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The Impact of Cooking of Beef on the Supply of Heme and Non-Heme Iron for Humans

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Abstract

Red meat contains a high proportion of heme iron (HI) which is absorbed at a far higher extent into the blood than the non-heme iron (NHI) found in plants. However, HI and NHI are expelled in the juice during cooking while a fraction of HI is converted into NHI, thus decreasing iron bioavailability. This paper relies on experiments and the use of modeling. The kinetics of the conversion of HI into NHI was measured and modeled in juice extracted from uncooked beef meat, and beef cubes were cooked to measure the variations of HI/NHI contents. In meat, HI/NHI ratio decreased from 2.0 when it was raw to less than 1.0 for the longest heat treatments and highest temperatures. The model was used to predict the effect of cooking conditions on the variations of the iron supplied by beef meat. The lowest contribution of meat to iron supply was found for under-pressure cooking at temperatures above 100˚C.

Keywords

Iron, Model, Meat, Transfer, Reaction

1. Introduction

Iron deficiency is identified as the most common nutritional problem in the world, affecting several billion people, mainly children, pregnant women and women of child-bearing age, both in developing countries and in Europe [1] [2] [3]. Iron deficiency can increase the mortality and morbidity of both mother and child at birth [4], decrease the mental and psychomotor development of children [5] and alter work performance and resistance to infection [6].

In humans of normal status, iron absorption is higher when meat is part of the diet for at least 3 reasons. Firstly, red meat supplies high amounts of iron, mainly HI as myoglobin. Secondly, far more HI is absorbed than NHI (15% - 40% versus 2% - 10%) [7]-[12]. Thirdly, meat favors NHI absorption, through the so-called "meat factor" which could be related to cysteine-containing peptides arising from muscle protein hydrolysis in the intestines [13] [14]. In contrast, NHI in the absence of the meat factor is poorly absorbed because many components of diets such as tannins and polyphenols inhibit its absorption [15] [16] [17]. Several descriptive models (statistical or compartmental) have been developed to predict iron bioavailability in various diets [12] [18] [19]. One of the main parameters affecting the quality of prediction is the HI content in diets and the changes in both HI and NHI content during cooking [12].

Red meats from beef, horse, and lamb generally contain high amounts of iron and especially HI. The effect of animal species and muscle type on HI and NHI contents has been reported in details in the literature [20]-[25]. In contrast, the effects of meat cooking on HI and NHI contents are less documented and much information is lacking (**Table 1**). It has been established that heating causes changes in HI and NHI contents in meat through several mechanisms. First, part of HI and NHI is expelled into the juice during cooking [26]. Second, heating over 60˚C induces the progressive denaturing of globin, which leads to an increase in insoluble HI in meat and juice [27] [28]. Third, part of HI is converted into NHI during meat cooking through oxidation of the porphyrin ring [23] [26] [29]. The relative contributions of these phenomena to iron cooking losses depend on many parameters including the type of cooking equipment, functioning, and control, the time-temperature treatment chosen, and meat cut geometry

Table 1. Effect of different cooking modes and conditions on the cooking yield, the HI and NHI contents, and on the HI/NHI ratio measured in literature for meat. Cooking yield is based on the variations of the sample weights recorded in the literature papers (ratio of the mass of the cooked meat piece to the mass of the raw meat piece multiplied by 100). The percentages of iron, of HI, and NHI contents are calculated by $100C_{Fe}/(C_{Fe})_{raw}$, $100C_{HI}/(C_{HI})_{raw}$, $100C_{NHI}/(C_{NHI})_{raw}$ respectively.

Cooking modes		Pieces size	Cooking yield (%)	Iron (mg/g DM)			% of iron content calculated on the raw meat basis			Ratio	References
				Total	HI	NHI	Total	HI	NHI	HI/NHI	
Raw	/	LD Steack (2 cm in thickness) 0.500 kg	100.0	72.1	57.1	15.0	100	100	100	3.8	[21]
Grilled	Core T˚: 70˚C		75.9	69.8	51.2	18.6	97	90	124	2.8	
Pan fried			74.5	70.1	47.6	22.5	97	83	150	2.1	
Roasted			71.6	69.5	46.2	23.3	96	81	155	2.0	
Raw	/	/	100.0	83.9	56.9	28.5	100	100	100	2.0	[26]
60˚C	Immersed in Water bath for 1 hour	ST Steak 2.5 cm in thickness (0.160 kg) beef and pork	76.9	72.5	41.8	31.1	86	73	109	1.3	
77˚C			65.1	69.7	37.5	31.6	83	66	111	1.2	
97˚C			55.9	79.6	41.8	37.6	95	73	132	1.1	
Autoclave	60 min	2.5 cm in thickness (0.160 kg)	52.3	69.2	28.9	36.2	82	51	127	0.8	

and size [23] [24]. Comparing the data on the quantification of iron losses and iron conversion is often difficult because measurements are performed on meat cuts of different shapes and sizes that are heated using various cooking modes and under different time-temperature conditions.

The application of experimental designs will always be limited to compensate for the lack of literature whereas combining modeling and experiments is a good way to better understand the respective effects of the different reactions and mechanisms observed experimentally and to predict non-measured data [30]. The purpose of this paper is to describe a method to improve the prediction of both HI and NHI losses in meat pieces due to juice expulsion and to the conversion of HI into NHI by the development of a mathematical combined heat-mass transfer and reaction model. This model, often mentioned in the following as "the transfer-reaction model", is used at the end of the paper to discuss the effect of cooking mode and time-temperature conditions on the iron supply for consumers of beef meat.

