

EFFECTS OF X-RAYS ON WHEAT GLUTEN

by

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## INTRODUCTION

The relationship of the molecular structure of wheat gluten protein to its physical and biochemical properties remains one of the most perplexing problems in the field of plant biochemistry today. Although a number of physico-chemical techniques such as ultracentrifugation, electrophoresis, and fractional precipitation, etc., have contributed to increased knowledge of the nature of this complex substance, any new approach which shows at least a little promise of producing new information is to be welcomed.

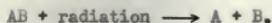
Since ionizing radiations appear to provide a means to introduce considerable energy into a very sensitive biological material such as gluten without appreciably affecting the temperature, and since considerable information has recently appeared on the effect of ionizing radiations on proteins of animal origin, it was deemed worthwhile to apply such treatment to gluten, with a view to ascertaining whether some clues might be obtained concerning the molecular structure and properties of this material.

Previous development in this laboratory of a method for the separation and partial purification of wheat gluten, as well as a viscometric technique to evaluate the molecular properties of gluten, provided the background for the present study of the influence of X-rays on this material.

## Historical Review

Direct and Indirect Action of Ionizing Radiations. Generally, two mechanisms dominate the theory of the fundamental reactions of radiation chemistry. One is that the ions produced by irradiation of a substance are themselves the agents which cause directly the reactions within the substance (direct "hit" theory). This theory is supported by a great deal of exact quantitative evidence. A second mechanism which has had only theoretical support but which has received popular acceptance in recent years was postulated to take place in dilute solutions whereby the effects of the radiation are brought about on the solute by reaction with free radicals and/or activated molecules produced in the solvent (indirect or activated solvent theory). In the radiation of dilute solutions, any direct action of radiations on the solute assumes minor importance since most of the radiation energy is absorbed by the water and is then either transferred to the solute by some mechanism not yet fully understood, or contributes to an increase in the energy state of the water molecules and is eventually dissipated as heat. In biological systems (organisms, etc.), the direct "hit" mechanism assumes more important proportions since the conditions (dilute concentrations of solute) necessary for the activated solvent mechanism are usually not fulfilled.

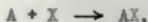
Weiss (44) gave an example of direct absorption by a "biologically active giant molecule (AB)" which was followed by dissociation, i.e., formation of radicals A and B according to :



There follows the possibility of either, recombination to form the original substance,



or reactions of the fission products to form new products with other substances (X and Y) which act as acceptor molecules according to the schemes:



The above three reactions represent the fundamental radiation process of the direct "hit" mechanism.

Primary Radiation Process for Water. Most authors agree as to the primary process involved in the decomposition of water by ionizing radiations.

As explained by Weiss (44), Allen (1) the absorption of penetrating radiations generally results in excitation and ionization as follows:



followed by



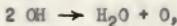
All of the above reactions are possible under the influence of radiation and thus, hydrogen atoms and hydroxyl-free radicals are eventually obtained.

Another mechanism for hydrogen and hydroxyl-free radical formation was also proposed by Weiss (43) in which the hydroxyl ion loses its electron by transference to a neighboring water molecule, which then dissociates to yield a free hydrogen atom and a hydroxyl ion.



This is usually followed by a recombination or decomposition products. This is especially true in solutions because the dissociation products primarily formed are held in close proximity by the solvent molecules. Such a "cage effect" was described by Rabinowitch and Wood (36) in which two molecules in the liquid state repeatedly collide with each other after an initial collision before one of them can escape the surrounding "cage" of molecules.

Actual decomposition of pure water can occur only so far as the subsequent reactions of H and OH free radicals to form molecular hydrogen and oxygen can compete with the reaction of the H and OH free radicals to reform water.



Effects of Solute. If there are substances present in the water which can react chemically with the hydrogen atoms and hydroxyl radicals initially formed, the radiochemical process is radically changed. The hydroxyl free radical is a strong oxidizing agent which, by accepting an electron is transformed into the hydroxyl ion, whereas the hydrogen atom is a powerful reducing agent. Practically all substances when

dissolved or dispersed in the water, will be attacked by these powerful reagents and will thus act as acceptors toward one or the other of the radicals formed.

For example, the acceptor action of molecular oxygen may be written:

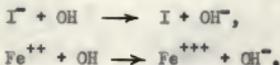


followed by the combination of two  $HO_2$  free radicals to form hydrogen peroxide and molecular oxygen which re-enters the process



Burton (7) points out that presence of oxygen causes the entrapment of free hydrogen atoms to yield  $HO_2$  radicals which are less reactive and are therefore longer range than hydrogen atoms or hydroxyl free radicals because they can travel farther within the solution before their energy is dissipated. Thereby the effectiveness of the radiation process is increased.

Other typical acceptor reactions are the oxidation of iodide or ferrous ions as follows:



It was pointed out by Fricke (20) that the latter reaction is important in the irradiation of oxyhemoglobin in which the production of methemoglobin is one of the reactions.

In the case of complex organic molecules (for example, tyrosine, enzymes, and proteins) there will always be a reaction of the solute with the hydroxyl radicals and possibly also with the hydrogen atoms thereby leading to decomposition or deactivation of the solute. If

no hydrogen or oxygen gas is evolved, one has to assume that both the radicals primarily formed have reacted with the solute.

Hydrogen Peroxide Formation. The production of hydrogen peroxide by irradiation of water has been thoroughly investigated. In 1929, Risse (39) announced that under irradiation by X-rays, pure water would not decompose at all, and he showed that hydrogen peroxide formation came as a result of oxygen dissolved in the water. This was confirmed by Fricke (22) and later by Bonet-Maury and Lefort (5). However, Allen (1) disclosed in a later publication that the irradiation of pure water with X-rays does result in  $H_2O_2$  production but that the reaction for the decomposition of  $H_2O_2$  causes a steady state concentration of hydrogen peroxide so low as to be scarcely detectable so that it appears as though X-rays do not decompose water.

Bonet-Maury and Lefort (6) carried out further investigation and found that the radiochemical yield (number of ion pairs produced per ml. per second) for X-radiation of aqueous systems depends largely on experimental conditions, increasing with temperature, intensity of radiation, and concentration of dissolved oxygen, and decreasing with dose and pH.

Influence of Ionizing Radiations on Proteins. According to the peptide theory, proteins are large molecules composed of amino acid residues joined through peptide bonds. The amino acid residues are believed by many to be oriented in definite patterns within the structure of the molecule and to be held in these patterns by hydrogen bonds and secondary valence forces between numerous polar functional groups.

Pauling et al, (37), indicate that the energy of formation of the hydrogen bond is of the order of 8 Kcal. per mole, indicating a relatively unstable bond when contrasted to the energies of formation of covalent bonds. Thus, it would be expected that the internal structures of labile molecules of protein would be disrupted by high frequency radiations which supply energies in the order of 1 million kcal. per mole.

It has been known for some time that the stability of proteins with respect to denaturation (as defined by aggregation) is lowered upon treatment with ionizing radiation. This was illustrated in 1939 by Fricke (20) who showed that the stability of egg albumin towards heat denaturation was lowered by X-irradiation prior to heating. Recently, he clarified the latter phenomenon more quantitatively (21). He calculated the energies of activation ( $E$ ) from measurements of first order velocity constants of the heat denaturation reactions of egg albumin in which irradiated and non-irradiated solutions were used,  $E$  was much lower in the case of the irradiated solution. He concluded that, "the irradiated protein is initially undenatured but contains relatively large quantities of (molecular) species of decreased thermal stability characterized by much reduced values of the energy of activation ....". For the mechanism of the lowering of  $E$ , he suggested the breaking of side-chain bonds or in extreme cases where very high dosages of radiation are used, the breaking of primary bonds in the polypeptide chain.

A great deal of the investigation of the effects of ionizing radiations on biological materials has been carried out using nucleoproteins and other intercellular proteins. In no instance has any information concerning irradiation of plant proteins been found.

Depolymerization of thymonucleohistone and sodium thymonucleate by X-radiation with dosages up to 120,000 roentgens was investigated viscometrically by Sparrow and Rosenfeld (41). A sharper decrease in viscosity and in birefringence for the nucleate was caused by equal dosages of radiation than for the nucleohistone. The decrease in these properties due to irradiation was interpreted as the partial scission or degradation of large asymmetric particles into shorter and more symmetrical segments.

Rozendaal et al, (40), carried out experiments of desoxyribosenucleoprotein fibers prepared from rabbit liver and calf thymus using cathode ray dosages up to 1,000,000 roentgens. It was found that release of desoxyribosenucleic acid was effected by the radiation in proportion to the dosage. No significant changes were found by varying the pH, or by the addition of hydrogen peroxide.

Stiff gels of nucleoproteins from chicken erythrocytes and carp sperm were irradiated by Errera (15). The gels gradually liquified. If the nucleoproteins were extracted after the intact cells were first irradiated with 50,000 r. of intense radiation, they formed liquid solutions instead of gels.

The effects of X-radiation up to 56,000 r. on solutions of sodium thymus nucleate were studied by Taylor, et al, (42). A decrease in the viscosity proportional to the dosage of X-rays and independent of the temperature was observed. Chemical and enzyme studies indicated that whatever the manner in which the particle had been damaged by X-radiation, it did not involve the splitting of primary linkages or a rearrangement of spatial

configurations necessary for its susceptibility to enzyme attack.

Matuo (32) irradiated the albumin sol of the yolk of hen egg with hard X-rays and observed that the viscosity was increased over a wide range of pH values. The increase in viscosity varied as the dosage used. He considered the mechanism of the radiation effect on the ovalbumin sol to be the same as that of heat.

Protective Effect of Cysteine and Other Substances. Small concentrations of reducing compounds containing the sulfhydryl group such as cysteine and glutathione cause flour doughs to soften appreciably due to direct action on the gluten protein. Cysteine also exerts a protective effect on organisms against the lethal effects of radiation. Because of this protective effect, some investigators have carried out experiments to determine the effects of cysteine and other substances on the properties of nucleoproteins and enzymes.

Feinstein (16) studied the protective effects of cysteine and other agents on thymus nucleoprotein sols. He found that cysteine, glutathione, cyanide, and thiocyanate greatly increase the viscosity of alkaline solutions of thymus chromosomes, while urea, and BAL do not do so. Hydrogen peroxide had no effect on the viscosity. If cysteine was added to the alkaline nucleoprotein solution before X-radiation, the expected reduction in viscosity was largely prevented. If it was added after X-radiation, it was much less effective in this respect. He suggested that this phenomena may be relevant to the question of the mechanism of cysteine in increasing resistance of organisms to X-ray lethality.

Patt, et al, (36) irradiated rabbit thymic cells with X-rays from

0 to 2,000 roentgens and determined their viability with a specific stain test. They found that the percentage of surviving cells decreased exponentially with increasing radiation dosage. The sensitivity of the thymic cells was decreased by a constant factor over a wide dosage range when cysteine was added to the suspension. The degree of protection was dependent upon the concentration cysteine as well as upon the time of addition relative to the period of irradiation.

Barron and Finkelstein (4) irradiated aqueous solutions of serum albumin, serum globulin, and egg albumin with X-rays and characterized the irradiated substances by ultraviolet absorption measurements, viscosity, sedimentation velocity, and electrophoretic mobility. An increase in optical density was caused by irradiation which was even more marked if the irradiated solutions were oxygenated. The changes in the absorption spectrum were attributed to oxidation of tyrosine residues and other oxidizable groups. Incorporation of salts into the solutions materially mitigated the effects of irradiation. The viscosity of the proteins increased slightly on irradiation with 50,000 r. but there were no marked changes in the electrophoretic mobilities or the sedimentation constants. Appearance of a second component taken to be a dimer was noted after irradiation with 100,000 and 200,000 r. Formation of the dimer was prevented by addition of cysteine but cysteine could not cause the protein to revert to its original properties once the dimer was formed. Precipitation of dilute solutions of serum albumin was produced by irradiation with 75,000 r. at 25°C. This did not occur when the solution was irradiated at 0°C., when the protein concentration was increas-

ed, or if salts were added to the solution.

Summary of Important Information Contained  
in the Literature

A number of facts can be discerned from the literature and are summarized here.

It is generally agreed that two distinct mechanisms are responsible for the observed chemical changes produced in matter by ionizing radiations: one is termed the "direct hit" theory by which the ions produced in a substance by the direct absorption of radiation are the agents causing the reactions, and the other, known as the indirect mechanism or activated solvent theory, is brought about by irradiation of aqueous solutions in which the energy absorbed by the solvent is transferred to the solute by a mechanism involving hydrogen and hydroxyl free radicals.

The primary irradiation process for water is the production of these free radicals which due to their great reactivity will cause chemical changes in almost any solute present.

Hydrogen peroxide is produced in significant levels of concentration by irradiation of water only when dissolved oxygen is present.

