni & Patureau-Mirand, 2003). Moreover, undigested proteins entering the colon are suspected of favouring carcinogenesis (Le Marchand, Donlon, Seifried, & Wilkens, 2002). Gastric digestion by pepsin, which takes place before the trypsin/chymotrypsin reactions, affects protein digestibility. Gastric digestion is a complex process in the course of which pH and pepsin concentrations vary during stomach residence. The residence time of a piece of solid food in the stomach ranges from 30 min to 4 h, depending on the composition of the meal, the size of the piece and physiological parameters. The pH in the stomach is approximately 2, but can vary from 1.5 to 4 depending on the time since eating, and on the concentration of the different types of foods in the meal. The concentration of pepsin in the stomach is not known, and probably varies widely from one consumer to another.

We set out to model the gastric digestion of processed beef, emphasising the effect of technological treatments, because meat composition only slightly affects digestion (Bax et al., 2013). We focused on the cooking of salted or non-salted meat, because aging and mincing have proved to have a negligible effect on the digestion rate of proteins, whereas this rate is affected by salting, and above all by heating temperatures (Bax et al., 2012; Hassoun, Sante-Lhoutellier, Lebert, Kondjoyan, & Daudin, 2011). This paper deals with the digestion of the extracted myofibrillar proteins, which contribute to 60% (Maruyama, 1985) of the beef meat protein content. The modelling of gastric digestion was assessed against *in vitro* experiments in which the pH and concentration of pepsin were controlled, in contrast to *in vivo* experiments Bax et al. (2013).

Several types of models are described in the literature that predict the rate of product formation from the initial concentrations of enzyme and substrate. Of these, the Michaelis–Menten model is the best known. More complex models can account for inhibitor effects, the presence of various substrates, affinity of an enzyme to substrate binding sites, and cooperative or allosteric phenomena (Van Boekel, 2009). These models are not best-suited to describing meat protein digestion, where proteins are thermally denatured and oxidised to various degrees from reactions with pepsin. In this case it is mainly the change in the number of sites available for the enzyme to cleave the protein that varies with time–temperature heating conditions, and so controls the kinetics of digestion. We developed a primary mathematical model linked to the number of available sites to predict the kinetics of *in vitro* digestion of myofibrillar proteins by pepsin. A secondary model was then added to take into account the effect of meat heating on this digestion, and then the model predictions were compared with measurements.

* Corresponding author. Tel.: +33 (0)473624492; fax: +33 (0)473624089.
E-mail address: alain.kondjoyan@clermont.inra.fr (A. Kondjoyan).

http://dx.doi.org/10.1016/j.foodchem.2014.08.110
0308-8146/

List of symbols used

A_s	surface coverage parameter
E	enzyme concentration
δ_{max}	product of E^*_{max} and the calibration constant of the spectrophotometer
E_T^{pH}	total active enzyme concentration, which depends on pH
E^*	concentration of enzyme that reaches the cleavage sites of the proteins
E_{eq}	concentration of enzyme at equilibrium
E^*_{eq}	concentration of enzyme at equilibrium at the cleavage sites of the proteins
E^*_{max}	maximum value of E^* (linked to the maximum number of cleavage sites available)
ES	concentration of the enzyme-substrate complex
IS	*Infraspinatus* muscle
K	pseudo rate constant of reaction, which depends on k_f, k_a and k_d (11)
K_{a1}	first constant of dissociation of pepsin (bi-acid behaviour)
K_{a2}	second constant of dissociation of pepsin (bi-acid behaviour)
K^*_d	kinetic rate constant of dissociation from the cleavage sites, $K^*_d = k_d/k_a$
k_a	kinetic rate constant of the absorption reaction on the cleavage sites
k_d	kinetic rate constant of the desorption reaction on the cleavage sites
k_f	kinetic rate constant of the formation of products (min^{-1})
OD	optical density absorbance at 280 nm
P	product concentration
pK_{a1} and pK_{a2}	equal to Log_{10} (K_{a1}) and Log_{10} (K_{a2}) respectively
S	substrate concentration
ST	*Semitendinosus* muscle
T	Temperature of the water bath during the heating treatment
t_{OD}	time of the pepsin digestion followed by the measurement of OD (min)
t_h	heating time of the meat at a given temperature prior to digestion experiment (min)
$\theta = E^*/E^*_{max}$	coverage rate of the enzyme on the sites
X	hydrophobicity or values of OD_{max} and k_f (Table 2)

Superscripts or subscripts

T	total concentration in contact with the myofibrillar proteins
end	value at the end of the evolution for long heating time
eq	value obtained at equilibrium
max	maximum value
0	initial value on the raw meat

We end with a short example illustrating how these models can be used to gain a fuller understanding of the gastric digestion of meat.

