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Response of the Extract-Release Volume and Water-Holding Capacity Phenomena to Microbiologically Spoiled Beef and Aged Beef

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ABSTRACT

JAY, JAMES M. (Wayne State University, Detroit, Mich.). Response of the extract-release volume and water-holding capacity phenomena to microbiologically spoiled beef and aged beef. Appl. Microbiol. **14**:492–496. 1966.—The aging of ground beef was effected by storing in gas-impermeable, sterile plastic bags with incubation at 7 and 15 C. Control meat from the same preparations was wrapped in aluminum foil and stored at the same temperature. In three experiments where control meat was tested, aged meat did not attain a log bacterial number of ca. 8.4 per gram until an average of 6 days after this level was reached in control meats. This degree of difference was shown in values for both extract-release volume (ERV) and water-holding capacity. The previously reported ERV value of around 25, which was found to correspond to an average log bacterial number of ca. 8.5 per gram for ground beef allowed to spoil in aluminum foil and freezer paper, was approximated for aged meats, which required an average of 9.7 days to attain this number compared with 4.1 days for unaged meats. Plate count methods indicated the predominant flora of aged beef to be gram-negative, facultatively psychrophilic rods.

For the rapid determination of beef microbial quality, two simple methods were presented recently from this laboratory. The first method, designated ERV (extract-release volume), is based upon the amount of aqueous extract released by an homogenate of beef when it is allowed to pass through filter paper for a given period. Beef of good organoleptic quality with a relatively low bacterial count releases large volumes of extract, whereas beef of poor organoleptic quality with larger numbers of bacteria releases progressively less extract (5, 6). The second method is based on a measure of water-holding capacity (WHC) by use of the modified filter-paper press technique of Wierbicki and Deatherage (11). By this technique, beef of good organoleptic quality and low bacterial numbers has a low WHC (high free-water area on pressing), whereas microbiologically spoiled beef has a progressively higher WHC manifested by a smaller free-water area (7).

If phenomena of the types in question are employed for judging meat microbial quality, it is necessary that they differentiate between aged and spoiled meats. Previous reports have shown that neither method responded in the same manner to chlortetracycline (CTC)-treated beef, in which spoilage was delayed, as they did to paired controls without CTC which spoiled sooner. In other words, when CTC was added as a preservative, both tests were shown to respond to microbial numbers and associated changes and not to aging or extended holding. It is known, however, that CTC possesses the capacity to chelate metals in meats and that metal ions affect the hydration capacity of muscle proteins (4). In this regard, it seemed desirable to attempt to effect meat aging in the absence of added substances which alone might directly or indirectly affect beef hydration capacity, and consequently one or both of these spoilage-detection tests.

Since gas-impermeable plastic packaging materials have been known for some time to extend the keeping time of various meat products, and since additives are unnecessary in their use, a

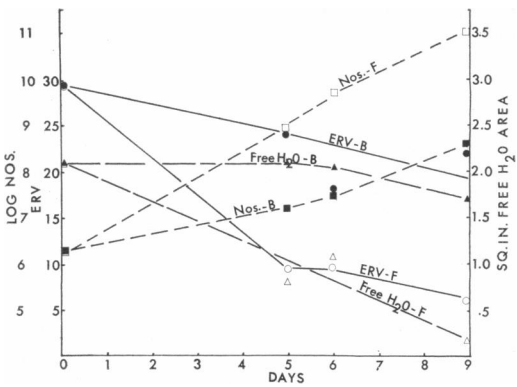

FIG. 1. *Log bacterial number per gram, ERV, and WHC from all-lean ground beef stored in foil and plastic bags and held for 9 days at 7 C. F = foil: B = bags.*

commercially available plastic bag was chosen. The utility of devices of this type for prolonging the keeping time of meat was demonstrated by Ayres (1), Kraft and Ayres (9), Halleck et al. (3), Jaye et al. (8), and Clauss et al. (2).

This study was made to determine whether ERV and WHC respond in any observable way to plastic bag-stored meat whose spoilage is delayed, as opposed to aluminum foil-wrapped meat which spoils much faster, other factors being equal. In other words, do ERV and WHC respond to changes brought about by large numbers of bacteria or to changes that occur in meats upon long-term storage in the absence of high bacterial counts?

