THE EFFECTS OF HIGH VOLTAGE CATHODE RAY IONIZING RADIATION ON SOME OF THE PHYSICAL AND CHEMICAL PROPERTIES OF WHEAT FLOUR PROTEIN

by

ELSIE BETH ALSUP

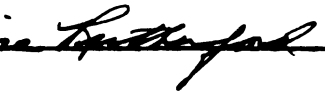
AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Foods and Nutrition

1959

Approved 

The use of ionizing radiations for insect deinfestation and sterilization of flour will not be commercially feasible until the limitations of the process have been thoroughly explored and the end products have been shown to be acceptable. The purpose of the present investigation was to determine the effect of irradiation on some of the physical and chemical properties of wheat flour protein. It included a study of the baking quality and palatability of an irradiated flour product, yields of crude gluten from irradiated flour, and electrophoretic analyses of some of the protein fractions of irradiated flour.

Triangle taste tests indicated that 50,000 rep was the dosage level at which the irradiation treatment of flour could be detected in baking powder biscuits. Taste panel scores for color, odor, and baking characteristics of biscuits made with flour treated with 100,000 rep or less of ionizing radiation, however, were not significantly different from the scores for the control biscuits. An off-odor and darkening in color of the biscuits were readily apparent at a dosage of 500,000 rep.

The weight of crude gluten extracted from flour was greater from flour exposed to dosages of 1,000,000 rep or less than from the control flour, but no differences were found in the volumes of the baked balls of crude gluten. This indicates a greater water retention with the irradiated flour. Very small yields of crude gluten at dosages of

3,000,000 rep or more indicated that definite changes had occurred in the protein structure.

Electrophoretic analyses by the moving boundary method were performed on acetic acid extracts of whole flour in a citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024. Electrophoretic patterns obtained from the non-irradiated flour indicated that there were at least six seperate protein components present, including one which was fast-moving and high in relative concentration, one which was slow-moving and medium high in relative concentration, and four relatively small components. Irradiation of the flour with 300,000 rep produced no apparent changes in the electrophoretic patterns obtained, but at 1,000,000 rep or more, the relative concentrations of the two largest components were changed--the slow-moving one becoming larger and the faster-moving one smaller with each increase in dosage. Also with increased radiation dosages, the peaks of the smaller components became less distinct until only the two main peaks were distinguishable at 10,000,000 rep.

THE EFFECTS OF HIGH VOLTAGE CATHODE RAY IONIZING RADIATION ON SOME OF THE PHYSICAL AND CHEMICAL PROPERTIES OF WHEAT FLOUR PROTEIN

by

ELSIE BETH ALSUP

A THESIS

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Foods and Nutrition

1959

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FORMS

THE EFFECTS OF HIGH VOLTAGE CATHODE RAY IONIZING RADIATION ON SOME OF THE PHYSICAL AND CHEMICAL PROPERTIES OF WHEAT FLOUR PROTEIN

INTRODUCTION

Insect infestation and fungal growth in stored grain, flour, meal, and cereal products result in enormous annual losses of these foods. Studies have shown that many of these losses may be eliminated by use of ionizing radiations which are capable of deinfesting, pasteurizing, or sterilizing cereal products--depending upon the dosage level applied. In experimental work, radiation-sterilized foods have not been found toxic, nor has any evidence of carcinogenicity appeared. Moreover, no radioactivity is induced in foods by the beta and gamma sources used in the radiation process. However, there has been evidence that certain chemical and physical properties of wheat protein are affected by irradiation. These chemical and physical changes may result in alteration of baking properties, palatability, and nutritional value of the wheat.

Before irradiation of wheat flour can become commercially feasible, the limitations of the process must be thoroughly explored and the end products shown to be acceptable. There have been several published reports concerning some of the physical properties and the biological value of wheat protein which has been irradiated, but there have been no reports on the actual nature of the chemical changes that occur.

In the present study, baking tests on biscuits made from flour irradiated at low dosage levels of 20,000 to 500,000 rep were used to determine the effect of irradiation on the baking quality and palatability of flour products. The next step was to determine the effect of irradiation (20,000 to 10,000,000 rep doses) on the quantitative yield of crude gluten which could be extracted from the flour. Then electrophoretic analyses were performed on some of the protein fractions of the irradiated flour in order to follow any chemical alterations which may have taken place during irradiation at dosage levels of 300,000 to 10,000,000 rep. This latter phase of the study was not an attempt to determine the definite composition of flour proteins but rather to make a comparative study of the proteins from non-irradiated flour and flour irradiated at several dosage levels.

REVIEW OF LITERATURE

Use of Ionizing Radiations

Insect infestation in stored grains and grain products results in at least a five percent loss of the annual grain production in the United States (White, 1953). The monetary value of the lost product combined with the cost of measures to combat these pests brings the annual bill to $300,000,000 (Cotton, 1952).

The best methods of insect control today depend on the use of various chemical agents which have been found exceedingly

effective when used on standing crops and stored unprocessed grains. Toxic chemicals cannot, however, be applied to foods that must undergo storage, during which the attacks of insects are costly. This has led to the consideration of radiant energy for the protection of stored products.

Types of Radiant Energy. X-rays are among the oldest known artifically-produced radiations and many investigators have studied their effect on biological materials. However, as pointed out by Robinson (1954), their use in food preservation is somewhat limited. Hard X-rays (produced by an accelerating voltage of 185 kilovolts or more) have a relatively large penetrating power, but low efficiency of generation which makes their use uneconomical for large scale food preservation. Soft X-rays (produced by an accelerating voltage of 100 kilovolts or less) have relatively low penetrating power which may be controlled by the design of X-ray units, but the over-all depth of treatment is still limited by the high absorption of the soft X-rays.

Hassett (1956) has reviewed the various forms of ionizing radiation, other than X-rays, which can be used to kill insects. Each form has its own characteristics, advantages, and disadvantages. (a) Electron accelerators, including the Van de Graaff electrostatic generator, which produce beams of electrons traveling at extremely high speeds have been found effective in insect control. Electrons penetrate only a few millimeters of most substances so that their use is limited to

relatively thin layers of material or to surface irradiation of thick objects. This disadvantage is balanced somewhat by the relatively light shielding required to protect personnel. Another advantage is that, unlike radioactive radiation, the electron beam can be shut off when it is not wanted. The terms electrons, cathode rays, and beta particles are used interchangeably to designate the flow of electrons. (b) Radioactive elements or radioisotopes are another very useful source of radiation. A number of cobalt-60 units of high intensity, rated in thousands of curies are now in use. Cobalt-60 emits strong gamma rays, which like X-rays, penetrate deeply so that large objects can be treated. A disadvantage of cobalt-60 is that heavy shielding must be provided to protect personnel from the effects of this radiation. (c) Means are being sought to develop practical ways of concentrating radioactive wastes from the operation of nuclear reacters, or piles. Both gamma and beta radiation can be extracted from these wastes, or the crude waste need only be concentrated and the total radiation used.

Units of Radiation. The units which are used for biological radiation dosage are derived from the roentgen unit (Friedlander and Kennedy, 1949). One roentgen unit, or r unit, is "that quantity of X or gamma radiation such that the associated corpuscular emission per 0.001293 g (weight of 1 cc of dry air at 0°C and 760 mm pressure) of air produces, in air, ions carrying 1 esu[1] of quantity of electricity of either sign." This means that one r produces 1.61×10^{12} ion pairs per gram

---

[1] esu = electrostatic unit

of air which corresponds to the absorption of 83.8 ergs of energy per gram of air. The r is a unit of the total quantity of ionization produced by gamma or X-rays, and dosage rates for these radiations are therefore expressed in terms of roentgens per unit time. Because of its definition, the r unit should not be used for radiations other than X- or gamma rays. Another unit, the roentgen-equivalent-physical, or rep, has been used to express ionization in tissues caused by other radiation (electrons, protons, alpha particles, neutrons). However, the rep is an unofficial unit and must be defined by each investigator since it is interpreted differently by various workers (Friedlander and Kennedy, 1949; Pomerantz, 1956). It is probably most often defined as the quantity of ionization produced when 93 ergs are dissipated by the radiation per gram of tissue. The rad is an official unit which is now commonly used in place of the rep. It is defined as the quantity of ionization produced when 100 ergs are dissipated by the radiation per gram of tissue (Gray, 1956).

Mechanisms of Action. The deinfestation or sterilization of foods by means of irradiation involves the radiation of the foods with sufficient energy to destroy the insects or bacteria. The killing of insects or bacteria is brought about by some chemical structural change induced by the radiation. Chemical changes in the food being irradiated also occur. Morgan (1955) has estimated that these changes affect only 0.0003 percent of all the compounds present at sterilization dosages for bacteria and therefore would affect even smaller amounts of the compounds

present at the lower dosages necessary for killing insects. Thus these changes are not likely to affect the nutritive value of the macronutrients which make up the major portion of the food but they may be recognized by alterations in odor, taste, and color of a food. Actual studies have verified this theory, with the result that acceptability is probably a more serious problem than is nutritive value of the macronutrients. However, extensive changes may take place in some of the vitamins, due to specific labilities.

Two main theories have been advanced to account for the changes brought about by irradiation. The first theory (target theory) suggested that the changes were due to direct "hits" by the radiant energy on the molecule of the food ingredients (Crowther, 1926; Lea, 1940). Later, many workers came to believe the effects of irradiation were due primarily to the action of the radiation products of the water present (radical or activated solvent theory) (Fricke, 1935; Johnson, 1957). Included in these radiation products of water were atomic hydrogen and hydroxyl radicals which are both very reactive and, in addition, secondary products of their reactions with dissolved molecular oxygen, eg., the O_2H radical and H_2O_2. Thus, the radical theory indicates that the effect of rays on matter is produced largely indirectly, the oxidative processes being then the most important unless the irradiation is carried out in the absence of oxygen.

Evidence for the radical theory was accumulated in investigations of the influence of irradiation on aqueous solutions of

amino acids. A given dose of radiation had more effect on a dilute solution than on a more concentrated one (Bhatia and Proctor, 1951; Proctor and Bhatia, 1952). This dilution phenomenon also was observed when carotene was dissolved in ether (Goldblith and Proctor, 1949). Therefore, other compounds must be formed by the irradiation of ether, which act in the same way as the hydroxyl radicals and hydrogen atoms of activated water.

In practice, probably both direct and indirect action of the radiant energy takes place in the same system. The indirect theory, however, offers a wider basis for chemical change (Johnson, 1957).

Effect on Insects. It was not long after the discovery of X-rays by Roentgen in 1895 that a number of investigators reported the effect of Roentgen rays on various insects. Davey (1917) has reviewed the very early papers that were published. He stated that most of these studies would have been hard to duplicate because the physical data relating to the X-ray dosage was so incompletely stated. However, they could be summarized by saying that X-rays may act upon an organism in one of three ways: (a) to produce a stimulus to growth, (b) to produce a destructive effect which takes place only after a certain latent period, or (c) to produce an instant destructive effect.

Even though most of the early studies indicated that ionizing radiations could be effective in the destruction of insects, there were some negative results reported also. For example, Hunter (1912) concluded after an extensive series of

experiments, that X-rays had no apparent effect upon the fertility or the development of the various stages of several insect species and thus, no practical utilization in the destruction of injurious species. Runner (1916), on the other hand, found that an X-ray dosage of .50 milliampere-minutes at 65 kilovolts and a distance of 7.5 inches could be successfully used in the treatment of tobacco infested with the cigarette beetle (Lasioderma serricorne). He found that in treating the egg stage, heavier exposures of radiation were required to sterilize eggs which were near the hatching point than was required to sterilize eggs newly laid. If the embryonic development was well advanced, the beetles were hatched from the eggs but failed to reach the adult stage. No effect on the length of life of adults was noted, but the adults were rendered sterile. When larvae were treated, activity and development ceased and eventually death occurred before the pupae stage was reached.

More recently with the coming into existence of the more powerful particle accelerators and radioactive sources, other workers have greatly extended the knowledge of radiation treatments to insects.

Hassett and Jenkins (1952) used a cobalt-60 source to demonstrate the killing power of gamma radiation on six species of insects including the Attagenus and Dermestes larvae and the Attagenus, Lasioderma, Sitophilus, Rhyzopertha, Tribolium, and Dermestes adults. The length of time between treatment and

death of the insects was shown to be dependent upon the radiation dosage. Dosages of 322,000 r killed nearly all insects instantly whereas dosages of 128,800 r had varying lethal effects but killed insects of all species in from seven to 26 days.

Baker (1953) and Toboada (1953) conducted tests using accelerated electrons which indicated that a dose of 10,000 rep would sterilize flour beetle (Tribolium confusum) and granary weevil (Sitophilus granarius (L.)) eggs and prevent adults from reproducing. In further studies, Baker et al. (1953, 1954) found that a dose of 500,000 rep was lethal to 100 percent of adult flour beetles immediately after treatment, whereas a dose of 250,000 rep was lethal to 100 percent of adult granary weevils immediately after treatment. A dose of 250,000 rep was lethal to 92 percent of adult flour beetles one week after treatment and a dose of 100,000 rep was lethal to 82 percent of adult granary weevils one week after treatment.

