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TECHNICAL REPORT

FD-23

STUDIES ON MEANS OF CONTROLLING OR INHIBITING BEEF TISSUE ENZYME SYSTEMS

by

W. A. Landmann
AMERICAN MEAT INSTITUTE FOUNDATION
Chicago, Illinois 60637

Contract No. DA 19-129-AMC-87(N)

September 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

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FOREWORD

Microbial spoilage of meat during prolonged storage can be prevented by treatment with ionizing radiation. Inactivation of the natural proteolytic enzymes in meat is not accomplished by the treatment required to produce sterility. During storage radiation sterilized raw meat deteriorates by proteolytic action, becoming progresively softer and finally mushy and acquiring a bitter flavor. The only known effective method for inactivating the natural proteolytic enzymes of meat is a heat treatment equivalent to cooking to medium rare. If radiation sterilized raw meats are to be provided for military use, some means other than heat are required for proteolytic enzyme inactivation.

The work covered by this report was performed by the American Meat Institute Foundation under Contract No. DA 19-129-AMC-87(N) during the period from May 1963 to May 1964. It represents an investigation of the effects of controlled pH combined with radiation treatment on the rate of proteolysis in beef muscle tissue. The investigator was W.A. Landmann. His collaborators were D. McIntosh, M.C. Worland, L.H. Harbers and R.I. Morrow.

The U. S. Army Natick Laboratories Project Officer was A.S. Henick, and the Alternate Project Officer was E. Wierbicki, both of the Food Division.

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ABSTRACT

Control or inhibition of proteolysis in meat as influenced by pH has been investigated over the pH range of 4.0 to 8.0. In non-irradiated meat inhibition has been achieved only when the pH of the meat is 4.0 to 4.5 and 6.8 to 7.0. In both cases the inhibition is slight. In Irradiated meat varying degrees of inhibition of proteolysis occur throughout the entire range studied. Maximum hydrolysis occurs in irradiated beef at pH 5.6 and in untreated controls. Inhibition in significant amount to be of some practical use occurs only at pH 4.0 to 4.5 in irradiated meat. Some indication has been obtained that combined effects of treatment with buffers and radiation may influence the inactivation of enzyme systems more profoundly than either factor alone. It is recommended that further studies be directed to examining at various dose levels of radiation meat samples treated with varying concentrations of buffer at a given pH - either 4.0 to 4.5 or 7.0 to 8.0.

The injection of buffers into large pieces of meat was studied to evaluate the method of stitch-pumping. Some difficulty was encountered in introducing enough buffer to maintain the pH of the meat at that of the buffer. However, at the pH which was achieved for each sample, the same hydrolysis pattern was obtained as was indicated by the ground meat samples. It was concluded that enough buffer can be introduced to be effective in altering hydrolysis but that the problems of achieving a desired pH remains. This may be solved by using more concentrated buffers along with more efficient injection and diffusion methods.

Ante-mortem control of pH was investigated in rabbit muscle by injection of epinephrine before slaughter. The pH was very effectively controlled in the meat from treated animals throughout the storage period at approximately pH 7. The inhibitory effect of the treatment on proteolysis was more pronounced in non-irradiated meat, although some inhibition was noted in irradiated meat, as well. The amount of inhibition was about the same as was observed for pH 7.0 buffered meat. In both cases the inhibition was considerably less than in irradiated meat at pH 4.0 - 4.5.

INTRODUCTION

Previous work[1/] had shown that the most effective way of inhibiting proteolytic enzymes in irradiated meat was through pH control. Acidic samples, pH 4.0-5.0, appeared to be most stable with respect to proteolytic breakdown on prolonged storage, although there was some evidence that samples with a pH of near neutrality were also hydrolyzed to a smaller extent than the corresponding controls.

As the first step in this study, the pH range of 4.0-8.0 was investigated, to establish an optimum pH for inhibition of proteolysis. Studies were initiated to examine the ability of various buffers to maintain the pH of meat at intervals of approximately 0.5 pH unit throughout the desired range. Two conditions were imposed: (1) The buffers must be effective when used in the ratio of 2 parts meat to 1 part buffer, and (2) The buffers must be non-toxic.

The buffers chosen for trial were prepared according to directions in "Data for Biochemical Research." [2/]

Fresh round steak was trimmed of all visible fats, ground through a 1/8-inch plate, and 4 grams mixed with 2.0 ml of buffer solution in a high speed mixer (Omni-Mix). The pH of the resulting slurry was measured with a pH meter immediately after mixing, and after 1 day's storage in a refrigerator. The pH values were stable and were as shown in Table 1.

The capacities of the buffers to control the pH of the meat to the desired levels were not predictable, as shown by the resulting pH of the meat-buffer mixtures. Repeated trials with meat samples show-

1/ Final Report, Contract DA 19-129-QM-1952, File #S-582, July 3, 1963.

2/ Dawson, R.M.C., et al, Editors,
Data for Biochemical Research, Oxford, Clarendon Press, 1959
pp. 192 - 209

ed that, while some pH values of the meat-buffer mixtures were far from the pH value of the buffer used, the final pH's were reproducible. However, none of the buffers was able to change the pH of the meat to 4.0 or to 8.0. In order to achieve these pH values, it was necessary to titrate the meat with acetic acid or sodium carbonate. It was found that 1.0 ml of 0.01 M acetic acid would be sufficient to bring the pH of 2.0 grams of meat to a pH of 4.0. In like manner, 1.0 ml of 0.1 M Na_2CO_3 solution was found to bring the pH of 2.0 g meat to 8.0.

