EFFECTS OF GAMMA RADIATION ON WATER HOLDING CAPACITY AND NITROGEN SOLUBILITY OF ISOLATED SOYBEAN PROTEIN

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HIROYASU TORIUMI

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ABSTRACT

EFFECTS OF GAMMA RADIATION ON WATER HOLDING CAPACITY AND NITROGEN SOLUBILITY OF ISOLATED SOYBEAN PROTEIN

By

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This study was initiated to investigate and to improve the water holding capacity of soybean protein for use in vegetable protein foods. The protein used (93% protein content) was obtained by Soxhlet extraction with ethyl ether of cracked soybeans followed by alkaline water extraction and acid precipitation. Final drying was by freeze dehydration. In this method, the protein was prepared without the use of high temperature in order to minimize denaturation.

Two methods, the filter paper method and the centrifugal method, for water holding capacity of the protein were investigated. Both methods gave identical values. The filter paper method, however, was preferred because it is easier to use. The protein was irradiated in dry and wet forms and the water holding capacity was measured. Irradiation of the dry form did not increase the water holding capacity. In the wet form, at high doses (10 and

20 Mrad) increased water holding capacity occurred. This increase, however, was not retained when the protein was dried subsequent to irradiation.

Irradiation of both wet and dry protein decreased the soluble nitrogen at pH 7 at doses of 1.5 and 10 Mrad.

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INTRODUCTION

Simulated meats from vegetable proteins are being developed in the growing realization that there is an economic advantage in the future to utilize directly protein-rich vegetable sources for human foods. There are, however, some problems with such products before this can be done. These problems include the following:

1. texture,
2. absorption of water and fat,
3. stability during storage,
4. nutritional value, and
5. ability to carry flavor and color.

These problems are primarily related to the carbohydrate and protein portions of these foods, and to a smaller extent, to the lipid. Certain problems are related to the physical structure secured by the processing utilized in manufacturing these foods.

The texture of meats and meat products are related in part to the interaction between water and the protein constituents. The structure of meat largely is due to the protein fibers of the animal muscle, whose properties in turn depend upon highly hydrated proteins. The

simulated meats from vegetable proteins require similar characteristics.

Simulated meats can be produced as follows: The proteins of 95 to 98% purity of any of several vegetable materials are dispersed in somewhat alkaline water, and the resulting dope is extruded through spinnerets into an acid coagulating-bath where fiber formation occurs. This process takes advantage of the fact that the vegetable protein is more soluble at alkaline pH's and has a minimum solubility at the isoelectric point. For soy protein, the isoelectric point is approximately pH 4.6. It can be seen, therefore, that a spun protein-food can be made by this process only at near the isoelectric point, where the solubility of the protein is minimal. At the isoelectric point, however, vegetable proteins have minimum water holding capacity (WHC). Muscle proteins such as myosin, actin and others have quite high WHC as present in meat. Vegetable proteins have greater WHC at higher pH's but at such pH's the protein is quite soluble, and fibers cannot be formed. One way to improve the low WHC of the fibers would be to use a highly hydrated binder such as a starch gel, agar, gelatin and so on. Such substances, however, do not give a meat-like structure to the product but one more of a cereal type.

Another way to overcome the low WHC of the fibers is to alter the normal properties of the protein, making up the fibers. This may be accomplished in at least two

ways: (1) to increase the cross linking of protein with ionizing radiation and in that way to build up a network of protein so as to increase water holding capacity; and (2) by irradiation, to reduce the solubility of the protein at pH's greater than the normal isoelectric point and, through use of higher pH's in the coagulating bath to secure improved water holding capacity. (Some cross-linking also could be involved.)

It was the purpose of this study to investigate the above two methods for improving the WHC of proteins suitable for making spun protein foods. This study was limited to soy protein.

LITERATURE REVIEW

Isolation of Soybean Proteins

The first published work on the isolation of soybean protein is that of Meissl and Bocker in 1883. These authors recognized three proteins, one of which they called casein. It was extractable by potassium hydroxide and comprised 30% of the soybean (moisture 10%) and more than 90% of the total protein content. The second protein fraction they termed albumin, because it was coagulable by heating the whey obtained from the precipitation of the casein. They recognized its difference from common albumin (ovalbumin) but likened it to pea albumin, and said it was possibly derived from the casein. The third fraction, comprising 7% of the soybean, was insoluble on repeated extraction, and was designated insoluble casein.

In 1898, Osborne and Campbell, gave the name glycinin to the globulin-like protein extracted from the soybean by 10% sodium chloride solution.

In 1921 and 1923 the most extensive early work in this field is that of Satow, who investigated the

separation and properties of soybean protein with a view to its industrial application. In addition to preparing glycinin by the procedure of Osborne, et al., he separated protein by extraction with water and various alkalies, acids or salts. In 1920, Muramatsu characterized the water-soluble portion as containing 84 parts globulin, 5.36 parts albumin, 4.36 parts proteose, and 6.03 parts non-protein nitrogen. In 1928 Tadokoro and Yoshimura designated the water-extracted dialysis-precipitated protein as glycinin A. They obtained glycinin B by extracting the residual meal with 10% sodium chloride. Glutelin was obtained from the residual from the second extraction by using 0.2% sodium hydroxide. Legumelin was derived from the filtrate containing glycinin A by precipitation with ammonium sulfate. The order of increasing content of free amino nitrogen was glycinin, glutelin, legumelin.

In 1931 among other properties given for glycinins A and B, glutelin and legumelin by Ivanov are amino acid content, nitrogen distribution, composition of silver salts, presence of free amino groups and behavior toward aqueous sodium hydroxide or sodium chloride. In 1932 Jones and Csonka separated five fractions from a 10% sodium chloride extract of soybean meal by adding ammonium sulfate at specific degrees of partial saturation. The fraction precipitated at 55% saturation of ammonium

sulfate most nearly resembled Osborne, et al., glycinin. It had an isoelectric point of pH 5.2 and was not coagulable even at boiling water temperature.

In 1945 Evans and St. John determined the percentage protein present in soybean meal as albumin (soluble in water), globulin (soluble in 5% potassium chloride), prolamin (soluble in 70% ethanol), glutelin (soluble in 0.2% potassium hydroxide), and as residual protein.

The foregoing discussion illustrates the state of confusion brought about by the failure to characterize unequivocably any particular protein species of the soybean. Glycinin would appear to be the fraction most nearly approaching a state of chemical individuality, but many differences in its properties are reported by different investigators. Another complicating factor is the observation of Csonka and Jones (1933) that the glycinin fractions from different varieties of soybean differ not only in amount, but also in nitrogen content and amino acid composition.

In 1945 Vickery, however, applied modern methods to a known variety of soybeans to isolate a protein most nearly like the glycinin described by Osborne and Campbell (1898). Although 91% of the nitrogen could be brought into solution by extracting the meal in a Waring Blendor with 6% sodium chloride (adjusted to pH 7.1 to 7.5 by barium hydroxide) to insure complete cell wall rupture, the suspension was turbid even after removing the

insoluble portion. It was not possible to clarify it further by centrifugation or filtration. Vickery (1945) states that an essential prerequisite to the preparation of a protein fraction free from non-protein contaminants is complete clarification of its solution. He achieved such clarification by the use of a saturated sodium chloride extraction of the meal.

In 1946, Smiley and Smith preferred the use of nitrogen content as the most convenient criterion for the purity of soybean protein fractions from a single source, in the absence of crystallinity or other distinctive physical properties. This preference was based on the belief that variation in nitrogen content of the main protein in soybean is due to non-protein impurities. They found that a higher nitrogen content of separated protein was obtained when the meal had been defatted with ethanol, even when alkaline extraction and acid precipitation were used instead of Vickery's procedure.

The recent literature specifically relating to preparation and properties of the isolate includes that of Smith, et al. (1938). About 92% of the protein of oil-free soybean meal can be extracted with distilled water at a pH of about 6.6. Contrary to the behavior of most vegetable proteins, low concentrations of neutral salts reduce the dispersion of the protein. For example, 0.1 N sodium chloride in water lowers the dispersion from 92 to 45%, and 0.0175 N calcium or magnesium chloride

lowers the dispersion of nitrogen components to 21%. This cation effect is overcome by increasing the concentration of the salt or by raising the pH of the system. Most industrial sources of water would be too high in salt concentration for extracting protein in good yield, and alkali must be used to overcome the salt effect. To obtain a high yield of protein, the meal is extracted by adding water and adjusting to about pH 9.0. The insoluble residue is removed in a centrifuge and the protein precipitated with acid in the pH range of 4.6 to 4.1.

