SLAGTERIERNES FORSKNINGSINSTITUT
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FRYSETØRRING
Manuskript nr. 281

Effect of EDTA and pH on Properties of Freeze-Dried Pork Muscle

II. Effect of Injection of EDTA and NaOH before Drying[a]

J. Wismer-Pedersen[b]

Dept. of Food Science, Michigan State University

East Lansing, Michigan

Summary

The effects of EDTA, pyrophosphate and elevated pH on rehydration capacity and texture of freeze-dried meat were studied. Samples of pork loin were injected with solutions of EDTA and pyrophosphate in a conc. of approx. 10 mM per 1000 g meat. After injection, the samples were frozen and freeze-dried. The treated samples had improved rehydration capacities and texture as measured with the Warner-Bratzler shear press. The main effect of EDTA appeared to be due to better penetration of water into the dried meat structure, whereas, the effect of pyrophosphate appeared to be mainly due to swelling of the already wetted areas. These observations were substantiated by the results of an experiment, in which the samples were frozen but not dehydrated. In this case, only pyrophosphate improved the water binding capacity. Application of EDTA in the rehydrating solution did not improve the rehydration capacity of the freeze-dried samples. The average pH of the meat was raised approx. 1 unit through injection of NaOH solution before drying. This treatment did not appreciably improve hydration capacity or texture. Aging the meat before treatment with EDTA and NaOH did not improve the results. EDTA exhibited the same effects upon beef as with pork.

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[a] Joumal Article 3388, Michigan Agricultural Experiment Station, East

[b] Present Address: Danish Meat Research Institute, Roskilde

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Übersicht

Die Wirkungen von EDTA, Pyrophosphat und erhöhtem pH in Verbindung mit Rehydrierungskapazität und Struktur von gefriertrocknetem Fleisch sind untersucht worden. Proben von Schweinekamm wurden mit EDTA und Pyrophosphat in einer Konzentration von ungefähr 10 mM pro 1.000 Gramm Fleisch injiziert. Nach der Injizierung wurden die Muster gefroren und gefriertrocknet. Die behandelten Muster zeigten verbesserte Rehydrierungskapazität und Struktur. Die Messungen wurden mit der Warner-Bratzler Scherpresse durchgeführt. Die Hauptwirkung der EDTA scheint eine Folge der Verbesserung der Wasserpenetrierung in die getrocknete Fleischstruktur zu sein. Die Wirkung von Pyrophosphat ist aber hauptsächlich eine Folge der Aufschwellung der schon gefeuchteten Stellen. Diese Beobachtungen wurden durch die Resultate eines Experimentes mit gefrorenen aber nicht dehydrierten Proben bestätigt. In diesem Falle wurde die Wasserbindungskapazität nur durch Pyrophosphat verbessert. Zusatz von EDTA zu der Rehydrierungslösung hat die Rehydrierungskapazität der gefriertrockneten Proben nicht verbessert. Das Durchschnitt von pH des Fleisches wurde Injizierung von NaOH-lösung vor der Trocknung ungefähr 1 Einheit erhöht. Diese Behandlung hat keine wesentliche Verbesserung von der Hydrierungskapazität oder der Struktur verursacht. Reifung des Fleisches vor der Behandlung mit EDTA und NaOH hat auch keine Verbesserung des Ergebnisses verursacht. Die Wirkung von EDTA war die selbe auf Ochsenfleisch als auf Schweinefleisch.

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Introduction

In the first part of this study observations were made on a model system consisting of freeze-dried myofibrils. Results suggested that removal of the cations, magnesium and calcium, by EDTA before drying and adjustment of pH to 7 improved rehydration if the rehydrating solution was allowed to diffuse into the dried fibril mass. The purpose of the present investigation was to extend the observations on the isolated myofibrils to intact muscle samples. This was attempted by injecting neutralized EDTA solution to block the effects of Ca and Mg on the muscle proteins and by altering pH through injection of dilute NaOH solution before dehydration.

Experimental

Experiment 1

The longissimus dorsi muscles from a pig weighing approximately 200 lbs. was used as the source of meat. The pig was slaughtered in the Michigan State University abattoir and was of known origin. The loin was dissected out 2 days after slaughter, and cut into 4 cm thick slices. Each slice was split in 2 pieces of approx. equal size by cutting parallel with the fibres and perpendicular to the slice. Eighteen of the most uniform shaped samples were selected and distributed at random into 3 groups. Group 1 served as the control and was wrapped in aluminium foil and frozen at approx. -30°C. Group 2 was injected with a solution neutralized to pH 7.0 with NaOH and containing 30 g EDTA per liter. Group 3 was injected with a solution containing 50 g of sodium pyrophosphate per liter. The amount of solution injected corresponded to 10% of the weight of the fresh sample, giving a concentration of EDTA and pyrophosphate of about 10 mM and 11 mM per 1000 g meat, respectively. Injection was done with a 10 ml hypodermic syringe as uniformly as possible. Immediately after injection, the samples were wrapped in alumininum foil. The wrapped samples were then stored at 4°C for 3 hours to allow the injected solution to spread throughout, and were frozen in circulating air at approx. -30°C. The frozen samples were stored a few days at this temperature before freeze-drying. Freeze-drying was carried out on a heated plate in a Stokes laboratory freeze-drier. The plates were heated to a maximum temperature of 42°C, and the air pressure during drying was approx. 135 μ. The drying time was about 25 hours. After drying the samples were stored under nitrogen at room temperature and examined within 4 weeks.

