SLAGTERIERNES FORSKNINGSINSTITUT
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FRYSETØRRING
Manuskript nr. 280 E

Effect of EDTA and pH on Properties of Freezedried Pork Muscle

I Effect of pH, Magnesium, and Calcium Ions on Freezedried Myofibrils[a]

J. Wismer-Pedersen[b]
Department of Food Science, Michigan State University
East Lansing, Michigan

Summary

To study the effect of pH and divalent cations such as Mg and Ca on rehydration of free-dried meat, a model system of freeze-dried myofibrils was designed. The fibrils were dried at pH 5.4, 6.0, and 7.0 with and without addition of Mg and Ca, in the concentrations found in meat. The freeze-dried fibrils were rehydrated (1) after homogenization in the rehydrating solution and (2) by diffusion of the solution into the dried fibril mass. When the fibrils were homogenized the cations increased the water holding capacity of the sedimented fibrils. An increase in water holding capacity with higher pH was apparent only after the cations were added. When the fibrils were rehydrated through diffusion, the cations decreased the hydration. Increased hydration capacity with higher pH was found with and without the cations.

Übersicht

Um die Wirkung von pH und divalenten Kationen wie Mg und Ca auf Rehydrierung von gefriertrocknetem Fleisch studieren zu können wurde ein Modellsystem von gefriertrockneten Myofibrilen konstruiert. Die Fibrilen wurden bei einem pH Von 5,4, 6,0 und 7,0 sowohl mit als ohne Zusatz von Mg und Ca getrocknet - in Koncentrationen wie sie im Fleisch vorkommen. Die friergetrockneten Fibrilen wurden rehydriert (1) nach Homogenisierung in der Rehydrierungslösung und (2) durch Diffusion der Lösung in die getrocknete fibrile Masse. Nach Homogenisierung der Fibrilen wurde die Wasserbindungskapazität des fibrilen Sediments durch die Kationen erhöht. Die Erhöhung der Wasserbindungskapazität mit höherem pH wurde erst nach Zusatz der Kationen sichtbar. Als die Fibrilen durch Diffusion rehydriert waren, verminderten die Kationen die Hydrierung. Erhöhte Hydrierungskapazität bei höherem pH wurde sowohl mit als ohne Kationen gefunden.

a. Journal Article 3393, Michigan Agricultural Experiment Station, East Lansing.
b. Present address: Danish Meat Research Institute, Roskilde.

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Introduction

Freeze-drying of foods frequently results in rehydrated products which are characterized as dry, spongy, tough, or of decreased acceptability (Brockman 1962). Rehydrated meat is often tougher and drier than the original meat, and in addition has a characteristic "woody" texture. These texture characteristics are usually ascribed to decreased ability of the meat proteins to take up moisture during rehydration (Connel 1957; Hamdy et al. 1958; Hamm and Deatherage 1960).

Penny et al. (1963) recently demonstrated that injection of adrenaline in beef and rabbits before slaughter improved rehydration, with more tender, juicy, and a less woody texture to the rehydrated meat. The effect is ascribed to adrenaline which gives a higher ultimate pH in the meat as a result of glycogen depletion.

The decreased ability of meat proteins to rehydrate is generally regarded as due to the formation of an excessive amount of electrostatic and hydrogen bonds between actin and myosin in the myofibrillar filaments (Hamm and Deatherage 1960; Connel 1962). Formation of these bonds might be counteracted by relaxation of the muscle, as well as by an increase of pH away from the isoelectric point. Thereby, the least possible overlapping of the actin and myosin threads should occur.

Achievement of decreased bonding should be possible by treatment of the muscle before drying with pyrophosphate or Ethylene-Diamine-Tetra-Acetic acid (EDTA) salts. Bozler (1958) has shown that these compounds cause relaxation of muscle fibers. With regard to EDTA, the action appears to be due to its ability to chelate diivalent metal ions, specifically Ca (Maruyama and Gergely 1961). There is no evidence that EDTA is bound to the fibrillar proteins (Ebashi 1961).

