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RELATIONSHIP BETWEEN HIGH ENERGY PHOSPHATES, pH AND VISCOSITY OF COW AND BULL MEAT SLURRIES

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ZUSAMMENFASSUNG

Methoden zur Messung der Qualitaet von warmen, gefrorenen Rindfleisch wurden entwickelt. Die Zeitspanne (Viscositaetspanne), welche vom Fleischbrei, bestehend aus pre-rigor Fleisch und Salzloesung, benoetigt wird zu eretarren, schwankt mit der Fleischqualitaet und correliert mit dem Gehalt an energie reichen Phosphaten des Fleisches.

SUMMARY

Methods were developed to measure the quality of hot boned, frozen cow meat. The lapse in time (viscosity lag phase) needed by a slurry of pre-rigor meat and salt solution to set up, varies with meat quality and correlates with the high energy phosphate content of the meat.

The use of hot bull meat is an old art in sausage making. Turner and Olson (1) found that rapid freezing preserves the quality of hot boned meat by stopping the physiological processes of actomyosin formation and high energy phosphate depletion. If such meat is ground in the frozen state and introduced frozen into the extracting salt solution, thaw rigor is avoided. This meat can then be used instead of hot boned unfrozen meat in sausage manufacture, without loss of its water binding and fat emulsifying capacity. Because of variations in time for different animals to go into rigor and because of slow freezing, variations in the quality of pre-rigor meat were found. It became, therefore, necessary to measure the progress of its incipient rigor with fair accuracy.

In pre-rigor meat the structual protein actomyosin is dissociated to varying degrees into actin and myosin. The greater solubility of this structural protein is characteristic of pre-rigor meat as compared with post-rigor meat, where actin and myosin are joined into actomyosin.

The dissociation of actomyosin in pre-rigor meat is due to the presence of high energy phosphates (HEP). These phosphates are gradually depleted after slaughter and disappear when rigor mortis sets in.

The two methods reported in this paper were developed to determine pre-rigor frozen meat quality by measuring the progress in actomyosin formation and concurrent HEP depletion.

It was observed that when pre-rigor meat, obtained at various times after slaughter, was blended with salt solution, the resulting slurry needed different lengths of times to set up. The slurry which may have an initial low viscosity becomes gradually stiffer and finally does not run out of an inverted beaker. Consequently the delay in time required for a slurry to set up or to reach a certain viscosity, can serve as a method to measure pre-rigor meat quality. This delay was called viscosity lag phase (VLP).

The other method of quality measurement was based on the disappearance of high energy phosphate from pre-rigor meat after slaughter, characterized by a corresponding increase in inorganic phosphate. The method was developed along the lines of that by Lowry and Lopez (14).

While the VLP method provides a direct measurement of pre-rigor meat quality, the phosphate method is an indirect method. To make the data meaningful a correlation had first to be established between the results of the two methods.

METHODS

Viscosity Lag Phase. The meat sample was frozen and held for 24 hours prior to the test at -28.9° C. (-20° F.). The sample was ground frozen through a 1/8" plate at 0° C. One hundred and fifty grams were blended at room temperature in a Waring blendor with 187.5 ml of a 7% salt solution of 0° C. Blending was carried out until a temperature of 10° C. was reached. The slurry was poured into a 400 ml glass beaker and the viscosity measured with a Brookfield Viscometer using spindle No. 6. The spindle should be lowered to a constant depth and the 0-point marked on the dial. The viscometer was turned on and the dial was allowed to make two complete turns. The brake was put on and the number inside the dial under the pointer was read and multiplied by 1000. This was the viscosity in centipoises. The beaker was then placed in a water bath at 10° C. and the measurement repeated every ten minutes. When the viscosity in centipoises

exceeded 100,000 the meat was considered as set up. The delay between the first and last measurement was called the viscosity lag phase and was found to correlate with the pre-rigor quality of the meat used in various sausage formulations. Standard deviation s for the method was 4.7 minutes.

