USING BEEF MEAT COLORIMETRICS TO PREDICT MICROBIAL LOADING FOLLOWING CHILLED-THEN-FROZEN STORAGE

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Abstract – This study evaluated the relationship between the changes in colorimetric measures and microbial loading of beef stored chilled (for 0, 2, 3 and 5 weeks) and then frozen (for 0, 4, 8 and 12 weeks). Forty-eight beef *m. longissimus lumborum* (LL) were randomly selected, sub-sampled (*n* = 128) and assigned to chilled-then-frozen storage treatments. Samples were then stored under simulated retail display conditions where colorimetric measurements (L*, a*, b*) and the ratio of reflectance at 630 nm and 580 nm (R630/580) were taken at 1 day intervals over a 3 day period (0, 1, 2 and 3 days). LL samples were removed at day 0 of display and analysed for loading of lactic acid bacteria (LAB), *Enterobacteriaceae* (ENT), *Clostridium perfringens*, *Brocothrix thermospacta* and *Escherichia coli*. LAB was found to have significant positive correlations to all colorimetric measures, L* an exception, with these correlations generally increasing as the display period increased. Only ΔE (the change in colorimetric measures over display period) was negatively correlated with ENT (*P* < 0.05). Note that these correlations, whilst significant, were relatively low at around 20-30% magnitude. At least some of the remaining variation in ENT and LAB loading, after allowing for colorimetric measurements, was related to storage duration (*P* < 0.001). Insufficient loading of the other microbial species limited further investigation. These results suggest that colorimetrics may have an application in monitoring the microbial content of beef, although the observed correlation strength would restrict any practical application without further investigation.

Key Words – Bacteria, chilling, colour, freezing, storage.

I. INTRODUCTION

Consumers often associate spoiled meat with discolouration [1] and therefore both colour changes and microbial loading of stored meat contribute to product discount or wastage at a retail level due to quality and food safety issues [2, 3]. The latter can be defined by unacceptable levels of hazardous or spoilage-causing microbes proliferating to a total viable count (TVC) above a designated threshold [1, 3]. Surface discolouration can be estimated using colorimetric parameters based on reflectance spectroscopy [4].

Past research found that bacterial enumeration was related to the discolouration that occurred when beef meat was kept under aerobic chilled storage for up to 10 days [1]. One of the implicated bacteria was *Enterobacteriaceae* (ENT), as well as several other microbial types [1]. However, beef is widely stored under anaerobic conditions (i.e. vacuum packaging) and is therefore susceptible to other anaerobic spoilage microbial types (i.e. lactic acid bacteria; LAB) which can proliferate throughout its storage duration [5] – which often exceeds 10 days and may include frozen storage [6]. This study therefore aimed to determine the relationship between the microbial loading of vacuum-packaged beef stored for varied durations (chilled, frozen and chilled-then-frozen) and their colour stability when kept under simulated retail display conditions for up to 3 days post-thawing. It was hypothesised that beef with increased discolouration would also have increased microbial loading, and storage duration (either chilled or frozen) would influence both discolouration and microbial loading.

II. MATERIALS AND METHODS

Forty-eight beef *m. longissimus lumborum* (LL) were randomly sampled from the boning room of an Australian beef abattoir, sub-sectioned into four (n = 128), and then vacuum-packaged. These were then assigned ageing durations as chilled (0, 2, 3 and 5 weeks at 1-2°C) followed by frozen (0, 4, 8 and 12 weeks at -12 or -18 °C) storage conditions (n = 8 samples per treatment). Following the completion of each experimental treatment, two fresh sub-samples were cut.

The first sub-sample was placed on a black foam tray for colorimetric analysis, overwrapped with PVC film and permitted to bloom in a chiller under simulated retail display conditions (mean 864 lux, 3-4°C) for 3 days, with CIE colorimetrics: L* (lightness), a* (redness) and b* (yellowness) measured daily over the 3 days (0, 1, 2 and 3 days) using a HunterLab spectrometer (aperture size 25 mm, illuminant D65, 10° standard observer) calibrated as previously described [7]. From this, an estimate of the myoglobin oxidation ratio was calculated using the light reflectance at 630 nm and dividing by the light reflectance at 580 nm, denoted as R630/580 [8]. The overall change in colour over 3 days of retail display was also calculated and denoted as ΔE [4].

