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Review Article

Mechanical and Biochemical Methods for Rigor Measurement: Relationship with Eating Quality

Carlos Álvarez,[1] **Lara Morán**,[2] **Derek F. Keenan**,[1,3] **Anne-Maria Mullen**,[1] **and Gonzalo Delgado-Pando**[1]

[1]*Department of Food Quality and Sensory Science, Teagasc, Food Research Centre Ashtown, Dublin 15, Ireland*
[2]*Lactiker Research Group, Department of Pharmacy and Food Science, University of the Basque Country (UPV/EHU), Álava 01006, Vitoria, Spain*
[3]*Pesticides Control Service, Department of Agriculture, Food and the Marine, Backweston Laboratory Complex, Young's Cross, Celbridge, Kildare, Ireland*

Correspondence should be addressed to Gonzalo Delgado-Pando; g.delgado.pando@gmail.com

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Meat quality parameters are affected by a complex series of interacting chemical, biochemical, physical, and physiological components that determine not only the suitability for consumption and the conditions for further processing and storage but also consumer acceptability. Deep understanding and careful manipulation of these intrinsic and extrinsic factors have to be taken in account to ensure high quality of meat, with better technological properties and increased safety for consumers. Among meat quality characteristics, meat tenderness has been perceived as the most important factor governing consumer acceptability. Therefore, being able to early predict meat texture and other related parameters in order to guarantee consistent eating quality to the final consumer is one of the most sought-after goals in the meat industry. Accurate measurements of both the biochemical and mechanical characteristics that underpin muscle and its transformation into meat are key factors to an improved understanding of meat quality, but also this early-stage measurements may be useful to develop methods to predict final meat texture. It is the goal of this review to present the available research literature on the historical and contemporary analyses that could be applied in early postmortem stages (pre-rigor and rigor) to determine the biochemical and physical characteristics of the meat that can potentially impact the eating quality.

1. Introduction

Since the 1970s, there has been an academic interest in the management of meat quality with the goal of reducing the inherent variability of the product at retail [1–3]. Consumer perspectives on meat quality are complex, and meat quality is a continuously evolving concept [4]; however, for decades, meat tenderness has been perceived as the most important factor governing consumer acceptability [5], although there is a growing acceptance that other factors, specially flavour, have greater influence than initially thought [6, 7]. More recently, other factors are known to play a role in this arena, such as sustainability [8], animal welfare [9, 10], or healthy lifestyle [11].

Despite breakthrough research and the implementation of the findings to the management of meat quality, surveys from 2017 indicated that considerable variation in the tenderness of beef cuts was still present and the same range of variation was found than in the previous surveys carried out in the past 15 years [12–16]. Reducing this variation is paramount in order to win consumers' confidence [6]. Meat quality in general, particularly textural characteristics, has become so important for the consumers that several countries have included a quality classification in their grading systems: Australia, Canada, Japan, and USA [17]. The quality assessment is usually done on the ribeye by means of marbling determination, but the Australian system (Meat Standard Australia) has also included a cook x cut

based model, taking into account the consumer perception on tenderness, juiciness, flavour liking, and overall liking [18].

There are many pre- and postslaughter factors affecting eating quality attributes of meat. Intrinsic preslaughter factors include genetics, breed, gender, type of muscle, and age of the animal, while the extrinsic encompasses nutrition/dietary management, animal health, animal welfare, stress management, and climate [19–21]. Postslaughter extrinsic factors include slaughter protocol, postslaughter interventions (e.g., temperature control, electrical stimulation, hanging method, or ageing), storage, conservation and display (e.g., packaging), and cooking time/type [22–24],which are some of the main factors.

In this regard, after slaughtering, the most important processes affecting the development of meat texture begin when the blood irrigation stops, triggering the development of the conversion process from muscle to meat [20]. This process has been divided into three main stages: (a) pre-rigor phase; (b) rigor phase; and (c) tenderisation phase (proteolysis by enzymes, such as calpains and cathepsins) [25]; however, the contribution of a fourth step (apoptosis, taking place before the rigor step) has been proposed by Ouali [26].

Briefly, during the pre-rigor and rigor phases, several changes occur within the muscle. Firstly, a depletion of the total available energy takes place in muscle, followed by a shift from aerobic to anaerobic metabolism, which, in turn, leads to the production of lactic acid. Subsequently, there is a rise in the ionic strength of the muscle, due, in part, to the lack of adenosine triphosphate (ATP) which prevents the work of the ion ATP-dependent pumps (calcium, sodium, and potassium) [27]. Finally, the cell is not able to maintain reducing conditions and calcium pumps stop working, inactivating the relaxation capacity of the muscle and establishing rigor mortis [28]. Regarding the tenderisation phase, two main theories have been proposed to explain the changes observed in meat structure and tenderness. The most widely accepted theory is based on the presence of endogenous calcium-activated proteinases (such as calpain and its regulator calpastatin, and cathepsins) which are able to hydrolyse the myofibrillar proteins, giving as result a loss of structure and further tenderisation [29]. To date, the proposed mechanisms of proteolysis have been comprehensively reviewed by others [29–31]. The other theory suggests that the accumulation of calcium ions in the sarcoplasm—due to the lack of activity of ATP-dependent pumps—increases the ionic strength, leading to a solubilisation of the myofibrillar structural elements which will be further degraded [32]. However, none of the theories is currently fully supported by empiric evidence. In any case, it seems clear that proteolysis is, at least in part, one of the main players for postmortem tenderisation. Recent research has demonstrated that degradation of structural proteins such as desmin, nebulin, or titin (which are the substrates of calpains) is highly correlated with the final tenderness [33, 34]. Finally, the role of apoptotic routes has been recently highlighted [35]. Briefly, apoptotic factors such as cytochrome C when overexpressed promote calpain activity, thus improving tenderisation on the aging phase. On the other hand, heat shock proteins (HSP) related to stress have been found to inhibit calpain activity when overexpressed, leading to lesser degree of tenderisation.