Irradiation of nucleoproteins results in a decrease in the viscosity of their solutions while irradiation of globular proteins such as egg albumin or serum globulin causes an increase in viscosity. Since viscosity depends chiefly upon the particle shape and increases with the degree of molecular asymmetry (30), these phenomena were interpreted to be due to

a depolymerization reaction in the case of the nucleoproteins and to a reaction mechanism causing the unfolding of the globular proteins resulting in more asymmetric molecules in the case of egg albumin and serum globulin.

#### Statement of the Problem

The present study of the influence of ionizing radiations on wheat gluten proteins was conducted by X-radiation of lactic acid dispersions of gluten prepared by a method outlined by Finney (18) using a viscometric technique as the principle criterion of effect. It was proposed to study the behavior of gluten and gluten sols when treated with X-rays under diverse environmental conditions of pH, temperature, concentration of protein, etc., and in the presence of sulfhydryl-containing reducing agents and to compare this behavior with that of other proteins which were similarly treated as reported in the literature.

#### MATERIALS AND METHODS

##### Sampling

Three separate samples of flour from which gluten was isolated were labeled A, B, and C. Flour A was a composite of approximately 50 different, experimentally milled flours obtained from the Hard Winter Wheat Quality Laboratory at Kansas State College and represented wheat

from every major wheat producing area in the United States. This composite was mixed in a mechanical tumbling device for one hour so that a homogenous sample was obtained. All of the flours contained good quality gluten based on evaluations made in the Hard Wheat Quality Testing Laboratory at Kansas State College (Finney - 17). The resultant composite was considered to be a representative sample of very high quality flour (protein content = 14.17%) from which gluten was prepared for use in radiation studies.

Flour B was a composite of flours used as a standard for quality evaluation of other flours by the baking test in the Hard Winter Wheat Quality Laboratory.

Flour C was milled at Kansas State College from hard red winter wheat grown in Kansas.

#### Viscosity Determination

Viscosities were determined by a method developed by Finney (18) and described by McCammon (33). The viscometer pipettes used were of identical design as those used by McCammon except that the water jacket was an integral part of the unit and the bulb in which the solution was contained was larger so that the accuracy of the method was increased. Two water thermostats were used to control the temperature of the viscometer assemblies. One capable of controlling the temperature to  $\pm .09^{\circ}\text{C}$ . was used for the preliminary viscosity determinations and the study on the influence of pH on viscosity. All other viscosity

determinations were made with a thermostat capable of controlling the temperature to within  $\pm .04^{\circ}\text{C}$ .

Protein content was determined by the Kjeldahl procedure according to the method outlined by the Association of Official Agricultural Chemists (2) with the exceptions that a mixture of sodium alizarin sulfate and methylene blue were used as an indicator for the titrations and the digestion period was shortened to 20 minutes since serious bumping occurred after this adequate length of time.

Controls consisting of ammonium oxalate solutions of known normality were employed with a number of the determinations at the same level of nitrogen content as the protein unknown. It was calculated from these that the standard error of the assay on the basis of five replications was - 0.27 percent.

All pH measurements were made with a Beckman Model G meter accurate to  $\pm 0.02$  pH units using a glass electrode and a calomel reference electrode. The electrometric titration of lactic acid contained in the lyophilized gluten preparations was made using the larger outside electrodes supplied for the pH-meter.

#### Preparation of Glutens

The technique used for isolation and purification of gluten from flour included washing the gluten from the flour, dissolving the gluten in lactic acid solution, purification by centrifuging at high angular velocity and finally, shelling and lyophilization of the purified gluten

solution. A more detailed explanation of these steps follows.

Isolation. Two hundred and fifty grams of flour (14% moisture basis) were introduced into a 2,000 ml. flask and 590 ml. of distilled water (2-4°C.) added and a uniform suspension effected by vigorously shaking the flask for one minute. The suspension was weighed into tubes and centrifuged at 1,800 r.p.m. for 15 minutes (International no. 2). The supernatant liquid was then combined with the doughy centrifugate from each tube and the dough kneaded in the liquid until a good separation of gluten and starch was effected (10 min.). After allowing the gluten to settle for 10 min., the mixture was passed through a 40-50 mesh sieve which allowed the starch suspension to pass through but retained most of the gluten. It was then collected and washed by kneading with six 40 ml. aliquots of cold distilled water ( 2 min. each washing) passing each aliquot through the sieve and combining with the starch suspension. The gluten was then weighed after pressing out as much water as possible and the value recorded as weight of wet gluten. The starch suspension was centrifuged for 20 minutes at 1,800 r.p.m. (International no. 2), after which the supernatant containing the water soluble portion was shelled, lyophilized and stored in the deep freeze for later study.

The shelling apparatus consisted of a motor driven device for slowly rotating a one-gallon jar at 2 r.p.m. in a horizontal position. The jar was partly immersed in a methyl-cellosolve and dry ice bath (-30° to -50°C) so that the solution was frozen in concentric layers on the walls of the jar. The solution in this form was in a suitable condition for lyophilizing.

Purification. The wet gluten obtained was sliced into small pieces and dissolved in 500 ml. of 0.1 N lactic acid by stirring in a covered beaker for 22 hours. The time required for effecting a solution was determined by taking 100 ml. aliquots of the mixture being agitated at various intervals of time ranging from 2 to 48 hours and centrifuging them at 2,300 r.p.m. (International no. 2). The volume of precipitate (which consisted mostly of starch particles) was found to be practically constant for all intervals beyond 20 hours. Twenty-two hours was chosen as a period for solvation because it was more adaptable to the overall time schedule.

The crude gluten solution thus obtained was centrifuged for 30 minutes in a Servall vacuum angle-centrifuge (SS-2) at 14,000 r.p.m. which corresponds to a relative centrifugal force of 33 kilograms per gram. Any starch particles or solid impurities trapped by the gluten meshwork and not removed by the isolation procedure were sedimented by the high centrifugal force developed in the centrifuge. The resulting supernatant was a relatively clear solution containing 3 to 5 percent protein.

The solution was then placed in a jar preparatory to shelling and allowed to stand at room temperature until its protein content could be determined by Kjeldahl analysis and a portion diluted to a 1 percent solution for viscosity determinations, after which it was shelled and stored in a deep freeze unit to await lyophilization.

Lyophilization. The purified gluten solutions were lyophilized by a method described by Yamazaki and Moser (45) except that a larger installation with certain improvements developed by Finney (18) was used.

which was capable of removing approximately 2.5 liters of water in a 24-hour period with a condenser of 5 liter capacity. The lyophilized gluten thus obtained was a light, flaky, and hygroscopic substance which was very water soluble.

#### Compositing of Lyophilized Glutens

A composite of several of these glutens was made up in order to have one large sample for use in the radiation studies. The composite (about 170 g. ) was made from 7 glutens prepared from flour A and one prepared from flour C. All of these samples had received the same treatment in their preparation. The composite was formed by sifting all samples through a stainless steel, 20 mesh screen into a large beaker and then mixing in a two gallon jar for an hour. The composite was divided into five equal parts and stored in numbered quart Mason jars at room temperature. Portions weighing  $1\frac{1}{2}$  grams were taken from each of the samples before compositing and stored at room temperature in small glass jars for a study on the stability of the protein towards aging. Later, during the course of the radiation investigations there was reason to doubt that the sample was homogenous since the viscosity of 1 percent solutions of the gluten taken from two separate jars proved to be significantly different. Kjeldahl analysis using 5 replicates showed that the protein content had not changed due to absorption of water from the air since the protein content of the composite assayed immediately after mixing was 78.4 percent. and after the discovery of the discrepancy ( a period of 45 days) assayed

as 78.6 percent. The whole composite was then reblended by rotating and tumbling for  $1\frac{1}{2}$  hours in a two gallon jar equipped with vanes which repeatedly sectioned and divided the protein bulk as the jar was rotated. After determination of the N-content, the composite was then divided again into four separate and equal parts for storage at  $10^{\circ}\text{C}$ . in quart Mason jars until used. The homogeneity was tested by determining the viscosity of 1 percent sols made up from samples from each of the four jars. Results are shown in Table I. The composite was considered to be homogeneous for practical purposes although not perfectly so. The variation in viscosities of the four samples was not significant since the standard deviation of the viscosity determination was approximately  $\pm .005$  centipoise.

Table I. Results of viscosity determinations demonstrating the homogeneity of the reblended gluten composite.

SAMPLE	:	VISCOSITY (centipoise)
1	:	1.250
2	:	1.258
3	:	1.247
4	:	1.249

#### Sample Holder and Tube Adapter

All samples of gluten sols or dry lyophilized gluten were irradiated in a glass weighing dish  $2\frac{1}{2}$  cm. deep and 5 cm. in diameter. The

same dish was used throughout the whole series of experiments so that conditions of dosage, etc., could be replicated. An adapter which would always hold the sample dish in precisely the same position in relation to the target of the X-ray tube was machined from brass stock. Plate I is an illustration of the adapter showing also the sample dish, the cover, and schematic diagram of the X-ray tube. The adapter consisted of an adapter ring and a base plate, which were threaded so that they could be disassembled to insert or remove the sample dish. The adapter was made water tight by a polyethylene gasket between the two parts. The whole assembly may be screwed into the tube housing which acts as its support. When a 25 ml. fluid sample is being irradiated, the distance from the center of the focal spot of the target to the center of the sample is 2-3/8 inches.

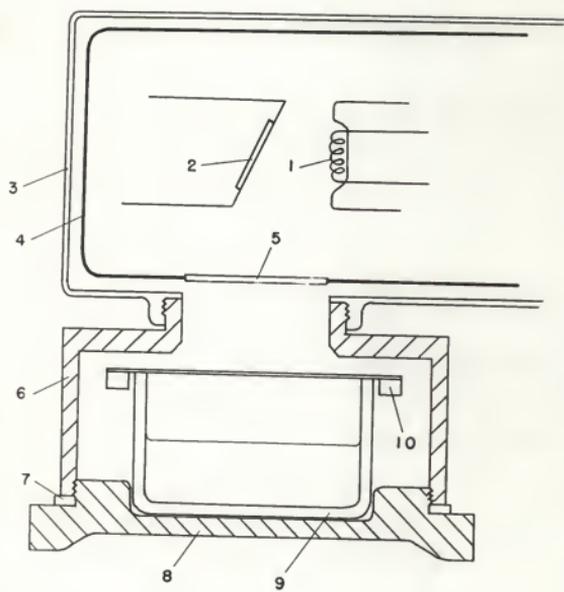
Temperature of the sample during irradiation was controlled by a constant temperature water bath or melting ice bath in which the assembly was immersed. Heat transfer through the brass base plate of the adapter and the glass sample dish was efficient enough to allow a temperature control to within  $\pm 0.5^{\circ}\text{C}$ . The adapter assembly served to hold all samples in the same position with respect to the tube target so that the output of radiation from the tube needed to be calibrated only for one position for a given voltage and tube current. The radiation dosage applied could then be controlled by the length of irradiation time. The holder had the added advantage of protection of personnel against radiation since the system was shielded by at least 1/8" of brass.

## EXPLANATION OF PLATE I

Cutaway view of brass adapter used for sample irradiation showing the glass dish and plastic cover with 25 ml. sample in place with a schematic drawing of the X-ray tube and target.

1. X-ray tube filament
2. Molybdenum target
- 3..X-ray tube housing
4. Glass X-ray tube envelope
5. Beryllium tube window
6. Brass Adapter ring
7. Waterproofing polyethylene gasket
8. Adapter base plate
9. Glass Sample dish
10. Plastic dish cover.

## PLATE I



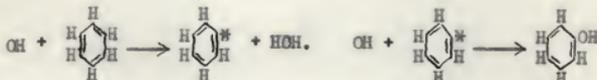
## X-ray Unit and Tube

A Picker low voltage industrial X-ray unit was employed equipped with a Machlett AEG-50-A Tube. The tube is a beryllium window, ground-anode tube designed to provide high intensity, long wave length X-radiation. Minimum inherent filtration of radiation was made possible by the thin beryllium window which is a part of the tube envelope.

The tube has a molybdenum target, employs a small focal spot of 1.5 mm, and is rated for continuous operation at 50 kilovolts peak and 20 milliamperes.

## Calibration of Tube Output

The chemical method of Day and Stein (11) was used to calibrate the output of the tube. The procedure is based on the production of free radicals in water by the action of ionizing radiation (10) which then react quantitatively with dissolved aromatic compounds to form phenols.



The dosage is directly proportional to the phenol produced in the presence of dissolved air up to 60,000 energy units where one energy unit is equivalent to 93.1 ergs/gm or to one roentgen for the case where the absorbing medium is air. The concentration of phenolic products

formed were assayed colorimetrically by use of Folin's phenol reagent. The color produced is compared with a standard curve based on the color produced by action of salicylic acid with Folin's reagent so that the amount of radiation absorbed is expressed as the concentration of salicylic acid per unit of energy absorbed. This relationship has been determined by Day and Stein to be  $6.04 \pm 0.15 \times 10^{-10}$  gm. salicylic acid per ml. per energy unit absorbed so that the standard curve may be calibrated directly in color produced versus radiation units. (11). The method has several advantages over measurement of dosage rates with an ionization chamber. It has been shown to be practically independent of the wave length of radiation, and is particularly suited for measurement of high dosage rates and total energy absorption by irregular volumes which are non-uniformly irradiated.

