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SOME MICROBIOLOGICAL AND CHEMICAL FEATURES OF THE
BEEF PACKED IN OXYGEN + CARBON DIOXIDE STORED AT 0°C.

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SUMMARY
Samples of Beef (*M. semitendinosus* and femoral biceps) removed from beef carcass were divided into two equal groups weighed and placed in bags. Ones bags were filled with air and others with 80% O₂ + 20% CO₂. Steaks were stored at 0°C for specified storage periods (0, 3, 6, 9, 12, 15, 17, 20 and 22 days).

At the end of each storage period, data were collected which included microbial counts, percentage of moisture, pH, total basic nitrogen (T.B.N.), thiobarbituric acid (T.B.A.), percentages of myoglobin and metmyoglobin and exudation.

The rounds stored under air, present higher microbial counts than those stored under 80% O₂ + 20% CO₂. The packaged steaks in controlled atmosphere exhibited higher weight losses, as well as a decrease in pH and lower values of T.B.N. and metmyoglobin than those packed under an air atmosphere.

It was concluded that packaging beef under an 80% O₂ + 20% CO₂ atmosphere makes an extension in the shelf life of cold stored meat possible.

INTRODUCTION
There is substantial economic incentive to lengthening the shelf life of fresh meats. This has been attempted through many techniques, some of which are refrigeration, irradiation, vacuum-packaging and most recently controlled atmosphere storage in gases such as CO₂, N₂, O₂ and air, as well as a mixture of several gases (1). The storage of meats in gas atmospheres may alter meat color and microbiology due to effects on the myoglobin pigment and growth rate of gram-negative psychrotrophic bacteria which are most often responsible for fresh meat spoilage (2, 3, 4). Myoglobin levels affect meat color. Heme pigments may be oxidized by CO₂ resulting in deleterious effects on meat color (5). However CO₂ slows growth of microorganisms which cause surface deterioration of meat especially *Pseudomonas* sp. (6). High concentrations of O₂ has been shown to extend the shelf life of the product (7), as well as maintain the bright cherry-red lean color of the retail cut, thereby preserving the appearance of beef marketed in this form.

The aim of this study was to determine the effect of 80% O₂ + 20% CO₂ atmosphere on the color, microbiology and shrinkage of beef steaks in an attempt to select the best controlled atmosphere for preservation of color and extension of shelf life.

MATERIALS AND METHODS
The samples were obtained from a flesh, called "Round roose", anatomically composed by muscles-semi-tendinosus and femoral biceps. This flesh was removed from beef carcass and divided into two equal parts. These parts were also cut in steaks of about 200g and packed into plastic bags of polyethylene/polyamide. These bags were filled with about 2.4 l of air (control) and 2.4 l of mixture 80% O₂ + 20% CO₂ (sample). The steaks were stored at 0°C for specified storage periods (0, 3, 6, 9, 12, 15, 17, 20 and 22 days).

At the termination of each storage period it was performed some microbiological and chemical analyses (2 bags at a time). The individual packages were aseptically opened after defrost period (30 min) at room temperature. The appearance and odor were observed. Odor was evaluated by sniffing vapors emanating from the sample source. The accompanying exudate volume was measured.

Small portions (10g) of steaks were removed to microbiological sampling. Using conventional dilution procedures (8, 9), viable counts were obtained from the suspension on Tryptone Glucose Extract agar (TGE; Oxoid), Deoxycholate, Rogosa & Sharp Medium (RSM), Rose Bengal with Kanamycin and Tetracycline (RB) and *Pseudomonas* Medium (PM; Oxoid). The TGE cultures were incubated at 30°C for 3 days (d) - (Total aerobic count) or at 10°C for 8 d (psychrotrophic count); Deoxycholate at 30°C for 1 d (coliforms); RSM at 30°C for 2d (lactic acid bacteria); RB at 22°C for 8 d (Yeasts and molds); PM at 30°C for 2 d (*Pseudomonas*). Mean bacterial counts were scored arithmetically then converted to logarithmic (log₁₀) values per g.

