BIOCHEMICAL CHANGES IN 140-DAY STORED VACUUM PACKAGED CHILLED BEEF AND POTENTIAL SHELF-LIFE MARKERS

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I. INTRODUCTION

During extended storage of vacuum packaged chilled beef (VPCB), lactic acid bacteria become the dominant microbiota before growth plateaus. Complex uncharacterized biochemical changes occur in the meat, which at some point render the product no longer acceptable. Current models suggest that a shelf-life of between 120 to 180-days is achievable for VPCB subject to strict storage temperature control (-1 ^{o}C). The point at which VPCB becomes unacceptable is subjective and normally determined by the "sniff test", where consumers use their nose to detect sensory changes in the raw meat odour. By definition, for there to be a perceptible sensory change in the pack odour, corresponding changes in the volatile profile or "volatilome" must occur. Changes in volatile signatures of VPCB stored under ideal conditions (- 1 ^{o}C) up to 140-days or 20-weeks were measured by gas chromatography-mass spectrometry (GC-MS) and proton transfer reaction mass spectrometry (PTR-MS). PTR-MS is a real-time gas phase analytical technique that does not require extensive sample preparation and may provide a more quantitative snapshot of the volatile composition. We also monitored semi-quantitative changes in key non-volatile metabolites in raw meat, such as nucleotides and free amino acids using electrospray mass spectrometry (LC-MS/MS).

II. MATERIALS AND METHODS

Striploins (*Musculus longissimus lumborum*) collected from pasture-fed Santa Brahman cross-steers were vacuum packaged according to a statistical design in high-shrink Cryovac polymer (oxygen transmission rate; 30 cc/m^2/ 24 hat 23 ^{o}C). Initial microbiologal counts were within specification for export VPCB; aerobic plate and lactic acid bacteria counts 2.25 and 2.05 $\log_{10}$ CFU/cm ^{2} respectively, with no *E. coli* or coliforms detected. VPCB packs were stored at ~ - 1 ^{o}C at CSIRO. Samples (n=10 per time point) were removed at zero, 84, 98, 120 and 140-days and frozen until analysis. Sensory assessment of packs was performed after opening and blooming. Volatile compounds from raw and grilled beef (220 ^{o}C, 2 min, clamshell grill) were homogenized in Milli-Q water (1:2) and extracted by headspace solid phase microextraction (SPME) (Carboxen/divinylbenzene/dimethylsiloxane, 2 cm) for 40 min at 37 ^{o}C and analysed by electron impact GC-MS (Shimadzu 2010-Plus, Tokyo, Japan). Slurries (20 mL) were equilibrated to 37 ^{o}C and the headspace was analysed by a single quadrupole PTR-MS (Ionicon, Austria). Methanolic extracts of underivatized raw meat were analysed by LC-MS/MS (Thermo Scientific, TSQ-Quantiva). Replicate data were subjected to ANOVA and principal component analysis (PCA) using GensStat (16th Ed).

III. RESULTS AND DISCUSSION

Sensory assessments indicated small increases in odor "intensity" and decreases in "fresh" odor over time after opening and blooming ($p < 0.001$). However, no consistent increase in spoilage attributes; "cheesy", "fruity" or "sulfur" odor were measured. SPME resolved more than 70 volatile compounds in the headspace of raw and grilled samples. Of these, only 18 volatiles changed significantly over the storage period in the raw samples and only 14 in the grilled samples. The largest volatile changes generally occurred between zero time and 84-days, with minimal changes thereafter. These included some typical fermentation related volatiles; ethanol, acetone and acetic acid.[1-2] Methyl butanoate and methyl heptanoate also increased. A number of unidentified alkane-like volatiles increased as well as lipid derived aldehydes, such as hexanal, heptanal and nonanal. Sulfur volatiles, methanethiol, dimethyl disulfide and dimethyl trisulfide have previously

been associated with meat spoilage and strong objectionable odours in meat.[2-3] No significant increases were measured in these sulfur volatiles in VPCB over time in the current study. The largest volatile change was an approximately 3-fold increase in ethanol that occurred from 120 to 140-days, measured by both SPME and confirmed by PTR-MS (Fig 1.). Further increases in ethanol may signal the limit for consumer acceptability of VPCB. An increase in free amino acids over time was indicative of ongoing proteolysis and an increase in tyramine was indicative of catabolism of tyrosine (Fig 1.). None of the volatile and non-volatile changes were consistent with spoilage, however further increases after 140-days may provide objective markers of shelf-life limits. In line with shelf-life prediction models and the sensory data, the chemical data indicated that the VPCB in this study could be expected to achieve a shelf-life of 140-days or longer.

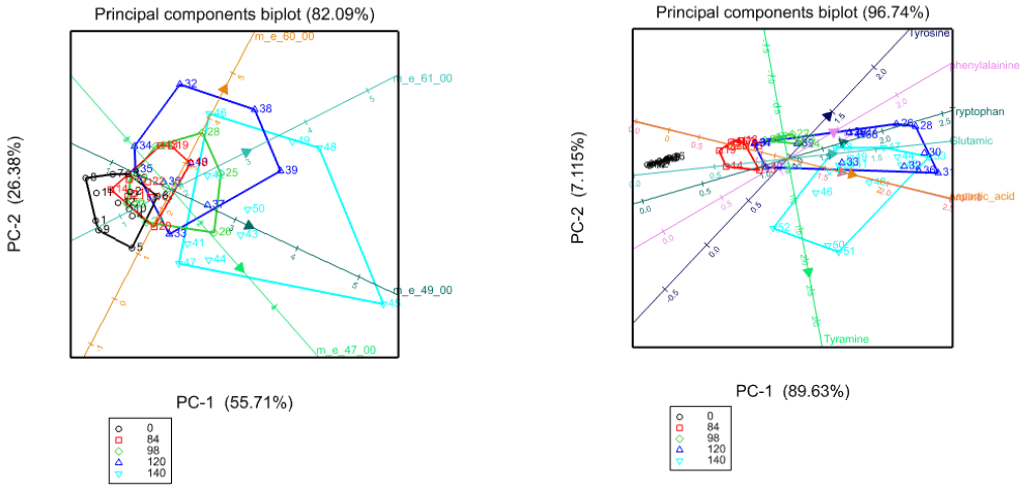

Figure 1. PCA biplot based on multiple packs at 0, 84, 98, 120 and 140-days for selected PTR-MS volatiles (Left) and selected non-volatile components (Right) measured by LC-MS/MS. Both volatile and non-volatile markers increased over time – left to right.

IV. CONCLUSION

Volatile and non-volatile changes measured in the VPCB samples, point toward objective chemical markers that could be linked to consumer acceptance information. Changes in key volatile and non-volatile markers may be useful to assist shelf-life prediction and may potentially form the basis of smart packaging technologies. Future research should include greater replication and more time points beyond 140-days, e.g. 160, 180 and 200-days, to further understand key changes that occur in VPCB with extended shelf-life.

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