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EVALUATION OF THE EXTRACT RELEASE VOLUME PHENOMENON FOR THE DETECTION OF BACTERIAL SPOILLAGE OF MEATS

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Introduction

The usual method for determining the bacteriological quality of meats involves the estimation of viable bacteria present in a sample by plate counting. These tests are time-consuming because at least 2 or 3 days are required for incubation. Since fresh meats have a relatively short shelf life, results obtained are usually of limited value. Jay (1) has reviewed various techniques which have been proposed for the estimation or determination of spoilage or proteolysis in meats. Most methods are based directly on bacterial content or on various biochemical changes brought about by the presence of microorganisms in meats, and are impractical as routine procedures because of complexity or lack of reliability and reproducibility.

Jay[2,3] has suggested that the extract release volume phenomenon (ERV) and the water-holding capacity (WHC) of meats can be used as simple, reliable, and rapid methods for determining microbial quality of meats.

The test is based on the observation that beef samples with high bacterial counts tend to produce viscous homogenates with water. When viscous homogenates are filtered, less fluid is released in a given period than from the less viscous homogenates, presumably obtained from meat samples with low bacteria counts.

E.R.V. was chosen in this study because it requires simple laboratory equipment, uses a large sample size (25 g) which is likely to be more representative than the smaller sample (0.5 g) used in the WHC test, and is said to give more reproducible results. If effective, it would therefore be prefered as a routine test for the detection of spoilage in meat.

It is conceivable that factors other than bacterial spoilage brought about by high bacterial counts may influence ERV. Jay (4) has shown that proteases will decrease the ERV of beef and that a rise in pH and a decrease in ERV normally occurs with bacterial spoilage. However, Jay (2,5) noted that increases in pH values observed as meats undergo spoilage are not great enough to account for the increases in WHC or the decrease in ERV which are normally associated with spoilage. While it is not known exactly, what causes increased water retention by meats as spoilage occurs, Jay (1,2) has clearly shown that numbers of bacterial cells per se do not result in greater water retention of meats. However, as shown in this paper, the effect of pH is more important than has previously been anticipated.

The increase in pH normally associated with spoilage of meats led us to believe that only spoilage which was largely putrefactive might be detected by the test and that ERV might not reflect spoilage due to a mixed bacterial population which included a large proportion of lactic acid bacteria.

The effect of packaging materials and storage conditions on the composition of the bacterial flora of ground beef has been illustrated by Jaye et al (6). It is clear that the type of spoilage which occurs is greatly influenced by the selective effect of the package environment on the bacterial flora. The following experiments were conducted to determine whether several types of spoilage induced by various conditions of packaging and storage can be detected by the ERV test.

MATERIALS AND METHODS

(a) Two lean beef rib cuts were obtained. One of the samples had been stored at 0°F under vacuum for 40 days and was wrapped in an air-impermeable Cryovac bag. The other sample was overwrapped with a permeable polyethylene film and had been stored at 0°F for about 3 months. The meat was ground through a 1/8" plate. Samples of 90 or 95 grams were packaged in aluminum foil and in vacuum-sealed Cryovac pouches. The samples were then stored at 7°C and 15°C.

(b) Lean pork muscle was selected to obtain separate samples of light and dark pigmented pork exhibiting low and high initial pH's respectively. These were ground and wrapped individually in aluminum foil and in vacuum-sealed Cryovac pouches. The samples were then stored at 7°C.

Samples were removed from storage periodically. After equilibrating at room temperature the ERV, pH, and bacterial numbers were determined as follows.

The ERV was determined by the method of Jay (4) using distilled water as the extractant. A 25 gram sample of meat was homogenized with 100 ml of distilled water for 2 minutes in a Waring blendor at 15,000 rpm. The homogenate was poured into a glass funnel equipped with a Whatman No. 1 filter paper. The extract collected in a 100 ml cylinder after 15 minutes at room temperature was recorded as the ERV. Duplicate determinations were averaged.

The pH was determined using a Beckman model N pH meter at room temperature on the 1:10 meat-distilled water homogenate prepared for bacteriological examination.

Bacterial counts were determined by the pour plate procedure and plates were incubated at room temperature (24 to 26°C) for three days using media as stated below:

a) Total bacterial count was obtained on Tryptone Glucose Yeast Extract Agar (Difco).

b) Lactic acid bacteria were estimated on LBS Agar obtained from the Baltimore Biological Laboratory, Inc.

c) Pseudomonads were determined by the method described by Silliker et al. (7) on King's Medium prepared in our laboratory. Plates were examined under ultraviolet light (General Electric Blacklight - F15T8BLB).

A determination of total bacteria counts was made to judge the overall bacteriological quality of the meat, whereas lactic acid bacteria were determined to see whether spoilage of meat occuring in vacuum packages with a dominant lactic acid flora, would result in different ERV changes than meats undergoing proteolytic spoilage (indicated by increasing pseudomonad counts) as observed in ground meats stored in aluminum foil.

Organoleptic acceptability of raw ground meat was judged by three laboratory technicians on the basis of odour and tactile response.

RESULTS AND DISCUSSION

1) Ground Beef Prepared From a Frozen Rib Cut

Jaye et al. (6) have shown that ground meats spoil due to the development of a mixed bacterial culture, regardless of the type of packaging. However, the type of packaging has an influence on the types of bacteria which become dominant and thus influence the spoilage pattern. Tabel I and Figs. 1, 2,3, and 4 show the changes in total, lactic acid and fluorescent pseudomonad counts obtained when ground beef was stored at 7 and 15°C both aerobically in aluminum foil and anaerobically in air-impermeable pouches under vacuum.

