SHELF LIFE EVALUATION OF REFRIGERATED VACUUM PACKAGED BEEF KEPT FOR EXTENDED STORAGE

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Background.
Vacuum package and refrigeration temperatures are used primarily to delay growth of microbes in order to minimize undesirable biochemical changes, and therefore to extend shelf life of meat cuts. Main microbiological objective of vacuum packaging, however, is the partial or total inhibition of the rapidly growing spoilage bacteria (aerobic and facultative Gram negative genera). Ideally, vacuum packaging should allow dominance of flora by lactic acid bacteria, which have a low spoilage potential (Gill, 1996). This type of packaging intends to provide an appropriate shelf life for an extended period that is necessary in overseas exporting conditions. Rodriguez et al. (1996) have reported that using Good Manufacturing Practices (GMP's), a low O_2 permeability film and adequate refrigeration storage, sirloins vacuum packaged can achieve a shelf life at least of 90 days. Reports on studies of shelf life, considering microbiological, biochemical and sensory conditions of vacuum packaging products for extended storage period are scarce.

Objective.
To determine the shelf life of refrigerated vacuum packaged *Longissimus dorsi* beef cuts intended for exportation to distant markets and fabricated under local commercial conditions.

Material and Methods.
Meat fabrication and sampling: *Longissimus dorsi* samples (n=80) were obtained from 20 Aberdeen Angus steers (20-22 months old). Animals were slaughtered and fabricated at a commercial facility using GMP's. Beef samples were vacuum packaged in a high oxygen barrier film (Cryovac, Sealed Air Corporation, Argentina) with an oxygen transmission rate of 16 cm^3/m^2 / 24 h/atm and stored in the dark. During storage registered temperature varied between 0 and 3°C with a mean of 1.15 ± 0.78°C. At each sampling time (0, 30, 60, 90 days) bacterial counts, pH measurements, off-odors detection, color and oxidative stability conditions, were performed on five samples.
Microbiological analysis: From each sample, an area of 22 cm^2 and 4 mm depth was removed in an aseptic manner, placed in a stomacher bag containing 50 ml of peptone water (0.1%), and homogenized for 2 min in a stomacher (Lab Blender 400, Seward Med, England). Suitable decimal dilutions in 0.1 % peptone water were prepared and bacterial groups enumerated in selective and non-selective media. Total Viable Count (TVC) on Plate Count Agar, *Pseudomonaceae* count (PC) on Cefixime-Fusidine-Cetrimide Agar, *Lactobacilleceae* count (LC) on MRS agar and *Brochothrix thermosphacta* (BtC) on streptomycin thallium acetate actidione agar (STAA), *Enterobacteriaceae* counts (EC) on Crystal-Violet Neutral-Red Bile Glucose agar were performed as described elsewhere (Rodríguez et al., 1993; Lasta et al., 1995).
Microorganism identification: The square root of the number of colonies growing on TVC plates was identified by simple biochemical tests according to Dainty et al. (1979) and Lasta at al. (1995).
Determination of pH: Determination of pH on the surface and in depth was done on a 1/10 slurry using a pHmeter (Metrohm 691 model, Switzerland) with a combined pH electrode.
Color and sensory evaluations: Color, odor and general appearance were evaluated at each sampling time. Color parameters (**L**, **a** and **b**) were determined by using a colorimeter (ByK Gardner Color View model 9000, USA) at a lamp temperature of approximately 2,854°K and a wide window (32mm).
Oxidative stability: TBA number tests was used to determine oxidative rancidity in raw and cooked samples and was performed according to Pensel (1990); where appropriate, statistical analysis were performed using SAS (1998).

