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# Microbiological Characteristics of Vacuum-CO<sub>2</sub>- Packaged Broiler Carcasses

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# Microbiological Characteristics of Vacuum-CO<sub>2</sub>-Packaged Broiler Carcasses

Bulk packaging of fresh broiler products (whole or part) in ice permits distribution from the South to major markets in urbanized areas throughout the United States, but shelf life of ice-packed poultry is short. Therefore, an alternative to ice packing is needed to (1) extend shelf life, (2) reduce transportation costs by replacing ice with salable products and (3) facilitate physical handling by eliminating melting ice.

Vacuum packing has been introduced as an alternative. Cantoni and Bolther (1973) identified several advantages of vacuum packaging, including reduction in weight loss due to dehydration, preservation of muscle color in its freshest state and elimination of external contamination. In addition,

vacuum packaging improves organoleptic acceptability of ground beef (Jaye *et al.*, 1962).

Arafa and Chen (1975) observed that vacuum packaging of cut-up fresh broilers did not prolong shelf life during refrigerated storage. Microorganisms responsible for the spoilage of vacuum-packed, cut-up fresh broilers were members of *Enterobacter* species, and large numbers of *Pseudomonas* species were found in control samples.

Treatment with carbon dioxide (CO<sub>2</sub>) is another way to improve packaging. CO<sub>2</sub> in the environment selectively inhibits growth of bacteria. Researchers have found that a combination of low temperature (0-10°C) and CO<sub>2</sub> in a controlled environment effectively extends the shelf life of red meat

(Baran *et al.*, 1970), poultry (Wabeck *et al.*, 1968) and pork (Adams and Huffman, 1972). Recently, Sander and Soo (1978) reported that substituting 95% CO<sub>2</sub> for the air in packages of fresh broilers limited the growth of aerobic and anaerobic microorganisms. They indicated that packaging broiler carcasses in Nylon/Surlyn film with carbon dioxide addition rates of  $3.6 \times 10^{-4}$  and  $7.22 \times 10^{-4} \text{ m}^3/\text{kg}$  carcass, respectively, extended shelf life to 22 and 27 days at 1.1°C.

The purpose of this study was to evaluate the effect of vacuum-CO<sub>2</sub>-packaging on the shelf life of broilers under two different storage temperatures.

## Procedure

### Sample Preparation and Storage

Ice packed and vacuum-CO<sub>2</sub>-packed broiler carcasses (with giblet packages) were obtained from a commercial processing plant. The ice-packed samples were prepared by placing broiler carcasses directly into crushed ice. The vacuum-CO<sub>2</sub>-packed samples were prepared by placing three broiler carcasses in 4.0 mil polyethylene pouches and sealing after drawing vacuum (44.4 cm or 17.5 in) and adding CO<sub>2</sub> at rates of  $3.54 \times 10^{-4}$  to  $3.97 \times 10^{-4} \text{ m}^3/\text{kg}$  carcass. Ten samples of each treatment were stored in -2.2 to 1.1°C and 1.1 to 3.3°C refrigerators in the Mississippi State University Poultry Science Laboratory. One sample from each storage condition was selected randomly for analysis at each testing period. Experiments were repeated.

### Drip Measurement

The vacuum-CO<sub>2</sub> packages were cut at the corner and drained for 30 seconds. The drip was weighed and converted to a percentage of original carcass weight.

### Sampling of Microorganisms

**Carcass**---Samples for microbiological study were obtained by swabbing the breast skin with a moist, sterile cotton swab (Falcon plastics, CA) for thirty seconds in a different direction. The skin area swabbed was 1 sq inch (6.45 cm<sup>2</sup>) circumscribed by a sterile aluminum foil template. The cotton tip was broken into a serial dilution containing 10 ml of nutrient broth, and the solution was shaken vigorously to disperse the cotton swab and thoroughly mix the microflora.

**Gizzard**---The gizzard sample

was weighed into a sterile, wide-mouth 250 ml Erlenmeyer flask. An equal weight of sterile nutrient broth was added, and the mixture was shaken vigorously for one minute.

### Plate Counts:

Plate counts were made in duplicate by using standard method agar (BBL). Plates were incubated for 72 hours at 20°C to determine the psychrophilic counts. For anaerobic counts, anaerobic agar (BBL) was used, and plates were incubated for 48 hours at 37°C. Average counts from the duplicate plates were reported as the number of bacteria per cm<sup>2</sup> of skin surface or number of bacteria per gram of gizzard.

