Osmotic pressure changes in postmortem bovine muscles: factors of variation and possible causative agents

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SUMMARY: The present paper described the high dependence of postmortem osmotic pressure changes on pH, muscle type and chilling conditions. Using bovine *Longissimus, Tensor Fascia Latae, Rectus abdominis and Masseter* muscles, it was found that pH drop might be the major cause of the large increase in osmotic pressure which was twice higher than in live muscles, two processes showing a comparable activation energy (ΔH=30.2 kJ). This pH effect might be mediated by the release of ions into the cytosol especially K, Na, Mg and Ca ions. Moreover, the extent together with the rate of this osmotic pressure enhancement was greatly variable with the chilling conditions applied to meat during rigor onset and with muscle type, the maximum value increasing with both temperature and muscle contraction speed.

INTRODUCTION : Several recent findings suggest that the process of meat tenderising probably involves two sets of mechanisms. The first to be established was proteolysis of muscle protein, and it is still considered as the primary mechanism of meat tenderization. At least two indigenous proteolytic systems, namely lysosomal (especially cathepsins B and L) and calcium dependent (calpains I and II) proteinases, are very likely implicated in this process. Besides and together with proteolytic enzymes, weakening of myofibrils associated with the improvement in meat tenderness may also be mediated by the large postmortem increase in muscle osmotic pressure and, hence, in muscle ionic strength. This second type of mechanisms which is physicochemical in nature, might affect in different ways muscle properties and consequently meat quality (For review see OUALI, 1990; MONIN and OUALI, 1991).

Osmotic pressure attained in postmortem muscles (WINGER and POPE, 1980-81; OUALI, 1990) was high enough to cause solubilization of myofibrillar proteins including myosin and, as suggested by *in vitro* studies, would make the hydrolytic action of the above proteinases easier (WU and SMITH, 1987), two important changes often associated with meat tenderization.

In the present work, the time course changes in muscle osmotic pressure in relation with pH, chilling conditions and muscle type were therefore investigated . We also attempted to identify the nature of the ions contributing mostly to these changes.

MATERIAL AND METHODS : *Longissimus* (L), *Rectus abdominis* (RA), *Tensor Fascia Lata* (TFL), and *Masseter* (M) muscles were excised from Friesan cull cows within 30 min post-exsanguination, vacuum packed and stored at 15° C until the completion of *rigor-mortis* and kept thereafter at 4° C for 8 days. Investigations dealing with the temperature effect on osmotic pressure were performed essentially on vacuum packed L muscle samples from another animal (one for each sampling time) soaked in a water bath at 10, 15, 20 and 30° C until the completion of rigor and then kept at 4° C for 8 days.

Muscle juice was obtained by centrifuging 5 g of minced tissue at 100 000 g for 20 min. Extracted juice was weighted and immediately used for osmolality determination. Osmotic pressure of muscle juice was determined by osmometry using an automatic Rœbling micro-osmometer. Osmolality expressed as mOsmoles/Kg of water was measured on 100 µl of sample. Osmotic pressure of intact muscle was obtained as reported elsewhere (BONNET et al., submitted) from the melting point of intramuscular water determined by Differential Scanning Calorimetry (DSC) a method highly related to the above micro-osmometer one ($r > 0.9$). Measurements of melting point of ice were performed with a DSC -111 Setaram calorimeter. Muscle samples (100 mg) were sealed in a hermetic stainless steel pan and frozen by decreasing temperature from +20°C down to -20°C afterwhich the melting of ice was recorded by heating the sample at a rate of 2°C/min.

For each sample, muscle juice was clarified by centrifugation at 100 000 g for 20 min and analysed for Na, K and Mg ions content. After adapted dilution of the juice in deionised water, analysis was carried out with a Perkin-Elmer 303 atomic absorption spectrometer.

Muscle pH was measured on the suspension obtained after homogenization of 1g of muscle in 10 ml of 5mM sodium iodoacetate using a Radiometer pH meter 26 equipped with a combined glass electrode.

Preparation of samples for electron microscopy was carried out as previously described (VIGNON et al., 1989). Discriminant Factor Analysis was performed using a program developed by J. KOPP and adapted to a Hewlett Packard HP 9816 computer.

RESULTS AND DISCUSSION

1 - Postmortem behaviour of muscle osmotic pressure

As rigor mortis proceeds, osmotic pressure of either extractable muscle juice or intact muscle assessed by osmometry or DSC respectively, increased and attained its maximum value at the completion of rigor (Fig 1). A parallel increase in the total extractable water was observed. Both events are highly related to pH drop (Table 1) which is probably one of the main causative agent. A similar behaviour was noted for all muscles investigated which differed from each other essentially by the rate at which this change takes place and by the maximum value attained.

