

# GELATION PARAMETERS OF ENZYMATICALLY MODIFIED SOY PROTEIN ISOLATES<sup>1</sup>

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## ABSTRACT

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Soy protein isolates that were enzymatically modified were tested for gelling characteristics by heating and cooling. The irreversible gels were subjected to pressure and the gel deformation was measured by a specially designed apparatus. Parameters pertaining to that deformation were obtained by computer analysis of the rate of deformation. The

parameters were then related to the percentage enzyme-treated protein in the gel and to the kind of enzyme used in the digestion. In general, it was found that treatment with plant proteases was producing better gels than treatment with animal enzymes. Ficin seemed to be the best enzyme for producing the irreversible gels.

The gelling properties of the soybean and of its proteins have been of interest to many researchers who have studied soy and its uses. In one of its most ancient applications in the Orient, soy was used in the form of gel—Tofu. In recent years, with the decrease in availability of animal proteins and their increased price, attempts were made to substitute soy and its proteins for those gelling components previously obtained from other sources employed in prepared foods.

In connection with these efforts, some basic studies on the gelation of soy proteins were undertaken. Circle *et al.* (1) reported that the viscosity of heat-set gels was not significantly altered by changes in pH close to neutrality. High content soy protein gels were examined by Aoki and other Japanese workers (2-9) with an attempt to elucidate the involvement of sulfhydryl-disulfide interchange during gel formation. Penetrometer data were correlated with mastication tests in these studies. Similar high content gels were investigated by Yasumatsu *et al.* (10). They checked various soy protein products (from flours to isolates), measuring gelation in sausage casings by tests on industrial texture measurement devices. Catsimpoolas and Meyer (11-13) devised an explanation for heat-gelling of soy proteins in terms of hydrogen bond formation. They studied reversible-nonreversible gel transformation. A reversible gel was produced by the Northern Regional Research Laboratory group by alcohol extraction of either soy protein (14, 15) or soy flakes prior to protein isolation (16).

One common characteristic of all of these reports was the size of the protein molecules tested. In all cases the proteins, whether isolated or not, were not subjected to major disruptive or hydrolytic attack. Up to now, no report has been seen on the gelation of an enzymatically modified soy protein isolate, nor has there been a description of any attempt to modify soy isolates by enzymatic digestion to simulate gels of animal proteins.

This study started with the aim of trying to duplicate the gelling properties of

<sup>1</sup>Presented at the 58th Annual Meeting, St. Louis, Mo., Nov. 1973.

egg white solid. With this in mind, it followed the clue supplied by Huggins *et al.* (17), who discovered that even a nongelling albumin such as bovine serum albumin can gel, provided its disulfide linkages are reduced and the molecule is unfolded by breaking the hydrogen bonds. Two things were therefore attempted: 1) reducing the size of the soy protein molecules to simulate those of egg white, and 2) introducing sulfhydryl groups into the molecules by interchange reduction of disulfide bonds. The postulate was that, once these two aims were achieved, one could, by unfolding the molecule during heating, produce adequate gels similar to egg white. Gelling proteins were produced. A way was found to measure their gelling ability in a quantitative manner, and optimum conditions for gelling were determined.

## MATERIALS AND METHODS

### Base Materials

Soy base material was generally an industrially prepared extracted soy flake of high protein solubility (Nutrisoy 7-B [ADM]). A commercial isolate was used occasionally (Promine-D [Central Soya]). Analysis of these products is shown in Table I.

### Enzymes

Ficin, bromelin, pepsin, and papain were obtained from Miles Laboratories. The activities and concentrations used in digestion are given in Table II. All other chemicals were analytical grade.

### Preparation of Isolates

The high protein solubility soybean flakes (hexane-extracted and flash-desolventized) were extracted twice by slow stirring with twenty times (first extraction) and ten times (second extraction) their weight of water, and brought to pH 9 with sodium hydroxide after mixing. Extraction times were 60 min (first extraction) and 30 min (second extraction). The supernate was separated from the exhausted flakes by centrifugation in a basket centrifuge. The extracts were

TABLE I  
Analysis of Materials

	Nutrisoy 7-B	Promine D
Moisture <sup>a</sup> (14.004) <sup>b</sup>	8.32	5.28
Crude protein <sup>c</sup> (46-10)	52.30	40.60
Fat <sup>a</sup> (2.049)	0.86	0.12
Ash <sup>a</sup> (2.049)	5.52	4.17
Fiber <sup>a</sup> (7.054)	3.13	0.60
Carbohydrate <sup>d</sup>	29.87	0
PDI <sup>c</sup> (46-24)	92.9	80.1
NSI <sup>c</sup> (46-23)	73.7	81.5

<sup>a</sup>Analyzed by AOAC Methods of Analysis, 1970.

<sup>b</sup>Numbers in parentheses refer to analysis numbers in footnotes a and c.

<sup>c</sup>Analyzed by AACC Approved Methods, August, 1973.

<sup>d</sup>By difference.

filtered through glass wool and brought to pH 4.7–4.8 at 43°C. The curds were left to settle and the supernate was siphoned off. The curds were then washed three times with water using ten times the weight of the flakes each time. The curd was separated from the wash by filtering through an orlon fine mesh screen with the aid of vacuum. It was then brought to volume with water (four times the weight of the flakes), the pH was adjusted as specified (if not specified, the pH was 9.6), and it was stored overnight in the refrigerator before digestion. Sometimes it was freeze-dried before this last step, after adjustment to pH 7.0.

#### Protease Digestion

The dissolved isolate was digested at a concentration which was set after preliminary experiments (see below) at 1.00% with the desired enzyme for the time designated at the temperature specified. In most cases, the pH of digestion was optimum for the enzyme as given by the manufacturer. After digestion, the protein was reprecipitated at pH 4.75, washed twice with five times the weight of distilled water, and adjusted to the desired pH before freeze-drying.

#### Measurement of Protease Strength

This was done by a modification of the method of Kunitz (18). Casein was used as a substrate and the Casein units (CU) were calculated by the equation:

$$\text{Casein units} = \frac{\text{mg protein solubilized} \times 50,000}{70 \times \text{enz. conc. (mg)}} \times \frac{60}{\text{time of digestion}}$$

#### Molecular-Weight Determinations

Molecular-weight determinations were made by the electrophoretic method of Bietz and Wall (19). Protein, 2.5%; sodium dodecyl sulfate (SDS), 10%; borate buffer, 0.125M (pH 8.9); mercaptoethanol, 1% heated at 100°C for 5 min.