Irradiation of five species of insects by gamma, cathode, and X-rays indicated that each type of radiation was equally effective (Proctor, et al., 1954). The insects studied were confused flour beetle (Tribolium confusum (Jacq. Duval)), yellow mealworm (Tenebrie molitor (L.)), sawtoothed grain beetle (Oryzaephilus surinaminsis (L.)), lesser grain borer (Rhizopertha dominica (Fab.)), and the cigarette beetle (Lasioderma serricorne (Fab.)). The differences in radiosensitivities of the several insect species were relatively small.

The rate of death was dependent on the dose used, higher doses causing death more rapidly. Low doses of 12,500 rep destroyed insects in all forms within a few weeks. In general, however, 300,000 to 600,000 rep were required to destroy the insects immediately. With doses of approximately 50,000 rep, all the insects died within 12 days of treatment; with doses of 100,000 rep, all the insects died within 10 days.

In most of the work reported thus far in this review, irradiated insects have all been killed, regardless of the metamorphic form treated. Very recently, two studies have been published where more attention was given to sub-100-percent-lethal doses in an effort to provide some clue as to the order of death of the irradiated insects and also some basis for comparison among species.

One of these studies was made in the United Kingdom (Cornwell et al., 1957) where the effect of gamma-radiation from a cobalt-60 source was determined for 17 species of insects including C. granaria, C. oryzae, R. dominica, T. castaneum, T. confusum, O. surinamensis, L. minutus, L. ferrugineus, S. cerealella, E. kühniella, E. elutella, and T. granarium. Irradiation prolonged the duration of larval stages; but doses greater than 20,000 rep were required to prevent the emergence of adults from irradiated pupae. It was possible to sterilize 13 species with a low dose of 6,000 rep and all adults of all species were sterilized at

50,000 rep. A dose of 50,000 rep produced no immediate "knockdown" effect on adults. The length of time required to kill the adults varied with the species with L. minutus and C. oryzae among the most susceptible and T. castaneum and R. dominica the most resistant.

Another investigation on sub-100-percent-lethal doses has been reported by Nicholas and Wiant (1959) on 12 grain-infesting pests, including the confused flour beetle (Tribolium confusum Duv.), rice weevil (Sitophilus oryza (L.)), granary weevil (Sitophilus granarius (L.)), sawtoothed grain beetle (Oryzaephilus surinamensis (L.)), cadelle beetle (Tenebriodes mauritanicus (L.)), Angoumois grain moth (Sitotroga cerealella (Oliv.)), Indian meal moth (Plodia interpunctella (Hbn.)), Mediterranean flour moth, (Ephestia kühniella Zell), lesser grain borer (Rhyzopertha dominica (F.)), yellow mealworm (Tenebrio molitor L.), flour mite (Acarus siro (L.)), and book lice (Psocoptera). The pests were treated with 1-Mev[1] electrons at various doses to determine the lethality of the treatment to the metamorphic forms and to determine sterility effects upon adults. The results suggested differences among species, graduated resistance within a species increasing with age of the insect and a more marked difference between the three moths and five beetles. The LD_{50}[2] (reduction, compared with controls, in

---

[1]Mev = million electron volts

[2]LD_{50} = dose lethal to 50 percent of the test organisms

number of emerged adults from the given irradiated immature form) for larvae for which complete data were presented, showed a range for the moths of 5,000-8,000 rep compared with a range of 1,200-4,000 rep for the beetles. The corresponding 99.9 percent-lethal doses were about 40,000-60,000 rep for moths and 10,000-20,000 rep for beetles.

Thus, data from these various studies indicate that a dosage of 10,000 rep ionizing radiation is generally sufficient in cereals to sterilize insect eggs and prevent the adult insects from reproducing. However, a 25,000 to 50,000 rep dose is recommended to provide a greater margin of assurance of egg and insect sterility. A dose of 300,000 to 600,000 rep is necessary to destroy insects instantaneously.

Wholesomeness of Irradiated Foods. Extensive work by Meinke (1954) and others has demonstrated that no induced radioactivity occurs through radiation preservation of foods with fast electrons or gamma rays below eight to 10 Mev.

Radiation-sterilized foods have not been found toxic, nor has any evidence of carcinogenicity appeared in the long-term animal experiments which have been conducted during the past few years. Swift and Co. (Poling et al., 1955) have studied the growth, reproduction, survival and histopathology of rats fed beef irradiated with electrons as two-thirds of their diet over a life span (2 years) and through three successive generations. The Swift data provide substantial

evidence that treatment of beef muscle with a sterilizing dose (2,000,000 rep) of electron radiation did not significantly impair the wholesomeness.

The University of Michigan (Burns et al., 1956a) has carried out a long-term feeding and breeding experiment with rats fed a diet receiving 4,000,000 rep of gamma radiation. In addition to growth and reproduction performance, data on pathology and blood cell counts were obtained on four generations of animals. The evidence collected indicated that aside from some destruction of vitamins, the radiation-sterilized diet was wholesome for rats.

Richardson and Brock (1958) reported that mortality rates and general appearance of four generations of rats on irradiated (3,000,000 rep gamma radiation) synthetic diets gave no indication that the irradiated diet was toxic.

The Quartermaster Food and Container Institute (Donald, 1957) has conducted a test with human volunteers in which a 100 percent diet of irradiated foods was consumed for 12 weeks without any adverse physiological effect.

Burns et al. (1956b) also have reported that there was no evidence indicating the presence of chronic or subacute toxicity apparent in an experiment wherein chickens were raised and maintained for 13 months on a wet-mash diet which had received a sterilizing dose (3,000,000 rep) of gamma radiation.

Work by the Wisconsin Alumni Research Foundation on

concentrates of food irradiated at 500,000-10,000,000 rad levels and fed to rats, injected subcutaneously, or painted on the skin of mice, has not revealed thus far any carcinogenicity (Tepley and Kline, 1956).

Effect on Nutritive Value of Food Proteins. Metta and Johnson (1956) and Metta et al. (1957) have studied the biological value of the proteins of beef, milk, peas, and Lima beans that have been heat- and radiation-sterilized and fed to growing rats. All four of these protein sources are deficient in sulfur amino acids. It was found that a 3,000,000 rep gamma irradiation dose did not affect the apparent or true digestibility of beef or of milk protein. Neither was there an effect on the biological value of beef protein, but the biological value of milk protein was reduced by eight percent upon irradiation as compared to a reduction of six percent due to heat sterilization. Pea proteins also were significantly reduced (six percent) in biological value after irradiation with 3,000,000 rep gamma-ray irradiation but only slightly reduced by heat treatment. In contrast to these effects, Lima bean protein was improved considerably by heat treatment but practically unimproved by radiation processing. It was believed that this simply indicated a greater destructive effect of heat than of radiation on the trypsin inhibitor present in Lima beans. Cystine and methionine seemed to be particularly sensitive to ionizing radiation in these studies.

Metta and Johnson (1959) also have investigated the effect

of gamma-ray irradiation and of heat-cooking on the nutritive value of corn protein and wheat gluten in which the limiting amino acids are generally lysine and/or tryptophan. Finely ground corn and commercial wheat gluten were suspended in water (35 percent concentration) before irradiation. They were then vacuum-dried and incorporated into balanced diets to provide 10 percent protein (N x 6.25) on a moisture-free basis. The radiation-sterilized corn and wheat gluten were found to be completely acceptable for the growing rat during a 20-day feeding period. Irradiation at 2,790,000 rad, like heat-cooking, did not affect either the digestibility or the biological value of corn protein or of wheat gluten. However, irradiation of corn at 9,300,000 rad lowered its digestibility by five percent but did not lower its biological value. The lysine content of wheat gluten did not change owing to heat cooking or irradiation.

In contrast to Metta and Johnson's study (1959) where wheat gluten suspensions were irradiated, Melehy (1958) investigated the nutritive value of whole ground wheat irradiated in the dry form. She found no significant differences in biological values or protein efficiency ratios among rat diets containing the ground irradiated wheat supplemented with vitamins, minerals, and corn oil. This lack of significance indicated that neither the availability nor the growth-promoting value of the wheat protein was affected by irradiation doses up to 1,000,000 rep cathode radiation.

Biochemical Effects on Proteins. The major effects of irradiation on proteins appear to be denaturation, degradation, and polymerization. The most obvious of these effects is denaturation. For example, upon irradiation of raw evaporated milk at 3,000,000 rep, coagulation and precipitation of the milk protein occur, whereas only a darkening of the milk occurs with heat sterilization (Johnson, 1957).

If one molecule is inactivated per primary ionization (Lea et al., 1944; Setlow, 1955), it can be calculated (Bellamy, 1955) that at 1,000,000 rep irradiation of an amino acid of molecular weight 150, one molecule in 2000 will be ionized (damaged) while for a protein of molecular weight 150,000, half the molecules may be altered. These alterations are difficult to demonstrate chemically but they do show up as changes in physical properties such as the absorption spectra, viscosity, heat stability, and solubility (Barron et al., 1952; Barron and Finkelstein, 1952). The indirect effect of the irradiation products of the water present have been shown to be the main cause of these alterations (Barron and Finkelstein, 1952). There was less alteration in the absence of O_2 (necessary for O_2H formation) than in its presence. However, addition of H_2O_2 in concentrations similar to those which would be formed by irradiation had no effect; thus the O_2H radical must be the effective agent causing the alterations. Low doses of irradiation have been shown to cause proteins to be more heat-sensitive and less soluble(Barron, 1955).

Precipitation occurred upon irradiation of aqueous solutions of serum albumin with 75,000 r but not with 50,000 r.

Changes also have been observed in the viscosity (Proctor et al., 1952; Arnow, 1935; Barron and Finklestein, 1952) and sedimentation (Barron and Finklestein, 1952) of proteins which are indicative of degradation or splitting and also of polymerization (crosslinking) of protein molecules. When using high irradiation dosages of 140,000,000 rep Bellamy (1955) reported that a series of proteins showed on chromatography marked breakdown to smaller units regardless of whether they were irradiated dry, in a 20 percent aqueous solution, or in a 20 percent solution of 0.1 N HCl. An example of polymerization was reported by Johnson (1957) as the formation of a faster component on sedementation of serum albumin irradiated with 100,000 r X-ray radiation. The presence of this faster component was prevented or reduced when cysteine was added to protect the protein by reacting with the irradiation products of water. This protection by cysteine indicated that the polymerization might consist of dimerization brought about by oxidation of the sulfhydroxyl groups of the protein with the formation of a dimer, protein-S-S-protein. Barron and his co-workers (Barron et al., 1952; Barron and Finkelstein, 1952) have observed that the sulfur linkages (—SH) and the —OH (eq., of tyrosine) are more susceptible to radiation rupture than are other groups (linkages) in the protein molecule.

Masking of these groups by sulfation reduced irradiation damage to the proteins (Bellamy, 1955).

Effect of Using Irradiated Flour in Baked Products. A previous study made at Michigan State University (Nicholas et al., 1958) showed that a dose of about 50,000 rep (cathode rays) was the level of irradiation at which distinguishable taste and/or odor changes were produced in bread made from irradiated flour and flour milled from irradiated wheat. Loaf volumes were not significantly different among treatments up to 500,000 rep with bread made from irradiated flour, but with bread made from milled irradiated wheat, dose levels in the range of 50,000 rep gave significantly higher volumes than at 250,000 rep.

Brownell et al. (1955) at the University of Michigan found no affect on baking, eating quality, and flavor of cake flour, all-purpose flour and bread flour which had been given a dose of 20,000 rep gamma radiation. Heavier dosages resulted in some decrease in cake and bread quality. The baking techniques which they used were essentially those used in a home rather than in a commercial bakery.

Brownell et al. (1955) explored the possibility that the gluten of bread flour could be modified by radiation doses higher than 50,000 rep in such a way as to make a softer crumb, but found cakes made with irradiated bread flour were progressively of poorer quality with increasing doses of gamma radiation. Cakes made with irradiated flour had low

total volume, were heavier, more compact, gummier, and had a darker yellow crumb color. The gluten appeared to lose its binding power and the flour had a drier or more starch-like texture in mixing. Some flavor changes were observed at a radiation dose of 50,000 rep and cakes made from flour given 100,000 and 150,000 rep doses were considered to have a different flavor in which the sweetness was increased, particularly in the crusts. Flour given 500,000 and 1,000,000 rep doses had an odor resembling extreme mustiness.

