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Abstract— Despite the industry's continued use of vacuum-packaging as a mechanism for extending shelf-life there has been little recent emphasis on determining the storage life of vacuum-packaged beef and lamb and the retail life of meat cuts prepared from the vacuum packs. Consequently current practices are based on data generated up to 2 decades ago. Improvements in processing technologies, hygiene, transport and refrigeration systems, and packaging technologies during that time highlights a need for a review of this area.

Four export establishments supplied vacuumpacked beef striploins and cube rolls for the project. Product was supplied to Food Science Australia (FSA) for storage at -0.5 ± 1 °C. Samples were taken where possible on days 3, 7, 10 and 14 of storage, and subsequently every 2 weeks from 6 weeks of storage. All samples were analysed for Total Viable Count (TVC), Enterobacteriaceae, Brochothrix thermospacta and Lactic Acid Bacteria (LAB) counts, and samples taken from week 6 of storage underwent sensory panel assessment and lipid oxidation analysis by thiobarbituric acid-reactive substances (TBARS).

There was a slight deterioration in visual appearance of the intact pack and the post-bloom primal, a slight increase in confinement odour, and a slight deterioration in visual appearance (greying of fat) of 3-day displayed steaks as the vacuum storage period increased. Concurrently, there was a slight increase in lipid oxidation over 3 days retail display, and a slight overall increase in mean LAB count, although microbial counts were very variable between cuts and processors. It was impossible to draw firm conclusions regarding the storage life of vacuum packed beef produced in Australia. However, the data gathered suggests that storage lives may well be substantially in excess of the currently recommended 10-12 weeks, assuming that appropriate production and storage conditions are met. Further work is required to ascertain whether this data set represents a true picture of the storage life of vacuum packaged beef produced within the Australian industry, and to identify processing practices and conditions that lead to such extended storage lives.

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Index Terms—beef; shelf-life; storage; vacuum packaging.

I. INTRODUCTION

acuum packaging of beef and lamb under chilled Vconditions remains an effective measure for extending the shelf life of such products and for the control of foodborne pathogens. The low oxygen concentration involved in vacuum-packaging has a selective effect on the microbial population and generally results in the proliferation of lactic acid bacteria (LAB). The predominant organisms include Carnobacterium divergens, Carnobacterium piscicola, Lactobacillus sakei, Lactobacillus curvatus, Leuconostoc gelidum, Leuconostoc carnosum and Brochothrix thermosphacta [1-5]. Under good processing and packaging conditions, the counts of LAB on the surfaces of primals at the time of packaging are very low (<100/cm²), however the numbers of LAB increase during storage and can be expected to exceed 10⁶/cm² after 2 to 3 weeks [6, 7]. The presence of high numbers of LAB may result in the presence of unacceptable odours and meat flavours in products stored for prolonged periods of time [6, 8]. Similarly the presence of LAB and other contaminating bacteria such as Enterobacteriaceae may subsequently cause spoilage, odour and flavour issues in retail packs prepared from vacuum-packaged meat.

Despite the industry's continued use of vacuum-packaging as a mechanism for extending shelf-life there has been little recent emphasis on determining the storage life of vacuum-packaged beef and lamb and the retail life of meat cuts prepared from the vacuum packs. Consequently current practices are based on data generated up to 2 decades ago. Improvements in processing technologies, hygiene, transport and refrigeration systems, and packaging technologies during that time highlights a need for a review of this area.

II. MATERIALS AND METHODS

Four export establishments supplied vacuum-packed striploins and cube rolls for the project. On arrival, product was immediately transferred to chill storage at $0^{\circ}\text{C} \pm 1^{\circ}\text{C}$. In the first two weeks of storage, primals were subjected to microbiological evaluation only, on days 3, 7, 10 and 14. Subsequent sampling occasions were fortnightly from week 6 to week 20, at which time the primals were subject to sensory evaluation, microbiological evaluation, MINOLTA colourimetry and lipid oxidation by TBARS assay.

1) Sampling procedure

On each designated sampling occasion, one pack of striploin and one of cube roll was removed from storage. Packs were assessed by a 6-member informal sensory panel, using a 9-point scale, for vacuum integrity; appearance of the intact pack; presence of confinement odour; and post-bloom appearance, 30 minutes after the pack was opened. Packs were opened carefully, and the drip measured. Using aseptic technique three excision samples were taken from the lean meat surface. Each excision sample was 10cm² in area, and each was processed separately.

