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Analysis of the juice and water losses in salted and unsalted pork samples heated in water bath. Consequences for the prediction of weight loss by transfer models

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ABSTRACT

This study has analyzed the effect of different factors on variation of meat weight due to juice loss, and variation of water content of pork samples heated in a water bath. The weight loss (WL) was influenced by initial water content of raw meat which can be connected to meat pH, muscle type, and by pre-salting. WL was also influenced by sample thickness and by nature of the surrounding fluid. These effects were significant at 50 °C and in thinner samples but decreased as meat temperature and sample thickness increased. WL showed no significant difference in response to prior freezing, applying a surface constraint during heating or varying meat salt content from 0.8 to 2.0%. The results were interpreted from literature knowledge on protein denaturation, contraction and, transport phenomena. Reliably predicting WL from water content variation during heating hinges on taking into account the loss of dry matter and the possible effects of meat pH, sample size or surrounding fluid.

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1. Introduction

When cooking meat, weight loss (WL) increases with cooking time until reaching an essentially temperature-dependent equilibrium state (Oillic, Lemoine, Gros, & Kondjoyan, 2011). Weight loss is due to the migration of juice out of the meat which affects both technological vield and meat quality (juiciness, tenderness, loss of nutritional components, etc.) (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003; Oillic et al., 2011). Meat processing often adds salt (cooked ham, sausages, etc.) but, industry is under pressure to reduce salt content for health reasons (Desmond, 2006). Understanding and predicting variations of the weight losses from unsalted or "lightly salted" meat is therefore a key challenge for both industry and consumers.

The two main theories to explain variation in raw meat waterholding capacity were reviewed by Puolanne and Halonen (2010) and, Huff-Lonergan and Lonergan (2005), who basically recapped earlier theories developed by Hamm (1972) and Offer and Knight (1988) based on electrostatic, osmotic forces, and a modification of the charge of proteins at lower pH caused by the production of lactic acid. Moisture transfer and WL can also be related to thermodynamics phenomena using the Flory-Rehner theory. The water flux is then connected to the variation of the swelling pressure which included three contributions: a contribution due to the mixing of proteins and water, a contribution of ions and polyelectrolytes and the elastic deformation of the crosslinked protein network (van der Sman, 2013). During heating, two phenomena contribute to juice loss: water

debinding and meat contraction. Water debinding is caused by protein structural changes due to decreasing water-protein bonding. Water debinding begin from 40 °C due to the structural changes of myofibrillar proteins (Davey & Gilbert, 1974; Promeyrat, Daudin, & Gatellier, 2013). Then, water migration is caused by meat contraction, principally due to collagen shrinkage. Indeed, meat contraction and variation in meat piece volume directly correlate to weight loss (Davey & Gilbert, 1974) and meat water content (Bouhrara, Clerjon, Damez, Kondjoyan, & Bonny, 2012). Meat contraction begin at 40 °C and accelerates at about 60 °C (Bouhrara et al., 2012), which corresponds to the collagen denaturation temperature measured by differential scanning calorimetry (DSC) (Tornberg, 2005).

Numerous studies have investigated the impact of raw material properties (sample dimension, muscle type, animal species) and process (cooking, injection rate, and type of packaging) on weight loss. Table 1 gives a short literature review. Many studies disregard the influence of heating kinetics on weight loss. Heating kinetics depends on both sample dimensions and on type and control of the heating







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Table 1

Subject	Authors	Conclusions
Impact of raw material		
Effect of dimension	Bouton et al. (1976); Oillic et al.	- By determining heating rate, sample size had a major effect on WL* for quick heating but had no
	(2011)	impact on equilibrium WL*
Effect of muscle type	Jeremiah et al. (2003); Rhee et al.	- WL* is dependent on muscle type, but the ranking of muscle types remains controversial between
	(2004)	studies
Effect of animal species	Oillic et al. (2011)	- Highly significant difference in water content of raw SM** (from 2.9 g/gDM for horse to 3.4 g/gDM
		for lamb) but minor difference between species in equilibrium WL* of SM**
Effect of sampling	Rhee et al. (2004); Oillic et al.	- Difference in CL* in a single muscle can reach 3% depending on the sampling location.
Effect of pre-freezing (-20 °C)	(2011); Utrera et al. (2012)	- Pre-freezing had no significant effect on WL*, nor on moisture content
Impact of meat processing		
Effect of injection rate	Desmond et al. (2002)	- Injection rate (20-35%) did not influence moisture content in cooked meat
	Boles & Shand (2001)	- Slight decrease in moisture content in cooked meat when injection rate increased from 10 to 25%
Effect of packaging	Cheng and Sun (2007)	- Ham cooked with a cooking bag had lower WL* than without a bag
Effect of type of cooking: water vs. wet air	Cheng et al. (2005)	- No difference in moisture content in cooked ham nor in WL* for water or wet-air cooking

* WL = Weight loss.

** SM = Semimembranosus.

equipment (Kondjoyan et al., 2014). When heating trials end before the equilibrium state is reached, the reported differences can often be explained by variation in the heating conditions leading to variation of the factor studied (Oillic et al., 2011). Looking at the effect of salt content, results differ between studies. A majority of studies show that reducing added salt content in meat from 1.5 to 1.0, 0.5% or 0.0% increases weight loss when the meat is cooked to a core temperature of about 70 °C. This conclusion applies to restructured pork ham (Lee & Chin, 2011), pork or beef sausages (Puolanne, Ruusunen, & Vainionpaa, 2001; Sikes, Tobin, & Tume, 2009), pork meatballs (Hsu & Yu, 1999), and pork or beef muscles (Baublits, Pohlman, Brown, Yancey, & Johnson, 2006; Detienne & Wicker, 1999; Vaudagna et al., 2008) cooked in a water bath, and has been verified for ground pork ham cooked in a microwave oven (Jeong et al., 2007). However, a few studies have found a much lower effect of changing salt content. Villamonte, Simonin, Duranton, Cheret, and de Lamballerie (2013) found no significant difference in weight loss between unsalted and 1.5%-salted pork, even if weight loss did tend to decrease.

