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Full Length Research Paper

Is the pH drop profile curvilinear and either monophasic or polyphasic? Consequences on the ultimate bovine meat texture

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In meat science, pH has always been considered as an important determinant of meat quality including juiciness and tenderness. Muscle acidification is generally believed to be a linear continuous and monophasic process. From examples provided in the literature, we showed that this was not the case and concluded that the pH profile is either exponential or sigmoidal but never linear. In addition these examples demonstrate that the profile is polyphasic. On this basis the pH drop profile was analysed in bovine *Longissimus* muscle from 100 animals of different age, gender and breed, a choice aiming at increasing the variability of the ultimate tenderness. The results clearly indicate that most if not all animals present one or two transient decreases in the rate of pH fall leading to the appearance of plateau-like discontinuities. These transient stability of the pH are always observed soon after death (<8-9h) and within a pH range of 6.2 to 6.8. In beef, ultimate toughness of *Longissimus* muscle from animals showing one pH stability step is significantly lower than that observed for animals showing two steps. Animals with one step further showed a lower initial rate of pH drop and a lower extent of pH fall. Animal age and sex affect tenderness which is higher for females and decreases with animal age. This relationship between the number of pH stability steps and the ultimate toughness of meat was confirmed using these other sources of variation in the ultimate quality of meat. Regarding the different groups of age, a significant and linear relationship was found between the ultimate meat toughness and the percentage of animals showing either one step or two steps (r = 0.92). Similarly, a significantly greater number of animals present one pH stability step in females than in males. Taken together, these results demonstrated that muscle from animals showing only one stability step will provide more tender meat irrespective of their breed, sex and age. The potential origins of the pH stability steps and their relationship with meat toughness were then discussed.

Key words: pH, muscle, pH stability steps, meat toughness, cell death.

INTRODUCTION

The rate and the extent of post mortem pH decline have been always considered to be of prime importance to ultimate meat qualities. According to Bendall (1973) and Warriss, Bevis and Ekins (1989), the characteristics of the pH decline are determined by the physiological condition of the muscles at the time of stunning, and can be related to lactate production, or to be more specific of the capacity of the muscle to produce energy in the form of

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Table 1. Bovine animals used in the present experiment: classification according to their breed, gender and age.

Breeds	Gender	Age (months)						Total
		15	19	24	54	78	102	
Aubrac	F				4	2	4	10
	M	3	2	7				12
Charolais	F				2	5	6	13
	M	4	4	7				15
Limousin	F				4	4	6	14
	M	3	4	6				13
Salers	F				2	1	6	9
	M	2	5	7				14
Total		12	15	27	12	12	22	100

of ATP. It has been shown that genotype as well as muscle type and amount of stress imposed on the animals in connection with pre-slaughter handling are major causes of the variations observed in pH decline. Consequently, the capacity of the muscle to produce ATP at the time of stunning is a result of an interaction between genotype, muscle type and environmental factors. (Monin et al., 1986; Rahelic and Puac, 1980-81; Fuji et al., 1991; Le Roy et al., 1990; Monin et al., 1986).

In vivo, ATP is the immediate source of energy for muscle contraction. Although a muscle fibre contains only enough ATP to power a few twitches, its ATP "pool" is replenished as needed. There are three sources of high-energy phosphate to keep the ATP pool filled, i.e. creatine phosphate, glycogen and cellular respiration in the mitochondria.

The pool of creatine phosphate in the fibre is about 10 times larger than that of ATP and thus serves as a modest reservoir of ATP. Skeletal muscle fibers contain about 1% glycogen. The muscle fibre can degrade this glycogen by glycogenolysis producing glucose-1-phosphate. This enters the glycolytic pathway to yield two molecules of ATP for each pair of lactic acid molecules produced. Not much, but enough to keep the muscle functioning if it fails to receive sufficient oxygen to meet its ATP needs by respiration. However, this source is limited and eventually the muscle must depend on cellular respiration.

After bleeding, very limited amounts of blood is present in muscle and oxygen supply from blood is very likely negligible. In such hypoxic environment, glycolysis and to a much lower extent, creatine phosphate, will constitute the last resort for energy production but cells cannot maintain anaerobic respiration for an extended length of time. Glycolysis generates lactic acid which is the major cause of the concomitant postmortem acidification of muscle tissue.

According to a large set of reports and in contrast to the conclusions of Bendall (1973), pH drop is mostly considered as a pseudo-linear process and the rate of pH fall was determined by linear regression analysis using the first experimental values (Henckel et al., 2000). Some others estimate the rate of pH fall by measuring the pH value at predifined times and most commonly at 45 min, 3 or 6 h postmortem (Klont et al., 2000). The two questions arising are (a) is the pH drop profile linear or curvilinear? and ((b) is it monophasic or polyphasic?

In the present report, we provide evidence supporting that the pH drop profile is curvilinear and polyphasic using data taken from published works and personal data. The consequence of the number of phase on ultimate beef toughness was further analysed and discussed.