**Coliform Most Probable Number (MPN)**

Methods for the coliform test as

described by Thatcher and Clark (1968) were followed.

Psychrotrophic count plates of the zero time and spoiled carcasses were used for selecting isolates under the two packing conditions. Carcasses stored for 28 days at 1.1 to 3.3°C were used as spoiled samples. All colonies from plates

containing 10 to 30 colonies were isolated without bias and were purified by a streak-plate technique.

*Identification of Microorganisms:*

The purified cultures were transferred to standard method agar slants and incubated at 20°C, and these fresh cultures were used

for identification. Gram stain motility, oxidative and fermentative metabolism, cytochrome oxidase and catalase tests of the cultures were made according to the methods described by Colli and Lyne (1970). The scheme Freeman *et al* (1976) was used to identify the isolates.

## RESULTS AND DISCUSSION

The log of total bacterial counts on 6.5 cm<sup>2</sup> of skin surface at 20°C, suggested by Elliott and Michener as a spoilage indicator, revealed that vacuum-CO<sub>2</sub>-packed broiler carcasses spoiled after 21 days of storage at 1.1 to 3.3°C. Ice-packed samples stored under the same conditions spoiled after 14 days of storage. Lowering the storage temperature to -2.2 to 1.1°C extended the shelf life for the vacuum-

CO<sub>2</sub>-packed and the ice-packed broiler carcasses about five and four days, respectively.

The finding of longer shelf life for broiler carcasses in a CO<sub>2</sub> atmosphere agreed with the results of Killeffer (1930) and Coyne (1932). They determined that meat and fish products can be kept fresh longer when refrigerated in a CO<sub>2</sub> atmosphere. Our results supported the findings of Sander and Soo

(1978) who indicate that, at storage temperature of 1.1°C packaging broiler carcasses Nylon/Surlyn film with 3.6x10<sup>-4</sup> and 7.22x10<sup>-4</sup>m<sup>3</sup> CO<sub>2</sub>/kg carcass extended the shelf life eight to ten days, respectively.

The growth of psychrotrophic microorganisms (Figure 1) was delayed by the absence of air and/or the presence of 3.54x10<sup>-4</sup> and 3.97x10<sup>-4</sup>m<sup>3</sup>CO<sub>2</sub>/kg carcass. Ps