2. Approach, Experimental Procedure and Mathematical Model

In the first step, the reaction kinetics of the thermal conversion of HI into NHI was measured in meat juice and modeled under a wide range of time-temperature conditions. Then meat cubes were cooked in a water bath to determine the variations of the HI and NHI contents due both to juice expulsion and to thermal conversion. During experiments, the heating of the samples was most of the time continued well beyond the usual cooking durations to be able to test the robustness and accuracy of the numerical model under these extreme conditions.

2.1. Experiments

2.1.1. Meat Samples

The meat came from muscles of 2 - 3-year-old Charolais cows, vacuumpacked and then aged for 12 days at 4˚C. Two muscles were used: *longissimus thorasis* and *semimembranosus* (named in the following LT and SM respectively). The pH was about 5.5. Muscles were frozen at −80˚C until the experiments were performed. Before the experiments, the meat was thawed at 4˚C for 48 hours and then cut into appropriate pieces to extract juice or to be cooked. Meat remaining after cutting was used to determine the initial contents of HI and NHI in raw meat. The dry matter contents of the samples ranged from 22% to 25% while the fat content ranged from 3% to 5%.

2.1.2 Measured Kinetics of the Conversion of HI into NHI in Meat Juice

Juice was extracted from SM pieces about 300 g (30 × 50 × 200 mm) according to the procedure of [31]. The pieces were frozen slowly to weaken the muscle cells through the formation of large ice crystals and then thawed before the pressing stage. Juice was extracted through three successive steps under 300 bars (the muscle sample was folded and placed again in the device to be squeezed 3

times) using a hydraulic press and a specific device to maintain the muscle (Figure 1). Juices were collected, filtered on a sintered glass under a low vacuum and then freeze-dried and stored at −80˚C. Fresh juice accounted on average for 31% of meat weight and contained 10.2% of dry matter. To determine the kinetics, juices were restored to a final density of 1.022 in distilled water. An aliquot of juice (15 mL) was poured into a test tube, closed hermetically. Tubes were heated in the following conditions: 50˚C for 7, 20, 40 and 60 min, 60˚C for 10, 20, 40 and 300 min, 80˚C for 10, 20, 60, 180 and 300 min, 89˚C for 10, 60, 180 and 300 min, 98˚C for 10, 60, 180, 300 and 900 min, 120˚C for 10, 60, 180 and 300 min. To correctly determine the kinetics parameters, it was necessary that the measurements reflect the evolution of the reaction rates at the different temperature levels. As these rates were initially unknown, a step-by-step approach was applied to determine the most suitable measurement times for each of the temperature levels starting from the lowest temperatures. This step-by-step approach explains why the measurement times were sometimes different from one temperature level to another. Times of 900 min and even 300 min are much longer than those commonly used for cooking beef meat, but these long experimental times were needed to precisely determine the model's parameters to predict the conversion of HI into NHI. The tubes were heated in a water bath (up to 98˚C) or an oil-bath (for 120˚C). They were then cooled in ice-water until the

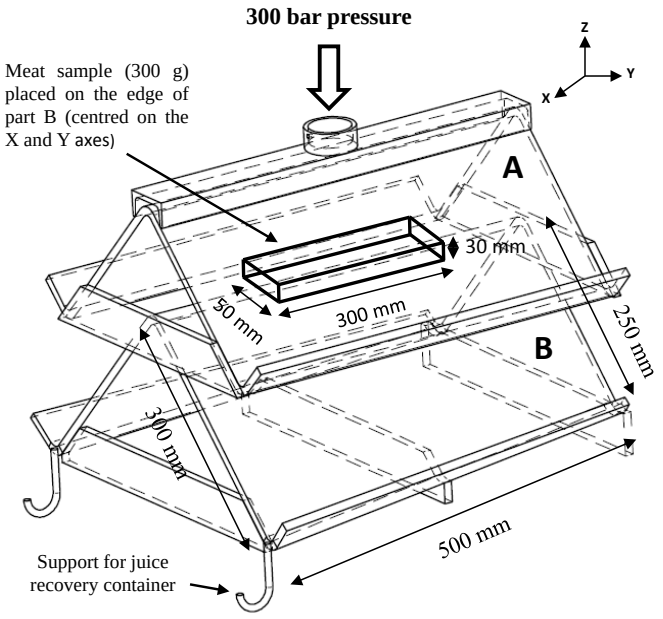

Figure 1. Schematic representation of the system of juice extraction (made in dichromate steel). This device is placed under a press generating a pressure of 300 bar (15 t). The meat sample is placed between parts A and B of the device as shown in the graph. Part B of the device has a slope of 10% which allows the juice to flow during pressing. During pressing, it is estimated that the surface area of the sample is multiplied by 5 (for an initial surface area of 150 cm^2, the final surface area is about 750 cm^2) but it remains much smaller than the total area of the part B of the device that is greater than 2500 cm^2.

temperature fell to 4˚C. Four tubes were heated to establish each kinetics point. One was used to monitor the temperature kinetics in the tube with a thermocouple and the 3 others to measure the HI and NHI contents in meat juice after heating. HI and NHI contents were also determined in triplicate in freshly restored juice (from the juice freeze-dried).

2.1.3. Measured Kinetics of HI and NHI in Meat Pieces

Thawed pieces of LT were cut into small cubes: 30 × 30 × 30 mm. Four cubes of meat were used for each point/time of the kinetics. One was used to measure the evolution of temperature in the sample and the three others for iron analyses after heating. Meat was heated in a water bath. The raw meat cubes were placed on racks and directly immersed in the water at 60˚C, 80˚C or 95˚C for 60, 180, or 300 min. Measurements were also performed after 30 min of heating at 80˚C and 95˚C to obtain a more accurate analysis of the kinetics. At the end of the heating time, the samples were quickly cooled in a freezer until the internal temperate fell to 4˚C. HI and NHI contents were determined in triplicate for each kinetics point to calculate the standard deviation.