MATERIALS AND METHODS

Animals and sampling

Amongst 164 bovine animals of different breeds, sex and age slaughtered at the abattoir of the INRA Research Centre for another experiment, 100 were used for the present study. The number of animals within each group (sex and age groups) is depicted in Table 1. After captive-bolt stunning, exsanguination and dressing, M. *Longissimus* was excised within 45-50 min after stunning. One *Longissimus* muscle was used for toughness measurement whereas biochemical and physicochemical parameters were assessed on the other muscle. Five 5 cm thick transversal slices (≈300 to 400 g each) were cut off from the last muscle. Two of them were used for pH, osmolarity and water retention measurements during the 10 first hours following animal bleeding (Day 0), one at times 1, 2 and 3 h post-mortem and the other at time 4, 6 and 8 h post-mortem. Four of the five cuts were immediately vaccum packed and immersed in a water bath at 12°C. From the last sample, we took 20 to 30 g piece of muscle and vacuum packed the remaining part. Three pieces of 1 g of muscle (3 replicates) were cut off from this sample, immersed in 9 ml of cold 5 mM iodoacetate and homogenised with a polytron homogeniser for subsequent pH measurement. This procedure was repeated at each sampling times. At 24 h all samples were transferred to the cold room at 4°C until subsequent use.

No difference in temperature was found between meat samples from one experiment to another and 12°C was reached on average

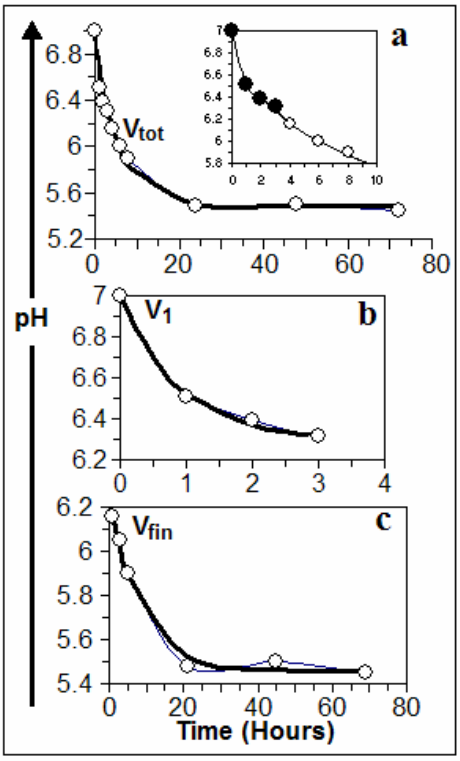


Figure 1. Curve fitting efficiency using an exponential decay of the pH for a muscle showing only one pH stability step (insert Figure 1a). (a) Fitting of the whole curve to determine the constant rate Vtotal. Open circles represent the experimental values and the thick black line is the fitted curve. The insert located the first pH stability step (closed circles) between 1 and 3 h post mortem. (b) Determination of V1 by fitting the curve at the level of the pH stability step. (c) Determination of Vfinal by fitting the rest of the pH drop curve after translocation of the points to time 0, a modification enabling the verification of the maximum value estimated by the exponential model.

at 80 ± 3 min after animal exsanguinations. This parameter was therefore no more considered.

pH measurement

1 g of muscle cut into small pieces with a scalpel was immersed in 9 ml of 5 mM cold iodoacetate and homogenised with a polytron homogeniser. Muscle pH was then measured with a combined glass electrode using a WTW 537 pH meter (Amilabo, Lyon, France).

Rheological assessment of meat toughness

Muscle toughness was assessed on raw meat according to Lepetit and Buffiere (1995). This measurement is independent on the collagen content enabling comparison of muscles with various amounts of connective tissue (Lepetit, 1991).

Fitting of the pH profile for rate constant determination

This was performed using the following exponential equation:

$$pH_{time\ t} = [(pH_{max}-pH_{min})^* \exp(-k_{pH} * t)]+pH_{min}$$

where pH_{max} and pH_{min} were the maximum and minimum pH values; k_{pH}, the rate constant of pH fall, and t, the length of post-mortem storage (hours). Rate constant were determined for all parts of the polyphasic pH drop curve (Figure 1) including the first (V1) and the second step (V2) when present, the part of the profile between the last step and 72 h post-mortem (Vfinal) and the whole profile (Vtotal). Fitting was carried out with the Microsoft excel XP solver using the least square method.

Assuming that glycolysis is the only one process taking place in post-mortem muscle, we can effectively expect a linear pH decline. However, this assumption does not agree with the modern view of cell biology which is far different from the scientific basis of the proposal of Bendall (1973). Our actual understanding of cell biology strongly supports the idea that glycolysis will be faced to antagonist processes which will preserve cells from acidification and fight for cell survival. Hence, considering that pH is a discontinuous and polyphasic process, we are allowed to determine the rate of pH fall within each phase identified even if the identity of the underlying active antagonist process remain unknown.

Data collection from the literature

Published profiles of pH decline were scanned and curves digitized using the UnScanIt program from Ritme Informatique (Paris).

Statistical analysis

Comparison of mean values was carried out using the Student's t test and differences were significant for $p<0.05$. ANOVA was performed using XLSTAT from Addinsoft (Paris).

RESULTS

Is the pH drop profile curvilinear and either monophasic or polyphasic?

To analyse these aspects, we first refer to published data found in the literature. Bond and Warner (2007) recently reported the pH drop profile observed in *Longissimus* muscle from lambs subjected or not (control) (n=6) to exercise (n=6) before stunning. Each point of the plot presented in Figure 2a is the mean value for 6 lambs. Both profiles are clearly not linear. The pH decrease is indeed either sigmoidal (control group) or exponential (exercised group). Refined examination of the profiles showed that in control animals, the pH value is quite similar between times 30 and 60 min (grey closed circles). The only one linear region of the profile was indicated by a square between 3 and 5 h post-mortem. In exercised animals, a transient change in the rate of pH fall can be detected between 120 and 150 min as compared to previous points (grey closed triangles). The slope of the curve increased between these sampling times.