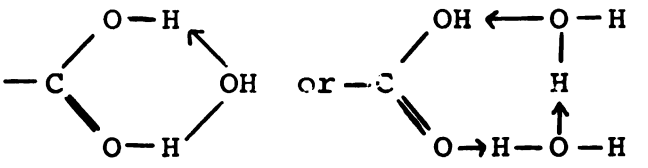
Protein isolated by this procedure is a mixture of globulins and glutelins. In laboratory-scale operation as much as 84% of the total protein of the meal is extracted. Thus, with dehulled, oil-free meal containing 50% protein, the yield will be 42% based on the weight of the meal. In large-scale processing, however, the yield will be much lower, owing partly to the lower water-to-meal ratio which is necessary in commercial operations and partly to loss of protein through chemical degradation into a more soluble form which is not precipitated upon acidification. A yield of 30% is considered good commercial practice.

Nature of Water Holding Capacity

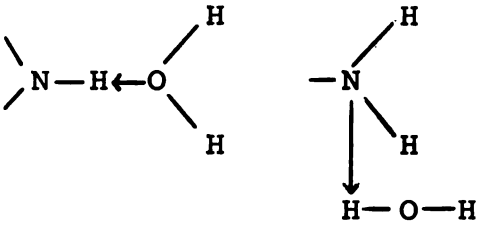
The water holding capacity of meat has been the subject of considerable study. For example, Hamm (1960) reviewed the biochemistry of meat hydration in which the

WHC of muscle tissue was reported to concern mainly actin and myosin of the complex actomyosin. Though there are no studies reported on the water holding capacity of soybean protein, some basic studies of water holding characteristics of proteins were reported. In 1940 Sponsler, et al., reported that the hydrophilic groups responsible for the fast binding of water by protein are of two types. One type includes the polar groups of the protein side chains, such as the carboxyl-, amino-, hydroxyl-, and sulfhydryl- groups. The other type is made up of undissociated carbonyl- and imido- groups of the peptide bonds in which the binding of water is due to the dipolar character of water. The water molecule is a dipole because of the unsymmetrical space distribution of the negative charge of oxygen and positive charge of hydrogen. This circumstance leads to attraction and association with polar groups in the protein. For example, the water molecule is bound by hydrogen bond (H), probably as follows:

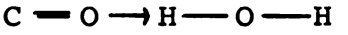
Carboxyl group



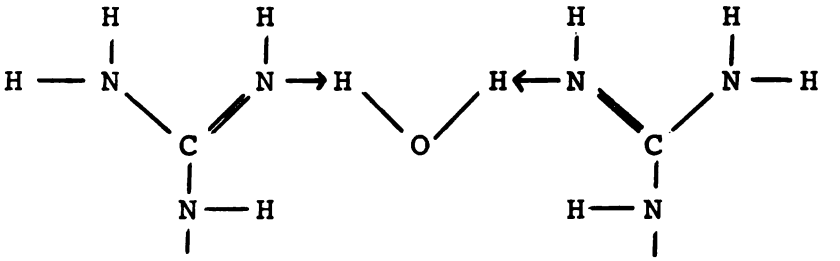
Amino and imidazol groups



Carbonyl groups



Guanidino groups



Olcott and Frankel-Conrat (1946) found that the activity of proteins for binding water is diminished by blocking polar groups by certain reagents, and proved in that manner that polar groups are at least partly responsible for the water binding. With the same technique, they showed that carboxyl groups play a less important role in hydration than do amino groups (Seehof, et al., 1953). At a very low relative humidity (about 5%) one molecule of water is bound by two amino groups. When the relative humidity is increased to 60%, one amino group binds 2-1/2 molecules of water. At that point, the ability of amino groups to bind water is exhausted.

According to Pauling (1945) the initial phase of binding of water by proteins consists in the binding of one water molecule by one polar group. The affinity for water of the different polar group varies. Therefore, water attaches first to the most active groups, and then to the less active. Whereas Pauling supposed that peptide groups do not play any role in binding water, Mellon, et al., (1947, 1949) found that the -CO-NH- group takes part in hydration. At 60% relative humidity, peptide groups appear to be responsible for about 45% of the vapor-phase water absorption by casein and 70% of the absorption by zein.

According to the hypothesis of Klotz (1958), non-polar groups can also have some influence on hydration, but are not concerned in the true binding of water.

Determination of Water Holding Capacity

Ultrafiltration was used by Lloyd and Moran (1933) for determining bound water in gelatin. This technique might be primarily applicable for homogenates of tissues, but has the disadvantage of requiring a long time to carry out the determination.

At first, pressing meat between two plates was used only for a qualitative judgement of wetness (Schonberg, 1937). Pusch (1950) screw-pressed pieces of muscle between two steel plates and measured the volume of juice squeezed out. Grau (1952) also used the pressure method for the estimation of the quality of frankfurters. In his method the meat was pressed in a sieve-ended cylinder by a moving piston and the juice was weighed. The water holding capacity of cooked meat was determined by press methods, particularly for study of the correlation between a subjective impression of "juiceness" and an objective test using "pressometers."

The press method was transformed to a quantitative technique by using filter paper. The more loosely the water bound, the better it is absorbed by filter paper. Grau and Hamm (1953, 1957) developed a quantitative method for determining the WHC of meat--a combination of the press technique and the filter paper technique. The method of Grau and Hamm was modified by several authors by applying a constant pressure or weighing the filter paper before and after pressing.

Mohler and Kiermeir (1953) used a sedimentation method for ground meat which was suspended in a calibrated cylinder. This method was used for studying the influence of certain salts on the swelling of meat. The advantages of this method are its simplicity and usability for serial-type experiments.

Wierbicki (1957) put a meat sample on a fritted glass disk in a special centrifuge glass tube and separated the loose water from tissue by centrifuging the juice at 1000 r.p.m. into a smaller graduated section of the tube.

Effect of Radiation of Proteins

The principal changes in proteins produced by ionizing radiations are explained by Bacq, as follows:

1. Main Chain Scission

This is related to a reduction in molecular weight. Since proteins are made up of a large number of identical or similar repeating units, there is an equal probability that a break is produced at almost every unit along the molecule. If all the molecules are of uniform molecular weight to start with, then radiation-induced breaks will product a non-uniform (or polydisperse) product. If the polymers are polydisperse at the beginning, then degradation by radiation does not change the character of the distribution but only the average molecular weight.

2. Crosslinking

This can be of two types. If two different molecules are joined together, this is inter-molecular crosslinking. As this process proceeds, more and more molecules are joined together. This is crosslinking of an insoluble gel network. Initially the network is so loose that the gel may be difficult to detect especially with polymers of high molecular weight. A certain number of crosslinks have to be formed before a sufficiently large network is formed to give a gel and consequently there will be a minimum radiation dose before any gel can be detected. When a large network is formed of molecules, they are no longer soluble, but only swell in solvents which dissolved the starting material.

If crosslinks can then be formed between different groups in the same molecule, this is intramolecular crosslinking. The effect of such intramolecular crosslinkins is to pull the molecule together so that it occupies a smaller volume in solution and this brings with it a reduction in viscosity without any change in molecular weight. In such randomly coiled long molecules intramolecular crossing will predominate, if the crosslinking reaction is carried out in very dilute solution; at higher concentrations the reaction will be predominantly between different molecules and give rise to gel networks.

3. Disruption of Secondary Structure of Macromolecules

Proteins in their native state are maintained in rigid steric configurations by secondary valency forces and do not assume in solution purely random configurations, as do most synthetic polymers. The main chains are constrained in fixed configurations by hydrogen bonds. Radiation disrupts this secondary folding. This type of effect has so far only been encountered in proteins.