#### Gelation Measurement

1. *Time of Gelation Method.* The protein was dissolved in distilled water at a specific concentration, pH, and ionic strength. Cysteine was added after solubilization was complete (using a magnetic mixer).

TABLE II  
Activity and Usage of Proteases

Enzyme	Specified Activity <sup>a</sup>	Digestive Power <sup>b</sup>	Concentration [ <sup>E</sup> /subst.]
Ficin	1300–1200 Nu/G	5000 Cu/G	0.001
Bromelin	1500 Nu/G	1500 Cu/G	0.001
Papain	1250 Nu/G	2000 Cu/G	0.001
Pepsin	3500 μ/G	10,000 Cu/G	0.0001
Trypsin	500 μ/G	10,000 Cu/G	0.0001
Pancreatin	3 × NF	15,000 Cu/G	0.0001
Acid fungal protease (AFP)	1800 μ/G	5,000 Cu/G	0.001

<sup>a</sup>Activity as specified by the manufacturer.

<sup>b</sup>Activity as analyzed in our laboratory (see MATERIALS AND METHODS).

The solution was placed in 3-ml portions into test tubes ( $200 \times 18$ ) and placed in a water bath at the specified temperature. Every 30 sec, a test tube was taken out and checked by cooling to see if it hardened. The gel strength is reversibly related to the time it took the protein to gel; or, gel strength =  $1/\text{time of gelation (min)}$ .

2. *Semi-Qualitative Method.* This method was used when a large number of variables were to be studied. The method was similar to 1) above, except that each test was run in many duplicates, time of heating was 30 min, and the strength of the gel is indicated by a number from 0 to 5 depending on the ease with which the gel could be shaken to break it up.

- 0—no gel (liquid)
- 1—very weak gel (viscous)
- 2—weak gel (clumpy)
- 3—medium gel (clumps stick together)
- 4—good gel (hard to break up), and
- 5—strong gel (withstands shaking)

3. *Quantitative Gelometer Measurements.* The gelling mixtures were prepared by dissolving the proteins in distilled water at the concentration desired. Salt was added ( $0.5M$ ) during the mixing. This solution was poured into identical beakers to the same height so that the gels were of equal physical dimensions. After heating at the required temperature for the designated time, the beakers were cooled in running water (room temperature). During heating, the beakers were covered to reduce evaporation. The gels were released onto filter paper pads by inversion and tapping. At times they were released from the walls with the aid of a thin spatula.

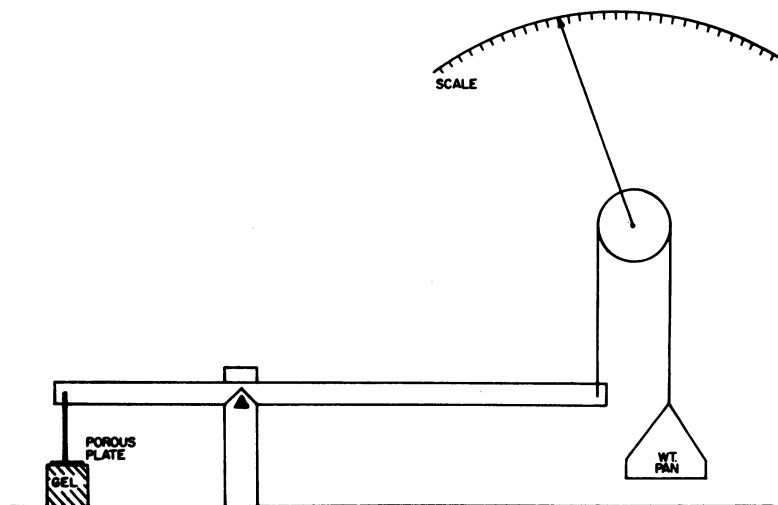


Fig. 1. Gelometer.

The cooled gels were tested in the gelometer (Fig. 1). The readings on the scale were equal to a 0.0909 mm depression of the plate per division. The value of depression was calculated per unit pressure applied in Barye (dynes/cm<sup>2</sup>), since it was found that depressions were proportional to the pressure in the range of weights used (10–20 g).

$$D = d/P \text{ [cm/Bar]}$$

where

D = specific depression,

d = linear movement of the pressure plate, and

P = pressure exerted on the plate.

The pressure plate was made of porous polyethylene. This reduced side slipping. The filter paper under the gel served a similar purpose. It also served to absorb any exudate formed during the depression. This enabled a qualitative evaluation of the "syneresis"—loss of water under pressure. No quantitative evaluation of this property was attempted.

The depression (per unit measure) was fitted to an equation (see RESULTS AND DISCUSSION) and the parameters were calculated by computer.

## RESULTS AND DISCUSSION

### Reversibility

In all of the gels reported here, the gelation was accomplished by heating to such a degree that on cooling a permanent gel was produced. In no case did we encounter the progel-gel relationship described by Catsimpoolas and Meyer (11). On cooling, a gel (or a clump) was formed which could not be liquefied on warming or heating. The only effect seen when these gels were heated was a drying of the surfaces with eventual cracking due to loss of water. Further heating resulted in browning or charring of the surfaces.

### Effect of Cysteine and Salt

In preliminary experiments, Promine D was digested by pepsin and isolated as

TABLE III  
Gelation of Pepsin-Treated Soy Proteins<sup>a,b</sup>

Sample	Additive			
	None	Cysteine 0.025 g/g Protein	Salt 0.01M	Cysteine + Salt
	Time, min, to gelation			
1	∞	30	30	15
2	∞	35	40	7

<sup>a</sup>Digestion at 5% protein, pH 1.9 to 2.5, 0.002% pepsin, 4 hr, 40°C.

<sup>b</sup>Gelation at 10% protein.

in the methods. The results in Table III show that, as we theorized, based on the work of Huggins *et al.* (17), a judicious addition of cysteine improved the gel. Also, we noticed that salt had a synergistic effect with cysteine. Protein recovery was only 12% of the starting proteins. Reproducibility was poor. This was found to be due to the neutralization procedure.

