

# CAUSATIVE FACTORS FOR GREENING OF VACUUM-PACKAGED LAMB

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## I. INTRODUCTION

Australian vacuum-packaged lamb has at times been rejected by export markets due to greening of the product after storage times of 6 weeks or more. Vacuum-packed lamb would normally be expected to have a chilled storage life of at least 8 weeks when stored at -1°C (1). Greening of vacuum-packaged beef was a major storage life issue when chilled beef was first exported from Australia. Nicol et al (2) determined that the green pigment was sulphomyoglobin formed from the attachment of hydrogen sulphide (H<sub>2</sub>S) to the myoglobin molecule. The H<sub>2</sub>S-producing bacteria were tentatively identified as *Pseudomonas mephitica* (now reclassified as *Janthinobacterium lividum*). The organism only produced H<sub>2</sub>S when the oxygen tension was about 1% and the pH of the meat was 6.0 and above. Subsequently, it was shown that greening could occur with high-pH beef (6.2 – 6.7) stored at 1°C in all packaging materials and in packaging with a relatively high permeability (73 and 92 cm<sup>3</sup> O<sub>2</sub>/m<sup>2</sup>/day/atm at 90% RH) with beef in the pH range 5.9 – 6.1 (3). Greening did not occur with low oxygen permeability of 23 and 25 cm<sup>3</sup> O<sub>2</sub>/m<sup>2</sup>/day/atm and meat pH below 6.2. There has been very little research reported into the causes of greening of vacuum packed lamb. The aim was to investigate the influence of pH, packaging material permeability and storage temperature on greening in sheep meat.

## II. MATERIALS AND METHODS

Lamb carcasses were measured for the ultimate pH of the loin at 24 h post-slaughter and 24 carcasses were selected to achieve equal representation (n=8 of each pH grouping) of 'Normal' (pHu ≤5.7), 'Intermediate' (pHu =5.71 – 5.99) or 'High' (pHu ≥6.0) pH loins. An 8-rib loin rack (*longissimus thoracis*) was removed from each carcass and transported, chilled, to the laboratory. Within each pH group, half of the loins were used as un-inoculated controls and the other half were inoculated with a cocktail of microorganisms expected to cause greening in vacuum-packaged meat. The cocktail included *Pseudomonas mephitica* and isolates of *Aeromonas salmonicida* and *Rahnella sp.* which had previously been isolated as dominant microflora of vacuum packaged lamb that had turned green. Each bacterial strain was grown independently in nutrient broth prior to diluting and combining to achieve a cocktail broth concentration of approximately 5.00 log<sub>10</sub>CFU/mL. All lamb racks were then packaged in either a high (OTR) film or a low OTR film and stored at either -1°C or +2°C. After storage for 4 weeks, each pack was inspected for evidence of greening of the meat or the exudate in the vacuum bag. Packs were then inspected at weekly intervals until 12 weeks of storage. When fluid in the packs was considered unacceptably green, the packs were opened and sampled for total viable count, by excising 1 x 10 cm<sup>2</sup> core from the exposed fat and meat surface. These cores were pooled, placed into a stomacher bag to which 100 mL of 0.85% saline was added, and stomached for two minutes. A decimal dilution series was prepared for each sample in 0.85% saline, and these were plated onto Petrifilm Aerobic count plates, incubated at 25°C ± 1°C for 72 ± 3 h and then TVC measured. Data presented is for each loin rack for the OTR, pH and storage temperature groups.

## III. RESULTS AND DISCUSSION

Greening first began to appear in the weep of vacuum packs containing inoculated and un-inoculated (control) samples, 4-5 weeks and 5-6 weeks respectively, after commencement of storage (Table 1). This generally occurred in the lamb racks packed in the higher OTR film and stored at +2°C. Interestingly, greening occurred in low pH loin racks, as well as in medium and high pH loin racks, albeit generally at a later time. In the case of control (un-inoculated) loin racks, greening still occurred, but at a later stage, relative to inoculated loin racks. This indicates that the naturally occurring population of bacteria included

those bacteria which cause greening. In samples stored at +2°C in vacuum bags of higher oxygen permeability, greening appeared at 5-7 weeks and 4-6 weeks in uninoculated and inoculated samples respectively. In uninoculated samples, the low storage temperature, even with the higher OTR bags, prevented greening if storage period was less than 10 weeks. Greening in the packs was often preceded or accompanied by the appearance of gas bubbles in the weep. The total viable counts (TVC) for samples that were sampled 1 to 2 weeks after showing indications of greening are also presented in Table 1. This indicates that packs that were sampled after 6 weeks storage (mainly those stored at high temperature and/or high OTR films) had already reached a similar microbial count to those that were not sampled until the end of the trial after 12 weeks storage.

Table 1. Time (weeks) for greening to appear, and total viable count when greening appeared, on control and inoculated lamb racks, with low (5.5-5.6), intermediate (5.8-5.9) or high (6.1-6.2) pH, stored in films of low or high oxygen transmission rate at temperatures of -1 or +2°C

	<b>Low OTR (9.9 cm<sup>3</sup>/m<sup>2</sup>/day)</b>		<b>High OTR (36 cm<sup>3</sup>/m<sup>2</sup>/day)</b>	
	-1°C	+2°C	-1°C	+2°C
<b>CONTROL LOIN RACKS, Time for greening to appear, weeks</b>				
<b>Low pH</b>	nm	10	12	7
<b>Intermediate pH</b>	>12	10	>12	5
<b>High pH</b>	11	>12	10	6
<b>INOCULATED LOIN RACKS, Time for greening to appear, weeks</b>				
<b>Low pH</b>	9	5	6	6
<b>Intermediate pH</b>	6	6	6	4
<b>High pH</b>	8	6	6	5
<b>TOTAL VIABLE COUNT (log<sub>10</sub> cfu/cm<sup>2</sup>)</b>				
<b>Low pH</b>	8.04	7.80	7.53	7.64
<b>Intermediate pH</b>	7.98	7.90	7.85	8.02
<b>High pH</b>	8.09	8.17	7.93	7.85

nm = not measured

#### IV. CONCLUSION

The results of this storage experiment show that regardless of the pH of the lamb loin, a storage time and shelf-life of up to 8 weeks at -1°C is generally achievable, with no development of greening. Most samples exhibited greening after 12 weeks storage. Storing the packs at a temperature of -1°C delayed greening by about 2 weeks in the high OTR film and up to 3 weeks in the low OTR film. Muscle ultimate pH had less influence on the susceptibility to greening than storage temperature. In contrast to beef data, greening can occur in vacuum packed lamb loins with low pH, when stored either at +2°C, or in high OTR (36 cm<sup>3</sup>/m<sup>2</sup>/day) packaging at -1°C.

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