It is apparent from a comparison of Figs. 1 and 3 and of Figs. 2 and 4 that storage temperature had little influence on the composition of the developing spoilage flora; but that, in vacuumpacked ground meats more fluorescent pseudomonads were evident at 7°C (Fig. 2) than at 15°C (Fig. 4). This phenomenon, may be due to the psychrophilic tendency of pseudomonads which would give them a competitive advantage at the lower storage temperature.

TABLE I

THE INFLUENCE OF PACKAGING AND STORAGE OF GROUND BEEF ON E.R.V., pH, AND BACTERIAL FLORA

Storage		Type of Packaging Material									
		Aluminum Foil					Cryovac - Vacuum				
Temp.	Days	Log Bacterial Count Per Gram					Log Bacterial Count per Gram				
		E.R.V. (ml.)	pH	Total	Lactics	Pseudo-monads	E.R.V. (ml.)	pH	Total	Lactics	Pseudo-monads
7°C	0	47.5	5.90	6.26	2.52	3.83	47.5	5.90	6.26	2.52	3.83
	2	50.0	5.90	6.78	5.95	3.95	49.5	5.85	6.34	4.22	5.00
	6	38.0	6.10	9.22	8.54	8.10	43.5	5.70	8.38	6.58	3.30
	9	22.0	5.90	8.94	6.76	7.48	42.0	5.60	8.48	7.23	3.48
	12	38.5	6.30	10.70	7.48	8.30	50.0	5.65	8.48	7.26	5.00
	14	29.5	6.25	9.23	7.39	7.30	50.0	5.75	8.49	7.44	5.00
	16	4.5	6.70	9.70	7.16	8.04	51.0	5.70	8.06	7.18	3.70
	19	11.5	6.95	9.69	7.19	7.81	53.5	5.70	8.25	7.64	3.85
15°C	2	44.5	6.25	9.07	6.67	7.85	49.5	6.00	8.65	6.62	2.70
	6	27.0	6.15	9.70	7.54	7.30	47.5	5.70	7.55	7.10	4.00
	9	33.0	6.25	9.23	8.19	7.60	49.0	5.60	8.49	7.84	< 1.00
	12	10.5	6.55	9.45	7.88	7.65	49.0	5.70	8.06	7.54	3.00
	15	3.5	7.05	10.19	8.18	8.11	43.5	5.90	8.62	7.88	< 1.00

Ground beef stored in aluminum foil (Fig. 1 and 3) spoiled proteolytically and was accompanied by a considerable increase in the fluorescent pseudomonad count, an increase in pH of approximately 1 unit and a considerable decrease in ERV as would be expected from the work of Jay.

In the vacuum-packed ground beef samples (Fig.2 and 4), there was no apparent increase in pseudomonads. However, lactic acid and total bacteria counts increased similarly to the aluminum foil wrapped meat. Instead of the increase in pH noted in the aerobically wrapped beef, the pH of the vacuum-packed sample did not change and ERV did not show that spoilage had occurred. In this case there appears to be no correlation between ERV and spoilage.

2) GROUND BEEF PREPARED FROM A RIB CUT WHICH HAD BEEN STORED UNDER VACUUM/

Table II and Fig. 5 and 6 illustrate that ERV will not reflect spoilage of ground meat from a rib cut which had been stored under vacuum in an air-impermeable pouch regardless of the type of packaging material. This was true at both 7°C and 15°C. This may be explained by the fact that lactic acid bacteria have become dominant while the rib cut was initially stored under vacuum. The observed change in pH is $< \frac{1}{2}$ of a pH unit and ERV does not reflect spoilage.

3) CHANGES IN ERV, pH AND TOTAL BACTERIA COUNTS OF DARK AND LIGHT PORK MUSCLE WHEN STORED AEROBICALLY AND ANAEROBICALLY/

In Fig. 7 and 8 it can be seen that samples of light and dark pork muscle with initial pH values of 6.2 and 6.7 respectively had relatively similar total bacteria counts (log 7.36 and 7.16). However, the initial ERV values were very different (29.0 and 16.7 ml respectively). Aerobic storage of both samples in aluminum foil (Fig. 7) resulted in an increase in pH and a decrease in ERV. Total bacteria counts for both samples increased similarly. Thus when dark and light pork muscle were stored aerobically, the ERV decrease reflected spoilage. Fluorescent pseudomonads (Tabel III) were observed to increase during storage. However, when light and dark pork muscle were stored under vacuum in air-impermeable pouches, (Fig. 8) although total bacteria counts increased; the pH value of the meat decreased slightly at first and then increased almost to its initial value.

Lactic acid bacteria were dominant in this case (Table III) and the change in pH was not sufficient to be reflected in a change in ERV. Samples became organoleptically unacceptable without a decrease in ERV.