In most published works, the starting pH value, i.e. the physiological value corresponding to time 0, which is assumed to be about 7.0, was almost always not included in the profiles. Inclusion of the starting physiological

value as the first data point will make it possible to estimate the initial speed of pH fall assuming an exponential decay (inserts Figure 2a). The transient changes in the rate of pH fall observed at times 30 min and 2 h for control and exercised animals respectively, are all the more realistic that each point is the mean value for 6 animals, a phenomenon known to tighten the differences.

A second example was found in the paper from Henckel et al. (2000) who analysed the effect of exercise and adrenaline on the pH drop in pig *Longissimus* muscle. For our purpose, we considered only the exercise effect and compared the profile of exercised and control animals. Figure 2b shows the plots of pH decline versus time for exercised and resting animals without (Figure 2b) or with (insert Figure 2b) the initial value, i.e. pH 7.0, respectively (adapted from Henckel et al., 2000). As depicted in Figure 2b, it can be stressed that the pH profile is overall not linear but rather exponential. In addition, the pH decrease seems to be regular and without any transitional change in the rate. Magnification of the first points region by expanding the time scale and by introducing the initial physiological value revealed that a transient increase in the rate of pH fall can be detected at 15 and 45 min for exercised and control animals, respectively (large insert of Figure 2b). Furthermore, the initial rate of pH fall appears much higher in the exercised animal as compared to the control animal (small insert of Figure 2b). Although our analysis concerned only one animal from each group, the present finding is different from the conclusion of these authors who reported a similar rate of pH fall for both groups of animals. The reason is very likely that, in the cited work, these rates were determined by using the first experimental values corresponding to 1, 15, 30 and 45 min post-mortem assuming a linear pH decline (Henckel et al., 2000), suggesting that all pH profiles must include the initial physiological value and the profile analysis must be carried out more accurately. Hence, we might suspect that differences between these two groups of animals are very likely larger than those reported by Henckel et al. (2000).

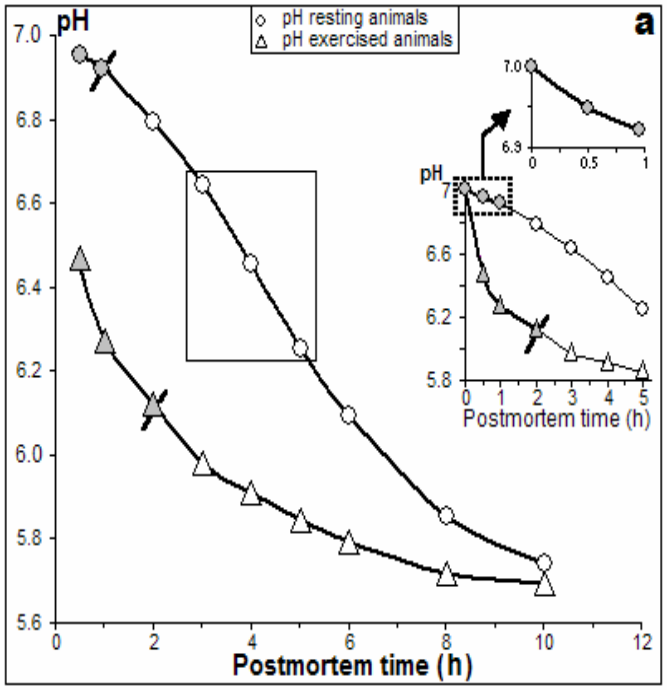
The next example was from Klont et al. (2000) who analysed the veal quality characteristics of animals classified into three groups (fast, intermediate and slow) according to their rate of pH decline (n=10 for each group). In that experiment, pH measurements were performed on *Rectus abdominis* veal muscle at 0.75, 3, 24 and 48 h post-mortem. Although a detailed analysis of the profile is impossible because of the low number of points, this example has been chosen to emphasize that in addition to a transient stagnation of the pH, a slight increase can be observed between 0.75 and 3 h post-mortem within the "slow" group (Figure 2c). Expansion of the time scale confirmed the pseudo-stability of the pH in the "slow" group between 0.75 and 3 h post-mortem (insert Figure 2c).

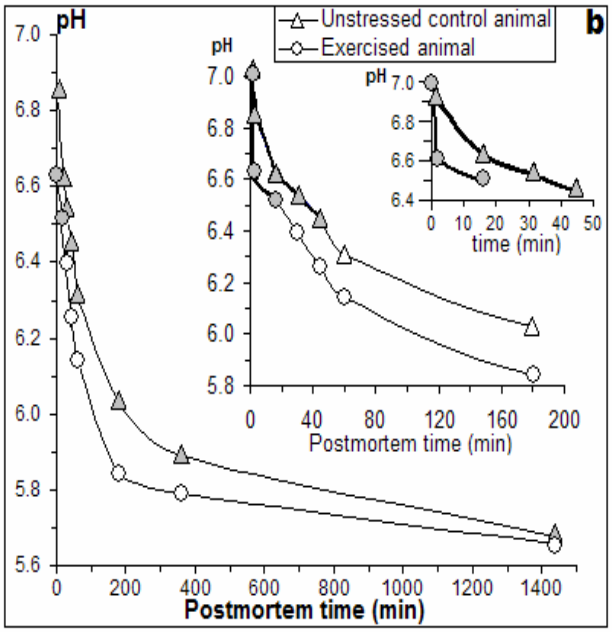
Finally, the last example concerned data published in 1996 by Zamora et al. dealing with a kinetic analysis of pH drop in three bovine muscles including M. *Longissimus* (L), *Triceps brachii caput longum* (TB) and *Supraspinatus* (SS). Measurement of pH was carried out at 2, 4, 7, 10, 15, 24, 48 and 72 h post-mortem. As shown in Figure 2d, the pH drop profile is sigmoidal for L and TB muscles and exponential for SS muscle. Furthermore, in L and TB muscles, pH does not move to much between 2 and 4 h post-mortem, a feature emphasized by expanding the time scale and including the initial physiological value (grey closed symbol in the Insert of Figure 2d). By contrast, a continuous exponential pH drop profile was obtained for SS muscle which is mainly composed of type I fibres.