Phenol concentration was assayed by a modification of a method appearing in Practical Physiological Chemistry (Hawk, et al - 24). Several attempts were made to produce a standard curve following the exact procedure given but results were erratic and a smooth curve could not be obtained. Good results were obtained, however, with the following modified method:

Ten ml. of standard or unknown solution were pipetted into a 25 ml. volumetric flask. Five ml. of Folin-Cicalteau reagent (2) diluted one part reagent to 2 parts water were added followed by 3 ml. of 14 percent sodium carbonate solution. The solution was then agitated for 30 seconds and heated in a boiling water bath for exactly 5 minutes at which time a precipitate of lithium carbonate formed. It was immediately cooled by

immersion in a cold running water bath, diluted to 25 ml. and filtered. Optical density was determined at a wave length of 765 m $\mu$  in a Beckman spectrophotometer (model DU) at a slit width of 0.5 - 0.6 mm. using a blank in which water was substituted for an unknown solution or standard solution.

The absorption spectrum of the product formed by reaction of salicylic acid (25 ppm.) with Folin's reagent according to the procedure described is shown in Fig. 1. The absorption spectrum of the substance formed by reaction of the radiation produced phenolic substance with Folin's reagent was found to have the same maximum as that produced with salicylic acid. Fig. 2 shows the standard curve produced by reaction of salicylic acid with the phenol reagent. The standard solutions contained 0.5 percent benzoic acid in addition to the salicylic acid. The concentrations of salicylic acid appearing on the graph are those of the standard solutions of which 10 ml. were used in the procedure so that the optical densities shown corresponded to that produced from 10 ml. aliquots of phenolic substance diluted to 25 ml.

Calibration of the dosage rate of the tube was carried out by irradiating three 25 ml. aliquots of 0.5 percent benzoic acid for  $3\frac{1}{2}$  minutes at 45 K. V. P. and 20 M. A. using the brass adapter assembly. It was necessary that the same conditions of sample size, shape and relative position with respect to the target be maintained for all samples since the method of dosimetry described was designed to integrate the energy absorbed within a volume defined by the size, shape and position of the sample used for the calibration of dose rate. A sample size of 25 ml. was

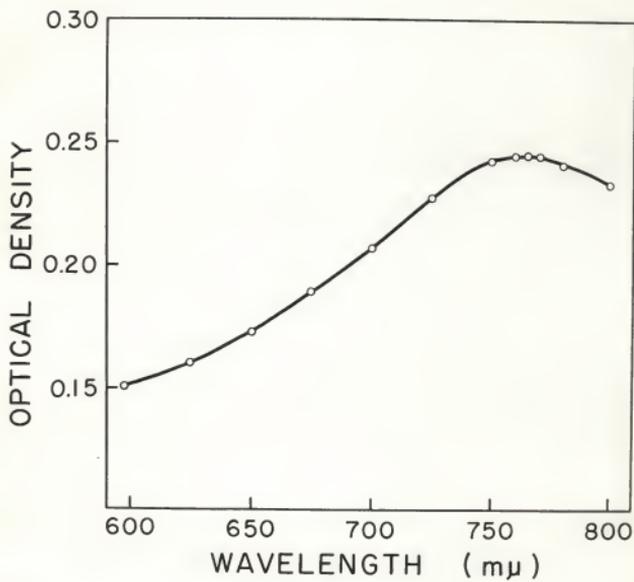


Fig. 1. Absorption spectrum of the substance produced by reaction of Folin Cicalteau reagent with salicylic acid.

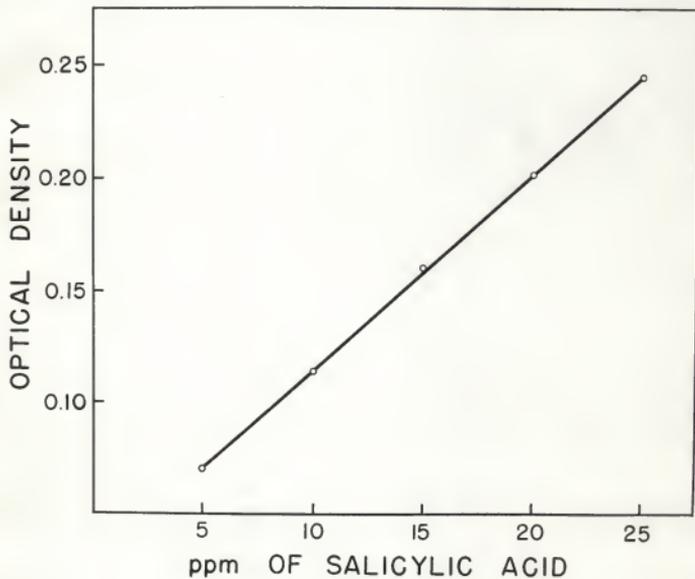


Fig. 2. Standard curve obtained by reaction of Folin Cicalteau reagent with salicylic acid. Multiplication of the equivalent salicylic acid concentration by 1656 gives the dosage of radiation absorbed in roentgens.

chosen since it was a convenient size for use in later radiation studies with gluten sols. Table 2 shows the data used to calculate the dose rate of the X-ray unit. It was assumed that the dosage rate of the X-ray unit remained constant after calibrating.

Table 2. Calibration data and dosage rate of X-ray unit using brass adapter and 25 cc. sample size. Irradiated  $3\frac{1}{2}$  minutes at 45 K.V.P. and 20 M.A.

Sample	Optical density	Equivalent salicylic acid concentration (ppm)	Dose rate (roentgens per min.)
1	.158	14.5	6860
2	.162	15.2	7200
3	.164	15.4	7290
Average	.161	15.0	7120

#### Irradiation of Samples

In order to eliminate error due to proteolytic activity as much as possible, all lactic acid sols of gluten were irradiated at 0°C. except those used in the study of the influence of temperature on irradiation effectiveness. It has been demonstrated by Olcott, et al, (35) that traces of proteolytic enzymes are adsorbed on gluten when it is isolated by the washing-out technique and that the loss in viscosity of dilute acetic acid dispersions of gluten is due to proteolysis.

Gluten sols dispersed in lactic acid were incubated at 0° and 30°C.

to determine the extent of proteolysis in terms of viscosity changes and to determine whether lowering the temperature of the sols would prevent viscosity loss. Two hundred and fifty ml. of 1 percent gluten sol were made up and immediately immersed in a constant temperature water bath at 30°C. Twenty-five ml. aliquots were taken at various times and the viscosity simultaneously determined in duplicate using two separate viscometer pipettes. The procedure was repeated substituting a melting ice bath for the 30° water bath. Fig. 3 shows the results. While the viscosity of the sol at 30°C. continued to fall, the viscosity at 0°C. leveled off after incubation for two hours and remained constant. The total loss in viscosity of the sol at 0° was only 0.01 centipoise.

The irradiation procedure consisted of making up the sols and immediately placing them in the sample holder which was already cooled to 0°C. by previous immersion in the ice bath. Non-irradiated controls were placed in the same ice bath in stoppered test tubes. The dosage of X-radiation applied was controlled by varying the time of exposure. After irradiation, the sols were kept at 0°C. for viscosity determinations or other tests.

Exposure of gluten in the lyophilized dry powdered form to X-radiation was carried out at room temperature using the same glass sample holder, cover and adapter used for irradiation of the gluten dispersions. A sample size of 0.7 g. was used throughout which, when leveled out and settled in the bottom of the container, was in a layer  $\frac{1}{4}$  inch deep. The same dosage rate as used for the 25 ml. liquid samples was used

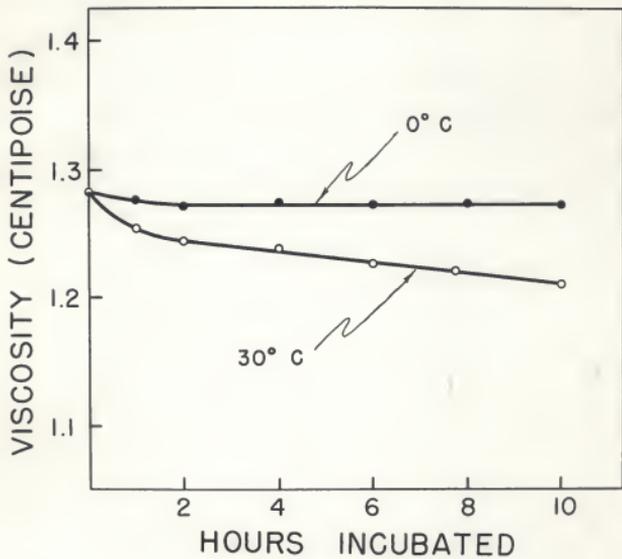


Fig. 3. Changes in viscosity of one percent lactic acid dispersions of gluten incubated at 0° and 30° C.

to calculate the dose for the solid samples.

Dosage of X-rays ranging from 0 to 700,000 r. were used since the effects of the radiation within this range could be adequately shown. A dose of 200,000 r. was chosen for those studies wherein the samples of irradiated and non-irradiated gluten were to be compared because this level of radiation was sufficient to cause a marked effect and could be applied in a relatively short period of time (ca.  $\frac{1}{2}$  hr.).

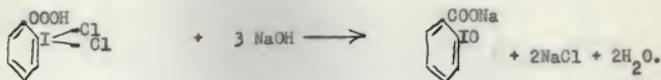
#### Synthesis of O-iodosobenzoic Acid

O-iodosobenzoic acid used in these studies as a quantitative reagent for determination of the sulfhydryl groups in gluten protein could not be obtained ready-made on the market, and had to be synthesized. The method of Lucas and Kennedy (31) for the synthesis of iodobenzene from iodobenzene was adapted to the synthesis of o-iodosobenzoic acid from o-iodobenzene. The synthesis was carried out in two steps. In the first, 0.12 moles of o-iodobenzene (Eastman) were chlorinated according to the procedure described by Lucas and Kennedy and the end product purified by washing twice with a little chloroform. The yield was 78.3 percent. The reaction involved was:



In the second step, the o-iodobenzene dichloride was treated with an excess of NaOH and NaHCO<sub>3</sub> in the cold to yield the sodium salt

of *o*-iodosobenzoic acid.



After filtering the solution, *o*-iodosobenzoic acid was precipitated by neutralization with HCl. The *o*-iodosobenzoic acid was separated by filtering through a Buchner funnel and purified by washing several times with cold water. The product (62.5 % yield) was dried over anhydrous  $\text{CaCl}_2$  for five days and the purity determined by iodometric titration of two samples. The reaction involves the oxidation of  $\text{I}^-$  to free iodine.



The method of synthesis resulted in an overall yield of 48 percent of theoretical of average purity of 97 percent.

#### Titration of Sulfhydryl Groups

A method of determining the sulfhydryl content of gluten was sought which was sufficiently sensitive to show a slight change in -SH content which could be caused by irradiation with X-rays. A first attempt was made to use a modification of the method of Hellerman, et. al (25) in which an excess of *o*-iodosobenzoate was added to a sol of gluten dispersed in 10 percent sodium salicylate at a pH of 7 and the excess de-

terained by iodometric titration. However, the starch-iodine complex used as an indicator was so unstable in the presence of the sodium salicylate that it was impossible to obtain clear endpoints. The amperometric titration method of Foulk and Bawden (19) was then applied as an alternative and was found to be much more accurate as a method for determining the excess iodosobenzoate. The method depends on the depolarizing action of iodine on the cathode employed in the circuit. The anode is kept depolarized by  $I^-$  or  $S_2O_3^{2-}$ . When the endpoint is reached, i.e., when an excess of iodine is present, the cathode is depolarized and a current is registered by a permanent deflection on the galvanometer in the circuit. Successive additions of more iodine cause still greater deflections and these are plotted against the ml. of iodine solution added. Extrapolation of the plot to zero galvanometer deflection (current flow) gives the endpoint.

The specific application of the method for the determination of the sulfhydryl content of gluten used in these studies was based on that employed by Larson and Jenness (29) for milk protein and was as follows: Ten ml. of 2 percent gluten sol were added to 10 ml. of water containing 2 ml. of molar phosphate buffer (pH 6.4) and 4 gm. of sodium salicylate (which was needed to prevent the precipitation of the gluten at that pH). An excess of iodosobenzoate solution was added ( usually 4 ml. of .005 N solution) and the mixture allowed to react for 2 mins. An even larger excess of .005 N  $Na_2S_2O_3$  (5 ml.) solution was then added followed by 3 ml. of 10 percent KI solution. The solution was then titrated with freshly standardized .005 N iodine solution using the amperometric method of Foulk and Bawden to determine the endpoint. The milli-

equivalents of -SH groups present were calculated by subtracting the milliequivalents of iodine titrated in a blank determination (in which water was substituted for the gluten sol) from the milliequivalents of iodine titrated when the protein sol was determined. This value was then multiplied by the milliequivalent weight of cysteine (.1211) to calculate the weight of cysteine present.