The purpose of this study was to investigate the effect of EDTA on whole muscle as well as on isolated myofibril preparations before freeze-drying. The purpose of using the myofibrils was to simplify the system under study, whereas, the complexity of whole muscle may make results difficult to interpret.

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Experimental

Preparation and freeze-drying of myofibrils

The myofibrils were prepared as described by Fukazawa et al. (1961). A 2000 g sample of longissimus dorsi muscle from pigs slaughtered at approximately 200 lbs. liveweight in the MSU Meat Laboratory was used as the source of material. In the course of preparation, the fibrils were treated with excess EDTA to remove Ca and Mg. Subsequent washing of the fibrils with 0.1 M KCl was performed so that the resulting fibril preparation was practically free of EDTA as well as Mg and Ca. The concentration of fibrils in the preparation was 8-10 mg fibrillar protein/ml. The preparation was divided into two equal parts. The first part served as the control while $MgCl_2$ and $CaCl_2$ (Baker analyzed-Reagent grade) were added to the other part. The concentrations were adjusted to bring the Mg and Ca conc. to the levels expected in muscle homogenates, that is 2 mM and 0.4 mM/l., respectively. Each part was then divided into 4 portions. The pH of the three portions was adjusted to 5.4, 6.0, and 7.0, respectively. The fourth portion was adjusted to pH 7.0 after sodium pyrophosphate was added in an amount corresponding to 2 % of the fibrillar dry weight. The portions were poured into aluminum moisture dishes (E.H. Sargent Co.), frozen in an air blast freezer at -29°C and stored for a few days wrapped in aluminium foil until freeze-drying could take place. The freeze-drying was carried out in a Stokes laboratory freeze-drier using heated plates. The plates were heated to a maximum temperature of 42°C and the air pressure during drying was 135 µ. The drying time was 25 hours. Along with the fibril preparations, a sample of unwashed muscle homogenate and a sample of the original meat was dried. After the drying the samples were stored under nitrogen until used.

Rehydration of the fibrils

During drying, the fibril mass in each dish was transformed into a spongelike, porous network. The rehydration studies were performed with this structure intact as well as after homogenization in the rehydration fluid. In the latter case, the fibrils were homogenized in 0.1 M KCl in a Waring blendor (Three short bursts). After 3 hr., the homogenized mass was divided into 8 portions containing about 7-8 g of the fibril suspension. These portions were adjusted to a pH between 4.6 and 7.0 by addition of 0.1 N HCl or NaOH. The portions

were left for three hours and then centrifuged for 20 min. at approximately 2000 G. At the time of centrifugation, the pH was recorded. The amount of water bound per g. of protein was taken as an indication of the water holding capacity of the fibrillar protein. The protein was determined by multiplying the nitrogen content by 6, after determining nitrogen by the micro Kjeldahl procedure.

The rehydration characteristics of the intact sponge-like fibril network was studied by letting the rehydrating fluid diffuse into the mass. Wedge-shaped sections of about 0.3-0.5 g were weighed on a microbalance and rehydrated for 3 hours at room temperature in a tube with 30 ml 0.1 M KCl. The number of sections and pH adjustment were as described above. The excess fluid was then carefully drained off and the rehydrated mass weighed again on the microbalance. The amount of water per g protein was taken as an indication of the rehydration capacity of the fibril sponge. The protein content was determined as above.

Fibrils were isolated from the freeze-dried meat sample and meat homogenate as described by Bendall and Wismer-Pedersen (1962), and the water holding capacity was measured as described above.

Results

Water-binding of sedimented fibrils

The impact of sarcoplasmic proteins on the water holding of freeze-dried myofibrils was studied by isolation of myofibrils from the freeze-dried meat and the freeze-dried meat homogenate. The water holding capacity of these fibrils were compared with that of the myofibrils freeze-dried with added Mg and Ca at the same pH as the meat (5.4). Results showed that these different preparations of fibrils on sedimentation had about the same ability to retain moisture. Microscopic examination of the fibrils showed no obvious difference in their tendency to aggregate. This observation is in line with that of Cole and Smithies (1960), who found very little difference in the electrophoretic pattern of sarcoplasmic protein as a result of freeze-drying. These results point out that any difference in the water holding capacity is probably due to alterations within the fibrillar proteins.