In order to measure the meat tenderness of the final cut, several methods have been developed. The most common include both objective (TPA and shear force) and subjective (sensory panel) measurements. However, an early prediction of eating quality during the pre-rigor and rigor phase will have a clear impact on processing, research, and production. In this regard, the present paper wishes to review the methods that have been employed or can be employed during the pre-rigor and rigor phase to evaluate meat characteristics and how they relate to the final meat eating quality, with main focus on textural parameters. We assessed how these methods could be potentiality used as early meat quality/tenderness markers.

These methods can be grouped into biochemical methods, which aim to determine the concentration and activity of molecules and enzymes involved in the glycolysis process during rigor mortis; physical/mechanical methods measuring the textural and mechanical properties of the muscle during this early stage; and finally, rapid non-destructive techniques performed online usually based on image analysis or spectroscopy.

2. Muscle Metabolism, Postmortem Glycolysis, and Meat Quality

In order to fully understand the principles and mechanisms underpinning these methods, it is important to review the biochemical processes at play in the early postmortem stage and the subsequent rigor mortis onset.

Despite the fact that carbohydrates make up a relatively small percentage of muscle tissue (0.5–1.5% of total muscle weight [36]), they play a major role in the process of transforming muscle into meat [37, 38]. Glucose and glycogen are the preferred molecules employed in muscle metabolism and can be both used in either an aerobic (oxidative phosphorylation) or an anaerobic (anaerobic glycolysis) environment [39]. By far, the most important one of the two energy sources is glycogen as its preslaughter concentration (500 mmol/kg dry muscle) is related with pH decline during anaerobic metabolism (due to the accumulation of lactic acid during the transformation of glycogen to ATP). It has been reported that about 45 mmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5 [40]. This value is quite constant, and this process stops when the pH of meat is 5.6 to 5.3 [40], due to the inactivation of involved enzymes; therefore, when the amount of residual glycogen after pH 5.5 has been reached, it can be used to determine the preslaughter glycogen concentration [41].

Glycogen can be found in two forms: (1) as proglycogen (PG), which is smaller (up to 400 kDa) and not soluble in perchloric acid ($HClO_4$) and (2) as macroglycogen (MG), which is larger (approx. 107 kDa) and is $HClO_4$-soluble [42]. The PG degradation rate is higher at normal or high glycogen levels compared to MG [43]. Other sources of energy are phosphocreatine, lactate, fatty acids, and triglycerides. However, lipids and lactate can be employed as source of

energy only under aerobic conditions. In the case of lipids, the efficiency of adenosine triphosphate (ATP) generation is very high, but the rate of conversion into ATP is twice as slow as compared to ATP synthesis from glycogen in aerobic conditions (1.5 vs 3 mmol/kg·s, respectively) [44]. When glycogen is used in anaerobic conditions, the conversion rate increases up to 5 mmol/kg·s [44].

The glycogen content of animals at rest, or just after slaughter, has mostly been estimated by the glycolytic potential (GP). The GP is defined as the ability of the muscle to generate postmortem energy and can be determined by measuring the content of glycogen, glucose, glucose-6-P, and lactate expressed as lactate equivalents as follows [45]:

$$[\mathrm{GP}] = [\mathrm{lactate}] + 2\,([\mathrm{glycogen}] + [\mathrm{glycogen}-6-\mathrm{P}] + [\mathrm{glycogen}]). \quad (1)$$

During muscle function in vivo, myoglobin is responsible for oxygen transportation within the muscle cell. However, the oxygen supply ceases after slaughter and the remaining oxygen is rapidly consumed, resulting in extreme hypoxia conditions. The lack of oxygen activates the anaerobic pathways. Immediately after the oxygen supply has ceased, the muscle goal is to keep the homeostasis and maintain ATP levels. The initial system of ATP production is via the phosphagen system [46], in which a phosphate is transferred from phosphocreatine (PCr) to ADP by the action of creatine kinase; as a result creatine and ATP are generated, but supplies deplete quickly [47]. As the ATP is consumed, the PCr is depleted, while ADP and phosphate are accumulated. As additional source of ATP, it can be generated by the myokinase reaction ($2\mathrm{ADP} \leftrightarrow \mathrm{AMP} + \mathrm{ATP}$). After that, the AMP is rapidly deaminated to generate inosine monophosphate (IMP) by means of AMP deaminase.

Glycogen is then metabolised via anaerobic pathways with the aim of generating the needed ATP but is considerably less efficient compared to aerobic conditions: 38 mol of ATP against only 3 mol of ATP from mol of glucose-1-P from glycogen. Nonetheless, as mentioned before, the conversion rate is better under anaerobic conditions. Glycogen phosphorylase seems to be the rate-limiting enzyme in glycolysis; glycogen concentration, glycogen structure, and glycolytic rate might have a positive association [48]. Energy sources within the muscle (creatine phosphate, glycogen, glucose, ATP, ADP, and adenosine monophosphate (AMP)) are depleted, and lactic acid (from glycogen) and hypoxanthine (from ATP) are produced and accumulated [48]. The combined action of all these enzymes increases the glycogen degradation, yielding lactate and H^+. Finally, the ATP hydrolysis, which yields ADP, phosphate, and H^+, accelerates the pH drop in muscle. All these factors in combination exert an effect on the pH decline, thus affecting the meat quality development [49]. As lactate accumulates, a concomitant drop in muscle pH takes place. Any reaction that modulates the postmortem metabolism by enhancing the ATP production and altering the pH decline will impact on the meat quality development [50].