Following post-bloom assessment, each cut was sliced into 1.5cm thick steaks and packaged in overwrap trays. The resulting retail packs were assessed using MINOLTA colourimetry, and the meat pH measured. They were then displayed in a retail cabinet at 3°C, under fluorescent light for three days. At the end of the three day display, the packs were again assessed by MINOLTA colourimetry, and by a 6-member panel for visual appearance. Samples were taken for lipid oxidation analysis, pH measurement and the volume of drip present in the retail tray measured.

2) Microbiological analysis

A 50 ml aliquot of 0.85% saline was added to each stomacher bag containing 10cm² of meat sample and stomached for 30s. A decimal dilution series was prepared in 0.85% saline, and these plated onto Petrifilm Aerobic®, Petrifilm Enterobacteriaceae® and STAA plates for TVC, Enterobacteriaceae count and *Brochothrix thermospacta* count respectively. The dilutions were also prepared in MRS broth and plated

onto Petrifilm Aerobic® according to the Petrifilm method for enumeration of Lactic Acid Bacteria. Microbial counts were converted to $log_{10}cfu/cm^2$, and the mean log of the three samples calculated using an Excel spreadsheet (Microsoft).

3) Lipid Oxidation

Lipid oxidation was assessed by the TBARS method of Witte et al. [9]. All meat samples (2 g) were heated at 75°C for 20 minutes in a water bath and then cooled in ice prior to determination. TBARS were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of 1,1,3,3-tetraethoxypropane (TEP), and calculated as mg MDA per kg sample.

III. RESULTS AND DISCUSSION

A. Sensory Evaluation

1) Visual assessment of intact pack

All cuts scored highly (above mean score 5) at all time points.

2) Confinement odour

Product scored above 4 (acceptable) on all but three occasions: Processor Z striploin, week 10 (2.00); Processor Z cube roll, week 10 (3.80) and Processor A striploin, week 16 (3.43).

3) Post bloom visual assessment

All cuts evaluated scored good to excellent (mean score greater than 5), except Processor A striploin, week 16 (4.14, acceptable). A slight decrease in score over time was evident, but this was not marked.

4) Retail pack visual assessment

Product from processor A scored more highly than product from the other processors. Retail packs scored highly (above 5) at all time points except Processor B cube roll, week 18 (4.90) and Processor A cube roll, week 20 (4.60).

B. Lipid Oxidation

In general, TBARS values increased between weeks 6 and 20 for both cooked and raw product. TBARS values for striploin tended to be greater than those from cube roll from the same processor at any time point. Greene and Cumuze [10] identified that untrained taste panellists began to detect off-flavours in cooked beef in the TBARS range 0.6 - 2.0. In the current project, the highest TBARS values in cooked beef were 1.714 (Processor A striploin, week 14) and 1.153 (Processor C striploin week 18).

TBARS are a measure of secondary oxidation products, mainly aldehydes, carbonyls or hydrocarbons, which contribute to off-aromas and flavours in meat [11]. Their concentration in the product may also begin to decrease over time. The rate of decrease varies with storage conditions, packaging, and fat content. The consequence of all of these

changing concentrations is that any attempt to evaluate the rancidity of a product will be difficult. Low aldehyde concentrations may be the result of limited oxidation or the aldehydes may have volatilized. In consequence, a low TBARS value is not an absolute indicator of fat quality. Aldehydes may have not yet formed or volatile aldehydes may have been lost during processing and storage. In these cases, sensory evaluations may be the key to understanding the data [12].

Studies have shown that TBARS values increase up to a certain point during the storage period, after which there is a decrease in these values [13, 14]. Igene and Pearson [15] stated that, during the evaluation of lipid oxidation in stored foods, decreases in TBARS values are probably due to interactions between malonaldehyde and proteins.

C. Colourimetry

There were no consistent changes in colour measurements over three days retail display in the product, which is broadly in accordance with the sensory panel assessment scores assigned to displayed packs.