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When the isolated fibrils were dried without Mg and Ca, pH made no difference to the sedimentation characteristics, and hence to the water holding capacity. Thus, the fibrils could be dried at either pH 5.4 or 7.0 without effect upon the fibrils as illustrated in fig. 1. Fig. 1 shows that the isoelectric point and shape of the pH-hydration curves are overlapping. When sodium pyrophosphate was added to the fibrils before drying, the rehydrated fibrils had an increased water holding capacity and the isoelectric point was shifted to a slightly lower pH. The shift in isoelectric point indicated that the pyrophosphate ions were bound to the proteins. However, the water holding capacity was not quite as high as that of pH 7 fibrils with added Mg and Ca. Before the water holding capacity of the pyrophosphate treated fibrils was measured, the fibrils were washed with 0.1 M KCl until no reaction for phosphate could be detected following the method of Allen (1940).

When Magnesium and Calcium ions were added to the fibrils before drying, the water holding capacity after rehydration was considerably increased. This was especially important in the fibrils dried at pH 7 as shown on fig. 2. The water holding capacity of the fibrils dried at pH 5.4 achieved a water holding capacity only slightly higher than the level of the fibrils dried without addition of Mg and Ca.

The level of water holding capacity of the original fibrils with and without added Mg and Ca is indicated in fig. 1 and 2. The added ions in the fresh fibrils increased water holding capacity. When the ions were not added the water holding capacity of the freeze-dried fibrils was below that of the fresh fibrils. However, addition of pyrophosphate increased water holding capacity above that of the fresh fibrils. With added Mg and Ca, the fibrils dried at pH 7 had the same water holding capacity as the fresh fibrils, while those dried at pH 5.4 had considerably less. When Mg and Ca were added, the positive effect of added pyrophosphate on water-holding capacity appeared only when pH of the rehydrating solution was 6 or higher.

Rehydration of the sponge-like fibril mass

The positive effect of Mg and Ca on the water holding capacity was found only when the fibrils were homogenized into the rehydrating solution. Fig. 3 and 4 demonstrate that the fibril mass with added Mg and Ca had reduced rehydration capacity at the two pH levels. The contrast in the results is probably due to

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an adverse effect of Mg and Ca on diffusion of the rehydrating solution into the dried mass and/or the wetability of the dried fibrils. When the dried mass was homogenized into the rehydrating solution, the fibrils sedimented on the bottom of the flask rather quickly. When the mass was rehydrated without homogenization, it tended to float on top of the solution and appeared to contain a considerable amount of entrapped air as occurs in rehydration of whole dried meat. This situation prevails whether the ions are added or not and illustrates the two different sets of conditions occurring. When the pyrophosphate is present, the negative effect of Mg and Ca on rehydration is balanced. Table 1 as well as comparison of fig. 3 and 4 shows that a higher pH during drying results in better rehydration regardless of whether the ions are added or not.

In order to find out which of the ions was responsible for the decrease in rehydration capacity, samples of fresh fibrils were dried with only one of the ions added in the concentration originally applied. The rehydration capacity of the samples at pH 7 are shown in table 2. Results indicate that Mg has given the full effect, whereas, the decrease in rehydration capacity of the fibrils with added Ca is much less.

However, as Mg was added in five times as high a concentration as Ca we may conclude that both ions participate in the effect.

Discussion

Rehydration of the sponge-like fibril mass with and without breaking of the original structure produced two sets of results with regard to the effect of magnesium and calcium ions. Results showed that addition of the ions resulted in an increase in water holding capacity for the sedimented fibrils, but a reduction in water holding capacity if the mass was not homogenized during rehydration.