When making biscuits with irradiated flour receiving 20,000 to 150,000 rep doses, Brownell and co-workers (1955) found it necessary to add 10 cc. more milk to obtain a workable dough. There were no marked changes in appearance of the biscuits made with flour exposed to gamma radiation, but there were some noticeable changes in eating quality and flavor. The judges thought that the off-flavor of irradiated flour might be accentuated by the presence of double-acting baking powder. They found (a) the biscuits made with irradiated flour were gummier, and (b) off-flavors were present in samples made with flour given dosages greater than 20,000 rep.

Loaves of bread made from bread flour given a 20,000 rep gamma radiation dose were equal in all respects except volume, which was slightly smaller, to those made with the non-irradiated flour (Brownell et al., 1955). Loaves made with flour given 500,000 and 1,000,000 rep had a smaller volume and darker crumb color. Bread made from flour given a dose of

1,000,000 rep was considered to have a definite off-flavor.

A study made at Kansas State University (Milner and Yen, 1956) was concerned with breadmaking and related properties of flour milled from normal dry wheat with gamma radiation at levels of 125,000 to 1,000,000 rep. The irradiation dosage range covered that required for the elimination of storage fungi as well as insects. Techniques employed were essentially those used for commercial pan bread. A 125,000 rep treatment level resulted in apparent improvement of all loaf properties over those of the control. Higher dosages caused some down-grading of crumb properties. However, all of the bread--even that from wheat treated with 1,000,000 rep--was acceptable with no easily detectable changes in flavor and odor. These results were in direct contrast to those of Nicholas et al. (1958) and Brownell et al. (1955) who reported detectable changes in flavor and odor at 50,000 rep and above.

Lee (1959) has reported that loaf volumes of bread baked from two grades of flour (patent and baker's) decreased with increasing levels (250,000, 500,000, and 1,000,000 roentgens) of irradiation of the flours with Co^{60} gamma rays.

Bauman et al. (1957) were interested in increase of shelf life of cake mixes by ionizing radiation. They obtained an appreciable reduction in number of bacteria with a dosage level of 50,000 rep in dry white and spice cake mixes. The white cake mix resulted in acceptable cakes through 50,000

rep. Dosages of 100,000 rep or more produced off-odors and darkening in the dry white mixes and compact structure, low volumes, and dark colored crumb in the baked cakes. The spice cake mix appeared to be more resistant to irradiation--withstanding 100,000 rep with no signs of deterioration in the cake mix or the baked product. At 500,000 rep the spice cake was slightly off-color, slightly off-odor, and compact.

Effect on Physical Properties of Flour. Lloyd, Milner, and Finney (1957) showed that the viscosity of sols made from irradiated dry gluten or irradiated gluten sols decreased with doses of X-rays up to 700,000 rep which is an indication that the wheat flour protein molecules were broken into shorter and/or more symetrical particles. The decrease in viscosity was greater for the irradiated sols than for the sols prepared from irradiated dry gluten.

Milner and Yen (1956) and Milner (1957) found gamma irradiation of wheat in a dosage range of 125,000 to 1,000,000 rep caused no immediate change in fat acidity, protein solubility, or fluorescence of acid extracts of the grain. However, significant effects on the flour milled from the irradiated wheat were found. A marked and regular increase in autolytic sugar production (maltose value), regular decrease in gluten imbibitional capacity (sedimentation value), and a drastic reduction in starch gelatinization

viscosity (amylograph value) occurred with increased radiation treatment. These workers pointed out the two possibilities that irradiation may either activate the beta-amylase or render the starch fraction more susceptible to amylase attack, with increased production of reducing sugars. The second explanation was favored from consideration of the reduced amylograph values. It was emphasized that the starch structure must have been drastically disrupted by the radiation dosages applied.

Lee (1959) also obtained a regular increase in maltose values of flours irradiated with 250,000 to 1,000,000 roentgens). In addition, he observed corresponding rises in the gassing power of doughs prepared from irradiated flours. He stated that this increased reducing sugar production must have arisen from some autolytic process since without incubation, the maltose values were very low for both control and irradiated flours. No rise in saccharifying power was found for papain extracts of the irradiated flours which indicated the absence of a possible activation of the beta-amylase in the flour. However, autolytic production of reducing sugars from mixtures of non-irradiated flour and from irradiated starch increased with increasing irradiation of the starch. Likewise, rises in the saccharifying power occurred for papain extracts of non-irradiated flour when dispersions of irradiated starch were used as substrate. These findings agree with those of Milner and Yen (1956) in indicating that

the increases in maltose value for the irradiated flours were due to some modification of the flour, starch, resulting in an enhanced susceptibility to hydrolytic cleavage by beta-amylase.

Pomerantz of the Quartermaster Food and Container Institute (1956) has reported several observations in working with doughs made from irradiated flours. Dough consistency, as measured by farinograph absorption, appeared to decrease with irradiation, except that with very high dosages an increase was apparent. Dough development time increased regularly with increased irradiation indicating a protein degradation. Mixing tolerance decreased at higher levels of irradiation, indicating further damage to protein and dough structure. Reduced elasticity and weakening of dough, as measured by farinograph, was apparent after irradiation.

Lee (1959) found that recoveries of crude gluten from samples of flour irradiated with 250,000 and 500,000 r were slightly decreased over the control, while recoveries from samples of flour irradiated with 1,000,000 roentgens were sharply decreased. He suggested that the decrease in crude protein recovery was an indication of changes in the protein which contribute to the deterioration in baking quality of the more highly irradiated flour.

By means of crude protein analyses, Lee (1959) determined that 1,000,000 r had very little, if any, significant effect on the nitrogen contents of various flour fractions extracted in

water or 50 percent alcohol. However, the water-soluble nonprotein nitrogen fraction did appear to be greater than the control which he thought might possibly mean that at an irradiation level of 1,000,000 r, some fragmentation of nitrogen-containing compounds may have occurred, giving rise to greater amounts of nonprotein nitrogen.

Composition of Wheat Proteins

Historical. Recorded studies on wheat proteins date back to the announcement by Becarri, an Italian professor of medicine, in 1728 that he had separated gluten from flour by washing a dough with water (Bailey, 1944). After detailing how gluten was recovered from a refined flour by kneading with water to form a dough, and washing the starch and solubles from this dough with water, Becarri proceeded to compare the gluten with animal products. For this reason, he is considered to have been among the first to recognize in plant material the occurrence of a substance having characteristics in common with those of animal protein.

After Becarri's announcement, numerous other investigators of the 18th and 19th centuries worked with the proteins of wheat but their reports were characterized by confusion in terminology and by a lack of agreement as to the number and nature of the individual components of gluten. A complete literature survey of these earlier studies was prepared by Osborne in 1907. In addition, detailed accounts of many of the

individual investigations have been given by Bailey (1944).

Protein Fractions. The so-called modern period of gluten protein investigations began with the comprehensive and systematic studies of Osborne and his associates which extended over a period of about 15 years. The results of these studies were published by Osborne in 1907. He characterized the proteins of the wheat kernel on the basis of differences in solubility. The five main fractions were listed as: gliadin, insoluble in neutral aqueous solutions, but readily soluble in neutral 70 percent alcohol; glutenin, soluble in very dilute acid and alkaline solution, but insoluble in dilute alcohol or neutral aqueous solutions; leucosin, an albumin-like protein, soluble in pure water; a globulin soluble in salt solution; and an ill-defined "proteose" present in small quantities. Globulin, albumin, and proteose were found chiefly in the embryo, and gliadin and glutenin formed nearly the whole of the protein of the endosperm, or over 80 percent of the total protein matter of the seed.

Osborne (1907) reported that the components of gluten, which had been obtained by washing flour with water, were chiefly protein, together with a little starch, fat lecithin, and phytocholesterin. The protein substance was thought to be an intimate mixture of two distinct individual proteins, glutenin and gliadin, present in nearly equal amounts. The glutenin was believed to form the nucleus to which the gliadin

adhered and thus the gluten proteins were bound into one coherent elastic mass.

Sandstedt and Blish (1933) and Stockelbach et al. (1938) reported fractionation studies which indicated that gluten was composed of three fractions, namely, gliadin, glutenine, and an intermediate they called mesonin. The glutenin fraction was found to be soluble in very concentrated acetic acid and gliadin in 50-70 percent alcohol or dilute acetic acid. Mesonin was less soluble in neutral (50-70 percent) alcohol, but was highly soluble in dilute acetic acid.

Later studies using comparatively recent and specialized techniques, instruments, and criteria that were not available to Osborne have made evident the inadequacy of his characterization of gluten as a simple mixture of two individual proteins. It is now thought that gluten is composed of several, if not many, protein components and that Osborne's "glutenin" is a derived, not a natural protein. However, even though the true character of gluten has not been fully ascertained, it has been convenient for purposes of identification and discussion to retain the terms "glutenin" and "gliadin" in referring to later studies which have been based largely on the earlier work of Osborne. Of the two protein types, much more is known about gliadin--soluble in dilute alcohol and dilute acids--than of glutenin, which is far less soluble than gliadin and more indefinite as to composition and properties.

Due to the presence of substantial quantities of starch, fat, and mineral matter, which cannot be removed by the conventional washing process, the term "crude gluten" should be commonly applied to the proteinaceous material recovered by washing a flour dough under water. Blish (1945) stated that crude gluten as isolated by the washing-out procedure contained an average water content of 65 percent, while its dry substances contained 70-80 percent protein, 5-15 percent residual carbohydrates (chiefly starch), 5-10 percent lipids, and a small quantity of mineral salts. Sullivan (1954) reported the composition of gluten to be 85 percent protein, 8.3 percent lipid, 6.0 percent starch, and 0.7 percent ash (dry weight basis). Some variation in water content of wet crude gluten has been noted by Blish (1945) and a high water-absorbing capacity is likely to be associated with desirable physical and mechanical properties.

Elements. Osborne's (1907) elementary analysis of gliadin and glutenin resulted in the following figures:

	Gliadin %	Glutenin %
Carbon	52.72	52.34
Hydrogen	6.86	6.83
Nitrogen	17.66	17.49
Oxygen	21.73	22.26
Sulfur	1.03	1.08

Since the two percentage values for nitrogen agree so closely, 5.7 serves as a factor for determining the quantity of protein in gluten or in various protein fractions prepared from it.

Amino acids. Blish (1945) has assembled the known amino acid compositions (as percentage of total protein) of gluten, gliadin, and glutenin as follows:

	Gluten %	Gliadin %	Glutenin %
Arginine	4.3	3.2	4.7
Lysine	2.1	0.6	1.9
Histidine	2.4	2.1	1.8
Tyrosine	4.2	3.1	5.1
Tryptophane	1.1	0.9	1.8
Phenylalanine	2.0	2.5	2.0
Cystine	1.9	2.3	1.7
Methionine	3.3	2.3	---
Serine	---	0.1	0.7
Threonine	2.5	3.0	---
Leucine and Isoleucine	6.0	6.0	6.0
Valine	3.0	3.0	1.0
Glutamic acid	36.0	46.0	27.2
Aspartic acid	---	1.4	2.1
Glycine	---	1.0	1.0
Alanine	5.5	2.5	4.4
Proline	11.0	13.2	4.4
Hydroxyproline	---	---	---
Ammonia	4.5	5.1	4.0

The outstanding features of gluten protein composition is the high content of glutamic acid, especially in gliadin, where glutamic acid constitutes nearly half of the entire protein substance. There is also a large proline content and relatively small amounts of the basic amino acids and of tryptophan.

Electrophoretic Studies on Wheat Proteins

The charge-density distribution on the surface of colloid particles and their resulting electrokinetic behavior may be studied by the moving boundary method of

electrophoresis. By this technique, the solution of the colloid is stratified under a suitable solvent and the behavior of the colloid/solvent boundary under the influence of an electrical field is observed by suitable optical methods.

Electrophoresis is one of the more recent techniques which has been used to show the inadequacy of Osborne's characterization of gluten as a simple mixture of two individual proteins. However, the "glutenin" portion of the gluten protein has not satisfactorily lent itself to electrophoresis because of failure thus far to find a suitable solvent in which it can be dispersed. Accordingly, most of the studies involving this procedure have been restricted to the more soluble "gliadin" fractions, and even in these instances there is sometimes doubt as to whether the protein is molecularly dispersed.

One of the first electrophoretic studies of gliadin, using the Tiselius electrophoresis apparatus and the techniques of Longsworth (1942), was reported by Schwert et al. (1944). Gliadin was prepared according to the alcohol method of Osborne (1907) and the acetic acid peptization method of Blish and Sandstedt (1926). Using acetate buffers at pH of 3.8 and ionic strength of .01, Schwert et al. (1944) found no significant difference between patterns for a gliadin prepared from a pure durum flour and that prepared from a commercial mixed flour. However, small differences observed among patterns

for different gliadin preparations from the same or different flours indicated that every preparation of gliadin was likely to show small variations in ratio of components.