The samples were rehydrated in 100 ml distilled water for 4 hours at room temperature. After rehydration the samples were heated for 30 minutes at 70°C. in distilled water containing 0,9% NaCl. The weight of the samples was recorded to the nearest 0.1 g before drying, before and after rehydration, and after heating. The rehydrated samples were plotted with Whatman No. 1 filter paper for about 1/2 minute to remove excess surface moisture. The texture of the samples was evaluated after heating and subsequent cooling to room temperature using the Warner-Bratzler shear. Two cores 12.5 mm. in diameter were cut out of the sample parallel to the fibers using a cork borer. Whenever, possible, 6 shear readings were taken on each sample.

Experiment 2

A loin similar to the one used in experiment 1 was divided into 18 samples, which were distributed into three groups as in experiment 1. Group 1 served as the control and was injected with distilled water. Group 2 was injected with a neutralized solution of EDTA and group 3 with a solution of sodium pyrophosphate. The concentration of the solutions and injection was the same as carried out in experiment 1. Immediately after injection the samples were wrapped in aluminum foil, held at 4°C for 3 hours and then frozen at approx. -30°C. After 2 weeks storage at this temperature, the samples were thawed at room temperature for 4 hours and heated for 30 minutes at 70°C in distilled water containing 0.9% NaCl. Weight changes and texture evaluations were carried out as previously.

Experiment 3

The material used in this experiment was beef instead of pork. The samples were cut from the central section of the longissimus dorsi muscle from at steer carcass of Choice grade after aging for 10 days following slaughter at approximately 4°C. The samples consisting of 6 slices 1 cm in thickness were split in two as in experiment 1. Each of the 12 samples was cross assigned to two groups so that opposite halves were in opposite groups. One group served as the control while the other was injected with a neutralized solution of EDTA. Concentration, amount of injected solution and the method of freeze-drying were the same as in experiment 1. Before rehydration, the two groups were split in two sub groups of 3 samples each. One group was rehydrated

in distilled water and the other half in a solution identical to the injected EDTA solution. Otherwise the samples were treated and studied the same as in experiment 1.

Experiment 4

Three loins similar to the one in experiment 1 were each cut in 4 pieces of approximately equal size. The pieces were then divided into 4 groups so that the different regions of the loin were presented as equally as possible in each group. The groups were aged at 4°C for 2, 4, 6, and 8 days after slaughter before being cut into samples. Each piece was cut into 4 samples as described in experiment 1, after 1-2 cm slices were discarded from each. The first sample served as the control, while the second sample was injected with the EDTA solution made as previously described, the third was injected with a solution containing 28 g NaOH per liter and the fourth was injected with the EDTA solution to which 28 g NaOH was added per liter. Amount of injected solution, further processing and treatment were the same as in experiment 1. The pH of the meat was measured with a Beckman Model G pH meter on comminuted samples in distilled water. In connection with this experiment, shear readings, as in the other experiments, were taken on fresh loins aged 2 and 8 days after slaughter. The shear readings were taken on samples from 8 loins similar to those in the other part of the experiment.

Results

Experiment 1

The average results of experiment 1 are presented in table 1. The variation in weight during drying, rehydration and heating of the samples is given as the percentage of the original weight. As absolute figures they have no value, but as the samples originate from the same muscle and the groups are equal in composition, the results reflect the relative effects of the treatments on the properties of the freeze-dried meat.

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The dried weights of the control and EDTA groups were the same percentage of the original weight, whereas, the samples in the pyrophosphate group had a slightly higher relative weight after drying. The difference between group 3 and the two other groups is statistically significant ($P < 0.05$). The reason for the difference is attributed to the effect of pyrophosphate on the water binding capacity of meat proteins (Bendall 1954; Hamm and Grau 1955). The average original weight of the samples in the pyrophosphate group was a little lower than the average for the other two groups. This should have favored drying of the samples containing pyrophosphate, other factors being equal. The correlation coefficient between the original weight, which varied from 43 to 89 g, and the percentage weight after drying for the samples in groups 1 and 2 was 0.60. This relationship is statistical significant ($P < 0.05$). With regard to the percentage weight after rehydration, groups 2 and 3 gave considerably higher values than group 1. This shows that the treatments improved the rehydration capacity of the freeze-dried meat. The difference between rehydration levels for the control group and the two other groups was highly significant ($P < 0.01$), whereas, the difference between groups 2 and 3 was not significant. The same results were obtained for the percentage weights after heating. Thus the effects of the treatments were not altered during the heating process. This is important as the higher rehydration capacity of the treated groups could be due to superficial binding of loose water.