TABLE II

THE INFLUENCE OF PACKAGING AND STORAGE OF GROUND BEEF ON E.R.V.
/ pH, AND BACTERIAL FLORA/

Storage Temperature	Days	Type of Packaging Material									
		Aluminum Foil					Cryovac-Vacuum				
				Log Bacterial Count Per Gram					Log Bacterial Count per Gram		
		ERV	pH	Total	Lactics	Pseudo-monads	ERV	pH	Total	Lactics	Pseudo-monads
7°C	0	38.5	5.80	6.99	5.31	3.74	38.5	5.80	6.99	5.31	3.74
	2	47.0	5.70	7.10	6.72	4.15	48.0	5.60	7.85	6.81	3.74
	3	47.5	5.65	7.73	6.68	3.18	54.5	5.52	7.78	6.95	3.74
	6	52.0	5.60	8.32	7.02	5.90	52.0	5.40	7.60	7.87	0
	8	44.3	5.75	8.78	7.33	6.74	54.0	5.70	8.04	7.14	4.48
	10	51.5	6.0	9.15	7.35	7.60	57.5	5.50	7.78	7.16	4.70
	13	50.0	6.0	8.95	7.65	7.18	58.5	5.55	8.11	7.54	5.00
15°C	0	38.5	5.80	6.99	5.31	3.74	38.5	5.80	6.99	5.31	3.74
	2	49.0	5.55	8.43	7.90	5.85	52.0	5.40	8.44	7.70	0
	3	51.5	5.55	8.00	7.54	6.22	59.0	5.55	8.25	7.74	0
	6	51.0	5.65	8.72	8.00	5.18	56.0	5.50	7.48	7.81	0
	8	-	5.8	8.22	7.98	0	49.0	5.70	8.22	7.98	0
	10	-	-	-	-	-	53.5	5.60	8.18	7.85	3.00
	13	-	-	-	-	-	55.5	5.90	8.40	7.88	0

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TABLE III

THE INFLUENCE OF PACKAGING OF LIGHT AND DARK PIGMENTED PORK ON THE E.R.V., pH AND BACTERIAL FLORA

Storage Temp.	Days	ALUMINUM FOIL									
		Light					Dark				
		ERV	pH	Total	Pseudo-monads	Lactics	ERV	pH	Total	Pseudo-monads	Lactics
7°C	0	29.0	6.2	7.36	5.3	3.54	16.5	6.7	7.16	5.18	3.48
	1	26.0	6.1	7.98	5.18	5.18	15.0	6.5	7.78	5.18	4.75
	4	25.5	6.2	9.01	5.60	6.74	14.5	6.55	7.44	7.48	5.70
	6	12.5	6.6	9.42	7.30	6.79	12.0	6.65	9.41	7.40	6.30
	8	14.0	6.65	9.40	7.30	7.20	7.0	6.75	9.34	7.54	6.40
	11	1.0	7.05	9.85	8.04	7.25	2.0	7.25	9.28	5.00	6.63
		CRYOVAC- VACUUM									
		Light					Dark				
		ERV	pH	Total	Pseudo-monads	Lactics	ERV	pH	Total	Pseudo-monads	Lactics
7°C	0	29.0	6.2	7.36	5.30	3.54	16.5	6.7	7.16	5.18	3.48
	1	27.5	6.1	7.93	4.70	5.10	18.5	6.4	7.78	3.95	5.16
	4	33.0	6.05	8.65	< 3.00	7.31	23.5	6.25	8.84	< 1.00	6.18
	6	31.0	6.15	8.45	4.18	7.02	25.5	6.35	8.32	3.00	7.00
	8	31.5	6.25	8.56	3.60	7.80	26.0	6.40	8.78	3.70	7.04
	11	30.5	6.25	8.82	3.18	7.88	24.0	6.25	8.69	3.60	7.30

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The relationship between ERV and pH is further illustrated in Table IV. Pork samples with relatively similar bacterial content were selected by color. Dark pork samples with high pH values (7.0 and 6.7 respectively) were found to have low ERV values (4.3 and 16.7 ml respectively) and thus would have been presumed to have spoiled according to the test suggested by Jay. Light coloured pork samples with low pH values (6.2 and 5.8 respectively) were found to have high ERV values (29.0 and 53.0 ml respectively).

TABLE IV

THE EFFECT OF THE INITIAL, NATURALLY ATTAINED pH ON ERV OF PORCINE MUSCLE WITH SIMILAR INITIAL TOTAL BACTERIA CONTENTS.

Color of Porcine Muscle	pH	ERV	Log of Total Bacteria per Gram
Light	6.2	29.0	7.36
	5.8	53.0	6.30
Dark	7.0	4.3	7.19
	6.7	16.5	7.16

In summary it may be stated that ERV appeared to be influenced by pH and that an increase in pH was always reflected by a decrease in ERV. However, if spoilage occurred in the presence of a dominant lactic acid bacteria flora no increase in pH was observed and ERV did not reflect spoilage of meats. Further, naturally high pH meat would automatically be classified by the proposed ERV test as spoiled, even in the complete absence of microbial growth. It can therefore be concluded that the ERV phenomenon cannot be regarded as a reliable test for the rapid detection of bacterial spoilage of meats.

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SUMMARY

The extract-release volume (ERV) phenomenon was evaluated as a means of detecting microbial spoilage of meats of bovine and porcine origin. The results obtained demonstrate that this phenomenon, in ground beef and pork, is influenced by the type of microbial flora and the concomitant changes in pH associated with different storage and packaging conditions. Ground beef prepared from a rib cut spoiled due to the development of a mixed bacterial flora, resulting in an increase in pH and a decrease in the ERV when stored in aluminum foil. Ground beef stored under vacuum spoiled due to the growth of a bacterial flora which consisted predomantly of lactic acid bacteria. The pH of vacuum-packed ground beef did not change greatly and the ERV did not reflect spoilage. Ground beef prepared from a rib cut which had been stored under vacuum spoiled without showing substantial changes in pH or ERV regardless of whether the meat was packaged aerobically or anaerobically.

The relationship of pH and ERV was further illustrated by the fact that porcine muscle with naturally attained pH values of 6.2 and 6.7 which had similar initial bacterial counts, exhibited very different initial ERV's.

It was shown that ERV may not always reflect bacterial numbers developing in meats during storage and that ground meats may spoil without an appreciable change in ERV. It was concluded that the ERV phenomenon is not a reliable test for the rapid detection of spoilage of meats.

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FIG. 1 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS OF GROUND BEEF WRAPPED IN ALUMINUM FOIL AT 7 °C.