The following treatments were used to prepare samples for irradiation and storage:

	Treatment	Final pH
Group I.		
	H_2O control	5.6
	Acetic Acid, 0.01 M	4.0
	Citric Acid - Sodium Citrate, pH 3.0	4.5
	Citric Acid - Sodium Citrate, pH 4.0	5.2
	Citric Acid - Sodium Citrate, pH 5.0	5.6
Group II.		
	NaH_2PO_4 - Citric Acid, pH 6.0	6.0
	Na_2CO_3 - $NaHCO_3$, pH 9.0	6.5
	Na_2HPO_4 - NaH_2PO_4, pH 7.6	6.8
	Na_2CO_3, 0.1 M	8.0
	H_2O control	5.6

Approximately 2500 g of round steak trimmed of visible fat were ground through a 1/8 inch plate and divided into 10 portions of 250 g each. 125 ml of water or buffer were added, the samples mixed well, and placed into 16 glass vials. Two of the 10 large portions were treated with water to serve as controls. Eight vials of each 16 in a treatment group were selected at random to be irradiated, and the other eight were reserved for storage without irradiation. All samples were frozen. Those to be irradiated were allowed to come to 0-5° for irradiation, and were held at this temperature after irradiation. The non-irradiated samples were allowed to come to 0-5° on the day the irradiated samples were received from the facility, and were stored at this temperature for the remainder of the 12-week period.

A third set of samples was prepared to study the effectiveness of controlling the pH of meat by ante-mortem treatment with epinephrine. The autolytic activity of meat slurry has been found to be minimal at pH of 6.8 - 7.4, essentially the pH of living muscle. Ante-

(continued)

mortem treatment with adrenalin has been used to maintain a high pH in meat. In the absence of other non-enzymatic effects the effect of treatment with epinephrine should be one of inhibition of proteolysis.

Rabbits weighing ca. 3 kg each were treated with epinephrine by injecting intraperitoneally a saline solution containing 1 mg epinephrine/ml. The dose level administered was 1mg/kg. After five hours the rabbits were slaughtered, bled, and held at 45°F. for 16 hrs. A control animal receiving only saline was also slaughtered at the same time.

The thigh muscles of the animals were removed, ground through a 1/8 inch plate, mixed, divided into individual sample portions, and frozen. One-half of the samples were randomly selected for irradiation and the other half were kept frozen until the irradiated samples were returned from the radiation facility.

A fourth group of samples was prepared to investigate the practicability of controlling pH by injecting buffers into meat. Beef rump roasts were cut into sections large enough to be later further divided into 16 cubes approximately 1½ inches on each side. Four large sections were stitch-pumped with water, pH 3 citrate buffer, pH 5 citrate buffer and pH 11 sodium carbonate buffer, respectively. Attempts were made to inject enough solution to equal half the weight of meat, but it was found impossible to retain the solution in the meat. Consequently the amount actually injected could not be determined. After the stitch-pumping had been completed, each meat chunk was divided into 16 cubes, which were placed in separate vials. The buffer and juices which had been squeezed out of the meat during cutting was divided equally among the vials so that each cube was in contact with some solution. Vials were closed and stored overnight at 0.5°C to allow juices to penetrate, and were then frozen. Half the vials of each group were selected at random to be irradiated, and the other half were kept frozen until the irradiated samples had been received. All samples were then stored at 0-5° during the storage periods.

The amount of proteolysis was followed by the increase in free amino groups determined by the ninhydrin method of Moore and Stein, as modified by Locker. 3/ Results were expressed as uMoles of alanine, which was used as the standard of color reference. pH measurements were made on all samples during the various storage periods for the epinephrine treated and stitch-pumped meat.

An analysis of variance was carried out on all results, using a crossed classification of a three factor, 2 x 2 x 4, factorial design.

3/ Locker, R. H. J. Sci. Food Agr. 11, 520 (1960)

Interactions were plotted, but because of difficulties in interpreting the results, it was decided to complete the study by determining regressions of proteolysis on storage and their covariance. For this, purpose, results from all studies, including those from previous phases of this investigation, were grouped according to pH where possible to increase the number of samples in each category and thus increase the reliability of each regression line. All control samples (non-irradiated and non-treated) were combined in this manner, and their regression curves of proteolysis on storage determined and used as a basis of comparison for all other treatments.

Results and Discussion

Results of the ninhydrin determination of free amino groups are shown in Tables 2 to 7, and the pH values observed during storage of beef chunks and rabbit muscle (epinephrine treatment) are listed in Tables 5 and 7, respectively.

While the pH values of the ground meat samples, which were mixed with half their weight of buffer solution, were quite stable, the pH values found in stitch-pumped meat did not remain constant. This was presumably due to the lack of complete diffusion of buffer, as well as perhaps insufficient amount of buffer in relation to the amount of meat. The pH values of the meat at day 1 indicated that the injected buffers were not as effective in changing pH as when the buffers were mixed with ground meat. For example, pH 11 buffer brought the final pH to 6.0, and the pH 3 buffer reduced the pH only slightly to pH 5.0. It is of interest, also, that after irradiation, the pH values of all samples were somewhat higher than the non-irradiated counterparts. During storage the pH values all tended to reach values which were close to the pH of untreated meat. The pH level of the meat chunks injected with pH 11 buffer tended, however, to remain slightly higher than the other samples, which could all be considered to be at the same pH level after the 4th week of storage.