2. Materials and methods

2.1. Muscle sampling

Two very different types of muscles were used to take into account a possible effect of the composition of the muscle on the digestion of the extracted myofibrillar proteins: the *Infraspinatus* muscle (IS): high pH, high content of oxidative fibers and of connective tissue and quite fat, and the *Semitendinosus* muscle (ST) lower pH, high content of glycolytic fibers, low content of connective tissue and lean rich in glycolytic fibers, where connective tissue content is low (Talmant, Monin, Briand, Dadet, & Briand, 1986; http://bovine.unl.edu/eng/index.jsp). Experiments were performed with samples from these muscles taken from one Charolais heifer (2 years old). The muscles were excised from the carcass, vacuum packed, aged 14 days at 4 °C and cut into thick slices, which were frozen at −18 °C. Before treatments, one piece was warmed to −2 °C and either cut into thin slices (2 mm thick) or minced (2 mm diameter grid). In the latter case slabs of the same thickness as the slices were made and placed on a support.

2.2. Technological treatments

Two successive treatments were carried out. Firstly, six slices or slabs of meat were steeped (bioreactor Labfors, 3L) for 20 h at 10 °C so that the targeted meat pH and NaCl content were reached. These values were checked (pH, Mettler InLab427- NaCl, Sherwood chloride analyser 926) on small samples. Secondly, the samples were cooked individually in vacuum bags. The bags were immersed in a stirred water bath to achieve the targeted (t_h, T) heating condition, and cooled in an ice-water bath. Owing to the thinness of the sample, the time needed for it to reach the water bath temperature and to be cooled at the end of the experiments was negligible (less than 45 s) in comparison with the total treatment time (t_h). After the thermal treatment the samples were frozen and stored at −80 °C for *in vitro* digestion measurements. Final salt contents in the sample were 0.7%, 1.5% or 2.0%, and pH either 4.5 or 5.5. A complete experimental plan was applied at the heating temperatures: 60, 70 and 90 °C, and some additional experiments were performed at 50 and 80 °C to complete the results. The reference conditions taken here come from the *Infraspinatus* muscle, salted at 0.7% and marinated at pH 5.5.

2.3. In vitro digestion by pepsin

In vitro pepsin digestion kinetics were measured on four samples for each treatment using the protocol described by Bax et al. (2012). Firstly Myofibrillar proteins were extracted after cooking according to the method of Pietrzak, Greaser and Sosnicki (1997). The protein concentration was measured using the BioRad RC-DC (RC: Reducing agent Compatible/DC: Detergent Compatible) protein assay method. Serial dilutions of bovine serum albumin (BSA) in solubilisation buffer (tris 0.25 M; SDS 4% (w/v), MCE 1% (v/v) ; glycerol 20% (v/v) pH 6,8) were used for the standard curve from 0.2 mg/ml to 1.5 mg/ml. Protein content was adjusted to 6 mg. Proteins were digested at 37 °C by porcine gastric pepsin (P7012, Sigma–Aldrich, France) at a set pH and set enzyme concentration in glycine buffer to simulate stomach digestion for 4 h. The enzyme concentration and pH were usually equal to 10 U mg^{-1} protein and 1.8, respectively. However, in some experiments pH varied up to 2.5 or 3.8. The pepsin concentration also varied between 6 and 50 U mg^{-1} during one set of experiments. Digestion was stopped by adding 15% trichloroacetic acid and cooling. After centrifuging (10 min, 4000*g*) the amount of hydrolyzed peptides (PM < 15 kDa) in the supernatant was measured by absorbance (OD) at 280 nm. The increase in OD was measured at 10 time