Weight loss in meat can be usefully modeled to predict the influence of process factors and avoid expensive technological trials. Several modeling approaches have recently been developed in the literature to predict weight loss in meat (Goni & Salvadori, 2010; Kondjoyan, Oillic, Portanguen, & Gros, 2013a; van der Sman, 2007, 2013). Whatever the approach, the prediction of the water transport is always based on the difference between water content in the cooked meat and the equilibrium water content (X_{eq}). Water content is considered to be at equilibrium when loss is no longer observed whatever the duration of treatment. Experimental X_{eq} are generally fitted using a sigmoid function (Goni & Salvadori, 2010; van der Sman, 2007, 2013) (Eq. (1)).

$$X_{eq}(T) = a_0 - \frac{a_1}{1 + a_2 \exp(a_3(T - T_r))}$$
(1)

T is the sample temperature, a_0 indicated the initial water content while T_r a reference temperature; a_1 , a_2 , a_3 , and T_r are unknown parameters which shall be determined from experimental results.

Reliable prediction of weight loss hinges on knowing the precise equilibrium water content value (Oillic et al., 2011). Analysis of transfer phenomena and literature results leads to the conclusion that equilibrium weight loss and equilibrium water content can be affected by raw meat properties, sample dimensions or presence of packaging. It had also been found during a previous study on unsalted beef meat that weight loss during cooking was affected by muscle type and unaffected by pre-cooking processes such as freezing and thawing (Oillic et al., 2011). Thus, a first set of experiment was performed on unsalted pork meat to check whether previous conclusions were also true for pork meat and whether the effect of muscle type can be explained by variations in the initial pH value and in the initial water content of the raw meat. These experiments were completed to analyze the effect of sample dimensions and packaged or unpackaged conditions on the equilibrium water content and weight losses. A third set of experiments was performed on salted samples (without addition of polyphosphates). The influence of salt content was studied both on thin samples, which were uniformly salted without tumbling, and on bigger samples, salted under controlled gentle tumbling conditions. All these results have been interpreted in the light of the literature theories which explain waterprotein debinding and water migration. They were also used to discuss the results of the literature models which use measured water contents to predict the loss of weight during cooking.

2. Materials and methods

2.1. Meat cuts

Experiments were performed on 4 different pork muscles from ten hams, i.e. Semimembranosus (SM), Biceps femoris (BF), Rectus femoris (RF) and Semitendinosus (ST), and one dorsal muscle, i.e. Longissimus thoracis (LT). The four muscles mentioned above were taken from ham of each animal. The meat came from a batch purchased from an industrial manufacturer and considered by him as being homogeneous (drawn at random from a batch for the production of cooked ham). The pigs used were "Piétrain" breed and had a carcass weight of 90 kg on average. The average mass of the hams (with bone) was 10 kg. The muscles were vacuum-packed as soon they were received in the laboratory. The majority of them was frozen before experimentation: whole muscles was slowly frozen at -20 °C and kept frozen less than a month. To study the impact of pre-freezing, three *Semimembranosus* muscles were kept fresh. Samples were cut in fresh or frozen meat (for one week) into 40x5 mm-thick discs (diameter 40 mm) with a slicer or 30 x 30 x 30 mm and 50 x 50 x 50 mm cubes (accuracy 1 mm) with a knife. Frozen samples were vacuum-packed and then immersed in a water bath at 14 °C to be thawed until their core temperature reached 10 °C. Pre-manipulations were performed locating a thermocouple at the center of the samples to determine the time needed to reach a core temperature of 10 °C depending on the samples dimensions. The meat was then cooked vacuum-packed in a water bath at 70 °C for 5, 15, 60 or 120 min.

Water content and pH were measured in six replicates per fresh or thawed raw muscle used. Water content in raw meat (X_0) was

determined by drying 3 to 10 g (5 g on average taken on scraps of muscles) of sample for 24 h in an oven at 104 °C (ED240, Binder, Germany) and by accurately weighing the sample before (m_{DM+w}) and after drying $(m_{DM}, \text{Eq. } (2))$. X₀ is expressed in g of water/g of dry matter (DM).

$$X_{0} = \frac{m_{DM+w} - m_{DM}}{m_{DM}}$$
(2)

 $m_{\text{DM}+\text{w}}$ being the mass of dry matter plus water and, m_{DM} the mass of dry matter.

pH was measured with a penetration sensor (Inlab® Solids, Mettler Toledo and MP230 pH-meter, Mettler Toledo, Switzerland) on 0.6 g of meat ground with 300 μ L of pure water.

To investigate the distribution of water in the same muscle, a 70 x 30 x 23 mm parallelepiped of pork *Semimembranosus* muscle was cut into 27 samples to measure water content.

2.2. Meat salting

Some of the experiments were performed on salted meat. Different salting processes were used to salt the raw meat, depending on the sample dimensions. All of these methods used brine composed solely of water and sodium chloride. Two methods were used to salt disc samples. In the first case, brine (6% by weight) was coated on each disc face for 50 hours, avoiding evaporation (each sample placed in a box covered by a lid), until the meat had absorbed all the brine. It has been empirically determined that salt content in brine has to be 90, 150 and 200 g/L to reach 0.8, 1.3 and 2.0% in meat, respectively. In the second case, the discs were immersed in a bioreactor (Labfors, Infors HT, Switzerland) for 17 h in brine containing 11 or 27 g/L sodium chloride to reach 0.8 and 2.0% in meat, respectively (the previously mentioned bioreactor was used to control pH, temperature and stirring). The brines were stirred at 200 rpm and kept at 5 °C and pH = 5.6. The pH in the bioreactor was automatically controlled by adding hydrochloric acid or sodium hydroxide which ensured that the target pH value (here 5.6) was reached in the meat. Moreover, the use of a bioreactor made it possible to artificially increase the water content in the raw meat in order to study the effect of this initial water content on the weight loss of the sample when heated afterwards in the water bath. Working with thin samples that are saltable without tumbling eliminated the tumbling effect. Concerning cubes, tumbling was inevitable to reduce the salting time. In this case, brines were formulated with 110, 165, 220 or 270 g of NaCl per liter to reach 0.8, 1.3, 1.6 and 2.0% in meat, respectively. Cubes were vacuum-packed with 10% (by weight) of brine and were intermittently tumbled (8 rpm for a total of 2,064 rotations at 2 °C, Inject Star, Austria).

Salt content in salted raw meat was measured by deducing chloride anions by ion chromatography (850 professional IC, Metrohm, France) for which 0.5 g of meat homogenized in 10 mL of pure water was centrifuged at room temperature (11,300 rpm, 10 min) 0.2 mL of the supernatant was collected and diluted in 10 mL of pure water to measure chloride content.

The influence of salt content on equilibrium state after cooking was studied on 40x5-mm discs and tumbled 30 x 30 x 30-mm cubes of *Semimembranosus* pork muscles cooked vacuum-packed at 50 to 90 °C.