Although there was little difference between the average hydration capacities of the treated groups, there was a difference in the relationship between the original weight of the meat and the percentage weight after rehydration, and after rehydration and heating. In general there was negative relationship between original sample weight and these two measures of % rehydration. Figure 1 depicts this relationship for groups 1 and 2. It shows that the decrease in rehydration with increasing sample weight was considerably less for the EDTA treated group than for the control. The correlation coefficients between original sample weight and percentage rehydration of the heating were -.26 and -.67 for the EDTA and control groups, respectively. Thus, the higher rehydration of the EDTA treated samples relative to the control was most apparent at higher sample weights. The EDTA appeared to have increased the speed and/or extent

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of water diffusion into the dried meat. On figure 2 is shown the same relationship for the control and pyrophosphate treated samples. The rehydration of the pyrophosphate treated samples fell faster with increasing sample weight than was the case for the control samples.

The correlation coefficients between original samples weight and percentage rehydration were -.84 and -.67 for the pyrophosphate and control samples, respectively. Thus, the higher rehydration capacity for the pyrophosphate group was especially marked at lighter sample weights. It appears that the extent of water diffusion into the dried meat treated with pyrophosphate is about the same for the two groups but the wetted areas swell to a considerably greater extent in the pyrophosphate treated samples. Thus, the rehydrated pyrophosphate-treated meat may at the same level of rehydration be farther from having regained the properties of the original meat than the meat in the EDTA group. In evaluation of the results, we must bear in mind that an almost complete absorption of water by the freeze-dried meat does not necessarily mean that the meat has regained its former structure (Brooks 1958).

The average Warner-Bratzler shear value was considerably higher for the control than for the treated groups. The difference was statistically significant ($P < 0.05$). The effect of pyrophosphate is in line with Connell's (1962) observation that addition of pyrophosphate slightly improves the texture of accelerated freeze-dried meat. The lower shear values which denote more tender meat appear to be due to improved rehydration capacity. Figure 3 shows the relationship between the percentage weight after heating and the shear value. The shear values were inversely related to rehydration capacity. The correlation coefficient was found to be -.80, which is highly statistically significant. Although the difference was not significant, there is a tendency towards the EDTA group having a slightly better texture than the pyrophosphate group.

When the significance of the texture differences as measured by shear values are considered, we must remember that fresh meat from one particular muscle does not

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have one definite shear value. However, there is a general range within which the shear values most often fall. Figure 4 shows block diagrams of all the individual shear readings for the fresh meat samples studied in experiment 4, and the individual values for the rehydrated samples of experiment 1. The fresh meat values are spread over a range between 4 and 11 lbs., with most values between 5 and 9. The rehydrated control samples show a limited number of values in this same range. These values are associated with the fully rehydrated areas of the samples. Most values are, however, in the range 10 to 13 lbs. These values are associated with the partly rehydrated areas of the samples which is responsible for the general toughness of the rehydrated meat. The rehydrated treated samples show most values within the range of normal meat, although a few observations lies in the range of 10 to 13 lbs. The pyrophosphate group show slightly more values in the tough range than the EDTA group. Such a distribution might be expected if the samples in former group should have more incompletely rehydrated areas than the EDTA-treated samples.

Experiment 2

As the freeze-drying process involves two steps (1) freezing of the meat, and (2) removal of the water from the frozen meat, the properties of the freeze-dried meat may be a function of changes occurring in either or both steps. In order to elucidate the effects of EDTA and pyrophosphate when dehydration is omitted, this experiment was carried out identically to experiment 1 but involved only freezing of the meat. Table 2 summarizes the results. The pyrophosphate treated samples showed less shrinkage after thawing as well as after heating than the EDTA and control samples. The differences between the pyrophosphate group and the two other groups were highly significant ($P \leqslant 0.01$). Results are in line with Australian observations on the effect of pyrophosphate on the water holding capacity of frozen and thawed meat (Howard 1960). It is, however, important to note that the EDTA treated samples gave values close to those of the control samples. This shows that the effect of EDTA is exerted during the second step in the process, namely, the removal of water from the frozen meat. The subsequent effect is to increase the ability of the dried meat fibers in absorbtion water on rehydration. The improved water holding capacity of the pyrophosphate treated samples in this experiment is probably the reason for the lower shear values recorded in group 3.