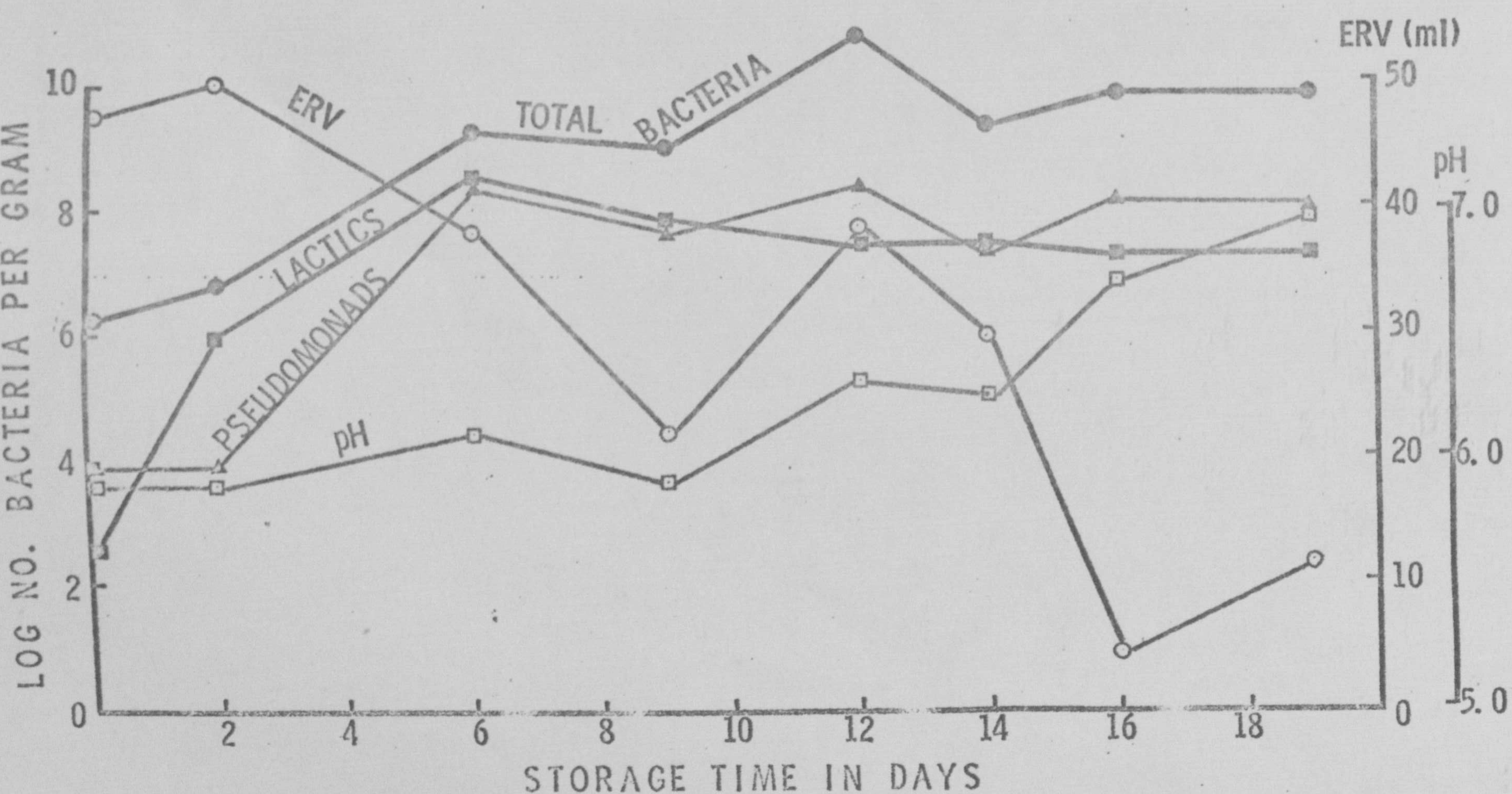

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FIG. 2 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS OF GROUND BEEF VACUUM-PACKED IN CRYOVAC POUCHES AT 7 °C.