2.3. Thermal treatments

Samples were suspended in a thermostat-controlled water bath at a constant temperature of 50, 55, 60, 65, 70, 75, 80, 85 or 90 °C (WNB 29, Memmert, Germany). In the bath water flow was due to free-convection. The samples were heated until the equilibrium time was reached. That means until the temperature of the sample and its weight remained constant. This equilibrium time depended on

sample dimensions *i.e.* 60 min for 40x5-mm discs, 120 min for 30 x 30 x 30-mm cubes and 240 min for 50 x 50 x 50-mm cubes. Premanipulations were performed locating thermocouples at the center of the cube samples to determine the time needed for the core temperature to reach the water bath temperature. For disc the shortest time needed to reach equilibrium was determined by calculations using a heat transfer model. However, this time was not enough to reach equilibrium since, as it had been observed in a previous work, mass transfer was much slower than the heat transfer (Oillic et al., 2011). Thus, equilibrium times were determined by weighting the samples until no more weight loss was observed. In practice an additional period was added to these time values to be sure to be at equilibrium. At the end of the experiment the samples were removed from the water bath and cooled for 10 min in iced water.

Presence of packaging influences the boundary conditions by changing the fluid in contact with the meat: when meat is cooked unpacked in a water bath, the surrounding fluid is water while it is meat juice when meat is cooked vacuum-packed (Multivac A 200/15, Multivac, France). Four packaging conditions were tested either with 40x5 mm discs or with 30 x 30 x 30 mm cubes: 1) meat samples were cooked unpacked in the water bath at a constant temperature; 2) samples were individually vacuum-packed (-0.9 bar, in 90 µm thick bags made of polyethylene and polyamide, and cooked in the water bath; 3) samples were individually vacuum-packed and placed in an aluminium mold (ensuring efficient heat transfer); 4) samples were individually vacuum-packed, placed in an aluminium mold and a 1 kg-deadweight was put on the meat.

Samples were wiped with absorbent paper and weighed before heating (m_0) and after heating and cooling (m_f) . Weighting loss (WL), expressed as a percentage, was calculated from Eq. (3).

$$WL = \frac{m_0 - m_f}{m_0} \times 100$$
 (3)

Cooking juice is often assimilated to water but this is not true as dry matter is also flowing out of the meat with the water. In the recent modeling studies of literature the mass transfer is calculated based on difference of water content in the raw and in the cooked meat (Feyissa, Gernaey, & Adler-Nissen, 2013; Goni & Salvadori, 2010; Kondjoyan et al., 2013a; van der Sman, 2007, 2013). In a previous study the following relation was used to calculate the weight loss from the initial water content in the raw meat X₀ and from the water content at a given time in the heated meat X, (Oillic et al., 2011):

$$WL = \frac{X_0 - X}{1 + X_0} \times 100$$
 (4)

In this relation the water contents are expressed on a dry matter basis and it is assumed that the variation of weight due to the loss of dry matter is negligible compared to the water loss. This assumption was correct on big cubes and for short or moderate cooking time. However, in present study it was measured during preliminary trials that up to 6.40% of dry matter was contained in the juice under the long heating of the 40x5-mm thick discs at 90 °C. In this case, the loss of dry matter in the juice was not negligible compared to the water loss. Moreover since the water content was expressed as a % of dry matter in the meat the water content in the heated meat X, was overestimated compared to the water content in the raw meat X₀. This led to under-predictions of the weight loss by relation (4). This was a problem because an important objective of this paper was to determine the effect of different factors on the weight loss and to discuss the ability of the transfer models to predict this weight loss. Thus, the measured value of the water content in the heated meat at equilibrium X_{eq}, was determined from the weight loss measurements in order to compensate for the dry matter loss and to be able to use the X_{eq} values in the future in

mass transfer models to predict weight losses without bias under conditions which will be different from those encountered in this paper:

$$X_{eq} = X_0 - \frac{WL}{100} \cdot (1 + X_0)$$
(5)

The loss of dry matter in the meat during heating (L_{DM}) was also theoretically calculated using the water contents measured (Eq. (2)) in the heated, X_m, and in the raw meat, X₀, by:

$$L_{DM} = 1 - \frac{DM}{DM_0} = \left(1 - \frac{\left(1 - \frac{CL}{100}\right) \cdot (1 + X_0)}{1 + X_m}\right) \times 100$$
(6)

To fit the experimental X_{eq} -curve with the sigmoid function in Eq. (1), the coefficients (a_0 , a_1 , a_2 , a_3 and T_r) were optimized by minimizing the sum of squared difference between measured and predicted equilibrium water contents at each temperature.

2.4. Statistics

The factors selected from literature as being likely to affect the weight loss were: temperature (50, 55, 60, 65, 70, 75, 80, 85 or 90 °C), sample dimension (40x5-mm discs, 30 x 30 x 30 mm or 50 x 50 x 50 mm cubes), presence of packaging, type of muscle (SM, BF, RT, ST or LT), initial water content and pH₀ in the raw meat, prior freezing and salting (0.8, 1.3, 1.6 or 2.0%) of the raw meat. Each trial was repeated at least three times, giving a total of more than 300 heated samples. Results are reported in the following as means \pm standard error of the mean (SEM). ANOVA was performed to compare means, and levels of statistical significance between groups were assessed using a Tukey test. Level of significance was set at 5%. Correlations were tested using the Pearson coefficient. Statistical analyses were performed using R versions 2.12.2 software.

3. Results and discussion

Weight loss increased with time until equilibrium (Fig. 1). Once equilibrium state was reached, weight loss and water content in meat plateaued. The results reported here are mostly at equilibrium state, *i.e.* equilibrium water content (X_{eq}), equilibrium Weight loss (WL_{eq}) and equilibrium DM loss (L_{DMeq}). The influence of studied parameters on weight loss was analyzed using the results obtained in literature on

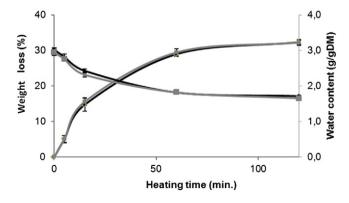


Fig. 1. Evolution of weight loss and water content during heating and impact of prior freezing on cooking loss curve and water content curve for 30 x 30 x 30-mm cubes of pork *Semimembranosus* muscle cooked vacuum-packed in a water bath at 70 °C. Black line: non-frozen samples; gray line: pre-frozen and thawed samples.

