MICROBIOLOGY AND KEEPING QUALITY OF REFRIGERATED VACUUM PACKED BEEF KEPT FOR EXTENDED STORAGE

H. Ricardo Rodríguez[1], M. Paula Suárez Rebollo[1], Adrián Rivi[2] and Jorge A. Lasta[1]

[1] Instituto Tecnología de Alimentos, CICV, INTA, CC 77, 1708 Morón, Buenos Aires, ARGENTINA.

[2] Grace Argentina S.A., Primera Junta 550, Quilmes, Buenos Aires, ARGENTINA.

Keywords: beef, shelf life, packaging, refrigeration

Background

International trade of high quality vacuum packed meat is a major business for several beef producing countries. Argentina has a long tradition of exporting overseas, mostly to the EU market, high quality vacuum packed beef. Shipment of beef to overseas markets, rely on an appropriate shelf life for extended periods. Early reports stated that, given refrigeration temperatures of -1 to 0°C beef can retain its quality for up to 10 weeks (D'Alessandria und Pagliaro, 1975). Several factors affect shelf life of fresh meat, most of them have been studied and reported (Dainty, 1983; Gill, 1986). In this sense, vacuum packing is the most common method used by the meat industry to extend shelf life and to protect the keeping quality of fresh beef. The meat cut is usually placed in a film of low O_2 permeability (<5cc $m^{-2}day^{-1}$ atm^{-1}) and, with good vacuum the package head space consists of <1% O_2 (V/V) and 10-20% CO_2 (V/V) produced from tissue and bacterial respiration. This condition will prevent spoilage by strictly aerobic organisms (ie. pseudomonades). Most studies on shelf life of refrigerated beef have been reported for storage periods from 30 to 60 days (Dainty et al., 1979; Gill, 1986). It is known that using Good Manufacturing Practices (GMP's), a low O_2 permeability film and adequate refrigeration storage, a shelf life of 90-100 days might be achieved (Pellegrino, 1995). Reports, however, on studies of shelf life and keeping quality of refrigerated vacuum packed beef stored for extended periods (more than 8 weeks) are scarce.

Objective

To investigate the shelf life and keeping quality of sirloins beef fabricated under commercial conditions and kept vacuum packed under refrigeration for an extended storage period.

Materials and Methods

A study was designed to simulate fabrication and storage following GMP's and abuse of such a practices under actual commercial conditions. Angus cross-breed steers (average live weight 530 and, 6 adult incisors) were slaughtered and fabricated at a commercial facility in the province of Buenos Aires. GMP's during slaughtering and fabrication were considered as those usually followed by the facility, which is approved to export to the EU market. Following a 48 h refrigeration period, carcasses were deboned and sirloins (*psoas major*) were fabricated and vacuum packed. Two different Cry-o-Vac type packaging materials (bags A and B) commonly employed by the industry were used (GRACE Argentina S.A.). Samples were kept at 0°C (±0.5°C), and visually examined on a weekly basis for lack of vacuum, and change in color. Any sample with such a deffect was eliminated. A total of 55 sirloins were used in this study. Fifteen samples were analyzed (bacterial counts and pH determination) at 0 day; 30 samples were analyzed after 90 days (regular refrigerated storage - 0°C) and 10 temperature abused samples at 100 days of storage. Temperature abuse was performed in sirloins that have been stored at 0°C for 65 days. These samples were stored in another cooler facility at 6°C for 35 days.

Microbiological Analysis: A surface area of 22 cm^2 and 4 mm depht was used, according to Rodríguez et al. (1993). Total Mesophiles Count (TMC), Total *Lactobacillus* Count (TLC), Count of Gram Negative organisms (GNC), Count of Yeast (YC) and Count of *Brochothrix thermosphacta* were performed as described elsewhere (Lasta et al., 1992 and1995). Isolated organisms ($n^{1/2}$, n=n° of colonies) from TMC and GNC were identified according to Lasta et al. (1995).

Determination of pH: Determination of pH from surface and depth (core) of sirloins were done in a slurry of 10 g of sample in 25 ml of distilled water and carried out in a Metrohm 691 pH meter (Metrohm, Switzerland) using a combined pH electrode (Metrohm 6.0218.010).

Statistical analysis: When appropriate, an analysis of variance was carried out using the GLM procedure of SAS (SAS, 1985).