#### Effect of "Exposure" to High pH and Salt Addition

Kelley and Pressey (20) found a beneficial effect on gelling by raising the pH of the soy protein isolates. In Fig. 2 we see the effects of "exposure" to different pH levels prior to neutralization and drying of the gelling protein produced by ficin digestion. At pH levels above 10.5, the protein after drying was not completely soluble, as noted by the sedimentation of material prior to heating. It was then decided to keep exposure constant at pH 10.5. Time of exposure was unimportant but for convenience it was left overnight. Temperature of exposure affected solubility; 6°C was chosen as the most practical means of eliminating this effect. In these experiments the protein was isolated from the flakes. Ficin was used in the digestion (0.001 g/g protein). Yields as high as 75% of total protein digested were obtained. Method two of gellation measurement was used. The reason for the nonreproducibility of the previous experiments was found in

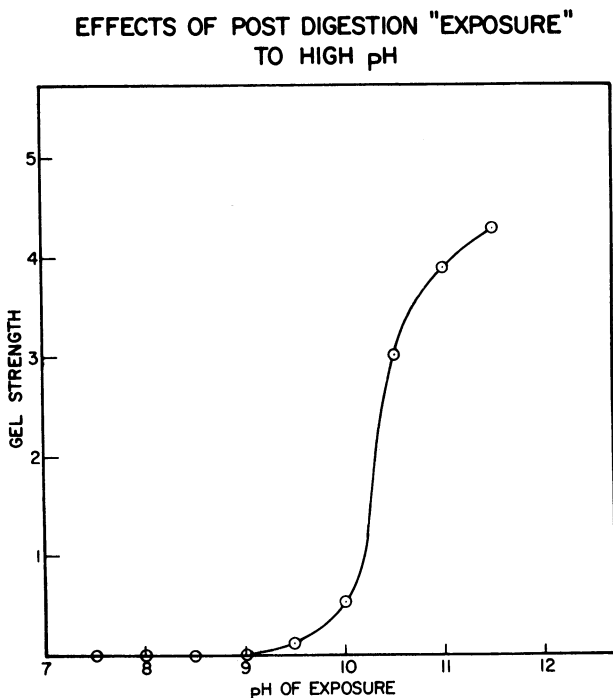


Fig. 2. Digestion—ficin/protein = 0.001/1; pH 9.0; 60 min at 32°C. Gelation—4% protein, 10 min at 90°C; measured by method 2.

the unevenness of neutralization. Local, excessive pH levels were apparently affecting the gelling properties irregularly. From this point on, neutralization was done carefully by dropwise addition with strong mixing to eliminate these local effects.

Ishino and Okamoto (21) have recently noticed that in intact soy protein isolates (undigested), treatment at high pH levels (over 11) unfolds the molecules and results in gel formation. It is quite possible that this effect is seen in digested, isolated soy proteins at milder pH levels. This would explain our significant improvement in gelation following "exposure" to pH 10.5.

During these experiments, it was also confirmed that salt addition improved the gelation of the gelling mixture. This is seen in Fig. 3. The increase in ionic strength had to be made in the gelling mixture itself. Incorporation of salt with the protein prior to drying did not result in similar improvement. We assumed that this addition prior to drying was resultant of a refolding of the molecule. Upon heating, the unfolding necessary to adequate intermolecular bonding accompanying gelation was not accomplished. When the salt was added to the gelling mixture, this refolding may not have gone to completion so that upon heating the unfolding of the molecules was adequate for gelation.

This effect of salt addition is in direct contradiction to the results obtained by

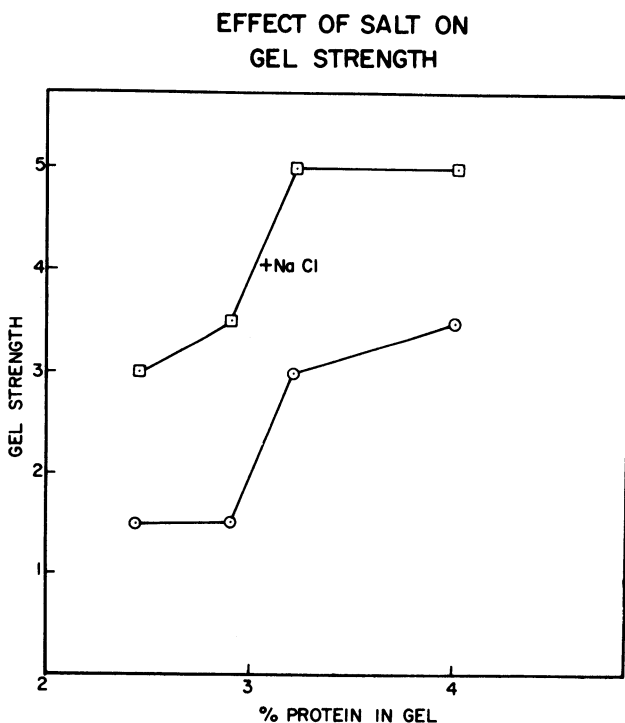


Fig. 3. Digestion—Ficin/Protein = 0.001/1; pH 9.6; 60 min at 40°C. Gelation—10 min at 90°C; measured by method 2.

Hermanson (22) on intact soy protein. He noticed that an increase in the ionic strength reduced the gelling of 10% Promine D dispersions. He measured the gelation by viscosimetry. The temperature of those experiments was only 75°C. Intact Promine D, however, is unique in this respect. It was observed in this laboratory that Promine D was the only isolate behaving in this manner. We assume that, by hydrolyzing this isolate and exposing it to high pH, we have reduced its size and shape to such an extent that NaCl effects on the refolding of the molecule are not the same as in intact Promine D.

#### Optimum Digestion Conditions

Optimization studies were undertaken using gelling test No. 2. The optimum digestion conditions were established as pH 9.6, time of digestion, 60 min at 40°C, substrate concentration, 1% protein, enzyme/substrate ratio, 0.001/1.

