# PREDICTION OF PURGE FROM OVINE SEMIMEMBRANOSUS USING RAMAN SPECTROSCOPY

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Abstract – A 671 nm hand held Raman device was used to predict the purge of 80 fresh intact ovine *semimembranosus*. This study demonstrated there was an ability to predict purge after 4 days ageing using Raman spectra collected at 24 h post mortem. This gave a 21% reduction in the prediction error (RMSPE<sub>cv</sub>) and a cross validated coefficient ( $R^2_{cv}$ ) equal to 0.42. Since ultimate pH (pHu) proved significant in determining purge (P < 0.05), it was hypothesised that Raman spectroscopy is able to predict purge indirectly through the relationship between pHu and purge by indirectly measuring the metabolic processes or metabolic substrates which are involved in the conversion of muscle to meat.

#### Key Words – lamb, Raman spectroscopy, purge

#### I. INTRODUCTION

Increased quality of Australian lamb has boosted the demand for chilled exports in recent years. However, high purge caused by fluid being drawn out of water channels between muscle fibres during vacuum packing is undesirable in products for chilled export markets as it is a cause of significant weight loss and detracts from the appearance of meat at retail [1]. Because lamb carcases are assessed for market suitability based on weight, age, gender and fat scores, it is currently not possible to identify carcases which are at risk of high purge and consequently better tools for online carcase assessment are required.

Raman spectroscopy (RS) is one optic technology based on the scattering of light, which has potential to measure meat quality traits in online situations as it is rapid and non-destructive [2]. Furthermore, recent research has demonstrated its ability to predict the drip loss of pork *m*. semimembranosus with good accuracy ( $R^2_{cv} = 0.73$ ) [3]. This research reports for the first time, the potential for a Raman spectroscopic hand held device to predict purge losses of fresh intact ovine *m. semimembranosus*.

#### II. MATERIALS AND METHODS

Eighty (80) lamb carcases were randomly selected and measured over 4 days (20 per day) from the same abattoir. Lambs were processed following standard abattoir procedures and were electrically stimulated with a mid-voltage unit [4]. At 24 h the topside [5] was removed from the carcase and the cap muscle (*m. gracilis*) and *m. adductor* were removed to leave the *m. semimembranosus* (SM) for measurement.

Raman spectroscopic measurements were conducted at 25 m (data not reported), 24 h and 5 days post mortem (PM) using a 671 nM hand held Raman device, as previously described [6].

Immediately prior to the RS measurement at 24 h, the pH of each SM was measured using a pH probe (pH<sub>24</sub>). Following RS measurements, SMs were weighed, vacuum packed and aged at  $-1^{\circ}$ C for 4 days until further measurement at 5 days PM.

At 5 d PM, SMs were removed from vacuum packaging, patted dry with paper towel and weighed to determine purge loss. SMs were then allowed to 'bloom' for 2 h before a finely cut surface was removed from the same face previously measured at 24 h and another Raman spectroscopic measurement was conducted. Once

measured with the RS device, a 1 - 2 g section was excised for determination of pHu [7].

Raw spectra for both experiments were prepared for chemometric analysis as previously described. [6]. However once spectra for each sample were averaged and reduced to a wavenumber range between  $500 - 1800 \text{ cm}^{-1}$  no other pre-processing methods were used. Models to predict traditional indicators of meat quality were fitted using partial least squares (PLS) regression analysis. Cross validation was completed using the Monte-Carlo K fold cross validation method [8]. Models to determine the relationship between pH and purge were fitted using simple linear regression.

### III. RESULTS AND DISCUSSION

Summary statistics for pHu,  $pH_{24}$  and purge are outlined in Table 1.

Table 1. Summary statistics for purge,  $pH_{24}$  and pHu.

Trait	Mean (± s. d)	Range
Purge (%)	2.7 (± 1.2)	1.1 - 6.4
$pH_{24}$	5.68 (± 0.15)	5.44 - 6.12
pHu	5.71 (± 0.10)	5.59 - 6.16

The results of the chemometric analysis indicated that using Raman spectra collected at 24 h PM gave a better ability to predict purge after 5 days ageing compared to using Raman spectra collected at 5 days PM (Table 2). This model yielded a 21.7% reduction in the error of the prediction (RMSPE<sub>cv</sub>) and a cross validated coefficient between predicted and observed values ( $R^2_{cv}$ ) of 0.42 (Fig 1).