Once the pH drops to the isoelectric point (pI) of the main proteins, especially myosin (pI = 5.3), a loss in the ability of these proteins to retain the water takes place [51]. The repulsion between the proteins decreases resulting in a tighter packing of the muscle fibres, reducing the space that separates myofibrils. Consequently, fluids between myofilaments are forced into the extramyofibrillar space, which is held in place by capillary forces [52]. The ability of meat to retain this fluid when a force is applied is termed as the water holding capacity (WHC) of meat [53], being an important attribute from a meat quality standpoint as it is related with textural and sensory characteristics such as tenderness and juiciness, among others. Factors that alter the gap between the fibres can affect the amount of water that is immobilised. The main extrinsic and intrinsic factors affecting the spatial order of the proteins include (1) pH changes; (2) the presence of divalent cations (Mg and Ca); (3) denaturing conditions (rapid pH declines while temperature is still high); and (4) the presence of plasticising agents such as ATP, enzymes as ATPase, and necessary cofactors that prevents myofibrillar protein crosslinking [28, 54]. Immediately after slaughter, meat has excellent WHC due to the combination of high pH and high levels of ATP. However, over the subsequent 12–24 h, WHC decreases because of the enzymatic breaking down of ATP and the muscle acidification due to lactic acid accumulation. Rapid postmortem glycolysis is led by alterations in carcass temperature (which can reach values of 42°C in the first 45–65 minutes after slaughter) and is related with high drip loss, poor WHC, and pale colour. These conditions together generate what is known as pale, soft, and exudative (PSE) meat. Even if the final pH is within the normal range, WHC is too low in these cases. It has been reported [55] that high stress conditions before slaughter decreased muscle glycolytic potential, leading to increases in plasma lactate, muscle temperature, cortisol, rate of pH decline, ultimate pH, and yellowness of meat [55].

With ATP becoming depleted, the ionic equilibrium in the muscle cell is disrupted due to Na/K being unbalanced and Ca pumps interrupting the cellular activity. A relaxed animal contains approximately 4–10 mmol ATP per kg of muscle [48]. After slaughter, the subsequent anaerobic glycogenolysis keeps the ATP concentration constant for a short time. When glycogen reaches around 50% of its original value, the ATP level starts to fall along with the pH [48]. When the temperature drops below 15°C and the ATP concentration is under a certain level, the calcium pump is unable to remove the calcium from the sarcoplasmatic reticulum, resulting in a build-up of calcium. This prevents proteins related with muscle movement—actin and troponin—from interacting, since the myosin will not be able to release from actin, impeding muscle relaxation, and therefore, the fibres will remain contracted. When the pH of meat drops to 5.9 and ATP is lower than 1 μmol/g, rigor mortis commences.

It is then clear how the conversion of muscle to meat is a key process that affects several meat eating quality attributes. A successful determination of the compounds involved in this conversion can lead to a better understanding of not

only the factors affecting the process per se but also the relationship with endpoint eating quality attributes such as juiciness, tenderness, and chewiness. In the next section, we review how these metabolites can be determined.

3. Biochemical Methods: Quantification of Molecules and Metabolites from the Glycolytic Pathway

In order to early predict meat quality characteristics such as texture, numerous methods have been developed with the aim of determining the concentration and activity of the molecules and enzymes involved in the glycolysis process. A scheme showing the relationships between the different metabolic pathways; analytes and enzymes involved; pH values; ATP, inosine, glycogen, glucose, and lactate levels; and meat defects is presented in Figure 1. The analysis of metabolites as a predictive tool to estimate the final tenderness is still not having high correlation values, as stated by Muchenje et al. [56] and Lahucky et al. [57]. For instance, these authors found poor correlation between objective texture measurements (Warner–Bratzler shear force (WBSF)) and glycogen amount premortem and at 1 h, 3 h, and 48 h postmortem. However, the molecules and metabolites from the glycolytic pathway involved in the rigor and pre-rigor are clearly related with meat quality as they have good correlation with both PSE and DFD (dark, firm, and dry) meats; they can be useful as meat quality defect biomarkers. The presence of meat defects causes important financial loss to the meat industry [13–16]; in this regard, DFD defects have been reported in the main meat-production species with exception of horse [58], while PSE meat has been reported in pig, cattle [59], turkey [60], chicken [61], and ostrich [62].

Numerous biochemical measurements have been developed with the aim of determining the concentration and activity of the molecules and enzymes involved in the glycolysis process, and these methods could be deemed indirect measures of meat quality, as they involve analysing biochemical markers of the ongoing muscle-to-meat conversion. Even though the analysis of these metabolites has not showed a significant correlation with objective tenderness, their involvement in the muscle-to-meat conversion cannot be neglected. While correlations of single metabolites have not been successful, better results are to be expected from a more holistic approach. Furthermore, in addition to their ability to predict meat quality defects, there is still room for research on their capacities to predict subjective eating quality measurements. Objective texture measurements do not explain full meat eating quality characteristics.

The most common methods to determine the glycolytic potential and the activity of the most important enzymes involved in the process of glycogen metabolism under anaerobic conditions are listed below. The methods are divided into three groups: (1) quantification of molecules and metabolites; (2) enzymatic activity assays (Tables 1 and 2, respectively); and finally (3) new online methods.