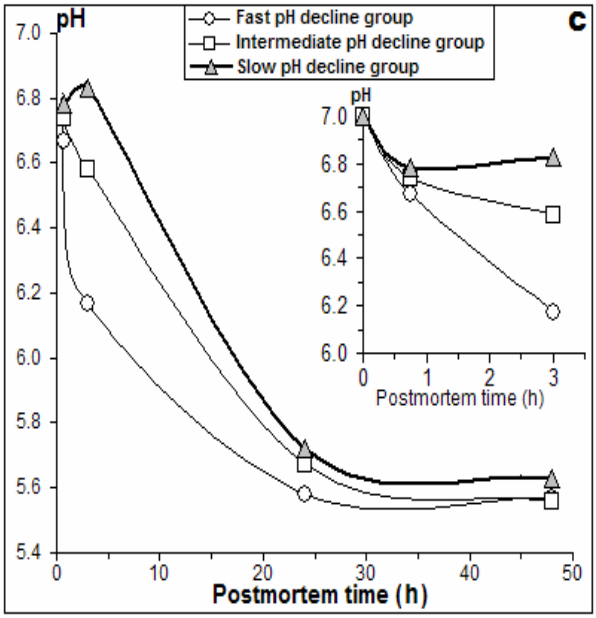
Taken together, the examples presented clearly demonstrate that, in most cases, pH decline is not linear. It is either exponential or sigmoidal. In addition, in most muscle examined, a transient stability of the pH and even a slight increase can be observed supporting that pH drop is a discontinuous process. On this basis, we attempted to analyse the pH drop profile of bovine *Longissimus* muscle from 100 animals of different age, sex and breed. Similar investigation was also carried out on five lamb muscles including M. *Semimenbranosus* (SM), *Briceps femoris* (BF), *Rectus femoris* (RF), *Longissimus lombarum* (Ll) and *Longissimus thoracis* (Lt). We further examine the consequence of the number of transient pH stability on the ultimate toughness of bovine *Longissimus* muscle assessed by a mechanical method (Lepetit and Buffiere, 1995).

Polyphasic profile of pH drop in beef longissimus muscle

Kinetic analysis of post-mortem pH drop in beef *Longissimus* muscle revealed a polyphasic process with one or two transient pH stability steps in the first hours post-slaughter (Figure 3a). This feature was observed in all animals analysed in that experiment irrespective of their breed, sex and age. Figure 3a showed the pH profile of 3 animals. These were a 54 months old Charolais cull cow (F-Ch-54), a 102 months Aubrac cull cow (F-Au-102) and a 24 months old Salers young bull (M-Sa-24). One transient pH stability step was observed between 2 and 3 h post-mortem for the 54 months old Charolais cull cows whereas the 102 months old Aubrac cull cows showed two steps between 1 and 3 h post-mortem for the first one and between 4 and 8 h post-mortem for the second. The Salers 24 months old young bull exhibited only one stability step between 1 and 3 h post-mortem. The initial rates (first step) of pH drop were estimated to be 2.7 (F-Ch-54), 1.1 (M-Sa-24) and 0.7 (F-Au-102) pH-unit/h. The configurations (1 or 2 steps) described here were observed for all breeds irrespective of animal age and sex. Analysis of the post mortem pH







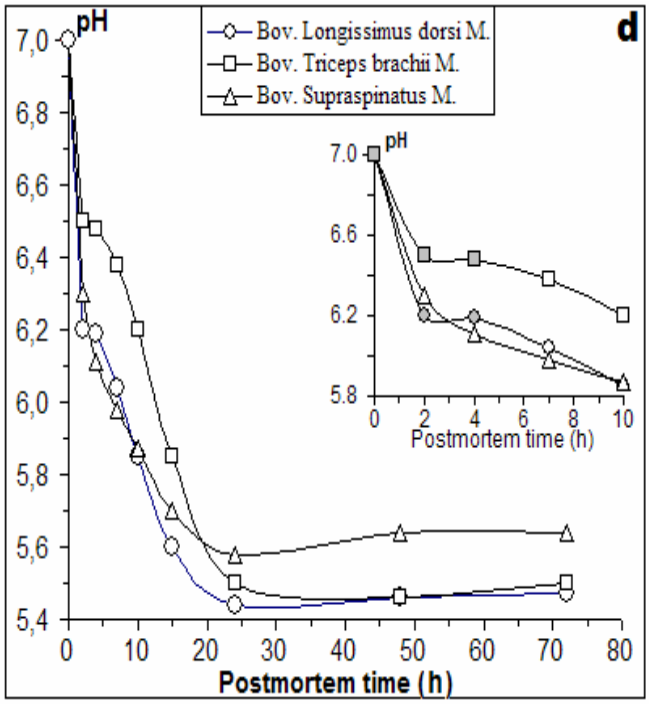


Figure 2. Profile of pH drop taken from the literature. (a) pH drop profile of lamb *Longissimus* muscle from resting and exercised animals. Inserts correspond to different expanded time scales for the first sampling times. Each point is the mean value for 6 animals (adapted from Bond and Buttler, 2000). (b) pH drop profile of pig *Longissimus* muscle from resting and exercised animals. Inserts correspond to different expanded time scales for the first sampling times (adapted from Henckel et al., 2001). (c) pH drop profile of bovine *Rectus abdominis* muscle from slow, intermediate and fast glycolysing animals. resting Insert corresponds to an expanded time scales for the first sampling times. Each point is the mean value for 10 animals. (adapted from Klont et al., 2000). (d) pH drop profile of bovine *Longissimus* muscle. Insert corresponds to an expanded time scales for the first sampling times (adapted from Zamora et al., 1996).

drop profile needs therefore a relatively high frequency of time course measurements.