-28.9° C. High Energy Phosphates. The frozen sample was ground at thirteen grams of the powder were blended in a micro-blender with 90 mls of 10% TCA solution at 0° C., for two minutes. After waiting for two more minutes for the foam to break, the suspension was filtered through a No. 1 Whatman filter paper. The filtrate passing through the paper during the first two minutes was retained. One half of one ml of the filtrate was transferred to a 50 ml graduated glass stoppered cylinder containing 6 ml of a 0.1 M sodium acetate solution. The cylinder was filled to 50 ml with pH 4.0 acetate buffer (1.6978 g sodium acetate and 3 g glacial acetic acid in 1 liter dist. water), the content mixed and held at 0° C. until needed. This sample served as control. Another 0.5 ml filtrate was hydrolyzed in a centrifuge tube with 0.5 ml 2 M HCl in the steam bath for 10 minutes and subsequently cooled in tap water to 25° - 30° C. The content of the tube was quantitatively transferred into a 50 ml graduated glass-stoppered cylinder containing 2 ml of a 1.0 M sodium acetate solution and filled up with pH 4.0 buffer to 50 ml. This sample was the test.

A blank was prepared by using the same solutions without substrate. The standard was prepared by using a solution containing 1.25 micrograms P per ml. Ten ml of each solution were pipetted into separate erlenmeyer flasks. To each flask one ml of a 1% ammonium molybdate solution (1 g $(NH_4)_6 MO_7 O_{24} \cdot 4H_2O$ in 100 ml 0.05 M H_2SO_4) and one ml of a 1% ascorbic acid solution were added while shaking the flasks. Optical density readings were carried out on a Beckman DU spectrophotometer at 700 mμ, after 5 and 10 minutes. If the sample readings differed from those of the standard, they were extrapolated to zero time. An optical density change in excess of 0.008 was considered unacceptable and a new sample was prepared. This was done also if optical density changes of standard and test were in opposite directions.

The percent high energy phosphate content was calculated by the equation:

$$\frac{\text{O.D. After Hydrolysis} - \text{O.D. Before Hydrolysis}}{\text{O.D. After Hydrolysis}} \times 100$$

The accuracy of the method was ± 2% HEP.

Experimental:

Pre-rigor Cow and Bull Meat. Hot cow rounds, (4 series) obtained on the kill floor about 15-20 minutes after killing, were brought immediately into the 0° C. cooler, freed from fat and connective tissue, and cut into 1" pieces. After mixing they were ground through a 1/8" plate, mixed again and spread out on trays between two polyethylene sheets about 1/4" thick. The trays were now placed into a big polyethylene bag to cut down evaporation. One tray was placed in the -63° C. freezer, and five trays in the +7° C. cooler. From this cooler they were removed one by one after 4, 24, 48 and 72 hours and placed in the -63° C. freezer. Hot bull meat (3 series) which makes up only a fraction of the hot boned cow meat, was not held over 24 hours at +7° C.

pH Determinations. One hundred fifty grams frozen meat powder were blended with 100 ml pre-cooled dist. water at 0° C. in a Waring blendor, until a slurry temperature of 0° C. was reached. A Beckman Model H2 pH meter with glass electrodes was standardized with pre-cooled pH 7.0 buffer at 0° C. and the slurry pH was read.

RESULTS

Figure 1 shows the decrease of VLP with holding time at 7° C. prior to freezing. The solid line refers to cow meat, the broken line to bull meat. The rate of change of VLP was the greatest during the first 24 hours. During the next 24 hours (cow meat only), the rate decreased to about zero. Results obtained with bull meat indicated that the initial VLP was considerably higher than that with cow meat but the rate of decrease was much faster. As it may be seen, VLP disappeared completely after 24 hours holding at 7° C. for bull meat as compared with 48 hours for cow meat.

Figure 2 presents the corresponding pH values. The rate of pH decline followed roughly the trend of the VLP. pH values obtained with bull meat started at a somewhat higher level but decreased at a slower rate than in cow meat.

Figure 3 demonstrates the change in labile phosphate during the test period. It may be seen that during the first 4 hours about half of the high energy phosphate was lost, while during the subsequent 20 hours about 60% of the remaining half was lost. From there on the rate decreased more evenly. Despite great VLP differences between pre-rigor cow and bull meats, no differences were found in percent HEP. The rate of HEP loss in bull meat closely paralleled that of cow meat.

In Figure 4 the VLP data were plotted against decrease in HEP. According to Bailey and Marsh (10) about 70% of all phosphate esters are hydrolyzable during 10 minutes in 1.0 M HCl. This amount was taken as 100 and the decrease in HEP was expressed in percent of the original amount.

DISCUSSION

According to various authors (2, 3, 4, 5, 6) muscle contains about 2-3 micromols ATP per gram and about 3-5 micrograms phosphocreatin (PC) per gram muscle (4, 7, 8, 9, 10). Of the about 70% acid hydrolyzable phosphate esters, about 76% correspond to ATP and PC of which 40% is ATP and 60% PC. Phosphocreatin does not dissociate actomyosin. Its main effect is the regeneration of ATP from ADP, and consequently disappears first from the cells. The steepness of the curve in Figure 3 during the first four hours is probably due to PC breakdown (10, 11, 12). It is evident that the first hours are the most important in the conservation of HEP.