3.1. Quantification of Molecules and Metabolites

3.1.1. Glycogen, Reducing and Phosphorylated Sugars, and Lactate. Table 1 gives a general overview of many of the methods currently used for analysing the products of postmortem glycolysis. As a general rule, samples must be immediately frozen using liquid nitrogen and then stored at temperatures around −80°C. This is to halt enzymatic reactions and to try to preserve the actual amount of glycogen, glucose, and other compounds of interest. Then, depending on the analyte, an extraction process must be carried out which typically involves a deproteinisation step using $HClO_4$. This is the underlying approach carried out by Choe et al. [63] modified from Dreiling et al. [64], in their determination of glycogen, who describe their extracted supernatants (0.4 mL) being combined with iodine solutions (2.6 mL) before determining concentrations from a glycogen standard curve. Alternatively, Chan and Exton [65] describe the measurement of initial glycogen through stoichiometry by determining both glycogen and glucose contents. In this two-stage approach, glycogen was extracted after washing in cold ethanol in stage one, while an enzyme digestion was employed to extract the glucose before being determined by Glucose PAP kit (spectrophotometric assay based on glucose oxidase and Trinder reaction where the end product is quinoneimine dye [66]). The determination of the two forms of glycogen (PG and MG) was described by Ferguson et al. [43]. After the extraction, the supernatant (100 μl) was used for the determination of MG and the pellet was used for PG. Further incubation, neutralization, and centrifugation steps occurred before the total glucose concentration of the two fractions was determined. Choe et al. [63] and Apaoblaza et al. [67] described the determination of lactate using commercial kits (Boeringer-Mannheim, Germany, and liquiform lactate, Labtest Ref. 116, respectively), while Henckel et al. [68] terminated the extraction prior to centrifugation by adding 1 ml of 2M $KHCO_3$, before measuring the lactate content by the method of Passonneau and Lowry [69]. For the determination of reducing and phosphorylated sugars, Aliani et al. [70] described the combination of two experiments to facilitate a common extraction procedure for sugars and nucleotides. This enabled one extract to be used for all these analytes, economising on chemicals and time. Aliquots of extracted chicken muscle filtrate (Table 1) were resin-treated to remove any interfering compounds and analysed (as described by Aliani and Farmer [76] using a modified resin, i.e., Marathon WBA ion-exchange resin). A secondary aliquot (1.5 ml) was used to analyse phosphorylated sugars by enzymatic treatment using sodium carbonate buffer (50 mM containing MgCl2 0.5 mM at pH 9.2) to improve peak separation.

3.1.2. Nucleotides, Glycolytic Rate, and Minerals. The nucleotide ATP is the main source of energy for the muscle and the driver of the Na/K and calcium pumps, powering contraction and relaxation of the muscle. In the very early postmortem stage, ATP concentration is apparently stable due to its formation from the creatine phosphate through

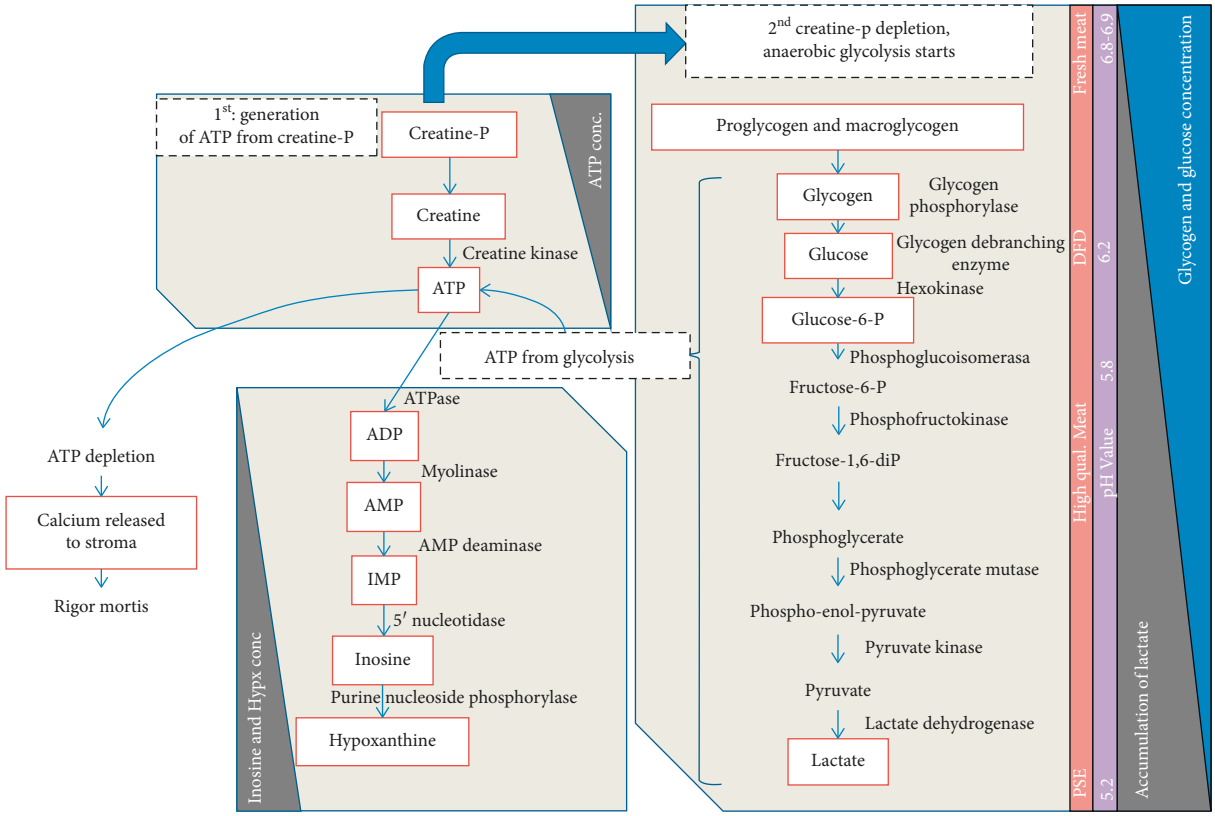

Figure 1: Compounds, enzymes, and metabolic pathways involved in the rigor mortis process. Enzyme activity currently assayed is given in blue letters and compounds currently quantified are in red border.