#### EXPERIMENTAL AND RESULTS

##### Analysis of the Gluten Composite

The gluten composite prepared from several samples of lyophilized glens, was analyzed for moisture, lactic acid content, total nitrogen content (reported as percent of total nitrogen), non-protein nitrogen, ether-extractable lipids, total lipids, and ash.

Moisture was determined by the 130°, 1 hour, air-oven method (2), total nitrogen by Kjeldahl analysis, and ash according to the procedure outlined in Cereal Laboratory Methods (9). All determinations were run in duplicate and the average taken.

Fifty ml. of a 1 percent solution of gluten was titrated electrometrically with 0.05 N KOH in order to determine the amount of free lactic acid present per gram of protein. When the change in pH per ml. of base solution titrated, ( $\frac{\Delta \text{pH}}{\text{ml.}}$ ), was plotted against the total after each addition of base solution, two peaks were obtained, the first of which was taken as the neutralization point for the lactic acid. Calculation

showed that 1.43 millimoles of lactic acid per gram of protein were present.

Ether-extractable lipids were determined by extraction of a 1 gm. sample of gluten with petroleum ether for 4 hrs. in a Goldfish extraction apparatus. The petroleum ether was evaporated and the residue dried to constant weight in an air oven at 100°C. The weight found was calculated as ether-extractable lipids.

Several investigators (Dill-14) have shown that only a small fraction of the lipids present in the crude gluten fraction of flour obtained by the "washing-out" technique can be extracted with petroleum ether. Olcott and Mechem (34) showed that when flour is wetted as in the washing-out technique, part of the ether-extractable lipids become bound to the gluten and consequently, cannot be extracted with ether. Treatment with absolute alcohol causes the bound lipids to be released. The lipids extractable with absolute ethanol and petroleum ether are termed total lipids in this report.

Total lipids were determined by a variation of the method described by Olcott and Mechem (34). A sample of gluten composite weighing 1.5121 gm. (11.32 percent moisture) was dried at 80°C. in vacuo for one hour whereupon it lost 2.37 percent moisture. A portion of the dried sample weighing 1.3337 gm. (dry weight) was extracted in a Goldfish extractor for 3 hours with absolute alcohol and then 4 hours with petroleum ether. The alcohol extract was evaporated and the residue dried for 15 minutes in an air oven at 100°C. The lipids were recovered from the dried residue by dissolving in petroleum ether followed by filtering. This fraction

was then added to the other petroleum ether extract, the ether evaporated, and the residue dried to constant weight at 100°C. The weight of the residue was taken as total lipids.

Non-protein nitrogen was determined as follows. One gm. of protein composite was dissolved in 25 ml. of water and 25 ml. of 35 percent trichloroacetic acid added and the mixture shaken intermittently for one-half hour. The precipitated protein was separated from the solution by filtering through Whatman no. 5 filter paper. The filtrate was still cloudy but this was considered to be due to suspended impurities such as carbohydrates rather than to unprecipitated protein. The nitrogen content of the filtrate was determined by Kjeldahl analysis of 2 - 15 ml. aliquots. The nitrogen in the filtrate thus obtained was expressed as percent of total nitrogen and was termed non-protein nitrogen.

Table 3. Analysis of the gluten composite used in all of the radiation studies. All results except non-protein nitrogen and lactic acid content reported on "as is" moisture basis.

Ether-extractable lipids	0.85%
Non-protein nitrogen (% of total N)	2.60%
Lactic acid (millimoles/gm of gluten)	1.43
Moisture	11.32%
Protein content	78.04%
Total lipids	4.47%
Ash	0.31%
Unidentified substances	5.86%
Total	100.00 %

Table 3, just preceding, shows the analysis of the gluten composite and accounts for 94 percent of the total constituents. The remainder most probably consisted of starch, insoluble carbohydrates and other impurities which were not separated from the gluten in the washing and purification processes.

#### Replicability of the Gluten Preparation Process

Replicability of the isolation and purification processes for preparing lyophilized gluten samples from a single flour (flour A) was tested viscometrically. The processes were described in the section entitled "Materials and Methods". The nitrogen contents of the freshly centrifuged gluten sols were determined by Kjeldahl analysis of five 10 ml. aliquots, the sols diluted to 1 percent protein and their viscosities immediately determined at 30°C.

Before the actual replication study was conducted however, several practice runs on the process were carried out in order that some uniform degree of proficiency be maintained throughout the procedure, especially for the washing-out technique which was entirely manual. Several samples of flours A, B, and C (see section entitled "Sampling") were used in conducting the practice procedure which was carried out entirely including the viscosity determinations in some cases. The results shown in Table 4 include the values for the weight of wet gluten obtained by the washing-out procedure and the value for the percent total nitrogen recovered from flour as gluten (calculated by the following equation) as well as the

viscosity and pH of the 1 percent solutions.

$$\% \text{ total nitrogen} = \frac{\text{Total N in flour}}{\text{N in purified solution}}$$

The value in the denominator was calculated as the product of the concentration of nitrogen in the purified solution and the total volume of crude gluten solution instead of the purified solution since losses of the solution were incurred during the purification step. Sample A<sub>2</sub> was suspended in acetic acid to test the stability of gluten in its presence during storage of the lyophilized product.

Table 4. Results of viscosity determinations on 1 percent solutions of gluten lactate in a preliminary study on replicability of isolation and purification techniques.

Sample	Average Viscosity (centipoise)	Wt.-wet gluten (gm.)	pH	% Total N <sub>2</sub> recovered as purified gluten
<u>1/</u>				
B <sub>1</sub>	--	75	--	--
B <sub>2</sub>	--	74	--	--
B <sub>3</sub>	1.459	78	3.04	69.5
B <sub>4</sub>	--	70	--	66.3
B <sub>5</sub>	1.426	70	3.01	69.5
C	1.371	90	3.18	70.0
<u>2/</u>				
A <sub>1</sub>	--	128	--	--
<u>3/</u>				
A <sub>2</sub>	1.347	135	--	78.6
A <sub>3</sub>	--	130	--	--

See explanation of groupings 1/, 2/, and 3/ following immediately.

Explanation of groups:

- 1/ Dissolved in 1 liter of 0.02 N lactic acid and centrifuged for 15 minutes at 12,000 r.p.m.
- 2/ Crude gluten solution kept at 2°- 4° C. in ice-box for 13 days before purification.
- 3/ Dissolved in 500 ml. of 0.1 N acetic acid.

The three flour samples responded differently to the initial washing-out procedure. The gluten of flour B tended to disintegrate more readily and was much less cohesive than the gluten of sample C or of A which was the most cohesive of all. This partially explains the differences in values obtained for percent of total nitrogen recovered as gluten shown in the last column of Table 4, since small gluten particles would pass through the sieve used to recover the gluten along with the starch suspension in the separation technique and thus would be lost.

Table 5 shows the results of the actual replication study carried out on five samples of flour A and is intended to demonstrate the replicability of the processes. Included in the table are the viscosities determined after the samples were lyophilized and 1 percent solutions made with the lyophilized products.

Table 5. Viscosity and pH of 1 percent gluten sols determined at 30°C. before and after lyophilization of the glutens. Glutens prepared from flour A.

Before lyophilization		After lyophilization	
Viscosity (centipoise)	pH	Viscosity (centipoise)	pH
1.246	3.24	1.280	3.23
1.249	3.22	1.277	3.22
1.251	3.18	1.277	3.21
1.254	3.23	1.276	3.23
1.268	3.22	1.281	3.20

The results show the viscosities of the sols after lyophilization to be on the average of 0.025 centipoises higher than those determined before lyophilization, a difference which statistical analysis proved to be significant at the .01 confidence level. The wide range of viscosity values obtained before lyophilization was due primarily to errors of measurement of the protein contents of the purified gluten solutions and unavoidable errors in the dilution of the lafter solutions to 1 percent.

#### Stability of Gluten During Storage

The viscosity and pH of 1 percent solutions of several glutens were determined after lyophilization of the glutens and also after a period of 140 days during which time the glutens were stored at room temperature in closed containers. Table 6 shows that neither the pH

nor the viscosities of most of the sols prepared using lactic acid as the dispersing agent had changed significantly. However, the values for sample A<sub>2</sub> which had been suspended in 0.1 N acetic acid during its preparation shows that lyophilized gluten is not stable in the presence of acetic acid.

Table 6. Viscosity (30°C.) and pH of 1 percent sols of gluten determined soon after lyophilization and 140 days thereafter during which time they were stored at room temperature in closed containers.

Flour from which gluten was prepared	:      Soon      :		:      140 days      :	
	: after lyophilization		: after lyophilization	
	: Viscosity :	: pH :	: Viscosity :	: pH :
	:(centipoise)		:(centipoise)	
C	1.426	3.14	1.472	3.16
<u>1/</u>				
A <sub>1</sub>	1.105	3.22	1.101	3.27
<u>2/</u>				
A <sub>2</sub>	1.246	1.00	1.192	1.00
A <sub>3</sub>	1.289	3.22	1.326	3.23
A <sub>4</sub>	1.280	3.23	1.284	3.23
A <sub>5</sub>	1.277	3.22	1.283	3.30
A <sub>6</sub>	1.277	3.21	1.285	3.23
A <sub>7</sub>	1.276	3.23	1.281	3.23
A <sub>8</sub>	1.281	3.20	1.292	3.30

1/ Crude gluten solution kept at 2 - 4°C. for 13 days before purification.  
2/ Crude gluten suspended in 500 ml. of 0.1 N acetic acid instead of 0.1 N lactic acid.

### Influence of pH on Viscosity

The pH of 1 percent solutions of gluten which had been prepared by McCammon (33) were adjusted by adding 0.1 N lactic acid or 0.1 N KOH before adjusting the volume of the solution to the mark. Viscosities were then determined along with the pH. Fig. 4 is a plot of the pH against viscosity and indicated that the viscosity of gluten sols is dependent upon pH. The determinations were not carried out beyond pH 4 since the gluten began to precipitate from solution above this pH.

### Influence of Temperature on Viscosity

One percent solutions of the same gluten preparation as used in the study of pH on viscosity were made up and the viscosity determined at temperatures ranging from 20° to 45°C. A plot of the viscosity against temperature is shown in Fig. 5. The curve was found to correspond closely to the equation,

$$\log V = \frac{A}{T} + B,$$

where V is the viscosity in poise, T is the absolute temperature, and A and B are constants which, calculated from the data by the method of least squares, were found to equal 870.2 and -4.841 respectively. The plot shows that the temperature coefficient of the viscosity of gluten sols is large and that consequently, the temperature must be accurately controlled for accurate determination.

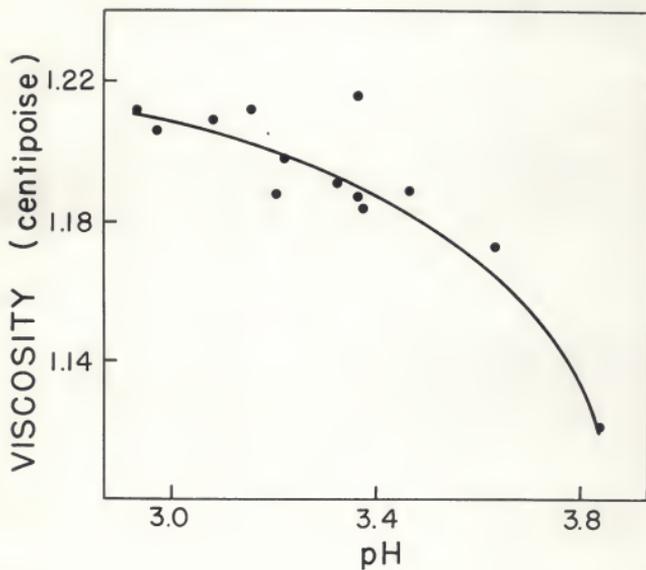


Fig. 4. Scatter diagram showing the decrease in viscosity of 1 percent gluten sols when the pH values of the sols are increased.