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Experiment 3

This experiment was performed to ascertain if the effect of EDTA also occured with beef as well as with pork and to determine the effect of EDTA in the rehydrating solution upon rehydration capacity. The results are shown in table 3. EDTA treatment before drying substantially increased the rehydration capacity before and after heating. The difference between the control and EDTA group was highly significant ($P < 0.01$). Thus, EDTA also improved the rehydration of beef as well as pork. When the EDTA was applied in the rehydrating solution, no increase in rehydration capacity was found, in fact, there was a tendency for rehydration capacity to decrease, which was most noticeable in the control group. Results show that EDTA per se does not increase rehydrating capacity, but is effective only when added to the meat before the drying process.

Experiment 4

Studies with the isolated myofibrils indicated that the water holding capacity on rehydration increased directly with the pH of the freeze-dried material. In this experiment injection of dilute NaOH solution in the meat prior to freeze-drying was used to study the effect of pH on the rehydration capacity of the meat slices. The NaOH treatment was used alone and in combination with EDTA treatment. The experiment was also designed to study the effect of aging on the effectiveness of the treatments. Arnold et al. (1956) found that during aging of beef, calcium ions are continuously released by the muscle proteins. Their results also indicated that magnesium ions are released during aging. It is conceivable that such an ionic shift might enhance the EDTA effect. The associated experiment with aging of the fresh samples showed that the texture measured by the shear became definitely more tender during aging from 2 to 8 days after slaughter. At 2 days, the average shear value was 7.0 lbs. compared to 4.9 lbs. after 8 days. The difference was highly significant ($P < 0.01$). Aging of the meat before drying might be an advantage if the increased tenderness could be carried over to the rehydrated meat. Table 4 gives the results of the experiment. The weight after drying in % of the original sample weight generally was higher for the alkali treated samples. The effect of the alkali treatment is highly significant ($P < 0.01$)

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and must be ascribed to the increased water holding capacity of the meat proteins at elevated pH. The pH determinations indicated that the treatment had increased the average pH of the samples from 5.3-5.4 to 6.4-6.9. When the relative rehydrated weights before and after heating were studied, there was a tendency for increased hydration capacity as a result of the NaOH treatment. Only the increased rehydration capacity brought about by the EDTA treatment was statistically significant ($P < 0.05$). With regard to texture, the shear press gave lower values only for the group treated with EDTA at normal pH. The difference between the average values of this group and the other groups was statistically significant ($P < 0.05$). Neither aging nor the interaction of aging x EDTA treatment had any appreciable effect on rehydration capacity or texture.

Discussion and Conclusion

Results indicate that injection of a neutralized solution of EDTA before freeze-drying does not affect the removal of water during dehydration but it increases the rehydration capacity of the freeze-dried meat. Improved rehydration appears to be due to the increased ease of diffusion of the water into the dried meat structure. The difference in rehydration capacity between the EDTA-treated samples and the controls is most pronounced at high sample weights. EDTA does not appear to improve the water binding capacity of the meat per se. The experiment with frozen meat showed that EDTA does not increase the water binding capacity of already hydrated meat. The effect of EDTA seems to take place in the meat before and/or during removal of water in the drying process. In light of the observations with the freeze-dried myofibrils, the effect of EDTA most likely is associated with removal of magnesium and calcium ions from the fibrillar proteins through chelation before drying. Actually the positive effect of EDTA on the meat samples shows that the results with the myofibril system may have practical value. In addition to this effect, EDTA may also act on the sarcolemma. Dennis and Rothstein (1955) thus found that 10% of the calcium content in muscle is

bound on the cell surface. Pyrophosphate gave an increase in rehydration capacity of the same average magnitude as EDTA. The results indicated, however, that the mechanism was swelling of the wetted areas rather than improvement of water diffusion into the dried meat. This swelling effect also decreased the efficiency of freeze-drying the meat samples. In contrast to the results with myofibrils, an increase in pH of the meat through injection of dilute NaOH did not appreciably improve rehydration capacity. The probable reason for failure of alkali injection to improve texture may be a result of high pH in isolated areas prior to diffusion throughout the tissues. The damage thus incurred in some instances gave rise to brown patches with disintegrated fibers in the core of the samples. Artificial regulation of pH in the meat through direct injection of NaOH can not replace the adjustment of pH through depletion of the glycogen depots of the live animal as was done by Penny et al. (1963). It is possible, however, that a milder method to artificially increase pH may produce the desired result.

The texture of the rehydrated control samples as judged by the Warner-Bratzler shear was predominantly tougher than fresh meat after identical heat treatment. Due to the close relationship between texture and rehydration capacity, the treatments with EDTA and pyrophosphate considerably decreased the number of shear readings falling outside the range of fresh meat.