The non-irradiated meat chunks, particularly those injected with pH3 buffer, had evidence of spoilage at the 4th week. Those badly spoiled, or having considerable bacterial and mold growth, were discarded. This resulted in an incomplete series for pH 3 samples. To avoid further spoilage in the other non-irradiated samples, Hutner's preservative was added to all vials. Since the loss of samples necessitated a change in the plan for storage, the Week 8 samples were not

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taken in order to provide enough samples for the final Week 12.

The texture and physical appearance of the meat chunks after irradiation appeared to be quite different from the non-irradiated samples. The meat had lost its resilient characteristics and was quite friable and tender. The fibers were easily broken and pulled apart and the connective tissue appeared to have been completely destroyed. Although no information regarding the temperature during irradiation was received from the Natick facility, the appearance of the meat and juices would indicate that temperatures were high enough to partly cook the meat. Instead of the bright red color usually seen after irradiation, the meat was brown and the juices surrounding it were brown and coagulated. In contrast the non-irradiated controls retained the appearance and texture of fresh meat, the juices were not coagulated, and the connective tissue was not easy to cut. After the samples were homogenized for analysis, all of the non-irradiated samples contained strings or clumps of connective tissue, while none of the irradiated samples did.

The pH of rabbit muscle was found to be quite stable, with the pH of the epinephrine treated muscle being uniformly higher than the controls throughout the storage period.

The raw data in Tables 2, 3, 4 and 6 indicate that proteolysis occurred during storage, but do not show clearly what trends are present, and whether or not inhibition occurred for any given treatment. An analysis of variance of each pH group was performed to study the effects of the treatment, irradiation and storage alone, as well as the combined effects of any two of these. Results of this study are shown in Tables 8, 9, and 10.

For the ground meat samples, Table 8, treatment with buffers alone at any of the pH levels does not appear to have any great influence on inhibiting proteolysis. The significant effects all appear to be due to combined influences of treatment and irradiation - the T x I interaction in Table 8. This is especially true at pH 4.0, 4.5, 5.2, 6.5 and 8.0, and would indicate that at these pH's there is an optimum combination of buffer concentration and irradiation dose which would give maximum inhibition. The treatment x storage interaction was expected, since the amount of proteolysis would be influenced by both. It is of lesser importance than the T x I interaction. The third interaction of storage and irradiation, is also expected and contributes to the variability found, but does not give any information regarding the control of hydrolysis.

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Because of these significant interactions, and particularly the three-way interaction, T x S x I, it is difficult to interpret the effect of treatment alone, or the T x I combined effect on proteolysis. It is therefore necessary to look at the trends in the data, by studying the regression curves, as will be discussed subsequently.

In Table 9, analysis of data from meat chunks shows the results to be much the same as for ground meat. The meat samples at pH 5.5 showed little effect from treatment, or from treatment and irradiation combined. They differ little from untreated controls, and where differences occur, they are probably due to the irradiation and storage combined effects. At pH 6.0 - 6.7, however, all effects are significant and the samples are different from the controls. Inhibition has probably occurred here, but the mechanism is not clear. Again a study of regression curves is necessary.

Table 10 indicates, from the high level of significance of all the individual factors and two-way combinations of factors, that epinephrine treated irradiated meat from the rabbit is definitely less hydrolyzed than untreated controls. The uniformly high significance values suggest that again a combined effect of epinephrine and irradiation may be important.

In examining the regression curves of proteolysis on storage, one can clearly observe and evaluate the effect of pH and irradiation on the hydrolysis of meat. The regression coefficients listed in Table 11 are actually the slopes of the lines representing the rate of proteolysis of meat samples over a 12-week period. Figures 1 and 2 show the actual regression lines, or rates of hydrolysis at each pH for non-irradiatedmeat (Fig. 1) and irradiated meat (Fig. 2). The slowest rate is exhibited by samples in the pH range of 4.0 - 4.5, which means that proteolysis is being inhibited. If the non-irradiated samples are compared to the non-irradiated control, the only treatments which cause inhibition are pH 4.0 - 4.5 and pH 6.8 - 7.0. If there were no additional effect in enzyme activity by irradiation, the irradiated samples should show the same results. However, when the irradiated samples are compared with the irradiated control, all pH treatments appear to cause inhibition of proteolysis in varying degrees. The differences reflect the interaction of treatment and irradiation. While the slopes generally become smaller (or curves flatter) with irradiation, at pH 5.6 and pH 7.0 the slopes tend to become steeper. In other words, irradiation alone increases proteolysis at these two pH levels. Thus, the unusual combined effects of irradiation and treatment can be observed

(continued)

in these curves. Figure 3 illustrates the same information in another way which makes the effectiveness of inhibition clear. Any points lying below the control point indicate inhibition, any above indicate increased proteolysis.

From this figure it will be noted that for non-irradiated meat, the inhibitory action of pH 4.0 and pH 7.0 buffers is slight, and that for irradiated meat the inhibition at all pH's appears to be somewhat more important. Since one of the purposes of this investigation was to find effective means of inhibiting proteolysis in irradiated meat, the comparison between irradiated control and irradiated treated meat is of primary concern. Thus, an evaluation of the significance of the observed inhibition must be made to be able to state with confidence which treatments actually caused inhibition and whether the amount of inhibition is of practical importance. This was accomplished by means of an analysis of covariance, the results of which are discussed subsequently for each treatment.