On the basis of the electrophoretic data which Schwert et al. (1944) obtained, it was apparent that gliadin was not an electrophoretically homogeneous material. It was equally evident that the components of which it was constituted did not migrate independently in solution under any of the conditions studied. Pronounced asymmetry of the patterns obtained for the ascending and descending boundaries and variation in mobilities and relative areas of the several fractions which were separated under various conditions were indications of the occurrence of complex formation. Electrical interaction (interionic) did not appear to account for the complex formation since increase in ionic strength of the systems failed to cause any noticeable decrease in the asymmetry or complexity of the patterns. Neither time of electrophoresis nor field strength influenced the mobilities of recognizable peaks in the gliadin patterns. Changes in ionic strength, protein concentration, nature of the buffer system and pH caused radical changes in the contour of the patterns obtained. Although it was found possible to obtain a distinct separation of fast and slow fractions from the main body of the complex, the fractions obtained were believed to be mixtures of components themselves. Isoelectric points

determined on samples of these fractions showed that the isoelectric point of the fast fraction was at pH 7 while that of the slow fraction was at pH 5.

Laws and France (1948) made a comparative study of some protein fractions of flour from various wheats by use of the moving boundary method of electrophoresis. They found it impractical to study the proteins in either alcoholic buffers or in aqueous alkaline buffers with a pH of 10.2. When the electrophoretic analyses were done in acetic acid and dialyzed against citric acid-disodium phosphate buffer, pH 2.15, there was marked asymmetry in the two boundaries, indicating very decided component interaction. The patterns showed that proteins from all flours studied apparently undergo the same type of interactions because all ascending boundaries were similar and all descending boundaries were similar. The patterns for protein extracted directly from the flour and for freshly washed gluten were very nearly identical except for the slow-moving component which was much more prominent on the patterns made from the protein extracted directly from the flour. It was suggested that this was probably due to the presence of part of the water-soluble protein present in the gluten suspension because of the method of sample preparation. The slow-moving "component" moved so very little that it could easily be taken for the well-known delta and epsilon anomalous boundaries except for the fact that the area under the remainder

of the peaks only accounted for about 80 percent of the protein present in the solution.

The electrophoretic patterns failed to reveal any significant differences in glutens from flours milled from three varieties of wheat which indicates that either there was virtually no difference in the composition of the gluten protein from poor and good quality flour, or the development and manipulation of the gluten during the sample preparation altered the protein in such a manner as to destroy existing differences. Moreover, baking tests with flour fortified with wet and dry gluten washed from the various flours appeared to confirm the results obtained by electrophoresis technique, namely, that the glutens from the flours studied were very similar in properties.

Kondo and his co-workers in Japan studied the electrophoretic behavior of both wheat-glutenin and wheat-gliadin. For the glutenin studies (Kondo and Chiba, 1951) the electrophoretic patterns were obtained in Kolthoff's buffer solution ($NaOH$-Na_2HPO_4), pH of 11.0 and ionic strength of 0.16 at a field strength of 4.863 volt/cm., for 6000 to 8000 seconds. From the patterns obtained, it was concluded that the glutenins isolated from five species of wheat were homogeneous, monocomponent proteins--identical in electrophoretic behavior. The mobilities of the single glutenin components ranged from 8.0 to 8.25 x 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ for the various flours. Even though the ascending and descending patterns agreed in

number and mobility of components, they were not identical in area. The peaks produced in the ascending patterns were much higher than those for the descending patterns.

For the studies of gliadin, Kondo and Owada (1952) used an acetate buffer solution of pH 3.8 and ionic strength of 0.01, at a field strength of 8.46 volts/cm. for 6630 seconds. The patterns obtained showed that the preparations from four species of wheat were identical in electrophoretic behavior though heterogeneous and abnormal. It was suggested such abnormal electrophoretic patterns may depend upon the fact that the gliadin may disperse heterogeneously in the buffer solution and the dispersed phase consisted of the various gliadin particles with different hydrations, electric charges, volume contractions and specific viscosities.

Fidanza et al. (1952) reported that gliadin from a hard wheat did not give an electrophoretic diagram characteristic of a pure substance in the Tiselius electrophoretic apparatus. They used solutions of 0.6 to 0.7 percent concentration in a acetic acid-sodium acetate buffer at pH 3.8.

Butler, et al. (1949) partially separated the gliadin fraction of gluten by means of a thermal fractionation process from 70 percent alcohol. The gliadin fraction was then examined electrophoretically in sodium chloride-acetic acid. In the order of increasing solubility, the fractions obtained were designated as a, b, and c. Fraction a was almost homogeneous, while fractions b and c exhibited the same

five peaks as did the whole gliadin, but in different ratios.

Mills (1954) separated gliadin into two fractions by means of a thermal fractionation process from 70 percent alcohol. He subjected both fractions to electrophoretic analysis in buffer systems consisting of sodium acetate-hydrochloric acid, glycine-sodium chloride-hydrochloric acid, and acetic acid-sodium chloride. In addition to these buffers, sodium acetate-acetic acid, potassium hydrogen phthalate-hydrochloric acid, sodium citrate-hydrochloric acid and citric acid-disodium phosphate were also used for fraction 2. It was found that results over a narrow range of conditions using any of the other buffers except the phthalate were comparable with those of sodium acetate-hydrochloric acid buffer under similar conditions. No significant difference in the patterns was observed with a variation of 2.5 to 3.5 pH. Their results indicated that the electrophoretic examination of gliadin is best performed in buffers of ionic strength of 0.04 at a protein concentration of 0.4 percent. At lower ionic strengths the descending boundary became very diffuse and in some cases precipitation of protein took place. At an ionic strength of 0.04 the "enantiography" of the patterns in the two limbs was greatly improved, although the descending limb became too diffuse to observe towards the end of the run.

During electrophoresis of whole gliadin and fraction 2 (Mills, 1954) in sodium acetate-hydrochloric acid at values of ionic strength of less than 0.03, patterns were produced

which showed a number of rapidly moving convective boundaries in the cathode limb at the end of 30 minutes at a field strength of 5.5 volts/cm. At ionic strengths between about 0.03 and 0.04, patterns were obtained in which five distinct peaks could be discerned in the ascending limb. Migration was cathodic and the boundaries moved fairly rapidly under a potential gradient of 4 volts/cm. The proportion of the leading component relative to the others decreased considerably as the protein concentration was reduced from 1.5 percent to 0.8 percent to 0.4 percent. At an ionic strength of 0.04, the maximum solubility of the protein fell off rapidly. Using sodium acetate-hydrochloric acid buffer at an ionic strength of 0.02, fraction 1 gave a pattern which showed a single, rapidly moving boundary which partly resolved into two peaks after 170 minutes at a field strength of 3.6 volts/cm. When the ionic strength was increased to 0.04 it was difficult to get sufficient protein into solution and the boundaries became very diffuse at the end of the run.

Lonti, et al. (1954) showed gluten to consist of three major and two minor fractions by electrophoresis of wheat gluten in 0.015 M lactic acid-sodium lactate buffer, pH 3.5, containing 0.1 percent 2-mercaptoethanol. Their findings were substantiated by the descending boundary procedure with gluten in 0.01 M formic acid-sodium formate buffer, pH 3.73, with 0.1 percent 2-mercaptoethanol.

No electrophoretic studies on irradiated wheat flour were found in the literature.

EXPERIMENTAL PROCEDURE

Commercial all-purpose flour (Gold Medal) to be used in this study was irradiated with a 1,000,000 volt, resonant-transformer, electron beam generator (Knowlton et al, 1953) located in the Agricultural Engineering Department, Michigan State University. Dose measurements were based on ionization chamber dosimetry.

The dosage levels ranged from 20,000 to 10,000,000 rep[1]. The lower levels used are capable of sterilizing insect eggs and preventing adult insects from reproducing. The higher levels used bring about instant destruction of insects and prevent fungal growth. It was hoped that this wide range of treatment levels would permit the observation of both physical and chemical changes in the flour protein.

Baking Tests on Biscuits

Materials. Four 10-pound lots of all-purpose flour to be used in making biscuits were exposed to varying doses of ionizing radiation while a fifth 20-pound lot served as a non-irradiated control as shown below:

Lot	Treatment
1	None - control
2	20,000 rep
3	50,000 rep
4	100,000 rep
5	500,000 rep

[1]One rep (roentgen equivalent physical) = the absorption of 93 ergs per gram of material of unit density

Two types of baking powder were used:

1. Tartrate powder (Royal).
2. Sulphate-phosphate powder (Calumet).

The fat was a hydrogenated vegetable shortening (Crisco).

Preparation. All ingredients except the milk were placed in a constant temperature box (25°C.) for at least 24 hours prior to use. The milk was brought to room temperature shortly before using. Each batch of biscuits were mixed by the formula (Brownell et al., 1955) shown in Table 1.

Table 1. Formula used in making biscuits with irradiated all-purpose flour.

Ingredients					
Flour	2 cups	225 gm.	Shortening	1/4 cup	50 gm.
Baking powder	3 tsp.	12 gm.	Milk	180 cc.	186 gm.
Salt	1/2 tsp.	2 gm.			

The flour, baking powder, and salt were sifted together once into a 2-quart mixing bowl. The fat was cut in with a pastry blender using 60 strokes. The bowl was given a one-sixth turn between strokes and the sides were scraped after the 15th, 30th, 45th, and 60th strokes. A cavity was formed in the center of the flour mixture and the milk was added all at once and mixed in lightly with a 4-tine fork, using 30 strokes. The dough was then formed into a ball and removed from the mixing bowl with a spatula and placed on a thin layer of flour on a bread board. The dough was rolled without compressing it in the flour, until the sticky surface was covered. The dough was kneaded 10 times lightly with the fingers and

rolled to 1/2 inch thickness with the aid of strips of wood. Ten biscuits were cut with a 2-inch biscuit cutter. Three to four teaspoons of flour in addition to the amount called for in the recipe were used on the bread board during the kneading, rolling, and cutting of the biscuits. The biscuits were baked at 218°C. (425°F.) for 10 minutes on an ungreased baking sheet. On removal from the oven, the biscuits were cooled on the baking sheet before evaluation.

Evaluation of the Dough and Biscuits. Evaluation was accomplished by means of two panels of tasters as well as by several mechanical tests. The tests were conducted over a three-month period with limits of three tasting days a week and two judgments a day at least 30 minutes apart. The biscuits were tasted after they had cooled to room temperature. Mechanical measurements on the raw dough were made immediately after the biscuits were rolled out and on the cooked biscuits within 1 1/2 hours after baking.

Three batches of biscuits made with untreated control flour and flour treated at two levels of irradiation and tartrate baking powder were baked on each judging day until each treatment level had been scored (1-7 range) four times for odor, color of the crust and crumb, flavor, tenderness, lightness, texture, moisture, and general eating quality by a panel of three judges. Thereafter, flour batches of biscuits made with the control flour and one treated flour and two types of baking powder were baked on each judging day. Two triangle tests were

then conducted each day with biscuits made with tartrate rapid-action baking powder being used in one and biscuits made with sulphate-phosphate double-action baking powder being used in the other. The panel was asked to select the odd sample and state whether they preferred it to the other samples in the triangle tests. Samples of the two types of score sheets are included in the Appendix as Forms I and II.

The original taste panel consisted of three persons who had not been informed of the irradiation treatment of the flour and had never worked with irradiated foods. They served as judges for both the scoring tests and triangle tests. Additional judgments in the triangle tests were provided by a panel of seven persons who were aware of the irradiation treatment.

Mechanical tests were made on six batches of dough and biscuits made with tartrate baking powder and flour from each level of irradiation. Triplicate measurements of compressibility and elasticity of the dough were determined immediately after the dough was rolled out by means of a "Micrometer Adjustment" Penetrometer (Sold by Arthur H. Thomas Co., Phila. Pa.). The compressibility of the dough was recorded as the change in height of a core of dough one inch in diameter and one-half inch in height caused by a load of 172 gm. acting on the surface for 15 seconds. Elasticity of the dough was recorded as the change in height 15 seconds after the load had been removed from the core of

dough. Specific volume of the biscuits was measured after the biscuits had reached room temperature in a Volumemeter (Manufactured by the National Manufacturing Co., Lincoln, Nebr.) by the method of rapeseed displacement using five biscuits as a unit.

Statistical Analyses. Analyses of variance were performed on the taste panel scores for the various characteristics of the biscuits and on the mechanical measurements of the dough and biscuits. When a significant difference (F-test) due to irradiation treatment of the flour was found the treatment level means were compared using Studentized multiple ranges (Duncan, 1955).

Levels of significance for the triangular taste tests were determined from published tables for both ability to choose the odd sample and to select the control as a preference (Roessler et al., 1948, 1956).