2.1.4. HI and NHI Measurements

HI was determined after extracting heme in acidified acetone according to the method of [32]. Samples of meat (2 - 4 g) or juice (4 mL) were homogenized for 15 seconds with a polytron in acidified acetone mixture (acetone/water/pure HCl: 40/9/1). The samples were placed in the dark for 20 hours before centrifuging at 2200 rpm for 10 min. The supernatants were filtered on Whatman paper and the absorbance was measured at 640 nm. The HI concentration was calculated using a standard curve made of hydrochloride-hemin in acidified acetone mixture.

NHI was determined using ferrozine as described by [23] and [33]. Briefly, samples of meat (2 - 4 g) and juice (4 mL) were mixed with 3 volumes of 0.1 M citrate-phosphate buffer, pH 5.5. The samples were homogenized with a Polytron for several seconds. Then, 1 mL of 2% ascorbic acid in 0.2 N HCl was added to 3 mL of homogenate and kept at room temperature for 15 min. Next, 1 mL of 11.3% TCA was added to precipitate proteins. Afterward, the homogenate was centrifuged at 3000× g for 10 min at room temperature. One mL of the supernatant was mixed with 0.8 mL of 10% ammonium acetate and 0.2 mL of ferrozine reagent. The absorbance was read at 562 nm against a blank. The NHI concentration was calculated using a standard curve made of $FeCl_2$ in 0.1 N HCl solution. Total iron was calculated by adding the HI and NHI contents.

The results were expressed as µg/g dry matter in meat and µg/mL in juice. Meat dry matter was determined by drying meat samples (about 2 - 5 g) in an oven at 105˚C according to the normalized method [34].

2.2. Mathematical Transfer-Reaction Modeling

The total model combined the calculations of the heat-mass transfer model pre-

viously described by [35] and those of the thermal reaction model developed in the present paper to predict the conversion of HI into NHI in the meat.

Meat is a multi-composite structure and juice expulsion during cooking is the result of complex phenomena. When the meat is heated, water begins to unbound to proteins and myofibers and collagenous tissues contract. This thermal contraction exerts a strong mechanical pressure on the juice located inside the fibers and between the different muscle bundles. This mechanical pressure expels the juice from the meat through multiple channels of different sizes that pass in between the fibers and in between the primary and the secondary bundles [36] [37]. This migration of juice under mechanical stress is anisotropic and leads to a reduction of the meat piece volume. Advanced heat-mass transfer models have been developed in the literature to predict the expelling of juice under mechanical pressure [38] [39]. However, they do not consider the multi-composite nature of the beef meat piece, the flowing of juice into channels of different sizes, etc. Thus, discrepancies remain between the predictions of these models and the water content profiles measured in the meat. Faced with this situation we have decided to describe juice expelling by an observation-based model using a simple relation and a few parameters, to have enough time: 1) to test it under different cooking situations, and 2) to determine the kinetics of the reactions responsible for the variations of the meat nutritional qualities. Our juice transfer model [35], is based on experimental observations and on the assumptions that: 1) the unbounding of water from proteins and the pressure effects exerted by collagen tissues on juice migration depend on the spatial variations of temperature inside the meat, 2) the water concentration at one point of the meat (expressed on a dry matter basis) can never be less than the equilibrium water content calculated from the maximum temperature reached that point of the meat, and 3) effects of crust formation on juice expelling can be neglected.

Model's parameters are given in **Table 2**. The variation of the concentration of Fe in meat (C_{Fe} being either C_{HI} or C_{NHI}) as the function of time depended on both juice expulsion and thermal conversion through the two mathematical terms $\left(\frac{\partial C_{Fe}}{\partial t}\right)_{exp}$ and $\left(\frac{\partial C_{Fe}}{\partial t}\right)_{conv}$:

$$\frac{\partial C_{Fe}}{\partial t} = \left(\frac{\partial C_{Fe}}{\partial t}\right)_{exp} + \left(\frac{\partial C_{Fe}}{\partial t}\right)_{conv} \quad (1)$$

A conduction model is used to calculate the space-time variation of temperature in the meat (Equation (2), **Table 3**). This result is used to calculate the variations of the concentrations through Equations (2)-(6) under the hypotheses detailed in **Table 3**. Our juice transfer model was based on a reaction-like equation. There was no water transport equation, and the effect of the water migration on the spatial water content in the meat was indirectly considered by varying the reaction rate constant, not only as a function of temperature but also as the function of the distance from the surface [35].

Table 2. Significance of the parameters used in the paper, symbol, and units. Parameters values used during the numerical simulations. *Units of the international system were used during the calculations.

Parameters	Symbols	Significance	values	Units used in the paper*	Reference
General	C_{Fe}	Concentration of iron (either HI or NHI)		In the text the concentrations are expressed in liquid	
	C_{HI}·C_{NHI}	Concentration Heme. or Non-Heme. Iron		as mg·(mL)$^{-1}$ in solid as mg·g$_{DM}^{-1}$ (µg = 10^{-9} kg)	
	DM	Meat Dried Matter		kg	
	R	Molar gas constant	8.314	J·mol^{-1}·K^{-1}	
	t	Time		s. or min. or h	
	Δ	Laplacian			
Heat Transfer	T	Temperature		˚C or K	
	D_T	Meat thermal diffusivity	1.2×10^{-7} (20˚C)	m^{2}s^{-1}	
Mass Transfer	A_j	First pre-exponential term	6.8×10^{-4}	m^{-1}s^{-1}	
	B_j	Second pre-exponential term	1.275		
	d	Distance to the nearest meat surface constant		m	[35]
	E_{aj}	Activation Energy	18,500	Jmole^{-1}	
	$k_{exp}(T, d)$	Rate of juice expulsion	2.8 - 3.2	s^{-1}	
	X	Water content in meat		kg water/kgDM	
	X_0	Initial water content in meat		kg water/kgDM	
	$X_{eq}(T)$	Equilibrium water content in meat at T		kg water/kgDM	
Reaction Conversion of HI into NHI	k_0	Pre-exponential factor	69,420 ± 5300	s^{-1}	
	E_a	Activation Energy	64,520 ± 210	Jmole^{-1}	
	k_{conv}	Constant rate of conversion of HI into NHI		s^{-1}	

Table 3. Assumptions and equations used in the combined model.