Results and Discussion

Shelf life and keeping quality of refrigerated vacuum packed beef were evaluated by visual examination, microbial and pH determinations. While visual monitoring was done on a weekly basis, sampling for microbiological and pH analysis was carried out after 90 days of storage, since this is the expected shelf life of this type of product. Four temperature abused samples were rejected during storage. Microbial counts at 0 day of storage showed (Table 1) similar values to those reported by Dainty et al. (1979) for beef chucks. Counts from samples of bags A and B were considered together at 0 day of storage, since these counts only represent initial microbial condition of the beef sirloins. Identification of isolated organisms from TMC showed a 27.3% of *Acinetobacter*, 27.3% of *Micrococcus*, 18.1% *Enterobacteriaceae*, 9.1% of *Staphylococcus*, 9.1% of *Lactobacillus* and 9.1% of *Aeromonas/Vibrio*. Identification of organisms coming from GNC showed a 54.6% of *Acinetobacter*, a 27.3% of *Enterobacteriaceae* and a 18.1% of *Aeromonas/Vibrio*. These findings are explained in terms of the microflora present at the time of packing and are cualitatively similar to those reported by Lasta et al. (1995) in subcutaneous beef fat except for pseudomonades. *Pseudomonas* was not identified from TMC and GNC colonies, and again, this may in part be due to microbial load at time of packing. The pH of samples showed no differences (P>0.05) between surface and depth (Table 2) at 0 day of storage. Values found are similar to those expected in what is considered normal pH beef.

Microbial counts found at 90 day of storage were good indicators from the shelf life standpoint - lower than 10^7 CFU/cm^2 (Table 1), since it is well accepted that meat showing a count of 10^7 CFU/cm^2 result in some sort of spoilage (Gill, 1986). No differences (P>0.05) were found between samples from different bags treatments. Results found in this study are lower than those reported by Dainty et al. (1979) particularly for TMC, GNC and LC for refrigerated beef stored up to 8 weeks. Moreover, growth of *B. thermosphacta* was not detected in the current assay. Differences may be due to packaging materials; bags A and B used in the current assay had an O_2 permeability of 19.2 and 7.2 cc m^{-2} day^{-1} atm^{-1} respectively. Film permeability plays a very important role in determining shelf life of vacuum packed beef; in this sense keeping quality increases as film permeability to O_2 decreases (Newton and Rigg, 1979). Absence of *Pseudomonas* among the isolate identifications, supports the evidence of using a very low O_2 permeability film. Among 801 isolated colonies (samples kept at 0°C), 17% were gram negative (93% *Enterobacteriaceae* and 7% *Acinetobacter*) and 80% were gram positive (95% *Lactobacillus* and 5% not identified) and 3% was not identified.

Regarding pH values, they were lower at 90 day than at 0 day of storage. No differences (P>0.05) were found between the surface and the depth of the sirloin. Low ultimate pH may not be explained only in terms of growth of *Lactobacillus*, since this group of organisms did not show high counts as expected (Dainty et al., 1979). No differences in pH (P>0.05) were also found from samples packed in bags A and B. All analyzed sirloins had a typical vacuum packed aged beef aroma (slightly acidic or sour), but not cheesy odor was detected when opening the bags for sampling. *B. thermosphacta* produces isovaleric and isobutyric acids from metabolism of leucine and valine (Gill, 1986). Lack of chessy odor in the samples of the current assay might be explained in terms of no growth of this bacterium. Temperature abused samples showed off-odors which

correlated with their high microbial counts (Table 1).

Extension of shelf life by vacuum packing is primarily due to a failure of *Pseudomonas* to grow in such number to produce spoilage. *B. thermosphacta* might become important as a potential spoilage agent when pseudomonades are supressed. No detection of *B. thermosphacta* may be due to an inhibition of some extent by CO_2 and particularly by growth of lactic acid producing bacteria. Moreover, this organism has a rather low spoilage potential under strictly anaerobic condition -low O_2 permeability film (Gill, 1986). Regarding vacuum packaging disadvantages, cut deformation and exudation from the meat are concerns; however, provided a low enough permeability to O_2 (< 1-4 mm Hg), it is possible to prevent metmyoglobin formation which gives an undesirable brown color (Renerre, 1990). Nonetheless, vacuum packing used in conjunction with refrigeration has many advantages. As shown in the current study, prolonged shelf life and keeping quality appear to be major factors that support the use of this technology.

Conclusions

High quality beef fabricated under GMP's, packed in a very low O_2 permeability film and, kept refrigerated at 0°C, achieved at least a 90 days of shelf life. Temperature abuse after 65 days of 0°C storage affected both microbiological and sensory (off-odors) characteristics of beef sirloins.

Acknowledgment

The authors express appreciation to Dr. Marcelo Masana for critically reviewing this paper and, to Dr. Celia Melamed for her technical assistance.

References

-D'Alessandria, A. V. H. und Pagliaro, A. F. 1975. Mikrobiologisches Verhalten von vakuumverpacktem gekühltem Rindfleisch bei längerer Lagerzeit und in Beuteln unterschiedlicher Sauerstoffdurchlässigkeit. Die Fleischwirtschaft, 55:1582-1584.