One or two pH stability steps: what consequences on the ultimate toughness?

In order to increase the variability in ultimate quality traits, especially tenderness, the present experiment was carr-

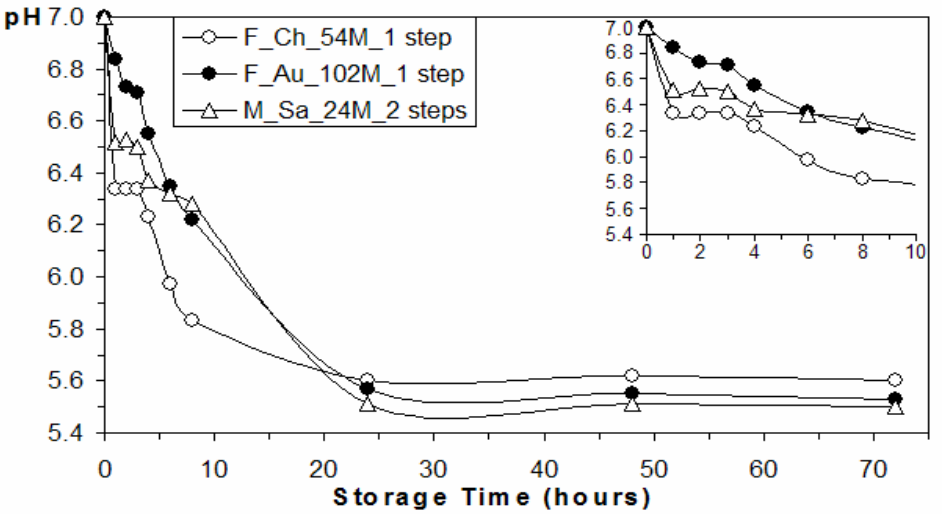


Figure 3. pH drop profiles in bovine muscle: pH profile of *Longissimus* muscle from three different animals including a Charolais 54 months old cull cow (F_CH_54M), an Aubrac 102 months old cull cow (F_Au_102M) and a 24 months old Salers young bull (M_Sa_24M).

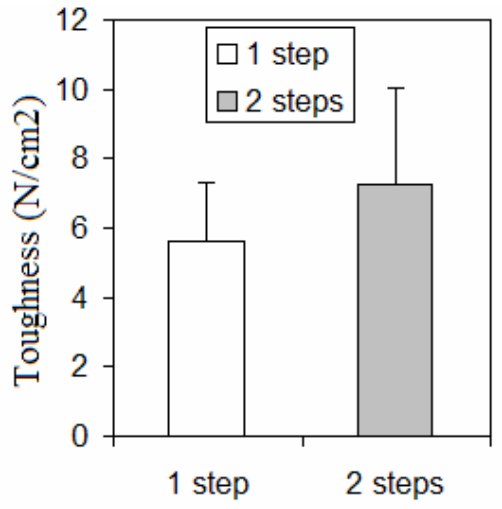


Figure 4. Toughness of bovine *Longissimus* muscle (mean ± SD) from two sets of animals showing one pH stability step (1 step; n= 53) or two pH stability steps (2 steps; n=47). The difference between the two groups is highly significant. (p <0.001).

ied out on 100 animals of different breeds, age and sex. Ultimate toughness of *Longissimus* muscle was estimated mechanically after 14 days of storage at 4°C and the mean value of the set of animals showing one pH stability step (n=53) was compared to the mean value of the other set with two pH stability steps (n=47).

As shown in Figure 4, muscles from animals with one pH stability step are significantly less tough after 14 days of ageing than those with two pH stability steps ($p<0.001$).

The question arising is to know what could be the relationship with the rate and extent of pH fall? To answer this question, different rate constants characterising the pH profile were determined by fitting the experimental curve to an exponential equation. The first one take into account the whole set of pH values from 1 to 72 h post-mortem (Vtotal). The second (V1) and the third (V2) rate constants correspond to the rate of pH fall during the first and second stability steps, respectively. The last value (Vfinal) represents the rate of pH fall after the last stability step up to 72 h. On the other hand we determined the extent of pH fall using the 24 h pH value as the end point of the rigor process since, for longer periods, the pH increased more or less.

As shown in Table 2, the set of animals with one pH stability step exhibited a significantly lower magnitude of pH drop (1.47 versus 1.50 for one and two steps respectively; $p<0.05$) and initial rate of pH fall (V1) (1.25 versus 1.61 for one and two steps respectively; $p<0.05$). By contrast no significant differences between the two groups were found for Vtotal and Vfinal. The data of Table 2 fit quite well with the above statements since animals with 1 step are more tender and exhibited higher ultimate pH and lower rate of pH fall in the first hours post-mortem than those with 2 steps.

