

## Microbiology of Meats in a Hypobaric Environment

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### ABSTRACT

Growth of total bacteria on fresh meat and heat-processed commodities was analyzed at refrigerated hypobaric and conventional cooler storage. When using the time required for bacterial levels to reach one million/in.<sup>2</sup> as the estimate of the shelf life, the refrigerated hypobaric storage system substantially extended the shelf-life of broiler chickens, pork loins, bone-in heat-processed hams, and lamb and beef carcasses, as compared to storage in conventional coolers. The dominant microorganism isolated from the surface of the bone-in heat-processed hams stored in hypobaric and conventional coolers was a *Streptococcus* which was resistant to the heat process and tolerant to salt and sodium nitrite. For the fresh meat products, *Pseudomonas* was the dominant microorganism isolated from these products stored in either hypobaric or conventional coolers.

Hypobaric storage refers to a controlled environment of low pressure, low temperature and high humidity ventilation. Table 1 describes the technical set-up for hypobaric storage that was designed by the Grumman Corporation (4). The system is divided into two parts: (a) a refrigeration subsystem and (b) a vacuum/humidity/ventilation subsystem. The refrigeration and subsystem consists of two closed loops. Ethylene glycol and water circulate through tubing located on the inner surfaces of the container. Due to lowering the pressure in the container by the vacuum system, the partial pressure of the water vapor in the container is lower than in the product, causing vaporization (cooling) from the product. This heat of vaporization is transferred to the ethylene glycol and water system which is connected to a heat exchanger containing Freon. The Freon is cooled outside the container by a standard refrigeration unit. The vacuum/humidity/ventilation system contains a two-stage vacuum pump used to lower the pressure in the container and to flush gases (ventilation) and water vapor. The water vapor is filtered, heated in a humidifier, mixed with filtered air and injected into the container as cool steam.

### DEVELOPMENT OF HYPOBARIC STORAGE

The theory of hypobaric storage developed by Dr. Stanley Burg in the early 1960s (6) showed that the storage life of fruits held within such an environment

TABLE 1. Technical set-up for the hypobaric system.

Subsystem	Technical set-up
Temperature	<ul style="list-style-type: none"> <li>A. Uses evaporative cooling.</li> <li>B. Ethylene glycol and water in aluminum tubing against inner surfaces of the container.</li> <li>C. Partial pressure differential.</li> <li>D. Heat of vaporization transferred to coolant on the walls.</li> <li>E. Heat exchanger outside container to Freon.</li> <li>F. Freon cooled by a standard refrigeration unit.</li> </ul>
Pressure/humidity/ Ventilation	<ul style="list-style-type: none"> <li>A. Two-stage vacuum pump to reduce the pressure and ventilation.</li> <li>B. Vented water vapor is filtered and heated in a humidifier.</li> <li>C. Filtered water vapor is mixed with filtered outside air and injected into the container as cool steam.</li> </ul>

would be extended. Low atmospheric pressure would enhance the release of ethylene and carbon dioxide from the fruit tissues (3) and ventilation would remove the gases from the immediate environment. Low temperature would reduce metabolic processes of both fruit and storage microorganisms and high humidity would retard drying. The partial vacuum would also reduce the oxygen concentration which also inhibits respiration and spoilage processes.

Table 2 lists the storage life of a few perishable fruits and vegetables in a hypobaric system (4). Usually strawberries are shipped via air freight with a transit time of <24 h. However, much spoilage occurs due to the high temperature (60 F) in the cargo areas of planes. By surface transporting in a hypobaric environment, the strawberries could be stored for 21 days and have a subsequent shelf life of 4-5 days under normal refrigeration. Limes under normal storage conditions last 2-4 weeks. Increasing the storage life would improve the marketing of this commodity. In hypobaric storage, limes could be stored for 71 days at 80 mm of Hg. Similar extended storage in hypobaric environments was calculated for papaya and bell peppers.

These are only a few samples of fruits and vegetables stored under hypobaric storage (6). Each commodity has

TABLE 2. *Four perishable products tested in hypobaric storage.*<sup>a</sup>

Commodity	Pressure (mm Hg)	Storage time (Days)	Relative humidity (%)	Subsequent storage under normal refrigeration (Days)
Strawberries	10	21	95	4-5
Limes	80	71	95	14-28
Papaya	20	28	92	5-6
Bell peppers	80	50	95	- <sup>b</sup>

<sup>a</sup> Extrapolated from an article published by Jamieson (2).

