COMPOSITION OF EXUDATES FROM MEAT DRIP LOSS AND MICROBIAL SPOILAGE DIFFERENCES BETWEEN VARIOUS PORK QUALITY CLASSES

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Abstract – This study aimed at elucidating the differences in the composition of meat exudates and determining which constituents contribute the most to microbial growth between five pork quality classes (DFD=Dark, Firm, Dry; RFN=Reddish-pink, Firm and Non-exudative; RSE=Red, Soft and Exudative; PFN=Pale, Firm and Non-exudative and PSE=Pale, Soft and Exudative). A total of 65 L*ongissimus* muscle samples (*n*=15/pork quality class; *n*=5 for DFD meat class) were analyzed in triplicate for glucose, glucose-6-phosphate, lactate and protein content, and microbial growth. Differences between pork quality classes were assessed using the MIXED procedure of SAS. Surprisingly, after storage at -80°C, the greatest pH value was observed in the purge of RFN pork (*P*<0.001), while lactate content of DFD pork tended to be lower (*P*=0.08) than the other pork quality classes. No differences were observed for glucose, glucose-6-phosphate and total protein values between pork quality classes (P>0.05). Volume of drip loss was a major limit with the methods used. High throughput mass spectroscopy is currently under investigation as a more effective tool to study drip loss composition and effect on microbial growth.

Key Words: microbial growth, purge composition, shelf life.

I. INTRODUCTION

Traditionally, raw pork has been classified into three quality categories, namely reddish-pink, firm and non-exudative (RFN), pale, very soft and exudative (PSE) and dark, firm and dry (DFD) meat, according to three main technological parameters, namely ultimate pH (pHu), colour (L* value) and water-holding capacity (WHC) or drip loss. Over the past few years, for a more reliable quality assessment taking into account the variation in either colour or exudate, additional quality categories, such as reddish-pink, firm and exudative (RSE) and pale, firm and non-exudative (PFN) meat, have been described [1, 2, 3].

RSE pork is a quality category with an acceptable colour, but with soft texture and exudation rate similar to PSE meat [1]. Due to its similarity in the exudation rate with PSE meat and its greater pHu [4, 5], RSE meat has been defined as a mild form of PSE [3, 6]. Additionally, Faucitano et al. [6] observed that among the meat quality classes with a pHu value lower than 6, RSE meat showed a greater microbial load after 35 days of storage under vacuum and refrigerated conditions when compared with the other meat quality classes. A higher pHu promotes microbial growth and shorter shelf-life due to a pHu higher than 6 has been well demonstrated in DFD meat [7], but the reasons for the greater susceptibility to bacterial spoilage in RSE meat during storage are still unknown. Previous studies have reported that the meat juice exuding from the meat during *post-mortem* storage is mainly composed of low molecular weight compounds readily available for bacterial growth [6, 8, 9]. However, the chemical nature and proportion in drip composition from the various pork quality classes and their contribution to microbial growth is still unclear. Therefore, the objectives of this study were to elucidate the composition of meat exudates obtained from all five pork meat quality classes and their contribution towards microbial growth and, consequently, to fresh meat shelf-life.

II. MATERIALS AND METHODS

Meat exudate

A total of 65 *Longissimus* muscle samples (*n*=15, for RFN, PFN, RSE and PSE; *n*=5 for DFD) were analyzed for the composition of frozen meat exudate collected from loins of five quality classes during drip loss measurement in a previous study [10]. As the volume of meat exudate samples was very small for some quality classes (*e.g.*, PFN=±0.4 and DFD=±0.2 mL/sample) samples were pooled by meat class in order to have sufficient volume of material to run the analysis. The pools were done by blending 4-5 samples of same meat class in order to have a similar pH for each pool. Thus, a total of 14 pools (PSE=4, RSE=4, DFD=1, RFN=2, and PFN=3) were used for the following analyses: exudate pH, enzymatic determination of glucose, glucose-6-phosphate, lactate, total protein and microbial growth. Even after pooling, the number of DFD exudates was so low that only one pool could be obtained.

Exudate pH and composition

The pH of the meat exudate was assessed in each sample before blending and in each pool, by meat quality class, using a portable pHmeter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a MicroProbe™ (Accumet, model: CP-620-96, Montreal, Canada).

Lactate content in the meat exudate was analyzed according to the method described by Monin and Selier [11]. Briefly, for the enzymatic determination of glucose and glucose-6-phosphate (G6P), 500 µl of meat exudate was transferred to a glass tubes and another 500 µl was transferred to regular eppendorf tube and kept at 4°C for the enzymatic determination of lactate. The 500 µl of meat exudate was then homogenized in acetate buffer containing *Rhizopus* amyloglucosidase to decompose glycogen to glucose and G6P. Then, glucose concentration was determined using a nicotinamide adenine dinucleotide (NAD), G6P, adenosine triphosphate (ATP) and enzymatic solution of hexokinase. Lactate concentration was determined with 100 µl of meat exudate using NAD and lactate dehydrogenase. Finally, total protein concentration was determined on 100 µl of meat exudate using the bicinchoninic acid protein assay kit (Pierce™, Rockford, IL).