Controls, ground meat, no treatment: Irradiation caused a significant increase in rate of proteolysis. This effect is probably due to the denaturation of the protein and the incomplete inactivation of the enzymes present, resulting in an over-all effect of increased activity.

pH 4.0 to 4.5, ground meat: Non-irradiated meat at pH 4.0-4.5 exhibits a noticeable inhibition of proteolysis, but with the data available, the treatment does not reduce the rate enough to distinguish the samples from the non-irradiated controls. Irradiation did not in itself cause any further reduction in proteolysis over that observed in non-irradiated meat at pH 4.0 - 4.5. However, when meat at pH 4.0 is irradiated, the rate of proteolysis is greatly reduced over that observed in untreated irradiated meat. This treatment was shown to be the most effective means used in this study to inhibit proteolysis of irradiated meat.

pH 6.0 to 6.5, ground meat: In this pH range, the rate of proteolysis of non-irradiated meat is greatly increased over that of untreated controls, indicating that the proteolytic enzymes in meat are at more optimal conditions at this pH than at pH 5.6. When the treated meat is irradiated, the hydrolysis rate is reduced to that of irradiated untreated meat. The over-all effect is therefore nil, and the treatment appears not to inhibit proteolysis of irradiated meat.

pH 6.8 to 7.0, ground meat: Treatment of meat with buffer to maintain pH at approximately 7 causes an inhibition of proteolysis. In non-irradiated meat the inhibition is slight, while in irradiated meat it is more noticeable. In both cases, it is statistically insignificant.

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Irradiation causes a slight increase in the rate of hydrolysis of pH 7.0 meat, but the increase is only about half of that observed in non-treated meat.

pH 8.0, ground meat: Treatment with Na_2CO_3 to bring the pH to 8.0 increases proteolysis of non-irradiated meat. Irradiation decreases the rate of proteolysis. The combined effect of irradiation and treatment brings the rate of proteolysis down to the level of non-irradiated meat, so that the net inhibition is zero. Differences in hydrolysis rates between irradiated and non-irradiated controls, and irradiated pH 8 meat are very slight.

Meat chunks, pH 5 Buffer, average pH 5.5: No significant effect could be noted, and the injected samples behaved like the controls. The factor contributing to the differences is primarily irradiation. Treatment with buffer and irradiation together may have a slight effect in reducing the proteolysis over that in non-irradiated meat, but appears to increase hydrolysis over that in irradiated untreated meat. This treatment would be unsatisfactory.

Meat chunks, pH 11 Buffer, average pH 6.1: No significant differences were demonstrated between irradiated controls and irradiated treated meat, but the difference between non-irradiated controls and non-irradiated treated samples approaches significance. Irradiation is an important factor in reducing proteolysis at this pH, as was shown in the ground meat samples of pH 6.0 - 6.5. When compared to non-irradiated meat, pH 6.1 meat after irradiation shows considerable and significant reduction of proteolytic activity. This indicates that a combined effect or interaction is responsible. However, the net result of this reduction in rate of proteolysis is to bring it to almost the same rate as that of the irradiated control, and no observable inhibition can be demonstrated. In general, results observed with the chunks of meat were similar to those obtained with the ground meat at pH 6.0.

Rabbit muscle, ante-mortem treatment with epinephrine: Epinephrine treatment effectively reduces post-mortem proteolysis in non-irradiated rabbit muscle. Proteolysis is also reduced in epinephrine treated irradiated rabbit muscle, but the effect is considerably smaller. Combined irradiation and treatment reduces proteolysis significantly over untreated non-irradiated meat. It would be expected that beef would behave similarly, although the observations on pH 7.0 ground beef are not quite the same, since in beef, the hydrolysis rate increased slightly when treated meat was irradiated. However, an over all effect of inhibition was observed, as with the epinephrine treated rabbit muscle. Neither inhibition is great in irradiated meat.

(continued)

Table 1. pH Values of Meat and Buffer Mixtures

	pH	pH, Meat + Buffer
Glycine - HCl	2.0	5.4
NaH_2PO_4 - Citric Acid	2.2	4.6
Citric Acid - Sodium citrate	3.0	4.5
Glycine - HCl	3.6	5.7
Citric Acid - Sodium citrate	4.0	5.2
Citric Acid - Sodium citrate	5.0	5.6
Citric Acid - Sodium citrate	5.6	5.8
NaH_2PO_4 - Citric Acid	6.0	6.0
Na_2HPO_4 - NaH_2PO_4	6.4	6.1
Na_2HPO_4 - NaH_2PO_4	7.0	6.2
NaH_2PO_4 - Citric Acid	7.0	6.2
Na_2HPO_4 - NaH_2PO_4	7.6	6.8
NaH_2PO_4 - Citric Acid	8.0	6.5
Na_2CO_3 - $NaHCO_3$	9.0	6.5
Glycine - HCl	9.3	6.2
Glycine - HCl	11.0	5.4
Glycine - NaOH	11.0	5.7
Na_2CO_3 - $NaHCO_3$	11.0	6.7

(continued)

Table 2. Proteolysis of Bovine Skeletal Muscle, Ground and Buffered at pH 4.0 to 5.6 (μM of Alanine/mg Protein)