The second sub-sample (n = 128), taken on day 0 of display, was immediately frozen at -80°C until analysis whereby a representative 10 g was taken aseptically and diluted with 90 mL peptone salt solution (0.1%) for 30-60 seconds for microbial loading, measured as TVC, of LAB, ENT, *Clostridium perfringens*, *Brocothrix thermospacta* and *Escherichia coli*. For LAB (catalase negative. Gram positive cocci, coccobacilli or rods), diluted samples were plated at 0.1 mg on MRS agar and incubated in an anaerobe jar with the addition of Campygen for 72 ± 2 hours at 30 ± 1 °C prior to counting. For ENT (dark red or dark purple, diameter 0.5 to 2 mm), 1 mL of each dilution was pour plated on VRBG agar and overlaid with the VRBG agar and incubated for 21 ± 3 hours at 36 ± 2 °C prior to counting. For *C. perfringens* (black colonies), 1 mL of each dilution was poured onto a petri dish and mixed with 10-15 mL of SC agar, overlaid with 10 mL SC agar and incubated aerobically for 20 ± 2 hours at 37 ± 1 °C. For *B. thermospacta* (oxidase negative, shiny, off-white and round colonies), diluted samples were plated on an STAA spread plate and incubated for 48 ± 4 hours at 22-25 °C. For *E. coli* (blue colonies with gas bubbles), 1 mL was inoculated onto a Petrifilm count plate and incubated for 24-48 hours at 37 ± 1 °C. All TVC are given as colony forming units (CFU) per g of meat. Microbial analysis was undertaken at a commercial laboratory.

The microbial loading data was left-censored at the limit of detection (LOD = 100 for LAB, 10 for ENT). The correlations shown are the r-likelihood coefficients from fitting a censored linear regression between the logarithm of each microbial response (LAB and ENT) versus each colorimetric measure (L*, a*, b*, R630/580 and ΔE) using the cenreg function in the NADA library [9] of R [10]. The relationships were also examined graphically for adequate assumption of linearity. In addition, the corresponding non-parametric Kendall correlations (not shown) were found to be similar to the reported correlations.

To assess whether the relation between microbial response and colorimetric measure was consistent across treatments, the above censored regression model was augmented with treatment effects (interactions of chilling and freezing duration and temperature), along with the interaction of treatments with the colorimetric measure. The significance of each of these additional terms was assessed using analysis of deviance.

III. RESULTS AND DISCUSSION

Microbial loadings were counted for each microbial species, though *C. perfringens* and *E. coli* did not present any contamination above the limit of detection (10 µg/mL) and only two samples presented *B. thermospacta* contamination above the limit of detection. Meanwhile, LAB was found to be viable in 42% of samples (> 100 µg/mL) and ENT in 30% of samples (> 10 µg/mL). Therefore, the only two microbial species included in the analysis were LAB and ENT.

All colorimetric parameters, excluding L* and b*, had significant correlations with LAB on day 0, with all these being positive correlations, except hue ($P < 0.05$). At day 3 of

display, all parameters excluding L* presented highly significant ($P < 0.001$) correlations with LAB loading measured on day 0. These correlation values are presented in Table 1.

Table 1. Correlations of lactic acid bacteria (LAB) and Enterobacteriaceae (ENT) with colorimetric values (L*, a*, b*, R630/580) over 3 days of display.

	Day	LAB	ENT
L*	0	0.11	0.04
	1	0.00	0.02
	2	0.04	0.01
	3	0.08	0.05
a*	0	0.25**	0.02
	1	0.34***	0.07
	2	0.42***	0.04
	3	0.41***	0.08
b*	0	0.13	0.05
	1	0.25**	0.12
	2	0.29***	0.06
	3	0.30***	0.16
R630/580	0	0.22*	0.04
	1	0.31***	0.10
	2	0.38***	0.08
	3	0.36***	0.12
ΔE		-0.07	-0.21*

ΔE refers to the overall colour change over 3 days display duration; * significant ($P < 0.05$); ** highly significant ($P < 0.01$); *** very highly significant ($P < 0.001$).