Number of pH stability steps in males and females: relationship with ultimate toughness

Although still very controversial, most studies suggested that females provide more tender meat than males. Hence, we tested here this assumption and found that *Longisimus* muscle from females was significantly more tender ($p=0.031$) than the same muscle from males (Figure 5a). As the above results suggested that animals with one pH stability step would give more tender meat,

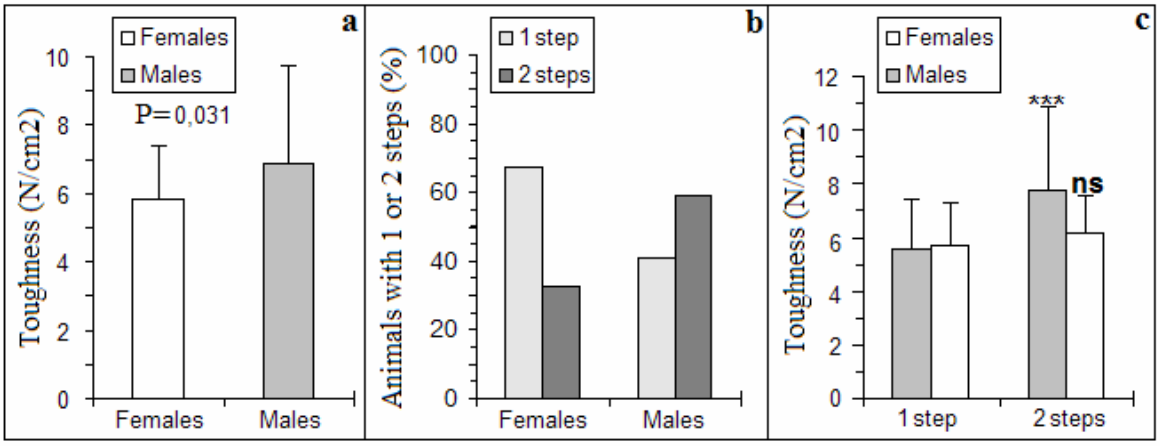


Figure 5. Toughness of *Longissiumus* muscle from female and male as affected by the presence of one or two pH stability steps. (a) Comparison of female (n=46) and male (n=54) ultimate toughness of *Longissimus* muscle; Male are significantly tougher than female ($p<0.05$). (b) Percentage of animals showing one or two pH stability steps within each group. Steps. A major proportion of females exhibited only one step and conversely a greater proportion of males showed two pH stability steps. (c) Toughness of bovine *Longissimus* muscle (mean ± SD) from two sets of animals showing one or two pH stability step within each group (females and males). *Longissimus* muscle from males with two steps are significantly tougher than the same muscle from animals with one step ($p<0.001$). No significant difference was observed in the female group.

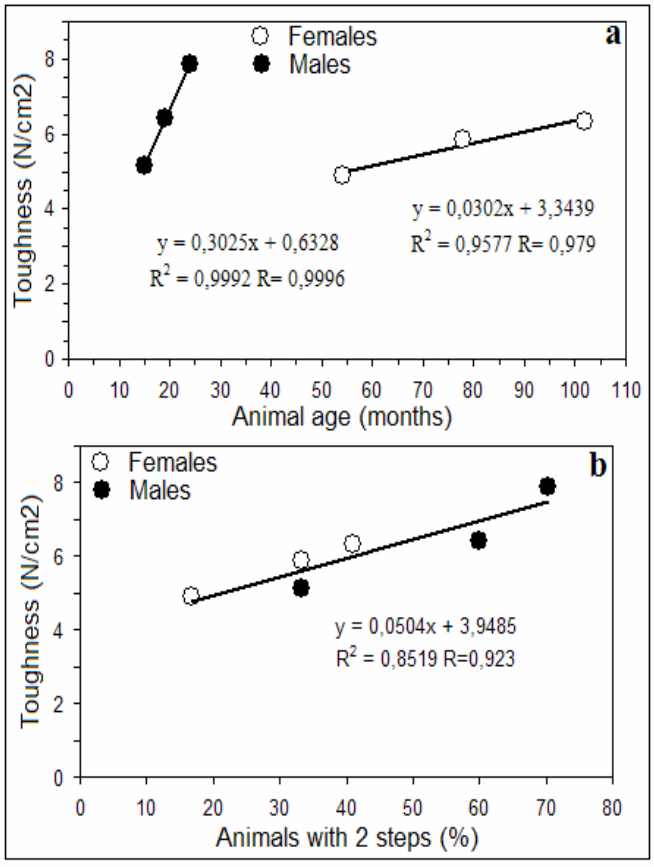


Figure 6. Toughness of *Longissiumus* muscle from female and male of different ages (a) and relationship with the percentage of animals showing two pH stability steps (b). .(a) as affected by the presence of one or two pH stability steps.

we tested whether the presence of only one pH stability step is more frequently observed in females than in males. As depictured in Figure 5b, 67% of the females showed only 1 pH stability steps versus 40% in males supporting that the presence of only one pH stability step is often associated with more tender meat. Accordingly, males with one step were significantly ($p<0.01$) more tender than those with two steps (Figure 5c). Although not significant, females with one pH stability step exhibited lower ultimate toughness (Figure 5c).

Variability in the number of pH stability steps with animal age

As for sex, in the present experiment we tested the hypothesis suggesting that ultimate tenderness of meat decreased with animal age. Results of Figure 6a comforted this statement since, for males and females, ultimate toughness of meat increases with animal age. The fact that this change occurs more sharply in males can be probably explained by the reduced range of age considered for male (15 to 24 months) as compared to females (54 to 102 months).

Plotting of the percentage of animal with two pH stability steps (Figure 6b) versus ultimate toughness showed a positive linear relationship with a high correlation coefficient ($r = 0.92$). In agreement with the results reported above, this would suggest a great impact of the number of pH stability steps in the first hours post-mortem on the ultimate meat toughness. The presence of one step would be synonymous of a better ultimate tenderness.