Meat microbiology

Microbial growth was performed by plating 100 µl of a 1:10 dilution (Phosphate buffered saline solution) onto growth medium (Lysogeny Broth) in duplicate for RSE, PSE and DFD meat classes. DFD and PSE classes are known to be permissive and non-permissive for microbial growth [12, 13], respectively, and were compared with RSE meat exudate. For the DFD meat exudate, an additional serial dilution (1:100) was necessary. Plates, including a negative control, were incubated at 37°C overnight and colonies were enumerated after 48 h. The mean of the duplicate plating was calculated and the data were tabulated as colony forming units (CFU) per ml.

Statistical Analyses

Differences in glucose, G6P, lactate and total protein data were analyzed using the MIXED procedure of SAS [14] in a one-way analysis of variance for the pork class effect and with the pool per meat quality class as the experimental unit. Spearman correlations were performed using SAS to determine relationships between the meat exudate components for each pork class. The meat microbiology results were transformed in Log CFU/ml and numerically compared between quality classes. A probability level of $P<0.05$ was chosen as the limit for statistical significance, whereas $P\leq0.10$ were considered to be a tendency.

III. RESULTS AND DISCUSSION

The microbial analysis showed that counts with PSE exudate (1.85 Log CFU/ml) were 2.45 and 0.45 Log below the results for DFD (4.30 Log CFU/ml and RSE (2.30 Log CFU/ml) exudates, respectively. Hence, DFD was the most spoiled samples whereas RSE was classified as intermediate compared to PSE. Greater counts in DFD exudates is not surprising based on its low concentration of lactic acid/lactate and the higher pHu [12, 15], both known to promote microbial growth at a faster rate. The greater availability of glucose on the PSE meat exudates delays the use of amino acid as a carbon source and thereby delays spoilage [12]. These results confirm the greater predisposition for bacterial spoilage of

RSE pork, which is only second to DFD pork, as reported in a previous study [6].

Faucitano et al. [6] suggested that the meat exudate composition or quantity may contribute to meat spoilage in pork presenting a pHu<6. Kim et al. [9] also highlighted the role of meat exudate as a suitable substrate for bacterial growth in meat during cold storage. However, in this study, the differences in the exudate components among the five meat quality classes were mostly numerical (Table 1). The small sample size may explain the lack of significant differences.

Table 1. Variation of lactate, glucose, glucose-6-phosphate, total protein content and pH in exudate by pork quality class

Variables	PFN	PSE	RSE	RFN	DFD[1]	SEM	*P* value[2]
G6P[3]	2.45	2.47	1.78	2.01	-	0.63	NS
Glucose[4]	11.54	13.73	11.55	8.46	-	1.70	NS
Lactate[5]	141^{AB}	150^{A}	150^{A}	142^{AB}	128^{B}	6.9	0.08
Protein[6]	99.7	98.4	97.6	106.0	115.0	7.0	NS
Exudate pH	5.28^{c}	5.29^{c}	5.90^{b}	6.45^{a}	-	0.11	< 0.001

[A,B] Different capital letters mean that values tend to be different (P<0.10).
[a,b] Different lower cap letters mean that values are significantly different (P < 0.05).
[1] Missing values are due to lack of DFD samples to run these analyses.
[2] NS = P>0.10.
[3] Glucose-6–phosphate (µmole/mL).
[4] Glucose (µmole/mL).
[5] Lactate (µmole/mL).
[6] Total protein (mg/mL).

Unsurprisingly, the lactate content of DFD meat exudate tended to be lower (P=0.08) compared to PSE and RSE meat exudates (Table 1). These results are consistent with the findings of Traore et al. [16], who reported greater lactate content in the exudate from exudative compared to non-exudative meats. This difference in lactate concentration likely led to differences in the pH of meat exudates (P<0.001) between meat classes. For meat to be classified as RFN, pH of the meat had to be lower than 6 by definition [10]. The pH of the RFN meat exudate is higher than 6 suggesting that either during the EZ-drip loss procedures at 4°C for 48h or during storage at -80°C, meat exudates composition was altered. Apart from DFD, the exudates from RFN and RSE pork classes showed the higher pH values compared to all the other meat classes (P<0.05). The pH from DFD meat could not be measured due to the low quantity of meat exudate available for the analysis.

No difference in protein concentration was found between the different pork exudates (P>0.10). These results disagree with Bowker et al. [17] who reported greater values for the drip protein concentration between dark and pale breast fillets. The lack of difference in protein content between pork quality classes in this study may be explained by the exudate storage conditions (-80°C) pending analysis that may have caused certain physicochemical qualitative changes in the samples, such as amino acid decarboxylation, formation of ice crystals resulting in protein denaturation [18]. No correlation between constituents, or ratio of constituents, with meat quality classes could be established firmly. Only the pH of the exudate can explain variation observed in microbial growth. To our knowledge, this is the first study on meat exudate composition from the different pork quality classes including PFN and RSE meats.

CONCLUSION

The results of this study showed that meat exudates contain low molecular weight compounds, such as glucose, G6P, lactate and protein that can be readily available for microbial growth. However, either the small size or the quality of samples available for this study prevented from identifying the contribution of these components to the variation in shelf-life between pork quality classes. New analytical techniques, such as high throughput mass spectroscopy coupled with machine learning strategies are now being tested as a mean to better profile meat exudate constituents when limited quantity of material is available for analysis such as in the case of meat exudates where microbial growth actually takes place on the surface of fresh intact meat.

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