2 - Muscle variability

Fitting of the time course kinetic changes of the osmotic pressure led to the following expression of muscle osmolality :

$$\text{Osmolality (Osm)} = P1 + (P2 * t) - [P4 * \text{Exp}(-t / P3)] \quad (1)$$

where P1 is the maximum value attained at the completion of rigor; P2, the slope characterising the slight decrease in muscle osmotic ssure occurring in post-rigor meat; 1/P3 and P4 the rate constant and the extent characterising the increase in muscle osmolality and t, the rage time (h), respectively. Of these variables two appeared highly influenced by muscle type, namely the maximum value P1 and the the e constant 1/P3.

As shown in Fig. 2, in accordance with earlier findings (OUALI, 1990; MONIN and OUALI, 1991), the maximum osmolality value ained at the completion of rigor increased as the contraction speed of muscles raised. Maximum osmolality values ranged from 450 up to 00 mOsmoles for slow-twitch oxidative muscles and from 550 to 580 mOsmoles for fast-twitch glycolytic ones. A linear and highly nificant relationship (r = 0.98) was thus observed between these characteristics.

Though to a lower extent, the rate constant k (k=1/P3) determined according to Equation 1 was also muscle type dependent and showed a ndency to decrease as muscle ATPase raised, values of 1/P3 ranging from 0.19 to 0.23. Though the difference between extreme muscle pes was limited, the relationship between rate constant values and ATPase activity was found to be highly significant (r = - 0.99).

3 - ***Temperature effect***

Changes in osmotic pressure were completed over the rigor onset period. For investigating temperature effect, muscle samples were herefore subjected to enter in rigor at different temperature ranging from 10 to 30° C. As reported in Fig. 3a, for a given muscle, namely L uscle, maximum osmolality values exhibit a complex relationship with temperature. Linear increase in this value was thus observed as mperature of rigor onset was raised from 15 to 30° C whereas at 10 ° C the osmolality was as high as or even greater than at 20° C. These ata may be interpreted to reflect the temperature dependence of the rate of pH fall which show a similar biphasic behaviour over the mperature range 2 to 30° C, this effect being negative below 13° C (JEACOCKE, 1977). According to this author, the lowest rate constant alue for pH fall was obtained for a temperature close to 13° C, a value not far different from that noted for muscle osmolality (15° C). lthough the exact origin remains still unknown, one possible explanation for the negative temperature effect below 15° C might be provided y cold-shortening which is known to set in with increasing velocity as the temperature is lowered under a limit value ranging between 10 and 5° C (LOCKER and HAGYARD, 1963).

By contrast, the rate constant determined using Equation 1 was linear over the whole temperature range 10 to 30° C (r = 0.98) and increased s temperature raised. When these data were converted to an Arrhenius plot (Fig. 3b), they thus conform to a single straight line relationship. The slope of the regression line gave an activation energy of 30.2 kJ / mol (7.2 kcal/mol). This is comparable to the activation energy of the rate of pH fall (40.0 kJ / mol; 9.5 kcal / mol) (JEACOCKE, 1977), a finding substantiating the close relationship between pH and osmolality changes aforementioned.

4 - ***Causes of muscle variability in osmotic pressure***

4.1. Preliminary statements

As previously shown (Fig. 2), maximum osmotic pressure attained at the completion of the rigor process was highly muscle type dependent. Increase in muscle osmotic pressure and, hence, in muscle juice osmolality resulted undoubtedly from an increase in the free water-soluble mineral concentration following very likely a previous delocalisation from their initial binding site. As specific assessment for each type of ions, within intact muscle, of their extent of release was difficult, we attempted to overcome this problem and to explain at least part of the postmortem changes in osmotic pressure together with its muscle variability by analysis of muscle juices for their mineral content. In this respect, only the predominant minerals were analysed.