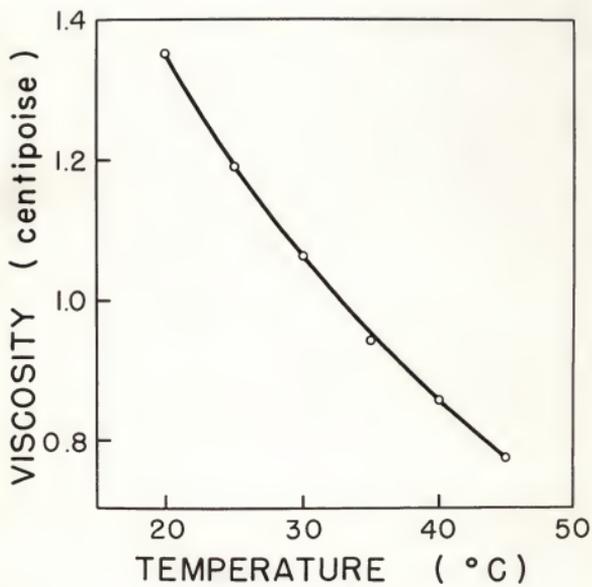


Fig. 5. Effect of temperature on the viscosity of 1 percent lactic acid suspensions of gluten.

## Effects of X-rays on Gluten and Gluten Sols

Dosage Study. Samples of gluten and gluten sols were given doses of X-radiation up to 700,000 roentgens. The dry gluten samples (0.7 gm.) were irradiated at room temperature. Immediately after irradiation, each sample was dissolved in water to make 1 percent sols and the viscosity of the sols determined in duplicate at 30°C.

Twenty-five ml. aliquots of 1 percent gluten sols were irradiated at 0°C. and their viscosities determined in duplicate at 30°C. as described in "Materials and Methods". Fig. 6 shows the results of the determinations after various doses of X-radiation were administered. The plot shows that the viscosity of the sols made up of the irradiated dry gluten decreases linearly as the amount of irradiation is increased while the decrease in viscosity of the irradiated sols with increasing dosage is a non-linear function. The drop in viscosity indicated that the protein molecules were broken up into shorter and more symmetrical particles and the greater drop in viscosity of sols irradiated in solution indicated that a separate and more efficient mechanism was responsible for the breakdown of the protein molecules in solution than for the case where the dry gluten was irradiated. This corroborated the results of other workers who have made similar radiation studies with animal proteins. Sparrow and Rosenfeld (41) showed that when 0.2 percent solutions of sodium thymonucleate and 0.4 percent solutions of thymenucleohistone prepared from calf thymus were irradiated with X-rays, progressively smaller decreases in viscosity of the solutions occurred with

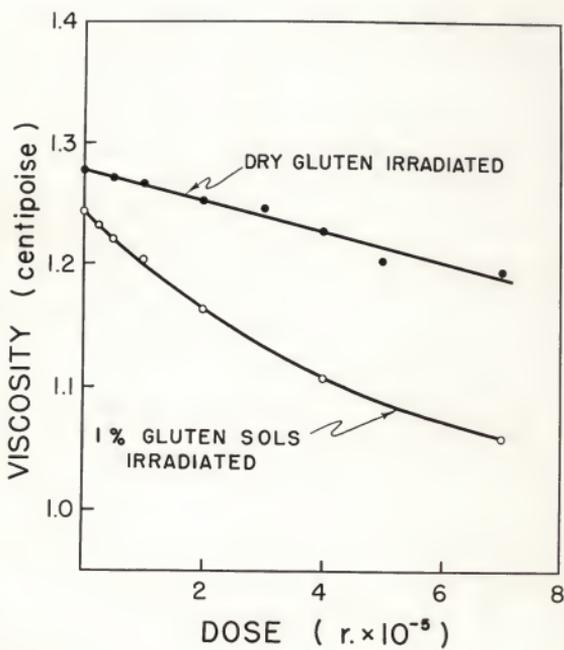


Fig. 6. Change in the viscosity of irradiated gluten sols and sols of irradiated gluten at varied levels of irradiation.

increasing dosage of X-radiation. Taylor, et. al., (42) studied the effects of X-radiation up to 50,000 r. on solutions of sodium thymus nucleate and noted a decrease in the viscosity proportional to dosage and independent of the temperature at which it was irradiated.

Effect of Incubation after Irradiation. A 25 ml. aliquot of 1 percent gluten sol was irradiated at 0°C. with 200,000 r. during which time the sol was incubated at 30°C. The viscosity of a non-irradiated control which received identical treatment was determined simultaneously with the irradiated sample. Fig. 7 shows that the irradiation had no apparent effect on the behavior of the sol except to lower the initial viscosity. The proteolytic enzyme(s) present were not inhibited at this level of irradiation since the viscosity of the irradiated and non-irradiated samples decreased at the same general rate.

In order to determine if irradiation of dry gluten had an inhibitory effect on the proteolytic enzyme(s), a 0.07 gm. sample of the lyophilized gluten was irradiated with 200,000 r. and the viscosity of its 1 percent solution determined at 30°C. at various periods of incubation up to 18 hours after irradiation. A non-irradiated sample was given identical treatment. The results, shown in Fig. 8 demonstrate that there is no inhibitive effect on proteolysis and that the principle effect is the initial lowering of the viscosity.

Irradiation of Dialyzed Sols of Ether-Extracted Gluten. In order to study the effects of impurities present in the gluten composite on the irradiation of gluten sols, a 2 gm. sample of the composite was ether-extracted and the sol made up from it was dialyzed before irradiation.

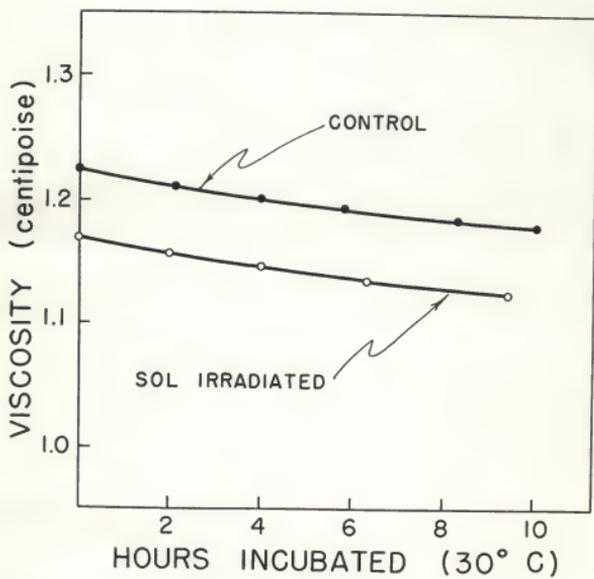


Fig. 7. Change in viscosity of a one percent gluten sol irradiated with 200,000 roentgens of X-radiation upon incubation at 30°C.

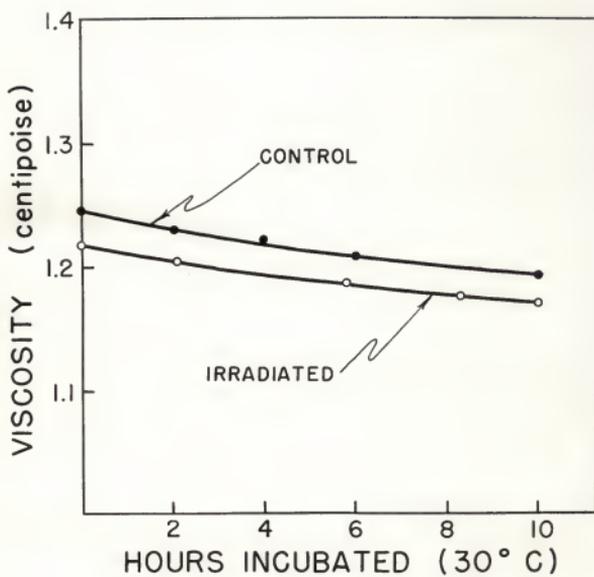


Fig. 8. Change in viscosity of a one percent sol of irradiated gluten (200,000 roentgen) upon incubation at 30°C.

iation. The sample was extracted with five 50 ml. portions of petroleum ether over a period of ten hours, alternately centrifuging and decanting the extract. The extracted material was then air-dried and a 2 percent solution made from it. It was dialyzed for 4 days at 2 - 4°C. against frequently changed distilled water. After dialysis, the sol was kept at 2 - 4°C. while the nitrogen content of the sol was determined by Kjeldahl analysis on two 5 ml. portions and was then diluted to 1 percent protein and 25 ml. portions irradiated with 200,000 and 400,000 r. at 0°C. The pH of the purified sols was 5.0 while that of the unpurified sols was 3.2. After irradiation, the pH was adjusted to 3.2 with concentrated lactic acid and the viscosity determined at 30°C. Table 7 shows a comparison of the viscosities of the sols made up of the gluten composite and of the purified and dialyzed sols at the levels of irradiation used. The data show that purified sols are more sensitive to X-radiation than are the unpurified sols. This difference may have been due to the difference in pH at which the sols were irradiated. The loss of viscosity which the gluten sol underwent during its purification was due to proteolysis during the dialysis period.

Table 7. Viscosities of 1 percent gluten composite sols and dialyzed sols of ether-extracted gluten composite after receiving various doses of X-radiation.

Dose (roentgens)	Viscosity (centipoise)	
	Standard composite	Ether-extracted and dialyzed samples
0	1.243	1.219
200,000	1.163	1.169
400,000	1.108	1.149

Effect of Temperature of Irradiation. This study was conducted to determine whether the temperature of the sol during irradiation had an effect on the radiation phenomena involved and to thereby determine whether the temperature of irradiation must be rigidly controlled in order to obtain accurate results.

The temperature of the sol during irradiation was controlled by a constant temperature water bath, and in one instance by a melting ice bath in which the brass adapter was immersed. The temperature of the contents of the sample holder corresponded to the temperature of the water bath to within  $\pm 0.5^{\circ}\text{C}$ . The sols were irradiated at different temperatures and all were given a dose of 200,000 r. Non-irradiated controls received the same treatment and were run simultaneously with the irradiated samples. Viscosities were determined at  $30^{\circ}\text{C}$ . immediately after irradiation. The results shown in Fig. 9 indicate that the radiation sensitivity of the sols is slightly greater at higher temperatures but that the effects of temperature on the sensitivity of the sols to X-rad-

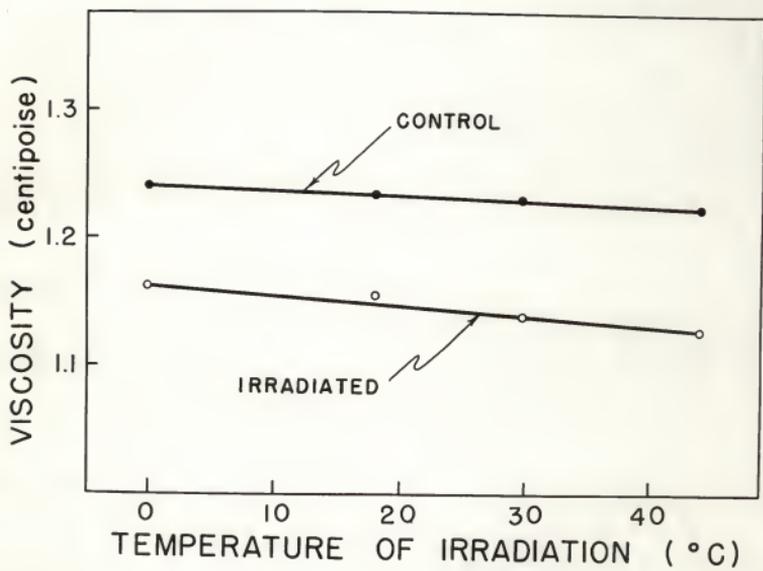


Fig. 9. Influence of the temperature of irradiation on the X-ray induced reduction in viscosity of one percent gluten sols.

iation is relatively insignificant so that slight variations in temperature are not a source of experimental error.

Effect of Gluten Concentration. Gluten sols were made up at concentrations ranging from 1 to 5 percent and were irradiated with 200,000 r. Immediately after irradiation at 0°C. they were diluted to 1 percent and their viscosities determined at 30°C. Non-irradiated controls received the same treatment and their viscosities were determined simultaneously with the irradiated samples. The pH of the gluten sols did not vary by more than 0.1 pH units from a mean of 3.2 units.

Results of the viscosities of the sols are plotted against the concentrations at which they were irradiated as shown in Fig. 10. The gluten sols are definitely more sensitive to X-radiation at the more dilute concentrations. This phenomena supports the "activated solvent" theory of the effect of ionizing radiations on aqueous solutions (- 13).

Effects of pH. In order to study the effects of pH on the sensitivity of gluten sols to viscosity reduction by X-rays it was necessary to adjust the pH of the sols to the value required for irradiation and after irradiation, to readjust the pH to a common value for the viscosity determinations since the viscosity of the sols are dependent on pH. Several attempts to reversibly adjust the pH of gluten sols by various means so that the sols which had been readjusted would have a common viscosity value were unsuccessful. The agents used to adjust the pH to the required value for irradiation were KOH,  $\text{NaHCO}_3$ ,  $\text{Ca(OH)}_2$ , and dialysis. After irradiation, all sols were readjusted to a pH of 3.2 by addition of concentrated lactic acid.