-Dainty, R. H.; Shaw, B. G.; Harding, Ch. D. and Michanie, S. 1979. The Spoilage of Vacuum-packed Beef by Cold Tolerant Bacteria. In: Cold Tolerant Microbes in Spoilage and the Environment. pag. 83. Ed. A. D. Russell and R. Fuller. Academic Press, London.

-Dainty, R. H.; Shaw, B. G. and Roberts, T. A. 1983. Microbial and Chemical Changes in Chill-stored Red Meats. In: Food Microbiol. Advances and Prospects. pag 151. Ed. Roberts and Skinner. Academic Press, London.

-Gill, C. O. 1986. The Control of Microbial Spoilage in Fresh Meats. In: Advances in Meat Research, Vol. 2, pag. 49. AVI Publishing Co.

-Lasta, J.; Rodríguez, R.; Zanelli, M. and Margaría C. 1992. Bacterial Count from Bovine Carcasses as an Indicator of Hygiene at Slaughtering Places. A proposal for sampling. J. Food Protect. 55:271-278.

-Lasta, J. A.; Pensel N. A.; Masana M. O.; Rodríguez, H. R. and García, P. T. 1995. Microbial Growth and Biochemical Changes on Naturally Contaminated Chilled Subcutaneous Beef Adipose Tissue Aerobically Stored. Meat Sc. 39:149-158.

-Newton, K. G. and Rigg, W. J. 1979. The Effect of Film Permeability on the Storage Life and Microbiology of Vacuum-packed Meat. J. Applied Bacteriol. 47:433-441.

-Pellegrino, J. M. 1995. Personal Communication. Meat Promotion and Fairs Office. Secretaría de Agricultura, Pesca y Alimentación. Argentina.

-Renerre, M. 1990. Review: Factors Involved in the Discoloration of Beef Meat. Internat. J. of Food Sc. and Technol. 25:613-630.

-Rodríguez, H. R.; Lasta, J. A.; Mallo, R. and Marchevski, N. 1993. Low-dose Gamma Irradiation and Refrigeration to Extend Shelf Life of Aerobically Packed Fresh Beef Round. J. Food Protect. 56:505-509.

-SAS. 1985. SAS User's Guide. Statistic Version, 5th Ed. SAS Institute Inc., Cary, NC.

TABLE 1: MICROBIOLOGICAL ANALYSIS OF BEEF SIRLOINS FABRICATED UNDER GMP's AND SUBJECTED TO TEMPERATURE ABUSE DURING STORAGE

Time of sampling(ds)/ Type of sampling	Microbiological Analysis[1]					
	TMC[2]	GNC[3]	LC[4]	EC[5]	YC[6]	BtC[7]
Day 0 Packaging A&B (n=15)[8]	4.20 (0.39)	4.10 (0.38)	2.39 (0.56)	3.05 (0.48)	<1.27	1.77 (0.17)
Day 90 -Regular Refrig.[9] Packaging A (n=15)	6.33 (0.32)	6.03 (0.28)	6.09 (0.31)	4.11 (0.31)	1.78 (0.60)	<1.27
Packaging B (n=15)	6.47 (0.34)	6.14 (0.36)	6.23 (0.37)	4.40 (0.66)	1.97 (0.79)	<1.27
Day 100 - Temp. Abuse[10] Packaging A (n=3)	8.18 (0.10)	7.69 (0.15)	7.53 (0.85)	6.19 (0.99)	NT[11]	NT
Packaging B (n=3)	8.01 (0.43)	7.54 (0.56)	7.94 (0.44)	3.97 (1.06)	NT	NT

(1) Average Log $_{10}$ CFU/cm^2, within brackets standard deviation, (2) Total Mesophiles Count, (3) Gram negative Count, (4) *Lactobacillus* Count, (5) *Enterobacteriaceae* Count, (6)Yeast Count, (7) *Brochothrix thermosphacta* Count, (8) Number of samples, (9) Storage at 0°C (0.5C), (10) 6°C (35 ds) after 65 ds of storage at 0°C, (11) Not Tested

TABLE 2: pH OF BEEF SIRLOINS FABRICATED UNDER GMP's AND SUBJECTED TO TEMPERATURE ABUSE DURING STORAGE

pH	Time of sampling (ds)/Type of sampling				
	Day 0	Day 90 - Regular Refrigeration		Day 100 - Temperature Abuse	
	Packaging A&B (n=15)	Packaging A (n=15)	Packaging B (n=15)	Packaging A (n=3)	Packaging B (n=3)
SURFACE	5.67 (0.07)	5.13 (0.09)	5.04 (0.21)	5.57 (0.37)	5.24 (0.22)
DEPTH	5.75 (0.03)	5.03 (0.16)	5.07 (0.16)	5.25 (0.30)	5.21 (0.15)