Gluten Yields

Crude gluten yields were determined for flour samples which had received the following doses of ionizing radiation:

Lot	Treatment	Lot	Treatment
1	None - control	6	None - control
2	50,000	7	300,000
3	100,000	8	1,000,000
4	250,000	9	3,000,000
5	500,000	10	10,000,000

Preparation of Gluten. Six replicates of crude gluten were extracted from samples of each lot of irradiated flour

by the following method (Meiski, 1957). Twenty-four milliliters of tap water were added to 30 grams of flour which had been placed in a glass bowl of an electric mixer (Kitchen Aid Model 3-C). The flour and water dough was mixed 2 minutes at speed 1, scraped down with a rubber spatula; mixed 2 minutes more at speed 1, scraped down again; and mixed a final 1 minute at speed 1. The total mixture was scraped from the bowl with a rubber spatula to Saran Wrap, which had been sprinkled with water, and was rested for 30 minutes at a constant temperature (25°C.). At the end of the rest period the dough was scraped from the Saran Wrap into a glass mixing bowl containing 750 milliliters of tap water at 25 ± 1°C. and washed at speed 1 for 2 minutes. The wash water was then poured through a wire sieve. The gluten retained in the sieve was squeezed 10 times under running tap water to remove more starch. The gluten was again placed in a bowl of water and received three more 2-minute washings at speed 2. The wash water was changed at the beginning of each 2-minute washing. The pH of the tap water was taken each day. The gluten was squeezed 10 times under tap water after each of the three wash periods. It was then placed on wire mesh in a desiccator containing a saturated solution of $Mg(NO_3)_2 6H_2O$ to maintain a constant humidity of 52 percent and the dessicator was placed in a constant temperature box set at 25°C. for 30 minutes.

At the end of the draining period, the gluten was weighed,

placed back in the mixing bowl and mixed 1 minute at speed 1 and 9 additional minutes at speed 4. It was weighed again after mixing and shaped into a ball with 10 folding strokes.

The gluten ball was baked 15 minutes in an oven set at 232°C. (450°F.), transferred to an oven set at 149°C. (300°F.) and baked an additional 35 minutes.

When gluten yields of less than 3 grams were obtained, the mixing times and baking times were shortened. In such cases mixing consisted of beating the gluten for 1 minute at speed 1 and 4 minutes at speed 4 and baking was carried out for 10 minutes at 232°C. and 20 minutes at 149°C.

Volume Measurements. The volume of the gluten ball obtained was determined by means of rape seed displacement in a pint-size plastic box.

Electrophoresis

Four 2-pound lots of all-purpose flour to be used in the electrophoresis studies were exposed to the following doses of ionizing radiation:

Lot	Treatment
6	None-control
7	300,000 rep
8	1,000,000 rep
9	3,000,000 rep
10	10,000,000 rep

Preparation of sample directly from flour. Twenty grams of flour were stirred in 250 ml of cold 0.07 N acetic acid by stirring for 5 minutes in a Waring Blendor. The suspension was then centrifuged for 10 minutes at 2000 rpm. to

remove traces of starch and to destroy the foam formed during stirring. Of the suspension thus obtained, 100 millliliters was heated to 92°C. and held there for 2 minutes to destroy the proteolytic enzymes present. This treatment does not injure the protein (Olcott et al., 1943). The 100 millliliters of suspension were then dialyzed against 2 liters of a citric acid-disodium phosphate buffer of pH 2.2 in the cold room (4°C.) for approximately 72 hours in order for the buffer and sol to reach equilibrium as shown by conductivity measurements. The suspension was centrifuged again just before being placed in the electrophoresis cell to remove the material which salted out. This last centrifugation was done at 12,000 rpm. for 30 minutes in a refrigerated superspeed centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.).

Preparation of sample from crude gluten. Crude gluten was prepared from 20 grams of non-irradiated flour as outlined in the previous section on Gluten Yields. The moist crude gluten obtained was then used to prepare the electrophoretic sample by the same procedure as described above for the whole flour sample.

Preparation of sample from gliadin. Crude gluten was prepared by the procedure outlined in the previous section on Gluten Yields. The gliadin was then extracted by the method of Blish and Sandstedt (1926). Thirty grams of crude gluten were treated with 500 millliliters of .07 N acetic acid

for 2 1/2 hours with vigorous shaking at frequent intervals. The suspension was allowed to stand 1 1/2 hours before siphoning off about 2/3 of the supernatant liquid. Approximately 250 milliliters of .07 N acetic acid was then added to the residue, with stirring, and the insoluble portion again allowed to settle before siphoning off the supernatant liquid which was added to the extract previously withdrawn. The total extract was then filtered water clear through a thick mat of filter paper pulp on a large Buchner funnel.

The clear liquid thus obtained was treated with 10 grams of lithium chloride. There was an immediate precipitation of gliadin, which readily collected on the stirring rod. This was readily dissolved in 250 milliliters of 60 percent ethyl alcohol. The gliadin was then reprecipitated by pouring into absolute alcohol containing ether and a little LiCl. It was redissolved in 60 percent ethyl alcohol, reprecipitated in water containing LiCl, again redissolved in 60 percent alcohol, and finally reprecipitated in absolute alcohol and ether.

The gliadin thus obtained was then suspended in .07 N acetic acid and prepared for electrophoresis in the same manner as used for the samples taken directly from flour.

Nitrogen Determinations on the Protein Extracts. Duplicate one milliliter aliquots of the protein extracts prepared for the electrophoretic determinations were analyzed by the micro-Kjeldahl method (Asso. of Off. Agr. Chemists, 1945). Protein content was then calculated as the product of the

nitrogen value and the factor 5.7.

Electrophoretic Procedure. An attempt was first made to use a filter paper electrophoresis cell of the ridgepole type (Spinco Model R, Specialized Instruments Corp.) with several different buffers at various levels of pH and ionic strength. However, none of these tests were successful since it was not possible to get the protein to move along the paper.

As a consequence, the Tiselius moving boundary method of electrophoresis was adopted since it offers one of the best means of detecting differences in proteins despite the lack of suitable neutral solvents. The electrophoretic apparatus, including 11-milliliter analytical cells, (manufactured by the Klett Manufacturing Co.) used was located in the Agricultural Biochemistry Dept., Michigan State University. The procedure followed is presented and discussed in a manual (Klett Mfg. Co., 1948) which accompanied the apparatus and is essentially as described in the literature (Abramson, et al., 1942; Longsworth, 1942). Runs were made at a temperature of 2.1°C. It required 1 1/2 hours at a field strength of 6.5 to 7.1 volts per centimeter to complete a determination in citric acid-disodium phosphate buffer[1] at pH 2.2, ionic strength 0.024. The electrophoretic patterns, as recorded on the photographic plates by the schlieren-scanning method, were enlarged

[1]The citric acid-disodium phosphate buffer was prepared by adding 40 milliliters of 0.2 M Na_2HPO_4 to 1960 milliliters of 0.1 M citric acid for each electrophoretic run.

by projection and their outlines were traced on graph paper. The various maxima of the gradient curve were separated by the minimum ordinate method (Longsworth, 1942). Then the area of each component was measured with the aid of a planimeter (manufactured by Keuffel and Esser Co., N.Y.)--an instrument which automatically integrates areas by tracing their outline with the stylus of the instrument. For estimating the relative concentration of each component, the size of its area was computed in terms of percentage of the total area. The calculation of the electrophoretic mobilities consisted of measuring the distance from the center of the starting position of the boundary on the enlarged tracings of the electrophoretic diagrams to the various maxima of the gradient curve. The over-all magnification represented by the product of the photographic and the projection magnification factors was taken into consideration in calculating the actual distances in the cell. The actual distances were divided by the potential gradient, F, and the time of electrolysis in seconds in order to arrive at a value for the mobility in terms of centimeter^{-2} second^{-1}. The potential gradient F, was computed from the formula $F = i/kq$, where i was the current in amperes, k was the conductivity of the system in the cell, and q was the cross-section of the cell in centimeter^2. The relative concentrations and mobilities reported were calculated for the ascending patterns only.

A sample form for recording data necessary for the calculation of mobilities is shown in Form 3 in the Appendix.

RESULTS AND DISCUSSION

Baking Tests on Biscuits

The tables containing the taste panel scores (Tables 12-20), mechanical measurement data (Tables 21-23), and statistical analyses (Tables 24-35) for the biscuit tests are found in the Appendix.

Organoleptic Tests. The various characteristics which were scored numerically (1 to 7) will be discussed first and the triangle tests later.

Table 2 gives the average taste panel scores for the biscuits made with irradiated all-purpose flour and tartrate baking powder. Each of these scores is an average of the scores of three judges, each scoring biscuits from four batches identical in treatment.

The average scores for odor of the biscuits ranged from 4.2 to 5.4. Statistical analysis indicated that the mean score for odor of the control biscuits was significantly higher than the mean scores for the biscuits made with flour irradiated at either 50,000 or 500,000 rep.

The color of the crust of the 500,000-rep biscuits was noticeably darker than that of the control, but the judges did not seem to object to it. The average scores for the color of the crust varied from 4.6 to 5.3, but the differences were not significant. The average scores for color of the crumb decreased significantly in the order of increase in

Table 2. Average taste panel scores (3 judges and 4 replications) for biscuits made with irradiated all-purpose flour and tartrate baking powder.

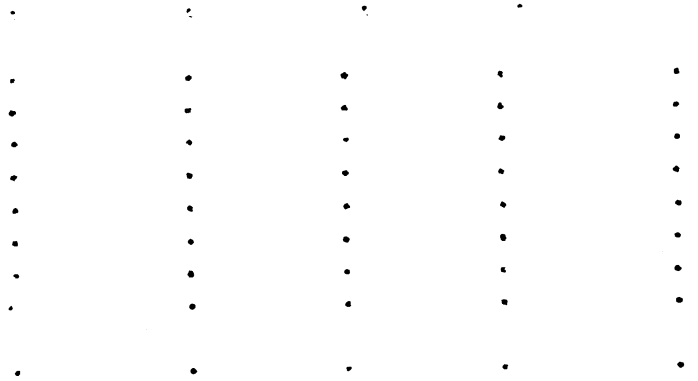
Characteristic	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
Odor	5.4[a]	4.6	4.2	4.8	4.2
Color - crust	5.1	5.3	4.8	4.6	5.0
Color - crumb	6.2[b]	5.9[c]	5.5[c]	5.1[c]	3.2
Flavor	5.0	5.0	4.5	4.7	3.9
Tenderness	4.8	4.8	4.8	5.3	4.6
Lightness	4.7	5.1	5.0	5.1	4.3
Texture	5.3	4.9	5.4	4.8	4.4
Moisture	5.2	4.9	5.2	5.0	4.7
General eating quality	4.8	4.8	4.7	4.8	4.1

[a]Significantly higher than the 50,000-rep and 500,000-rep biscuits at the .05 level.

[b]Significantly higher than the 100,000-rep and 500,000-rep biscuits at the .01 level.

[c]Significantly higher than the 500,000-rep biscuits at the .01 level.

dosage of ionizing radiation applied to the flour. These decreases, however, were not significant except for the highest level of irradiation. The grayish-tan color of the crumb of the 500,000-rep biscuits was quite noticeable compared to the creamy-white color of the control products. The mean score for color of the crumb of the 500,000-rep biscuits was significantly (.01 level) lower than the scores of the control biscuits and biscuits from all other treatment levels. The mean score for color of the crumb of the 100,000-rep biscuits also was significantly (.01 level) lower than the mean score of the untreated control, but not of the 20,000-rep and 50,000-rep biscuits.



There were no significant differences due to treatment for the average scores of the other characteristics--flavor, tenderness, lightness, texture, moisture, or general eating quality, but several observations were noted. The average scores for flavor tended to decrease with increase in dosage of ionizing radiation, with a range of 5.0 for the control products down to 3.9 for those made with flour treated at 500,000 rep. The average score for tenderness was slightly higher for the 100,000-rep biscuits than for any of the others (5.3 as compared to 4.6-4.8). The average scores for lightness ranged from 4.3 to 5.1 while those for texture varied from 4.4 for the 500,000-rep biscuits to 5.4 for the 50,000-rep biscuits. Average moisture scores only varied from 4.7 to 5.2. The average scores for general eating quality were practically identical for the control biscuits (4.8) and those made with flour given dosages of 20,000 rep, 50,000 rep, and 100,000 rep (4.8, 4.7, and 4.8, respectively), while the average score for the 500,000-rep biscuits was somewhat lower (4.1).

The triangle test results are shown in Table 3. The judgments of the three panel members who were unaware of the irradiation treatment (designated as Panel I) were small in number, but nevertheless indicated that these judges were able to select the odd sample a sufficient number of times at the 50,000-rep and 500,000-rep levels of irradiation to show that the selection was not due to chance alone. The number

Table 3. Triangle taste tests for biscuits made with irradiated all-purpose flour.