creatine kinase and anaerobic glycolysis routes. Once these sources are exhausted, ATP concentration starts to drop by converting to other compounds such as ADP, AMP, IMP, inosine, and hypoxanthine. Whereas ADP, AMP, and IDP are intermediates and their concentrations are negligible after 24–48 h and inosine and hypoxanthine experience a substantial increase as muscle is aged. Depletion of ATP in PSE muscle is very rapid, and as commented before, the rate is even faster at high temperatures. England et al. [75] reported that a decreased activity in AMP deaminase could extend the postmortem glycolytic process, which results in a lower pH. Initially, the measurement of pH at 45 min postmortem was considered as a valid detector for PSE meat but, it is now clear, that using this measurement alone leads to gross classification errors. As a result, several methods based on the analysis of nucleotides and nucleosides have been developed in order to predict meat quality. Traditionally, ion-exchange chromatography was used to analyse ribonucleotides and related compounds (e.g., ATP, ADP, AMP, IMP, inosine, hypoxanthine, and creatine), but this was time-consuming and the resolution was poor. Currently, HPLC coupled to reverse-phase columns have given good analytical separations and recoveries [68, 71].Common to these studies was the use of low molarity phosphate buffers and methanol as solvents and similar column lengths (150–250 mm). Henckel et al. [77] described the use of an isocratic method using a flow gradient (0.5–1.5 mL/min), while Battle et al. [71] and Aliani et al. [70] used solvent gradients for their separations. It has been shown that the ATP level in PSE meat at 4 h postmortem is almost ten times lower than that of normal meat; as a consequence, IMP, inosine, and hypoxanthine are found in higher quantities in PSE meat. Rapid methods based on the measurement of the *R* value have been developed (Table 1). These methods analyse the ratio between inosine-related and adenosine-related compounds, which can be easily measured in meat extracts at 250 and 260 nm [63]. Furthermore, they have shown good correlations with meat quality when meat is analysed within the first 8 hours postmortem. In the absence of ATP, the cessation of the calcium pumps results in a build-up of calcium in the sarcoplasmic reticulum, making muscle relaxation impossible, i.e., onset of rigor mortis. Young & Lyon [32] discussed the extraction and determination of calcium ions using atomic absorption spectroscopy.

3.2. Enzymatic Activity Assay. As it has been stated, besides analysing the content of metabolites which have a role in the postmortem process, determining the activity of the enzymes involved in the transformation of such molecules is essential to understand what the real state of the muscle is, during its transformation into meat. Not all the enzymes involved in the process can be currently analysed; however, available methods for some of them have been reported, for example, glycogen debranching enzyme [67, 74], glycogen

Table 1: Direct chemical measurements of molecules and metabolites involved in post-mortem glycolysis.

Molecule	Physical treatment	Solvent	Centrifuge	Neutralised	Detection (nm)	Ref.
Glycogen	Homogenised: 30–45 s	10 mL $HClO_4$ (9%)	15,000 g 4°C		460	[63, 64]
	Incubated: 100°C–15 min	400 μl KOH (30%)	—			
	Digested: amyloglucosidase (1 mL) Incubated: 37°C–2 h	$C_2H_3NaO_2$ buffer pH 4.8	—		505	[65, 66]
Proglycogen and macroglycogen	Incubated: on ice 25 min	3M $HClO_4$	1900 g 20 min 4°C	2 M Trizma base		[43]
Lactate	Homogenised: 30 s	2 ml of 1 M $HClO_4$		KOH (2 M)	340	[63]
	Homogenised	20 mM citrate, 50 mM phosphate buffer, 2.5 mg/ml EDTA, pH 6.8.				[67]
	Incubated: on ice (30 min)	600 μl of 3M $HClO_4$	1500 g 10 min 4°C			[68, 69]
Reducing and phosphorylated sugars	Homogenised: 2 min Ultra Turrax	6 ml of $HClO_4$ (0.6 M), deionised water (1.0 ml); 0.5 ml 20 mM rhamnose and 10 mM cytidine as IS for sugar and ribonucleotide	3000 g 15 min 4°C	KOH (~0.8 ml of 4 M) (supernatant)		[70]
Ribonucleotides: ATP, ADP, AMP, IMP	Homogenised: 4 min, 4°C masticator	15 mL cold 0.6 M $HClO_4$	15000 g 20 min 4°C	Solid potassium carbonate	254, 280	[71]
	Incubated: on ice (15 min)	600 μl of ice-cold 0.6 M $HClO_4$ containing bromthymol blue and phenolphthalein 0.004%	10,600 g 5 min 4°C	540 μl of ice-cold 0.8 M KOH and 25 ml ice-cold 25% KH_2PO_4 buffer	210	[68]
	Homogenised: 2 min full-speed Ultra Turrax	6 ml $HClO_4$ (0.6 M) deionised water (1.0 ml)	3000 g 15 min 4°C	KOH (~0.8 ml of 4 M) (supernatant)	254	[70]
	Homogenised: 1 min full-speed Virtis Macro 45	20 ml $HClO_4$ 0.9 M	3000 g 10 min	2M potassium hydroxide	Several wavelengths	[72, 73]
Glycolytic rate	Homogenised: 5000 rpm for 90 s (Ace homogeniser AM-8)	6% $HClO_4$	3000 g 10 min 4°C		Ratio of absorbance: 250/260	[63, 73]
Creatine, creatine phosphate	Incubated: on ice (15 min)	600 μl of ice-cold 0.6 M $HClO_4$ containing bromthymolblue and phenolphthalein 0.004%	10,600 g 5 min, 4°C	540 μl 0.8 M KOH + 25 ml 25% KH_2PO^4 buffer ice-cold	210	[68]
Calcium	Ashed: furnace 450°C	HNO_3 and $HClO_4$ digestion			Atomic absorption spectrometry	[32]

phosphorylase [67, 75], AMP kinase (AMPK) [67], phosphofructokinase, pyruvate kinase, AMP deaminase [75], or phosphoglucoisomerase [78]. These methods are summarised in Table 2. Most of these methods are based on the extraction of the active enzyme in an appropriate buffer providing the optimum medium (co-factors and pH stability) to observe the activity of extracted enzymes. A measurement of the "in vitro" activity of these enzymes is then performed using specific subtracts from each of the particular enzymes under study. Subsequently, either the substrate depletion or product formation can be monitored by means of colorimetric methods or by HPLC analysis.