<sup>b</sup> No data available.

its own pressure, temperature and relative humidity ranges that allow for the longest storage without product deterioration. Various kinds of commodities including the above-mentioned fruits and vegetables, as well as seafood, floral products and meats, have displayed an extended storage time under hypobaric storage versus normal refrigerated storage. The remainder of this paper will involve the discussion of hypobaric storage of various meats and meat products.

### EQUIPMENT

The hypobaric trailer is an internationally standardized cargo container that can be transported by truck, rail and/or cargo ship. The trailer is 40 ft long, 8 ft wide and 8 ft high. When operating in the hypobaric mode, the container will maintain temperatures of 28 to 56 F  $\pm$  1.5 F, a relative humidity of 80-100%  $\pm$  3% and an absolute pressure of 10-80 mm of Hg  $\pm$  2.0 mm of Hg. It requires about 90 min to reach the desired vacuum, depending on the load density. For the studies conducted by Armour, the container was preset for 31 F, 90-95% relative humidity and a pressure of 10 mm Hg. We found temperatures to vary generally from 28-31.5 F, humidity ranged between 85-95% and pressure varied between 5-20 mm Hg.

### BROILERS

Figure 1 shows the proliferation of total bacteria on broilers placed in hypobaric and conventional refrigerated storage. Broilers were removed from a packing line at a plant and divided into three treatment groups: (a) 320 broilers were packed in ice-pack boxes but were not iced, (b) 340 broilers were packed in ice-pack boxes with a polyethylene liner bag which was folded over the top of the birds but not iced and (c) 200 broilers were packed in ice-pack boxes and covered with 20% ice nuggets by weight. Treatment groups 1 and 2 were placed in the hypobaric trailer, the environmental conditions established within 2 h after loading. The broilers from treatment 3 were placed in conventional refrigerated storage. At each sampling period, two boxes (20 broilers/box) of each groups were removed from storage. The birds were evaluated for appearance and odor, and five birds (2 from the top, 1 from the middle and 2 from the bottom of the box) from each box were swabbed (2 in.<sup>2</sup> of outer thigh) for microbiological evaluation.

Tray-pack data are also included in Fig. 1 as an example of the normal shelf-life of fresh poultry parts. The tray-pack data were derived from a previous study conducted on birds from the same source as the ice-pack and hypobaric broilers. The thighs were removed from birds received whole, packaged in styrofoam trays with a polyethylene overwrap and placed in a 32-F cooler. Fresh broilers could be stored or transported in a hypobaric container for about 18 days without initiation of bacterial growth, as compared to 7 days in a standard ice-pack. There is some indication that once growth is established, the rate of proliferation is slightly greater under hypobaric conditions than in the ice-pack. Thus the time required for bacterial levels to reach 10<sup>6</sup>/in.<sup>2</sup> on the birds stored hypobarically was 26 days, compared to 18 days for those in ice-pack.

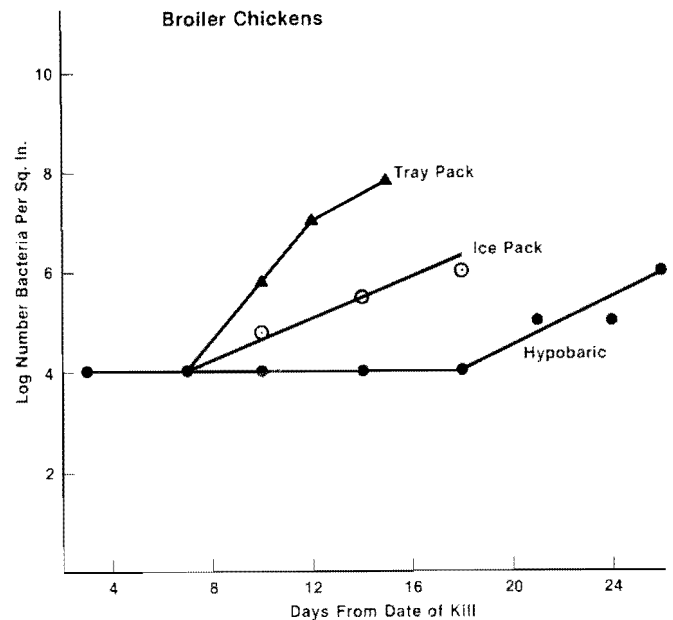


Figure 1. *The growth of total bacteria on broiler chickens placed in refrigerated hypobaric, conventional and showcase environments.*

### PORK

Figure 2 illustrates the bacterial growth on fresh pork loins wrapped in Kraft waxed paper and stored in the hypobaric trailer or in a cooler at 32-33 F. At designated time periods, 1 in.<sup>2</sup> of one pork loin from each variable was swabbed at three sites (inside rib, chine bone and sirloin). Again, the hypobaric environment delayed onset of bacterial proliferation, although the 7-day delay found

with pork loin is much less than the 18-day delay demonstrated with broilers (Fig. 1). Possibly the Kraft waxed paper wrap negated some of the influence of hypobaric low pressure, or the cut surface of the loins simply provided a better environment for microbial growth than the broiler skin.