Mineral content of bovine muscle was shown to be greatly influenced by animal age, diet and muscle type (KOTULA and LUSBY, 1982). According to these authors and expressed on a fat-free basis as mg/100 g of wet tissue, the major ion in muscle tissue was K (330-400 mg/100 g) followed by Na (36-50 mg/100 g), Mg (20-24 mg/100 g) and Ca (3.3-3.6 mg/100 g). Though Zn content (3.0-5.5 mg/100 g) was slightly higher than Ca, this last ion was preferred to Zn and the investigation therefore restricted to the three major ions aforementioned plus calcium. Regarding muscle juice content in calcium ions, for unknown reasons and though determined in muscle juice with the same atomic absorption method, very variable results were obtained. Hence, this ion has not been considered in the subsequent statistical analysis of the data. Nevertheless, a parallel electron microscopic investigation of the postmortem changes in intra and extracellular calcium distribution supported a major role of this bivalent cation in the increase in muscle osmotic pressure. For instance, as reported earlier (VIGNON et al., 1989) and as shown in Fig. 4, following a progressive release of this ions from both the sarcoplasmic reticulum and the mitochondria, calcium electron-dense precipitates largely increased in the cytosolic compartment between at death (Fig. 4a) and 24 h postmortem (Fig. 4b).

4.2. Discriminant Factor Analysis of the data

Discriminant Factor Analysis (DFA) performed on quantitative variables led to the assignment of a discriminant power index to each of them which is proportional to their respective efficiency to discriminate classes of homogeneous elements. In the present experiment four classes were defined each of corresponding to one muscle type, i.e. L, TFL, RA and M. On the other hand, the six variables considered were Na, K and Mg concentration in muscle juices together with the osmolality value of these juices (OSM), the pH of meat (pH) and the amount

of extractable water (JUICE), all variables being determined at the different postmortem sampling times depicted in Fig. 1.

Correlation matrix between these variables reported in Table 1 shows that, as it could expected from Fig. 1, osmolality of muscle juice was significantly related to muscle pH (r = - 0.865) and to the amount of extractable juice (r = + 0.684). More surprising was that a significant relationship was only observed with K ions content but neither with Na nor Mg ions content and this despite the fact that, though to a lesser extent than K, the concentration of both Na and Mg ions in muscle juice also increased with time. Does this means that these last cations did not contribute to the increase in muscle osmolality? This is probably not the case and one possible explanation would be that the variability between muscles in the level of Na and Mg extractable ions (Coefficients of variation : 15 and 7 % respectively) was greater than that observed with time within muscle (Average coefficients of variation within muscles : 7% and 4% respectively), an assumption well supported by their ability to discriminate muscles (See Table 2 and below).

According to the power indices assigned to each variables, Na thus appeared to be the most efficient to discriminate muscle classes followed by Mg, OSM and K (Table 2). The contribution of pH and JUICE was not significant. Hence, DFA was further performed on a reduced set of variables comprising only the most discriminant, i.e. K, Mg, Na and OSM. DFA performed on the last set of variables extracted two axes accounting for 98.6% of the data variance shared into 58.5% and 40.1% between axes 1 and 2, respectively. Axis 1 encompassed the following variables : OSM (r = -0.83) and K (r = - 0.95) with negative loading (r = value of the correlation coefficient with this axis) and Na (r = + 0.99) with positive loading while Mg showed preferential loading on axis 2 (r = + 0.95). Using this set of variables, 93.3 % (28 among a total of 30 muscle samples) of the samples were well grouped in there respective muscle classes. Distribution of muscles in the plane defined by these axes shown in Fig. 5 thus indicated that only L1 (L muscle 1 h postmortem) and L4 (L muscle 24 h postmortem) were ranged in TFL and RA classes, respectively. Muscles were ranged on axis 1 from the left to the right according to their decreasing osmolality. Moreover, TFL and RA were mainly distinguished from each other on axis 2 on the basis of their Mg content. Another noticeable feature was that, on axis 1, discrimination of these last two muscles was mainly assumed by their Na content rather than their osmolality (See Fig. 2). This emphasized the large variability between muscles of the variables Na and Mg previously suggested.

CONCLUSION: The findings reported clearly demonstrate that during rigor onset and though to various extent, osmotic pressure increased in all muscles of probably all species. They further suggested that pH drop accounted for a large part of this change very likely through alteration of proteins and/or structure to which ions are normally bound. Less clear are the consequences of this osmotic pressure enhancement on meat quality. However, a weakening of myofibrils through both solubilization of some proteins and the increase in their susceptibility to proteolysis together with a modification of the water holding capacity of meat could be expected. There is therefore a need for further work in this field which have to take into account the muscle type variability of the myofibrils sensitivity to high ionic strength.