MATERIALS AND METHODS

The ERV test, bacterial numbers, WHC, per cent moisture, *p*H, and ninhydrin-positive substances were determined as previously described (5, 7). Samples of meat to be aged were placed in sterile 6- or 18-oz 3-mil polyethylene bags (Whirl-Pak by Nasco; distributed by Fisher Scientific Co., Pittsburgh, Pa.). Duplicate ground-beef samples of 26 g each were employed, and either wrapped in sterile aluminum foil or placed in bags, or both. Meats employed were obtained in retail trade; more freshly slaughtered beef was avoided to gain more normal refrigerated beef flora. All determinations were made in duplicate, and the reported values are averages of the duplicate determinations.

RESULTS AND DISCUSSION

Figure 1 presents bacterial numbers per gram, ERV, and WHC from all-lean ground beef stored in foil and bags and held for 9 days at 7 C. The foil-wrapped meat showed almost a 5-log cycle increase in bacterial numbers over the 9-day period, while similar samples stored in bags showed only a 2.5-log cycle increase over the same period. The initial ERV of 29 dropped to 7 in foil-wrapped meat and to only 22 in bag-stored samples. The data on free-water area are seen to be quite similar to the foregoing where the initial free-water area of 2.13 square inches decreased to 0.19 in foil-wrapped and only to 1.75 square inches in bag-stored meat. The foil-wrapped meat showed definite signs of organoleptic spoilage by the 6th day, being quite tacky and possessing strong odors. The bag-stored meat displayed unpleasant odors on the 9th day but little or no tackiness. Jaye et al. (8), employing a Saran film, reported that they were unable to detect either off odors or slime formation (tackiness) after 12 days of storage of ground beef in gas-impermeable bags. Kraft and Ayres (9) and Clauss et al. (2), on the other hand, did observe off-odor development in their use of a gas-impermeable bag. The latter authors were unable to demonstrate tackiness on ground beef stored in gas-tight bags. In the present study, when bag-stored meats were exposed to air for a relatively short period of time, the objectionable odors disappeared and were replaced by a somewhat pleasant offness which one often associates with aged beef. This is not meant to imply that bag-stored meats did not undergo spoilage, but by comparative organoleptic evaluations these meats did not spoil to the same degree as the foil-wrapped samples. Testing bag-stored meats with forceps showed that the ground particles had retained much of their original firmness. It was possible for one to move a sample from one area to another by picking up each particle with forceps. This, of course, could not be done with the foil-wrapped meat after 9 days of holding, as the once firm particles had almost "melted" to give a highly slimy preparation. The per cent moisture was determined on this meat on the 4 test days over the 9-day holding period and found to be essentially the same in the foil-wrapped and bag-stored samples (foil samples: 70, 69, 69, and 69%; bag samples: 70, 69, 71, and 72%). The above-mentioned features of both foil- and bag-stored meat preparaions were in general seen in all others included in this report.

Table 1 presents data from ground-beef chuck containing approximately 10% fat. In foil-wrapped samples stored at 15 C, the initial bacterial count per gram of log 6.70 increased to log 9.00 over a 6-day period, whereas it onlv attained log 8.26 over a 9-day period in bag-stored samples under the same conditions. At 7 C over the 9-day period, total numbers in bag-stored meat were about the same as those at 15

TABLE 1. *Log bacterial number per gram, ERV, and WHC from ground beef chuck containing approximately 10% fat wrapped in foil, stored in bags, and incubated at 7 and 15 C*[a]

Days held	ERV			WHC			No./g		
	15F	15B	7B	15F	15B	7B	15F	15B	7B
0	30	30	30	1.13	1.13	1.13	6.70	6.70	6.70
1	29	36	35	0.72	0.82	1.00	8.11	7.66	7.00
2	20	35	30	0.71	0.75	0.91	8.51	7.91	7.67
5	10	33	33	0	0.63	0.68	8.85	8.20	7.83
6	5	—	—	0	—	—	9.00	—	—
7	—	—	31	—	—	0.57	—	—	7.60
9	—	25	30	—	0.50	0.77	—	8.26	8.08

[a] Storage of foil-wrapped and bag-stored meat at 15 C is indicated by 15F and 15B, respectively; 7B indicates bag storage at 7 C; — = no data.