Level of irradiation rep	Correct selection of odd sample	Preference for untreated sample
	Panel I	
20,000	1/6	1/6
50,000	8/11**	5/11*
100,000	7/12	5/12
500,000	6/6***	----
	Panel II	
20,000	11/26	9/26*
50,000	17/28**	17/28***
100,000	17/28**	16/28***
500,000	14/14***	----
	Combined Totals for Panels I and II	
20,000	12/32	10/32
50,000	25/30***	22/39***
100,000	24/40***	21/40***
500,000	20/20***	----

*Significant at the .05 level.
**Significant at the .01 level.
***Significant at the .001 level.

of correct selections of the odd sample at the 100,000-rep level was just short of being significant. Since the darker color of the 500,000-rep biscuits was disguised by use of a red light, it appears that the correct selection of the odd sample may have been due to odor alone since Panel I found no significant differences in any of the other characteristics when they were scoring the biscuits. The odor scores were significantly lower for the 50,000-rep and 500,000-rep biscuits which were the same treatment levels which were shown to be significant in the triangle tests. However, there is no

obvious explanation as to why these judges were able to select the odd sample more often at the 50,000-rep level than at the 100,000-rep level. The triangle test results of the other seven judges (designated as Panel II) who knew about the irradiation treatment indicate that these judges were able to identify the odd sample a sufficient number of times at all levels of irradiation of 50,000 rep and above to show that the selection was not due to chance alone. Thus the triangle test data from both panels indicate that the treatment level of irradiation at which distinguishable changes were produced in baking powder biscuits made with irradiated flour was approximately 50,000 rep which agrees with the bread data of Nicholas et al. (1958). There was a definite difference, however, in preference of the two panels for the untreated product (Table 3). Although Panel I could ascertain a difference between the control and irradiated products, they did not prefer it to the irradiated product whereas Panel II preferred the control biscuits to any of the biscuits made from flour irradiated at the various levels.

The individual judgments of the 10 judges in the triangle tests are shown in Table 4. All judgments as to the odd sample were correct at the 500,000-rep level of irradiation, but there was a great deal of variation among judges in ability to select the odd sample at the three lower levels of irradiation. One judge (E.J.) who had worked with irradiated products previously was correct in all tests. Among the remaining

Table 4. **Triangle taste test data for biscuits made with irradiated flour showing individual correct choices of the odd sample by the 10 judges.**

Judge	Level of irradiation - rep			
	20,000	50,000	100,000	500,000
		Panel I		
B. S.	0/2	3/3	3/4	2/2
R. A.	1/2	4/4	3/4	2/2
S. S.	0/2	1/4	1/4	2/2
		Panel II		
E. R.	2/4	3/4	1/4	2/2
E. J.	4/4	4/4	4/4	2/2
B. T.	0/4	1/4	2/4	2/2
M. Ma.	1/4	3/4	2/4	2/2
B. B.	0/4	2/4	3/4	2/2
B. S.	2/4	2/4	2/4	2/2
M. Mo.	2/2	2/4	3/4	2/2

judges, two of those who did not know of the irradiation treatment chose the odd sample just as many or more times than the judges who were aware of the treatment.

A comparison of the triangle test data for the two types of baking powders--tartrate, rapid-action and sulfate-phosphate, double-action--is shown in Table 5. In contrast to the suggestion made by Brownell *et al.* (1955), this data indicates that the double-action baking powder may have tended to cover up rather than emphasize the changes brought about by irradiation. At dosage levels of 50,000 rep and 100,000 rep, the judges correctly identified the odd sample more often when tartrate baking powder was used than when sulfate-phosphate powder was used.

Table 5. Triangle taste test data for biscuits made with irradiated flour comparing use of tartrate and sulfate-phosphate baking powders.

Level of irradiation rep	Correct selection of odd sample		Preference for untreated sample	
	Tartrate	S-P	Tartrate	S-P
20,000	6/16	6/16	6/16	4/16
50,000	13/19**	12/20*	13/19***	11/20***
100,000	14/20***	10/20	11/20***	10/20**
500,000	10/10***	10/10***	----	----

*Significant at the .05 level.
**Significant at the .01 level.
***Significant at the .001 level.

Mechanical Tests. Average values for the mechanical measurements on the dough and biscuits made with flour at each level of irradiation are found in Table 6. The average compressibility of the dough made with flour irradiated with

Table 6. Average compressibility, elasticity, and volume measurements on dough and biscuits (6 batches) made with irradiated flour and tartrate baking powder.

Mechanical test	Level of irradiation - rep				
	control	20,000	50,000	100,000	500,000
Compressibility of the dough (1/10 mm.)	98.5[a]	96.4[a]	96.9[a]	96.8[a]	90.2
Elasticity of the dough (1/10 mm.)	18.8	18.2	16.7	17.5	14.9
Volume of the biscuits (cc.)	279	275	282	275	268

[a]Significantly higher than the 500,000-rep biscuits at the .05 level.

500,000 rep ionizing radiation was significantly less than that of the doughs made from untreated flour or flour irradiated at the three lower levels. The compressibility of the other three irradiated flour doughs were practically identical and were less than that of the control but the difference was not significant. Mean measurements for elasticity or recoil of the dough varied slightly, but the differences were not significant.

Mean volumes of the baked biscuits did not vary significantly between the controls and those made with irradiated flour at any of the levels used.

Gluten Yields

Tables giving the data on yields of crude gluten (Table 36) and volumes of the baked gluten balls (Table 37) for each flour sample, and statistical analyses of these data (Tables 38 and 39) may be found in the Appendix.

Weight of crude gluten. The mean crude gluten yields obtained by the "washing out" process from non-irradiated control and irradiated wheat flour are shown in Table 7. These are the weights taken immediately after the draining period.

Irradiation of the flour with dosages of ionizing radiation up to 1,000,000 rep resulted in increased yields of crude gluten above the yields produced from the nonirradiated control flour. The mean crude gluten yield for control I

Table 7. Mean yields of crude gluten extracted from non-irradiated and irradiated all-purpose flour.

Irradiation dosage - rep	Weight - grams
Control I	9.4[a]
50,000	10.1
100,000	10.2
250,000	10.4
500,000	10.9
Control II	9.6[b]
300,000	10.7
1,000,000	10.5
3,000,000	0.6[c]
10,000,000	0.0

[a]Significantly less than the 250,000 rep, 300,000 rep, 500,000 rep, and 1,000,000 rep at the .01 level and the 50,000 rep and 100,000 rep at the .05 level.

[b]Significantly less than the 300,000 rep and 500,000 rep at the .01 level and the 250,000 rep and 1,000,000 rep at the .05 level.

[c]Significantly less than all others at the .01 level.

was significantly less than those of all the other treatment levels below 3,000,000 rep (250,000 rep, 300,000 rep, 500,000 rep, and 1,000,000 rep at the .01 level; 50,000 rep and 100,000 rep at the .05 level). The mean crude gluten yield for control II was significantly less than those of the 300,000-rep and 500,000-rep samples at the .01 level and of the 250,000-rep and 1,000,000-rep samples at the .05 level. This was in contrast to the results of Lee (1959) who reported a decrease in recoveries of crude gluten from samples of flour irradiated with .25 and .50 million roentgens over the control and sharply decreased recoveries from

samples of flour irradiated with 1.0 million roentgens. However, in this study, there was a sharp decrease in crude gluten recovered when the flour sample had been irradiated with 3,000,000 rep and no yield at all when the flour had been irradiated with 10,000,000 rep. At these higher levels, manipulation of the flour under water did not bring about gluten formation to any degree so that the flour was just dispersed in the water and most or all of it went right through the sieve.

Volume of baked gluten balls. The mean volumes of the baked gluten balls made with non-irradiated control and irradiated flours are shown in Table 8.

Table 8. Mean volumes of baked gluten balls made with non-irradiated and irradiated all-purpose flour.

Irradiation dosage - rep	Volume - cc.
Control I	83.7
50,000	90.7
100,000	91.2
250,000	83.7
500,000	83.0
Control II	89.2
300,000	82.8
1,000,000	95.7
3,000,000	4.7[a]
10,000,000	0.0

[a]Significantly less than all others at the .01 level.

No significant differences due to irradiation dosage of the flour samples was found except at the two higher dosages--

3,000,000 and 10,000,000 rep--where crude gluten yields had been small or non-existent. At the lower levels of irradiation there was as much variation in the volume of gluten balls made from flour at any one level of irradiation as between flours at different levels of irradiation (See Appendix Table 36). No logical explanation for this is known.

Thus the crude gluten yields indicate that at high dosages of irradiation of 3,000,000 rep or more, there was a definite indication of changes in the gluten protein of wheat flour. Since there were no significant differences in the volumes of the baked gluten balls at the lower levels of irradiation, it may be that the crude gluten weights were greater than those for the control samples due to greater water retention rather than due to greater protein content. In the baking tests, it was found that the biscuit doughs made with irradiated flour were less moist in handling than the doughs made with control flour even though the same amount of liquid was added.

Electrophoresis

Nitrogen anlyses indicated that the all-purpose flour used was 12.0 percent protein. Enough acetic acid was added to the whole flour samples to produce an initial concentration of 0.91 percent protein in the dispersions prepared for electrophoresis. After centifugation and dialysis against citric acid-disodium phosphate buffer, pH 2.2, for 72 hours

the mean concentrations of the dispersions at the various irradiation levels ranged from 0.78 to 0.83 percent as shown in Table 9. (Concentrations of the individual samples are found in Appendix Table 40.) These differences due to irradiation dosage were not significant (See Appendix Table 41). It can be seen that large mean percentages (86.1 to 91.2) of the protein did remain in the dispersions used for electrophoresis.

Table 9. Mean initial protein concentrations of acetic acid dispersions of flour and mean final protein concentrations after centrifugation and dialysis for 72 hours against citric acid-disodium phosphate buffer, pH 2.2.

Irradiation dosage - rep	Protein concentration - %		
	Initial	Final	Final/Initial
None - control	0.91	0.80	88.3
300,000	0.91	0.83	91.2
1,000,000	0.91	0.82	89.7
3,000,000	0.91	0.78	86.1
10,000,000	0.91	0.79	87.2

At the beginning of the electrophoretic study, determinations were made on the non-irradiated flour to compare protein extracted directly from the flour with protein extracted from crude gluten. The electrophoretic patterns for protein extracted directly from the flour (Plate I, Fig. 1) and from crude gluten (Plate II, Fig. 1) were very similar except that the slow-moving component a was more prominent on the patterns made with the protein extracted directly from the flour. Laws and France (1948) have reported similar

EXPLANATION OF PLATE I

Representative electrophoretic patterns of wheat protein extracted from non-irradiated flour and flour irradiated at four dosage levels. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C; time 90 minutes.

Fig. 1. Non-irradiated control flour.

Fig. 2. 300,000-rep flour.

Fig. 3. 1,000,000-rep flour.

Fig. 4. 3,000,000-rep flour.

Fig. 5. 10,000,000-rep flour.

PLATE I

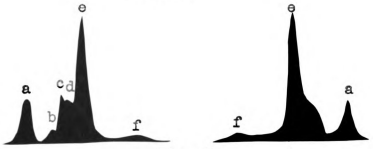


Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

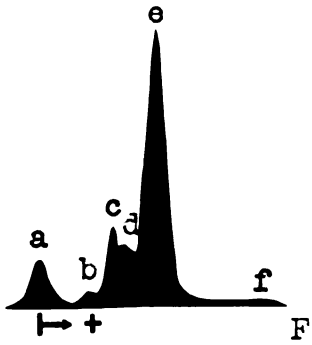
EXPLANATION OF PLATE II

Electrophoretic patterns of wheat protein extracted from crude gluten and from gliadin from non-irradiated flour. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

Fig. 1. Crude gluten

Fig. 2. Gliadin

PLATE II



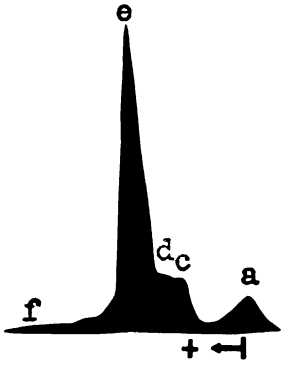
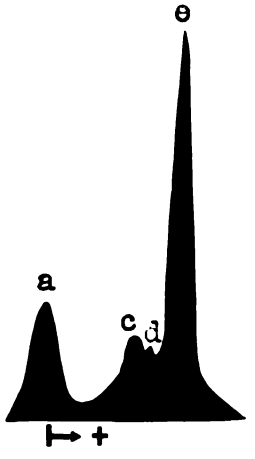


Fig. 1



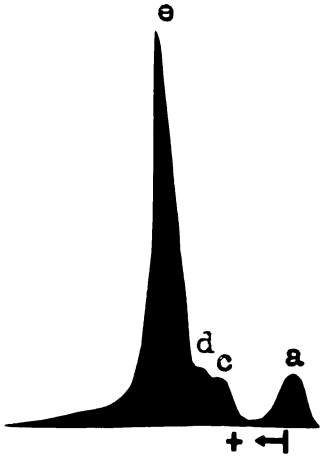


Fig. 2

results when using the same buffer and suggested this was probably due to the presence of part of the water-soluble fraction in the suspension since they had found that the main component from the water-soluble protein also had a very low mobility. There would naturally be less water-soluble protein present in the crude gluten suspension due to the method of sample preparation. Since there was so little difference in patterns between these two methods of extraction, subsequent determinations made use of the less time-consuming method of extracting the protein directly from the flour. A further reason for using the whole flour extract was that crude gluten yields were so very small at the higher levels of irradiation.