Apparently, the added ions may affect the packing density of the sedimented fibrils in the centrifuge flask whereby they retain more water. The same effect was also found with the fresh fibrils before freeze-drying and is in line with observations by Wierbicki et al. (1957), who studied the effects of salts on the water binding capacity of ground meat using shrinkage after heating to 70°C to evaluate the water binding capacity. They found that the positive effect of Mg and Ca was neutralized by EDTA. Swift and Ellis (1956) found that Mg increased but Ca decreased the water binding capacity of ground meat. It is interesting to note that the fibrils dried without addition of the cations have a lower water holding capacity after rehydration and sedimentation than the corresponding fresh fibrils, regardless of the pH before drying. However, when the ions were added, the pH before drying influenced the water holding capacity after rehydration. At pH 7.0, the rehydrated fibrils have the same water holding capacity as the corresponding fresh fibrils. From these observations, one may speculate that the decreased water holding capacity of the sedimented fibrils is due to formation of hydrogen and electrostatic bonds during the drying process, with the extent increasing as pH approaches the isoelectric point. Mg and Ca appear to counteract the formation of these bonds so that when the pH is 7, there is no decrease in water holding capacity. When the pH is 5.4, the cations are not as effective in preventing the decrease in water holding capacity. Hamm and Deatherage (1960) suggested that freeze-drying causes formation of new electrostatic and/or hydrogen bonds in the isoelectric pH range of the meat and causes cleavage of linkages between the bivalent cations and the proteins. Their theory implies that the divalent cations do not have a negative effect on the rehydrated meat, and in this study a positive effect was found with the sedimented fibrils.

From the point of view of maintaining the original structure upon rehydration of freeze-dried meat, results from rehydration of the sponge-like fibril mass is of the greatest interest. Results show that the added ions have a negative effect on the rehydration capacity of the mass. This may be due to a negative effect on the wetability and/or diffusion of water into the dried protein structure. Problems of this kind in water penetration into dried meat has been touched on by Luyet (1962). He has thus shown that in whole muscle several obstacles to the penetration of the rehydrating solution exist such as occasionally occurring water repelling surfaces and impermeable membranes.

On adding cations, a general increase in the rehydration capacity of the sedimented fibrils with higher pH was found. The same phenomenon occurred for the fibril mass as the pH was increased, which supports the findings of Penny et al.(1963). The effect of pH on the fibril mass may partly be a consequence of better wetability of the proteins and an increased swelling of the wetted areas. By comparison of the hydration-pH curves of the intact fibril mass with the water holding capacity-pH curves of the sedimented fibrils, it seems that the curves at higher pH values are not as steep for the former as for the latter. Thus, an increase in overall swelling with high pH in the rehydrating solution did not appear as marked for the fibril mass as for the sedimented fibrils. When intact freeze-dried meat is rehydrated the hydation-pH curve may be even flatter than those for the fibril mass as indicated in fig. 3. The pH of the rehydrating solution had little effect on the rehydration of freeze-dried slices of pork loin according to Suden et al. (1964). The flatness of the curve may be due to a closer structure in the original meat after freeze-drying than occurred in the fibril mass. The looser structure of the fibril mass appears to give considerably more physically bound water than is true in rehydrated meat.

General experience in freeze-drying suggests that a loose or open structure gives better and more thorough rehydration. The problems with diffusion and wettability may therefore carry more weight for freeze-dried meat than for the fibril mass. Because of the relationship between the freeze-dried meat and fibril mass results suggest that a high pH before drying and removal of divalent cations, such as Mg and Ca, might bring about a better rehydration of freeze-dried meat.