PROCEDURES

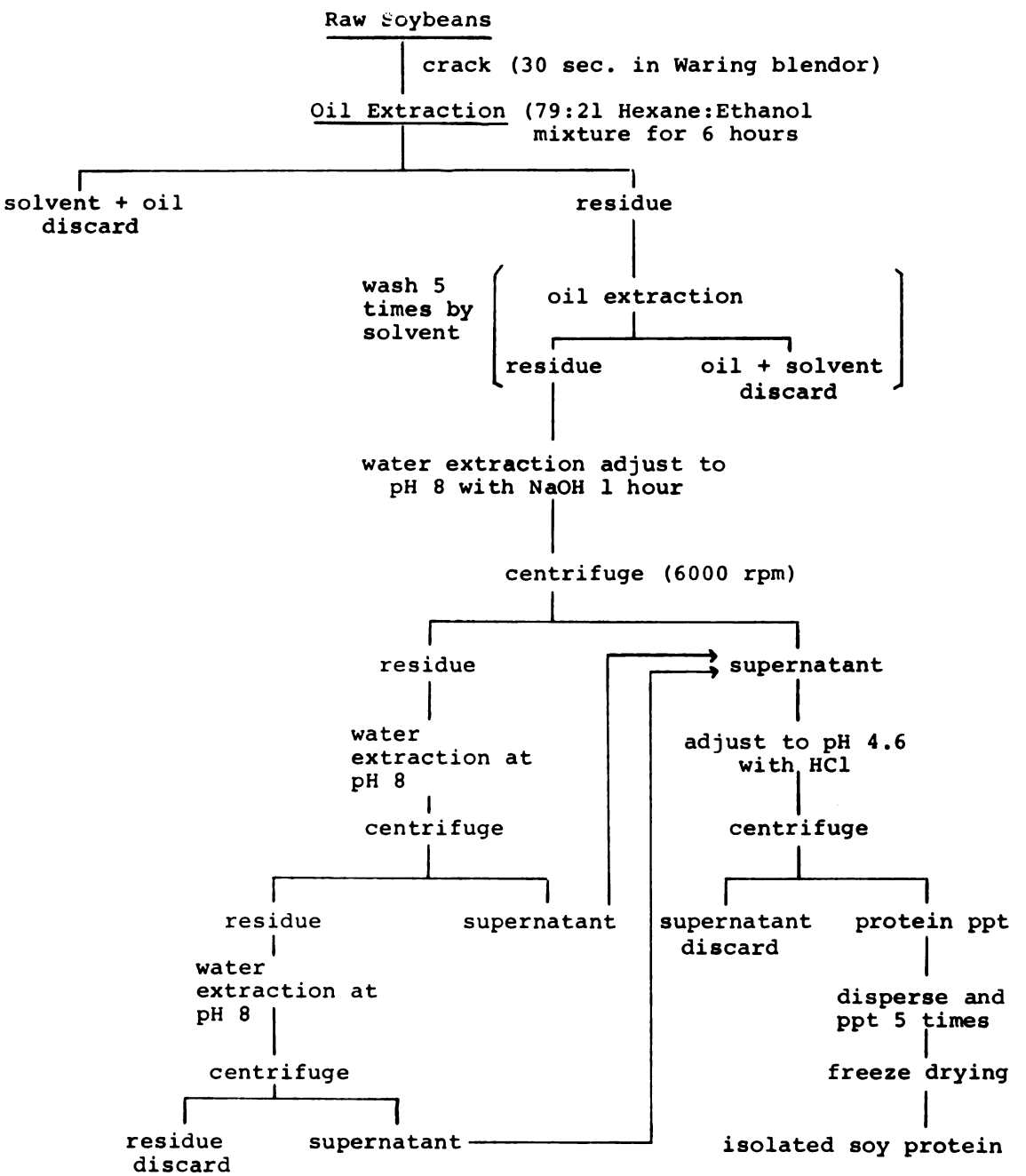
Preparation of Isolated Soy Protein

1. Batch Method with Hexane-Ethanol Mixture

Approximately 100g of raw soybeans (var. Harosoy 63) produced in Michigan, were cracked in a Waring Blendor for 30 seconds and passed through a 100-mesh screen. The crushed full-fat soybeans were then extracted with hexane-absolute-ethanol azeotropic mixture (79:21). A ratio of three parts by weight of solvent to one of beans was employed. The mixture was stirred for six hours without heating. Stirring was stopped and the mixture allowed to stand for one hour. The supernatant containing some suspended solids was removed and centrifuged at 6000 r.p.m. for 20 minutes in a Sorvall Superspeed RC2-B centrifuge with a GSA Rotor. The clear oil-containing solvent thus obtained, was discarded. The solids were added to those obtained by decanting the original mixture.

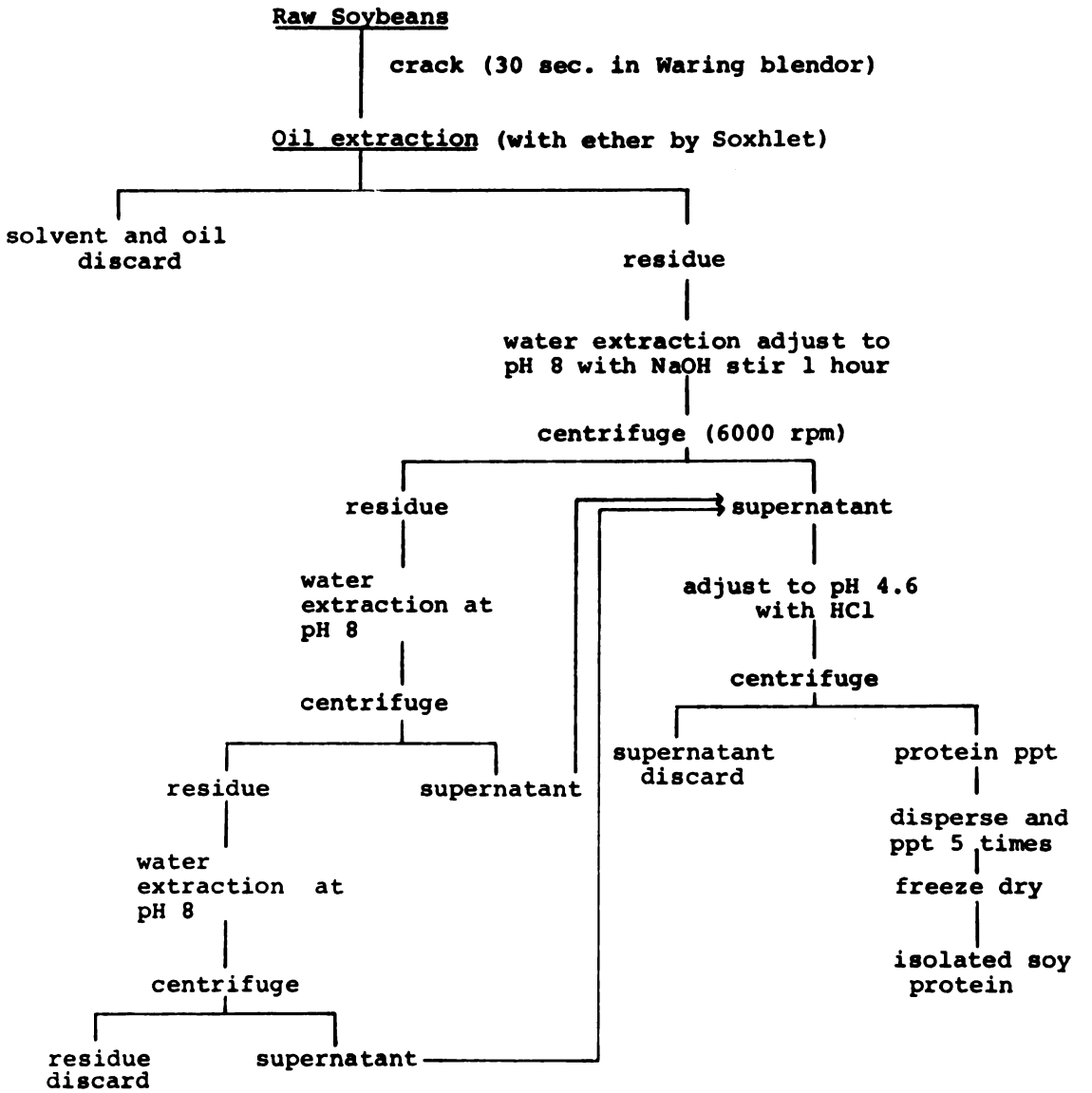
The combined solids were extracted five times with the hexane-ethanol mixture for one hour periods in order to get essentially oil-free residues. The solids so obtained,

were extracted with water with five times the weight of the solids. The mixture was adjusted to pH 8 with sodium hydroxide. The solution obtained after one hour of stirring contained the soluble protein, carbohydrate and mineral constituents. These were separated from the insoluble material by centrifuging at a speed of 6000 r.p.m. for 20 minutes. After removing the supernatant, the residue was again dispersed in the alkaline solution of pH 8, and another protein-containing supernatant was obtained in the same way. These two portions of supernatant were combined and acidified to pH 4.6 with hydrochloric acid. This precipitated the globulin fraction. The resulting curd containing liquid was then centrifuged to obtain the insoluble protein, and the supernatant portion was discarded. This curd was washed by dispersing it again with water at pH 8.0 obtained with sodium hydroxide and precipitated again at pH 4.6 in the same manner. This washing procedure was repeated three times. The purified protein so obtained was freeze-dried. The freeze-dried product was converted to a particulate form by placing it in a Waring Blendor for 10 seconds. Product so obtained and when passed through a 100 mesh screen was used in some of the studies reported below.