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Table 1. Effect of EDTA and pyrophosphate on weight and texture of freeze-dried pork after rehydration. Average results of experiment 1

Group	Treatment	Original weight in g	Weight after drying % of original	Weight after rehydration % of original	Weight after heating % of original	Warner-Bratzler shear in lbs.
1	control	65.7	27.8	73.5	58.2	9.8
2	EDTA	65.7	27.7	83.4	62.4	6.5
3	pyrophosphate	60.7	29.1	85.0	64.3	7.8

Table 2. Effect of EDTA and pyrophosphate on weight and texture of frozen pork after thawing. Average results of experiment 2.

Group	Treatment	Original weight in g	Weight after thawing % of original	Weight after heating % of original	Warner-Bratzler shear in lbs.	Average pH
1	control	64.1	94.9	71.3	6.4	5.53
2	EDTA	61.8	94.0	69.8	7.9	5.65
3	pyrophosphate	62.0	99.7	78.3	3.3	5.75

Table 3. Effect of EDTA treatment before freeze-drying and during rehydration on weight of freeze-dried pork after rehydration. Average results of experiment 3.

Group	Treatment before drying	Rehydrating solution	Original weight in g	Weight after drying % of original	Weight after rehydration % of original	Weight after heating % of original	Average pH
1 a	control	control	55.5	29.9	81.1	59.6	5.74
1 b	control	EDTA	52.3	30.7	74.4	56.8	6.14
2 a	EDTA	control	56.8	31.5	90.2	61.9	5.76
2 b	EDTA	EDTA	57.1	31.4	88.5	63.2	6.14

Table 4. Effect of alkali, EDTA treatment and aging of the meat before freeze drying on weight and texture after rehydration. Average results of experiment 4.

Aging time in days	Treatment	Original weight in g	Weight after drying % of original	Weight after rehydration % of original	Weight after heating % of original	Warner-Bratzler shear in lbs.	pH of the rehydrated meat before heating
2	control	53.0	28.5	67.5	51.0	16.0	5.29
	EDTA	56.3	28.1	61.6	49.3	15.9	5.34
	alkali	49.1	29.7	62.9	53.0	17.7	6.93
	EDTA+alkali	53.9	31.6	63.9	55.1	20.3	6.68
4	control	49.2	29.7	58.4	48.6	15.8	5.33
	EDTA	45.1	29.2	76.9	56.9	7.0	5.34
	alkali	57.0	30.0	59.2	51.0	15.0	6.43
	EDTA+alkali	50.0	30.1	63.8	52.2	14.7	6.53
6	control	59.9	28.8	58.5	51.0	14.7	5.45
	EDTA	51.3	29.1	66.0	55.9	12.2	5.34
	alkali	53.3	29.9	57.4	51.2	15.9	6.61
	EDTA+alkali	54.8	29.8	59.6	52.3	15.5	6.45
8	control	51.2	29.4	61.9	50.9	15.3	5.40
	EDTA	48.4	30.2	71.0	54.2	11.3	5.35
	alkali	52.1	30.7	64.1	54.8	13.2	6.43
	EDTA+alkali	51.8	30.6	70.9	59.2	11.0	6.60