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Table 1 Rehydration of fibril mass, without breaking structure, in 0.05 M succinate at pH 5.6 in g H_2O/g protein

	With added Mg and Ca ions	Without added Mg and Ca ions
Fibrils dried at pH 5.4	5.70	6.95
Fibrils dried at pH 6.0	6.87	7.82
Fibrils dried at pH 7.0	7.62	9.14

Table 2 Rehydration capacity of fibril mass without breaking of structure

	g H_2O/g protein
Fibrils without added ions	9.98
Fibrils with 0.4 mM Ca/kg	9.64
Fibrils with 2 mM Mg/kg	9.05
Fibrils with 0.4 mM Ca and 2 mM Mg/kg	9.08

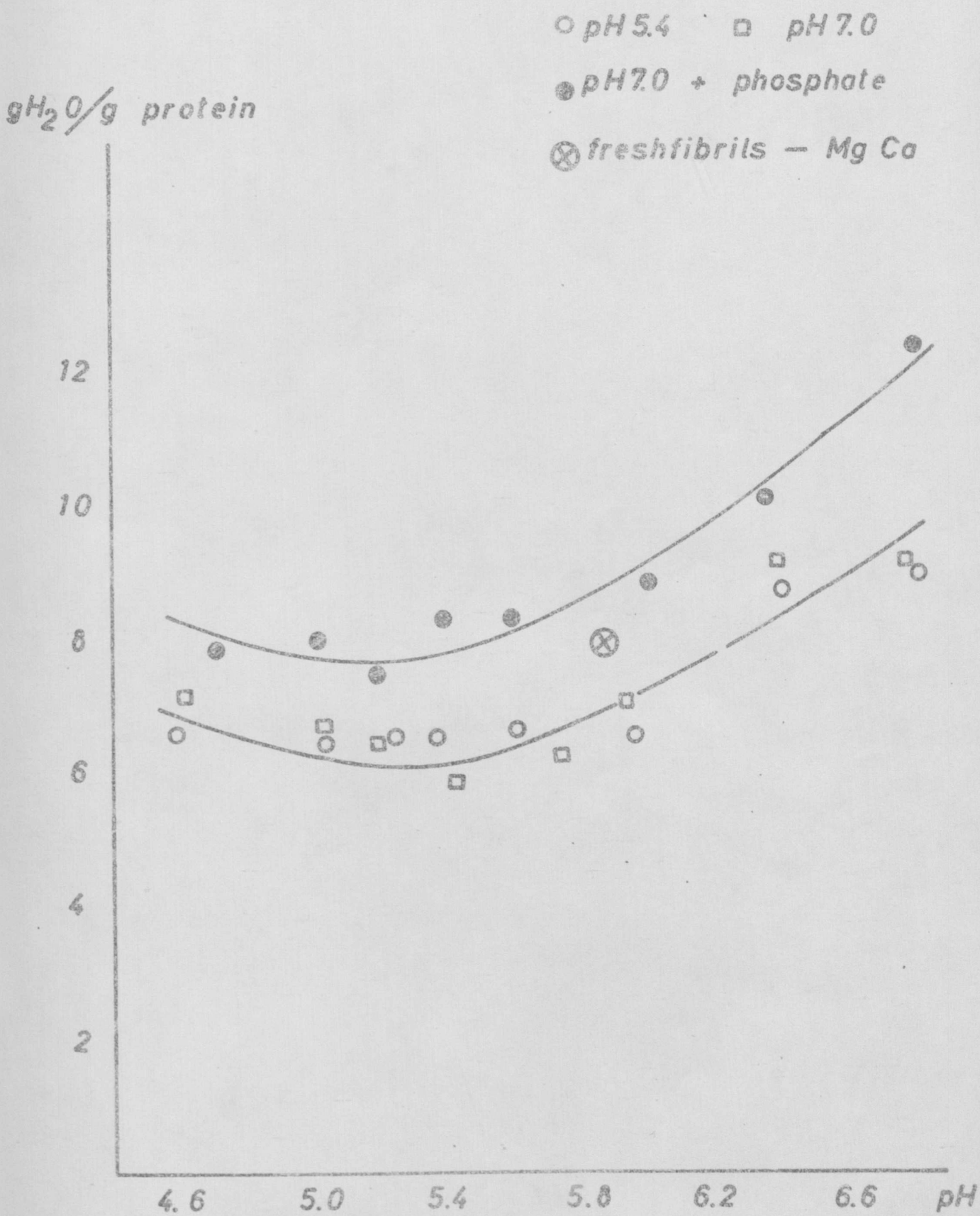

Figure 1

Water binding capacity of sedimented fibrils

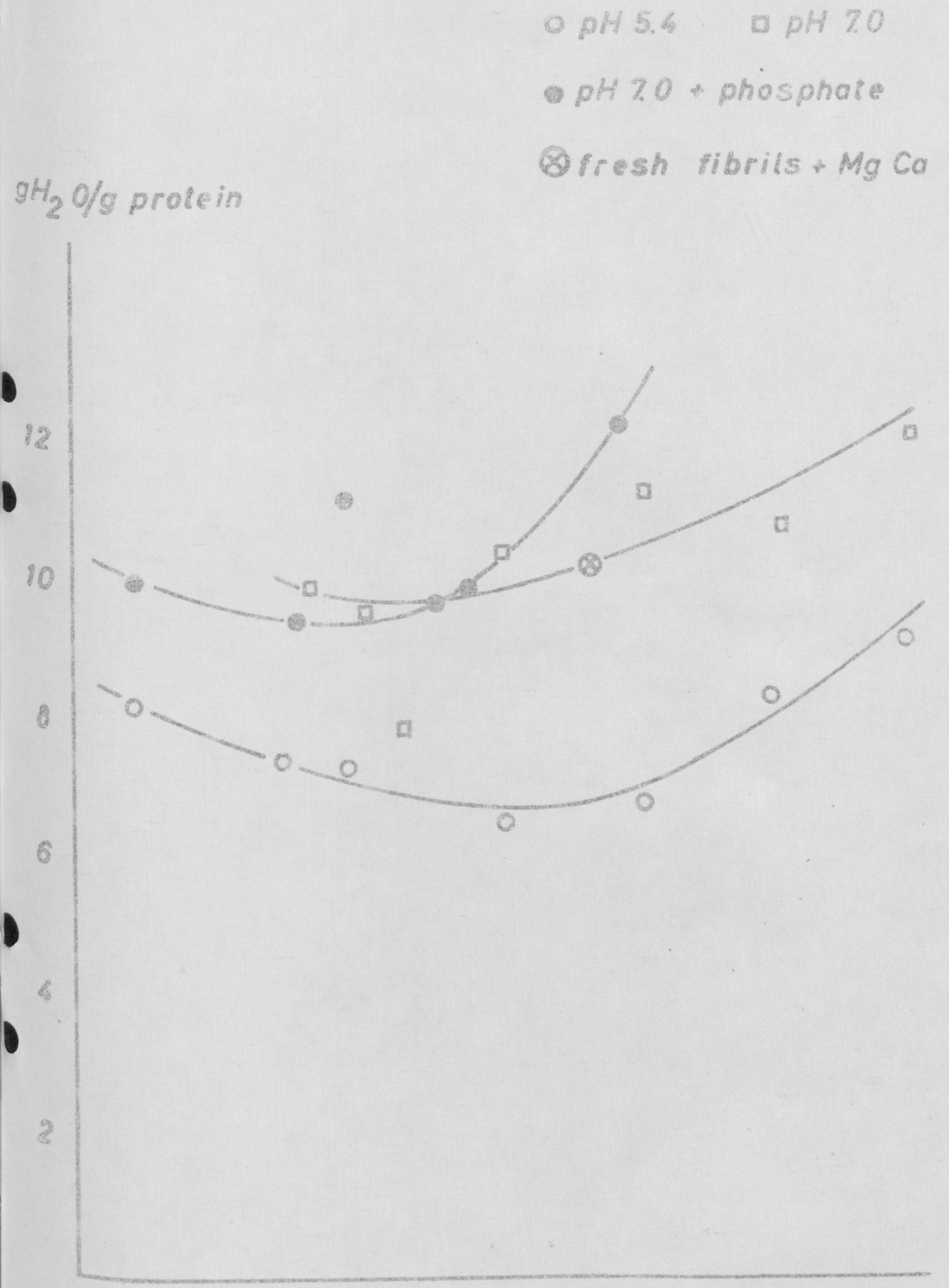

Figure 2