Table 2. The chemometric results for the prediction of purge using Raman spectra collected 24 h and 5 days post mortem.

Raman Spectra	Relative Reduction in RMSPE <sub>cv</sub> (%)	$R^2_{cv}$
24 h	21.7	0.42
5 days	18.3	0.33



Figure 1. Cross validated correlation between purge (%) predicted using Raman spectra and observed values measured after 4 days ageing.

Given that a significant relationship was found between pHu values and purge ( $\beta = -0.029\% \pm 1.2$  s. d., P < 0.05), it is plausible that Raman spectroscopy is predicting purge indirectly through the effects of pH on metabolic processes or via metabolic substrates which are involved in the conversion of muscle to meat that determine the biophysical and biochemical characteristics including pH. However, prediction of purge using Raman spectra measured 24 h PM ( $R_{cv}^2 = 0.42$ ) was more accurate than prediction using pHu alone ( $R^2 = 0.10$ ).

This agrees with previous research on the water holding capacity of meat, which suggests that the links between early PM events and the rate and extent of pH decline are critical in determining the ability of meat to retain moisture [9]. Indeed, studies conducted on pork suggest that the prediction of drip loss using Raman spectra is based on substrates from early PM metabolic processes including lactate, glycogen and organic phosphates [3, 10].

A tentative band assignment of SMs with the highest and lowest purge suggests that with increasing purge at 24 h there is a reduction in inorganic phosphate (875 cm<sup>-1</sup>), creatine (1042 cm<sup>-1</sup>)[10],  $\alpha$ - helical proteins (920 cm<sup>-1</sup>) [11], CH deformation signals (1448 cm<sup>-1</sup>) [11] and COO<sup>-</sup> (1560 cm<sup>-1</sup>) [12] which may reflect the concentration of lactate or pyruvate. Overall, this may suggest that samples with higher purge may have reached pHu values earlier and therefore may have entered rigor at higher temperatures causing greater amounts of protein degradation [9].

Yet it is important to acknowledge that as pork is characterised by a greater concentration of type IIB muscle fibres that have a greater glycolytic potential [13], deviations in drip losses of pork may be more related to accelerated pH decline or the on- set of rigor at earlier times post mortem and therefore at higher temperatures [14]. Consequently, it is difficult to directly compare between early PM pork and lamb at 24 h to determine which metabolic processes are contributing to the prediction of purge.

Like rapid pH decline, the development of low pHu has been related to high purge [1]. It is hypothesised that both accelerated pH decline and low pHu cause greater purge as there is a higher amount of protein denaturation, particularly if temperatures are higher at the development of pHu, as a result of cell structural changes which facilitate the movement of immobilised water through extracellular spaces [1, 9]. Once the pH of the muscle post mortem reaches the isoelectric point of the major proteins, the repulsion of the structures within the myofibril is reduced causing them to pack closer together, reducing space [9, 15].

In conjunction with these changes, formation of actin and myosin cross bridges and any shortening of the sarcomere as the muscle enters rigor results in longitudinal and lateral contractions, further reducing the space in the myofibril available for water [16]. Consequently, immobilised water may be forced into extracellular compartments permitting it to be lost as purge [1].

## IV. CONCLUSION

This study demonstrates the potential application of a Raman spectroscopic hand held device at 24 h post mortem to predict purge of fresh intact lamb at 5 days *post mortem*. Comparison of spectra from samples with high and low purge revealed that the amount of purge was discriminated using a variety of molecular vibrations including  $\alpha$ - helical proteins (920 cm<sup>-1</sup>), CH deformation signals (1448 cm<sup>-1</sup>) and COO<sup>-</sup> signals (1567 cm<sup>-1</sup>) which characterise metabolic substrates. However, further research is required to determine which biochemical processes contribute to this prediction in lamb.

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