Phenomenon	Hypotheses	Equations
Heat transfer	• heat transfer by conduction	$\partial T/\partial t = D_T \Delta T$ (2)
Mass transfer Iron expulsion in the juice	• No iron expulsion through evaporation • Simplified mass transfer model of [35] • HI and NHI soluble in juice • C_{Fe} varies with time and is equal to its local concentration in the meat	$\left(\frac{\partial C_{Fe}}{\partial t}\right)_{exp} = -k_{exp}(T,d)\left(\frac{X - X_{eq}(T)}{1+X_0}\right)C_{Fe}$ (3) $k_{exp}(T,d) = A_j d^{B_j} \exp\left(\frac{-E_{aj}}{RT}\right)$ (4)
Reaction Conversion of HI into NHI	• HI conversion into NHI follows a first order kinetics • Thermal conversion occurs at the same rate in extracted meat juice and meat • Rate constant of this reaction depends on the local T using Arrhenius relation	$\left(\frac{\partial C_{Fe}}{\partial t}\right)_{conv} = -k_{conv} C_{HI}$ (5) $k_{conv} = k_0 \exp\left(\frac{-E_a}{RT}\right)$ (6)

Using the equations of Table 3 the Equation (1) became:

$$\frac{\partial C_{Fe}}{\partial t} = -k_{exp}(T,d)\left(\frac{X - X_{eq}(T)}{1+X_0}\right)C_{Fe} - ek_{conv}C_{HI}, \text{ while } X > X_{eq}(T) \quad (7)$$

with e being equal to +1 for C_{HI} and to −1 for C_{NHI}. Juice expulsion was stopped as soon as $X = X_{eq}(T)$ then the variations of C_{HI} and C_{NHI} were only due to thermal conversion. The parameters values of the heat-mass transfer model detailed in Table 2 are those used in [35].

Equations (2), (4), (6) and (7) constituted the combined system to be solved to obtain the space-time variations of C_{Fe} in the meat. This combined transfer-reaction model was implemented in COMSOL Multiphysics® 3.4, which solves systems of nonlinear differential equations by the finite element method. When the cooking methods and conditions were closed to that of our previous paper as for immersion cooking, stewing, roasting under pure steam conditions, or mixed air-steam conditions, or even under dry air cooking for roast beef meat piece, Neumann boundary conditions were used in the heat transfer model and the values of the heat transfer coefficients, and the other parameters, were those used previously under the same conditions [35]. For different air velocities and/or more important radiation conditions an effective transfer coefficient was calculated as in [40] [41]. In the case of contact heating, a 100˚C Dirichlet boundary condition was simply applied on the contacting surface.

The numerical procedure and numerical mesh were the same as in [35] [42]. Spatial C_{Fe} values calculated by the model in the meat cubes were averaged at each cooking time to obtain the average concentration values in a given volume $\overline{C}$; these calculated values were compared afterward to the iron content measured in the same volume and at the same time.

The predictions of the total quantity of juice expelled from the meat by the model had been compared to experimental measurements, in a previous paper, for beef meat cubes and cuboids heated in water bath from 50˚C up to 90˚C [35]. The transfer model was also tested for steaks and roasts of different dimensions cooked in an oven under 10% steam injection or pure steam injection condi-

tions, and under dry air conditions at a temperature of 90˚C or of 250˚C. Despite its simplicity, the transfer model proved to be able to predict the mass of juice that was expelled from the meat under all these situations [35]. In the case of contact, the model had not been validated and the calculated values were considered as more approximate than those obtained under the other cooking situations.

The parameters of the reaction of conversion of HI into NHI (k_0 and E_a) were the only one which had not been determined in [35]. Thus, they have been identified in the present paper from the experiments performed in the extracted meat juice by minimizing the sum of squared differences between the experimental and the calculated results.

2.3. Estimations of the Iron Supply Related to Beef Meat Consumption

The amounts of the HI and NHI contents in the cooked meat were assumed to result from a 100 g raw meat portion completely eaten by the consumer. This is to reflect some typical French meals when beef meat is consumed without other important sources of iron coming from plant foods. As the French consume only 46 g of butcher's meat per day on average this typical French meal did not occur every day anymore. The potential amount of iron absorbed by the consumer (PAIA) during these typical French meals was calculated as followed:

$$\left[\text{PAIA}\right] = \left[\text{HIweight} \times \text{HIabs}\right] + \left[\text{NHIweight} \times \text{NHIabs}\right] \quad (8)$$

The absorbed proportion of HI and NHI: HIabs and NHIabs, were chosen to be equal to 0.25 and 0.05 respectively. These values are means of what is reported in the literature for both iron forms estimated for many diets in various experimental conditions for humans with normal iron status [8] [9] [12] [15]. The PAIA was calculated here as mg of absorbed iron. It is widely accepted that adult men and menstruating women must absorb 0.9 and 1.6 mg iron per day, respectively, to cover their iron requirements [43] [44]. The ratio between the PAIA and these two values of 0.9 and 1.6 indicated the contribution in percentage of each meat portion of these typical French meals in covering the daily requirement for an adult man and a menstruating woman. The PAIA was calculated for 2 beef muscles: *longissimus thoracis* (LT), and *semimembranosus* (SM), as the former is used for grilling and roasting, while the latter is an example of a tougher muscle that can be braised, stewed or even pressure cooked. HI and NHI contents in 100 g of the raw muscles were respectively: 1.56 ± 0.23 mg and 0.66 ± 0.06 mg for LT, 1.74 ± 0.22 and 0.64 ± 0.07 mg for SM.