Water binding of sedimented fibrils with Mg, Ca

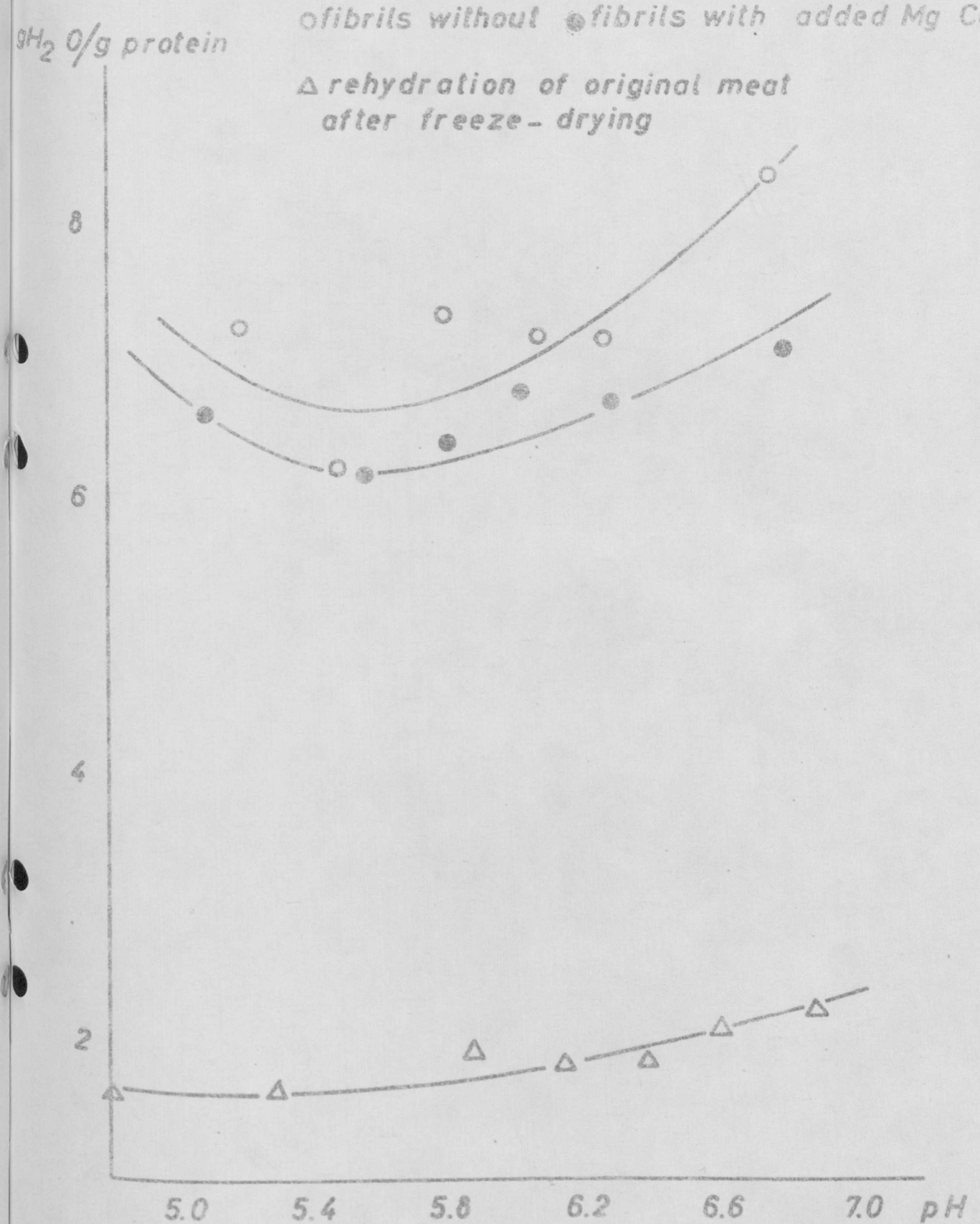

Figure 3

Water binding of freeze-dried pH5.4 fibril mass

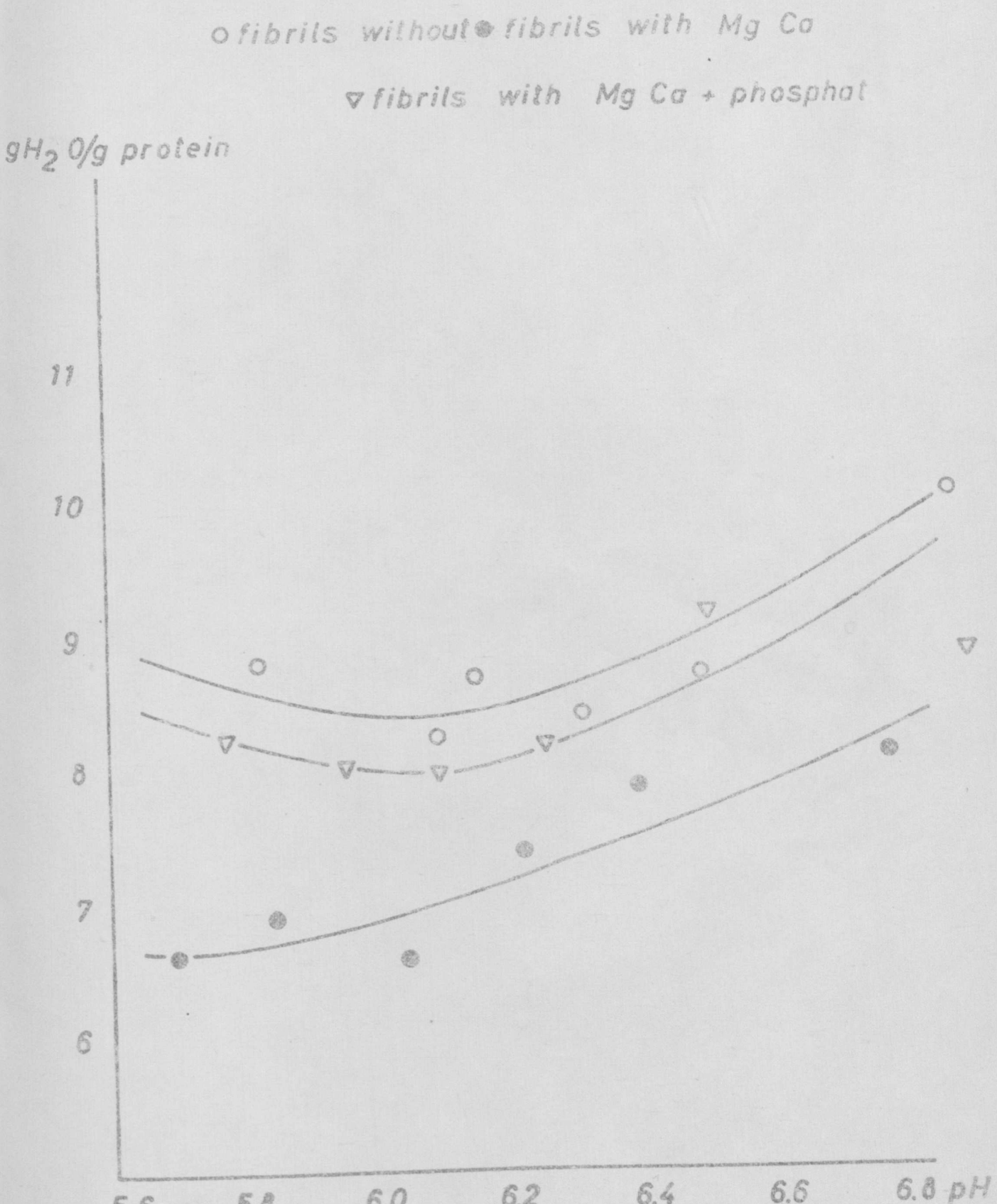

Figure 4

Water binding of freeze-dried pH7 fibril mass