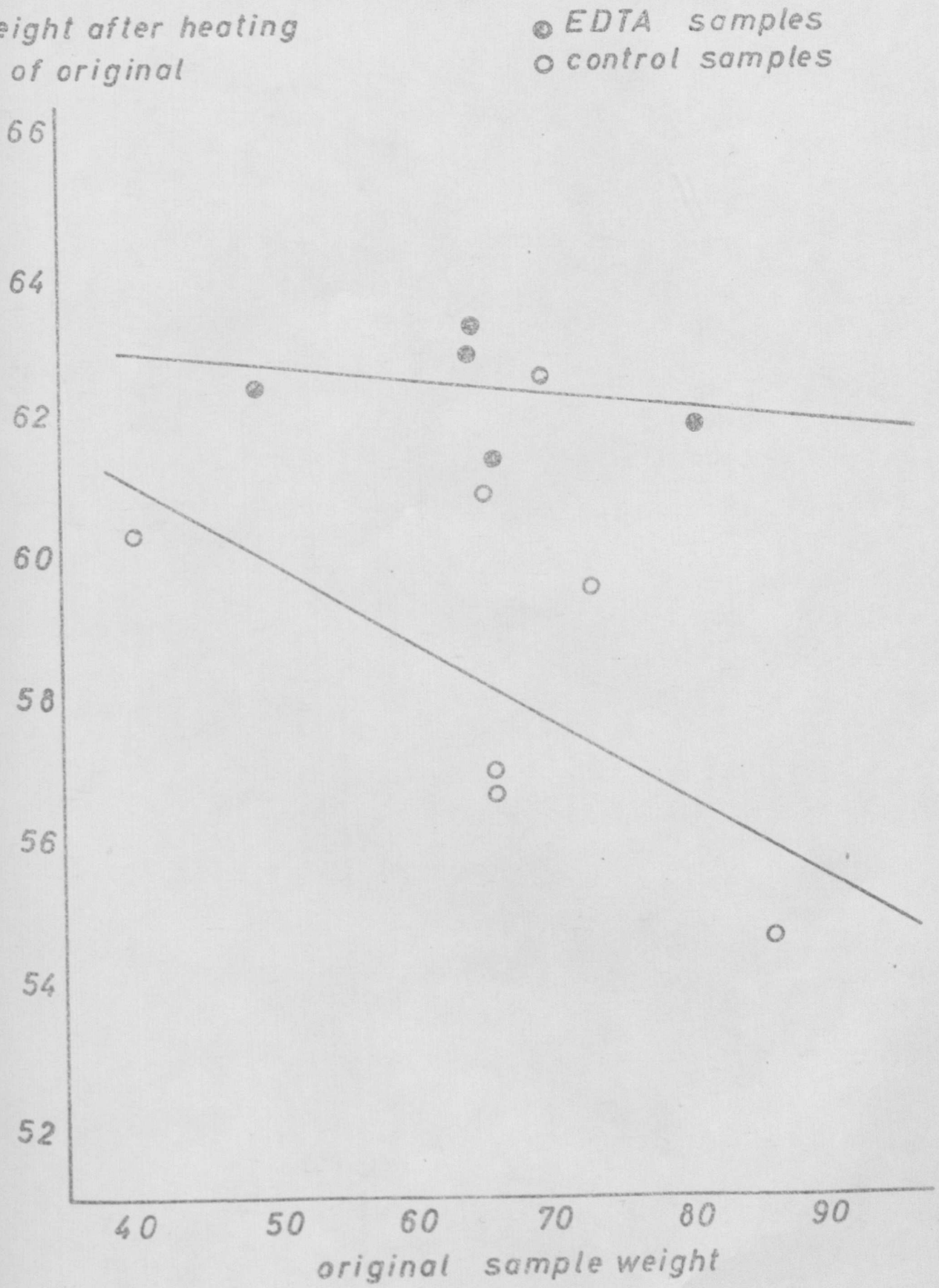

Figure 1

Relationship between original sample weight and rehydrated weight for control and EDTA samples