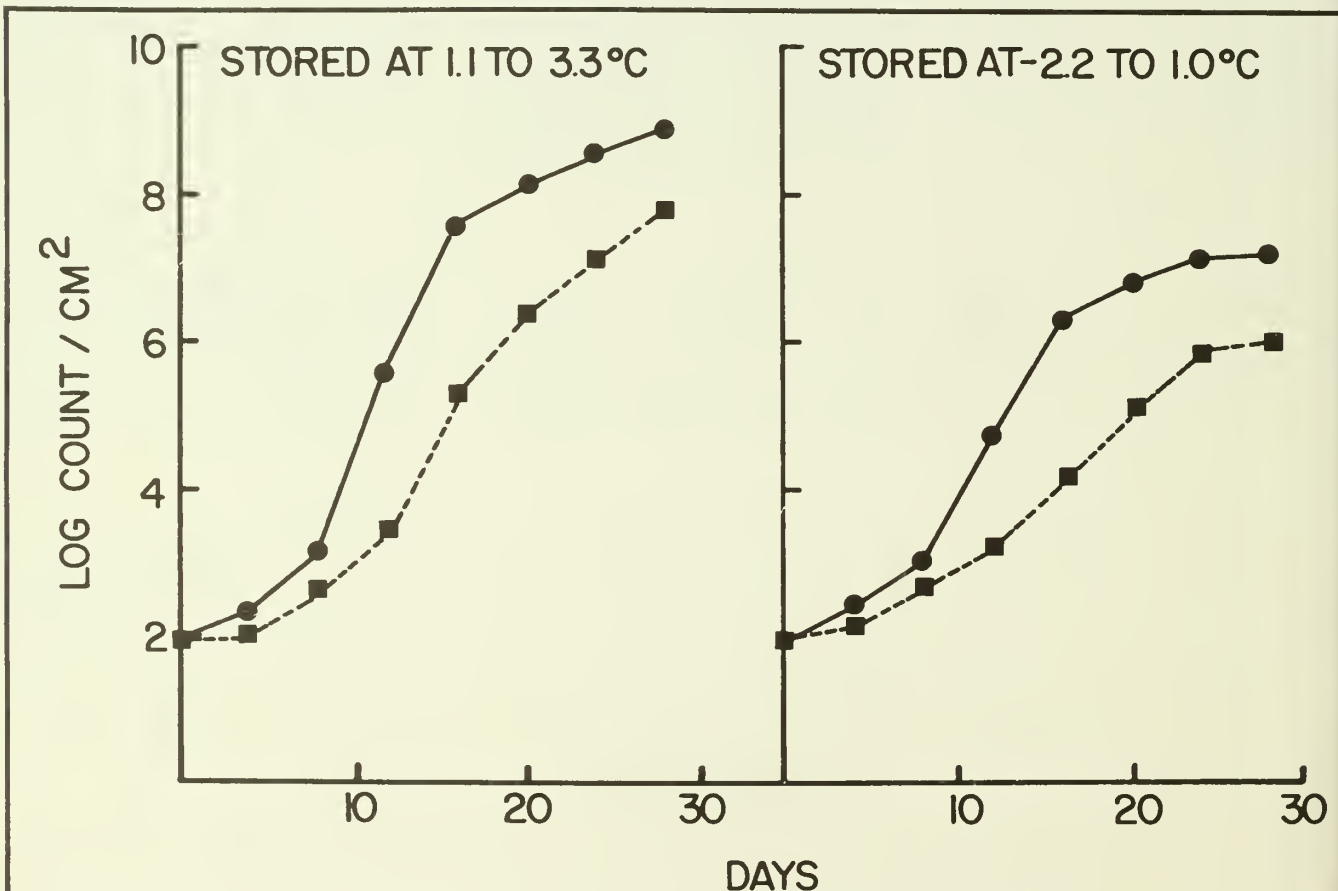


Figure 1. Psychrotrophic counts of vacuum-CO<sub>2</sub>-packed ■ and ice-packed ● broiler carcasses.

psychrophilic counts were consistently lower for the vacuum-CO<sub>2</sub>-packed samples than for the ice-packed controls. A longer growth lag phase was observed for the vacuum-CO<sub>2</sub>-packed samples than for the ice-packed control. Similar trends were observed at both storage temperature ranges. Total aerobic counts for both packaging methods were lower for samples stored at -2.2 to 1.1°C than for those stored at 1.1 to 3.3°C. Similar trends were reported by Bailey *et al* (1979) for broilers stored at 2°C and 5°C.

The initial psychrophilic counts for both types of broiler carcasses were lower than those of the psychrotrophic counts (Figures 1 and

2); however, the psychrophilic counts were slightly higher than the psychrotrophic counts after 16 days of storage.

Anaerobic counts increased steadily for both treatments. Lower storage temperature resulted in lower anaerobic counts for the carcasses. Lower anaerobic counts were observed for the vacuum-CO<sub>2</sub>-packed than for the ice-packed controls (Figure 3).

A log microbial count of 7.49/gram suggested by Charoenpong and Chen (1979) as detectable spoilage level for refrigerated gizzards, indicated that gizzards packed in giblet bags and stuffed in broiler cavities spoiled after 17 days of storage at 1.1 to 3.3°C. Log

total counts/gram of gizzard were higher than log counts/cm<sup>2</sup> of the broiler skin. Thus, the vacuum-CO<sub>2</sub>-packaging condition affected the growth of microorganisms on gizzards more than it did those of the carcass (Figures 1 and 4).

Under the same storage conditions, vacuum-CO<sub>2</sub> packaging extended the shelf life of gizzards in giblet bags about eight days beyond that of ice pack. Gizzard samples from the vacuum-CO<sub>2</sub>-packaged carcasses stored at -2.2 to 1.1°C had counts of less than 1.4x10<sup>5</sup> cells/g of gizzard at 28 days of storage (Figure 4). No off-odor was detected for these gizzard samples.