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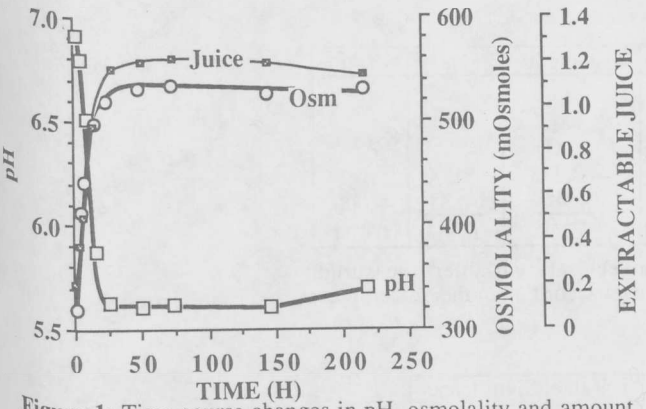

Figure 1: Time course changes in pH, osmolality and amount of extractable juice (g/5 g wet muscle) in postmortem bovine TFL muscle.

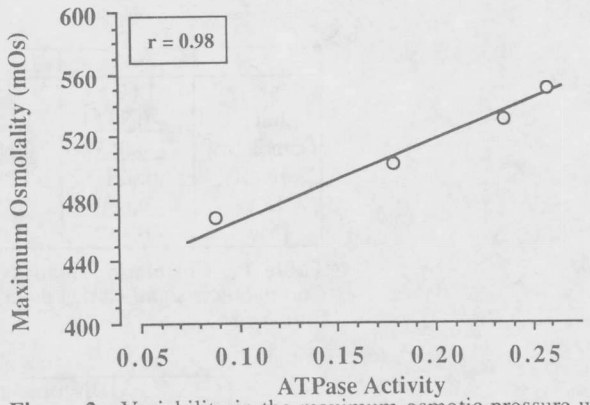

Figure 2 : Variability in the maximum osmotic pressure with muscle contraction speed assessed through ATPase activity

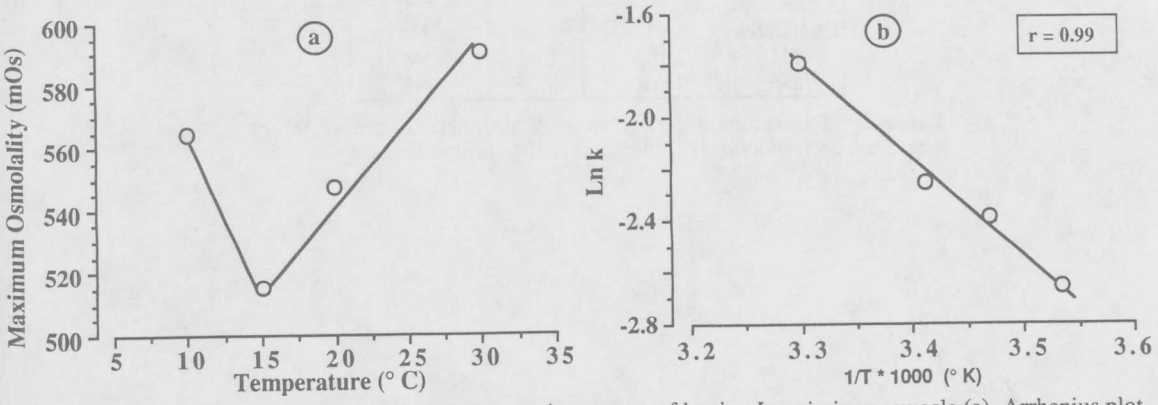

Figure 3 : Temperature effect on the maximum osmotic pressure of bovine *Longissimus* muscle (a). Arrhenius plot (b) of the rate constant characterizing the increase in the osmotic pressure of the same muscle.