D. Microbiology

1) First two weeks of storage

Packs were opened, where possible (i.e. if received from the processing establishment), on days 3, 7, 10 and 14, in order to determine the microbiological status of the product. However, only one processor supplied product in time for the day 3 evaluation, a second achieved the day 10 evaluation, while the remainder only met the day 14 evaluation. TVC and LAB counts were surprisingly low on day 14 (mean 0.5-2.53 log₁₀cfu/cm²). From published literature, TVC and LAB counts in vacuum packaged beef aged for 2 weeks would be expected to reach levels of around 6 log₁₀cfu/cm². Blixt and Borch [7] recorded increases in Aerobic bacteria from 3 log₁₀cfu/cm² to 6 log₁₀cfu/cm² and LAB from 1 log₁₀cfu/cm² to 6 log₁₀cfu/cm² in the first two weeks of storage of vacuum packed beef loin. while Sakala et al. [2] recorded increases from 3-3.5 log₁₀cfu/cm² to 5.5-7 log₁₀cfu/cm² over 2 weeks vacuum storage for a number of LAB species on beef. Leisner et al. [6] recorded increases in inoculated Carnobacterium maltaromicus from 3 log₁₀cfu/cm² to 5 log₁₀cfu/cm², Lactobacillus sake from 2 log₁₀cfu/cm² to 6.5 log₁₀cfu/cm², and Leuconostoc gelidum from 3 log₁₀cfu/cm² to 4.5 log₁₀cfu/cm² on beef slices stored under vacuum for 2 weeks. It is interesting to note, however, that none of the samples collected in the current project yielded microbial levels as high as those cited in the literature on day 14. It may be that limited flora on the current project's product at day zero contributed to the low counts observed at day 14.

Enterobacteriaceae were detected on two samples only, at the detection limit (0.5 log₁₀cfu/cm²), suggesting that hygienic conditions of production were good at all participants' premises. *Brochothrix thermospacta* were not detected in any sample.

2) Weeks 6 onwards

Again, some startling results were obtained. Total Viable Counts (TVCs) were highly variable, between 0.40 and 7.04 log₁₀cfu/cm², and there was no obvious consistent trend over time or across processors and primals. Processor A cube roll TVCs were particularly low (range 0.40 to 2.73 log₁₀cfu/cm²). Similarly, LAB counts were highly variable ranging from 1.00 to 6.76 log₁₀cfu/cm², and again surprisingly low, particularly in the case of Processor A. LAB were not detected in all samples, nor from all packs (Detection limit: 1.00 log₁₀cfu/cm²). In general, however, the frequency of detection and the mean count detected increased over storage time. From published literature [2, 6, 7], total counts and LAB counts on beef would be expected to plateau at around 6-7 log₁₀cfu/cm² after three weeks of vacuum storage, and be maintained at that level. It is possible that the highly variable counts observed in this project are a result of the small sample size in each sampling point, leading to data belonging to outliers or limit-of-range product. However, it is also possible, given the low initial microbial load at packaging suggested by the results of the day 14 samples, and the strict temperature control during storage, that such variability, and the presence of surprisingly low counts later in storage are in fact a feature of current commercially produced vacuum packed chilled beef. Enterobacteriaceae were detected on occasion, more often from processor Z than from other processors. Mean counts when detected were in the order of 0.70 to $2.85 \log_{10} \text{cfu/cm}^2$. Brochothrix thermospacta were recovered from nine samples only, at levels of 3 log₁₀cfu/cm² or less. The low detection rate for Enterobacteriaceae suggests that hygiene during production was good, while low detections of Brochothrix thermospacta are consistent with beef of normal pH, packaged in films of low Oxygen Transmission Rate (OTR). The pH of the product was determined at FSA on each sampling occasion, and found to be consistently in the range of pH 5.5 to 5.7.

E. SUMMARY

Overall, there was a slight deterioration in visual appearance of the intact pack and the post-bloom primal, a slight increase in confinement odour, and a slight deterioration in visual appearance (greying of fat) of 3-day displayed steaks as the vacuum storage period increased. Concurrently, there was a slight increase in lipid oxidation over 3 days retail display as indicated by increasing TBARS values, and a slight overall increase in mean LAB count. Low detections

of Enterobacteriaceae and *Brochothrix thermospacta* indicated that processor hygiene and packaging integrity were very good. It is possible that the highly variable counts observed are a result of the small sample size in each sampling point, leading to data belonging to outliers or limit-of-range product. However, it is also possible, given the low initial microbial load at packaging suggested by the results of the day 14 samples, and the strict temperature control during storage, that such variability, and the presence of surprisingly low counts later in storage are in fact a feature of current commercially produced vacuum packed chilled beef.