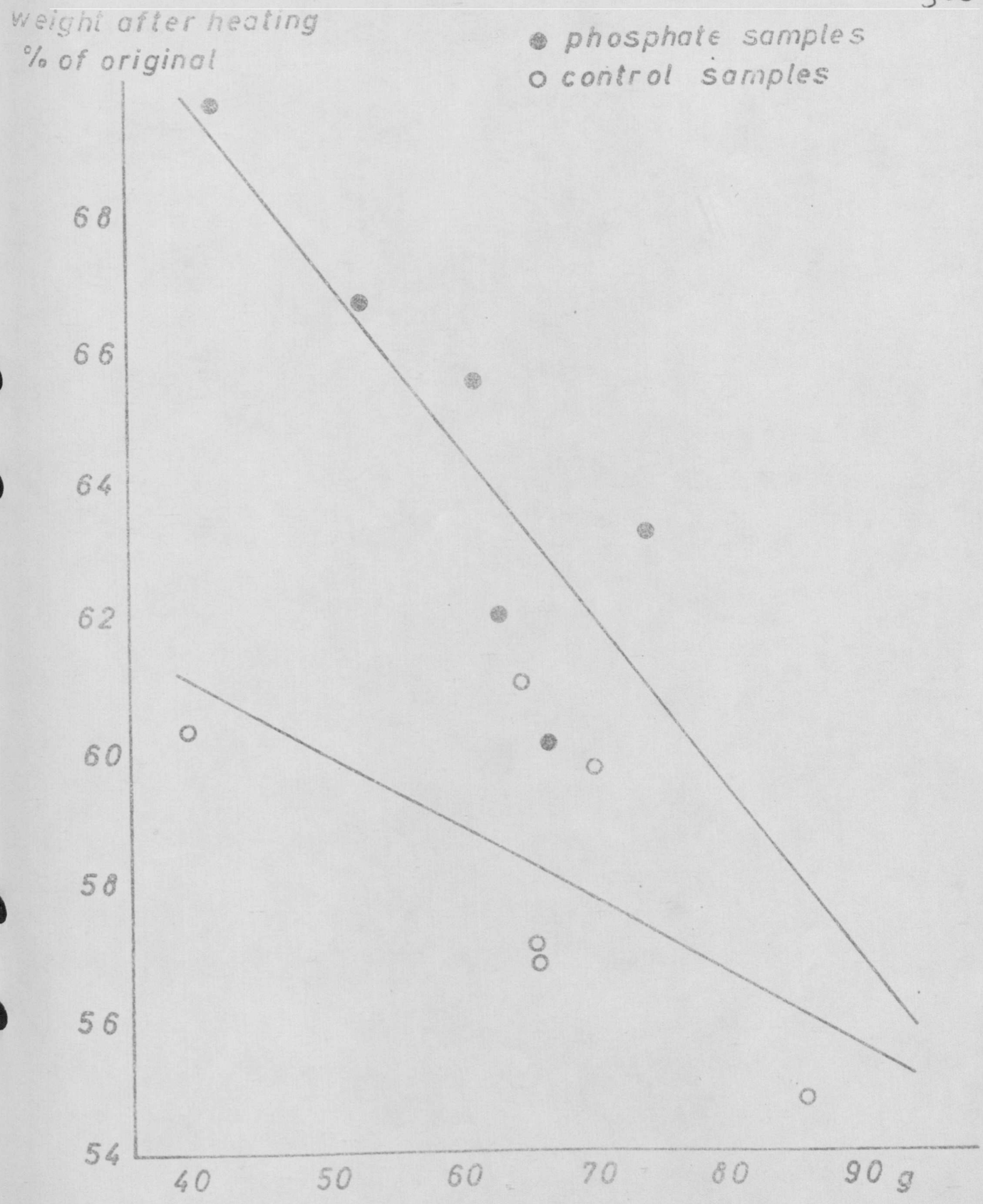

Figure 2

Relationship between original sample weight and rehydrated weight for control and phosphate samples