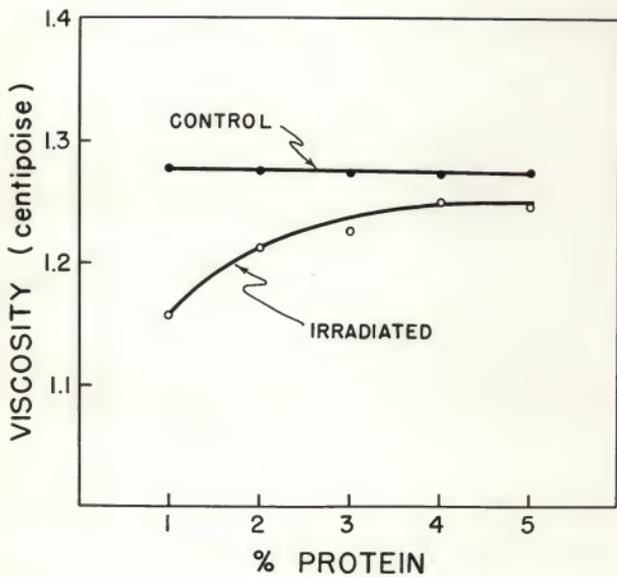


Fig. 10. Influence of the concentration of gluten on the sensitivity of gluten sols to viscosity reduction by X-rays (200,000 roentgens). Viscosities determined on the sols after dilution to one percent.

Table 8 shows the results of the determinations in which KOH and  $\text{NaHCO}_3$  were used to adjust the pH values. All sols were made up by adding the agent to a sol which contained more than 1 percent protein and then diluting to 1 percent (50 ml. samples). Twenty-five ml. of the sol were then given a dose of 200,000 r. at 0°C. and the other 25 ml. were treated as a non-irradiated control. Immediately after irradiation, the pH of the sols were readjusted to a common value of 3.2 and the viscosities determined.

Table 8. Effects of irradiation on the viscosity of 1 percent gluten sols with 200,000 r. at various pH values.

Agent used to adjust pH	pH at which irradiated :	Viscosity of non-irradiated control : (centistokes)	Viscosity of irradiated sample : (centistokes)
KOH	4.1	1.128	1.098
KOH	4.5	1.100	1.090
KOH	10.7	1.016	1.003
$\text{NaHCO}_3$	3.9	1.129	1.087
$\text{NaHCO}_3$	4.2	1.065	1.019
$\text{NaHCO}_3$	4.6	1.115	1.093

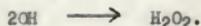
No valid conclusions could be drawn from the data since the viscosities of the control samples indicated that the protein sols were influenced as much or more by the agents used to adjust the pH as by the irradiation. However, the viscosity of the sols were decreased by significant amounts in all instances.

Influence of Dissolved Oxygen. Bonet-Maury and Lefort, (5) showed that

X-rays produce hydrogen peroxide in water only if oxygen is present dissolved in the water. The overall reaction postulated involves the primary action of X-rays on water to form H and OH free radicals,



and a secondary reaction of the free radicals with dissolved oxygen to form hydrogen peroxide,



In some systems of aqueous solutions of proteins and other biological materials, the hydrogen peroxide produced has been shown to have no observable effect on the solute (3), since it is produced in insignificant levels or decomposes as fast as it is produced.

In order to determine whether radiation-produced hydrogen peroxide was responsible for the observed effects of X-rays on gluten sols, samples were saturated with oxygen and nitrogen before irradiation. One sample was mixed in air.

Water was boiled for 15 minutes to drive off dissolved air and the flask immediately flushed with nitrogen several times by alternately producing a partial vacuum in the flask and then bubbling nitrogen in through the water. An exact amount of gluten composite needed to make a 1 percent sol was weighed into a 100 ml. volumetric flask and the atmosphere in the flask was freed of oxygen by repeated dilution with nitrogen. A stopper fitted with a two-way stopcock was placed in the neck of the flask so that alternately, a partial vacuum could be produced in the flask using an aspirator, and then nitrogen introduced through the

other connection. This procedure was repeated ten times. A partial vacuum was then produced in the flask and the oxygen-free water sucked in until the protein was diluted to the mark. Twenty-five ml. were then taken with a pipette flushed with nitrogen and, under a stream of nitrogen, were put into a sample holder used for irradiation and the cover put in place and sealed with stopcock grease. The sample was then given a dose of 200,000 r. at 0°C. Twenty-five ml. were similarly placed in a test tube under nitrogen, stoppered, and kept at 0°C. as a non-irradiated control. The same procedure was used to produce an oxygen-saturated sol except that the volumetric flask which contained the protein was flushed with oxygen only twice. Immediately after irradiation the viscosities of the samples were determined at 30°C.

Table 9. The influence of X-radiation (200,000 r.) on the viscosity of 1 percent gluten sols in the presence and absence of dissolved oxygen.

Treatment	Viscosity (centipoise)	
	Irradiated	Control
Air Saturated	1.163	1.243
N <sub>2</sub> Saturated	1.160	1.269
O <sub>2</sub> Saturated	1.204	1.230

Table 9 shows the results of the irradiation of the sols in the presence and absence of oxygen. The mixing of the gluten sols in the presence of pure nitrogen or oxygen resulted in higher viscosities than when the sols were mixed in air. The radiation-induced decrease of viscosity

was greater when all oxygen was excluded from the system than when the system was saturated with oxygen strongly indicating that the hydrogen peroxide formed was not involved in the mechanism responsible for the viscosity reduction of gluten sols by X-rays.

Influence of Reducing Agents. It has been widely reported that reducing agents have a mitigating influence on the effects of ionizing radiations on proteins and related substances. Feinstein (16) studied the protective effects of cysteine, glutathione, and other substances on the viscosity of nucleoprotein sols. He found that if the reducing agent was added before irradiation, the expected reduction in viscosity was largely prevented while this was not so if the reducing agent was added after irradiation. Patt, et. al. (36) found that the sensitivity of rabbit thymic cells to X-rays was decreased by cysteine added prior to irradiation. A study of the influence of cysteine - HCl, glutathione, and 2 - mercaptoethanol on the radiation sensitivity of gluten sols was undertaken to ascertain if similar effects would be produced.

Gluten sols (1 %) to which cysteine - HCl, glutathione and 2 - mercaptoethanol had been added prior to irradiation to make the solutions 0.05 molal were irradiated at 0°C. with 200,000 r. The pH of the sol containing cysteine - HCl was 1.8 while the pH of the others were unaltered by the reducing agents and remained at 3.2.

The odor of hydrogen sulfide was readily noticeable in all radiated samples which contained reducing substances. The liberation of hydrogen sulfide from aqueous solutions of cysteine and glutathione by the action of X-rays has been demonstrated by Dale and Davies (12).

After irradiation, the sols were incubated at 30°C. and the viscosities determined at periods up to ten hours. Non-irradiated controls were run simultaneously with the irradiated samples under the same conditions of temperature and reducing agent concentration.

Another series of 1 percent sols were given the same treatment except that the reducing agent was added after irradiation. This was done in order to provide a basis for comparison with the sols which had been treated with reducing agents before irradiation. Results of these experiments are shown in Figs. 11, 12, and 13. The most apparent effect shown by the plots is the drastic reduction in the viscosity of the sols upon treatment with the sulfhydryl-containing reducing agents and especially by treatment with cysteine - HCl which had an immediate effect. It is interesting to note that the viscosities of all of the samples treated with reducing agent approached 1.00 to 1.03 centipoises as a final value indicating that a common reduction mechanism was responsible. It has been shown by Olcott, et. al. (35) that the reduction of the viscosity of dilute acetic acid dispersions of gluten upon treatment with reducing agents is due to the reaction of the reducing agent with the protein rather than by increased proteolytic activity caused by activation of proteases present.

No protective effect against radiation-induced viscosity reductions by the presence of reducing agents during irradiation was apparent except perhaps where cysteine - HCl was used since the plot indicates that the sol which contained cysteine during irradiation did not incur as great a loss of viscosity as that which contained no cysteine.

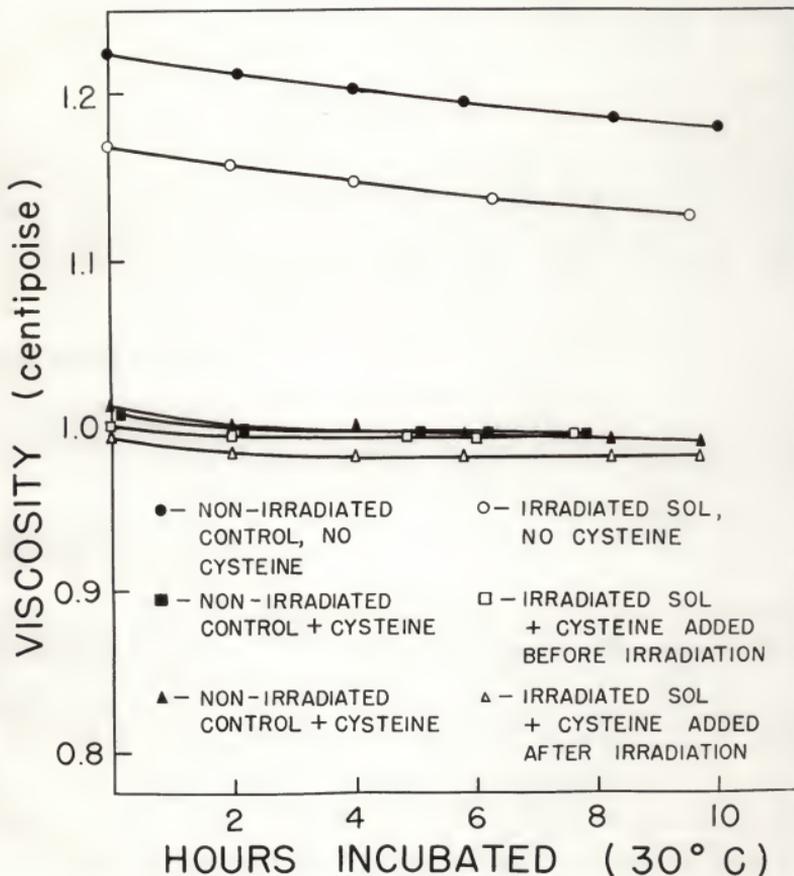


Fig. 11. Influence of cysteine hydrochloride on the viscosity of irradiated gluten sols incubated at 30°C. after irradiation. Cysteine hydrochloride was added before or after irradiation to make the solution .05 molal.

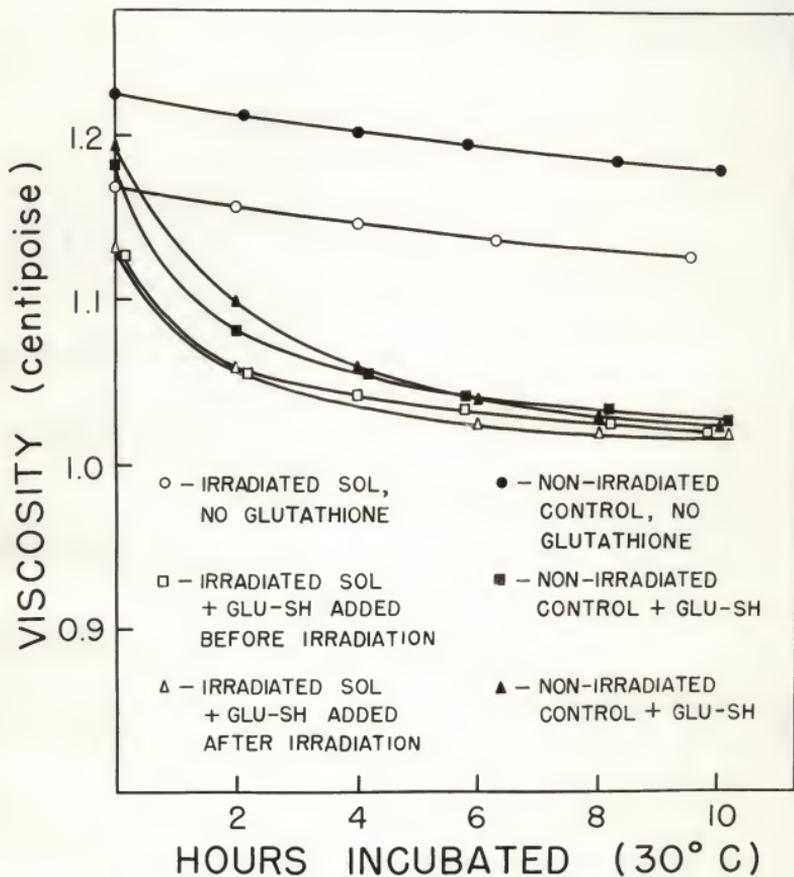


Fig. 12. Influence of glutathione on the viscosity of irradiated gluten sols incubated at 30°C. after irradiation. Glutathione was added before or after irradiation to make the solution .05 molal.