Treatment	Day O	Week 4	Week 8	Week 12
Non-Irradiated Control	.159	.140	.120	.148
	.158	.145	.142	.124
Acetic Acid pH 4.0	.160	.180	.162	.167
	.158	.243	.182	.156
Citrate, pH 4.5	.121	.156	.173	.131
	.151	.150	.160	.129
Citrate, pH 5.2	.154	.201	.124	.142
	.152	.236	.141	.121
Citrate, pH 5.6	.139	.137	.130	.142
	.156	.145	.123	.158
Irradiated Control	.171	.138	.245	.160
	.160	.146	.145	.139
Acetic Acid pH 4.0	.176	.142	.156	.143
	.155	.146	.144	.136
Citrate, pH 4.5	.130	.133	.128	.129
	.143	.158	.124	.148
Citrate, pH 5.2	.183	.150	.066	.145
	.237	.176	.063	.136
Citrate, pH 5.6	.172	.162	.130	.135
	.163	.160	.120	.119

(continued)

Table 3. Proteolysis of Ground Meat Buffered at pH 5.6 - 8.0
(μMoles of Alanine/mg Protein)

Sample	Week 0	Week 4	Week 8	Week 12
Non-irradiated				
H_2O pH 5.6	.115	.189	.240	.306
	.133	.217	.243	.296
pH 6.0 (PO_4-Citr)	.139	.175	.238	.254
	.126	.157	.273	.286
pH 6.5 ($CO_3^{=}$-HCO_3^{-})	.114	.212	.234	.296
	.139	.197	.199	.234
pH 6.8 ($HPO_4^{=}$-$H_2PO_4^{-}$)	.119	.174	.224	.318
	.120	.260	.243	.234
pH 8.0 (Na_2CO_3)	.125	.189	.200	.256
	.148	.206	.206	.228
Irradiated				
H_2O pH 5.8	.084	.160	.139	.188
	.123	.156	.153	.156
pH 6.0 (PO_4-Cit)	.108	.195	.122	.162
	.094	.168		.164
pH 6.5 ($CO_3^{=}$-HCO_3^{-})	.078	.179	.160	.230
	.125	.184	.154	.216
pH 6.8 ($HPO_4^{=}$-$H_2PO_4^{-}$)	.144	.156	.162	.162
	.121	.197	.178	.162
pH 8.0 (Na_2CO_3)	.121	.167	.177	.180
	.141	.178	.195	.178

(continued)

Table 4. Proteolysis of Meat Chunks, Stitch-Pumped with Buffers
(μMoles of Alanine/mg Protein)

Sample Description	Week 0	Week 4	Week 12
Non Irradiated Control (H_2O)	.128	.232	.625
	.188	.161	.623
Non Irradiated pH 3	.161	.187	
	.176	.183	
Non Irradiated pH 5	.152	.162	.581
	.136	.445	.595
Non Irradiated pH 11	.130	.127	.390
	.140	.148	.526
Irradiated Control (H_2O)	.146	.587	.267
	.147	.517	.289
Irradiated pH 3	.128	.366	.280
	.138		
Irradiated pH 5	.238	.159	.248
	.132	.212	.296
Irradiated pH 11	.162	.143	.281
	.113	.166	.279

(continued)

Table 5. pH of Meat Chunks.

Sample	Week 0	Week 4	Week 8	Week 12
NI Control (H_2O)	5.4	4.9	5.2	4.9
NI Control (H_2O)	5.5	5.2	5.1	5.0
NI pH 3	5.0	6.9	4.9	
NI pH 3	5.0			
NI pH 5	5.6	5.4	5.3	5.0
NI pH 5	5.5	5.3	5.4	5.0
NI pH 11	6.0	5.1	5.5	5.3
NI pH 11	6.1	5.2		5.3
I Control (H_2O)	5.8	5.7	5.8	5.7
I Control (H_2O)	5.8	5.7	5.8	
I pH 3	5.2	5.3	5.6	5.5
I pH 3	5.3	5.3	5.6	
I pH 5	5.5	5.5	5.8	5.7
I pH 5	5.5	5.5	5.8	5.7
I pH 11	6.7	6.0	5.9	5.9
I pH 11	6.6	5.9	5.9	5.8

I = Irradiated
NI = Non-irradiated

(continued)

Table 6. Proteolysis of Rabbit Muscle treated with Epinephrine, Ante-mortem. (μMoles of Alanine/mg Protein)

Sample	Week 0	Week 4	Week 8	Week 12
NI Control	.086	.185	.262	.222
NI Control	.098	.161	.214	.234
NI Epinephrine	.078	.134	.120	.116
NI Epinephrine	.076	.132	.133	.206
I Control	.063	.166	.130	.158
I Control	.078	.156	.121	.208
I Epinephrine	.119	.130	.124	.192
I Epinephrine	.097	.146	.120	.180

Table 7. pH of Rabbit Muscle, Controls and Treated Ante-Mortem with Epinephrine

Sample	Week 0	Week 4	Week 8	Week 12
NI Control	6.0	5.9	6.0	5.9
NI Control	6.0	5.7	6.2	5.7
NI Epinephrine	6.7	6.6	6.7	6.3
NI Epinephrine	6.7	6.6	6.7	6.3
I Control	6.0	6.0	5.8	6.0
I Control	6.0	6.0	5.8	5.9
I Epinephrine	6.8	6.5	6.7	6.8
I Epinephrine	6.9	6.6	6.5	6.6

I = Irradiated
NI = Non-irradiated

(continued)

Table 8. Results of Analysis of Variance. Significance Levels (P) of Various Factors in Proteolysis of Ground Meat Treated with Buffer

Factor	pH of Meat 4.0	4.5	5.2	5.6
Buffer (Treatment)	.25	.25	--	--
Irradiation	--*	--	--	.100
Storage	--	--	.001	--
T x I	.025	.10	.05	.25
T x S	--	.25	.001	.05
I x S	.25	--	.25	--
T x S x I	--	.25	.005	.25

Factor	6.0	6.5	6.8	8.0
Buffer	.25	--	--	--
Irradiation	.001	.001	.001	.001
Storage	.001	.001	.001	.001
T x I	--	.05	--	.001
T x S	--	--	--	.01
I x S	.001	.025	.005	.001
T x S x I	.05	.250	--	.10

* - indicates the effect is not significant
I = irradiation
T = treatment
S = storage

(continued)

Table 9. Results of Analysis of Variance Significance Levels (P) of Various Factors in Proteolysis of Meat Chunks Stitch-Pumped with Buffers.