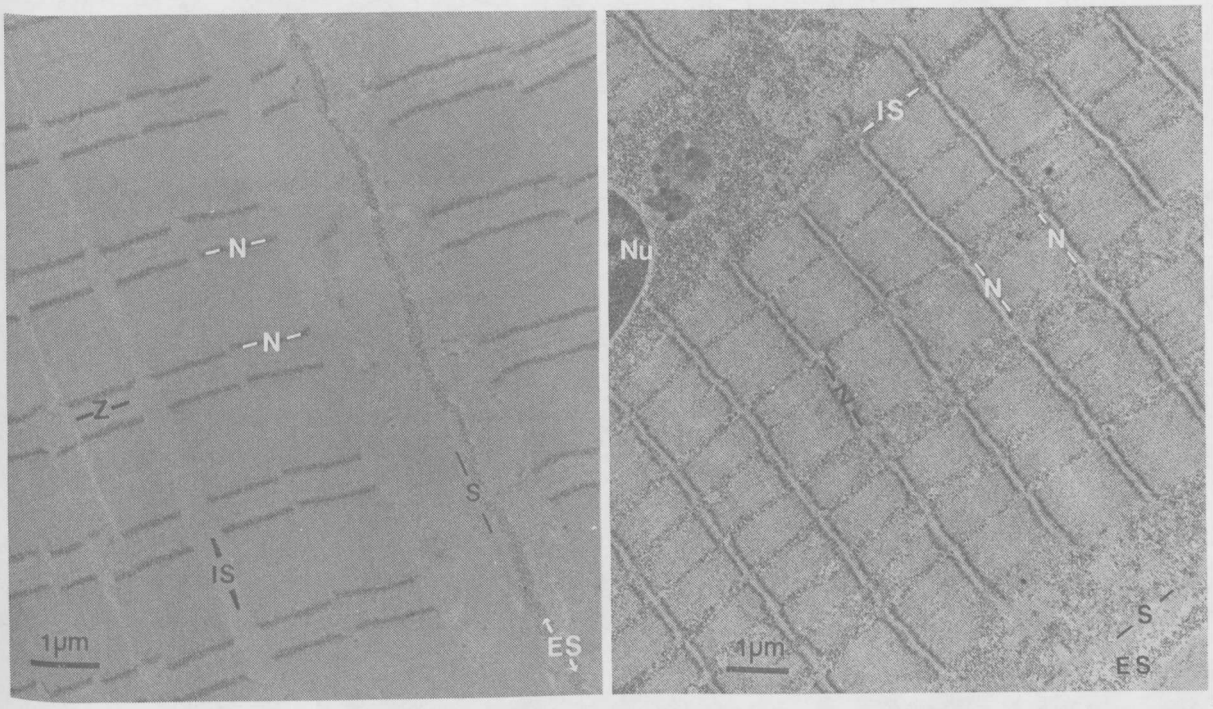

Figure 4 : Calcium ions localization in *Longissimus* bovine muscle as seen by Electron Microscopy after precipitation with potassium pyro-antimonate. Calcium is observed as electron-dense precipitates in these sections. (a) : muscle fixed within 1 h post slaughter. Calcium precipitates are mainly observed on N-lines of myofibrils (N) and in extracellular space (ES). Spaces between myofibrils are precipitate free (IS). (b) muscle fixed 24 h postmortem. In addition to the previous precipitates on N-lines and extracellular space, strong calcium precipitates appeared in intermyofibrillar spaces (IS). S: sarcolemma; Nu : nucleus; Z: Z-line.

	pH	Juice	Osmolality	[Na]	[K]
pH	1.000				
Juice	**- 0.882**	1.000			
Osmolality	**- 0.865**	**0.684**	1.000		
[Na]	0.021	0.092	- 0.278	1.000	
[K]	**- 0.650**	**0.515**	**0.761**	**- 0.517**	1.000
[Mg]	- 0.117	- 0.068	0.329	0.111	0.134

Table 1 : Correlation matrix between all variables measured. Correlation is significant at the 5% level according to the Studen-t test for $r \geq 0.361$.

	Discriminant power	F Value	Significance
Na	7.151	24.64	***
Mg	6.094	14.78	***
K	4.795	8.53	***
Osmolality	3.937	5.95	**
pH	1.103	1.11	NS
Juice	0.864	0.85	NS

Table 2 : Discriminant power indices assigned to the variables measured, corresponding F values and statistical significance.

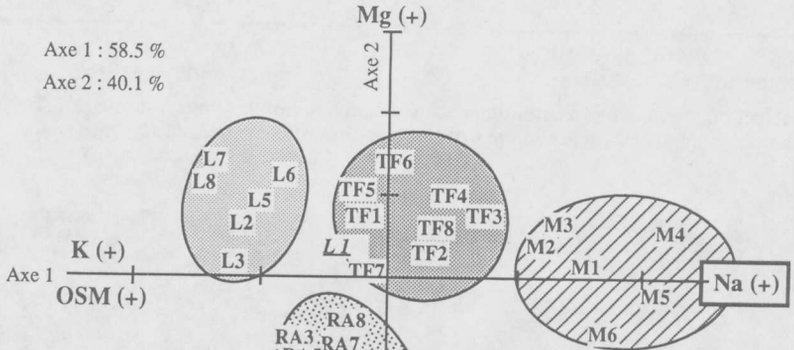

Figure 5 : Discriminant Factor Analysis: distribution of muscles on the two first discriminant axes 1 and 2. Variables loading on axis 1 are OSM (osmolality), K and Na while Mg is preferentially loaded on axis 2. Muscles are indicated by their initial (see text) followed by a number which indicates the range order of postmortem sampling times.