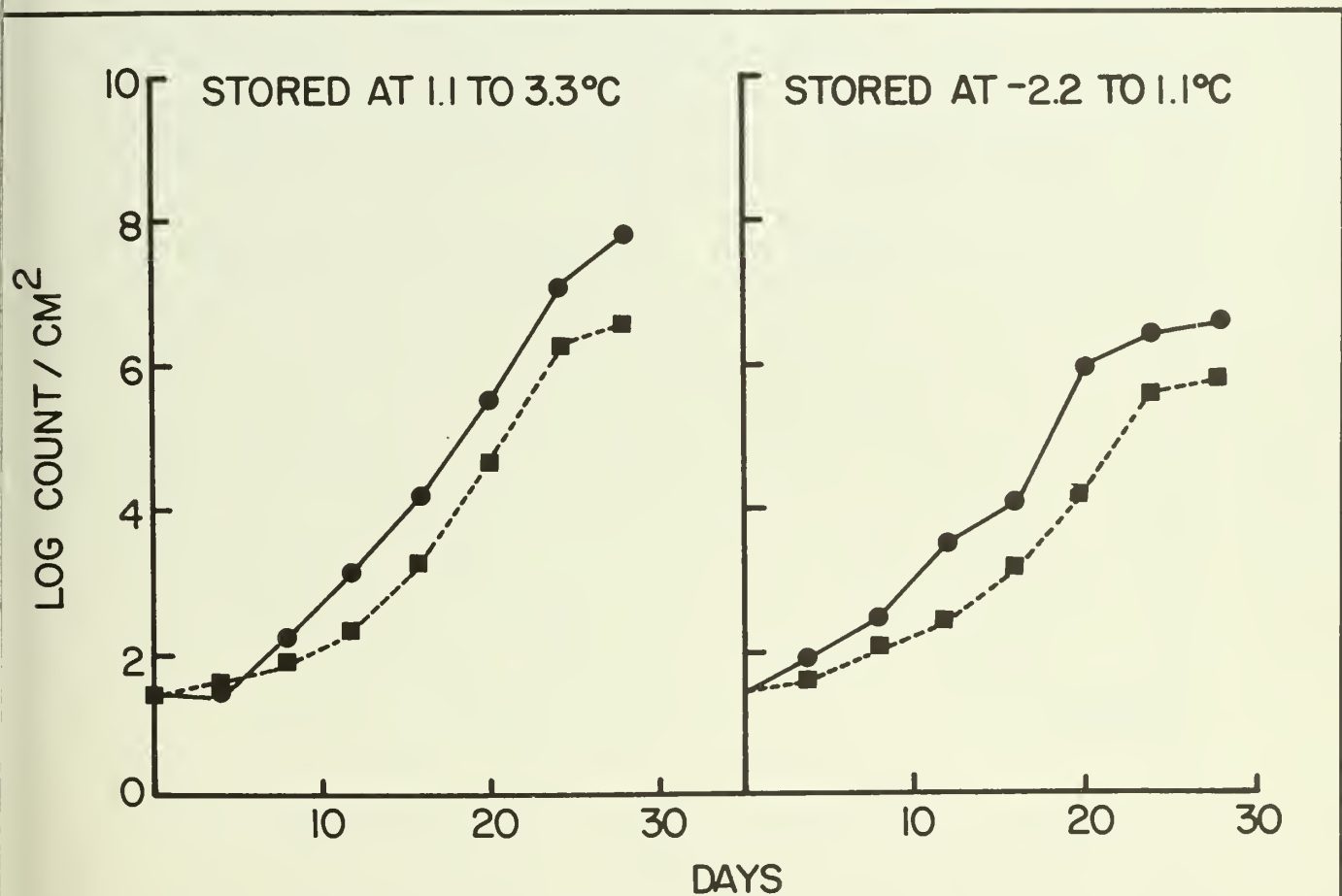


Figure 2. Psychrophilic counts of vacuum-CO<sub>2</sub>-packed ■ and ice-packed ● broiler carcasses.