Raw Soybeans
crack (30 sec. in Waring blendor)
Oil Extraction (79:21 Hexane:Ethanol mixture for 6 hours
solvent + oil discard
residue
wash 5 times by solvent
oil extraction
residue
oil + solvent discard
water extraction adjust to pH 8 with NaOH 1 hour
centrifuge (6000 rpm)
residue
supernatant
water extraction at pH 8
adjust to pH 4.6 with HCl
centrifuge
centrifuge
residue
supernatant
supernatant discard
protein ppt
water extraction at pH 8
disperse and ppt 5 times
centrifuge
freeze drying
residue discard
supernatant
isolated soy protein

2. Method with Ethyl Ether, Using Soxhlet Apparatus

Approximately 100g of raw soybeans (var. Harosoy 63) produced in Michigan, were cracked in a Waring Blendor for 30 seconds and passed through a 100-mesh screen. The cracked full-fat soybeans were extracted with ethyl ether by Soxhlet apparatus for 30 hours. The defatted soybeans were treated in the same way as with the batch method in order to secure isolated soybean protein.



Determination of Water Holding Capacity

1. Water Holding Capacity by Filter Paper Method

The filter paper method by Sherman (1961) for the determination of percentage water retention was used. Two grams of isolated soy protein were weighed into a tared centrifuge tube (50 ml capacity) and 20 ml of distilled water added. After thorough mixing with a glass rod, the tube was stoppered and mixture kept for three hours at room temperature. The stopper was removed and the tube was centrifuged at 3000 r.p.m. by Lourdes Model AX centrifuge for 20 minutes. The supernatant fluid was decanted through a presoaked and drained filter paper resting in a glass funnel into a 10 ml graduated bylinder that could be read to within 0.05 ml. The mixture was drained into the filter paper for 10 minutes, and the amount of liquid passing the filter was measured. The water holding capacity of the sample expressed as percentage water retention per gram of soybean protein was calculated as follows:

Percentage of WHC =

$$\frac{\text{vol (ml) fluid added minus vol (ml) fluid not absorbed}}{\text{weight (g) of protein}} \times 100$$

3. Water Holding Capacity by Centrifuge Method

The procedure used for determining WHC in this present study was a modification of the method reported by Wierbicki, et al. (1957). A specially constructed tube was used for this determination. Overall length was 180 mm; the top section was 30 mm in diameter, 100 mm in length

and the bottom section was 18 mm in diameter, 80 mm in length. The quantity of expressed water was determined in the graduated bottom section calibrated in 0.1 ml division. A medium coarse fritted glass disc was placed at the intersection of the large and small diameter sections of the tube. One gram of the isolated soybean protein and 10 ml of buffered solution which was adjusted to a specified pH with hydrocloric acid or with sodium hydroxide, were added and mixed well in upper portion of the tube. After 30 minutes, the mixture was centrifuged for 20 min at 1000 r.p.m. by International Refrigerated Centrifuge Model PR-6. The water holding capacity was calculated from the following relationship:

Percentage of WHC =

$$\frac{\text{vol (ml) of fluid added minus ml fluid not absorbed}}{\text{wt (g) of protein}} \times 100$$

Determination of Soluble Nitrogen Index

The method is a modification of the method for the dispersible nitrogen in soybean products by A.O.C.S. (1946) Tentative Method Ba 11-65. Two grams of the soybean protein were weighed into a 400 ml beaker. A small portion of 200 ml of distilled water was added and the protein thoroughly dispersed with a stirring rod. The remainder of the 200 ml of water was added and the mixture stirred. The pH of the protein-water mixture was adjusted to selected pH's either with hydrochloric acid or sodium hydroxide. This solution was stirred by a mechanical stirrer for 120 minutes at

room temperature, and transferred quantitatively to a 250 ml volumetric flask and additional water added to make 250 ml. After a few minutes about 40 ml were drained into a 50 ml centrifuge tube, and centrifuged for 10 minutes at 1500 r.p.m. Then the supernatant was decanted through a funnel containing a plug of glass wool and collected in the 100 ml beaker. Twenty-five ml of the clear liquid were pipetted into a Kjeldahl flask. The standard procedure for Kjeldahl nitrogen determination was followed. Soluble nitrogen index is expressed as follows:

$$\% \text{ Soluble Nitrogen Index} = \frac{\text{water soluble nitrogen}}{\text{total nitrogen}} \times 100$$

Nitrogen Determination

All nitrogen determinations were made by the micro-Kjeldahl method outlined by the American Instrument Company (1961). Nitrogen values were determined in duplicate.

Moisture, Fat and Ash Determination

The methods of the A.O.A.C. (1965) were applied for moisture (13.070), fat (13.074) and ash (13.071) determinations.

RESULTS AND DISCUSSION

Preparation of Isolated Soy Protein

It was reported by Honig, et al., (1969) that dehulled soybean flakes, defatted with pentane-hexane, could be further extracted with an azeotropic mixture of hexane and ethanol in order to remove free and bound lipids including phospholipids, sterols and triglycerides. In their procedure, however, lipids in soybeans were extracted in a Soxhlet which is designed to do a continuous operation by raising the solvent mixture to a boiling point.

Generally five physical factors affecting the extraction of protein from soybean meal are important: (1) size of meal particle; (2) solvent-meal ratio; (3) temperature; (4) time; and (5) separation of meal residue from extractant. The size of meal particles is a very important factor and 100-mesh or finer meal is needed for maximum extraction. In this study, the soybeans were cracked in a Waring Blendor for 30 seconds and passed through a 100-mesh sieve.

During the cracking in the Blendor, heat is generated. Since such heat can cause denaturation of the protein, a limit of 30 seconds for the operating of the Blendor was employed. With this limitation the soybeans were heated only slightly above room temperature.

Although batch-type of extraction is not used in the industry because of the small throughput per man hour and the outlay of equipment necessary to handle moderately large tonnage, it was applied in this study when the hexane-ethanol solvent was used. With batch-type extraction it was possible to keep the extraction temperature close to room temperature and to cause a minimal heat denaturation of the protein. Using the convenient Soxhlet operation method with this solvent would require quite high temperature (above 70°C). This would cause significant denaturation of the protein.

In the batch operation employed, the solvent and soybean meal were mixed in a polyethylene beaker and stirred for six hours. After settling, the liquid portion was decanted from the solid. The oil-extracted residues were washed five times with the solvent mixture in order to reduce the oil content further. Finally, the residue was freed of solvent by air drying at room temperature. The results of analysis of the extracted meal are shown in Table 1. Although a relatively high percentage of protein was obtained (89.1%), it was not adequate. Isolated soy protein is defined as not less

Table 1.--Analysis of Isolated Soybean Protein Extracted by Batch Method with Hexane-Ethanol Mixture.

Moisture	2.5%
Protein (N x 6.25)	89.8%
Ash	4.1%
Fat	3.1%

than 90% protein. It was realized that this method of extraction led to a quite high fat content (3.1%), being higher than commercial products by a factor of about ten. It was concluded, therefore, that this batch-type method was not suitable, since too much oil remained in the meal. It is conceivable that this resulted from the low temperature during extraction.

Because of this failure of the solvent used with the batch system, a second system was employed. This was the continuous extraction method, employing the Soxhlet apparatus with anhydrous ethyl ether as the solvent. Although ethyl ether is not a practical commercial solvent for the extraction, it is a most effective solvent for lipid extraction and, for the purpose of this study, is attractive because it boils at only 35°C. This temperature is not likely to cause denaturation of the protein. As shown in Table 2, the isolated soy protein was obtained with a higher percentage protein content (93.2%) and with very low-percentage fat content (0.2%). The Soxhlet extraction method, with

ethyl ether as the solvent, therefore, was used to prepare isolated soybean protein for this study.

TABLE 2.--Analysis of Isolated Soybean Protein Extracted in Soxhlet Apparatus with Ethyl Ether.

Moisture	2.2%
Protein (N x 6.25)	93.2%
Ash	4.2%
Fat	0.2%

Water Holding Capacity of Soy Protein

Two methods were employed for measuring the WHC of soy protein. Both methods, filter paper method and centrifuge method, yielded quite similar results for the WHC of non-irradiated soy protein. The influence of pH on the WHC of non-irradiated soy protein is shown in Figures 1 and 2. It is clear that WHC is dependent on pH value.