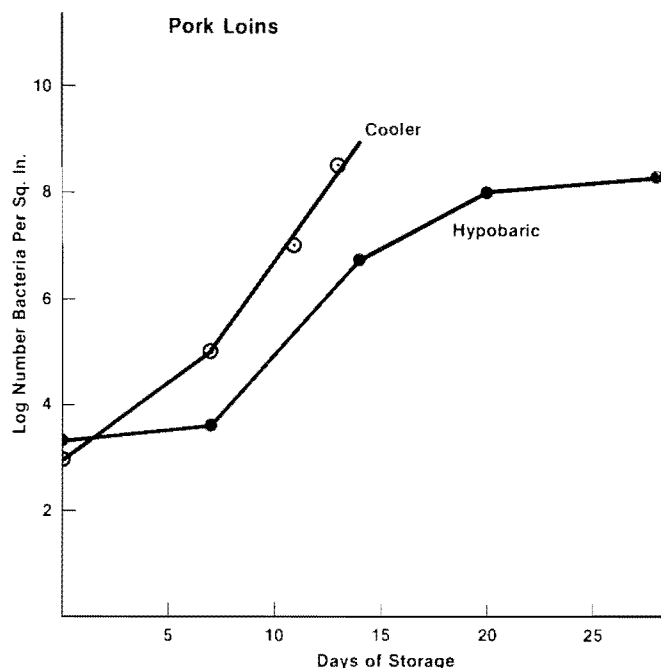


Figure 2. The proliferation of total bacteria on pork loins stored in refrigerated hypobaric and cooler containers.

To provide an extensive examination of the bacteriological growth on loins during hypobaric and cooler storage, 20 pork chops were fabricated from 5 loins and swabbed at various intervals. Pork chops fabricated from loins held in the hypobaric trailer had lower initial surface bacteria counts than chops derived from cooler-stored loins. If the maximum allowable surface count of pork chops immediately after fabrication should be 100,000/in.<sup>2</sup>, this limit could be achieved from loins held for 16 days under a hypobaric environment or from loins held about 1 week under conventional refrigerated storage (Fig. 3.). However, the subsequent shelf-life of the pork chops in a display case was dictated by the initial counts on the pork chops rather than the method of loin storage (data not presented). For example, chops having an initial surface bacteria count of 100,000/in.<sup>2</sup> would have the same shelf life regardless of whether they were fabricated from hypobaric- or cooler-stored loins.

Bone-in processed hams were also evaluated in the hypobaric system. These hams contained both salt and sodium nitrite and were fully heat-processed. As shown in Fig. 4, the hypobaric system delayed onset of surface bacterial growth by about 2 weeks and reduced the rate of growth substantially. As would be expected from the nature of the product, gram-negative psychrotrophs which do not survive the heat process were not present in detectable numbers on products stored hypobarically or conventionally. The dominant organism isolated from the hams used in this study was a *Streptococcus* (1,8),

which was resistant to the heat process and somewhat tolerant to salt and sodium nitrite.

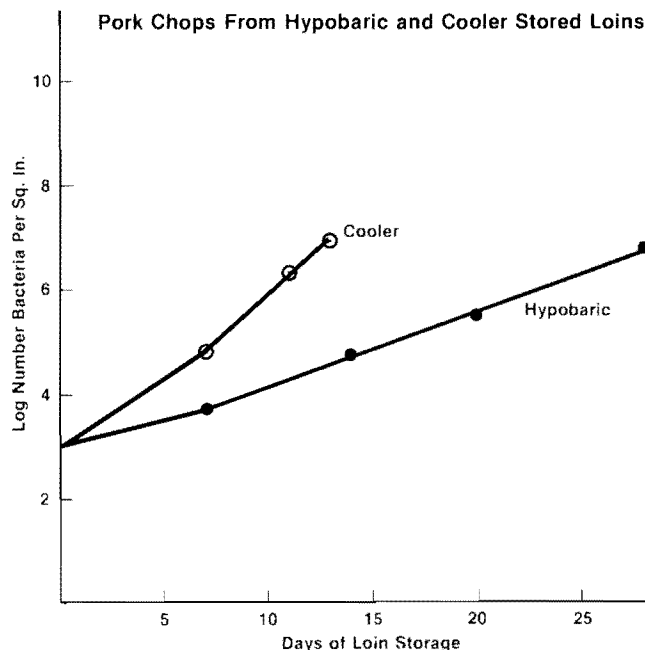


Figure 3. The bacterial analysis of pork chops fabricated from pork loins from refrigerated hypobaric and conventional cooler environments.