Results and Discussion.
Mean viable counts for microbial groups of *Longissimus dorsi* samples are shown in Table 1. Low TVC value at day 0 of storage (mean 2.58 Log UFC/cm^2) were characteristic for an overseas exporting meat operation and lower than TVC counts observed for other meat cuts under similar conditions (Rodriguez et al, 1996). TVC at 30, 60 and 90 days did not exceed 7 Log CFU/cm^2. These values were below the 10^8 CFU/cm^2, which is considered as the spoilage limit for vacuum packaged meat (Egan and Shay, 1982; Bell and Garout, 1994). *Pseudomonaceae* increased from an initial contamination of 1.92 Log CFU/cm^2 to a maximum level at 60 days. *Brochothrix thermosphacta* counts were similar to those reported by Rodríguez et al. (1996) for a similar product with a maximum level attained of 3.43 Log CFU/cm^2 at 60 days. The *Enterobacteriaceae* group as counted on VRBD did not markedly grow. *Lactobacillaceae* become the predominant organism at 30 days of storage. On a similar product, intended for extended storage, Bell and Garout (1994) also observed that *Lactobacillus* spp. predominated in the microflora. This predominance could be expected as the effect of inhibition on other spoilage microorganisms (Dainty et al., 1979).
Identification of isolated organisms from TVC at 0 day is presented in Figure 1. A large variety of bacterial groups at time of packaging was observed. Initial flora composition on TVC was similar to that found in sirloins (Rodríguez et al, 1996) with predominance of *Staphylococcus* and *Moraxella*, while at 90 days, *Lactobacillus* was the main group (>57.7%) indicating the selection due to oxygen-depleted atmosphere (Figure 2). Surface pH diminished from 5.45 to 5.07 at the end of storage, whereas in the same period pH on the depth of the tissue remained constant. Acidic or sour aromas, with no off-odors, typical for vacuum packaged beef were detected in samples opened at 90 days of storage. This agree with Bell and Garout (1994) who found that the refrigerated vacuum packaged beef could be stored more than 100 days without noticeable production of off-odors except in the case of a significant growth of *Enterobacteriaceae*.
Regarding color evolution, an increase of **L**, **a** and **b** parameters from their initial values was observed at day 30, both in surface and depth of samples. From that sampling time onwards **a** parameter values from surface begin to diminish up to below of its initial value; parameter **a** values from depth, however remained fairly constant along storage.

Before 90 days there were no color changes, from the characteristic purple red of vacuum packaged meat, which could be considered as a rejection cause. However after 105 days 8/57 samples showed few and limited dark brown areas. The unattractive brown pigmentation may be due to the formation of metmyoglobin from myoglobin at low oxygen concentrations (Renerre, 1990). The discoloration observed could not be attributed, in principle, to metabolic products from predominant lactic acid bacteria (Taylor et al, 1990) There was no significantly difference ($p>0.05$) along 90 days of storage considering TBA values. Cooked samples also showed a rather constant although higher, resulting from expected oxidative compounds generated from exposition to high temperatures (cooking).

Conclusion.

Considering microbiological, biochemical and sensory parameters vacuum packaged *Longissimus dorsi* beef cuts were acceptable for 90 days in refrigerated storage.

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TABLE 1. MICROBIOLOGICAL ANALYSIS AND pH OF REFRIGERATED VACUUM PACKAGING *Longissimus dorsi* KEPT FOR EXTENDED STORAGE

Storage time (days)	Microbiological analysis[1]					pH	
	TVC[2]	PC[3]	BtC[4]	LC[5]	EC[6]	Surface	Depth
0	2.58 (0.52)	1.92 (0.36)	1.67 (0.00)	1.67 (0.00)	1.02 (0.60)	5.45 (0.06)	5.40 (0.06)
30	6.17 (0.40)	4.46 ((0.69)	2.44 (1.07)	5.29 (0.94)	2.15 (0.80)	5.35 (0.10)	5.54 (0.07)
60	6.10 (0.41)	5.19 (0.80)	3.43 (1.61)	4.76 (1.31)	2.52 (1.35)	5.26 (0.10)	5.51 (0.06)
90	6.98 (0.86)	4.29 (1.49)	1.97 (0.68)	5.56 (1.37)	2.45 (1.21)	5.07 (0.16)	5.34 (0.09)

(1) Average Log_{10} CFU/cm^2 of 5 samples, within bracket standard deviation, (2) Total Viable Count, (3) *Pseudomonaceae* Count, (4) *Brochothrix thermosphacta* Count, (5) *Lactobacillaceae* count, (6) *Enterobacteriaceae* Count

Figure 1. Percentage distribution of microbial groups in TVC in vacuum packaged *Longissimus dorsi* cuts stored under refrigeration for 0 (a) and 90 days (b).