Practically, however, the filter paper method is easier to use than the centrifuge method. In the centrifuge method, a fritted glass disc was placed between the upper portion and the bottom portion of the glass tube. This disc, however, does not hold its position properly during the centrifugation, and moves so that the sample in the upper part of the tube leaks into the graduated bottom part. Paraffin can be used to seal the fritted

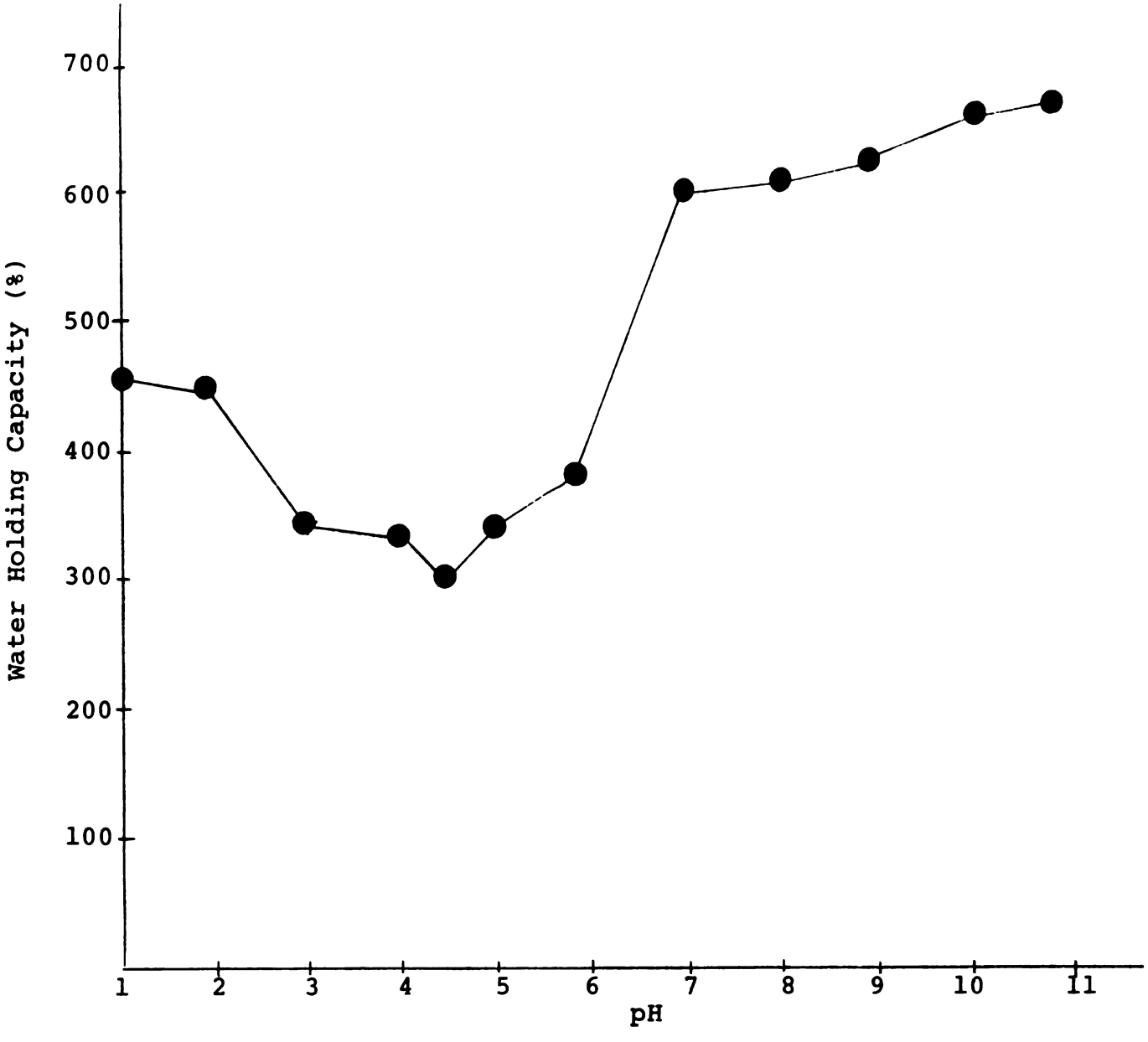


Figure 1.--Relation between pH and Water Holding Capacity (%) of Non-Irradiated Soybean Protein--Centrifuge Method.

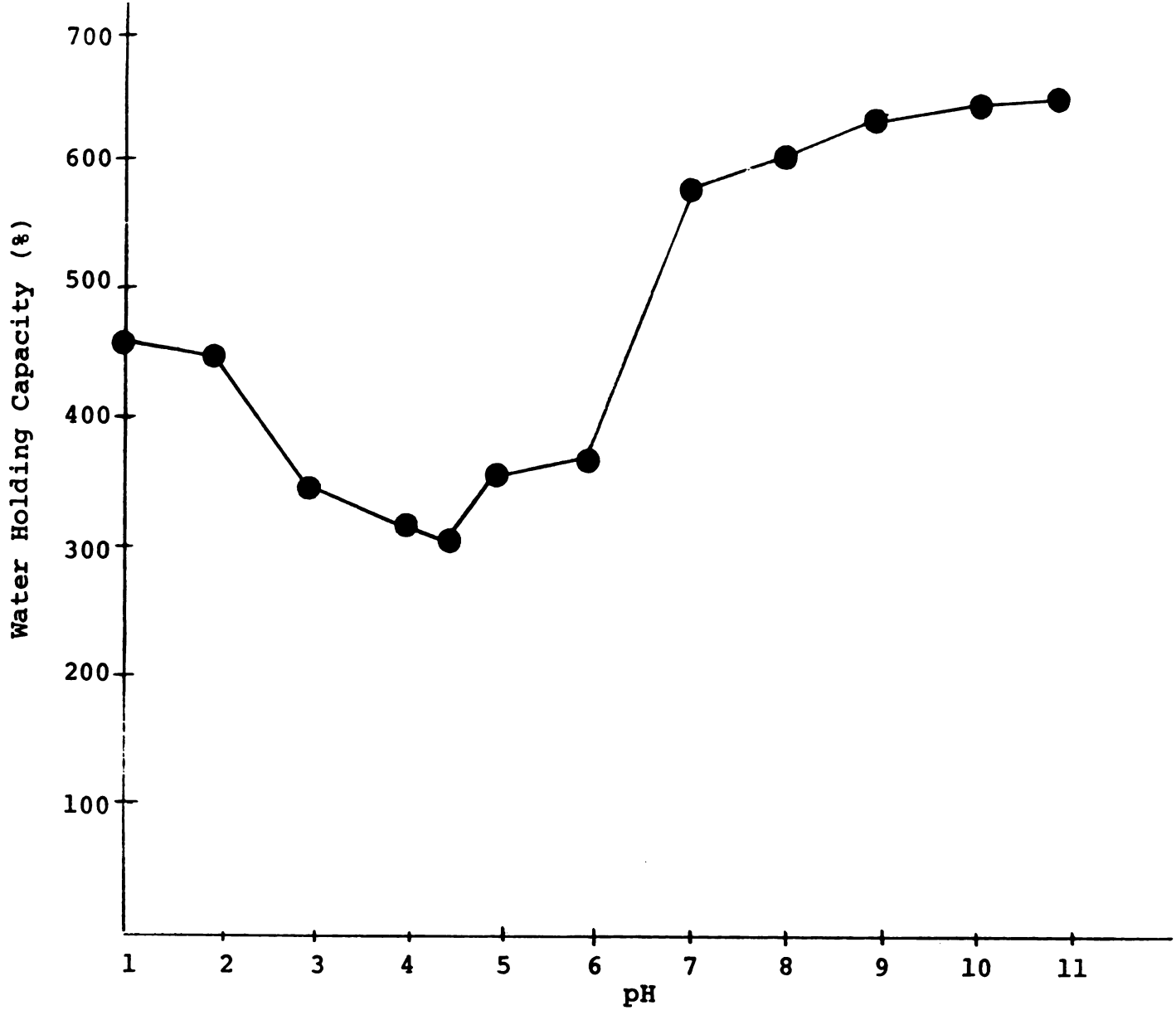


Figure 2.--Relation between pH and Water Holding Capacity (%) of Non-Irradiated Soybean Protein--Filter Paper Method.

glass disc to the glass tube. This, however, was inconvenient.