3.3. Online Techniques of Metabolic Pathways Analytes Involved in Pre-Rigor and Rigor Stages. The main problem with

Table 2: Methods employed for enzymatic activity assays.

Enzyme	Homogenization/other physical treatements	Buffer	Assay	Detection (nm)	Ref.
Glycogen debranching enzyme	20 min, 18,000 rpm	137 mM NaCl, 1 mM $MgCl_2$, 1% NP^{40}, 10% glycerol, 2 mM PMSF, 10 mM $Na_4P_2O_7$, 2.5 mM EDTA, 10 μg/ml Aprotinin, 10 μg g/ml Leupotinin, 100 nM·NaF	Glucose release	PAP kit	[67]
	Ultra tTurrax + centrifugation	0.05% $KHCO_3$ + 0.004 M EDTA (pH 7.8 at 25 °C)	Glucose release	Iodine reagent, Abs. 525	[74]
Glycogen phosphorylase	50 mM Tris–HCl buffer, (pH 7.6).	50 mM Tris (pH 7.6), 5 mM imidazole, 2 mM EDTA, 10 mM KH_2PO_4/Na_2HPO_4 (pH 7.6), 1.4 mM mercaptoethanol, 0.6 mM NADP, 0.05 mM glucose-1,6-diphosphate, 2 mM AMP, 0.65 U phosphoglucomutase, 0.28 U G-6-P dehydrogenase	Abs. increase	Abs. 340	[67]
	100 mM K_2HPO_4 ice-cold, pH 7.4	50 mM K_2HPO4, 2 mg/mL glycogen, 1.3 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM NADP, 200 mM MES (pH 6.8), 1 U/mL phosphoglucomutase and 1 U/mL G-6-P dehydrogenase	Abs. increase	Abs. 340	[75]
AMP- kinase					
Phosphofructokinase	100 mM K_2HPO_4 ice-cold, pH 7.4	120 mM MES (pH 6.5), 3.2 mM MgSO4, 2 mM ATP, 0.15 mM AMP, 1 mM NADH, 3 mM fructose 6-phosphate, 2 U/mL triosephosphate isomerase, 1 U/mL glycerol-3-P dehydrogenase and 1 U/mL aldolase		Abs. 340	[75]
Pyruvate kinase	100 mM K_2HPO_4 ice-cold, pH 7.4	120 mM MES (pH 6.5), 100 mM KCl, 10 mM $MgCl_2$, 1.25 mM ADP, 1 mM NADH, 0.5 mM PEP and 2 U/mL lactate dehydrogenase	PEP conc. decrease.	Abs 340	[75]
AMP deaminase	Ffrozen powdered muscle + 90 mM K_2HPO_4 pH 6.5, 180 mM KCl, and 0.1 mM dithiothreitol	200 mM 2-(*N*-morpholino) ethanesulfonic acid 150 mMKCl, and 10 mMAMP at pH 6.5	AMP level	HPLC	[75]
Phosphogluco isomerase	100 mM K_2HPO_4 pH 7.4	200 mM MES, 10 mM Na_2HPO_4, 5 mM MgCl2, 60 mM KCl, 0.5 mM NAD^+, 25 mM glycogen, 25 mM carnosine, 30 mM creatine, 10 mM gG-6-P and 10 mM $C_2H_3NaO_2$ at pH 5.5–6.0	F-6-P level		[75]

the traditional biochemical methods discussed above are that they are time-consuming, and biomarkers should be easy and fast to determine and preferably online. In this regard, later studies based on spectroscopy indicate the possibility to determine early postmortem metabolic analytes using Raman spectroscopy. The research of Scheier et al. [79] on porcine semimembranosus muscle indicates a good agreement between measured biochemical parameters and simulated Raman. In this regard, in the pre-rigor and rigor frame, they were able to find clear signals of phosphocreatine, ATP, IMP, and α-helical proteins (correlated with glycogen to lactate). Sheier et al. [79] provided a first semiquantitative description of the early postmortem Raman spectra of meat which show potential for fast detection of the metabolic state of meat and hence for the identification of meat quality defects.

4. Mechanical Methods

As the muscle enters rigor mortis, it loses its ability to relax (until further enzyme activity degrades the complex), which is of critical importance for meat quality since it increases the toughness of the meat. It is clear that the tenderness and other palatability characteristics depend directly on the rigor mortis state. For this reason, an early mechanical measurement of this phenomenon will help to predict the final meat characteristics, in this case mainly texture-related characteristics. Contrary to biochemical methods, mechanical methods provide a direct

measurement of the meat toughness "at rigor" that is directly related with the final texture and palatability of the steak [80, 81]. Physical methods have been used or have the potential to be used to determine the texture during onset and progress of rigor mortis include elasticity, ultramicroscopic observation, tensile and adhesive properties, myotonometry, isometric tension, NMR, NIR, and sonoelasticity.