Factor	Original pH of Meat after stitch-Pumping	
	5.5 to 5.6	6.0 to 6.7
Buffer (T)	-- *	.001
Irradiation	.25	.25
Storage	--	.001
T x I	.25	.25
T x S	--	.025
I x S	.01	.001
T x S x I	.10	.01

Table 10. Results of Analysis of Variance, Significance Levels (P) of Various Factors in Proteolysis of Rabbit Muscle Treated Ante-mortem with Epinephrine.

Factor	P
Epinephrine (T)	.005
Irradiation	.10
Storage	.001
T x I	.005
T x S	.10
I x S	.10
T x S x I	--

* - indicates the effect is not significant
I = irradiation
T = treatment
S = storage

(continued)

Table 11. Proteolysis during Storage of Meat Samples at varying pH. Composite Results of all Experiments.

pH	Regression Coefficient b*	
	Non Irradiated	Irradiated
Control, pH 5.6, Comp.	3.53	6.63
4.0 - 4.5	2.38	2.00
6.0 - 6.5	11.63	5.98
6.8 - 7.0	3.33	4.88
8.0	8.00	4.80
Rabbit, Control, pH 6.0	18.57	8.10
" , Epinephrine pH 6.8	6.15	5.45
Beef Chunks, Control pH 5.5	40.9	4.5
" " , pH 5.6	36.8	14.3
" " , pH 6.1	28.8	12.4

*µMoles of Alanine produced per g protein per week.

(continued)

Figure 1. Effect of Storage on Hydrolysis of Non-Irradiated (NI) Meat at Various pH Levels

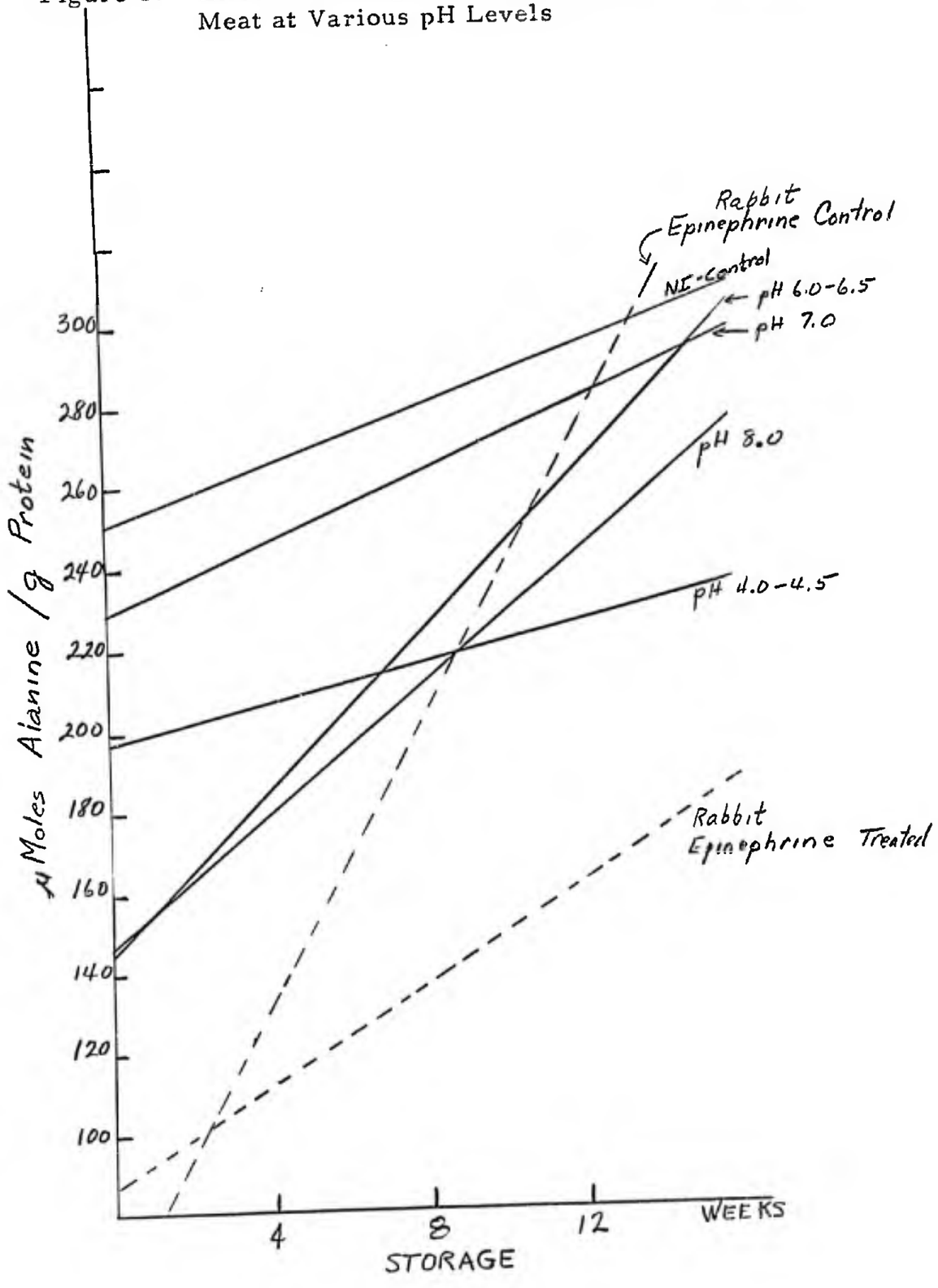


Figure 2. Effect of Storage on Hydrolysis of Irradiated (I) Meat at Various pH Levels