The curves in Figures 1 and 2 give a good picture of the relative electrochemical conditions of the protein with changing pH. When pH increases or decreases from the isoelectric point (4.6), the protein net charge increases, and consequently attraction of water to the peptide chains increases. Therefore, the protein hydration increases. On the other hand, at the isoelectric point, the protein net charge is zero, and hydration bonds between the peptide chains caused a tightening of the protein network. Therefore, the WHC at the isoelectric pH is at a minimum.

Since the filter paper method was convenient and effective, this method was used for the determination of WHC in this study.

Two different methods by which a chemical change affecting WHC can be brought about by ionizing radiations were considered possible in this study. One is direct action in which the molecule undergoing change itself becomes ionized or excited by the passage through it of an electron or other energy particle. The other is indirect action in which the molecule in question does not itself directly absorb the energy but receives it by transfer from another molecule or molecular fragment.

In this study, when soy protein is irradiated dry, the action or the soy protein is essentially direct. The data on WHC of the soy protein irradiated in dry form are shown in Table 3. It is clear that the WHC is

quite independent of the irradiation doses, and in respective pH ranges, it is similar to the WHC of non-irradiated soy protein.

TABLE 3.--Water Holding Capacity (%) of Protein Irradiated in Dry Form, and Measured at Indicated pH's.

	Dose - rad							
pH	k 5	k 50	k 100	k 500	1 M	5 M	10M	20M
3	330	340	330	335	340	320	340	330
4.5	300	320	310	320	320	310	300	310
5	380	365	386	370	370	370	360	370
6	365	380	370	380	380	350	365	370
7	605	600	585	590	590	595	585	585
8	600	605	580	590	595	600	595	605

When soy protein is irradiated in water, the action of the radiation can be due to both direct and indirect effects. The WHC of the protein in the wet form was determined over a pH range of 2 to 11, and for irradiation doses from 0.5 to 20 Mrad. The curves given in Figure 3 for 0.5, 1 and 5 Mrad, are all similar, showing the minimum water absorption at near the isolectric point and higher values at higher pH ranges. For 10 and 20 Mrad, the curves are quite different. The WHC's at these doses are significantly greater, particularly above pH 7. Above pH 7, the WHC's reach the value of about 1000%, and all water added was absorbed in the protein.

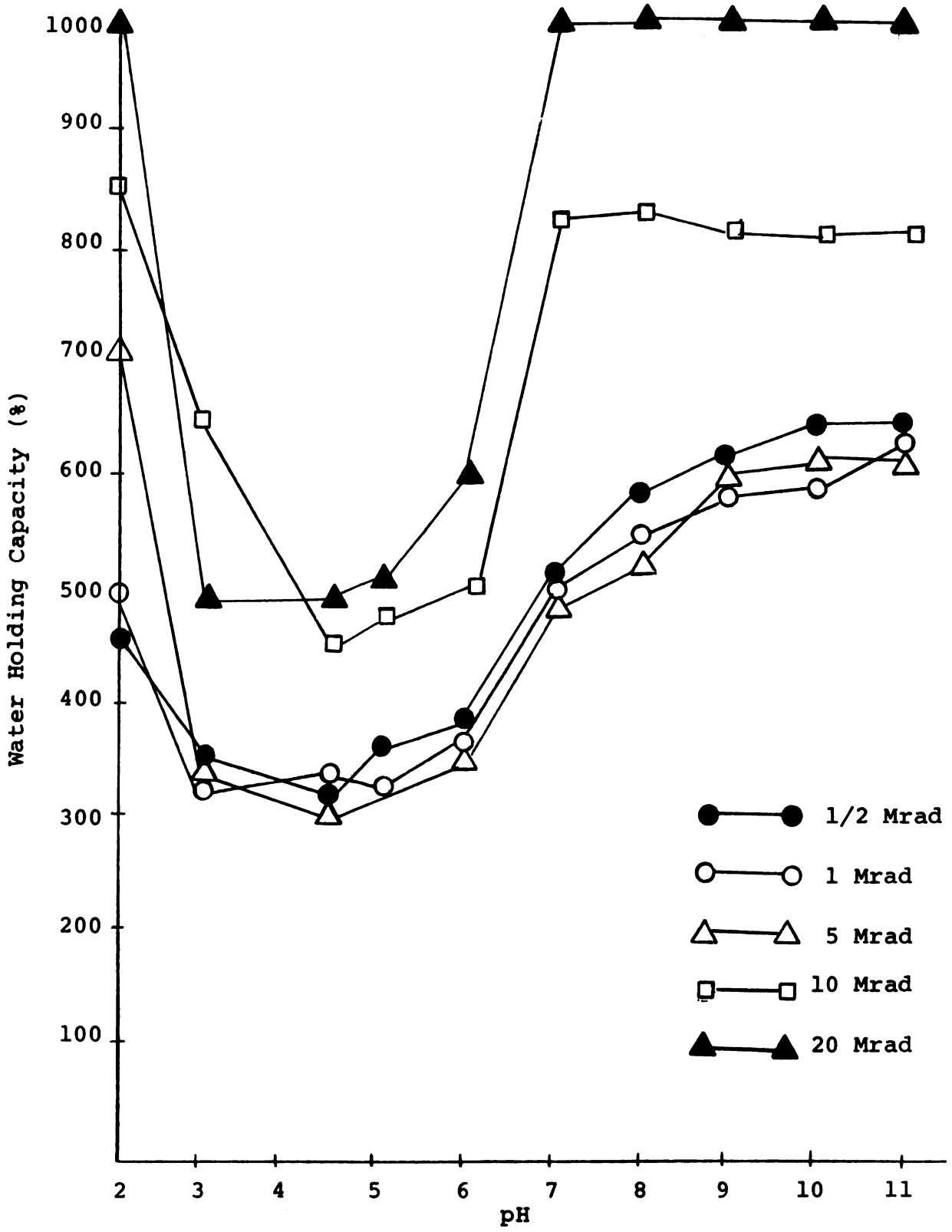


Figure 3.--Relation between pH and Water Holding Capacity (%) of Protein Irradiated in Wet Form.

At this state, gels were characteristically formed. It is conceivable that the crosslinkings responsible for the aggregation of the proteins, were set up by this rather large amount of gamma radiation.

It is clear that irradiation of wet form is quite effective in increasing WHC of the protein. This could be due to the change of chemical bonds in the protein or to simple physical change in the structure in which water is absorbed, as in a sponge, without being chemically bonded.

In an effort to determine which of these explanations might be correct, proteins which had been irradiated at 10 and 20 Mrad, were freeze-dried, ground, and passed through 100 mesh sieve. The WHC was again measured. The curves for the WHC values over the pH range 2 to 11 are given in Figure 4. They are essentially identical with the one given in Figure 2 for the protein which was not irradiated. It is believed, therefore, that the increase of WHC observed for the irradiation in the wet form with 10 and 20 Mrad resulted in water held in the structures without true chemical bonding.

Effect of Gamma Radiation on Nitrogen Solubility

One of the chemical and physical changes produced by ionizing radiation is an alteration of solubility of protein. The purpose of a determination of soluble