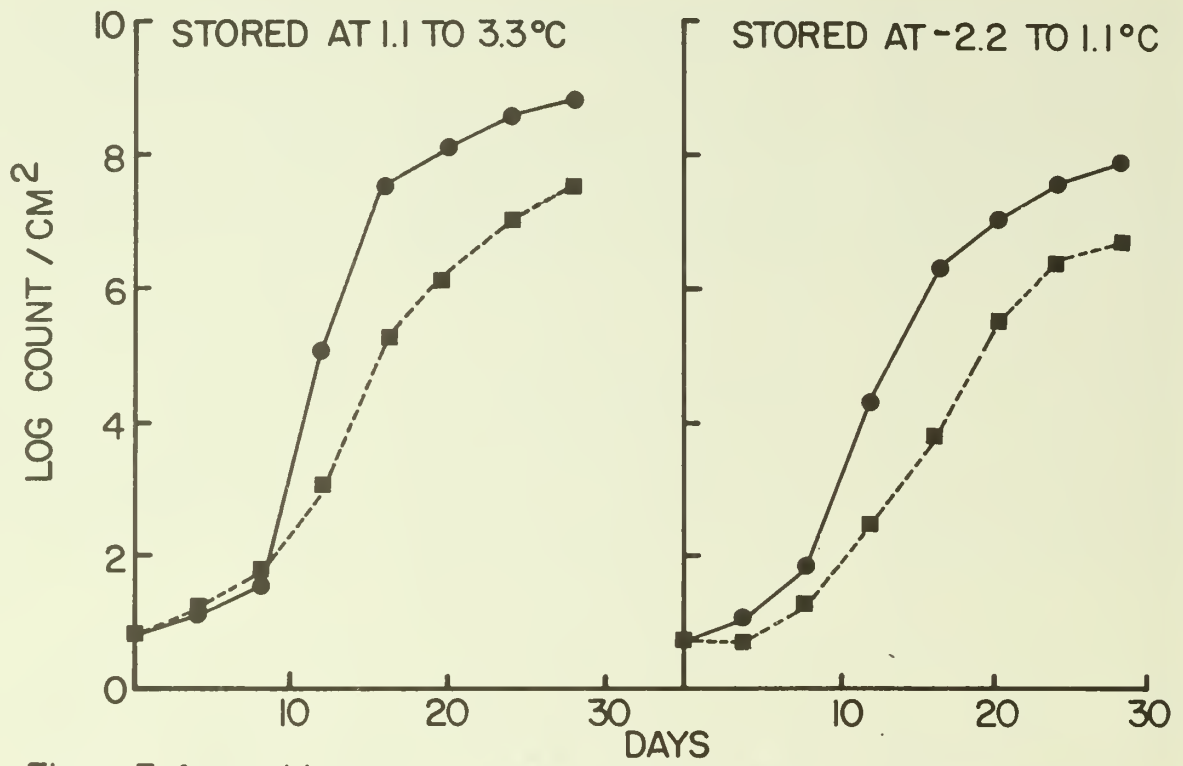


Figure 3. Anaerobic counts of vacuum-CO<sub>2</sub>-packed ■ and ice-packed ● broiler carcasses.

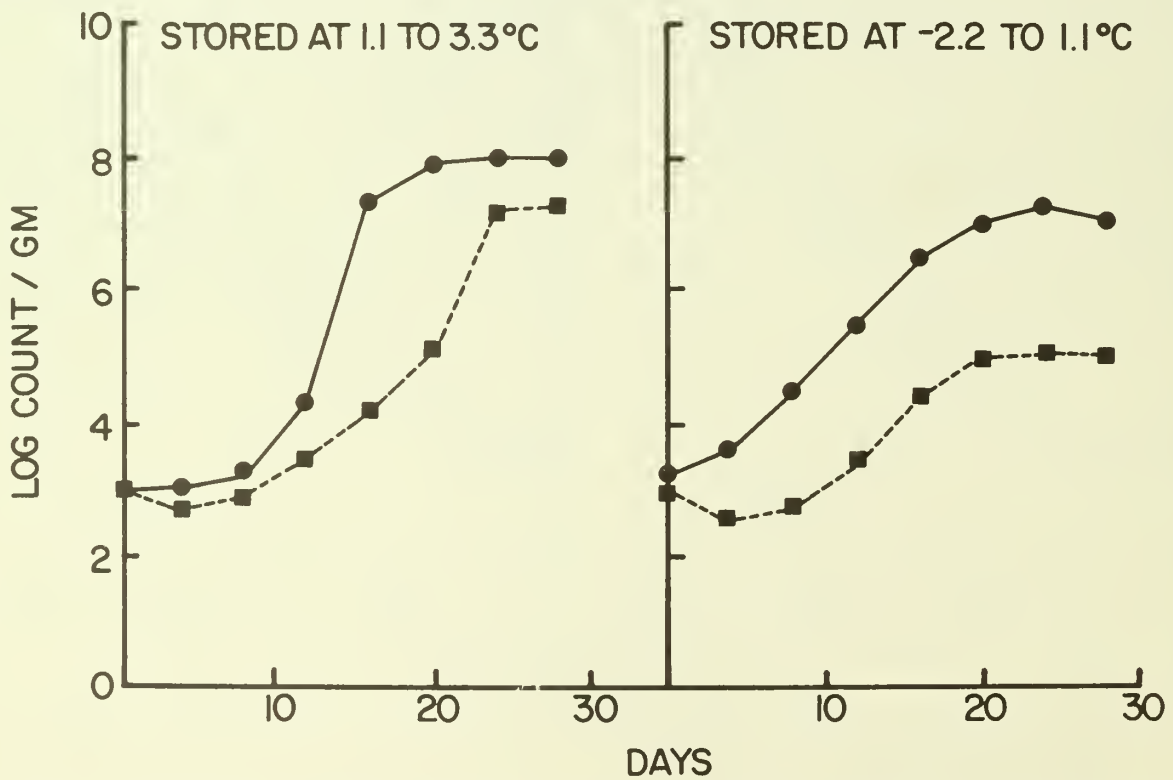


Figure 4. Psychrotrophic counts of vacuum-CO<sub>2</sub>-packed ■ and ice-packed ● gizzards