IV. CONCLUSION

It was impossible to draw firm conclusions regarding the storage life of vacuum packed beef produced in Australia. However, the data gathered suggests that vacuum packed chilled beef produced in Australia can confidently be stored for 20 weeks or more, assuming that appropriate production and storage conditions are met. Further work is required to ascertain whether this data set represents a true picture of the storage life of vacuum packaged beef produced within the Australian industry, and to identify processing practices and conditions that lead to such extended storage lives.

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REFERENCES

- 1. Fontana, C., P.S. Cocconcelli, and G. Vignolo, Direct Molecular Approach to Monitoring Bacterial Colonization on Vacuum-Packaged Beef. Applied and Environmental Microbiology, 2006. **72**(8): p. 5618-5622.
- 2. Sakala, R.M., H. Hayashidani, Y. Kato, T. Hirata, Y. Makino, A. Fukushima, T. Yamada, C. Kaneuchi, and M. Ogawa, *Change in the composition of the microflora, on vacuum-packaged beef during chiller storage.* International Journal of Food Microbiology, 2002. 74(1-2): p. 87-99.
- 3. Jones, R.J., Observations on the succession dynamics of lactic acid bacteria populations in chill-stored vacuum-packaged beef. International Journal of Food Microbiology, 2004. 90(3): p. 273-282.

- 4. Ercolini, D., F. Russo, E. Torrieri, P. Masi, and F. Villani, *Changes in the Spoilage-Related Microbiota of Beef during Refrigerated Storage under Different Packaging Conditions*. Applied and Environmental Microbiology, 2006. **72**(7): p. 4663-4671
- 5. Nissen, H. and O.D.R. Sorheim, Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. Food Microbiology, 1996. 13: p. 183-191.
- 6. Leisner, J.J., G.G. Greer, B.D. Dilts, and M.E. Stiles, *Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air.* International Journal of Food Microbiology, 1995. **26**(2): p. 231-243.
- 7. Blixt, Y. and E. Borch, *Comparison of shelf life of vacuum-packed pork and beef.* Meat Science, 2002. **60**(4): p. 371-378
- 8. Borch, E., M.L. Kant-Muermans, and Y. Blixt, *Bacterial spoilage of meat and cured meat products.* International Journal of Food Microbiology, 1996. **33**(1): p. 103-120.
- 9. Witte, V.C., G.F. Krause, and M.E. Bailey, *A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage.* Journal of Food Science, 1970. **35**: p. 582-585.
- 10. Greene, B.E. and T.H. Cumuze, *Relationship between TBA numbers abd Inexperienced panelists' Assessments of Oxidized Flavor in Cooked Beef.* Journal of Food Science, 1981. **47**(1): p. 52-54,58.
- 11. Igene, J.O., K. Yamauchi, A.M. Pearson, J.I. Gray, and S.D. Aust, *Evaluation of 2-thiobarbituric acid reactive substances (TBARS) in relation to warmed-over flavor (WOF) development in cooked chicken.* Journal of Agricultural and Food Chemistry, 1985. **33**: p. 364-367.
- 12. NPAL (2008) What is the Best Way to Measure the Quality and Stability of Fats and Oils? **Volume**,
- 13. Gokalp, H., H.W. Ockerman, P.F. Plimpton, and W.J. Harper, *Fatty acids of neutral and phospholipids, rancidity scores and TBA values as influenced by packaging and storage.* Journal of Food Science, 1983. **48**: p. 829-834.
- 14. Babji, A.S., S.Y. Chin, M.Y.S. Chempaka, and A.R. Alina, *Quality of mechanically deboned chicken meat frankfurter incorporated with chicken skin.* International Journal Food Sciences Nutrition, 1998. **49**: p. 319-326.
- 15. Igene, J.O. and A.M. Pearson, *Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems.* Journal of Food Science, 1979. **44**: p. 1285-1290