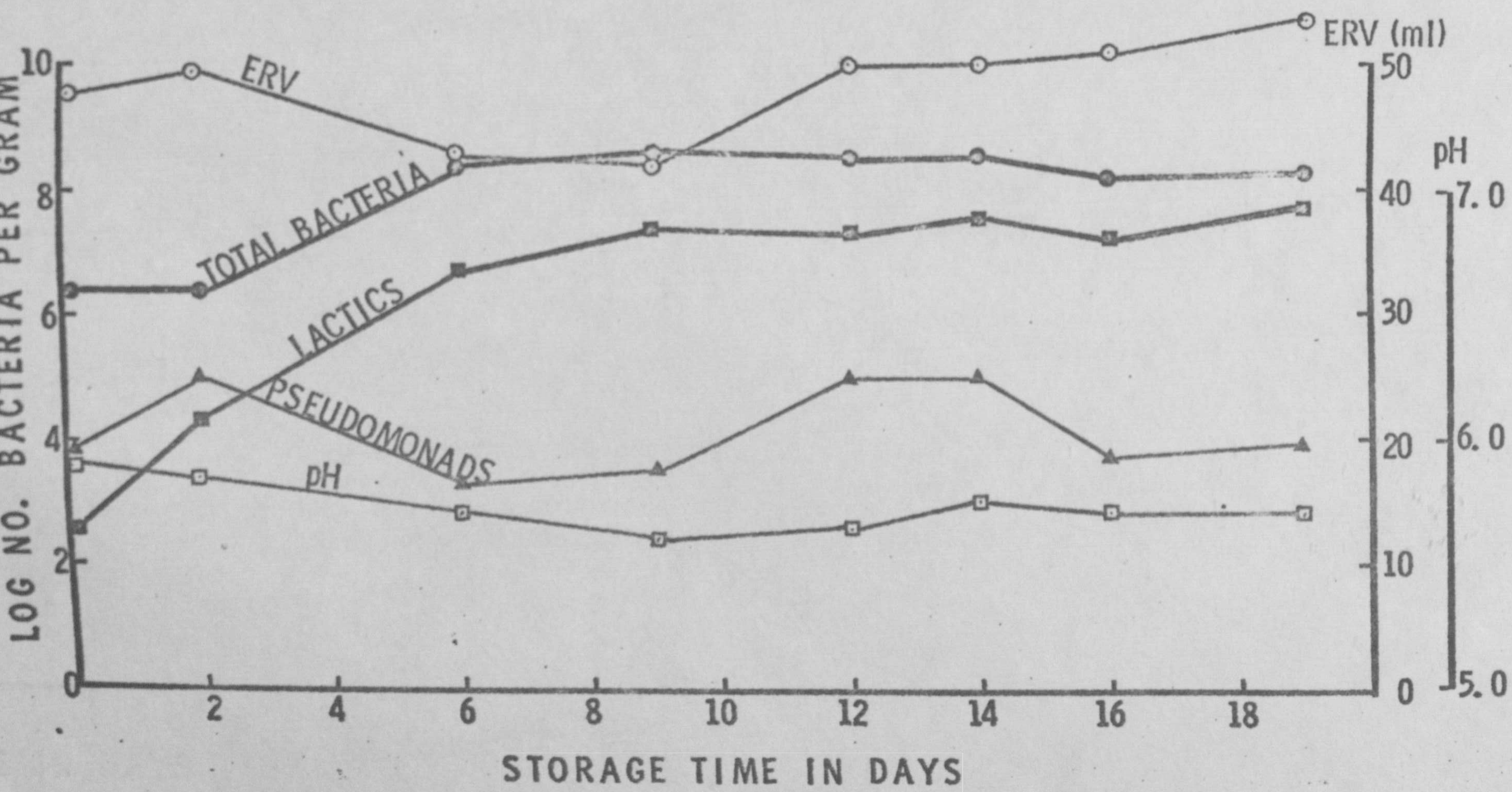

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FIG. 3 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS OF GROUND BEEF WRAPPED IN ALUMINUM FOIL AT 15°C.

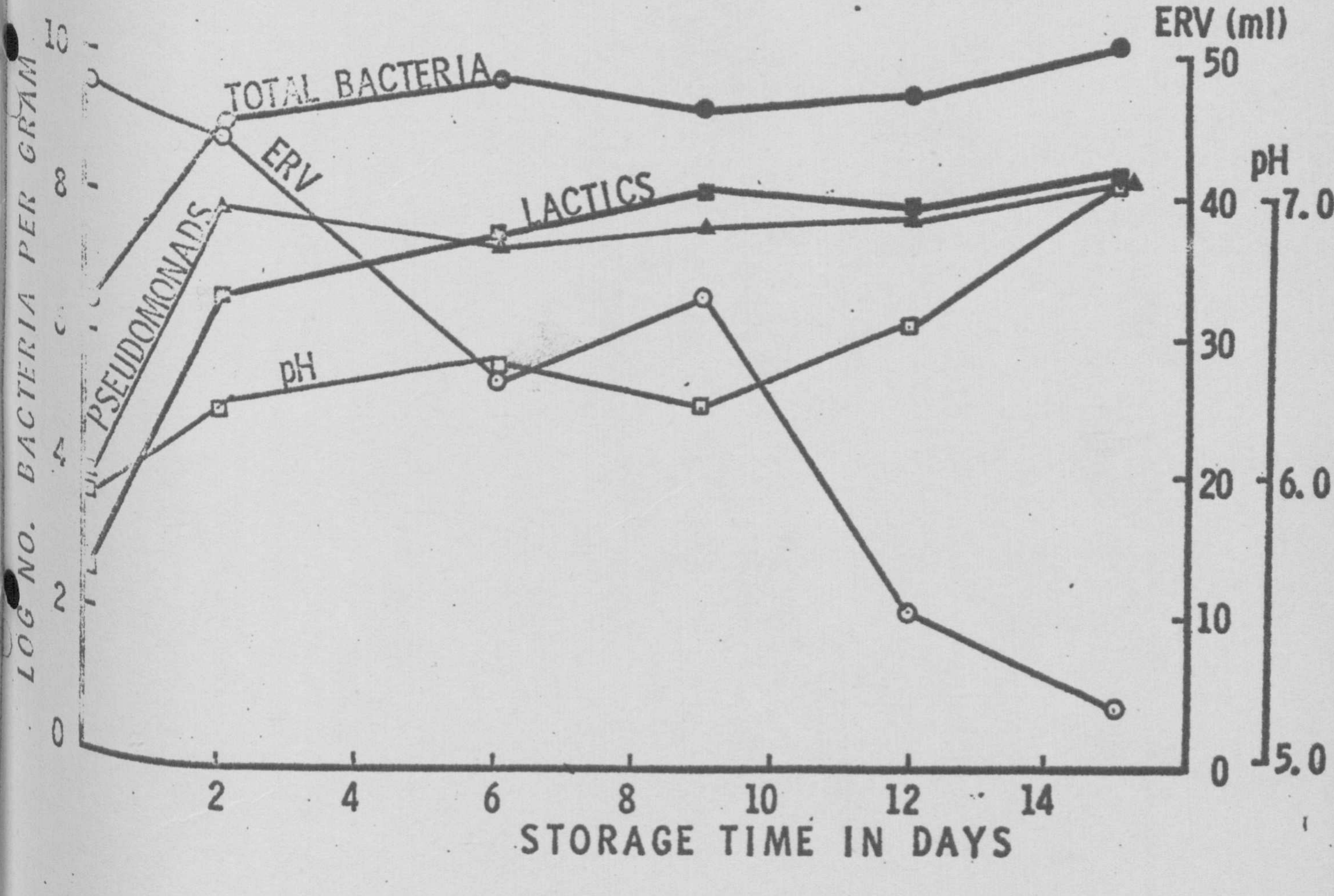

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FIG. 4 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS OF GROUND BEEF VACUUM-PACKED IN CRYOVAC POUCHES AT 15°C.