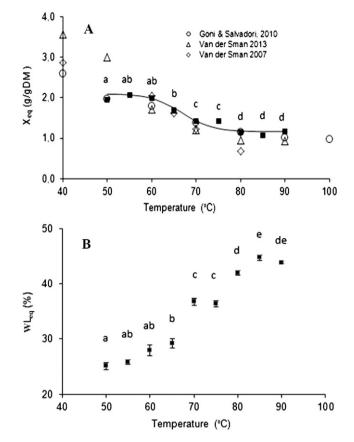


Fig. 2. Impact of temperature on A) equilibrium water content (X_{eq}) and B) equilibrium cooking loss (WL_{eq}) in 5-mm discs of pork *Semimembranosus* muscle heated unpacked in a water bath. Black line: sigmoid curve (Eq. (1)) fitting the experimental data. Letters indicate temperature effects on experimental data (p < 0.05).

the osmotic-like pressure in meat, the debinding of water from proteins and the migration of water in the product.

3.1. Basic transport phenomena: effect of temperature, dimension and surrounding media

Equilibrium states were determined every 5 °C from 50 to 90 °C in unsalted 5-mm semimembranosus discs heated unpacked in the water bath. WL_{eq}, X_{eq} and L_{DMeq} were significantly dependent on temperature (p = 0.000). Fig. 2A shows that the X_{eq}-curve on cubes was sigmoid, with a decrease of Xeg between 60 and 80 °C. WLeg showed almost symmetrical evolution to X_{eq} (Fig. 2B). The X_{eq}-curve was fitted with the sigmoid function used in the literature (Eq. (1)), with $a_0 = 2.10 \text{ g/g DM}$, $a_1 = 0.94$, $a_2 = 9.90$, $a_3 = -0.29$ and $T_r = 58.9$ °C. These parameters were specific of the studied muscle. Meat lost approximately 16% of DM when its final temperature was between 50 and 75 °C, the total loss of DM reached 21% when the final temperature was between 80 and 90 °C (for discs). Any direct comparison with Xeq values from the literature always warrants caution as they are dependent on several parameters (studied in detail below), including surrounding fluid conditions, dimensions, pH in raw meat, etc., as well as on the DM basis used to express X_{eq} . However, the sigmoid shapes of the X_{eq} - and WLeq -curves are similar to those found for beef (Davey & Gilbert, 1974; Goni & Salvadori, 2010) and rabbit (Combes, Lepetit, Darche, & Lebas, 2004). Fig. 2A reports the equilibrium water content values found in the literature for beef (Goni & Salvadori, 2010; van der Sman, 2007) and chicken filets (van der Sman, 2013). There are great difference between studies for T \leq 50 °C but closer values among different species for $T \ge 60$ °C, indicating that for increasing temperatures the

overall mechanism should be the same whatever the species. However, even if the differences in X_{eq} between studies first appear small in Fig. 2A, they can lead to great differences in the calculated WL. For example, after cooking at 80 °C, $X_{eq} = 1.0$ g/g DM for chicken fillets and 1.2 g/g DM for beef meat, yet predicted weight loss was 50 and 45%, respectively (with $X_0 = 3.0$ g/g DM).

The DSC studies reported in the literature reveal that the first protein to denature when heated is myosin (peak transition at 54–58 °C) (Tornberg, 2005). Hence at low temperatures (50 and 55 °C), the slight weight loss should essentially be due to myosin denaturation, resulting in water debinding. It is known in literature that during heating, myosin structure changed – myofibrillar and sarcoplasmic solubility decreased (Davey & Gilbert, 1974) and protein hydrophobicity and aggregation increased (Promeyrat et al., 2013).

According to many authors, protein oxidation might play a major role in the decrease of water holding capacity of meat (Bertram et al., 2007; Huff-Lonergan & Lonergan, 2005; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). For Bertram et al. (2007) and Lund et al. (2007), inter protein cross-links (disulfide or dityrosine cross-links), which lead to protein aggregation, may influence negatively the water holding capacity. Moreover, by generating cross-links, myosin oxidation strengthens the myofibrillar structure and increases shrinking of the overall muscle cell (Lund, Christensen, Fregil, Hviid, & Skibsted, 2008). In addition to oxidation, the increase of protein surface hydrophobicity, observed when increasing temperature (Chelh, Gatellier, & Santé-Lhoutellier, 2006), generates non-covalent protein aggregation and reduces water binding to protein. All these physico-chemical modifications of meat proteins should participate to juice loss.

Meat begins to contract strongly when its temperature reaches 55-60 °C (Bouhrara et al., 2012; Davey & Gilbert, 1974), which coincides with the beginning of a sharp increase in the weight loss (Fig. 2). Meat contraction occurred in the same range of temperature as collagen denaturation. This comfort the mechanisms which proposed that collagen denaturation is a major factor in meat contraction (Miles, Avery, Rodin, & Bailey, 2005; Tornberg, 2005). Within the same muscle, heated collagen follows different denaturation patterns according to its location in the different layers of connective tissue (epimysium, perimysium or endomysium) (Wu, Dutson, & Smith, 1985). During cooking, in a first stage collagen fiber which is located around the myofiber is free to contract. After this first free-contraction stage the contraction of the collagen is limited by the resistance of the myofiber (forced contraction stage). It is assumed in literature that during this forced contraction stage, all other things being equal, the pressure developed by connective tissues is directly related to the stress exerted by collagen fibres (Lepetit, 2008). So, it is expected that the higher the amount of cross linked chains, the higher the thermal contraction of collagen fibres (Lepetit, 2008). The collagen network starts to contract when the temperature reaches 55-60 °C but then this contraction continues over a broad temperature interval that can explain the observed increase in the meat weight loss. When the heating temperature is smaller than 50 °C, it is the water located in the inter-fascicle spaces which is expelled out the sample. When the temperature increases, because of the pressure exerts on the myofibers by collagen water migrates from the intra-myofibril space to the inter-fascicle space (Realini et al., 2013). This migration is likely to bring denatured proteins out of the sample, which would explain the increase in the DM loss at higher temperatures. The partial destruction of cell membranes at high temperatures (Silva, Orcutt, Forrest, Bracker, & Judge, 1993) could also promote DM loss in the cooking juice.