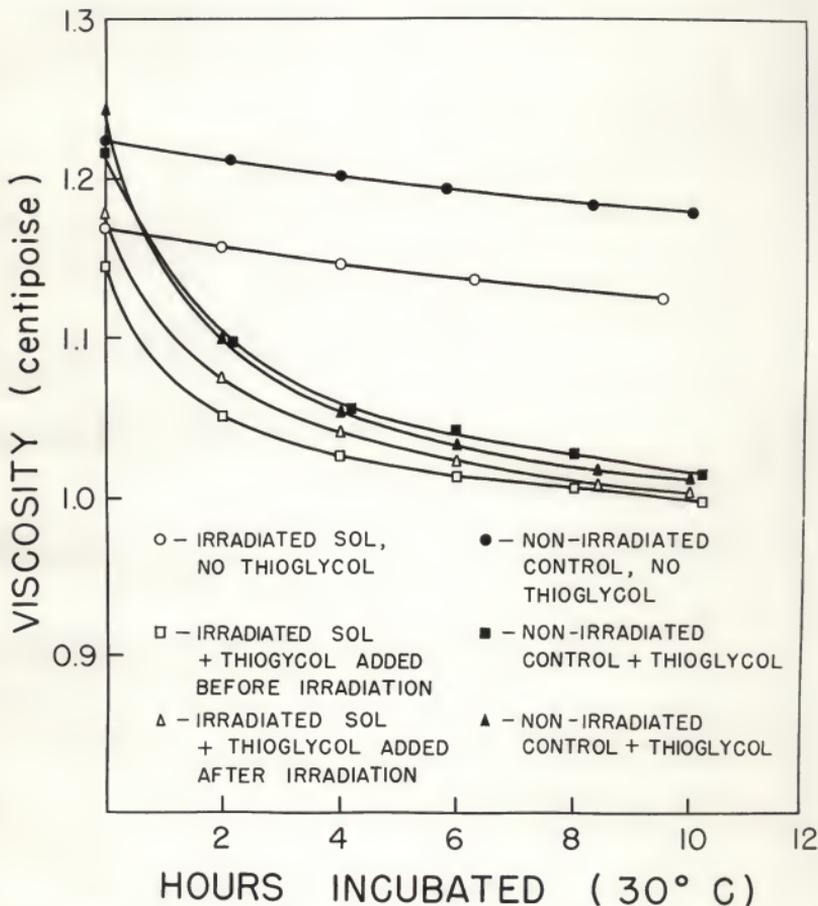


Fig. 13. Influence of 2-mercaptoethanol (monothioglycol) on the viscosity of irradiated gluten sols incubated at 30°C. after irradiation. Monothioglycol added before or after irradiation to make the solution .05 molal.

In order to determine the effects of the reducing agents in mitigating the reduction of the viscosity of gluten sols by X-rays, a study was conducted wherein the concentration of reducing agent (2-mercaptoethanol) added prior to irradiation was varied. One percent sols were treated with monothioglycol so that the concentration of reducing agents was 0.05, 0.10, and 0.15 molal after which they were irradiated with 200,000 r. at 0°C. Viscosities were determined after irradiation up to 10 hours and the sols incubated at 30°C. during that period. Non-irradiated controls were determined simultaneously with the irradiated samples as before. The results shown in Fig. 1h indicate that the concentration of reducing agent had no significant effect on the shape of the viscosity-incubation time curves of the irradiated samples. The shape of the curves for the non-irradiated controls indicate that monothioglycol is more effective in reducing the viscosity at higher concentrations.

Effects of X-rays on the Ultraviolet Absorption Spectrum of Gluten.

Two-hundred and fifty ml. of 1 percent gluten sol were centrifuged at 20,000 r.p.m. for 15 minutes in a Servall (SS-2) angle centrifuge in order to render the sol clear enough for determination of the ultraviolet absorption spectrum. The high relative centrifugal force (64 kilograms per gram) produced in the centrifuge caused 3.7 percent of the protein initially present to be sedimented as determined by Kjeldahl analysis of the centrifuged sol. Twenty-five ml. aliquots of the centrifuged sol were then given doses of 1, 2, 4, and  $6 \times 10^5$  r. of X-radiation at 0°C. After irradiation, 2 ml. of the sols were diluted to 25 ml. and

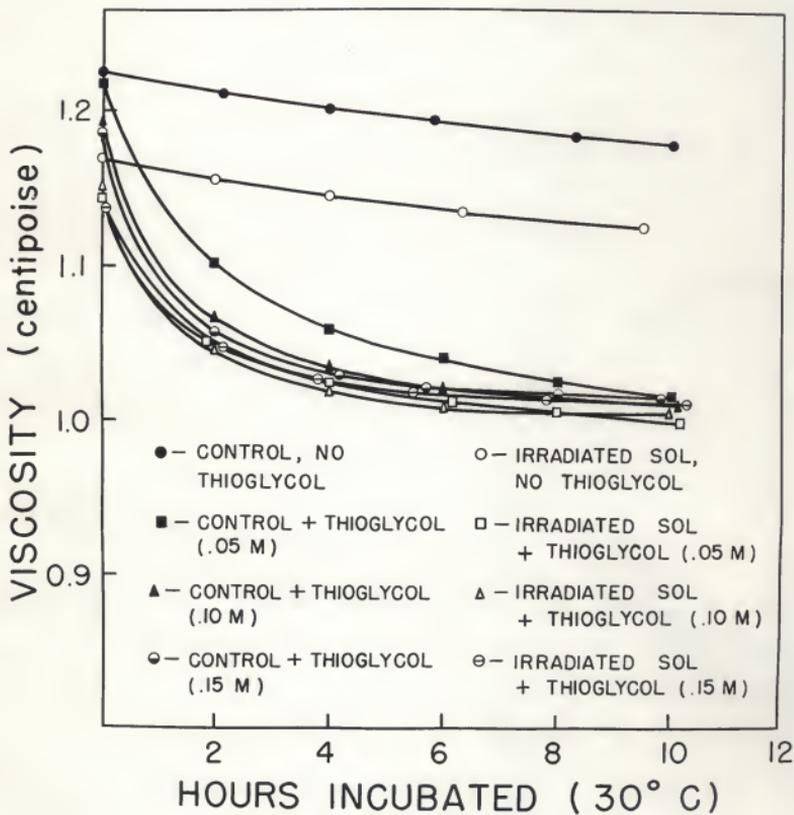


Fig. 11. Influence of 2-mercaptoethanol at varied concentrations on irradiated gluten sols incubated at 30°C.

their absorption spectra determined in a Beckman (model DU) spectrophotometer between the wave lengths of 240 to 330 m $\mu$  at a slit width of 0.7 mm. The diluted sols contained 0.077 percent protein and were quite clear. Water containing lactic acid equivalent to that contained in the sols was used as a blank.

The data, plotted in Fig. 15 failed to show any consistent changes in the spectrum although the absorption of light by the irradiated sols was generally greater than the absorption by the non-irradiated control in the region of wave lengths from 240 to 280 m $\mu$ .

#### Preliminary Investigation of the Sulfhydryl Content of Gluten

It was proposed to test the effects of X-rays on the free sulfhydryl groups of gluten. A suitable method for their detection was devised for this purpose from published information. Only the results of experiments on the sulfhydryl determinations of non-irradiated gluten are reported.

The technique employs *o*-iodosobenzoate, a specific reagent which is irreversibly reduced by the sulfhydryl groups of intact proteins to *o*-iodobenzoate while the sulfhydryl moieties are assumed to be oxidized to the corresponding dithio compounds according to the following reaction:



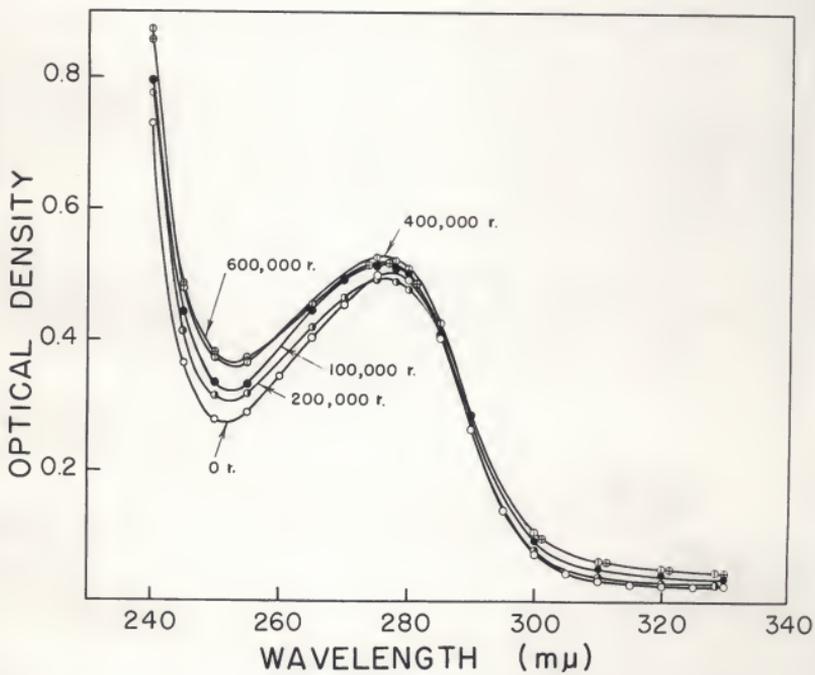


Fig. 15. Influence of varying doses of X-rays on the absorption spectra of gluten sols.

Titration of Crystalline Egg Albumin Sols. In order to evaluate the modified method of sulfhydryl determination, solutions of freshly prepared crystalline egg albumin were titrated and the value for percent cysteine compared with that published by Hellerman, et. al. (26).

Crystalline egg albumin was prepared from fresh egg white obtained from eggs not over 12 hours old, by the method of Kekwick and Cannan (28), and twice recrystallized from sodium sulfate solution. The crystalline material was then dissolved in distilled water after which filtration rendered the solution completely clear. Kjeldahl analysis of three 15 ml. aliquots showed the solution to contain 1.64 percent albumin ( $N \times 6.85$ ).

Three successive titrations of five ml. aliquots of the albumin sol by the described method yielded values of 2.780, 2.780, and 2.814 ml. of .005085 N iodine solution while two titrations on blanks gave 0.710 and 0.704 ml. From these data, the cysteine content of the egg albumin was calculated to be 1.56 percent. The value thus obtained was considerably higher than the value of 1.29 percent cysteine published by Hellerman et. al. (26).

Titration of Gluten Sols. Ten ml. aliquots of 2 percent gluten sols, some of which had been treated with denaturing agents, were titrated to determine their sulfhydryl contents. One sol was heat-treated by immersion in a boiling water bath for 10 minutes before titrating and another sol was treated with 2 gm. of urea which was added to the mixture of buffer, sodium salicylate, and the gluten sol before titration and allowed to stand for approximately 15 minutes. The results of the deter-

minations shown in Table 10 indicate that the sulfhydryl content of the gluten composite was so low as to be practically negligible and that treatment with denaturing agents failed to increase the reactivity of the sulfhydryl groups at all.

Table 10. Sulfhydryl contents of untreated and heat and urea treated gluten sols. Titrations made with .005 N iodine solution.

Treatment	: Blank : titrated	: Sol : titrated : (ml.)	: Cysteine : ( % )
Untreated	0.924	1.03	0.03
Untreated	0.924	1.07	0.05
Untreated	0.924	1.07	0.05
Heated 10 min. at 100° C.	0.924	1.06	0.04
Treated with 2 gm. of urea	0.924	1.12	0.05

Recovery of Cysteine Added to Gluten Sols. A solution of cysteine hydrochloride was standardized by the method described for titration of sulfhydryl groups of gluten with the exception that the sodium salicylate was withheld. The solution was found to be .00396 N (average of 3 titrations). Five ml. of the standard cysteine solution were added to each of two 10 ml. aliquots of 2 percent gluten sols and the mixture titrated. Calculations showed that of the .01980 milliequivalents of cysteine added, an average of .01903 were recovered by titration so that the average recovery was 96.3 percent.

## DISCUSSION

An analysis of the gluten composite prepared from flour A by the washing-out technique and purified by centrifugation of its lactic acid suspension followed by lyophilization indicated that the preparation was at best in a semi-purified state since it contained appreciable amounts of lipids and other unidentified substances presumed to be carbohydrates. Most of the lipids present were in the "bound" form since they were unextractable with petroleum ether but could be removed with ethyl alcohol. A high content of non-protein nitrogen indicated that considerable proteolysis of the gluten had taken place during its preparation due to proteases present.

The viscosities of 1 percent solutions of 5 samples of gluten prepared by the same isolation, purification, and lyophilization techniques from a common flour differed from one another by only 0.005 centipoises indicating that the techniques used could be accurately replicated. Since viscosity is an index of molecular asymmetry, the viscosity values obtained for the five replicates showed that the gluteins were composed of molecules or molecular aggregates of very similar average axial ratios and that other properties which affect the viscosity of aqueous solutions of proteins (such as charged groups on the surface of the molecules and the swelling of the molecules due to hydration) were also very similar.