The pH measurements were performed in steak homogenate using a digital Chemtrix type 60 A. The moisture was calculated by weight loss by drying (10). T.B.N. was determined by Conway's microdiffusion method (10). T.B.A. was performed by Tarladgis' method (11), myoglobin by Wilson's method (12) and metmyoglobin by Hornsey's method (12).

RESULTS AND DISCUSSION

The bacterial growth on steaks stored at 0°C in air, or in 80% O₂ + 20% CO₂ is summarized in Figures 1 and 2.

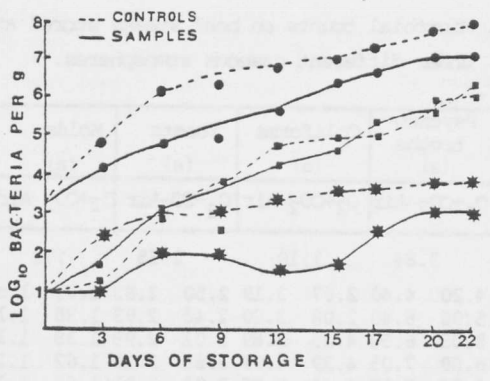


Fig 1. Microbial counts on beef steaks stored at 0°C in (---) air and in (—) 80% O₂ + 20% CO₂. Total aerobic count (●), *Lactobacillus* (■), *Pseudomonas* (*).

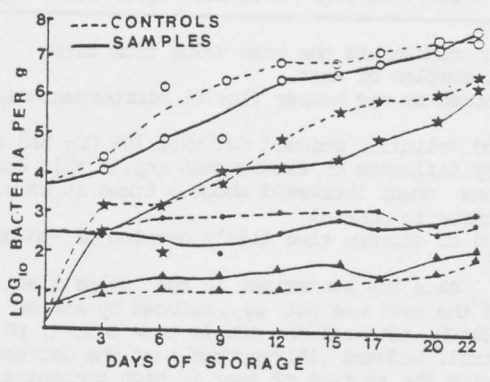


Fig 2. Microbial counts on beef steaks stored at 0°C. Psychrotrophic (○), Coliforms (★), Yeast (●), Molds (▲).

For the most part, the bacterial growth was higher in control, than in sample and along the time the development grown-up in coliforms and lactobacilli. Tables 1 and 2 show the bacterial count on different substrates.

Table 1. Microbial counts on beef steaks stored at 0°C under different gaseous atmospheres.

Stor. time (days)	Total aerobic counts (a)		Lactobacillus (a)		Pseudomonas (a)	
	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air
0	3.19		1.00		1.00	
3	4.21	4.84	1.69	1.30	1.17	2.75
6	4.84	6.23	3.23	3.01	1.00	2.00
9	4.92	6.29	2.61	3.79	2.00	3.10
12	5.65	6.83	4.34	4.87	1.50	3.47
15	6.36	6.92	4.76	4.95	1.54	3.60
17	6.59	7.34	5.49	5.27	2.60	3.65
20	6.97	7.71	5.79	5.85	3.13	3.70
22	6.98	7.94	6.36	6.15	3.00	3.74

Each value represents the mean value from three different samples of meat.

(a)- Reported as the number (log 10) microorganisms/g.

The growth of lactic acid bacteria was stimulated by packaging and the count was higher in steaks stored at O₂ and CO₂. Mean values for total aerobic count, coliforms, pseudomonas and yeast counts from O₂ and CO₂ packaged steaks were lower than were counts on steaks stored in air. The inhibitory effect of CO₂ on aerobic spoilage bacteria is well documented (13, 14).

Table 2. Microbial counts on beef steaks stored at 0°C under different gaseous atmospheres.