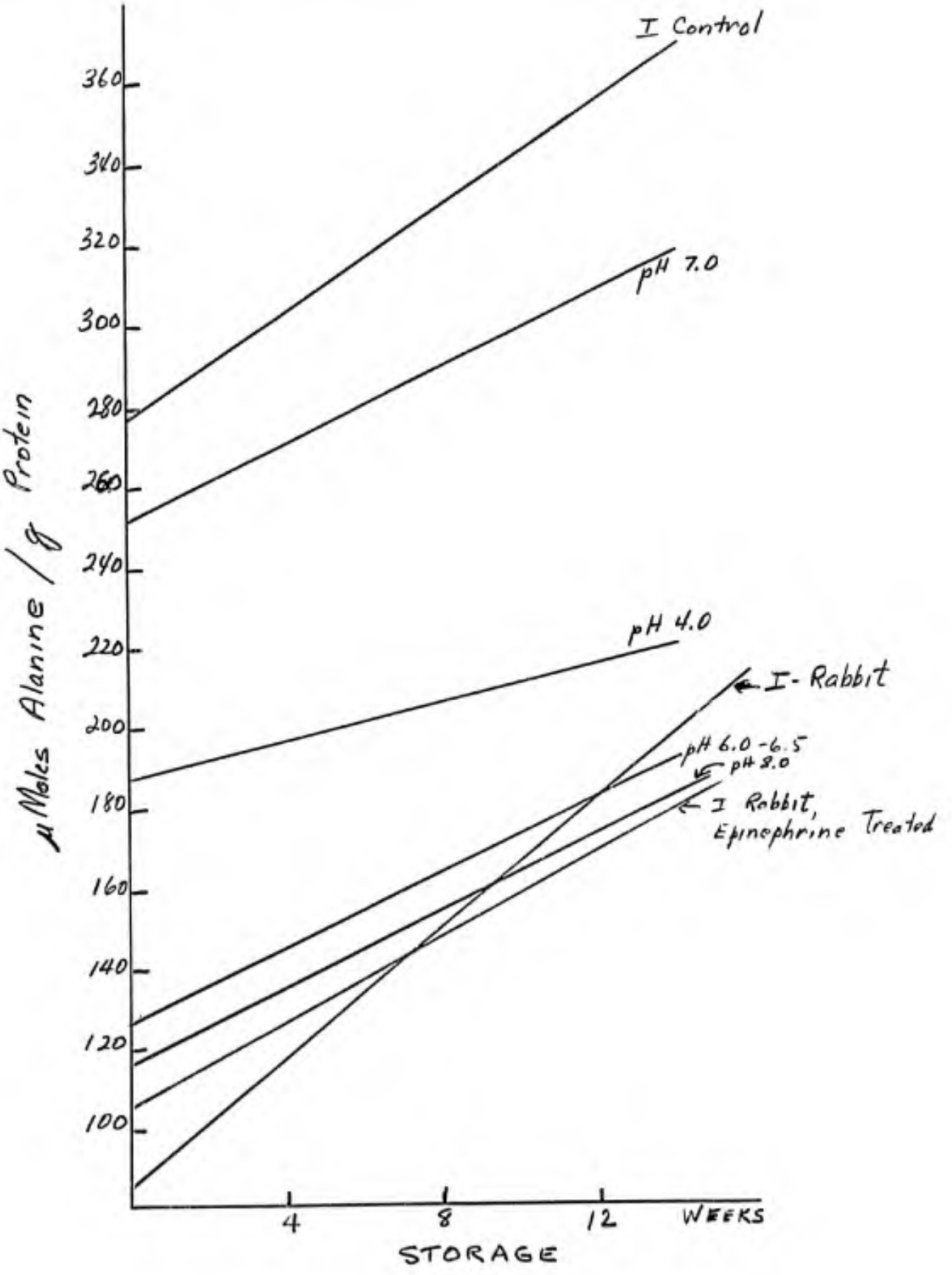
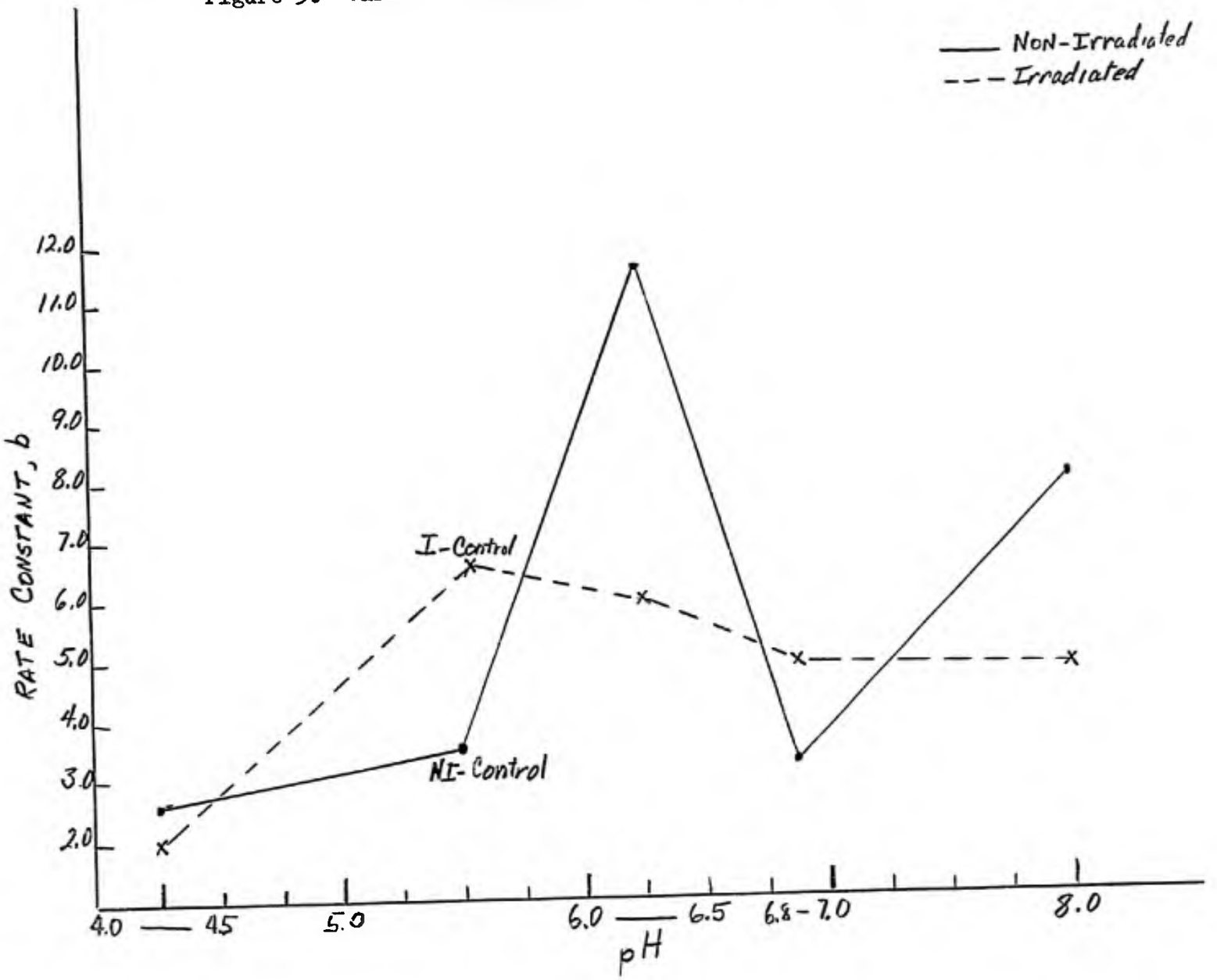