Unsalted SM with three sample dimensions – 40x5-mm discs, 30 x 30 x 30-mm cubes and 50 x 50 x 50-mm cubes – was cooked unpacked at 50, 70 and 90 °C until the equilibrium state was reached (*i.e.* for 60, 120 and 240 min, respectively). Dimension had a significant effect (p = 0.000) on equilibrium state (Fig. 3). Equilibrium water content was lower in discs than in cubes, meaning higher equilibrium weight loss. Differences in equilibrium water content in both sizes of cubes were only significant at 50 °C (P < 0.05). At higher temperature,

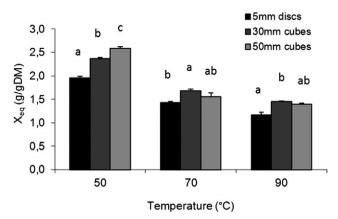


Fig. 3. Impact of sample dimensions on equilibrium water content (X_{eq}) in pork *Semimembranosus* muscle samples cooked unpacked in a water bath. Letters indicate dimension effects (p < 0.05) at constant heating temperature.

equilibrium water content was identical in all cubes whatever the sample dimensions (Fig. 3). Moreover, loss of DM in meat was systematically significantly higher in discs than in cubes (P < 0.05), while no difference was observed between cubes (not shown). Except at low temperature (50 °C), dimension did not influence equilibrium state after heating the cubes, as shown elsewhere for beef meat (Bouton, Harris, & Shorthose, 1976; Oillic et al., 2011).

Influence of the surrounding medium (water or juice) was studied on 40x5-mm discs and 30 x 30 x 30-mm cubes heated unpacked or vacuum-packed in a water bath. The presence of the packaging introduced and additional resistance to the heat transfer and thus modifies the temperature kinetic in the product. However, this resistance did not affect the equilibrium temperature which was equal to the water bath temperature. This was checked during preliminary simulations based on measured heat transfer coefficient values. Since the results in Table 2 have been measured long after the meat temperature has reached the water bath temperature there were not affected by the difference in the heat transfer conditions due to the packaging. However, difference of mass transfer at equilibrium (WL_{eq}, X_{eq} and L_{DMeq}) between the packed and unpacked conditions were significant (p < 0.05) for both the heated discs and cubes (Table 2). Unpacked samples had significantly higher weight loss (P < 0.05), lower water content and higher DM loss at equilibrium than packed samples. The influence of the surrounding fluid was higher at low temperature and on small samples (Table 2). The strong difference in weight loss observed between unpacked and packed samples at 50 °C occurred in the range of temperature of myosin denaturing which suggested a probable connection between the two phenomena. The difference in weight loss could be due to the difference in ion diffusion between the two treatment conditions. Meat heated in a sous vide bag is kept in contact with the cooking juice. Juice is coming from the water in the meat. This water phase contains ions which contribute to the osmotic pressure in the meat. There is no reason why these ions shall not flow out of the meat in the juice. Thus one can reasonably assume that there is almost the same ion concentration in the juice as in the meat piece, this would mean a close to zero ion diffusion. In the case of unpacked samples, ions are directly expelled into the water bath used as heating medium. This means an infinite dilution boundary condition for meatreleased ions and thus an acceleration of the ions diffusion out of the sample. These variations in the concentration of ions into the meat can have affected the protein denaturing. So, Hamm and Deatherage (1960) have demonstrated that divalent cations bound to muscle proteins can be released during heating like free ions and have also shown that some cations $(Mg^{2+} and Mn^{2+})$ have the ability to protect proteins from thermal denaturation.

At low heating temperature, the previously described ion diffusion process predominated, which would explain the higher differences in

Table 2

Influence of boundary conditions on equilibrium weight loss (WLeq), equilibrium water content (Xeq) and equilibrium dry matter loss (L_{DMeq}).

Dimension, X ₀ , pH ₀	Temperature (°C)	Boundary conditions	WL _{eq} (%)	$X_{eq} (g/gDM)$	L _{DMeq} (%)
Disc - 5 mm	50	Unpacked	$21.7\pm0.5a$	$1.99\pm0.02a$	$15\pm3a$
$X_0 = 2.8 \text{ g/gDM}$ pH ₀ = 5.8		Vacuum-packed	$10.9\pm0.9b$	$2.41\pm0.03b$	$2\pm 2b$
Disc - 5 mm	70	Unpacked	$35.1 \pm 0.2a$	$1.39 \pm 0.01a$	$11 \pm 1a$
$X_0 = 2.7 \text{ g/gDM}$ pH ₀ = 5.6		Vacuum-packed	$31.6\pm0.7b$	$1.52\pm0.02b$	$11\pm0a$
Disc - 5 mm	80	Unpacked	$40.6 \pm 0.5a$	$1.38\pm0.02a$	$18\pm0a$
$X_0 = 3.0 \text{ g/gDM}$ $pH_0 = 6.2$		Vacuum-packed	$35.9\pm0.1b$	$1.56\pm0.01b$	$10\pm0b$
Disc - 5 mm	90	Unpacked	$43.9\pm0.3a$	$1.24 \pm 0.01a$	$21\pm1a$
$X_0 = 3.0 \text{ g/gDM}$ $pH_0 = 6.2$		Vacuum-packed	$39.4\pm0.0b$	$1.42\pm0.00b$	$13\pm0b$
Cube - 3 cm	50	Unpacked	$17.1 \pm 0.1a$	$2.25\pm0.00a$	$12 \pm 1a$
$X_0 = 2.9 \text{ g/gDM}$ $pH_0 = 5.6$		Vacuum-packed	$11.1\pm0.5b$	$2.48\pm0.02b$	$5\pm0b$
Cube - 3 cm	70	Unpacked	$32.1 \pm 0.5a$	$1.76 \pm 0.02a$	$8\pm2a$
$\begin{array}{l} X_0 = 3.1 \ g/gDM \\ pH_0 = 5.8 \end{array}$		Vacuum-packed	$29.5 \pm 1.0 a$	$1.87\pm0.04a$	$3\pm 2a$

Packaging effect: letters indicate significant difference in a column for each temperature/'dimenion-X₀' pair.

With X₀ and pH₀, the initial values of water content and pH of the samples.

weight loss observed at 50 °C. Ion diffusivity in raw meat can reach 5.0 10^{-10} m².s⁻¹ (Sharedeh, Favier, Auberger, Portanguen, & Daudin, 2012), *i.e.* 1.8 mm².h⁻¹. Therefore, in our range of experimental conditions. Thus, ions diffusion should only have affected a limited portion of the product. This can explain why greater differences in mass transfer were found between packed *vs* unpacked discs rather than between packed *vs* unpacked cubes. For instance, at 50 °C, the difference in weight loss between the unpacked and packed samples was 1.8-fold higher for discs than for cubes (10.8% *vs* 6.0% respectively, Table 2).