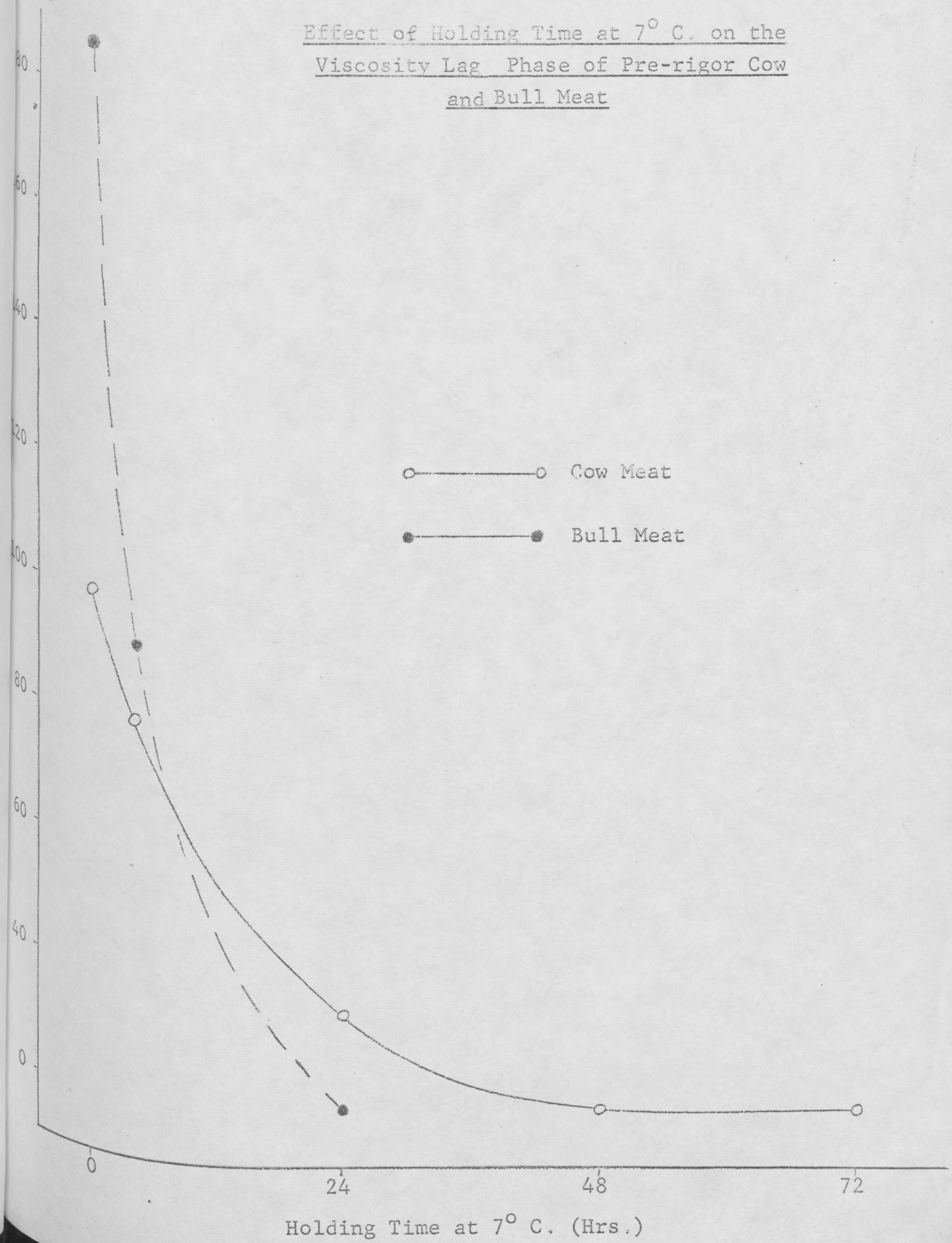
A similarly shaped curve was obtained with VLP (Fig. 1). The similarity of the curves in Figures 1 and 3 suggest that a close relationship between these sets of data exists. When VLP values were plotted against the decrease of % HEP as shown in Figure 4 two curves of completely different shape were obtained. It is interesting to note that during the first four hours of holding at 7° C. the HEP decreased in cow meat at a much higher rate than the VLP. This is demonstrated by the nearly horrizontal shape of the first section of the curve in Figure 4. The PC which makes up the major part of the HEP is broken down while part of it is used to regenerate ATP. Hamm (11) and Marsh (12) found that after slaughter there is a "delay period" of several hours during which ATP breakdown is slow, while PC disappears fast. Applying this theory to the curve in Figure 4 it is possible that VLP does not change markedly while rephosphorylation of ATP is going on. From four to 24 hours the easily hydrolyzable part of HEP disappeared together with the VLP. In the case with bull meat the rate of decrease in VLP and HEP was nearly constant which might suggest a lower myosinase activity in that meat (13).

