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The application of biosensors for drip loss analysis and glycolytic potential evaluation

Wiesław Przybylski [a,*], Barbara Sionek [a], Danuta Jaworska [a], Véronique Santé-Lhoutellier [b]

[a] Department of Food Gastronomy and Food Hygiene, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C str., 02-776 Warszawa, Poland
[b] INRA, UR370 QuaPA, 63122 Saint-Genès-Champanelle, France

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ABSTRACT

The aim of this study was to evaluate the relationship between glucose and lactate measured by biosensors in drip loss (strip method) with muscle glycolytic potential and their compounds. On the samples taken from *Longissimus dorsi* of 24 pigs (pure Neckar hybrid line) the following meat quality traits were determined: pH at 24 h, meat color according to CIE $L^*a^*b^*$ system and drip loss. The highest correlations were found between glucose in drip loss and glycogen ($r = 0.84$) or glycolytic potential ($r = 0.81$) in muscle. A significant positive relationship between lactate measured in muscle by enzymatic method and by biosensor in drip loss was established ($r = 0.76$). Glycogen, glucose, lactate and glycolytic potential with meat quality traits as ultimate pH, lightness, b^* value and drip loss were significantly related. Results of multiple regression between glucose as well as lactate measured in muscle drip loss with muscle glycolytic potential showed the possibility of its prediction ($r = 0.87$ with $P_\alpha \leq 0.001$).

1. Introduction

Glycogen is the main source of energy for the reconstitution of ATP during anaerobic *post mortem* muscle metabolism when it is converted into lactic acid. The amount of glycogen in muscle depends on genetics and environmental factors that can be divided into the *ante mortem* and *post mortem* (Larzul, Le Roy, Monin, & Sellier, 1998; Meadus & MacInnis, 2000; Rosenvold et al., 2001; Scheffler & Gerrard, 2007). Environmental factors influencing glycogen content and pork quality include· breeding conditions, nutrition, transport conditions, stress, weather conditions and the methods of slaughter. Limiting the stress factors is essential for improving the quality of pork. Muscle glycogen content is key determinant of ultimate pH and other quality traits such as drip loss, color, cooking loss and thus sensory properties of meat (Enfält & Hullberg, 2005; Hamilton, Miller, Ellis, McKeith, & Wilson, 2003; Meadus & MacInnis, 2000; Nanni Costa et al., 2009). The evaluation of glycogen content in live muscle means to operate a muscle biopsy *ante mortem*. In 1985 Monin and Sellier proposed a formula to determine glycogen content in muscle, namely the glycolytic potential (GP) expressed in μmol of lactate, which is the sum of all compounds that are formed in muscle during *post mortem* anaerobic glycolysis as glycogen, glucose-6-phosphate, glucose and lactate. Glycogen is generally analyzed by acid or enzymatic hydrolysis followed by glucose determination (Keeton, Benli, & Claflin, 2009). Maribo, Støier, and Jørgensen (1999) showed that glycolytic potential could be evaluated with the same accuracy both just after slaughter as well as 24 h later. Indeed the impact of glycogen content and its hydrolysate compounds such as glucose on meat quality has been often reported (Enfält & Hullberg, 2005; Hamilton et al., 2003; Meadus & MacInnis, 2000; Nanni Costa et al., 2009). Glucose content was reported to affect the sensory quality, the volatile compounds, the antioxidant activity and the browning level of grilled and fried pork (Buła, Jaworska, & Przybylski, 2015; Meinert, Andersen, Bredie, Bjergegaard, & Aaslyng, 2007; Meinert et al., 2008; Meinert, Schäfer, Bjergegaard, Aaslylg, & Bredie, 2009; Namysław, Buła, Jaworska, Grzywacz, & Przybylski, 2013).

Some studies showed a difference in muscle glucose level after *post mortem* glycolysis which could be attributed to differences in activity of the glycogen debranching enzyme, temperature of muscle or genotype (Meadus & MacInnis, 2000; Scheffler, Kasten, England, Scheffler, & Gerrard, 2014; Ylä-Ajos, Ruusunen, & Puolanne, 2006; Ylä-Ajos et al., 2007). Hamilton et al. (2003) reported a coefficient of variability for free muscle glucose content *post mortem* equal to 74.3% while for GP either measured *in vivo* or *post mortem* the variability is around 22.3% and 24.4% respectively. During glycogenolysis free glucose is liberated by glycogen debranching enzyme that hydrolyses α-1-6 bounds of glucosyl branch (Pösö & Puolanne, 2005). According to Meléndez, Meléndez-Hevia, and Cascante (1997) glycogen molecule is constructed

* Corresponding author.
E-mail address: wieslaw_przybylski@sggw.pl (W. Przybylski).

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from two linear glucose chains named A and B. Each chain contains 13 units of glucose bound together with α-1,4-glycosyl bonds. At the fourth and eighth glucosyl units of the B-chain there is an α-1,6-glycosyl bond initiating a new A-chain, and the total number of chains is $1 + 2^{12}$, *i.e.* about 4100. Information that glycogen molecule contains about 55,000 glucose residues and that some part of glycogen is not degraded (called residual glycogen) allows us to formulate a hypothesis that free glucose could be equal to about 5% of total amount of glycogen. Lundström and Enfält (1997) showed a possible application of glucose determination in meat juice of meat to detect pig carrying RN-gene. Based on the rapid enzymatic hydrolysis of muscle glycogen to glucose a method for early determination of meat ultimate pH was elaborated and patented by Young, Thomson, Merhtens, and Loeffen (2004a). Hargreaves, Barrales, Barrales, Riveros, and Peña (2009) elaborated a rapid and inexpensive method for glycogen determination based on muscle glucose determination. Hamilton et al. (2003) found that the free glucose concentration in the exudate from *Longissimus* muscle was more strongly related to fresh pork quality than glycolytic potential and was quicker and less expensive to measure. Analysis of sarcoplasmic protein in drip loss was also applied by some authors for meat quality evaluation. Bowker, Eastridge, and Solomon (2014) and Żelechowska, Przybylski, Jaworska, and Santé-Lhoutellier (2012) showed that muscle exudates may be a good source of protein markers that are useful in the development of rapid, noninvasive methodologies for predicting pork quality (pH, meat color, drip loss, juiciness) and beef tenderness respectively. Di Luca, Elia, Mullen, and Hamill (2013) stated that muscle exudate provided valuable information about the pathways and processes underlying the *post mortem* ageing period. Therefore we can hypothesize in this study that muscle exudate reflects the changes occurring in meat during aging and its composition. Thus, the objective of this study was to evaluate glucose and lactate measured by a simple method in drip loss as potential markers of muscle glycolytic potential.