Table 2. Significance of the difference between animals showing 1 or 2 pH stability steps in the extent of pH drop (amplitude), the rate of pH drop over the whole rigor Process (V_{total}), the rate of pH drop over the first pH stability step (V_1) and over the second stability step (V_2) and finally over the last part of the curve between the last pH stability step and 72h post-mortem (V_{final}).

	Amplitude	V_{total}	V_1	V_2	V_{final}
1 step	1.47 ± 0.07	0.12 ± 0.07	1.25 ± 0.79		0.17 ± 0.11
2 steps	1.50 ± 0.06	0.13 ± 0.08	1.61 ± 0.62	1.22 ± 0.82	0.21 ± 0.15
p	0.022*	0.51 ^{NS}	0.0129*		0.125 ^{NS}

Table 3. Variance analysis of the effect of gender, breed and age on the magnitude of pH drop at 1h post-mortem. ANOVA performed with XLSTAT (Addinsoft, Paris).

Source	DDL	Sum of squares	Mean of squares	F	Pr > F
Gender	1	0.258	0.258	6.439	0.012
Breed	3	0.374	0.125	3.116	0.028
Age	4	0.202	0.051	1.265	0.286

DISCUSSION

The conversion of muscle into meat starts with the onset of the rigor mortis, a phase during which energy stores (phosphocreatine, ATP and glucose derivatives) are exhausted. Rigor is associated with a drop of the pH and a loss of the muscle elasticity which reached its maximum toughness (Bendall, 1973). Muscle acidification has always been considered as a major determinant of meat quality traits including juiciness and tenderness. However, this characteristic assessed through pH measurement, is the consequence of complex and not well understood processes.

As soon as blood flow ceases, muscle cells will be subjected to hypoxia and quickly after to hyperosmotic conditions. The cell responses to these stresses are multiples. First of all, the cytosolic pH drops from pH 7.4-7.5 to 6.7-6.8 within 15 min after exposure to either hypoxia or hyperosmotic conditions or both (Satre et al., 1989; Pintsch et al., 2001). When a tissue experiences hypoxia, small amounts of oxygen can exert a vitally important influence on cellular energetics (Wilson et al., 1988; Gnaiger et al., 1995). Gnaiger et al. (2001) thus reported that oxidative phosphorylation is more efficient under oxygen limitation suggesting that mitochondria will be more efficient to produce ATP. Another feature reported under low oxygen content is the phosphoryation of myosin light chain after activation of the myosin light chain kinase (Kuwayama et al., 1996, 1998; Oyama, 1996), a change noticed in post-mortem muscle and associated with tender meat (Bouley et al., 2004). In cancer chemotherapy, hypoxia-induced acidification may even cause resistance to the drugs used for this purpose (Greijer et al., 2005). All these events probably occur in post-mortem muscle.

With the development of hypoxic conditions after bleeding, the first modification will be very likely a rapid decline of the pH. This feature is probably the major cause of the amplitude of the pH drop always observed upon the first sampling time (in general 30 to 60 min post-mortem). The pH value at the first sampling time is highly variable and depends on numerous factors including animal species, individuals, muscle type and others. Assuming an initial physiological pH of 7.0, a pH drop ranging from 0.1 to 0.44 pH Unit can be calculated at 1 min post-mortem from the data reported by Henckel et al. (2000). The variability depends on the muscle considered and the treatment the animals were subjected to before stunning (exercised or not). For the three muscles studied (M. *Longissimus*, *Semimembranosus* and *Biceps femoris*) the magnitude of the pH drop were, on average, 0.16 ± 0.07 and 0.39 ± 0.06 pH Unit for the control and the exercised groups of pigs, respectively. In the present experiment, mean values for each group of animals in the □pH at 1 h post-mortem (different sex, age and breed groups) ranged between 0.32 to 0.50 pH Unit. Variance analysis indicates a significant effect of sex (p = 0.012) and breed (p = 0.028) on the □pH at 1 h post-mortem whereas no effect was observed for age (p=0.286) (Table 3). In that case, the very fast drop of pH immediately after bleeding can probably not be ascribed to glycolysis and the subsequent accumulation of lactic acid. The high level of protons originated very likely from hydrolysis of ATP to ADP, one proton released being associated to the hydrolysis of one ATP, suggesting an intense metabolic activity of the cell just after death. This was supported by the increase in the level of enzymes involved in the oxidative and the glycolytic pathways within 20 min after slaughter, two pathways providing the energy needed to preserve cells from death and/or to setup the program cell death machinery (Jia et al., 2006a,b). The findings of Jia et al. (2006a, b) further demonstrate that protein syn-

thesis takes place after animal bleeding and prove the intense metabolic activity of post-mortem muscle cells suggested before.

Although not exhaustive, the few examples from the literature included in the present paper clearly indicate that pH decline is not linear but rather exponential or sigmoidal with a linear part limited to few experimental points. It is therefore incorrect to estimate the rate of pH decline assuming a linear decline. This discontinuity in the time course profile is strongly supported by one of these examples (Klont et al., 2000) which showed a slight increase in the pH of beef muscle between 45 min and 3 h post-mortem. Such transient limited increases in the pH were sometimes noticed in our experiment. Evidences provided therefore mostly agree with transient changes in the rate of pH fall and transient stability of this variable called plateau. It can be further stressed that the causes of muscle acidification are multiple and proceeds through a series of antagonist processes acting towards or against pH drop. Hence, the mechanisms established in the 70's must be reviewed in the light of the recent improvements in our understanding of cell biology.