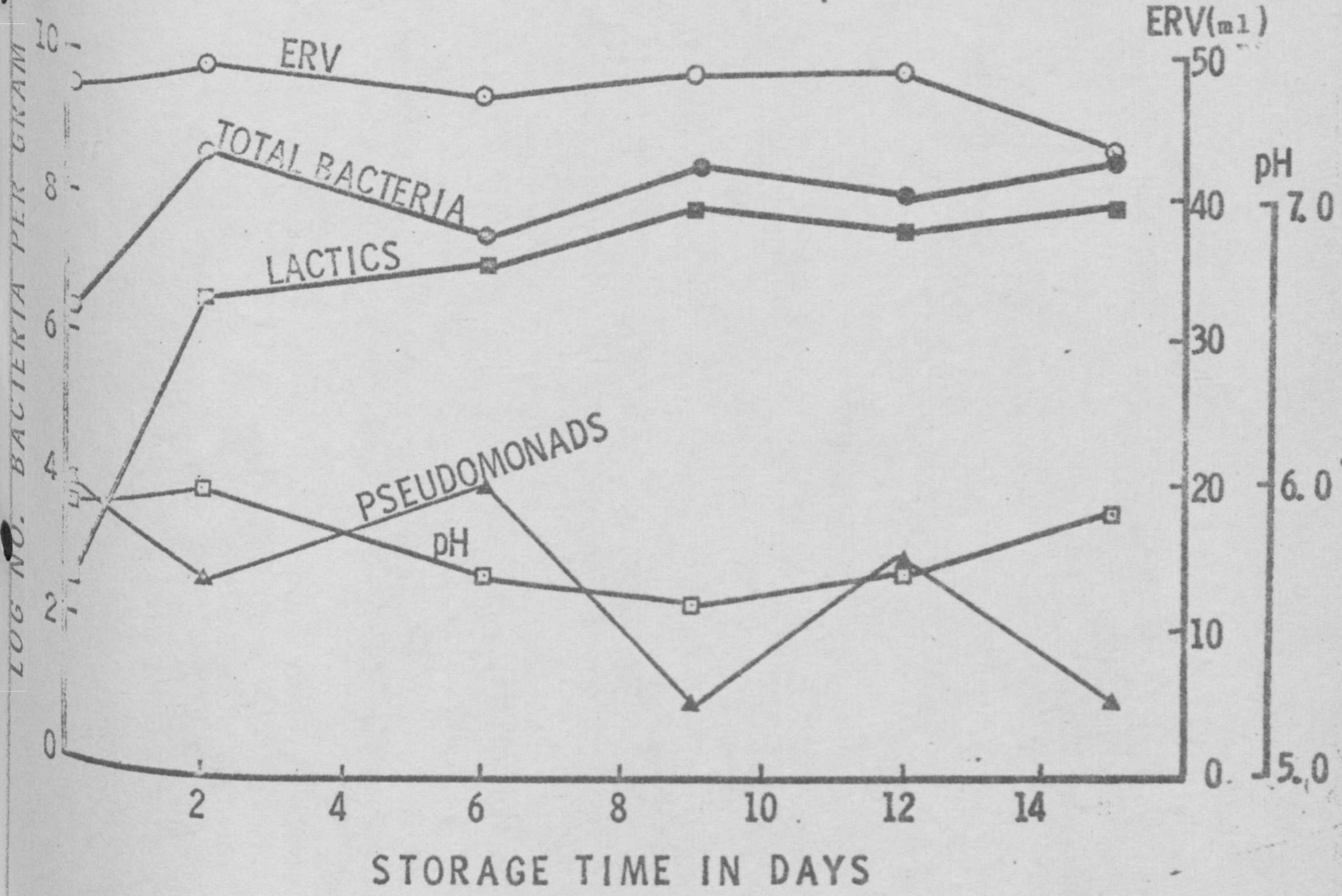

FIG. 5 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA COUNTS OF GROUND BEEF, PREPARED FROM A VACUUM-PACKED RIB CUT, WHEN STORED AT 7°C AND WRAPPED IN ALUMINUM FOIL.