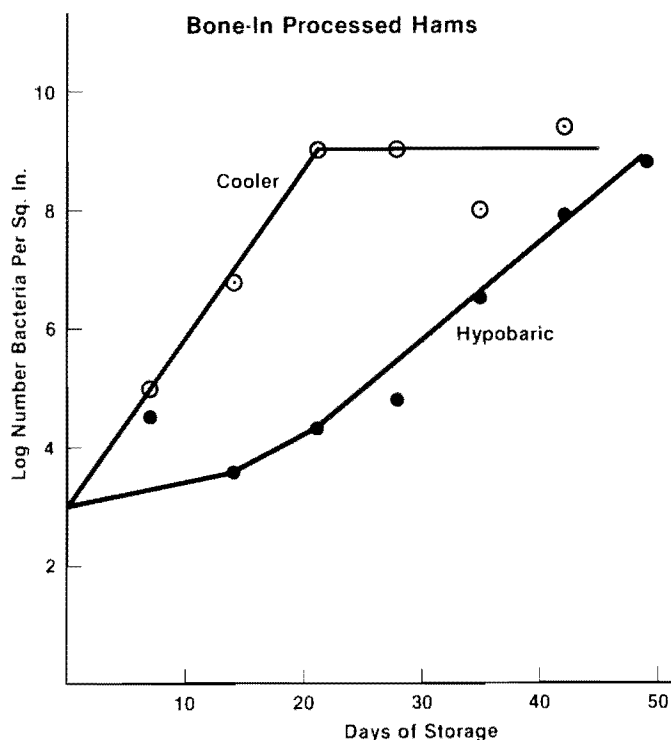


Figure 4. The bacterial growth on bone-in heat processed hams placed in refrigerated hypobaric and conventional cooler storage.

## DISCUSSION

The predominant bacteria dictating the shelf life of a commodity depends on whether the product was heat-processed or fresh rather than the type of storage conditions. As mentioned previously, streptococci, which

are able to survive the normal ham processing temperatures, were the dominant microorganisms on four types of hams throughout the storage in hypobaric and conventional coolers. However, for a fresh meat commodity, the major spoilage microorganisms for conventional and hypobaric storage were gram-negative, oxidase positive psychrotrophs belonging to the genus *Pseudomonas*. Since *Pseudomonas* is the dominant bacterium that grows on fresh meats stored in aerobic coolers and refrigerated showcases (5), the small but constant amount of oxygen (5-20 mm of Hg) present during normal hypobaric operation and/or the frequent opening of the container door could be responsible for causing this type of bacterium to grow with a delayed onset in hypobaric storage. *Lactobacillus* and other types of bacteria that are the dominant microorganisms in refrigerated vacuum-packaged fresh meat products (2,7) were present in low numbers on fresh meat stored in refrigerated hypobaric storage (data not presented). Therefore, since streptococci do not grow as well as *Pseudomonas* at temperatures near freezing (9), temperature control would appear to be the most critical factor in prolonging the shelf life of ham, whereas reduction of atmospheric oxygen appears most critical for fresh meats.

The Grumman hypobaric system has been shown to provide an excellent environment for long-term storage of meats. The hypobaric trailer was used to successfully transport fresh pork via ship to Honolulu which involved a total of 7 days of storage. The pork was inspected by state and USDA personnel and judged superior to that shipped conventionally. Also fresh lamb carcasses, beef and veal were transported in the hypobaric container via ship to Iran which involved a total of 42 days of storage. Again, the load was judged to be in excellent condition.

The data presented in this paper are obviously very applied in nature. Research involving studies somewhat more basic in nature still has not yet been initiated using the hypobaric transport system. Some of these research projects could involve the following questions. What is the influence of a hypobaric environment in production of extra-cellular protease and lipase by low temperature spoilage microorganisms? Can the hypobaric system be used to age meats at higher-than-usual temperatures for shorter-than-usual times without the proliferation of gram-negative enterics? From an engineering viewpoint, could a hypobaric system be adapted to retail meat cases or fast-food operations to reduce loss from throw-away conversion to lower cost items?

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