A peculiar rise was noticed in the gel strength of the protein digested above pH 7 (Fig. 4). This rise was not investigated any further in these experiments. Molecular-weight comparisons did not show extensive differences between the samples. We can only postulate that at these basic conditions the unfolded

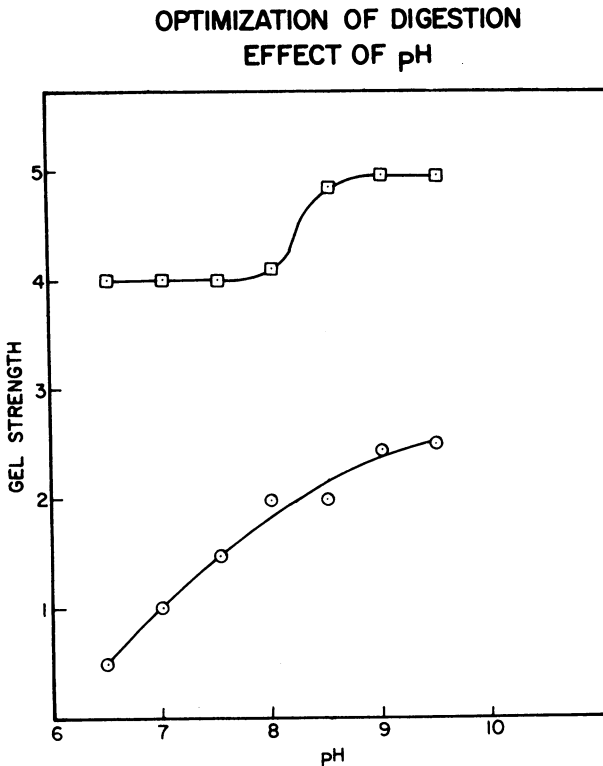


Fig. 4. Digestion—Ficin/Protein = 0.001/1; 60 min at 40°C. Gelation—○ = as is, □ = + NaCl 0.5M; 4% protein; heated 10 min at 90°C.



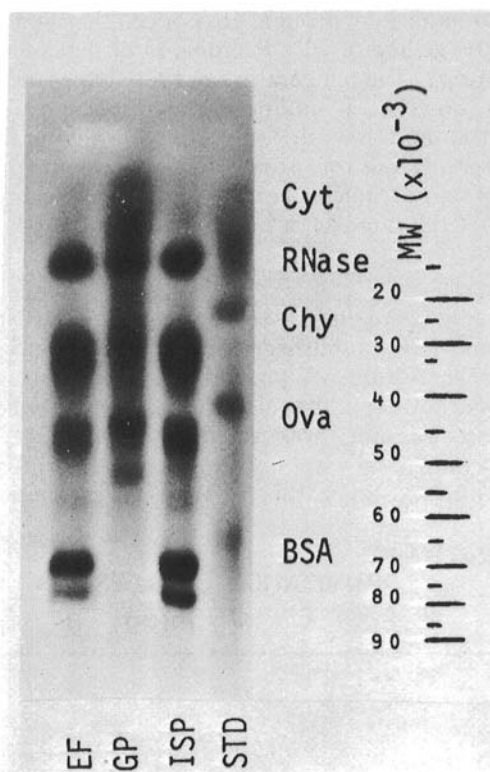


Fig. 5. Molecular-weight (MW) estimation by gel electrophoresis. Protein, 2.5%; SDS, 10%; 0.125M borate buffer, pH 8.9; 1% mercaptoethanol; heated at 100°C for 5 min. STD—standard proteins mixture; ISP—isolated soy protein (prepared as in the text) GP—gelling protein (ficin digest E/S = 0.001/1; 60 min at 40°C with cysteine 2.8 mg/g protein); EF—total extract of the soy flakes (prepared as in the text). CHY—chymotrypsin; OVA—ovalbumin; BSA—bovine serum albumin; CYT—cytochrom C.

TABLE IV  
Gelation at Different pH Values<sup>a,b</sup>

Temperature of Heating	Gelation Values (Method 2)			
	pH 7.57	pH 7.00	pH 6.90	pH 6.50
60°C	0	0	5	2
90°C	0	1	5	2

<sup>a</sup>Digestion: Ficin/protein = 0.001/1, pH = 9.6, 60 min at 40°C.

<sup>b</sup>Gelation: Cysteine 0.0028 g/g protein. Protein 10%, NaCl 0.1M.

protein molecules have certain bonds available to enzymatic attack so that, although the size of the molecules produced may not differ, their amino acid distribution does. This difference may result in different refolding on drying and better gelation when heated.

Cysteine addition at first was found to be needed in pepsin digested protein for improved gelation. When ficin, bromelin, or papain was used with cysteine added during digestion, no cysteine had to be added to the protein at a later stage; in fact, as the theory of Huggins *et al.* (17) postulated, cysteine added after protein re-isolation (thereby becoming excess) would mask the sulfhydryl group and inhibit gelation. This was found to be so. The question (unanswered as yet) is why the cysteine was not eliminated from the isolated digested protein with the enzyme during the repeated washing it underwent in its preparation.

#### Properties of the Gelling Protein

The protein was reduced to smaller size subunits during the digestion as seen in Fig. 5. The larger subunits disappeared and a heterogeneous assembly of lower molecular-weight pieces appeared. A large fraction of these were similar in size to egg albumin.

An interesting aspect of this protein was the dependence of gelation on pH. As seen from Table IV, small changes in pH markedly affected the strength of the gels. No reason for this effect can be given at this time. In undigested soy isolates

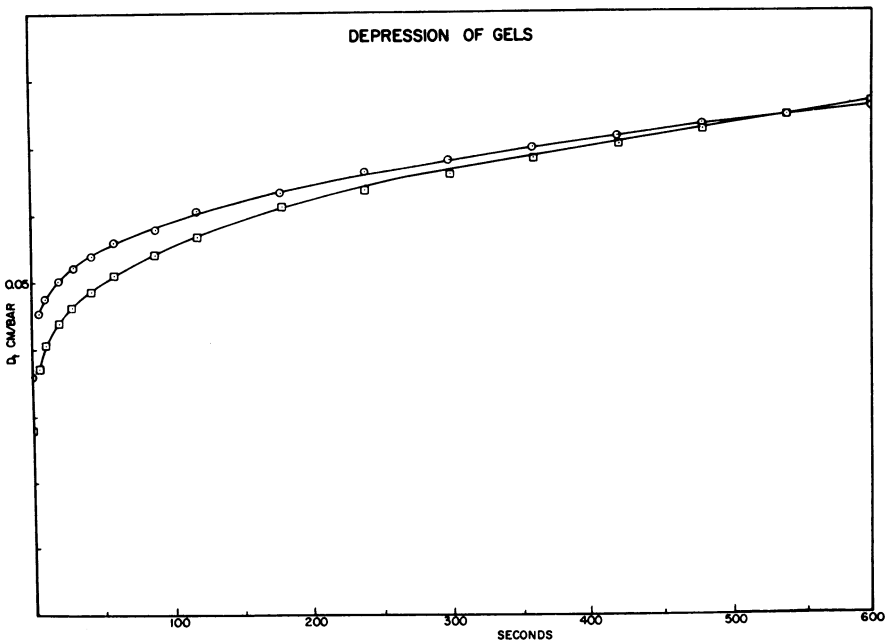


Fig. 6.  $\circ$  = Egg white solids (EWS);  $\square$  = digested soy protein (GWP); ficin digest E/S = 0.001/1; 60 min at pH 9.6 and 40°C; gels—10% protein heated 10 min at 90°C.

these differences were much smaller. Still, the pH 6.9 gels were slightly firmer than gels at the other pH levels.