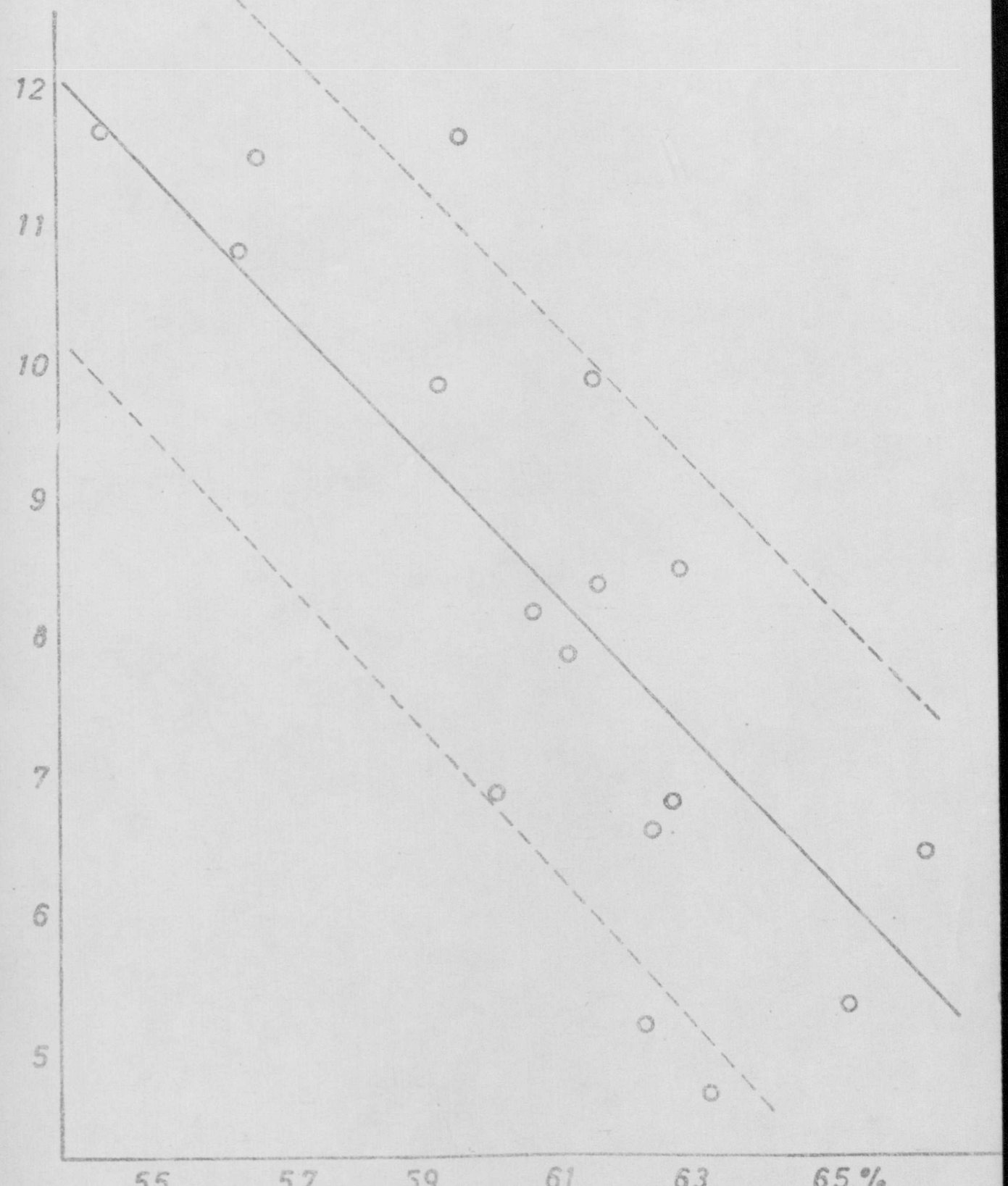

Figure 3

Relationship between rehydrated weight and shear value

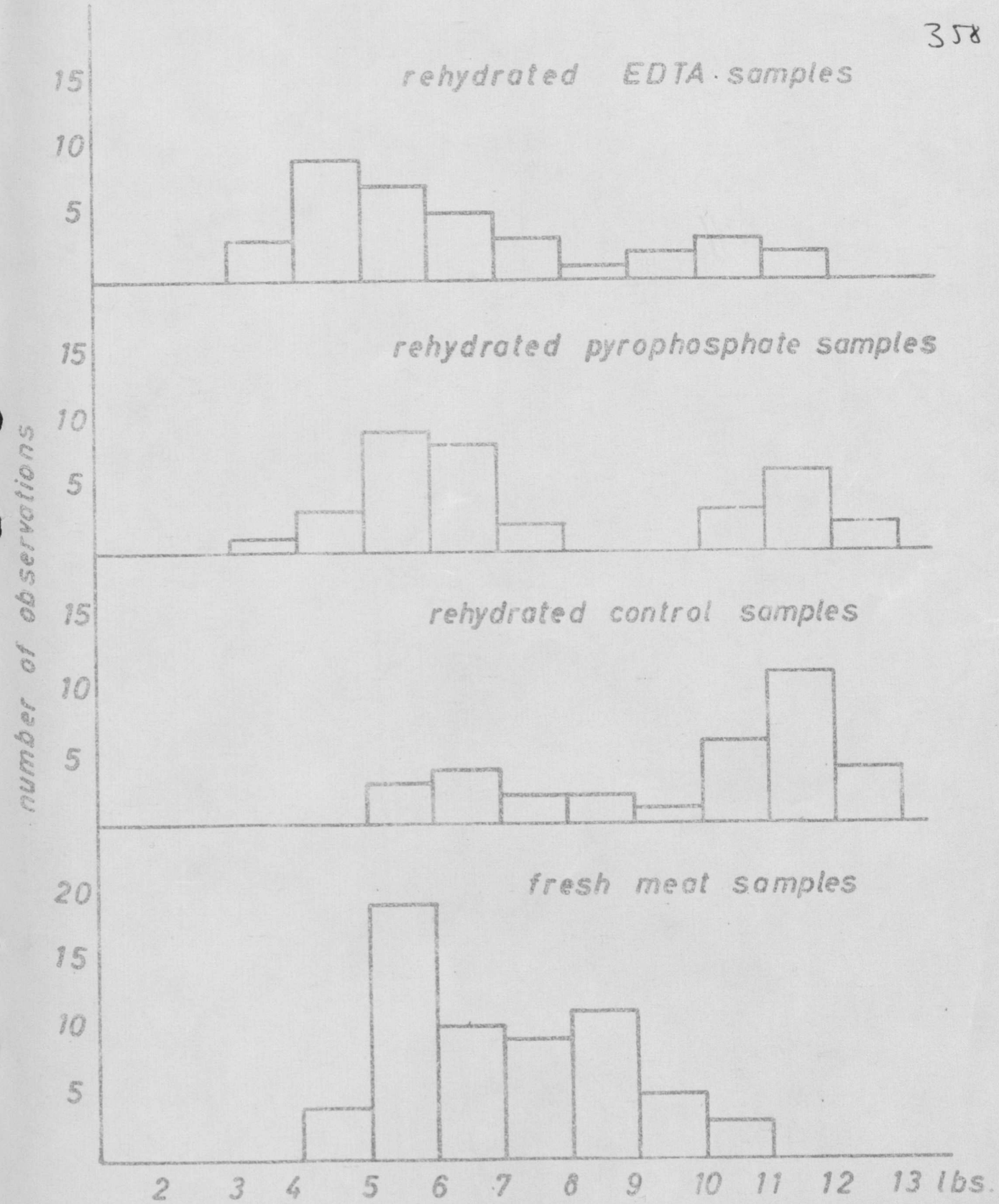

Figure 4

Distribution of individual shear values for fresh meat and rehydrated samples