The effects of storage temperature on the growth of microorganisms on gizzards differed from the effects on broiler carcasses. The stationary phase microbial counts for the gizzards stored at -2.2 to 1.1°C were lower than those stored at 1.1 to 3.3°C, and growth of microorganisms in ice-packed gizzard samples stored at -2.2 to 1.1°C was faster than on gizzards stored at 1.1 to 3.3°C.

In most cases, the coliform MPN's of vacuum-CO<sub>2</sub>-packaged samples remained low and were slightly higher than those of the ice-packed controls (Figure 5). The data supported the findings of Vabeck *et al* (1968) and Arafa and Chen (1976) but contradict those of

Sander and Soo (1978), who indicated that coliforms grew rapidly on ice-packed chickens and that the presence of CO<sub>2</sub> significantly reduced the rate of growth.

Percentages of drip in vacuum-CO<sub>2</sub>-packaged broiler carcasses increased slightly as storage time increased (Figure 6). Storage temperature affected the drip formation of packaged broiler carcasses. Vacuum-CO<sub>2</sub>-packaged carcasses stored at 1.1 to 3.3°C generally had similar or slightly higher drip percentages than those stored at -2.2 to 1.1°C. The increase in drip formation of vacuum-CO<sub>2</sub>-packaged broiler carcasses was found to be greater than that reported by Lentz and Rooke (1958)

and Baker (1959). These researchers indicated that the greatest loss of moisture under refrigerated storage of packaged broiler carcasses occurred within the first 2 hrs and stabilized after 24 hrs. A slight reabsorption of drip was observed after 24 days of refrigerated storage at both temperatures. This reabsorption of drip agreed with Arafa and Chen (1978).

The incidence of major microbial species when broiler carcasses arrived in the laboratory was similar for the samples packed by both methods. Gram positive cocci were the dominant microorganism followed by members of *Acinetobacter* and oxidative