The progressive decline in pH in both meats follows a parallel curve. Although the initial pH in bull meat was higher than in cow meat, the same difference was maintained during the 24 hours while the test with bull meat was run (Figure 2).

The big variations between tests make, however, pH measurement unsuitable for quality determination in frozen, pre-rigor cow and bull meat.

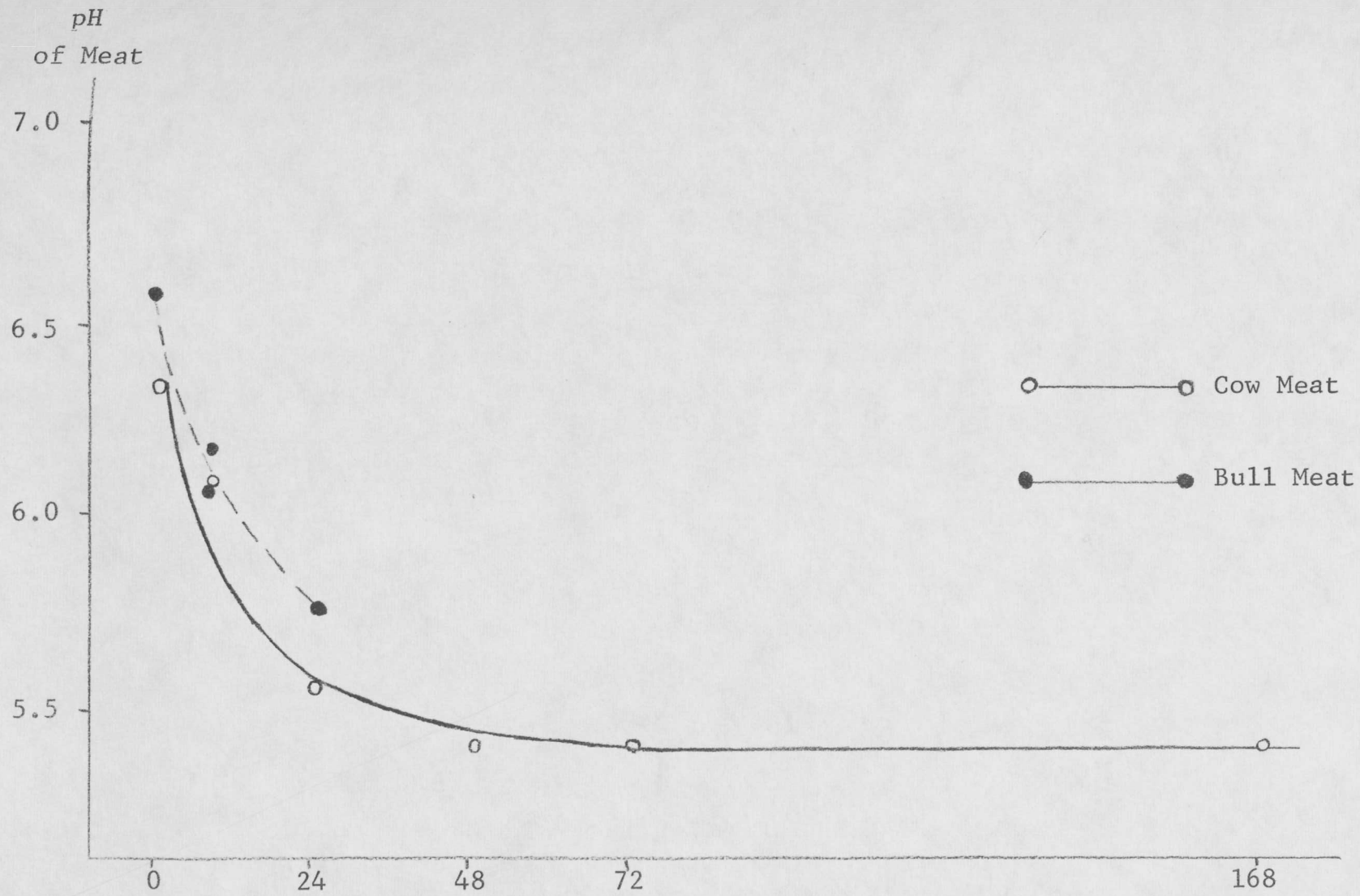
The results obtained with HEP determinations confirmed those of the VLP tests, which allows the conclusion that the VLP method is reliable enough to determine the quality of frozen, pre-rigor meat.