These studies have given us a profound understanding of rigor mortis in skeletal muscles, especially "red" muscles, and a technological guidance to control meat quality [82]. However, these studies are usually done on single muscle fibres or single muscle bundles, and thus, it is difficult to give an accurate depiction of rigor mortis for an intact muscle. The advantage of many of the techniques discussed here is that they are not destructive. Damez and Clerjon [83] published a review gathering available biophysical methods related to meat structure. In the present article, we expand the array of mechanical methodologies used for meat quality prediction during rigor mortis.

4.1. Elasticity. Measuring the elastic properties of the muscle was firstly investigated in the 1930s, culminating with the development of a "rigor-meter" instrument. The method is based on the accurate measurement of muscle length extension when a fixed amount of force is applied to the muscle and the extent to which the original length is recovered after the force is removed. Meat rich in ATP is able to endure significant stretching, while maintaining its ability to recover its original shape. However, when ATP becomes depleted, the muscle is only very slightly extensible. Muscle strips are maintained in an anaerobic atmosphere to prevent aerobic surface resynthesis of ATP [84]. Despite this method being created to concretely to evaluate the rigor phase, there have been not studies relating rigor elasticity with the final meat texture.

4.2. Isometric Tension. The principle of isometric tension is the application of a load to a muscle that is too great to be moved, and in response, the muscle generates increasing tension as it attempts to shorten. The isometric tension (IT) is expressed as force per unit area and the muscle shortening (SH) as percentage decrease in the length of the muscle. The isometric tension test was developed by Rheologica Instruments (Lund, Sweden) in conjunction with the Swedish Meat Research Institute. It is a unique analytical instrument (RigoTech Muscle Texture Analyser) with the aim of determining the optimum cooling conditions for meat tenderness. Tornberg et al. [85] describe the collection of data with strips (35 mm; 1.5 ± 2 g) of muscle tissue (M. *longissimus dorsi*). The isometric and isotonic recording components record the readings every 5 min in a closed chamber at a controlled temperature. Regarding the potential of isometric tension measurements as predictive method for final meat quality, a good correlation has been established between isometric measurements and other parameters such as myofibrillar and sarcomere length, a good predictor of meat tenderness [86]. On the other hand, trials comparing muscles with different shortening levels due to differences in temperature found a good correlation between isometric measurement and toughness even after 14 days of storage on some muscles [87]. On the contrary, Devine et al. [88] found that meat going into rigor at around 15°C is equally tender at 14 of ageing, independently of isometric measurements (restrained and unrestrained muscles) implying higher enzymatic importance at these temperatures.

4.3. Myotonometry. Vain et al. [89] describe the use of a myotonometer, a handheld instrument used to grade rigor mortis. The instrument delivers a mechanical impact against the muscle to which there is a damped vibratory response. This information is received and treated mathematically, i.e., the period and logarithmic decrease/decay of the vibrations are found to change with the development of rigor mortis, yielding quantitative information on muscular stiffness. It is particularly useful in determining the time of death. To-date, there is no study relating this technique with final meat quality.

4.4. Ultrasound Technologies. Ultrasonic properties have been used to assess physicochemical properties of many foods including meat. For the quality evaluation of muscle foods, mainly two methods have been employed: ultrasonic spectral analysis [90] and ultrasonic elastography [91]. The former is based on the differences in some ultrasonic parameters, such as velocity, attenuation, and backscatter intensity, due to differences in muscle composition and structure which generate the differences in wave propagation. Ultrasound has been applied to determine meat quality of different individual muscles [92–94], but it has also been used for predicting meat quality in live animals [95, 96] and carcasses [97, 98]. The ultrasound probe placed in the inside of the carcass has proved to reveal promising results about intramuscular fat determination in pork carcasses [98].

Ultrasound elastography originates from the medical diagnostic field; however, it is beginning to emerge as a diagnostic tool for evaluation of the skeletal muscle mechanical properties. Shear wave elastography (SWE) is an ultrasound-based technique that uses the propagation of remotely induced shear waves to characterise the mechanical properties of the tissue. Using the shear wave propagation and the tissue density, the shear modulus is calculated and can be correlated with tensile load. The main drawback of this promising technology is the cost, which at this point is too high for industry application. Eby et al. [99] described an experimental setup combining traditional materials testing techniques with SWE. The tensile test was applied to porcine *brachialis* whole muscle tissue at 1.15% of the initial length per second with simultaneous ultrasound measurements. The orientation of the ultrasound transducer to the muscle fibre direction played a key role in the importance of the obtained data; at 45° and perpendicular to the fibres, the shear waves did not propagate properly, whereas when parallel there was a positive correlation between SWE and the tensile load.

4.5. Sonoelasticity. Sonoelasticity is a nondestructive method that combines ultrasonic waves with Doppler imaging to indicate differences in biological tissues. Ayadi et al.

[100] used this technique on *biceps femoris* muscle of beef to monitor changes during rigor and ageing. The authors applied perturbation (80 and 100 Hz) and ultrasound waves (5 MHz) and recorded different sonoelastic parameters, including velocity and attenuation. In addition, the authors also took linear compression measurements and hourly pH determinations. When these were compared with sonoelasticity parameters, significant and higher correlations were obtained (coefficient of correlation up to 0.97). In the same study, the authors were also able to detect the mechanical textural changes during ageing with the sonoelasticity parameters. To our knowledge, there are no studies relating the results obtained by sonoelasticity in pre-rigor and rigor with final muscle tenderness or other meat quality characteristics.

4.6. Laser Air-Puff Systems. This technique has been adopted from the fruit processing industry, where it is applied for measuring the firmness of peaches or kiwis [101]. The potential application of a laser pressurised air system to assess poultry meat tenderness was investigated by Lee et al. [102]. Lee et al. [102] carried out experiments where raw chicken breasts (deboned at 1.25, 4, or 24 h postmortem) were longitudinally scanned on a conveyor belt by a laser distance sensor in order to obtain overall shape profiles; subsequently, they were scanned again with a pressurised source of air (206.8 kPa). The two resulting profiles were superimposed to quantify the amount of deformation caused by the application of pressurised air. Five parameters including the height and length of each fillet were calculated and used to establish a model to predict tenderness. Even though this technique has only been applied to chicken fillets, there is potential for developing it for implementation in the abattoir line.