When meat was heated above 70 °C, weight losses increased strongly and were accompanied by a smaller difference in packed *vs* unpacked loss. This suggested a change in the transport phenomena which evolved from diffusion to a contraction -driven mechanism; meat contraction having been observed on beef meat at this temperature (Bouhrara et al., 2012). Fig. 3 shows that there was also little difference in weight losses between discs and cubes heated directly in water at 70 °C, where contraction is observed in literature.

Tests were performed to determine whether applying constraint forces to the meat surfaces has an effect on sample weight loss. When vacuum-packed cube samples were cooked with or without an additional 1 kg deadweight, WL_{eq} and X_{eq} were the same whatever the pressure applied.

3.2. Impact of the properties of the raw unsalted sample

X₀ varied from 2.8 to 3.0 g/g DM depending on location in the muscle. X₀ and pH₀ then measured on 104 different semimembranosus muscles varied from 2.5 to 3.3 g/g DM and 5.5 to 6.1 respectively, with 50% of muscles having water content in the range 2.9-3.1 g/g DM and pH₀ in the range 5.6–5.8. Thus, if the difference observed inside the sampled muscle is representative of what exists inside each of the muscles, the variability in X₀ in the same muscle remained lower than the interanimal variability. Among raw SM muscles, X₀ and pH₀ were significantly (p = 0.000) and positively (r = 0.38) correlated. To identify whether this heterogeneity in X₀ could influence equilibrium state in cooked meat, 40x5-mm discs cut in Semimembranosus of varying raw water content (from 2.5 to 3.0 g/g DM) were cooked in a water bath at 65, 75, 80 or 90 °C. As water content in the raw meat was not controllable, it was impossible to achieve this manipulation in other conditions. The water content of 2.5 g/g DM was highly unusual for pork Semimembranosus muscle. After being heated at 65 or 75 °C, the samples with the lowest X₀ and pH₀ had lower X_{eq} and higher WL_{eq} than the other samples (Table 3). At higher temperatures (80 and 90 °C), differences in WL_{eq} disappeared while differences in X_{eq}, although reduced, remained significant (P < 0.05). Moreover, when meat was cooked above 75 °C, the samples with the lowest X_0 and pH_0 lost more DM than the other samples (Table 3). When comparing $X_0 =$ 2.8 and 3.0 g/g DM, the differences in X_{eq} and WL_{eq}, although still persistent, were nevertheless weaker, in particular at high temperature (Table 3). When salting was performed by brine coating (pH of the raw meat not controlled by immersion), the observed effect on X_{eq} and WLeq could be mostly due to the variability of the pH in the raw meat: indeed it is known that weight loss decreases as meat pH moves away from the pI of meat protein (5.0) (Hamm & Deatherage, 1960). During cooking, meat pH increased from 0.2 to 0.4 and this increase was dependent both on cooking temperature and, when meat was salted by brine coating, on the pH₀ of the raw meat (Hamm & Deatherage, 1960). Hence the reduced effect of X₀ and pH₀ on WL_{eq} at high temperature (80-90 °C) could be due to both the increased pH and the higher meat contraction.

Table 4 summarizes the raw meat characterization (X_0 and pH_0) of the 5 studied muscles: RF, ST, SM, LT and BF. Raw-meat water content varied from 3.0 to 3.6 g/g DM depending on muscle type. Through muscle type, X_0 was significantly (p = 0.002) correlated with pH_0 (r = 0.65): muscles with high water content tended to have higher pH_0 . The equilibrium state (X_{eq} , WL_{eq} and L_{DMeq}) was dependent on

Table 3

Influence of raw meat characterization (water content X_0 and pH_0) on equilibrium weight loss (WL_{eq}), equilibrium water content (X_{eq}) and equilibrium dry matter loss (L_{DMeq}) in 5-mm discs of pork *Semimembranosus* muscle cooked unpacked in a water bath at different temperatures.

	Heating temperature (°C)	Sample 1	Sample 2	Sample 3
X ₀ (g/gDM) pH ₀		$\begin{array}{c} \textbf{2.47} \pm \textbf{0.08a} \\ \textbf{5.47} \pm \textbf{0.02a} \end{array}$	$\begin{array}{c} 2.76 \pm 0.03 b \\ 5.75 \pm 0.04 b \end{array}$	$\begin{array}{c} 3.02 \pm 0.02c \\ 5.98 \pm 0.04c \end{array}$
X_{eq} (g/gDM)	65	$1.34\pm0.02a$	$1.57 \pm 0.03b$	$1.98 \pm 0.02c$
	75	$1.13\pm0.02a$	$1.36\pm0.02b$	$1.63 \pm 0.03c$
	80	$1.02\pm0.03a$	$1.29\pm0.02b$	$1.38\pm0.02b$
	90	$0.91\pm0.01a$	$1.16\pm0.07b$	$1.24\pm0.01b$
WL _{eq} (%)	65	$32.6\pm0.6a$	$30.6\pm0.9a$	$26.1\pm0.6b$
	75	$38.8\pm0.5a$	$36.7\pm0.6ab$	$34.9\pm0.7b$
	80	$41.4\pm0.8a$	$40.9\pm0.5a$	$40.6\pm0.5a$
	90	$44.8\pm0.2a$	$43.6\pm0.5a$	$43.9\pm0.3a$
L _{DMeq} (%)	65	$16 \pm 3a$	$18 \pm 1a$	$16 \pm 1a$
	75	$20 \pm 2a$	$15 \pm 1b$	$15 \pm 1b$
	80	$26 \pm 1a$	$19 \pm 1b$	$18 \pm 0b$
	90	$28 \pm 1a$	$17 \pm 4b$	$21 \pm 1b$

Letters indicate significant differences (p < 0.05) in a row.

Table 4

Influence of muscle type on characterization of raw meat and on equilibrium water content (X_{eq}) and equilibrium weight loss (WL_{eq}) in 30 x 30 x 30-mm cube samples cooked unpacked in a water bath (120 min).