Figure 1

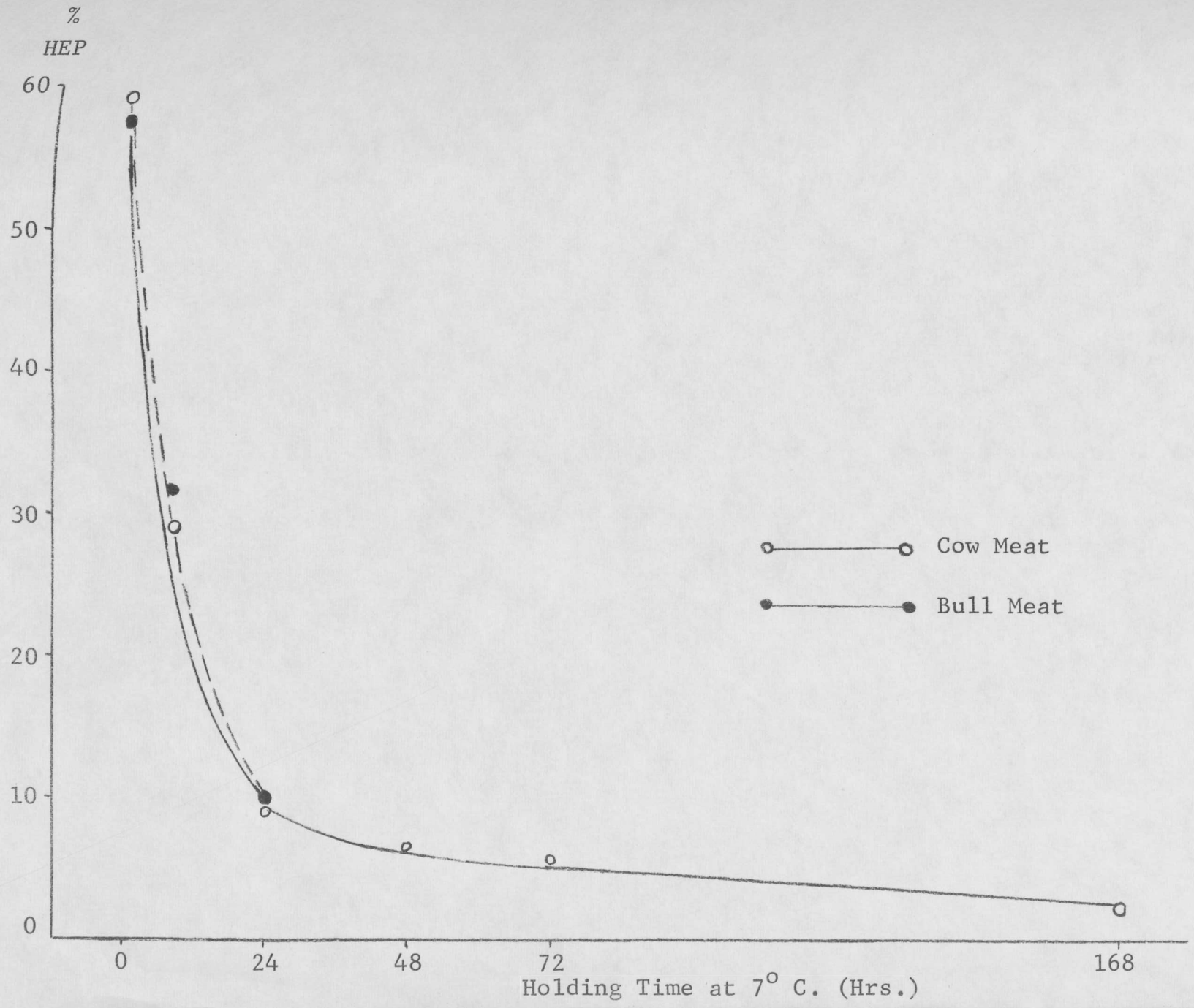


Effect of Holding Time at 7° C. on the Viscosity Lag Phase of Pre-rigor Cow and Bull Meat