#### Depression of Gels and Gel Parameters

The measurement of the depression of the gels was considered as the principal aim for this whole study in helping to substitute plant proteins for egg white protein. It is well known that heated egg white gels, when used in foods, are expected to support within their matrix other ingredients such as starches and fillers. Therefore, a strong plant protein gel, capable of withstanding larger pressures without collapse (lower specific depressions), would presumably be an adequate substitute to egg white solids. The aim was therefore to reduce by whatever means available the specific depressions of the soy protein gels. This was accomplished in 10% protein gels by heating at 90.5°C.

The specific depression  $D$  (cm/Bar) was measured by method 3. All of these proteins, unless otherwise stated, were prepared by using ficin (0.001/1 enzyme/protein). Figure 6 shows a comparison of egg white solids (EWS) and GWP (a gelling protein made by a ficin digestion of soy protein isolate).

At this concentration (10%), EWS gels had higher depression ( $D_t$ ) values than the digested soy protein. At first glance, the curves seem similar to the theoretical curve described by Mohsen and Morrow (23) of the dependence of Uniaxial Creep Compliance vs. time.

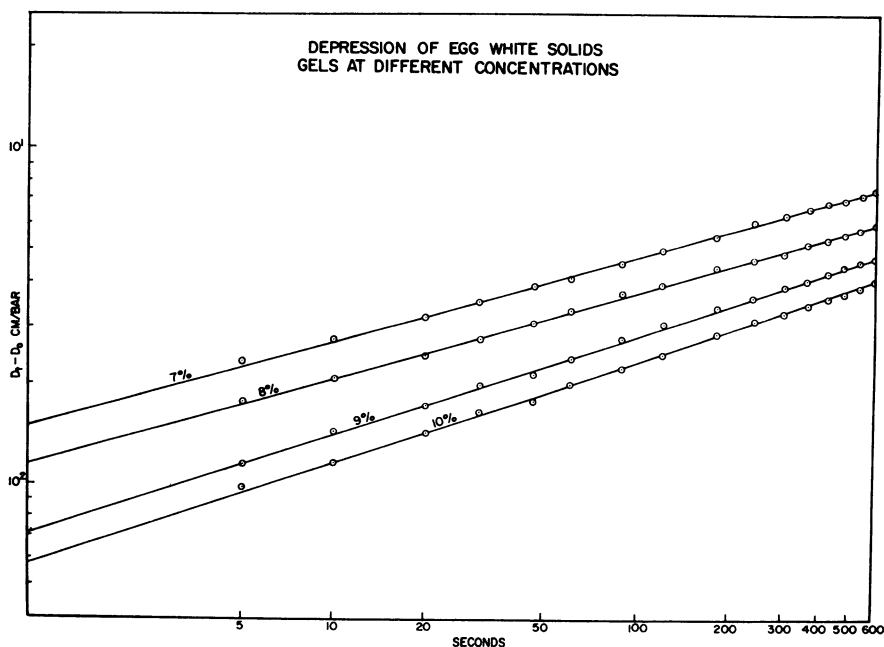


Fig. 7. All gels heated at 90.5°C, 10 min; percentages refer to percentage protein in the gel.

$$D_{(t)} = D_o + D_r (1 - e^{-t/T_{ret}}) \quad (1)$$

where  $D_{(t)}$  is the Uniaxial Creep Compliance at time  $t$ ,  $D_o$  is the instantaneous Uniaxial Creep Compliance,  $D_r$  is the retarded Uniaxial Creep Compliance, and  $T_{ret}$  is retardation time in min. However, a closer analysis proved that the data did not actually fit this equation but obeyed the phenomenological equation:

$$D_t = D_o + K_1 t^{K_2} \quad (2)$$

The fits were found by using a computer program with a stepwise reduction of  $D_o$  from  $D_5$  (5-sec depression) until the best linear fit was obtained for the linear equation:

$$\log (D_t - D_o) = \log K_1 + K_2 \log t$$

where  $D_t$  is the specific depression at time  $t$  (sec); and  $D_o$ ,  $K_1$ , and  $K_2$  are the parameters of that gel at the particular conditions of gelation. The linearity of the plot of  $\log (D_t - D_o)$  against  $t$  is the measure of the fit (highest  $r$  value).  $K_2$  is the slope and  $\log K_1$  is the intercept.

As seen from Figs. 7 and 8, plotting the depression as above for EWS gels and GWP gels yielded linear relations ( $r > 0.9990$ ) at various protein concentrations (90.5°C heating, 10 min).