	Raw meat	Raw meat		Heated meat				
			X _{eq} (g/gDM)		WLeq (%)			
Muscle	pHo	$X_0 (g/gDM)$	50 °C	70 °C	50 °C	70 °C		
RF*	$6.13\pm0.06a$	$3.55\pm0.04a$	$3.24\pm0.18a$	$1.74\pm0.06a$	7.4 ± 4.0b	$39.7\pm0.5a$		
ST*	$6.26 \pm 0.06a$	$3.24 \pm 0.08b$	$2.71 \pm 0.28b$	$1.70 \pm 0.05a$	$10.1 \pm 2.5b$	38.2 ± 1.1 ab		
BF*	$5.76 \pm 0.04 bc$	$3.05 \pm 0.03 bc$	$2.21 \pm 0.01b$	$1.43 \pm 0.01b$	$21.1\pm0.3a$	$39.9\pm0.1a$		
LT*	$5.43\pm0.04c$	$3.02 \pm 0.04 bc$	$2.32 \pm 0.01b$	1.58 ± 0.01 ab	$18.8\pm0.3a$	$34.7 \pm 0.3 bc$		
SM*	$5.79 \pm 0.05b$	$3.03 \pm 0.04c$	$2.36\pm0.04b$	$1.69\pm0.03a$	$17.1\pm0.7a$	32.8 ± 0.9c		

Letters indicate significant differences (p < 0.05) in a column.

With X₀ and pH₀, the initial values of water content and pH of the samples.

* RF = Rectus femoris, ST = Semitendinosus, SM = Semimembranosus, LT = Longissimus thoracis, BF = Biceps femoris.

muscle type (p = 0.000). At 50 °C, muscles with higher pH₀ and X₀ (RF and ST) had lower WL_{eq} than the other muscles (Table 4), generalizing the impact of raw meat properties (X₀ and pH₀) on the weight loss observed in the same muscle type (SM) at low temperature. At 70 °C, ST, RF and BF lost more juice than LT and SM (Table 4). BF and ST have higher collagen content than LT and SM (Wheeler, Shackelford, & Koohmaraie, 2000), which should lead to greater meat contraction and thus more expulsed juice. This interpretation is in accordance with that of Brunton, Lyng, Zhang, and Jacquier (2006) who mentioned the previous works of Offer and Trinick (1983) and Bendall and Restall (1983).

3.3. Impact of prior freezing

Water content and pH in raw muscle was not pre-freezingdependent (p = 0.292), measuring 3.0 ± 0.1 g/g DM and 5.6 ± 0.1 regardless of whether the meat had been pre-frozen. The quantity of thawing juice was not measured here but has been estimated between 0.9 to 6.8% in the literature and is dependent on freezing and thawing rate (Yu et al., 2010), frozen temperature, and meat pH (Mortensen, Andersen, Engelsen, & Bertram, 2006). Assuming the highest thawing loss (7%), raw-meat water content would be 2.7 g/g DM after thawing if thawing juice was pure water. However, as thawing juice is a mix of water and roughly 16% DM (Thyholt & Isaksson, 1997), raw-meat water content after thawing would be 2.9 g/g DM – a value contained within the experimental variation of the water content in the raw meat (*i.e.* \pm 0.1 g/g DM). Thawing juice thus had a negligible impact on raw-meat water content due to DM loss in the thawing juice. According to literature, rapid freezing forms numerous small ice crystals while slow freezing forms bigger crystals which can alter the cell membrane, leading to higher juice loss (Hardman, 1989). In the present case, the freezing of the muscles was slow and the frozen muscles were stored for a short period (less than one month) before being cooked. Under these conditions pre-freezing did not influence water content in the cooked pork nor the weight losses (Fig. 1), as shown elsewhere for beef muscles (Oillic et al., 2011). The lack of effect of frozen storage could be linked to the fact that a short frozen storage (less than a month) did not influence myofibrillar protein denaturation (Paredi, Pagano, & Crupkin, 2010; Xia, Kong, Liu, & Liu, 2009). However, further research is needed to relate the structural change of meat and the protein denaturation to the weight losses of pre-frozen meat samples. For experimental convenience, the other experiments were performed on pre-frozen meat.

3.4. Impact of salting

Raw-meat water content can be enhanced by injection or by immersion in salted water. Two salting methods were used to salt discs: immersion and aspersion. Immersion led to a drastic increase (p = 0.000) in water content from 2.99 ± 0.04 g/g DM in raw meat to 4.40 ± 0.08 g/g DM in raw salted meat, *i.e.* an average weight gain

of 35%. This gain was concordant with the data of Wu et al. (2006) who observed a 25 to 45% weight gain during 48 h immersion in brine. When samples were salted by aspersion, water content was the same in unsalted and salted meat (p = 0.932). Meat pH was between 5.6 and 5.7 and X_{eq} differed strongly between immersed and non-immersed samples at 50 °C, ($\Delta X_{eq} = 1.03$ g/g DM) but was no longer significantly different at 70 °C (ΔX_{eq} = 0.10 g/g DM). At 70 °C, the decrease of water content was faster for higher initial water content samples, rapidly (5 min) reaching the same water content as "non-enhanced samples". A drastic increase of initial water content had an effect on water content in cooked meat, but only when temperature was lower than the temperature at which meat started to contract (i.e. 55-60 °C): the same quantity of water gained was lost as soon as the meat contracted. In industrial meat processing, brine injection is often used to salt products, which increases in raw-meat water content. Then muscles are tumbled.

Salt content significantly influenced WLeq and Xeq but not LDMeq. Results for cubes were similar for 40x5-mm discs and are presented in Fig. 4. Equilibrium weight losses were higher for unsalted than salted samples, but without significant difference in equilibrium weight loss between sample salting conditions. In the literature, any reported differences in weight loss due to 0.5% differences in salt content remain small. To be quantified, they would require repeating the experiments with the same muscle type, having the same pH and the same X_0 , and using a wide range of temperature conditions, and the analyst would also have make doubly sure that the sample is uniformly salted. However, these conditions are not always verified in literature reports. Here, the experiments were heavily repeated between 50 and 90 °C using the same muscle type, controlling pH, and measuring the X₀ value of each sample. We did not observe any statistical effect of salt content as soon as the sample was salted. This was true whatever the temperature, even if trends started to emerge at 70 °C. In presence of salt (0.8-2%), the increase of salt content led to an increase in osmotic-like pressure in the meat (Offer & Knight, 1988) and an increase in protein denaturation (Villamonte et al., 2013). These two phenomena had contradictory effects - increasing water retention and increasing water debinding - that could have cancelled each other out, which would explain the absence of differences in weight loss in our experiments.