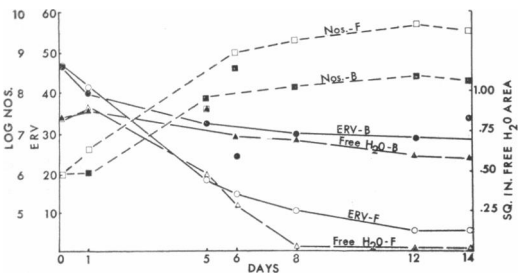

FIG. 2. *Log bacterial number per gram, ERV, and WHC from commercial ground beef containing 20% fat and stored in foil and plastic bags at 7 C.*

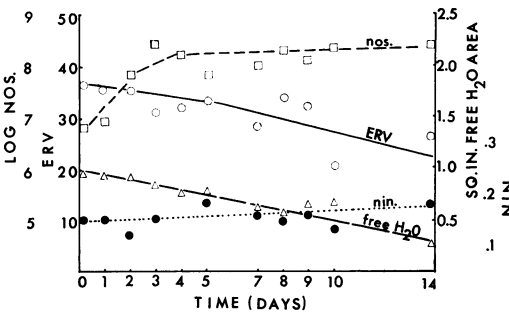

FIG. 3. *Log bacterial number per gram, ERV, WHC, and ninhydrin-positive substances from four different samples of all-lean ground beef stored in plastic bags at 7 C over a 14-day period. Nin = ninhydrin-positive substances: nos. = log bacterial numbers per gram.*

C. In foil-wrapped meat, ERV dropped from an initial of 30 to 5 over a 6-day period at 15 C, whereas in bag-stored meat over a 9-day period it declined to only 25 at the same temperature. In the case of bag-stored meat held at 7 C, there was no change in ERV values from the initial to the 9th day. It can further be noted from Table 1 that, at 15 C, ERV values increased after the first several days of storage in bags. Just what accounted for this is not yet known. It is seen rather consistently when ground beef is allowed to spoil at temperatures above the refrigerator range. With respect to WHC, the initial free-water area of 1.13 square inches decreased to zero in 5 days in meat stored in foil at 15 C but to only 0.5 and 0.77 square inches in bag-stored meats at, respectively, 15 and 7 C.

Results of the same general type are presented in Fig. 2 where commercial ground beef containing around 20% fat was employed and held at 7 C. In foil-wrapped samples, as bacterial numbers increased from log 6.00 to 9.50 over a 14-day period, ERV declined from 46 to 5.5 over the same period, and free-water area from 0.84 square inches to zero over an 8-day period. With the bag-stored samples, on the other hand, the initial bacterial number per gram of log 6.00 increased to only log 8.32 over the 14-day period, while ERV declined from 46 to only 34 and free-water area from 0.84 to 0.58 square inches over the same period. The growth-retarding effect of bag storage can be seen in Fig. 2 to have been quite evident in prolonging the lag phase to a greater degree than the overall rate of growth compared with foil-stored meat.

Figure 3 presents average data from four different samples of ground beef stored in bags and held in a refrigerator for 14 days. The bacterial number per gram increased from log 6.80 to log 8.40, while the initial averaged ERV of 37 declined only to around 27. The initial averaged area of free water decreased from 0.90 to 0.25 square inches, while ninhydrin-positive substances remained essentially unchanged, showing a rather slight increase over the 14-day period. The latter value has been shown already to decline in beef with the attainment of very large numbers of bacteria and definite spoilage, and its