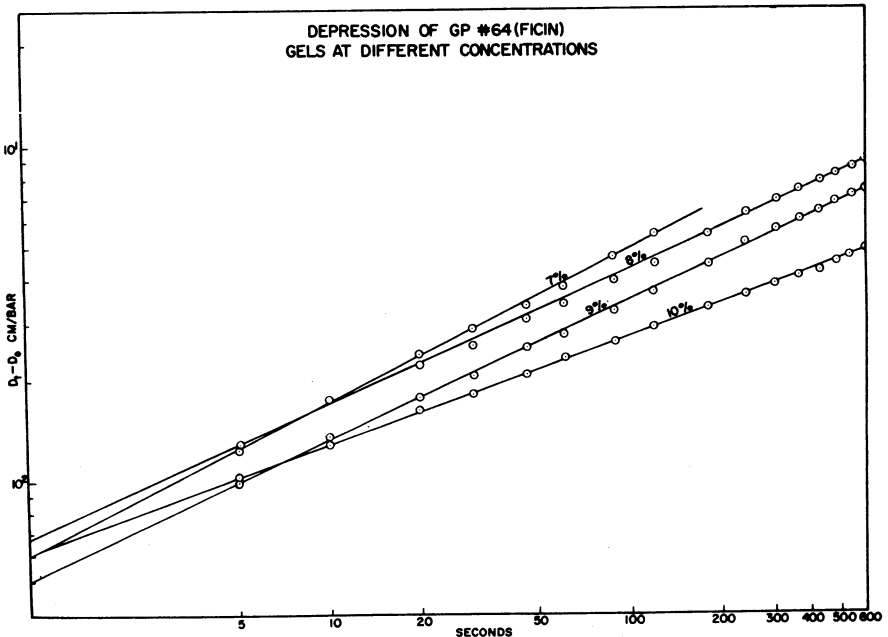


Fig. 8. All gels treated as in Fig. 7.

The physical meaning of  $D_0$  and  $K_1$  can be deduced from the comparison of equations 1 and 2.  $D_0$  is apparently equivalent to the Instantaneous Creep Compliance. We call it the Instantaneous Creep Coefficient. Intuitively, we relate it to an index of hardness. The harder the gel, the less deformation it suffers under the initial impact of pressure, hence a lower  $D_0$ .

$K_1$  we consider to be the Time Dependent Creep Coefficient similar to the Retarded Creep Compliance in equation 1. Intuitively again, we equated it with an index to the yielding power of the gel under steady pressure effects. Higher values of  $K_1$  indicate poor resistance to constant pressure and therefore poorer gels.

$K_2$  is the most difficult to define accurately. From our observations we deduced that it increases with "syneresis" (loss of water from gel under pressure). One can therefore hypothesize that it may be related to the binding of water within the gel matrix (coagulum). The higher  $K_2$ , the less tightly the water is bound within such a protein matrix. Examination of gels with additives tending to break hydrogen bonds may prove whether this hypothesis is correct.

#### Concentration of Gelling Protein and Gel Strength

EWS gel hardness ( $D_0$ ) decreased linearly ( $r = 0.997$ ) with increased concentration (Fig. 9). The slope was very shallow ( $-3.27 \times 10^{-2}$  cm/Bar/%).

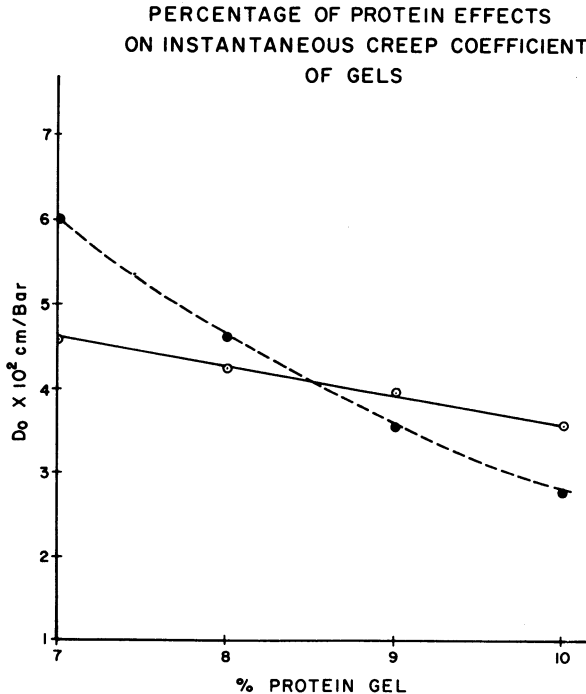


Fig. 9. All gels treated as in Fig. 7. Solid circles = GWP gels; open circles = EWS gels.

GWP gel hardness also decreased; linearity was not as strict, and the average slope was 2.5 times that of EWS gels. That means that at lower concentration the EWS gels will be much harder than GWP gels.

The other two parameters showed a different pattern (Fig. 10). The Retarded Creep Coefficient of EWS ( $K_1$ ) did follow the pattern set by the hardness coefficient. It decreased linearly ( $r = 0.985$ ). The slope was similar, too ( $-0.0271$  cm/Bar/sec/%). For GWP this coefficient did not change with concentration, and all across the range (7–10%) it was lower than that of EWS.

$K_2$  increased slightly with protein concentration for EWS gels. This increase was linear ( $r = 0.946$ ) but very small (slope =  $0.02/\%$ ). The biggest difference was seen in  $K_2$  for GWP, which was much higher than those for EWS (except for 10% gels) and was strongly dependent on concentration ( $r = 0.990$ ; slope =  $0.043/\%$ ).

In terms of our objective—producing an EWS-like protein from soy—this means that at 7% protein concentration and 90°C a pressure of 20 sec will increase the linear yield in EWS gels by  $4.2 \times 10^{-2}$  cm/Bar (or by 90% of the initial depression). For GWP, that value will be  $5.2 \times 10^{-2}$  cm/Bar (or 940% of the initial depression). It is apparent that at the lower concentration the GWP gels are inferior to those of EWS in terms of sustaining constant pressure.

**Temperature of Gelation Effects on Gel Strength**

The differences between EWS and GWP are manifested also in the effects of

PERCENTAGE OF PROTEIN EFFECTS ON GELATION PARAMETERS

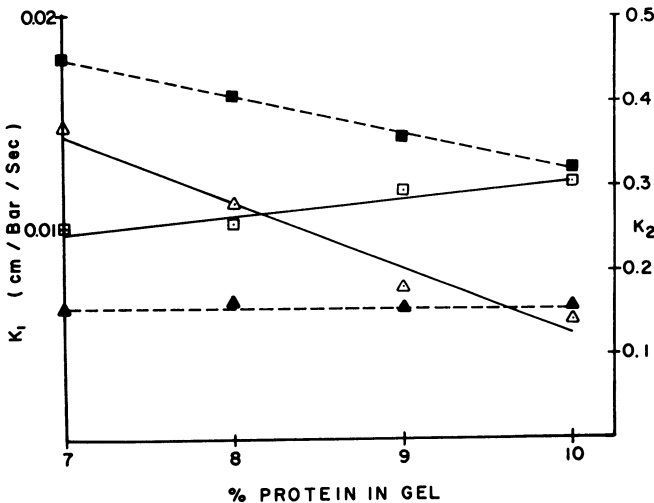


Fig. 10. All gels treated as in Fig. 7. — = EWS gels; - - - = GWP gels;  $\Delta$  =  $K_1$ ;  $\square$  =  $K_2$ .

temperature on gelation (Fig. 11). The hardness indicators were constant for both kinds of gels (standard deviations were 0.8% for EWS and 0.3% for GWP). GWP hardness was only slightly lower in these 10% protein gels.