3.5. Effect of previous results on the prediction of the weight loss

The results showed that the primary factor influencing weight loss was cooking temperature. At temperatures below the 55–60 °C range weight loss is small while it increases greatly above this threshold. This can be connected to what is known in literature on the denaturation and contraction of the major proteins which constitute the muscle. Below the previous temperature threshold myosin denatures, while above this threshold collagen contracts. According to Hardman (1989), the denaturation of myosin starts about 40 °C ending at about 60 °C, followed by sarcoplasmic proteins and collagen denaturation. Finally, the myofibrillar protein actin or actomyosin denatures between

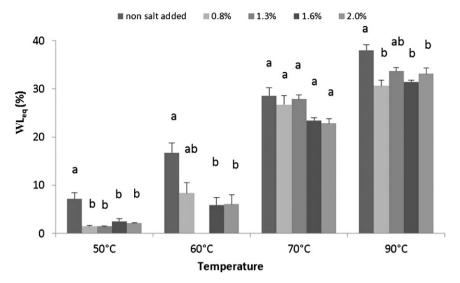


Fig. 4. Influence of salt content on equilibrium weight loss (WL_{eq}) from 30 x 30 x 30-mm cubes of pork *Semimembranosus* muscle cooked vacuum-packed in a water bath at 50, 60, 70 or 90 °C. Lowercase letters indicate salt content effects (p < 0.05) at constant heating temperature.

75-80 °C. A handful of factors tested had no significant influence on weight loss: pre-freezing the meat, mold pressure during cooking, and varying salt content from 0.8% to 2.0%. The other factors tested (pH_0) and X_0 , boundary conditions, size, water enhancement) had a strong impact at 50 °C but less if any effect above the temperature triggering collagen contraction. Testing the boundary conditions revealed a strong impact of ion diffusion on water debinding in the thin-sliced meat samples. Raw material variability had effects on subsequent weight losses: low pH_0 (close to 5.5), which was correlated with low initial water content X_0 , led to higher weight losses than the meat with the higher pH_0 (6.0). This was probably due to the fact that the proteins move closer to their pI which has been found to be close to 5.0 on unsalted minced pork meat (Offer & Knight, 1988). French industry practice selects meat based on pH: the average pH accepted for the ham industry is 5.6-5.9, and ham is usually cooked at a temperature of 70 °C. Our results showed that an increase of pH from 5.8 to 6.0 in raw meat could lead to a decrease of between 1.8% and 4.5% in weight loss at a temperature of 70 °C. The muscle-type effect was explained by an effect of pH_0 and X_0 coupled with an effect of different composition at 50 °C and by different meat contraction dynamics at 70 °C.

4. Conclusion

This study, working under fully controlled experimental conditions, highlighted the key influential factors of the weight losses and water content in water bath heated meat. The effects of these different factors have been interpreted using the knowledge of literature on the denaturation and contraction of proteins, or on water migration but without measuring these phenomena.

Weight loss can be actually predicted from mass transfer models based on the water content components by taking certain precautions. The water content in meat can be determined from the difference between water content at a given time and equilibrium water content $(X - X_{eq})$ (Kondjoyan et al., 2013a). However, the water content calculation needs to integrate dry matter loss, Eq. (4) clearly shows that predicted weight loss was highly sensitive to X_0 which was pH₀-dependent and had an impact on X_{eq} . The analysis of raw meat shows that the X_{eq} determined in order to predict weight loss has to be measured on samples with pH₀ and X_0 values not too far from the average (e.g. for pork SM: $X_0 \sim 3.0$ g/g DM and pH₀ ~ 5.7). The prediction will then be accurate however for a limited range of X_0 and pH₀. This is coherent with the simulation of a real production of cooked hams since industry selects

raw meat based on pH₀ (at 5.6–5.9). For "unusual" pH₀ and X₀, prediction will nevertheless be able to highlight the relative influence of processes on weight loss. X_0 and X_{eq} can also be measured for the new range of pH or for the new composition of the product. X_{eq} has however to be determined on several temperatures, and a smoothing function (Eq. (1)) makes it possible to assess X_{eq} at each temperature. We investigated the influence of dimension-related variation of Xeq in the prediction of WL_{eq} (Table 5). Predicted WL_{eq} from the mean X_{eq} found on cubes (30 x 30 x 30 mm cubes plus 50 x 50 x 50 cm cubes) led to a maximal absolute difference of 2.7% compared to the WLeq predicted from the measured X_{eq}. The maximal difference was reached at 50 °C and was lower at higher temperature. X_{eq} has to be determined on cubes to be able to predict weight loss whatever the meat dimension. Determining X_{eq} on small samples, as Goni and Salvadori (2010) did, could introduce a bias in the predicted weight loss that may reach 15% at low temperature (Table 5). Because X_{eq} was dependent on muscle type, on cooking process and on presence of salt, predictions have to factor in conditions such as cooking in-bag or directly in water ("courtbouillon"), and presence or absence of salt.

On the contrary, weight loss is unaffected by constraints applied at the meat sample surface or whether or not the sample is pre-frozen, and is also probably unaffected by the shape (disc or cube) or size of this sample. Unsalted and 0.8% salt content samples showed differences in water content and weight loss under our experimental conditions. However, samples with salt contents varying from 0.8 to 2% showed no quantifiable differences. If this finding is confirmed for

Table 5

Influence of the determination of X_{eq} on the prediction of the weight loss from Eq. (4), with $X_0=3.0$ g/gDM.

	T (°C)	Experimental X _{eq} (g/gMS ₀)	WL _{eq1} * (%)	WL _{eq2} ** (%)	WL _{eq1} - WL _{eq2} (%)	WL _{eq3} *** (%)	WL _{eq1} - WL _{eq3} (%)
30 mm	50	2.36	15.9	13.2	-2.7	26.0	10.1
cubes	70	1.69	32.9	34.5	1.6	39.4	6.5
	90	1.45	38.8	39.5	0.7	45.9	7.0
50 mm	50	2.58	10.4	13.2	2.7	26.0	15.6
cubes	70	1.56	36.0	34.5	-1.6	39.4	3.3
	90	1.39	40.2	39.5	-0.7	45.9	5.7

Eq. (4) used for calculations: $WL = \frac{X_0 - X}{1 + X_0} \times 100$.

* WL_{eq1} predicted from experimental X_{eq.}

** WL_{eq2} predicted from the mean of X_{eq} for cubes.

*** WL_{eq3} predicted from the X_{eq} for discs.

injected and tumbled meat muscle, it would enable industry processors to reduce product salt content without having to use additives like polyphosphates.

Nomenclature

DM	dry matter
Х	water content
WL	weight loss
L _{DM}	loss of dry matter
SEM	standard error of the mean
WL	weight loss

Muscle type

SM	Semimembranosus
BF	Biceps femoris
DE	Pactus famoris

- RF Rectus femoris ST Semitendinosus
- LT Longissimus thoracis
- LI LONGISSINUS INOTUCI

Subscripts

0	value for the raw sample
ea	value at the equilibrium state

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Conflict of interest

There is no conflict of interest.

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