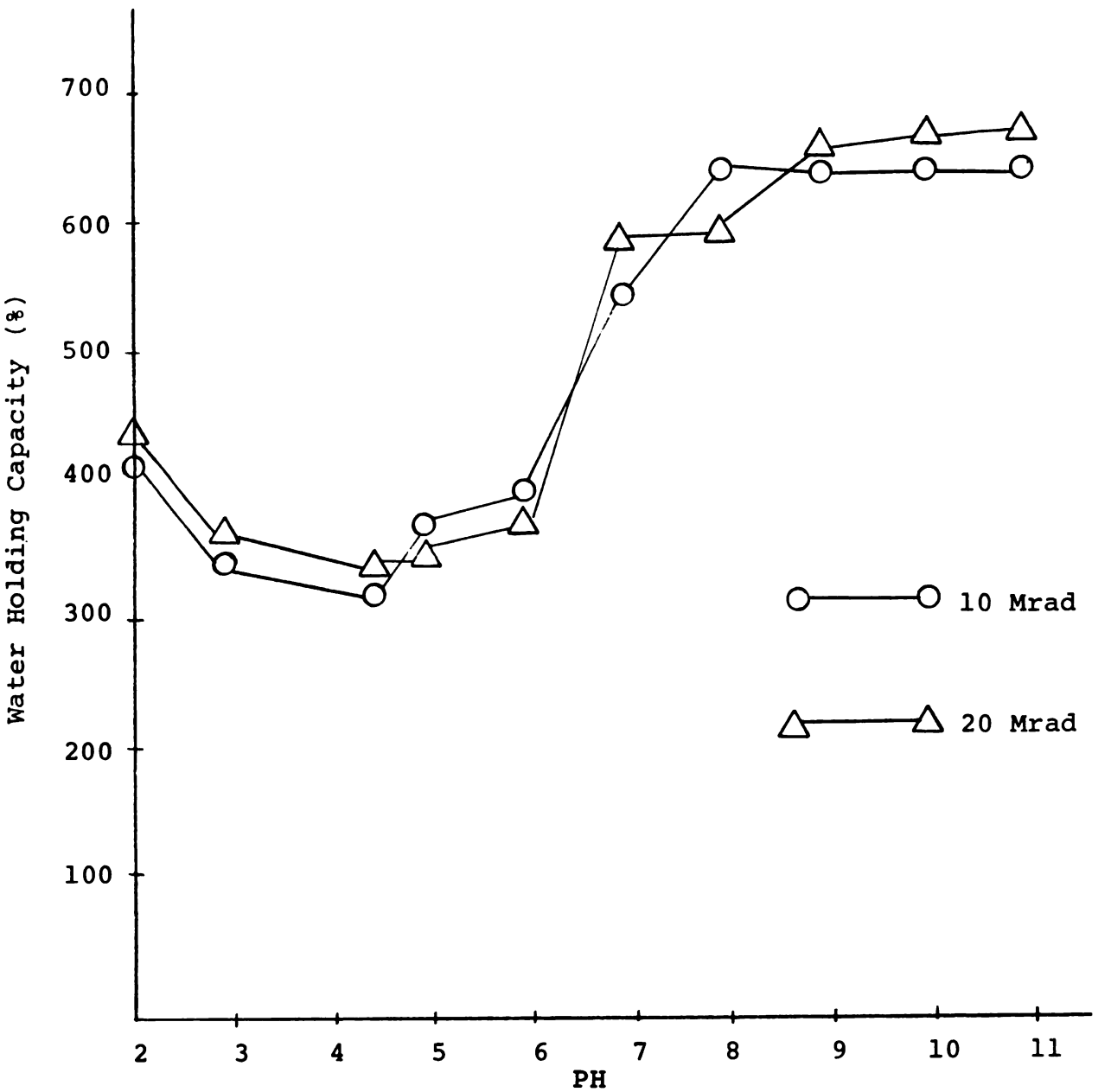


Figure 4.--Water Holding Capacity (%) of the Isolated Protein Sample that was Irradiated in Wet Form at the Doses of 10 and 20 Mrad and Freeze Dried.

nitrogen of the soy protein in this study was to find the amount of the decrease at various pH's and radiation doses. It was hoped that the irradiated protein would display a reduced solubility at a higher pH and at the same time would have a WHC greater than non-irradiated protein.

In Figure 5, the soluble nitrogen index of non-irradiated soy protein is shown for the pH range 1 to 11. In accord with theory, minimum solubility lies around isoelectric point. On both the acid and alkaline sides of the isoelectric point, the solubility is quite high. As seen in Figure 6, the solubility of soy protein which was irradiated in the dry form, decreases, giving the minimal solubility near the isoelectric point. At alkaline pH's, higher irradiation doses give lower solubilities but at acid pH's irradiation clearly did not affect the solubility. In Figure 7 data for the solubility of the protein which was irradiated in wet form, are shown. Quite different curves from those of the previous two graphs (Figures 5 and 6) were obtained. Again the higher doses decrease solubility. The most characteristic change of the solubility with wet irradiation, however, is that the solubilities around pH 7 are very low even at 1, 5 and 10 Mrads. This result is interesting when it is considered with the results shown in Figures 2, 3 and 4. In Figure 3, the WHC of the doses 1 and 5 Mrads

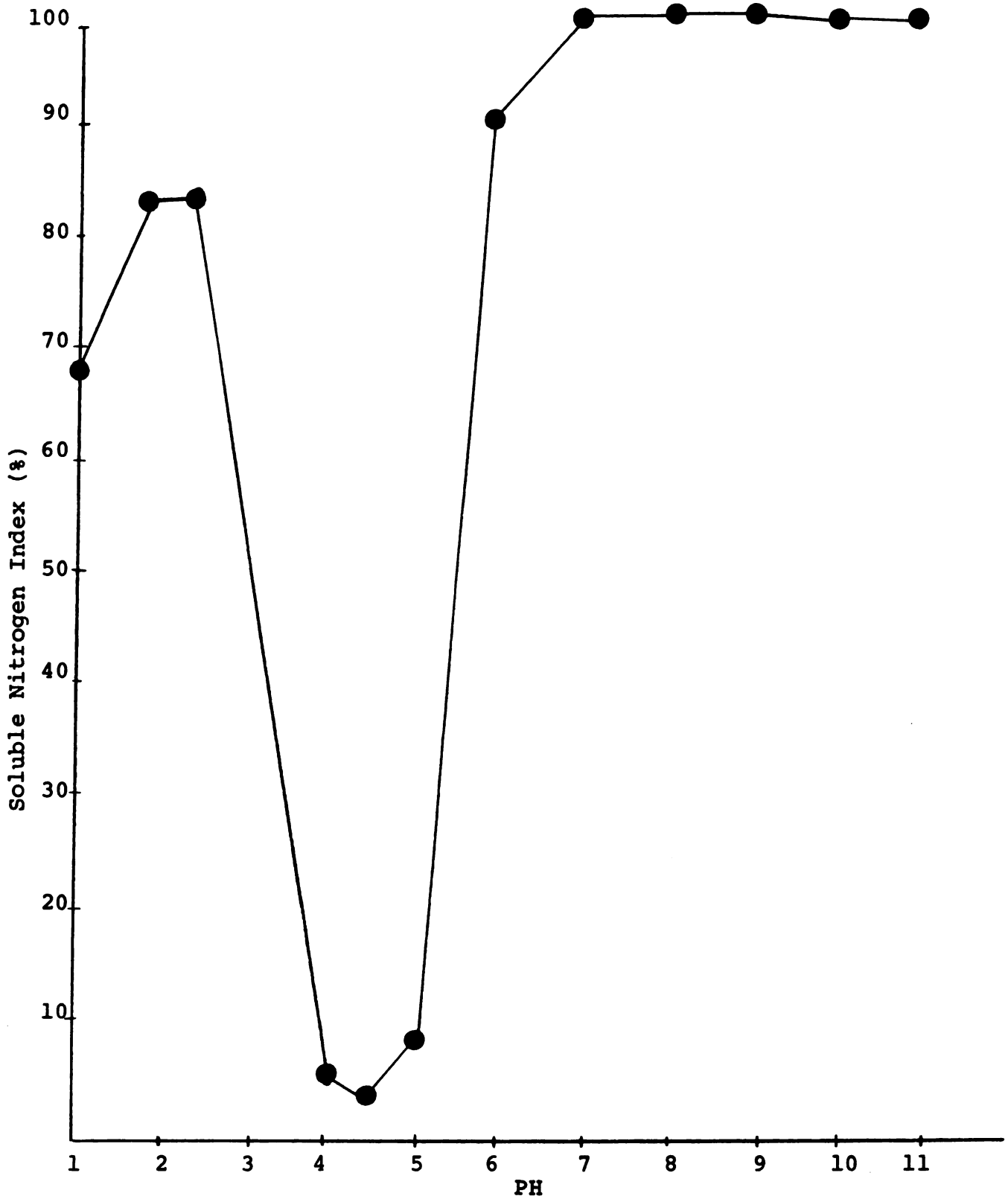


Figure 5.--Soluble Nitrogen Index of Isolated Soy Protein Without Irradiation.