Storage experiments carried out on samples of several lyophilized gluteins showed that gluten is stable at room temperatures in the pre-

sence of small amounts of lactic acid but not in acetic acid.

An experiment in which the pH of gluten sols were adjusted by additions of lactic acid or of potassium hydroxide prior to dilution to 1 percent showed that the viscosity of gluten sols are sensitive to changes in pH. The wide variation in the viscosity values obtained as shown by the scatter of points on the plot may have been due to denaturation of the gluten caused by localized concentration of the 0.1 N KOH solution as it was added. The decline in the viscosity of the sols may be attributed to a reduction in the volume concentration of protein caused by aggregation of the gluten molecules at the higher pH values.

A linear relation between the amount of X-radiation administered to dry gluten and the viscosity of 1 percent sols made from the irradiated material was demonstrated, whereas the relation between the amount of X-radiation administered to 1 percent sols of gluten and their viscosities was shown to be an exponential function indicating that different mechanisms were responsible for the degradation of the protein in each case. Since the viscosity of solutions of macromolecules is considered to be an index of the asymmetry of the molecules, the lowering of the viscosities by the action of X-rays is interpreted as the scission of large asymmetric protein molecules into shorter, more symmetrical segments.

Since most of the radiation absorbed by the aqueous solutions of gluten (roughly 99 percent) was absorbed by the water molecules, it is apparent that the energy was transferred to the solute or protein present in solution, since this method was more efficient in reducing viscosity than the method where the energy was absorbed only by the protein. The

energy transfer mechanism in aqueous solutions has been postulated to take place through a series of reactions involving free hydrogen and hydroxyl radicals produced by the action of the ionizing radiations on water (7) (13). This theory of the indirect action of ionizing radiations on aqueous solutions is borne out here where it is seen that the more effective utilization of absorbed radiation has taken place in the aqueous solvent system.

The viscometric behavior of gluten solutions is analagous to that of nucleoproteins when subjected to ionizing radiation since the viscosities are reduced in both cases. Solutions of egg albumin or serum albumin, unlike the former, undergo increases in viscosity when irradiated followed by decreases upon further irradiation. These differences in behavior are explained by considering that X-rays degrade the asymmetric molecules of gluten or nucleoprotein to more symmetrical forms with a subsequent decrease of viscosity. However, when globular or spheroproteins are irradiated, they are first mostly unwound which causes an increase in the asymmetry combined with an increase in the viscosity. Extended irradiation breaks the molecules into shorter segments causing losses in viscosity.

It is suggested that the failure to obtain consistent and interpretable results in the study of the influence of pH on the sensitivity of gluten sols to X-rays was due primarily to the differences in ionic strength of the buffers used to adjust the pH. The control samples showed that the buffering systems used affected the viscosity of the samples in different manners so that no basis for comparison with the irradiated

samples was afforded. Ionic strength affects the electrical properties of proteins which determine protein-protein interaction, aggregation, and solubility properties, and consequently, the viscosity of their solutions (23).

Radiation of gluten sols in the presence and absence of dissolved oxygen indicated that hydrogen peroxide formed by interaction of X-ray-induced free radicals with dissolved oxygen was not responsible for the reductions in viscosity incurred since the viscosity of the sols irradiated in an oxygen atmosphere was not reduced below that of a sol irradiated in a nitrogen atmosphere. The mixing of the gluten sols in the presence of pure nitrogen or oxygen resulted in higher viscosities than when the sols were mixed in air. This may have been due to oxidation in the case where pure oxygen was used.

Irradiation of gluten sols at different concentrations showed that the irradiation process was more efficient in degrading the protein in the more dilute solutions. Assuming that the concentration of water molecules activated by the fraction of ionizing radiation absorbed to be constant over the time irradiated and constant at the different concentrations of solute used, the ratio of activated molecules to solute molecules is greater at the lower concentrations of solute. Therefore, it would be expected that a greater percentage of protein molecules would be altered by irradiation in the more dilute solutions with a resultant greater drop in viscosity.

The sensitivity to X-ray-induced reduction in viscosity of a purified sol of gluten was shown to be less than that of an unpurified sol.

No explanation is given for this difference in behavior except that it may have been due to the differences in hydrogen-ion concentrations at which the sols were irradiated. The influence of pH on the viscosity reduction due to X-radiation is as yet an unknown factor since experiments on this interrelation failed to provide clearly interpretable results.

Glutathione, cysteine - HCl, and 2-mercaptoethanol provided no significant protective effect in preventing expected viscosity reductions as has been reported for other protein sols. On the contrary, it was observed that the reducing agents had a drastic action in reducing the viscosity of gluten sols probably due to the functional sulfhydryl group contained in all three substances. It was concluded that viscosity is not an adequate criterion for measurement of a possible protective effect of reducing agents on gluten sols and that molecular weight measurements by osmotic or sedimentation methods would be more suitable.

The method for determination of thiol groups of intact proteins by use of o-iodosobenzoate applied to gluten sols showed that the gluten preparation used in the radiation studies was practically void of reactive thiol groups. Further, denaturation of the sols by heat and treatment with urea did not cause any increase in the titratable thiol groups. Investigations on the sulfhydryl content of flour slurries by Holme and Spencer (27) indicated that the sulfhydryl content of native gluten is considerably higher than the values found here. In view of this and the evidence supported by the experiments

in this laboratory, it is suggested that the treatment which the glutens received in their preparation resulted in the conversion (perhaps by oxidation reactions) of the thiol groups presumed to be initially present, to species of compounds which were unreactive towards the titrating agent used.

#### SUMMARY

An investigation of the influence of X-rays on gluten and gluten sols under various environmental conditions was conducted using viscosity as the principle criterion of effect. The results were correlated with those of similar experiments recorded in the literature and are as follows:

1. A composite of lyophilized glutens, prepared by washing-out from flour and purified by high speed centrifugation of the lactic acid solution, contained appreciable amounts of impurities which consisted of a small fraction of ether-extractable lipids, a larger fraction of "bound" lipids which could be removed with ethyl alcohol, considerable amounts of non-protein nitrogen believed to have been caused by proteolytic activity during its preparation, and other unidentified substances presumed to consist mainly of carbohydrates. The gluten preparation was therefore considered to be in a semi-purified state.

2. The isolation, purification, and lyophilization techniques used for the preparation of gluten could be accurately replicated to yield products whose solutions possessed almost identical viscosities.

3. Lyophilized gluten, stored at room temperature in closed containers, was stable over an extended period of time in the presence of lactic acid but not in the presence of acetic acid.

4. The viscosity of gluten solutions was shown to be dependent on the pH of the solution and the temperature coefficient of the viscosity was shown to be large, indicating that variations in temperature could be a considerable source of error in viscosity determination.

5. The loss of viscosity of sols prepared from irradiated dry gluten was directly related to the dose administered while the relation between dosage and the viscosity of irradiated sols were exponential indicating that separate mechanisms were responsible in each case. Irradiation of the aqueous solution proved to be more efficient in decomposing the gluten, indicating that part of the energy absorbed by the water was transferred to the solute.

6. The viscosities of gluten sols irradiated at different pH's were decreased in all instances. Failure to obtain clearly interpretable results was believed to be due to buffers of different ionic strengths, a factor which influences the viscosity of protein solutions.

7. The proteolytic enzyme(s) associated with gluten in its preparation were not observably inhibited by X-radiation (200,000 r.) of the lyophilized or dissolved preparations.

8. A sol of gluten purified by ether-extraction and dialysis was shown to be less sensitive to X-ray reduction of viscosity than an unpurified sol when administered doses of 200,000 and 400,000 r.

9. The sensitivity of gluten sols to X-rays was found to be prac-

tically independent of the temperature at which the sols were irradiated.

10. An investigation of the effects of the concentration of solute on the irradiation of gluten sols showed that progressively greater percentages of protein were decomposed as the concentration of solute was lowered. This phenomenon supports the activated solvent theory of the action of ionizing radiations on solutions which was postulated to be most efficient for dilute solutions.

11. Irradiation of gluten sols in the presence and absence of dissolved oxygen showed that hydrogen peroxide, produced in aqueous systems by X-rays only when dissolved oxygen is present, was not involved in the mechanism responsible for the viscosity reduction observed.

12. Glutathione, cysteine-hydrochloride, and 2-mercaptoethanol drastically reduced the viscosity of gluten sols and provided no protective effect in preventing expected viscosity reductions due to X-radiation as has been reported for other protein sols.

13. No significant changes were observed in the ultra-violet absorption spectra of gluten sols which had been cleared by centrifugation at high angular velocity and then given doses of X-radiation up to 600,000 r.

14. The sulfhydryl contents of untreated, heat denatured and urea denatured gluten sols were found to be practically negligible. It was concluded that some factor present in the manipulative processes used for preparation of the gluten caused a change in the normal reactivity of the

free sulfhydryl groups assumed to be initially present in the native protein.

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## SUGGESTIONS FOR FUTURE RESEARCH

It is of first importance in fundamental research, to work with systems which have been isolated as far as possible from extraneous and uncontrolled factors. Therefore, it is suggested that more highly purified preparations of gluten be used in future investigations on the physical and chemical properties of gluten proteins. The isolation of gluten from fat extracted flour, the manipulation of crude gluten solutions at low temperatures or by some other means to avoid or eliminate proteolytic effects, the use of dialysis to remove unwanted low molecular weight solutes, and, perhaps the use of chromatography and electrophoresis are techniques which might contribute to the realization of this end.

Since viscosity measurements have been the sole criteria on which conclusions concerning the action of X-rays on gluten and gluten sols have been based, it is suggested that other methods be used to corroborate these conclusions. For the evaluation of the changes in the physical properties, such methods might include measurements of infra-red absorption spectra, molecular weight by osmotic methods, sedimentation rate constants, hydration capacity, birefringence of flow, and solubility. Also, the changes brought about in the functional groupings of the protein by each manipulative process of the isolation and by X-rays might be measured by titration of -SH groups with o-iodosobenzoate by the method presented here and by formal titration of carboxyl groups.

EFFECTS OF X-RAYS ON WHEAT GLUTEN

by

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The interaction of gluten sols with X-rays under diverse environmental conditions was studied principally with the use of viscometric techniques with a view towards developing or opening a new approach to the problem of the relationship of wheat gluten protein to its physical and biochemical properties.

Samples of lyophilized gluten were prepared from three separate flours. That which was used in the radiation studies was prepared from a representative sample of high quality flour milled from wheats obtained from every major producing area in the United States. Several of the glutes were composited to form a single sample which was used in all of the radiation studies. A brass irradiation chamber was fabricated and used to irradiate samples of dry gluten sols. The output of the X-ray unit employed was calibrated by a chemical method of dosimetry.

Analysis of the composite showed that it contained appreciable amounts of free and bound lipids, non-protein nitrogen as well as other unidentified substances. The isolation, purification, and lyophilization techniques could be accurately replicated to yield glutes whose suspensions possessed almost identical viscometric properties.

Viscosity measurements on several gluten samples before and after they were lyophilized and after storage at room temperature for 140 days showed that the lyophilization process used in their preparation materially changed the viscometric properties of the proteins, and that lyophilized gluten is stable over long periods of time in the presence of lactic acid but not in the presence of acetic acid.

Viscosity of gluten sols was shown to decrease with increases of pH and the temperature coefficient of the viscosity was shown to be large.

A linear relation existed between the effects of a radiation dose administered to dry gluten and the viscosity of 1 percent sols made from it, while an exponential function existed for the relation of dose to viscosity of irradiated 1 percent sols indicating that separate mechanisms were responsible for the effects produced.

The viscosity losses in gluten sols due to irradiation were practically independent of the temperature of the sol during irradiation in the range of 0° to 50°C. with a slight increase in the loss of viscosity at the higher temperatures.

The lowering of the viscosity of gluten sols by X-rays was shown to be more effective when the gluten was irradiated in dilute solution. This fact supports the activated solvent theory which holds that the energy absorbed by the solvent is transferred to the solute by a mechanism involving free radicals and activated molecules.

Hydrogen peroxide produced by X-radiation of aqueous solutions with dissolved oxygen present was shown to play no part in the mechanism responsible for X-ray induced reduction in viscosity of gluten sols.

Irradiation of sols in which sulfhydryl-containing reducing agents were dissolved showed that no protection against radiation-induced viscosity reductions was afforded as has been reported for other proteins, principally nucleo-proteins.

Irradiation of sols clarified by centrifugation at very high speed

caused a general increase in the absorption of light by the sols between the wave lengths of 240 to 280 m $\mu$ .

Titration of the sulfhydryl groups of gluten were carried out using the o-iodosobenzoate technique in preliminary studies of development of a method to determine changes in thiol groups of irradiated gluten. No appreciable amounts of thiol groups were found in samples of untreated and denatured gluten.