3. Results and Discussion

Model Equations (1)-(7) were applied for all the types of cooking methods considered in this paper; these equations are uncompleted when the heating is due to microwave treatments (not considered in this paper). The model parameters connected to the heat and mass transfers inside the meat had been determined in

a previous study [35]. Thus, the only unknown parameter values were those of k_0 and E_a which were determined from the HI kinetics measured in the heated meat juice. Model predictions in meat were validated by comparing calculations to the measurements obtained in meat cubes heated in the water bath. Finally, the boundary conditions were adapted to predict the variations of the HI and NHI contents in meat pieces cooked under different cooking methods and time-temperature conditions than in the water bath.

3.1. Kinetics of the Thermal Conversion of HI into NHI in Meat Juice

When the temperature of the bath ranged from 50˚C to 98˚C, the kinetics of temperature in the test tube was fast and the juice temperature in the tube reached 90% of the bath temperature in less than 10 min. When the oil bath temperature was 120˚C, the juice temperature kinetics was slower and 20 minutes were needed for the juice temperature in the test tube to reach 90% of the bath temperature.

The non-cooked rehydrated juice contained 10.2% of DM and 18.7 ± 0.7 µg total iron/mL. HI represented 15.1 ± 0.3 µg/mL, accounting for 80.6% ± 3.8% of the total iron content. At a bath temperature of 50˚C, the HI content in the juice was constant throughout the heating experiment. At 60˚C, it was still 92% of its initial value after 300 min of heating. Over 60˚C, the decrease of the HI content in the juice was much higher with only 4% of its initial value remaining after 300 min of heating at 120˚C (**Figure 2**). The variation in HI content in the juice during heating was calculated using the Equations (5), (6). The values of k_0 and E_a were determined by minimizing the differences between the experimental and calculated results, either using the water bath temperature, or the temperature measured in the test tube. No significant differences in the determination of k_0 and E_a and the prediction of the experimental data were observed when the bath temperature ranged from 50˚C to 98˚C, while test tube temperature measurements were required to accurately predict HI conversion when the bath temperature was 120˚C. The k_0 and E_a values determined using test tube measurements were 69,420 ± 5300 s^{-1} and 64,520 ± 210 $Jmole^{-1}$, respectively. The average difference between measurements and predictions using these parameter values was 0.7 µg/ml (**Figure 2**). The decrease of the HI content at all the bath temperatures was indeed associated with a simultaneous increase of the NHI content in the juice. The analyses of these simultaneous variations, illustrated in **Figure 3** for the 120˚C treatment, were used to check that we were able to accurately monitor the conversion of HI into NHI. Literature data on the conversion of HI into NHI are generally measured in the meat for product temperature of less than 100˚C and heating durations of less than one hour [28] [45]. This explains why this conversion is most often limited (less than 20% of the initial HI content) which is consistent with our results (conversion of HI into NHI during 1 hour of heating at 98˚C is about 20% in **Figure 2**).

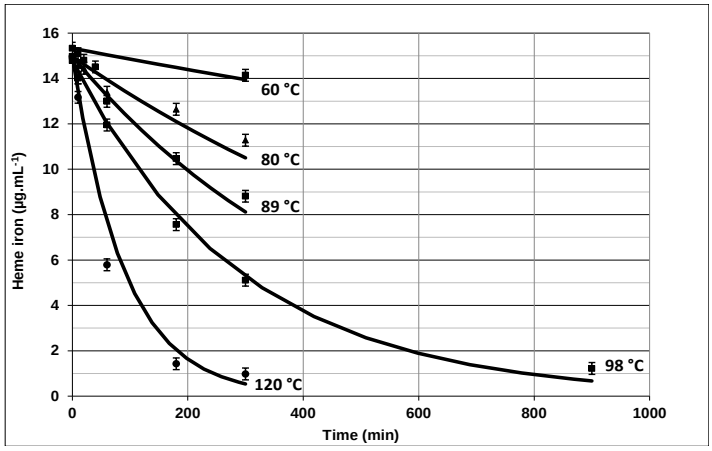

Figure 2. Kinetics of the decrease of heme iron (HI) due to its conversion into non-heme iron (NHI) in juice extracted from SM muscle and heated at different temperatures (symbols). Comparison of these measurements with the values calculated using Equations (5) and (6) with k_0 = 69,420 s^{-1} and E_a = 64,520 $Jmole^{-1}$ (full lines). For small SD, error bars can be hidden by the size of the dots. During the experiments, the heating of the juice was continued well beyond the usual cooking durations to be able to test the robustness and accuracy of the numerical model under these longest conditions.