Ideally, electrophoretic patterns obtained from the ascending and the descending limbs of the apparatus should be identical in all details, such as the number of components, their mobility, and their relative concentration. In other words, the two diagrams should be mirror images of each other. This ideal case is, however, never realized in actual practice. Usually the peaks of the diagram obtained from the ascending boundaries are steeper than those from the descending boundaries and the mobilities, as measured in both limbs, are also rarely identical. Longsworth and his colleagues (1946) prefer the values obtained from the descending pattern in their work with blood sera; other workers base their conclusions on the ascending boundaries; and still other

investigators average the results obtained on ascending and descending diagrams. In this study, the peaks of some of the components were readily distinguishable in the ascending patterns but tended to be rounded off and merge together in the descending patterns. Therefore, the mobilities and relative concentrations were based on the ascending limb only. Patterns are shown for both limbs.

Representative electrophoretic patterns for the control flour and flour irradiated at 300,000 rep, 1,000,000 rep, 3,000,000 rep, and 10,000,000 rep are shown in Plate I. (The patterns obtained for all three samples at each treatment level may be found in the Appendix, Plates III to VII).

There was little evidence of component interaction in this study since the patterns were nearly symmetrical in both legs of the cell. This is in contrast to the work of others (Schwert et al., 1944; Laws and France, 1948) who reported component interaction which was weakened somewhat by increasing the ionic strength of the buffer.

In electrophoretic analyses of blood sera, it has been commonly observed that there is usually an anomalous or "false" peak at the initial starting point. This boundary anomaly is designated as delta in the ascending limb and epsilon in the descending limb. Likewise, with the non-irradiated flour in this study, a slow-moving "component" moved so very little that it could easily be taken for the delta and epsilon anomalous boundaries. However, it is

believed that the first peak in these patterns indicates a true component as reported also by Laws and France (1948). The area under the first peak increased as the dosage of ionizing radiation was increased while the other main component decreased. Thus the first peak must represent a portion of the protein present and a shift from components c, d, and e to a (first component) has occurred as a result of irradiation.

The electrophoretic patterns for the untreated control flour (Plate I, Fig. 1) show that there are at least six separate protein components present. The mean distribution in percent of the total protein among the various components of the control flour and of the irradiated flours are given in Table 10. (See Appendix, Table 42 for distribution of components for each sample analyzed).

The proteins were identified by means of their mobility. The mobility of each component was determined from patterns of untreated flour. For subsequent patterns of the irradiated flours, the mobilities were calculated, and the protein was identified with the component of the control flour having the same mobility. Table 11 gives the mean mobilities of the six established components of the untreated control and irradiated flours. (Mobilities calculated for each sample analyzed may be found in the Appendix, Table 43). It is recognized that some discrepancy exists in the assignment of mobilities. This discrepancy can be accounted for in part by recognizing

Table 10. Mean distribution of the protein among the various electrophoretic components of untreated and irradiated flour (ascending patterns).

Irradiation dosages - rep	Relative concentration - % Components					
	a	b	c	d	e	f
None - control	19.6	3.6	10.9	8.2	51.8	7.0
300,000	19.0	2.6	10.8	8.3	52.3	6.9
1,000,000	22.0	2.9	8.5	9.6	50.2	6.8
3,000,000	24.6	2.9	7.6	11.1	47.9	5.7
10,000,000	35.3	3.4	-	-	57.5	3.8

Table 11. Mean electrophoretic mobilities of the protein components of untreated and irradiated flour (ascending patterns).

Irradiation dosage - rep	Electrophoretic mobility, $cm^2\ volt^{-1}\ sec^{-1} \times 10^{-5}$ Components				
	b	c	d	e	f
None - control	1.96	2.76	3.28	4.45	9.05
300,000	1.89	2.65	3.25	4.21	8.59
1,000,000	1.99	2.78	3.39	4.38	8.99
3,000,000	1.96	2.80	3.33	4.31	8.79
10,000,000	1.65	-	-	3.84	8.67

that obviously in the case of high levels of irradiation, certain components have disappeared or shifted. In the absence of some components, the mobilities of other components remaining may be altered.

The patterns for the 300,000-rep flour (Plate I, Fig. 2) were essentially the same as those for the untreated control flour. The first noticeable change in the patterns occurred at 1,000,000 rep (Plate I, Fig. 3) where although the patterns looked quite similar to those of the control, the relative

concentrations of the two largest components were beginning to change. The mean relative concentration of component a had increased from 19.6 to 22.0 percent while component e had decreased from 51.8 to 50.2 percent in mean relative concentration. At 3,000,000 rep (Plate I, Fig. 4), component d was hardly distinguishable and component c was rounded off at the top. There was an even greater change in mean relative concentration at 3,000,000 rep than was true at 1,000,000 rep, with the mean relative concentration of component a increased to 24.6 percent and that of component e reduced to 47.9 percent. At 10,000,000 rep (Plate I, Fig. 5), radical changes had occurred--components b, c, and d were no longer distinguishable as separate peaks and a much larger mean percentage (33.7) of the total protein had remained stationary as component a.

The electrophoretic patterns show no evidence of extra components appearing as radiation dosage was increased, but components occurring in the normal patterns have disappeared. This observation does not eliminate the possibility of protein hydrolysis. The hydrolysis products may not have remained in suspension as the samples were being prepared. This observation simply points to the fact that no hydrolyzed products accumulated in sufficient quantities to be detected by the electrophoresis technique unless they were of low mobility and therefore remained stationary. It would seem that irradiation of the flour with 3,000,000 rep or less has not altered

the proteins sufficiently to cause a significant change in the components which have not disappeared (Table 10). A complete disappearance of some components without formation of new components would result in a lower value for total nitrogen. The total nitrogen, however, remained approximately the same (see Table 9) with increasing dosages of irradiation so it appears that the loss of components is a shift from one component to another, rather than a complete disappearance of them from the flour.

After completion of the electrophoretic determinations on the whole flour extracts of non-irradiated and irradiated flours, an electrophoretic pattern also was obtained for a sample of gliadin (soluble in dilute alcohol) extracted from the non-irradiated flour (Plate II, Fig. 1). A definite similarity was observed. The main differences were the absence of components b and f for the gliadin sample. As mentioned earlier, component f was smaller in area in the pattern for the crude gluten extract (Plate II, Fig. 1) also indicating that with both the crude gluten and the gliadin, this small fast-moving component must have been removed in the extensive washing procedure. The absence of component b, in addition to component f, indicates the presence of just four separate fractions in the gliadin sample--compared to six in the whole flour extract.

SUMMARY AND CONCLUSIONS

Nine lots of wheat flour were exposed to cathode ray ionizing radiation. A tenth lot served as a control. Biscuits were made using control flour and flour irradiated at four dosage levels (20,000, 50,000, 100,000, and 500,000 rep). Crude gluten yields were measured for the control flour and flour irradiated at eight dosage levels (50,000, 100,000, 250,000, 300,000, 500,000, 1,000,000, 3,000,000, and 10,000,000 rep). Electrophoretic patterns were obtained for acetic acid extracts of protein from the control flour and flour treated at four dosage levels (300,000, 1,000,000, 3,000,000, and 10,000,000 rep) in a citric acid-disodium phosphate buffer, pH 2.2, ionic strength 0.024, field strength 6.80 volts/centimeter, temperature 2.1°C., time 90 minutes.

Triangle taste tests showed that 50,000 rep (recommended dosage for deinfestation) was the dosage level at which irradiation treatment of flour could be detected in biscuits. Taste panel scores for color, odor, and baking characteristics of biscuits made with flour treated with 100,000 rep or less, however, were not significantly different from those for the controls. A sterilizing dosage of 500,000 rep brought about marked changes in odor and color of biscuits.

Dosages of 1,000,000 rep or less resulted in increases in weight of crude gluten extracted from irradiated flour but did not affect the volumes of baked gluten balls. The

increased weight, therefore, must have been due to greater water retention rather than to protein. The very small crude gluten yields at dosages of 3,000,000 rep or more indicated definite changes were occurring in the wheat flour protein at these levels.

Electrophoretic patterns for the control flour indicated that there were at least six separate protein components present, including one which was fast-moving and high in relative concentration and another which was slow-moving and medium high in relative concentration. Irradiation of the flour with 300,000 rep produced no changes in the electrophoretic patterns obtained, but at 1,000,000 rep or more, the relative concentrations of the two largest components were changed--the slow-moving component becoming larger and the faster-moving component smaller with each increase in dosage. Also with increased radiation dosages, the peaks of the smaller components became less distinct until only two main peaks were distinguishable at 10,000,000 rep. Irradiation apparently brought about a shift of several of the components to the slow-moving component rather than a complete disappearance of them from the flour.

The results of this study confirm the idea that changes in palatability and physical properties of irradiated flour are apparent at the low dosage levels required for deinfestation and sterilization, but that alterations in flour protein are difficult to demonstrate chemically except at very high dosage levels.

ACKNOWLEDGMENTS

The writer is deeply grateful to Dr. B. Elaine Rutherford for advice during the experimental phases of this study and for guidance during the preparation of the manuscript. She also wishes to thank Dr. R. M. Grimes, Assistant Professor of Agricultural Chemistry, for his valuable help in the use of the electrophoretic equipment; Mr. W. E. Wiant, Professor of Agricultural Engineering, for irradiating the flour; Miss Mary Morr, Assistant Professor of Foods and Nutrition, for her counsel in the palatability phase of the study; and members of the faculty and graduate students in the Department of Foods and Nutrition who served on the taste panels.

LIST OF REFERENCES

Abramson, H. A., Cohn, E. J., Davis, B. D., Horsfall, F.L., Longsworth, L. G., MacInnes, D. A., Mueller, H., and Stern, K. G. Electrophoresis. Ann. N.Y. Academy of Sciences 39:105-212 (1939).

Arnow, L. E. Physicochemical effects produced by the irradiation of crystalline egg albumin solutions with alpha particles. J. Biol. Chem. 110:43-59 (1935).

Assoc. of Off. Agr. Chemists. Official and tentative methods of analysis (6th ed.) Wash., D.C. p. 805 (1945).

Bailey, C. H. The constituents of wheat and wheat products. Reinhold Pub. Corp.: New York (1944).

Baker, V. H. Some effects of electromagnetic energy and subatomic particles on certain insects which infest wheat, flour, and beans. Unpublished Ph.D. thesis. Michigan State University (1953).

Baker, V. H., Taboada, O., and Wiant, D. E. Lethal effect of electrons on insects which infest wheat flour and beans. I. Agr. Eng. 34:755-758 (1953).

Baker, V. H., Taboada, O., and Wiant, D. E. Lethal effect of electrons on insects which infest wheat flour and beans. II. Agr. Eng. 35:407-410 (1954).

Barron, E. S. G. The effect of ionizing radiations on systems of biological importance. Ann. N.Y. Acad. Sci. 59:574-593 (1955).

Barron, E. S. G., and Finkelstein, P. Studies on the mechanism of action of ionizing radiations. X. Effect of X-rays on some physicochemical properties of proteins. Arch. Biochem. and Biophys. 41:212-231 (1952).

Barron, E. S. G., Seki, Louise, and Johnson, Phyllis. Studies on the mechanism of action of ionizing radiations. VIII. Effect of hydrogen peroxide on cell metabolism, enzymes and proteins. Arch. Biochem. and Biophys. 41:188-202.

Bauman, H. E., Baeder, Helen, Stein, Jolyon A., Harrel, C. G., and Larsen, R. A. Effect of gamma irradiation on cake mixes at high and low moisture levels. Food Technol. 11:193-196 (1957).

Bellamy, W. D. Unpublished data. (1955). Reported by Johnson, B. C. (1957).

Bhatia, D. S., Proctor, B. E. Effect of high-voltage cathode rays on aqueous solutions of histidine monohydrochloride. Biochem. J. 49:550-553 (1951).

Blish, M. F. Wheat gluten. Advances in protein chem. 2:337-359(1945).

Blish, M. J., and Sandstedt, R. M. An improved method for the preparation of wheat gliadin. Cereal Chem. 3:144-149 (1926).

Brownell, L. E., Harlin, H. A., and Nehemias, J. V. A preliminary study of the effect of gamma radiation on the baking quality of flours. Food Technol. 9: 620-623 (1955).

Burns, C. H., Brownell, L. E., and Eckstein, H. C. Wholesomeness of a gamma-irradiated diet fed to rats. Federation Proc. 15:228 (1956a).

Burns, C. H., Brownell, L. E., and Eckstein, H. C. Wholesomeness of a gamma-irradiated diet fed to chickens. Federation Proc. 15:910-917 (1956b).