Figure 3. Variation in Rate of Proteolysis with pH



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13 ABSTRACT

Control or inhibition of proteolysis in meat as influenced by pH has been investigated over the pH range of 4.0 to 8.0. In non-irradiated meat, inhibition has been achieved only when the pH of the meat is 4.0 to 4.5 and 6.9 to 7.0. In both cases the inhibition is slight. In irradiated meat varying degrees of inhibition of proteolysis occur throughout the entire range studied. Maximum hydrolysis occurs in irradiated beef at pH 5.6 and in untreated controls. Inhibition in significant amount to be of some practical use occurs only at pH 4.0 and 4.5 in irradiated meat. Some indication has been obtained that combined effects of treatment with buffers and radiation may influence the inactivation of enzyme systems more profoundly than either factor alone. It is recommended that further studies be directed to examining at various dose levels of radiation meat samples treated with varying concentrations of buffer at a given pH -- either 4.0 to 4.5 or 7.0 to 8.0.

The injection of buffers into large pieces of meat was studied to evaluate the method of stitch-pumping. Some difficulty was encountered in introducing enough buffer to maintain the pH of the meat at that of the buffer. However, at the pH which was achieved for each sample, the same hydrolysis pattern was obtained as was indicated by the ground meat samples. It was concluded that enough buffer can be introduced to be effective in altering hydrolysis but that the problems of achieving a desired pH remains. This may be solved by using more concentrated buffers along with more efficient injections and diffusion methods.

(continued)

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STUDIES ON MEANS OF CONTROLLING OR INHIBITING BEEF TISSUE ENZYME SYSTEMS

13. ABSTRACT (continued)

Ante-mortem control of pH was investigated in rabbit muscle by injection of epinephrine before slaughter. The pH was very effectively controlled in the meat from treated animals throughout the storage period at approximately pH 7. The inhibitory effect of the treatment on proteolysis was more pronounced in non-irradiated meat, although some inhibition was noted in irradiated meat as well. The amount of inhibition was about the same as was observed for pH 7.0 buffered meat. In both cases the inhibition was considerably less than in irradiated meat at pH 4.0 to 4.5.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Control	8		4			
Inhibition	8					
Proteolysis	9					
pH factor	10					
Beef	5		9			
Irradiated	0					
Non-irradiated	0					
Hydrolysis	9		4			
Injection			8		8	
Buffers			8			
pH control			10		4	
Stitch pumping			10			
Epinephrine					8	
Ante-mortem					0	
Rabbits					9	
Muscles					9	

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