These results differed to previous studies also testing colorimetrics and microbial loading in beef [1, 7], and this is thought to be based upon the use of anaerobic vacuum storage which favours a microbial profile dominated by LAB [3, 6].

Increased concentrations of LAB have previously been linked to meat discolouration and off-odours due to the production of hydrogen sulphide gas at excessively high levels above 8 $\log_{10}$ CFU/g [5]. Despite using anaerobic storage, it has been reported that psychrotrophic bacteria increased in concentration as a* and b* decreased, although no relationship with LAB has been found [6].

For ENT loading, only ΔE presented a significant negative correlation ($P < 0.05$). The generally lower TVC of ENT (Table 2) could explain its lack of relationship with colorimetric parameters (Table 1). The mean TVC for ENT was found to be below the threshold level for adequate food safety of 5 $\log_{10}$ CFU/cm^2 [11], as shown in Table 2.

The linkage between discolouration and microbial loading is thought to arise from the consumption of surface oxygen by microbial species, leading to a loss of bloomed colour prior to typical myoglobin oxidation to metmyoglobin which follows prolonged storage duration [2]. It has been reported that as a* decreased (-0.72) and metmyoglobin increased (+0.66), LAB was significantly related despite a low TVC [1]. In the present study, an opposite, albeit much lower, effect was seen for LAB (Table 1), relating increased concentrations of LAB to more bloomed meat (higher a* and R630/580), which can be related to this study keeping samples under fluorescent light rather than darkness [1].

There were significant interactions of chilled and frozen storage treatments on microbial loading after allowing for differences in colorimetric measures (Table 2; $P < 0.001$). However, there were few significant interactions of treatment with colorimetric measures ($P > 0.05$), suggesting little or no evidence that the relationship between microbial response and colorimetric measures varied between treatments. No previous studies have investigated chilled-then-frozen storage effects on this relationship; therefore any comparisons must be made with research testing only chilled or frozen storage. The influence of frozen storage has been found to result in decreases in ENT [11], while anaerobic chilled storage has been found to increase psychrotrophic bacteria [6]. Potential for anaerobically stored meat to discolour has also been previously explored, with purplish meat resulting from deoxymyoglobin formation linked to anaerobic storage [2].

Therefore, the use of anaerobic vacuum-packaged storage could be another potential effect for the observations made in this study, which in a prior study was found to result in improvements in lamb meat colour over 3 days simulated retail display when compared to aerobic storage, although microbial loading was not measured [12].

Despite these findings, it is clear from Tables 1 and 2 that the correlation data be interpreted with care due to the relatively low correlation and TVC scores despite the high significance levels.

IV. CONCLUSIONS

Improvements in several colorimetric parameters of beef meat have been associated with increased loading of LAB in this study, with this relationship linked to anaerobic storage of meat. ENT loading, however, was not related to colorimetric measures, although it was found to be related to meat discolouration (negative ΔE) over three days of simulated retail display.

Table 2. Influence of chilled-then-frozen storage duration upon total viable count (TVC) of lactic acid bacteria (LAB) and Enterobacteriaceae (ENT) species (log_{10} CFU/g).

	Freezing duration (weeks)							
	0		2		4		8	
Chilling duration (weeks)	LAB	ENT	LAB	ENT	LAB	ENT	LAB	ENT
0	2.95	1.00	3.48	2.64	-	1.00	-	2.00
2	4.46	2.49	-	-	2.78	1.20	2.74	1.10
3	5.27	2.43	-	1.00	-	1.00	2.85	1.63
5	3.26	1.00	3.41	1.48	3.41	2.79	4.17	1.98

"-" refers to insufficient loading of the corresponding bacterial species (< 1 log_{10} CFU/g).

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