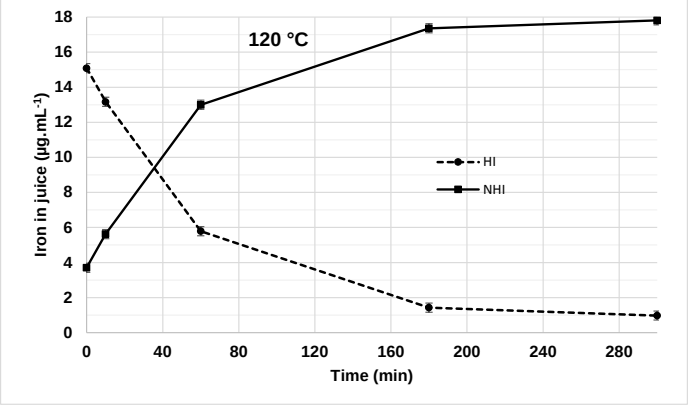

Figure 3. Measured time-related variations of HI and NHI in juice extracted from SM muscle and heated at 120˚C (lines are not predicted values but just connections between the measured points). For small SD, error bars can be hidden by the size of the dots.

3.2. Use of the Transfer-Reaction Model to Analyze Iron Variations for the Meat Cubes Heated in Water Bath

The values calculated from Equations (5) (6) (k_0 being 69,420 s^{-1} and E_a 64,520 $Jmole^{-1}$) were added to those issued from the heat-mass transfer model (Equations (2) to (4)) to predict the variations of the local HI and NHI contents in the heated meat cubes due to both thermal conversion and juice expulsion. Temperature gradients inside the 3 cm sided cube were high only during the first 30 min, as afterward the temperature could be considered as homogenous within it [35] [46]. The raw meat used during the experiments on the 3 cm side cubes cut from the LT muscle contained 2.54 ± 0.03 mg total iron/100g raw meat and 1.81 ± 0.02 mg HI, which represents 71.0% ± 0.9% of the total iron. These values were

close to those found in the literature which showed that HI in beef is composed of between 60% - 80% total iron [23] [45] [47]. In the following iron content is expressed for our results on a meat dry matter basis to consider both the variations due to juice expulsion and to thermal conversion.

As expected, the HI content measured in the meat tended to decrease with time; this decrease was more pronounced at 80˚C than at 60˚C (**Figure 4**). A further increase of the water bath temperature up to 95˚C led to more complex kinetics. The measured HI content in the meat at 95˚C decreased from 0 to 30 min then remained steady between 30 and 60 min and then decreased again between 60 and 300 min.

The HI kinetics predicted by the model at 60˚C and 80˚C ((1) and (2) in **Figure 4**) agreed with the measurements at these two temperatures whereas the calculations underestimated the HI content in the meat at 95˚C (curve 3 in **Figure 4**). It was possible during the calculations to separate the part of the HI loss due to juice expulsion from that which came from HI conversion into NHI (first and second terms in Equation (7)). These separate calculations show that the decrease of HI in the 3 cm side cubes was mainly due to juice expulsion during the first 30 min whatever the heating temperature, and totally due to the conversion of HI into NHI after 60 min of heating at 95˚C. The fact that the combined transfer-reaction model (1 - 7) was able to predict the kinetics obtained at 60˚C and 80˚C supports the assumptions on which the model relied for these two temperatures, *i.e.* the fact that HI was expelled in the juice at a concentration proportional to its local concentration in the meat while part of the HI remaining in the meat was converted into NHI at a rate which corresponded to the conversion observed in the juice and described mathematically by Equations (5, 6) (**Figure 2**). The failure of the model during the heat treatment after 60 minutes at 95˚C was due to a phenomenon not previously considered in the model, namely the loss of heme protein solubility which was visually observed by [26], by a change of color of the expelled juice that occurred between 77˚C and 97˚C. Like us, these authors also measured a higher HI content (on DM basis) in the meat after 1h of heating at 97˚C than after 1 h at 80˚C. This stopping of HI decreases in the meat after 30 min at 95˚C, clearly visible in our measured kinetics, indicated heme protein coagulation which occurred during heating (**Figure 4**). After 1 h of heating, the variations of the HI content in the meat were due only to the thermal conversion of HI into NHI. Considering the experimental errors, the NHI variations in the meat were the same for the three water bath temperatures (60˚C, 80˚C, 95˚C). An average of these variations is given in **Figure 5**. NHI content decreased during the first 30 min, remained steady between 30 - 60 min and then showed a moderate increase (**Figure 5**). The model was used to calculate the expulsion of NHI in the juice and its formation through the conversion of HI which remained in the meat into NHI. The predictions calculated at 60˚C and 80˚C reproduced these temporal variations which reflect the slowing down and then stopping of the HI and NHI expelled in the juice due to the end of meat protein contraction, and of the conversion of the HI

content which remained in the meat into NHI, which continued after the expulsion of the juice (Figure 5). The calculated quantity of NHI expelled in the juice and the conversion rate of HI into NHI were different at 60˚C and 80˚C, but their balances were similar, leading to similar NHI curves. The similarity of the NHI kinetics measured at 95˚C with that measured at 60˚C and 80˚C, suggested that the balance between NHI expulsion and formation was also similar above 80˚C.

The HI/NHI ratio was 2.0 in the raw meat and its decrease was different between 60˚C and 80˚C. The decrease was less pronounced at 60˚C than at 80˚C where it reached 1.5, 1.1 and 0.9 after 1 h, 2 h and 5 h of heating, respectively. [26], who measured a ratio of 2.0 in raw meat, found a ratio of 1.2 and 1.1 after 1 h of heating at 77˚C and 97˚C, respectively (Table 1).