Stor. time (days)	Psychrotrophs (a)		Coliforms (a)		Yeasts (a)		Molds (a)	
	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air
0	3.84		1.10		2.65		.70	
3	4.20	4.40	2.67	3.19	2.50	2.63	1.23	1.00
6	5.06	6.40	2.08	3.20	2.48	2.93	1.38	1.15
9	5.03	6.50	4.25	3.89	2.01	2.98	1.38	1.15
12	6.59	7.05	4.39	4.99	2.64	3.10	1.62	1.12
15	6.77	7.07	4.46	5.72	2.93	3.01	1.65	1.34
17	6.91	7.37	4.92	6.11	2.70	3.20	1.47	1.45
20	7.49	7.51	5.49	6.02	2.74	2.69	1.88	1.53
22	7.83	7.58	6.39	6.62	3.27	2.85	2.17	2.14

Each value represents the mean value from three different samples of meat.

(a)- Reported as the number (log10) microorganisms/g.

Enfors and Molin (15) pointed out that 10% CO₂ had an inhibitory influence on Pseudomonas spp. In this study, Pseudomonas count increased about 3 times in sample and 3.7 times in control. At the end of storage time didn't develop off-odours.

Chemical data are summarized in the Tables 3 and 4. The pH of the meat was not very reduced by air or 80%O₂ + 20%CO₂, although the sample show a lower pH than control. Ledward (16) reported that the decrease in pH during the storage of meat in high concentration of CO₂ may explain why autooxidation of myoglobin is accelerated at lower pH values, while enzymatic reduction is retarded.

In this study, the percentage of oxygen can be reversed these effects.

Table 3. Comparison of some chemical data of beef steaks stored at 0°C in different gaseous atmospheres

Stor. time (days)	pH		Moisture (g/100g)		T.B.N. (mg/100g)		T.B.A. (mg/Kg)	
	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air
0	6.12		76.0		9.56		.10	
3	6.05	6.09	76.2	76.2	10.6	13.4	.39	.25
6	6.10	6.16	75.1	75.5	12.8	12.9	.43	.11
9	6.12	6.16	76.0	76.0	12.9	14.6	.88	.11
12	6.12	6.18	73.5	75.5	13.0	16.8	.75	.39
15	6.16	6.18	75.7	75.7	15.7	16.6	.89	.12
17	6.15	6.20	75.9	75.9	17.7	19.4	.78	.35
20	6.18	6.33	74.8	74.9	19.8	21.0	.99	.28
22	6.25	6.30	73.8	74.9	21.0	24.6	1.10	.37

Each value represents the mean value from three different samples.

Data of T.B.N. show an unfavourable rise, though sampler values are lower than those of control. T.B.A. index is higher in steaks stored under O₂+CO₂ perhaps by phospholipid oxidation by oxygen. Myoglobin values are not different during the experiment. Metmyoglobin data show higher values in control than in sample.

The formation of metmyoglobin increases when the oxygen concentration decreases and the presence of carbon dioxide didn't promote the formation of metmyoglobin, what would have happened if it had been used alone.

Table 4. Comparison of some chemical data of beef steaks stored at 0°C in different gaseous atmospheres.

Stor. time (days)	Exudate (ml)		Myoglobin (mg/g)		Metmyoglobin (mg/g)	
	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air	O ₂ +CO ₂	Air
0	0		1.32		.14	
3	0	0	1.41	1.43	.12	.16
6	1.1	0	1.40	1.41	.11	.12
9	2.0	.5	1.38	1.42	.12	.13
12	2.5	.5	1.15	1.39	.15	.16
15	3.0	.7	1.39	1.49	.13	.13
17	3.0	1.0	1.32	1.32	.10	.13
20	2.5	1.0	1.32	1.20	.11	.16
22	2.9	1.2	1.34	1.39	.10	.17

Each value represents the mean value from three different samples.

The steaks under controlled atmosphere show more natural exudate than those with air. This can happen because N₂ content is useful to minimize exudate loss.

CONCLUSIONS

Carbon dioxide inhibits the microbial growth by its bacteriostatic effect. High concentration of oxygen doesn't aid the increase of aerobic flora.

In our experimental conditions the predominance of lactic acid bacteria can inhibit the development of other carbon dioxide resistant organisms which may, compared with lactobacilli, have a more deleterious effect on the meat.

Based on the results of this study, it appears that packaging beef in an 80%O₂ & 20%CO₂ atmosphere allows an extended shelf-life of fresh meat.

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