Retarded Creep Coefficient is much higher for EWS gels than for GWP gels over most of the range studied (80°–93.5°C), with a very negative slope for EWS (slope =  $-0.00162 \text{ cm/Bar/}^\circ\text{C}$ ) and a constant  $K_1$  for GWP between 82.5° and 93.5°C. A rise at lower temperatures was seen in  $K_1$  for the GWP gel.

The “syneresis” coefficient ( $K_2$ ) increased sharply with temperature for EWS gels. We noticed the increase in water released from these high temperature gels of egg white solids (slope =  $0.0161/^\circ\text{C}$ ). For GWP, the syneresis decreased with temperature. In actual values these effects can be summarized as follows: at 80°C, the parameters indicate a slight superiority of the EWS to GWP.

It was therefore understandable that, at less than 7% protein content and 70°–80°C heating, GWP gels should be inferior to EWS gels since in both initial depression and time dependent depression the former will exceed the latter. In the time dependent ‘yield to pressure’ the difference between the two is large. Therefore, resistance to extended pressure should not be expected for GWP.

This was found to be true. When custards of EWS and GWP were made (at 5.5–6.5% concentration and 75°C), and GWP preparation could not support the other ingredient in the matrix as the EWS did, it collapsed.

#### Gelation of Various Enzyme Digests of Soy Proteins

The many enzymes tested and treatments given the digested products

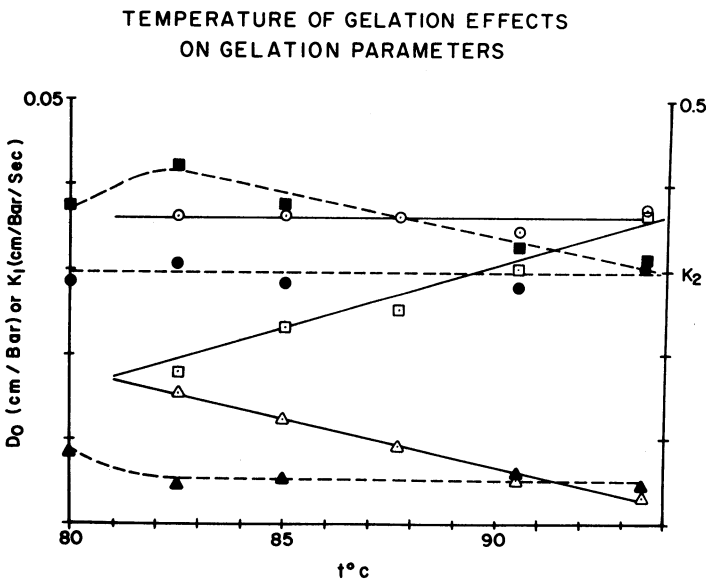


Fig. 11. All gels at 10% protein heated for 10 min. Solid line and open symbols = EWS gels; broken lines and dark symbols = GWP gels. O =  $D_0$ ;  $\Delta$  =  $K_1$ ;  $\square$  =  $K_2$ .

produced proteins of varying gel characteristics. Of the plant proteases tested, bromelin was very similar to ficin (Fig. 12). Papain was slightly less effective in having a higher initial depression (see Figs. 12 and 13).

Of the animal proteases studied, trypsin was least effective in producing gelling proteins. At all enzyme concentrations and gelling concentrations, its digests resulted in gels too weak to support their own weight. Pepsin was slightly better, but only pancreatin produced acceptable gels (Fig. 14).

An interesting characteristic of the pancreatin gel was the low Instantaneous Creep Coefficient. It also had very low  $K_2$  values and showed reduced syneresis. On the other hand, the Retarded Creep Coefficient was so high ( $3.6 \times 10^{-2}$  cm/Bar/sec) that in total gel strength it was deficient. A 20-sec pressure already produced depressions in excess of  $5 \times 10^{-2}$  cm/Bar. This is greater than any initial depression of the plant-proteases-digests-gels of the same concentration and heating temperature.

A mixture of the ficin digest and pancreatin digest for the purpose of reducing the syneresis in the latter (1:1) did not behave in an additive manner. Its

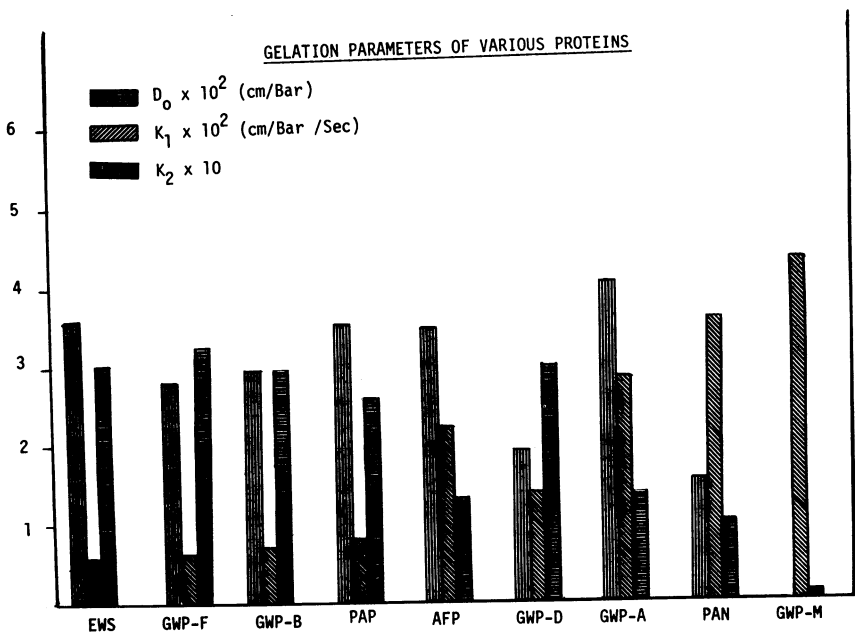


Fig. 12. All gels at 10% protein heated 10 min at 90.5°C; EWS = Egg white solid; GWP-F = ficin digests as in Fig. 5; GWP-B = bromelin digest at the same conditions; PAP = papain digest at the same conditions. AFP = acid fungal protease [E/S = 0.0001/1], other conditions similar to GWP-F; GWP-D-dialyzed GWP-F (2 hr against distilled water with two changes); GWP-A = alcohol-treated GWP-F [95% ethanol wash, ethanol/protein = 10/1, air-dried]; PAN = pancreatin digest at same conditions as GWP-F; GWP-M = a 1:1 mixture of GWP-F and PAN.



characteristics were closer to the pancreatin digest (Figs. 12 and 14). The main difference was that the Instantaneous Creep Coefficient was 0. It had remarkably low syneresis. However, its Retarded Creep Coefficient was very large and the gels could not therefore support any foreign substance in the matrix.