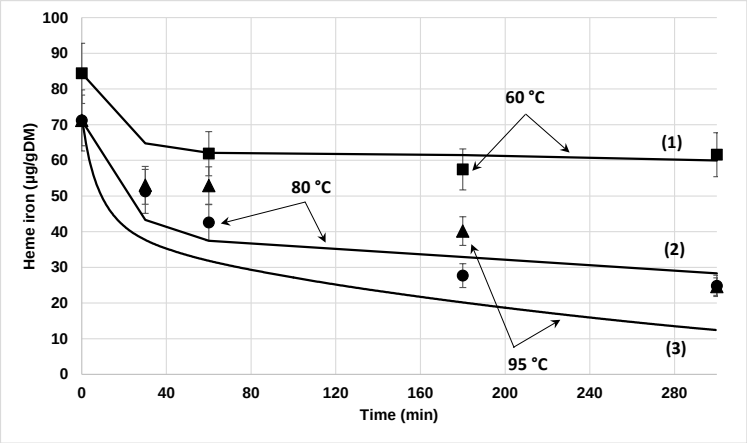

Figure 4. Comparison between the time-course of the HI concentration measured in the 3 × 3 × 3 cm meat cubes immersed in the water bath at 60˚C, 80˚C or 95˚C (square, circle and triangle symbols respectively) and the values predicted by our combined transfer-reaction model at the same water bath temperatures: 60˚C (1, line), 80˚C (2, line) and 95˚C (3, line).

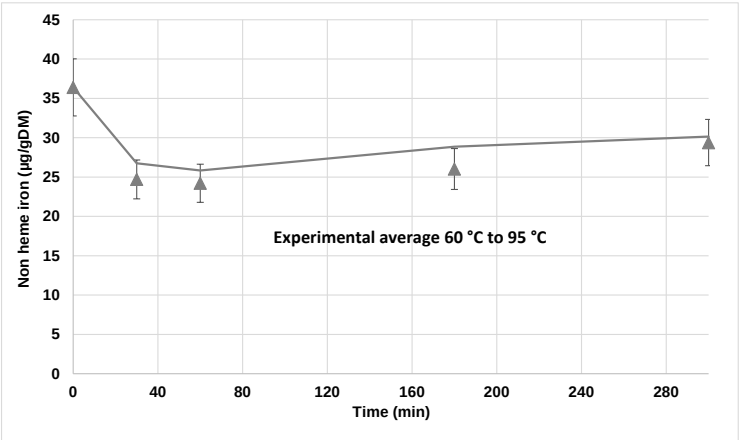

Figure 5. Comparison between the time-course of the average NHI concentration measured in the 3 × 3 × 3 cm meat cubes immersed in the water bath measured at 60˚C, 80˚C and 95˚C (symbols) or predicted by the transfer-reaction model at 60˚C and 80˚C and then averaged.

4. Effects of Cooking Mode and Time-Temperature Conditions on the Iron Supply Related to Meat Consumption

The combined transfer-reaction model was used to predict the variations of HI and NHI and their potential nutritional impacts for meat pieces cooked according to the most widely culinary practices in France (microwave cooking being excluded here). The equations of the model and the values of the parameters were those of Table 2 and Table 3. HI expelling was stopped as soon as the average temperature of the meat exceeded 80˚C to consider the effect of heme protein coagulation. The boundary conditions and the sample dimensions were changed according to the type of cooking methods and meat cuts commonly used in practice. Details on the application of the boundary conditions can be found in [35] [40] [41] [42]. In practice, for the same cooking method, the equipment can be different and a range of boundary conditions has to be applied to consider these variations. Since the dimensions of the sample can also be different this leads to a range of juice loss and cooking yield as shown in Table 4. This table is only a selection of some of the results obtained during a wider set of calculations, the purpose of this selection being to give an order of magnitude of the nutritional impacts of the different cooking modes and time-temperature conditions used in France. In the following, the iron expelled from the meat piece into the juice was supposed to be lost for the consumer. However, it should be noticed that in some recipes part of the expelled juice and its iron content is consumed.

HI and NHI losses can be calculated from the values reported in Table 4 by comparing the initial HI and NHI contents in the raw meat to the contents in the meat pieces subjected to different cooking conditions; the losses being expressed as percentages of the initial HI or NHI contents for a 100 g portion of the raw meat. Unsurprisingly, the calculations showed that the shortest cooking conditions (less than 5 min) and the lowest cooking temperatures (less than 55˚C - 60˚C) lead to the smallest iron losses. For the steaks cooked rare, the losses are on the average 12% of the initial HI or NHI content in the raw meat. These variations can be compared to the animal-to-animal variability assessed by the ratio of the standard deviation of the iron measured in the same raw muscle for different animals to the average iron content measured on all the animals. Under the shortest cooking conditions, animal variability was of the same order of magnitude as the variation of iron due to cooking. The values issued from (3, 4) and from (5, 6) were also used to compare the relative contribution of expulsion and conversion to the global losses. This comparison shows that under the shortest cooking conditions almost all the HI losses were due to juice expulsion. Roasting bigger meat pieces at higher temperatures increases irons losses and conversion of HI into NHI and thus decreases the contribution of the meat portion to the PAIA. For example, such a portion of meat, issued from a big very well-done roast contributes only to 29% - 37% and 16% - 21% of the PAIA for an

Table 4. Nutritional impacts of cooking on the estimated Potential Amount of Iron Absorbed (PAIA) and on the contribution of 100 g of raw beef meat cooked in different ways to the daily iron requirement for adult man (0.9 mg/day) and menstrual woman (1.60 mg/day). These values have been estimated from model calculations under the different assumptions detailed in the text of the paper. When the meat was cut in steaks or in roasts calculated results depended on the dimensions of the meat pieces. The values given in this table have been calculated for a steak of 20 × 70 × 70 millimeters and for a 60 × 60 × 110 mm meat roast. PAIA was estimated considering HI and NHI absorption rate were 25% and 5% respectively. The formula was PAIA = (0.25 × HI weight + 0.05 NHI weight) for each cooked or raw meat, in this formula, NHI and HI weights were expressed in mg.