On the basis of these different observations supporting a non linear and discontinuous drop of pH, we analysed here the pH profile of *Longissimus* muscle from 100 bovine animals of different age, sex and breed and try to clarify the consequences of the plateaux on the ultimate tenderness of meat assessed mechanically after 14 days of storage. The selection of animals of various age, sex and breed in the present experiment aimed at increasing the variability of ultimate meat tenderness.

In bovine *Longissimus* muscle, we observed one or two pH stability steps occurring essentially in the first ten hours post-mortem and in a pH range of about 6.2 to 6.8. Animals with one step showed a significantly lower magnitude of pH drop ($p<0.05$) and a lower initial rate of pH fall ($p < 0.05$). Results of previous studies about the relationship between early-postmortem muscle pH and extent of pH fall with beef tenderness have been inconsistent (reviewed in Monin and Ouali, 1990; Eilers et al., 1996). In the present study, higher initial rates of pH fall determined by curve fitting were found to provide tougher meat, a result in agreement with those of Zamora et al. (1996 and 2005). This contrasted with the controversial and inconsistent relationship between pH 3h post-mortem and ultimate meat toughness (Eilers et al., 1996). This discrepancy might originate from the fact that pH 3 h underestimates the initial rate of pH drop since this point is, in most cases, included in the first pH stability step. Similarly to the findings of Zamora et al. (1996; 2005), a greater extent of pH fall leading to more acidic meat cuts provides also tougher meat. In addition, animals with one stability steps provide significantly more tender meat irrespective of sex, age and breed (($p<0.001$).

To comfort this finding, two other sources of variability in the ultimate meat toughness were then analysed including sex and animal age. Although very controversial, meat from female cattle was found to be more tender than males while tenderness seemed to decrease with animal age. The number of reports supporting these assumptions is quite similar to the number providing converse findings (see Prost et al., 1975). Hence, faced to these uncertainties, we tested the effect of age and sex on ultimate meat tenderness. Ultimate toughness was thus found to increase with age and to be lower for female than for male. On this basis, we then analysed the effect of age and sex on the number of pH stability steps in *Longissimus* muscle.

Regarding the animal gender effect, the percentage of animals showing one pH stability step is much greater for females than for males comforting our previous findings suggesting a greater tenderness in animals with only one stability step. For males, the proportion of animals with two pH stability steps is even much higher than those with one step. Although the biological causes of this difference between males and females are not clearly known (reviewed in Monin and Ouali, 1990), a better understanding of the origins of these stability steps would be very likely helpful.

The second source of ultimate toughness variability investigated was animal age. It is well recognized that animal age is an important determinant of meat quality including tenderness (reviewed in Monin and Ouali, 1990). Our results clearly showed that, for both males and females, ultimate toughness increased with animal age. The plot of ultimate toughness versus the percentage of animals with two pH stability steps, a characteristic previously related to tougher meat, showed a high positive correlation between these variables ($r=0,92$). The present findings therefore provide a new predictor of meat tenderness based on the transient pH stability steps, one step corresponding to more tender meat and conversely, two steps are indicative of tougher meat.

How the presence of pH stability steps in the first hours post-mortem can be explained? On the basis of the current understanding of the conversion of muscle into meat, it is difficult to find out answers to the related questions. Indeed, the current concept is based on the following view. When phosphocreatine stores are exhausted, the required energy is mainly produced through the anaerobic degradation of glycogen by glycolysis. The rate of the process first depends on the efficiency of the glycolytic pathway, on the level of glycogen stores and on the buffering capacities of muscle cells. As long as glycolytic enzymes are not inhibited by acidic pH, acidification will proceed regularly and proportionally to the glycolytic pathway efficiency. The discontinuity in the pH fall observed in the present experiment cannot be explained by a transient reduction in the activity of phosphocreatine kinase and other enzymes of the glycolytic pathway but rather by a modification of either the buffering capacity of muscle cells or a transient more effi-

cient control of muscle cell pH. A more efficient control of the pH is supported by the fact that a transient slight increase in the pH was sometimes observed instead of a flat plateau.

In recent review papers, we proposed that the first step of the conversion of muscle into meat is not the rigor mortis but the onset of the cell death programme or apoptosis (Ouali et al., 2006; Herrera-Mendez et al., 2006). The onset of the apoptotic process is energy dependent and includes the synthesis of a series of proteins involved in different cellular regulation pathways and necessary for the completion of the cell death process. These include caspases, a large set of kinases, HSPs, etc (Jia et al., 2006ba). This was supported by the clear shift in energy metabolism in the post mortem muscle resulting from an increase in enzymes involved in the glycolytic pathway as well as in the tri-carboxylic acid cycle indicative of greater aerobic and anaerobic energy metabolism in the first hours post-slaughter (Jia et al., 2006b). Amongst these over-expressed proteins, some contributes to cell defense and survival whereas others act for the progression of the apoptotic process, the balance between these antagonist pathways shifting rapidly to the onset of apoptosis. In addition, the intense metabolic activities of muscle cells just after exsanguination need a precise regulation of the intracellular pH which could counteract the acidification process. We further suggested, that the membrane polarity inversion might led to the replacement of acidic charges (phosphatidyl-serine) by basic charges (phosphatidyl-ethanolamine, phosphatidyl-choline) counteracting the acidification process. In addition this membrane polarity inversion will cause very likely important modifications in the membrane fluidity which will become more permeable to salts and small metabolites molecules. Membrane fluidity is indeed a strictly controlled process (Simkiss, 1998; Vigh et al., 1998).

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