Another gel was produced by an acid fungal protease (AFP) digest. The optimum enzyme concentration in this case was only 0.01% (of substrate protein). It too had lower syneresis than ficin digests, but its  $D_0$  was similar and it had a high Retarded Creep Coefficient. The gel was therefore not stable to prolonged pressure (Fig. 14).

Alcohol washing of the ficin digests did not improve its gelling parameters as expected from alcohol improvement of gelation of undigested soy proteins (14,15). The alcohol treatment resulted in proteins very similar to the pancreatin digests except for higher Instantaneous Creep Coefficients.

A slightly better gel was produced by dialyzing the ficin digest (GWP). It had a lower instantaneous depression and  $K_2$ , but its  $K_1$  was comparatively high ( $1.394 \times 10^{-2}$  cm/Bar/sec for a 10% gel). It could not compare with the starting material (GWP) on overall gel strength after prolonged pressure (see Fig. 13). The small proteins in the ficin digest therefore seem to contribute to the gelation matrix in some manner. Different ion effects on this gel are expected to clarify the role of these components on gel strength.

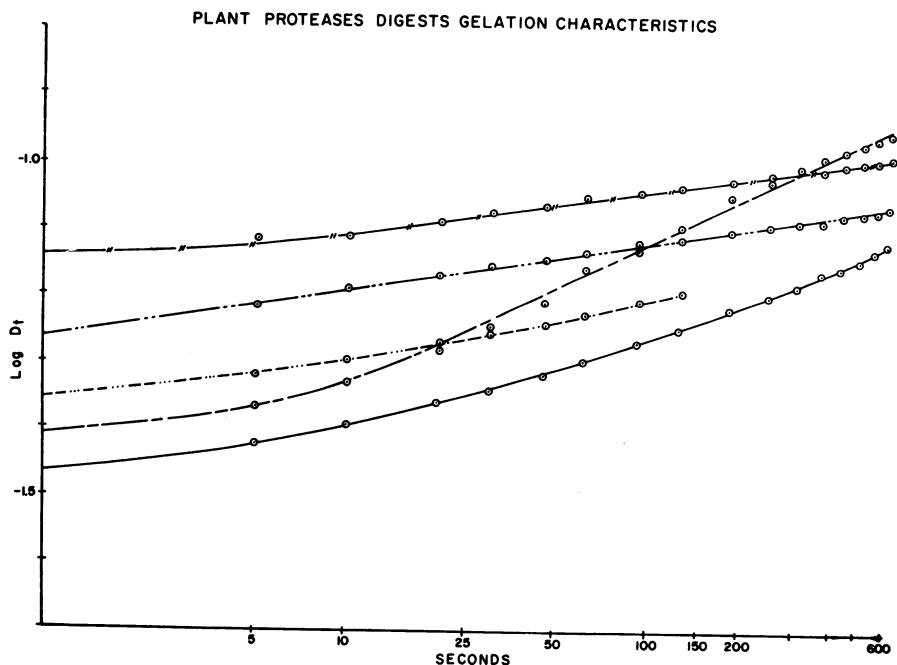


Fig. 13. All gels as in Fig. 12. ——— GWP-F; - - - - GWP-D; — · — · — PAP; · · · · · AFP; // GWP-A.

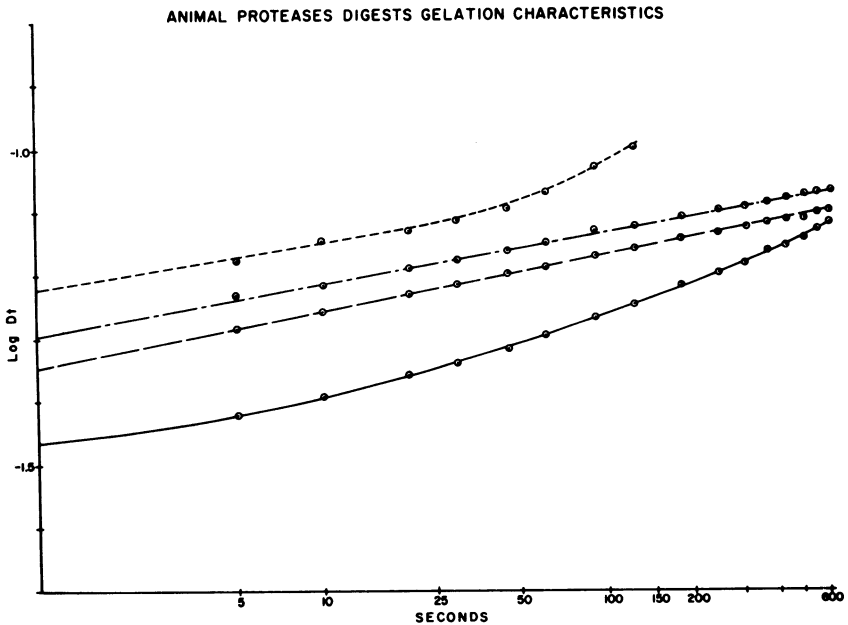


Fig. 14. All gels as in Fig. 12. — GWP-F; — - — PAN; - - - pepsin digest; — — — GWP-M.

In summary we conclude that, although we did not produce a product equal in gelling properties to egg white solids, we did discover, by studying these enzymatic digests of soy protein isolates, a means whereby such gels can be compared to each other. It is therefore hoped that further research in this area will make use of the procedure discovered here to yield products equal in gelling power to egg proteins.

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