Muscles	Cooking methods	Cooking yield (%)	HI in meat portion (mg/100g raw meat)	NHI in meat portion (mg/100g raw meat)	PAIA (mg/100g raw meat)	Daily iron requirements from 100 g of raw meat	
						Adult man	Adult woman
Longissimus thoracis	Raw	100	1.60	0.70	0.43	47	27
	Steak grilled rare	90 - 95	1.40	0.60	0.38	42	24
	Grilled or roasted rare	80 - 85	1.20	0.60	0.34	37	21
	Grilled or roasted welldone served hot	70 - 75	1.10	0.60	0.29	32	18
	Big roast very welldone or served cold	65 - 70	0.90	0.50	0.26	29	16
Semi-membranosus as an example of a tougher muscle	Raw	100	1.70	0.64	0.46	51	29
	Stewed or Braised	60 - 70	0.90	0.39	0.25	28	16
	Under-pressure > 115˚C	50 - 70	0.30 - 0.50	0.30 - 0.50	0.10 - 0.14	11 - 15	6 - 9

adult man and woman, respectively (Table 4). In this case, the degree of doneness and the size of the meat piece can affect the contribution of food portions to daily iron requirement more than the biological variability between animals.

In traditional French culinary practice some pieces of beef meat cut from muscles, or part of muscles known to be tougher, can be braised and/or stewed at temperatures close to 80˚C for more than one hour to ensure tenderness. These conditions can increase both the iron loss into the juice and the conversion of HI into NHI. An important decrease in both HI and NHI content in meat was observed, reducing the PAIA and the contribution of meat portions to the daily iron requirement. Thus, one hour of stewing an SM portion decreased the amounts of both HI and NHI in the meat portion: −47% and −39% respectively. PAIA was reduced by 45%. The contribution of a stewed meat portion to the daily iron requirement of an adult man and an adult woman fell to 28% and 16%, respectively. The decreases in both PAIA and the contribution to DIR can be higher when meat is stewed for several hours due to the ongoing conversion of HI to NHI.

The model was also used to assess the variation of HI and NHI contents during a one-hour pressure cooking at 118˚C (the highest temperature that a domestic pressure cooker can reach at 1.8 bar). In that case, the average meat temperature raised well above 80˚C, leading to the coagulation of heme protein which stopped the expelling of HI into the juice. Heme protein coagulation was

not included in the model which rendered the model more limited in that case. However, this phenomenon was simulated in the calculations by stopping the flow of iron in the juice as soon as the meat average temperature exceeded 80˚C. Hence, the quantity of HI expelled in the juice depended on the time needed for the meat piece to reach 80˚C, which was connected to the pressure increase in the cooker and the size of the meat piece. The differences in time needed to reach 80˚C have led to the different values of the cooking yield given in Table 4 (either 50% or 70%). Afterward, the meat temperature reached 118˚C where it stayed during the rest of the cooking, leading to the conversion of HI into NHI. In the pressure-cooking situation, the calculations showed that the amounts of both HI and NHI fell dramatically: −76% and −38%, respectively. Consequently, the PAIA was reduced by about 45% and the contributions of the SM meat portion to the daily iron requirement of an adult man and an adult woman were low: 11% - 15% and 6% - 8%, respectively.

Previous results should be considered in epidemiological studies on nutrition for certain sensitive populations, notably women during puberty, menstruation, and pregnancy, and elderly persons of both genders, which can have recourse to the most impacting cooking methods. It is well known as a general trend that in Western countries these populations tend to eat less meat while their iron needs can be the same or even higher than those of adult men. Using higher time-temperature cooking conditions to avoid tough meat (since tender meat is more expensive or because older people can have masticatory problems), or possible microbial safety problems, or simply for reasons of personal taste, can lead to anemia for sensitive populations if not compensated by other iron supplies. These considerations are not new but they can be better quantified and understood using the proposed modeling approach and results.

These results strongly suggest that cooking meat at low temperatures for a long time preserves heme iron content and bioavailability as illustrated by results in Figure 4. Cooking in these mild conditions could be helpful to prevent or correct iron deficiency in populations known to be specifically exposed to this problem in Western countries, such as adult women and poor and/or old people who tend to eat less meat than middle-aged men.

5. Conclusions

The effect of cooking on meat iron content is linked to both the loss of iron (HI and NHI) from the meat piece by juice expulsion and the conversion of HI into NHI in the meat piece. When the meat temperature was under 80˚C, HI was expelled in the juice. Above 80˚C, heme proteins coagulated and HI expulsion was stopped while the conversion of HI into NHI remained. Due to these phenomena, the HI/NHI ratio decreased from 2.0 when it was raw to less than 1.0 for the longest heat treatments and highest temperatures. The model was used to assess the effect of cooking on the contribution of 100 g of raw beef meat issued from two different muscles to the daily iron requirement for men and menstruating

women. Shortest cooking durations and lowest heating temperatures have almost no effect on the iron supply while roasting big meat pieces, braising and stewing at higher temperatures decreased this contribution. The lowest contribution of meat to iron supply was found for under-pressure cooking at temperatures above 100˚C, often used in practice to avoid tough meat or possible microbial safety problems. During our calculations, the iron expelled from the meat piece into the juice was supposed to be lost for the consumer. However, it should be noticed that in certain recipes (stews or casseroles) part of this released iron will be consumed, thus increasing the iron supply.

The paper was focused on iron supply and thus on the nutritional consequences of cooking. However, the present results and model can also help to better quantify the effect of cooking on the sensorial and toxicological properties of cooked meat due to oxidation if they are associated with more complex reaction schemes [48]. All these works will contribute to the design of tailor-made diets, containing meat, to ensure sensorial pleasure, balanced nutrition, and optimal health.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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