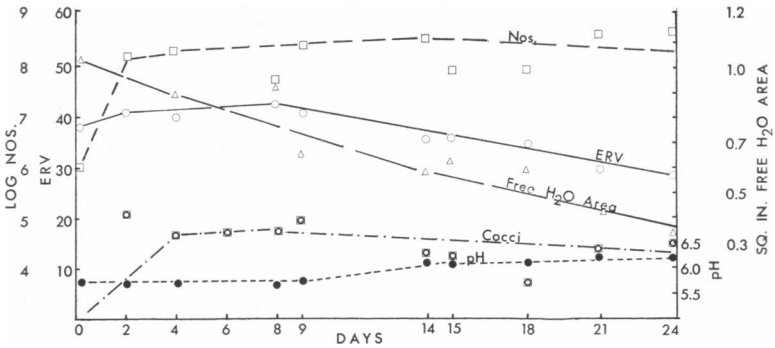

FIG. 4. *Log bacterial numbers; ERV, WHC, cocci, and pH from all-lean ground beef roast stored in plastic bags at 7 C for 24 days.*

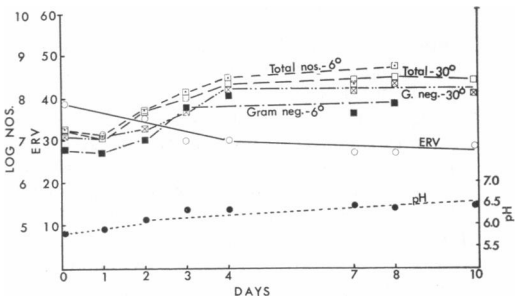

FIG. 5. *Total bacterial numbers and total number per gram of gram-negative bacteria at both 6 and 30 C along with ERV and pH from all-lean ground hindquarter muscles stored in bags at 7 C and held for 10 days.*

slight increase here can be taken to substantiate the relatively small increase in bacterial numbers in bag-stored meats and the lack of frank spoilage.

Data from a sample of beef which was held for 24 days in bags at 7 C are presented in Fig. 4. While bacterial numbers increased from log 6.00 to log 8.78 over the 24-day period, ERV decreased from 38 to only 28, and free-water area, from 1.1 to 0.38 square inches over the same period.

In an effort to determine whether significant changes in bacterial flora occurred in the bag-stored meats, homogenates were surface-plated onto Mannitol Salt Agar and MacConkey Agar (Difco) and incubated at 6 and 30 C. All colonies that developed within 24 hr on Mannitol Salt Agar at 30 C were taken to be cocci. This assumption, however, was not confirmed by microscopic examination. As can be seen in Fig. 4, the number never exceeded 100,000/g over the 24-day period. This is the same pattern displayed by this group in foil-wrapped meats when meat is held at refrigerator temperatures

TABLE 2. *Bacterial numbers from bag-stored meats illustrated in Fig. 1 to 5 and Table 1, corresponding to an ERV of 25, or the lowest above this value over the holding period*

Sample	ERV	Log bacterial no./g	Days of holding
Fig. 1	25	8.5	4
Fig. 2	28	8.4	12
Fig. 3	26	8.4	14
Fig. 4	28	8.5	9
Fig. 5	28	8.4	10
Table 1	30	8.1	9
Mean	27.5	8.4	9.7

(*unpublished data*). Data presented in Fig. 5 show that total numbers developing on tryptone-glucose-yeast extract-agar (Difco) at 6 C were only slightly higher than at 30 C and that the total gram-negative bacterial flora developing on MacConkey Agar was a little higher at 30 C than at 6 C. These findings can be interpreted to mean that the predominant bacterial flora which developed in bag-stored ground beef consisted of gram-negative, facultatively psychrophilic organisms. This finding and conclusion tend to substantiate the report of Ogilvy and Ayres (10), who showed that slime-forming bacteria proliferated on cut-up chicken stored under certain levels of carbon dioxide even though higher levels of carbon dioxide suppressed these types. The organisms in question were reported by these authors to be pseudomonads and *Alcaligenes*, two groups of gram-negative bacteria common in ground beef. The difference between the total counts and gram-negative counts in Fig. 5 is most likely accounted for by lactic acid bacteria which were found by Jaye et al. (8) to be an important group of gram-positive bacteria in beef stored under these conditions. Other gram-

positive groups such as micrococci and aerobic sporeformers do not grow well at the temperatures employed. The beef employed in Fig. 5 consisted of all-lean hindquarter portions, and was held at 7 C. The decrease in ERV was from 39 to 28 over the 10-day period while the bacterial counts increased approximately 1.5 log cycles. As can be seen from both Fig. 4 and 5, only a relatively slight increase of *p*H occurred during the holding period.