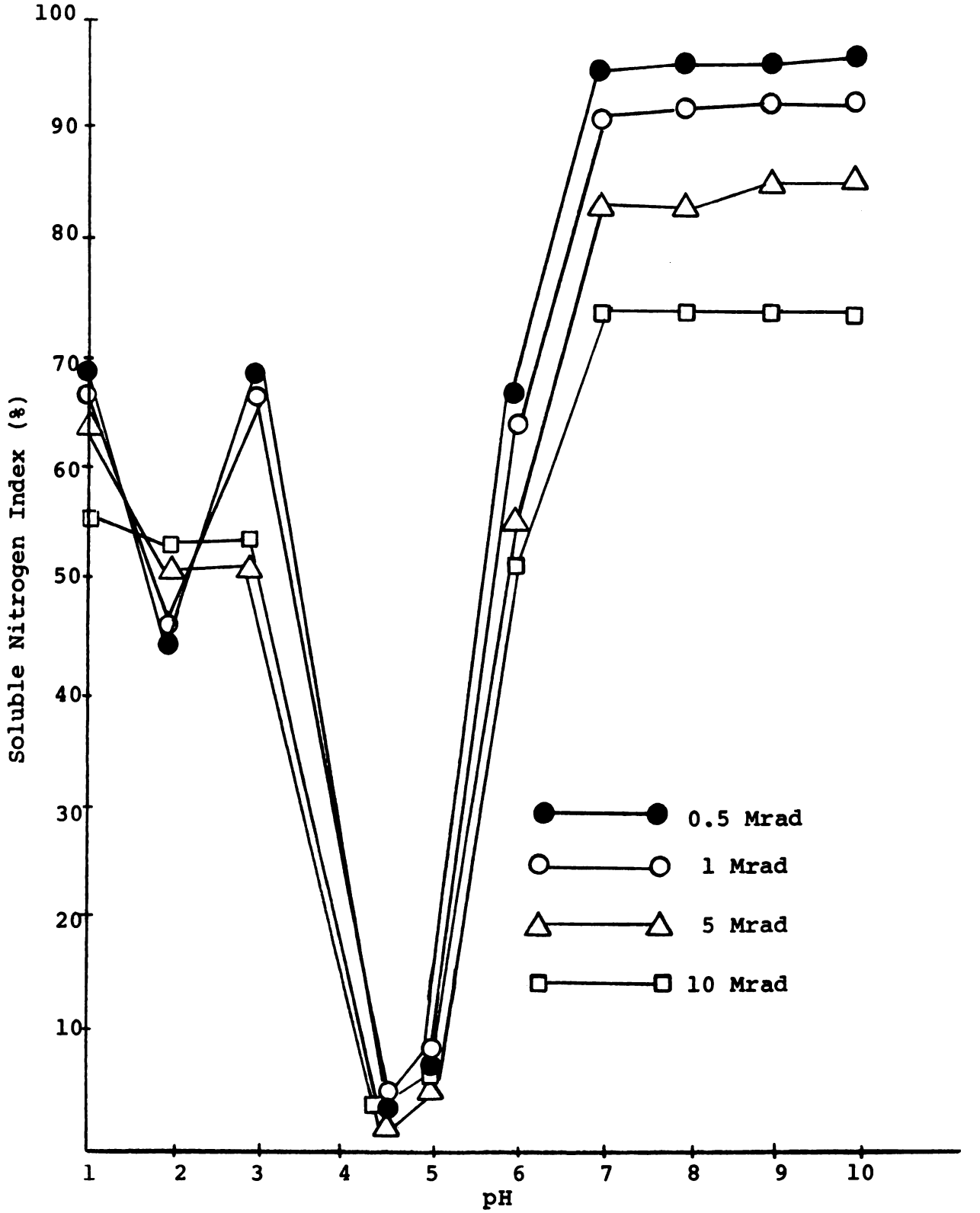


Figure 6.--Soluble Nitrogen Index of Isolated Soy Protein with Dry Irradiation.

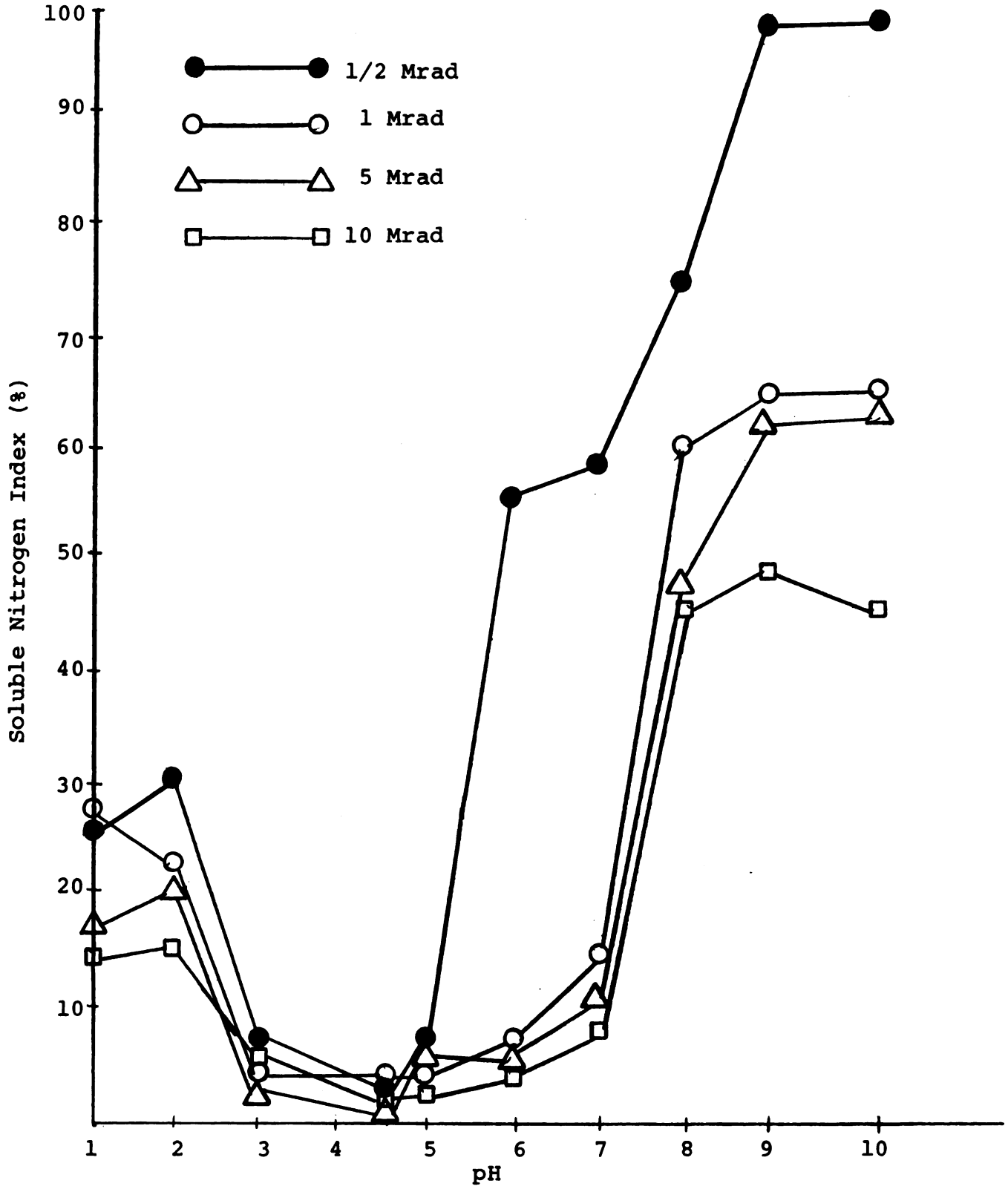


Figure 7.--Soluble Nitrogen Index of Isolated Soybean Protein with Wet Irradiation.

is given as approximately 500% and 10 Mrads about 825. In Figure 4, the WHC of the protein which was irradiated in a wet form and dehydrated again, is about 530% at pH 7. In Figure 2, the water holding capacity of unirradiated protein is shown about 625% at pH 7. From these results, one may conclude that if the protein is irradiated in wet form at about pH 7, the soluble nitrogen will be low and the WHC high. This could result in an improved spun protein food.

SUMMARY

In order to obtain a soy protein isolate with minimum denaturation, two methods of oil extraction were evaluated. The first, a batch method, in which hexane-ethanol mixture was used at room temperature did not remove sufficient oil, as evidenced by the analysis, which showed 3.1% oil and 89.1% protein. The second method, in which ethyl ether was used in a Soxhlet apparatus yielded a product analyzing 0.2% oil and 93.2% protein. This method was employed in this study.

In the determination of water holding capacity (WHC), two methods were tested. Both methods yielded similar results. The filter paper method was more convenient than the centrifuge method and was used for this reason. The minimal values of WHC were observed near the isoelectric point. Gamma radiation was applied to soy protein isolate, both dry and dispersed in water. Irradiation in the dry form did not change the WHC, whereas irradiation in the wet form caused some changes. At relatively high doses only (10 and 20 Mrad) irradiation in the wet form increased the WHC substantially, especially at the

higher pH's. Freeze drying of the irradiated protein restored the WHC to the values before irradiation. From this, it is concluded that the radiation-induced increase in WHC involved only absorption of water in the physical structure and was not due to significant chemical alteration of the protein. Changes in the soluble nitrogen index due to irradiation of the protein both dry and dispersed in water were measured. Only irradiation in the wet form produced an increase.

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