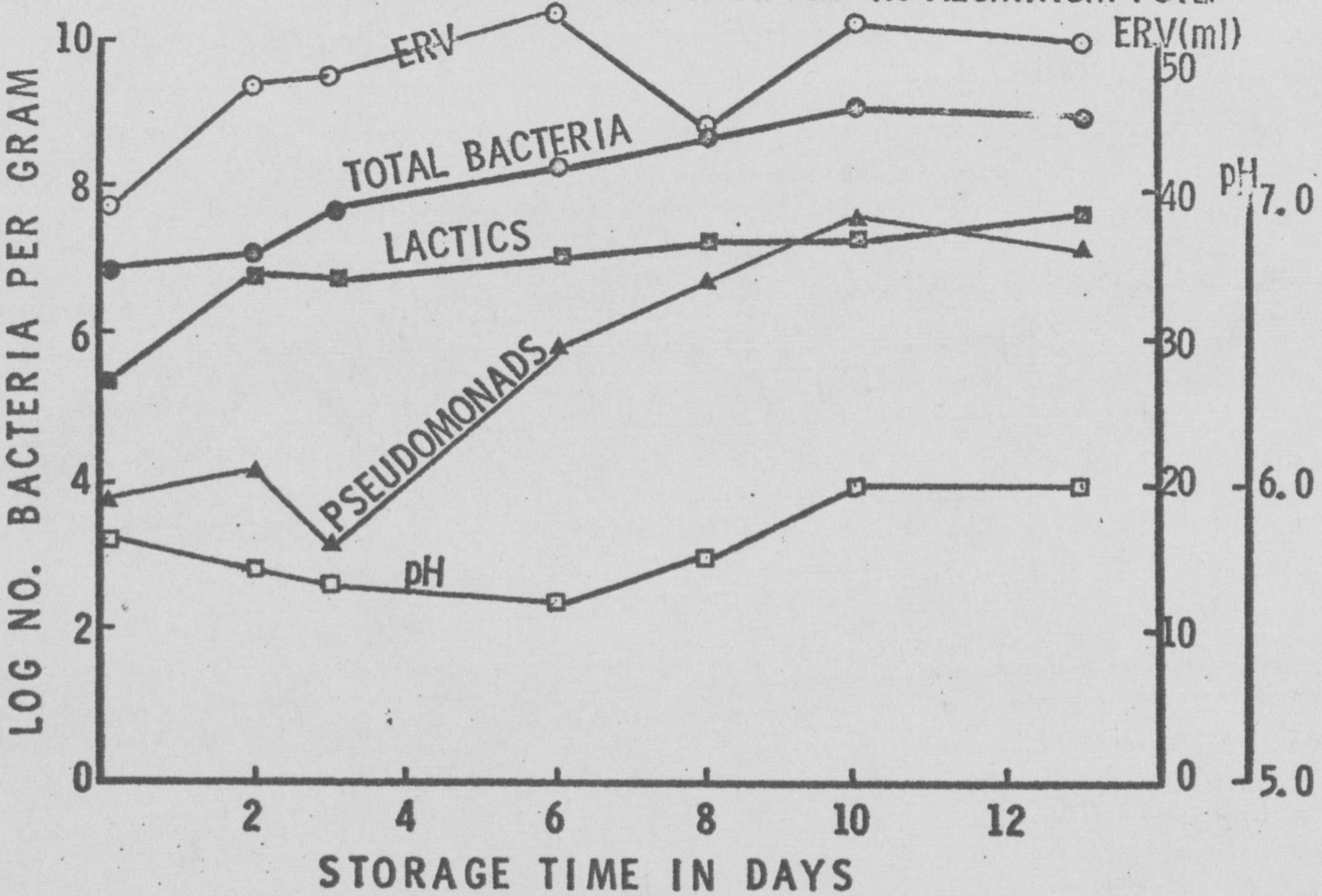

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FIG. 6 - CHANGES IN ERV, pH, TOTAL BACTERIA, PSEUDOMONADS AND LACTIC ACID BACTERIA OF GROUND BEEF, PREPARED FROM A VACUUM-PACKED RIB CUT, WHEN STORED UNDER VACUUM IN CRYOVAC POUCHES AT 7°C.