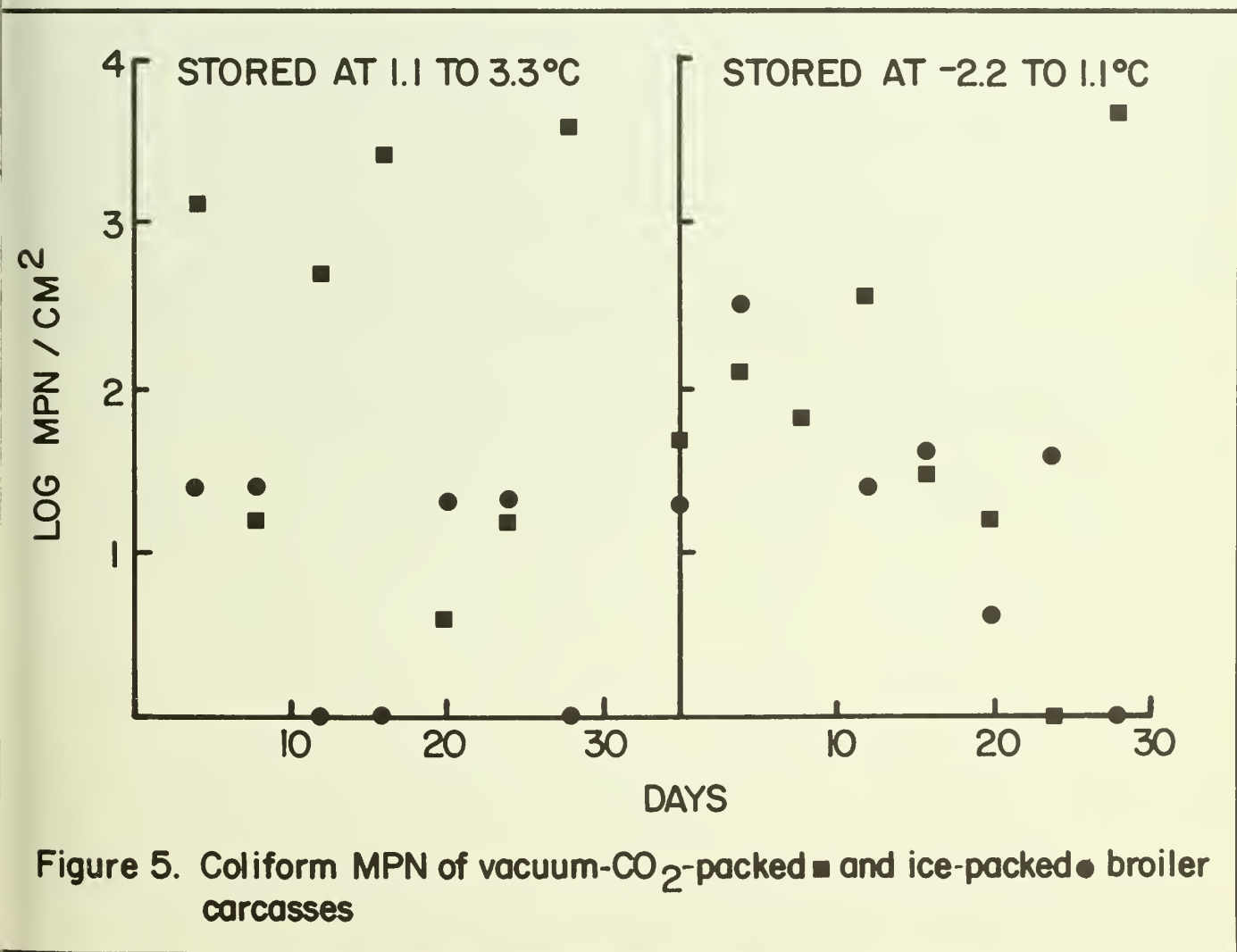


Figure 5. Coliform MPN of vacuum-CO<sub>2</sub>-packed ■ and ice-packed ● broiler carcasses



*Moraxella* (Table 1). The vacuum-CO<sub>2</sub>-packaged broiler carcasses had higher *Acinetobacter* counts than the ice-packed controls. In addition, no Gram positive rod type microorganisms, *Micrococcus*, *Pseudomonas* or fermentative *Flavobacterium* were isolated from the vacuum-CO<sub>2</sub>-packed samples. Higher percentages of gram positive cocci on fresh broiler carcasses also were reported by Arafa and Chen (1977).

The initial incidence of the major microbial group was similar for the ice-packed and the vacuum-CO<sub>2</sub>-packaged broilers, but major groups of microflora responsible for spoilage of these two types of broiler samples were different (Table 2). The major microbial groups isolated from the spoiled ice packaged samples were members of the *Pseudomonas* genus, but *Lactobacillus* species were found to be the dominant microflora on the vacuum-CO<sub>2</sub>-packaged samples after 28 days of storage at 1.1 to 3.3°C.

Species of *Pseudomonas* also were isolated from the vacuum-CO<sub>2</sub>-packaged samples after 28 days of storage at 1.1 to 3.3°C, but