4.7. Texturometer Analysis. The use of texture analysers is well documented for meat texture determination in lab [103–105]. However, Li et al. [106] used a texture analyser (TAXT2i, Stable Micro Systems Ltd., Godalming, UK) to monitor changes during rigor mortis or thaw rigor in chicken breast muscle. Plastic film-wrapped samples were penetrated by a cylindrical probe to a distance of 2 mm at a velocity of 1 mm/s and a trigger force of 0.02 N. The probe was held for 48 h, and data were acquired at 0.1 points per second. Representations of the force as function of time provided information of the differences in rigor mortis and thaw rigor at two different temperatures (4°C and 15°C). It was found that, even at lower temperatures where the process of rigor mortis takes longer times, the final contracture reached the same extent than at the higher temperature. When the rigor commences, the sarcomere shortens and the muscle fibre diameter increases and hence the force is higher. When the rigor finishes, the sarcomere length increases and the diameter decreases producing a decrease in the force. Texturometer analyses at pre-rigor and rigor phases have been used exclusively to monitor the process of rigor, and no experiments have been found relating these early measurements with final meat quality parameters.

5. Rapid Nondestructive Techniques Performed Online

The use of rapid nondestructive technologies can be applied to early predict meat quality parameters. They offer the ability to increase the control checks during meat processing and retailing but can also present an opportunity to evaluate meat quality right after slaughter. Apart from the use of Raman spectroscopy (section 3.3.) to determine biochemical parameters, there are a few other technologies based on imaging and spectroscopy that have being recently implemented or are under investigation for the evaluation/prediction of final texture.

5.1. X-Ray Analysis. Two main technologies based on X-ray have been used for meat quality analysis: computed tomography (CT) and dual X-ray absorptiometry (DXA). CT is widely used in the medical field, but it has also been used as a measuring tool of animal body composition (lean, fat, and bone) since the early 80s [107]. It has also been employed for meat quality determination in pigs and lambs by means of intramuscular fat [108, 109]. The main drawbacks of this technique are the generated radiation and the small aperture of the actual devices that make it impossible to scan full bovine carcasses.

DXA couples the information acquired at two energy levels (high and low) and was initially designed for the measurement of bone mineral density but later on was used for body composition. Kröger et al. [110] used DXA to predict tenderness in steaks obtaining a correlation of 0.69. A few years later, a patent was filed regarding the use of X-rays to evaluate meat tenderness in meat products [111]. According to the author of the invention, the meat sample is analysed through an X-ray beam and the amount of radiation transmitted can be related to a characteristic of the shear force measured by Warner Bratzler. Even though this technique has been only applied to meat products, it could be potentially used on carcasses to have an earlier prediction of meat tenderness.

5.2. Video Image Analysis (VIA). VIA systems are based on the differences in light intensity received by a video camera and were developed specifically for beef carcass evaluation. Nonetheless, some specific systems have been also utilised to predict tenderness and marbling in beef [112] and pigs carcasses [113]. Usually in these systems, the camera is located at some distance from the rib eye section.

5.3. NIR and Vis-NIR Spectroscopy. Spectrophotometric methods use probes in contact with or penetrating into the meat [114]. Earlier pre-rigor analysis was applied between 2 and 4 hours postmortem by Rodotten et al. [115] using near-infrared spectroscopy (NIR), and their results showed a poor correlation coefficient with the final texture after 7 days ageing (0.47–0.55). Hoving-Bolink et al. [116] investigated the ability of visible-NIR spectroscopy (1 h postmortem in pork) to predict meat

quality. Their results indicate a poor correlation between NIR and meat quality measurements. Vis-NIR spectroscopy has been also used to predict ultimate pH in beef [117], obtaining a prediction model able to correctly segregate 90 % of the carcasses with high (>5.8) or normal pH (<5.8).

5.4. Hyperspectral Imaging (HIS). HIS is a relatively modern technique that combines imaging and spectroscopic data. Applied on the ribeye surface of a hanging carcass, it was able to predict 14-day aged cooked beef tenderness with an accuracy of 86.7% [118]. pH and drip loss have been also assessed in different muscles and species [119, 120]. As this technique stores enormous amount of data, the processing is paramount. With new data processing techniques and hardware and software improvements, this technique could be a potential technique for meat quality determination at an early stage; however, few experiments have been done during the onset and rigor stages. A thorough review of the application of HIS in red meat has been published by Xiong et al. [121].

6. Conclusion

It is long established that proteolysis is responsible for a considerable portion of postmortem tenderisation. However, of great importance are the stages that precede, namely, pre-rigor and rigor mortis stages. Increasing accuracy and sensitivity of chromatography systems have led to more accurate measurements of the biochemical compounds that underpin the transformation of muscle into meat, leading to a better understanding of their connection with the final meat eating quality attributes, namely, tenderness. Furthermore, the use of increasingly nondestructive mechanical or spectroscopic methods to monitor the physical changes brought on by rigor mortis could be used to evaluate and predict a different array of meat quality attributes. However, despite the high number of techniques reviewed in the present manuscript, there is no particular method (chemical or physical) that can accurately predict the final meat eating quality characteristics in the early postmortem period. Therefore, further research is needed where combinations of the methods here described, the incorporation of emerging tools as proteomics and metabolomics, or their correlation with sensory properties will be of benefit to accurately predict meat eating quality attributes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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