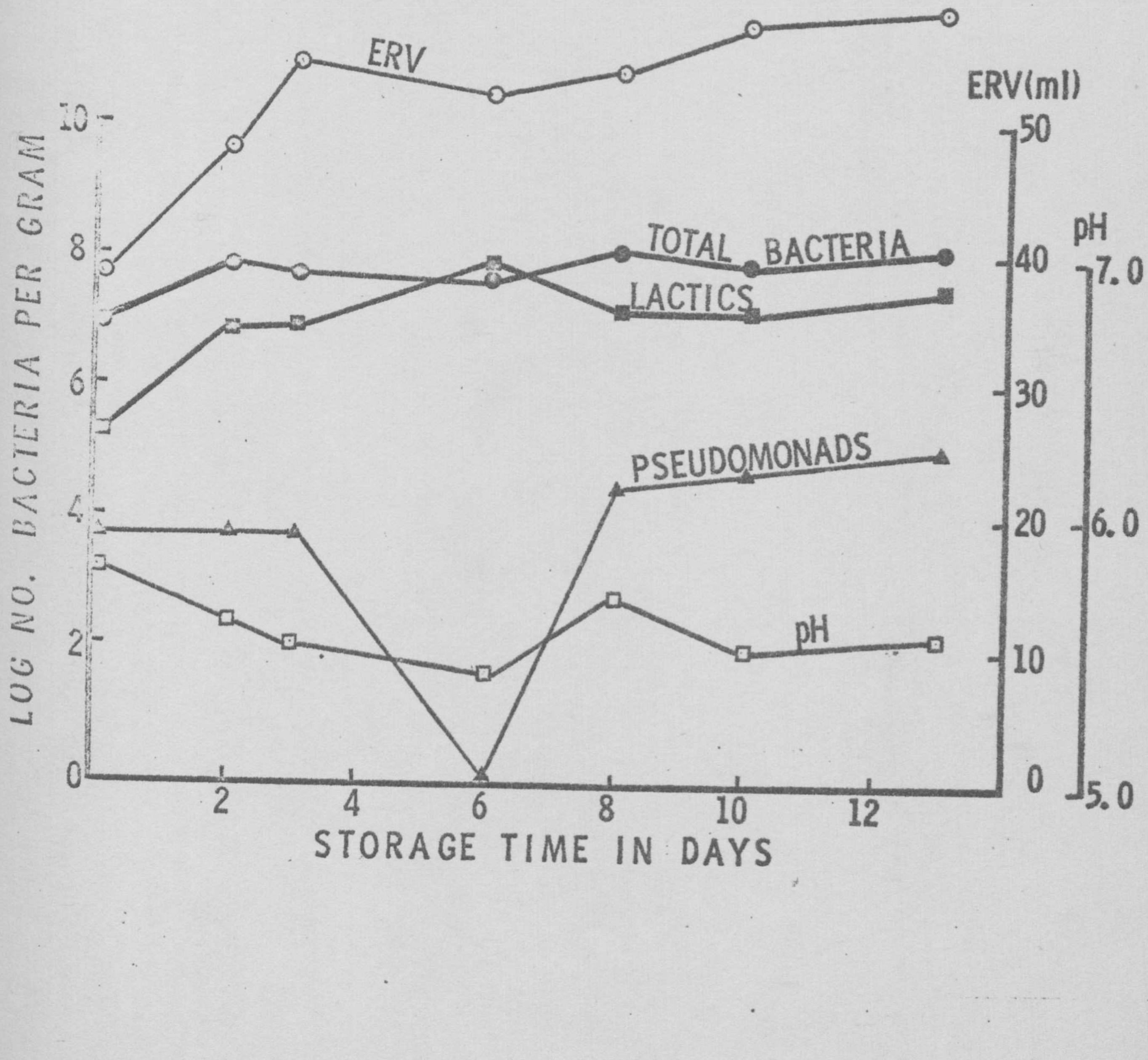

FIG. 7 - A COMPARISON OF CHANGES IN ERV, pH AND TOTAL BACTERIA COUNTS OF GROUND PORK, PREPARED FROM LIGHT AND DARK PORCINE MUSCLE WITH LOW AND HIGH INITIAL pH's RESPECTIVELY, WRAPPED IN ALUMINUM FOIL AT 7°C.

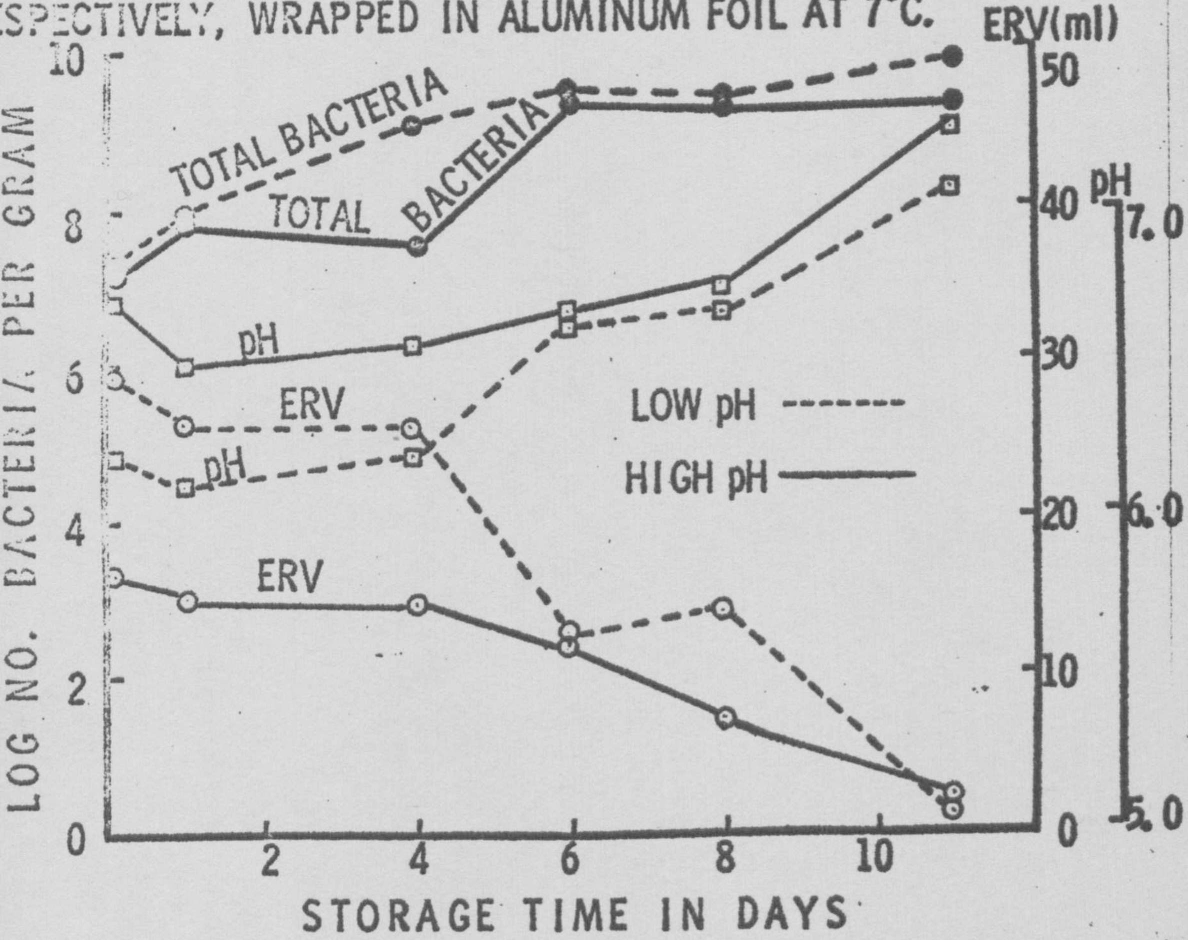

FIG. 8 - A COMPARISON OF CHANGES IN ERV, pH AND TOTAL BACTERIA COUNTS OF GROUND PORK, PREPARED FROM LIGHT AND DARK PORCINE MUSCLE WITH LOW AND HIGH INITIAL pH's RESPECTIVELY, STORED UNDER VACUUM IN CRYOVAC POUCHES AT 7°C.