2. Materials and methods

2.1. Materials

The research was carried out on 24 pigs (gilts) of pure Neckar line. The Neckar line was produced by PenArLan (France). The Neckar line is characterized by high daily gain and good carcass conformation (especially a large area of the loin "eye"), which enables the production of heavy pigs with high meatiness. Pigs originating from the herds were included in the program of elimination of disadvantageous genes' influence on meat quality (RYR1T and RN-). All animals came from the same farm and were kept under identical environmental conditions. The animals were slaughtered at 100 kg live weight, in the slaughterhouse 50 km far from the farm in accordance with legally binding procedures, according to the following conditions: 2 h resting before slaughter, automatic electric stunning and exsanguinations in the horizontal position, carcass was chilled in fast cooling system.

2.2. Meat quality traits

Samples were taken from *Longissimus dorsi* (LD) muscle behind the last rib (in the lumbar region) within 24 h after slaughter. The following meat quality traits were determined: pH at 24 h *post mortem* with a WTW 330i pH meter (Weilheim, Germany), meat color according to CIE $L^*a^*b^*$ system using CR310 Minolta Chroma Meter with D_{65} light source (Osaka, Japan). The drip loss from muscle tissue was determined 48 h after slaughter according to Honikel (1998). A 50 g sample of meat (10 cm × 5 cm × 1 cm) cut perpendicularly to muscle fibers was taken 24 h *post mortem*, placed in a plastic bag, and kept at the temperature of 4 °C. After 24 h, the sample was removed from the bag, dried on absorbent paper, and reweighed. Amount of drip at 48 h *post mortem* was expressed as a percentage: % drip loss = [(initial weight − final weight)/initial weight] * 100.

2.3. Enzymatic method of glycogen, glucose, glucose-6-phopsphate and lactic acid determination

About 1 g of muscle tissue was homogenized with 10 ml of 0.5 M perchloric acid and homogenate (0.5 ml) was taken for determination of glycogen, glucose and glucose-6-phopsphate according to Dalrymple and Hamm (1973) by enzymatic hydrolysis of glycogen with amyloglucosidase. Lactic acid was determined in the supernatant after centrifuging the homogenate according to Bergmeyer (1974) method. The glycolytic potential (GP) was calculated according to the formula from Monin and Sellier (1985) and expressed as micromoles of lactate equivalent per gram of fresh tissue. All measurements were done in duplicates.

2.4. Determination of glucose and lactic acid by using of biosensors

Twenty four hours after slaughter the samples of LD muscles were placed in a plastic bag and kept at 4 °C for another 24 h for drip loss collection. Drip loss was collected for the glucose and lactate measurements. The determinations of muscle glucose (mmol/l) were made with the Accu-Chek Active® glucometer, that is normally employed for measuring blood glucose in human (Accu-Chek Sensor Comfort®, Roche, Germany). Reactive strips were used for the quantitative determination of glucose with the glucometer in the value range between 0.6 to 33.3 mmol/l. Test results were ready provided 60 s after placing a drop with an approximate volume of 20 μl of drip loss on a reactive strip.

Lactic acid (mmol/l) was measured with the strip method using Accutrend^R Lactate type 3012522 (Roche, Mannheim, Germany). Samples were diluted with distilled water 1:10 to reach a lactate concentration in the following range: 8.7 to 26 mmol/l. The test results also were provided in 60 s after placing a drop with an approximate volume of 20 μl of diluted drip loss on a reactive strip. Then, the results were converted into concentration in undiluted drip loss. All measurements were made in duplicate.

2.5. Statistical analysis

Data were analyzed using the Statistica 10.0 software package (StatSoft, Inc., 2011). For each quantitative variable, the normality was tested using skewness and kurtosis method. The simple Pearson linear correlations and multiple regression between measured traits were calculated as studied sample was homogenous (one breed and sex of

Table 1
Characteristics of meat quality, glycolytic potential and its components in *Longissimus dorsi* muscle and glucose, lactate in drip loss.

Traits	Mean	Min.	Max.	SD
Meat quality traits				
pH_{24}	5.50	5.31	5.68	0.11
Color parameters				
L^*	54.88	49.97	57.29	2.26
a^*	16.23	14.29	17.66	0.88
b^*	5.25	3.14	7.86	0.87
Drip loss (%)	3.51	1.53	6.03	1.03
Muscle longissimus dorsi				
Glycogen[a] (μmol/g)	7.41	2.00	16.62	4.04
Lactate (μmol/g)	106.08	95.27	116.61	5.55
Glycolytic potential (μmol/g)	120.90	102.01	141.82	12.08
drip loss – muscle juice				
Glucose (mmol/l)	6.67	4.50	11.44	1.89
Lactate (mmol/l)	148.96	117.00	179.00	16.29

[a] As sum of glycogen + glucose + glucose-6-phosphate.