To determine whether the plastic bags contained antibacterial substances, broth cultures of *Proteus vulgaris* were grown in sealed bags and in ordinary culture tubes. No difference in lag or log phases of growth was found, thus ruling out specific antibacterial action of the bag material.

To determine whether conditions within bags containing 26-g samples of meat became anaerobic over varying periods of time, tryptone-glucose-yeast extract-agar slants were streaked with *Clostridium perfringens* and *Bacillus subtilis*, and placed separately and without cotton plugs in bags containing ground beef. No growth of *C. perfringens* occurred, while growth of *B. subtilis* did occur, indicating that the inner environment of bags with meat was aerobic. In addition, reduced tubes of methylene blue failed to remain in the reduced state when added to test tubes and placed in bags with meat as above. This finding indicated that delayed spoilage of bag-stored meats was probably not the result of inhibition of growth of aerobic bacteria.

Previous reports from this laboratory on the use of ERV as a rapid method of detecting spoilage in beef have shown that an ERV value of around 24 to 25 corresponded to an averaged log number per gram of around 8.5 bacteria in ground beef with 20% fat, or less. When ERV values from bag-stored meats of 25 or the lowest above this value from Fig. 1 to 5 and Table 1 are associated with corresponding bacterial numbers on the given days, the average is found to be 27.5, corresponding to a log bacterial number of 8.4/g (Table 2). In all cases except Fig. 1, ERV did not fall as low as 25, in which case the lowest above this value was selected. The average holding time corresponding to these values was 9.7 days for these bag-stored meats compared with 4.1 days for 40 different samples of ground beef allowed to spoil under the same conditions as controls in the present study (6). It might be speculated that, had the bag-stored meats been held long enough to produce ERV values of 25, the bacterial numbers would accordingly have increased more in line with the values for fresh ground beef allowed to spoil in foil.

On the basis of the foregoing findings, it seems clear that ERV and WHC do indeed respond to microbial numbers or associated changes in beef, or both, and not to aging per se.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. AYRES, J. C. 1951. Some bacteriological aspects of spoilage of self-service meats. Iowa State Coll. J. Sci. **26**:31–48.
2. CLAUSS, W. E., C. O. BALL, AND E. F. STIER. 1957. Factors affecting quality of prepackaged meat. I. Physical and organoleptic tests. C. Organoleptic and miscellaneous physical characteristics of product. Food Technol. **11**:363–373.
3. HALLECK, F. E., C. O. BALL, AND E. F. STIER. 1958. Factors affecting quality of prepackaged meat. IV. Microbiological studies. B. Effect of package characteristics and of atmospheric pressure in package upon bacterial flora of meat. Food Technol. **12**:301–306.
4. HAMM, R. 1960. Biochemistry of meat hydration. Advan. Food Res. **10**:355–463.
5. JAY, J. M. 1964. Release of aqueous extracts by beef homogenates, and factors affecting release volume. Food Technol. **18**:1633–1636.
6. JAY, J. M. 1964. Beef microbial quality determined by extract-release volume (ERV). Food Technol. **18**:1637–1641.
7. JAY, J. M. 1965. Relationship between water-holding capacity of meats and microbial quality. Appl. Microbiol. **13**:120–121.
8. JAYE, M., R. S. KITTAKA, AND Z. J. ORDAL. 1962. The effect of temperature and packaging material on the storage life and bacterial flora of ground beef. Food Technol. **16**:95–98.
9. KRAFT, A. A., AND J. C. AYRES. 1952. Post-mortem changes in stored meats. IV. Effect of packaging materials on keeping quality of self-service meats. Food Technol. **6**:8–12.
10. OGILVY, W. S., AND J. C. AYRES. 1951. Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol. **5**:97–102.
11. WIERBICKI, E., AND F. E. DEATHERAGE. 1958. Determination of water-holding capacity of fresh meats. J. Agr. Food Chem. **6**:387–392.