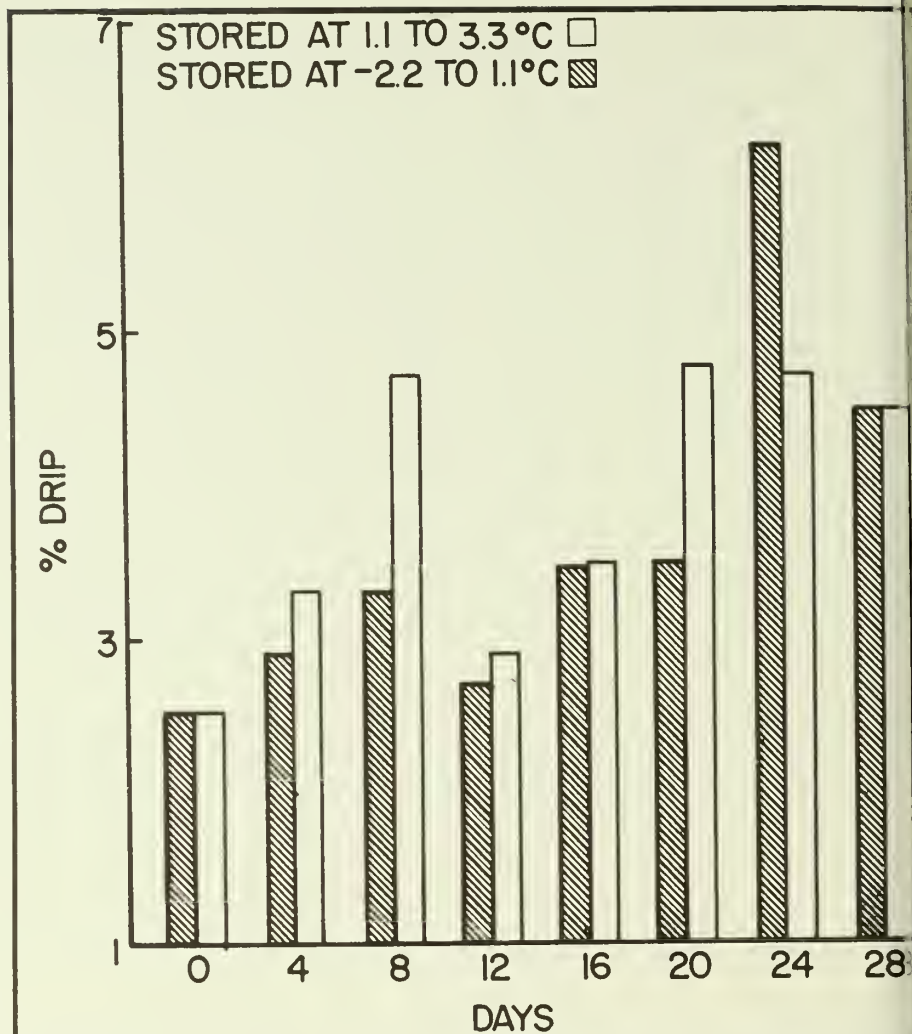


Figure 6. Percent drip from vacuum-CO<sub>2</sub>-packaged broiler carcasses.

Table 1. Incidence of microflora on ice-packed and vacuum-CO<sub>2</sub>-packed fresh broilers<sup>1</sup>

Gram-stain and Morphology	Ice packed		Vacuum-CO <sub>2</sub> packed		Microbial groups
	No. of isolates	% of isolates	No. of isolates	% of isolates	
G+ cocci	34	57.6	29	52.7	<u>Staphylococcus</u>
	31	52.5	29	52.7	
	3	5.0	--	--	
G+ rod	4	6.7	--	--	B ( <u>Lactobacillus</u> sp.) Others
	2	3.3	--	--	
	2	3.3	--	--	
G- rod	21	35.5	26	47.2	E ( <u>Pseudomonas</u> sp.) J ( <u>Moraxella</u> ) K ( <u>Acinetobacter</u> ) L ( <u>Moraxella</u> - oxidative) M ( <u>Flavobacterium</u> - fermentative) N ( <u>Flavobacterium</u> )
	2	3.3	--	--	
	2	3.3	2	3.6	
	9	15.2	18	32.7	
	5	8.4	4	7.2	
	1	1.6	--	--	
	2	3.3	2	3.6	

<sup>1</sup>Microbial groups as described by Freeman et al. (1976) were followed.

Table 2. Incidence of spoilage microflora on ice-packed and vacuum-CO<sub>2</sub>-packed, spoiled broilers<sup>1,2</sup>

Gram-stain and Morphology	Ice packed		Vacuum-CO <sub>2</sub> packed		Microbial groups
	No. of isolates	% of isolates	No. of isolates	% of isolates	
G+ rod	2	3.2	40	74.0	B ( <i>Lactobacillus</i> sp.) Others
	1	1.6	40	74.0	
	1	1.6	-	-	
G- rod	59	96.7	14	25.9	E ( <i>Pseudomonas</i> sp.) L ( <i>Moraxella</i> - P ( <i>Putrefaciens</i> ))
	57	93.4	12	22.2	
	2	3.3	-	-	
	-	-	2	3.7	

<sup>1</sup>Samples were stored at 1.1 to 3.3°C for 28 days.

<sup>2</sup>Microbial groups as described by Freeman et al. (1967) were followed.

the percent incidence of *Pseudomonas* was much lower than for the ice-packaged samples (22.2% vs 93.4%). No oxidative *Moraxella* were isolated from the vacuum-CO<sub>2</sub>-packaged samples. The high incidence of *Pseudomonas* species on the spoiled ice-packed broiler carcasses agreed

with results reported by Wabeck *et al* (1968) and Arafa and Chen (1975). Arafa and Chen (1975) reported that 95.5% of the microorganisms in vacuum-packaged broiler parts after 28 days of storage at 2-4° were members of *Enterobacter* species while the other 4.1% were members

of *Pseudomonas*. Results of this study indicated that the presence of CO<sub>2</sub> resulted in the growth of *Lactobacillus* species instead of *Enterobacter* species in the vacuum-CO<sub>2</sub>-packaged broiler carcasses.

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