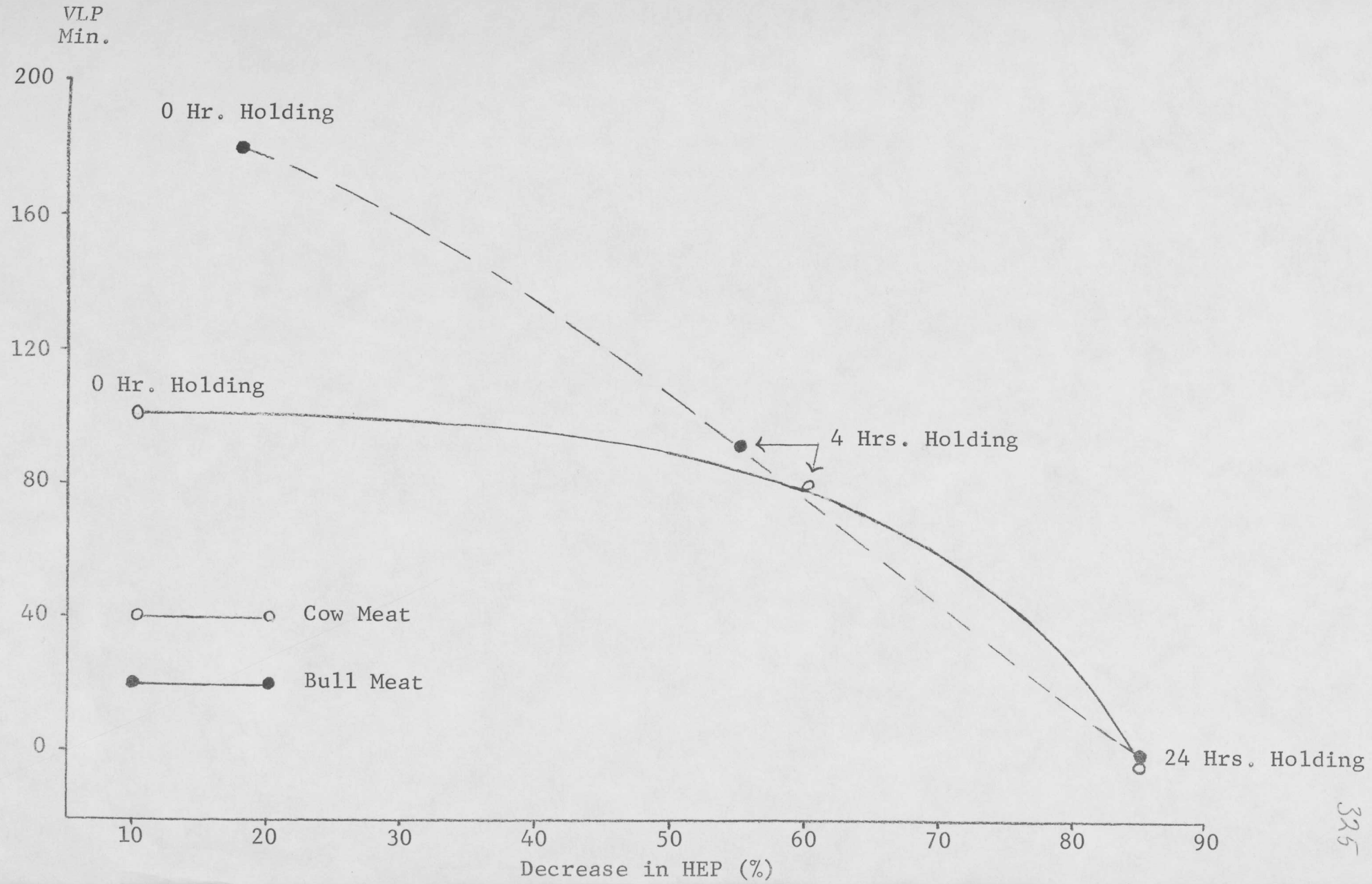
Change in pH in Pre-rigor Cow and Bull Meat
During Holding the Meat at 7° C. for Various Times



Effect of Holding Time at 7° C. on the Decrease of High Energy Phosphates in Pre-rigor Cow and Bull Meat



Relationship Between Viscosity Lag Phase and Decrease in High Energy Phosphate in Pre-rigor Cow and Bull Meat



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