Butler, J. A. V., Creeth, J. M. and Mills, G. L. Abs. 1st Int. Congress Biochem. pp. 117-118 (1949). Original not seen. Abstract in Chem. Abstracts 46:7141b (1952).

Cornwell, P. B., Crook, L. J., and Bull, J. O. Lethal and sterilizing effects of gamma radiation on insects infesting cereal commodities. Nature 179:670-672 (1957).

Cotton, R. T. Insect pests of stored grain and grain products. Burgess Publ. Co.: Minneapolis p. 1 (1952).

Crowther, J. A. The action of X-rays on Colpidium colpida. Proc. Roy. Soc. (London) 100B:390-404 (1926).

Davey, W. P. The effect of X-rays on the length of life of Tribolium confusum. J. Exp. Zool. 22:573-592 (1917).

Donald, G. E. Food irradiation makes strides. Food Eng. 29(12)57-59 (1957).

Duncan, D. B. Multiple range and multiple F tests. Biometrics 11:1-42 (1955).

Fidanza, F., Scardi, V., and Virgilio, A. Electrophoretic behavior of gliadin. Boll. Soc. Ital. Biol. Sper. 28: 1316-1318 (1952). Original not seen. Chem. Abstracts 48:753i (1954).

Fricke, H. The decomposition of hydrogen peroxide by the irradiation of its aqueous solution with X-rays. J. Chem. Phys. 3:364-365 (1935).

Friedlander, G., and Kennedy, J. W. Introduction to radiochemistry. John Wiley and Sons, Inc.: New York. p. 172 (1949).

Goldblith, S. A., and Proctor, B. E. Effect of high-voltage X-rays and cathode rays on vitamins (riboflavin and carotene). Nucleonics 5 (Aug.) 50-58 (1949).

Gray, L. H. The transition from roentgen to rad. Brit. J. Radiology 355-358 (1956).

Hassett, C. C. Current status of insect control by radiation. Science 124:1011-1012 (1956).

Hassett, C. C., and Jenkins, D. W. Use of fission products for insect control. Nucleonics 10(12):42-46 (1952).

Hunter, W. D. Results of experiments to determine the effect of roentgen rays upon insects. J. Econ. Entomol. 5:188-193 (1912).

Johnson, B. C. Effects of irradiation sterilization on nutritive value of protein, carbohydrate, and fat. Atomic energy and agriculture. AAAS Pub. 49, Wash., D. C., pp. 391-414 (1957).

Johnson, B. C., and Metta, V. C. Effect of irradiation sterilization on nutritive value of protein and energy of food. Federation Proc. 15:907-909 (1956).

Klett Mfg. Co. The Klett electrophoresis apparatus. New York (1948).

Knowlton, J. A., Mahr, G. R., and Ranftl, J. W. The resonant transformer: a source of high-energy electrons. Nucleonics 11(11):64-66 (1953).

Kondo, Kinsuke and Chiba, Hideo. Studies on proteins 53. Electrophoretic behavior and fluctuation of wheat glutenin. Bull. Research Inst. Food Sci., Kyoto Univ. No. 6, pp. 88-92 (1951).

Kondo, Kinsuke and Owada, Yutaha. Studies on protein 54. Abnormal electrophoresis of wheat gliadin. Bull. Research Inst. Food Sci., Kyoto Univ. No. 10, pp. 19-28 (1952).

Laws, W. D., and France, W. G. A comparative study of some protein fractions of wheat flour. Cereal Chem. 25:231-243 (1948).

Lea, D. E. Determination of the sizes of varuses and genes by radiation methods. Nature 146:137-138 (1940).

Lea, D. E., Smith, K. M., Holmes, B., and Markham, R. Direct and indirect actions of radiation on viruses and enzymes. Parasitology 36:110-118 (1944).

Lee, C. C. The baking quality and maltose value of flour irradiated with Co^{60} gamma rays. Cereal Chem. 36:70-77 (1959).

Lloyd, N. E., Milner, Max, and Finney, K. F. Treatment of wheat with ionizing radiations. I. Some effects of X-rays on gluten and gluten sols. Cereal Chem. 34:55-62 (1957).

Longsworth, L. G. Recent advances in the study of proteins by electrophoresis. Chem. Rev. 30:323-340 (1942).

Longsworth, L. G. Optical methods in electrophoresis. Ind. and Eng. Chem. Anal. Ed. 18:219-229 (1946).

Lontie, R., Crevecoeur, A., and Dulcino, J. Electrophoretic study of the proteins of wheat gluten. Mededel. Vlaam. Chem. Ver. 16:53-57 (1954). Original not seen. Abstract in Chem. Abstracts 48:102421 (1954).

Meinke, W. W. Does irradiation induce radioactivity in food? Nucleonics 12(10):37-39 (1954).

Meiske, Donna. The effect of kind and concentration of sugar on gluten formation and character. Unpublished M.S. thesis, Michigan State University (1957).

Melehy, Catherina P. The effect of high voltage cathode ray ionizing radiation on the biological value of wheat protein. Unpublished M. S. thesis, Michigan State University (1958).

Metta, V. C., and Johnson, B. C. The effect of radiation sterilization on the nutritive value of foods I. Biological value of milk and beef proteins. J. Nutrition 59:479-490 (1956).

Metta, V. C., and Johnson, B. C. Radiation sterilization of foods, biological value of gamma irradiated corn protein and wheat gluten. J. of Agri. and Food Chem. 7:131-133 (1959).

Metta, V. C., Norton, H. W., and Johnson, B. C. The effect of radiation sterilization on the nutritive value of foods. II. Biological value of pea and lima bean proteins. J. of Nutrition 63:143-154 (1957).

Mills, G. L. Electrophoresis of gliadin. Biochem. et Biophys. Acta. 14:274-281 (1954).

Milner, Max. Some effects of gamma irradiation on the biochemical storage, and breadmaking properties of wheat. Cereal Sci. Today. 2:130-131 (1957).

Milner, Max, and Yen, Yin-Chao. Treatment of wheat with ionizing radiations. III. The effect on breadmaking and related properties. Food Technol. 10:528-531 (1956).

Morgan, B. H. Proc. Symposium Radiation Sterilization of Foods. Quartermaster Food and Container Institute, Chicago, June 8-10 (1955).

Nicholas, R. C., Meiske, Donna P., Jones, Mary F., Wiant, D. E., Pflug, I. J., and Jones, Evelyn M. Radiation-induced changes in bread flavor. Food Technol. 12:52-54 (1958).

Nicholas, R. C., and Wiant, D. E. Radiation of important grain-infesting pests: order of death curves and survival values for the various metamorphic forms. Food Technol. 13:58-62 (1959).

Olcott, H. S., Sapirstein, L. A., and Blish, M. J. Stability of wheat gluten dispersions toward reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. 20:87-97 (1943).

Osborne, T. B. The proteins of the wheat kernel. Carnegie Inst. Wash. Publ. 84 (1907).

Poling, C. E., Warner, W. D., Humburg, F. R., Reber, E. F. Urbain, W. M., and Rice, E. E. Growth, reproduction, survival and histopathology of rats fed beef irradiated with electrons. Food Research 20:193-214 (1955).

Pomerantz, Reuben. Atoms strike the cereal industry. Cereal Sci. Today. 1:23-29 (1956).

Proctor, B. E., and Bhatia, D. S. Effects of high-voltage cathode rays on aqueous solutions of tryptophane, tyrosine, phenylalanine and cystine. Biochem. J. 51: 535-538 (1952).

Proctor, B. E., Coleman, M. T., and Goldblith, S. A. Effect of high energy cathode rays on the catalase activity of Micrococcus pyogenes var. aureus. J. Bacteriol. 63:337-339 (1952).

Proctor, B. E., Lockhart, E. E., Goldblith, S. A., Grundy, A. V., Tripp, G. E., Karel, M., and Brogle, R. C. The use of ionizing radiations in the eradication of insects in packaged military rations. Food Technol. 8:536-540 (1954).

Richardson, L. R., and Brock, Ruth. The nutritional value of a synthetic diet sterilized by gamma rays, as measured by reproduction and life span of rats. J. Nutrition 65:353-360 (1958).

Robinson, R. F. Some fundamentals of radiation sterilization. Food Technol. 8:191-194 (1954).

Roessler, E. B., Baker, G. A., and Amerine, M. A. One-tailed and two-tailed tests in organoleptic comparisons. Food Research 21:117-121 (1956).

Roessler, E. B., Warren, J., and Guyman, J. F. Significance in triangular taste tests. Food Research 13:503-505 (1948).

Runner, G. A. Effect of Roentgen rays on the tobacco, or cigarette, beetle and the results of experiments with a new form of Roentgen tube. J. Agr. Res. 6(11):383-388 (1916).

Sandstedt, R. M., and Blish, M. J. A new characterization of the gluten proteins. Cereal Chem. 10:359-366 (1933).

Schwert, G. W., Putnam, F. W., and Briggs, D. R. An electrophoretic study of gliadin. Arch. Biochem. 4:371-387 (1944).

Setlow, Richard. Radiation studies of proteins and enzymes. Ann. N. Y. Acad. Sci. 59:471-483 (1955).

Stockelbach, Lois S., and Bailey, C. H. The amino acid distribution in proteins resulting from the thermal fractionation of wheat gluten. Cereal Chem. 15:801-811 (1938).

Sullivan, Betty. Proteins in flour. Review of the physical characteristics of gluten and reactive groups involved in change in oxidation. J. Agr. Food Chem. 2:1231-1234 (1954).

Taboada, Oscar. Some effects of radiant energy on the beetles, Tribolium confusum, Duv, Sitophilus granarius, (L.): and Ascanthoccelides obtectus (Say). Unpublished M. S. thesis, Michigan State University (1953).

Teply, L. J., and Kline, B. E. Wholesomeness and possible carcinogenicity of radiated foods. Federation Proc. 15:927-929 (1956).

White, G. D. Weight loss in stored wheat caused by insect feeding. J. Econ. Ent. 46(4):609-610 (1953).

APPENDIX

SCORE CARD
BAKING POWDER BISCUITS

FORM I

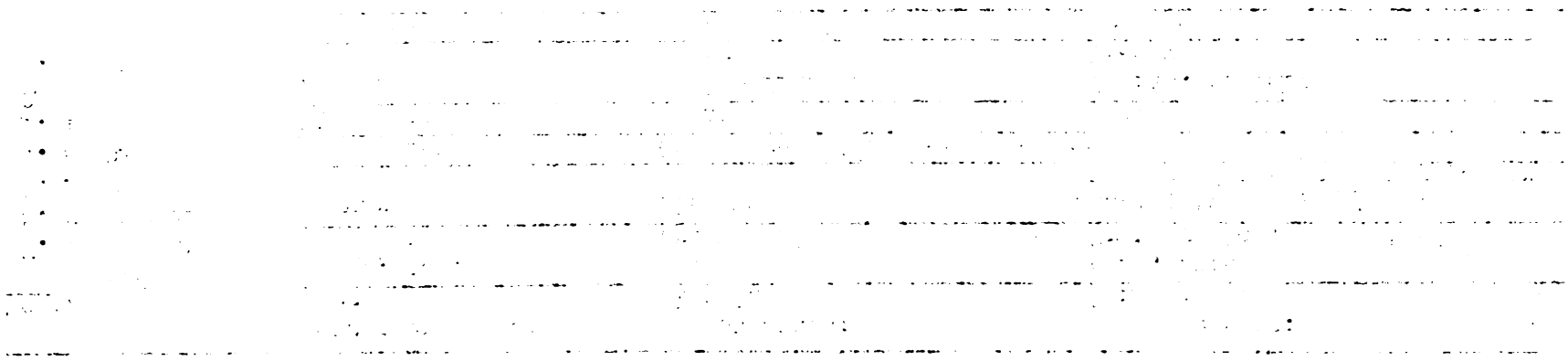
Name_____ Date_____

CHARACTERISTICS	SAMPLES					
Odor						
Color Crust						
Crumb						
Flavor						
Tenderness						
Lightness						
Texture						
Moisture						
General Eating Quality						
Comments						

Key:

7. excellent
6. very good
5. good
4. medium
3. fair
2 poor
1. very poor

Characteristics:	Standard:	Defects:
Odor	Pleasing	Rancid; foreign
Color, outside	Golden brown	Pale; dark
inside	Creamy white	Gray, yellow, or spotted
Flavor	Pleasing	Rancid; foreigh, flat or tastes of fat or baking powder
Tenderness	Crust cresp & tender	Crust tough and hard
Lightness	Light	Heavy
Texture	Medium fine grain	Coarse, crumbly
	Flaky	Bready
Moisture	Slightly moist	Dry; soggy



FORM II

TRIANGLE TEST
BAKING POWDER BISCUITS

Name_____ Date_____

Two of these samples are identical and one is odd.

1. Which is the odd sample?

A B C

2. Do you prefer the odd sample?

yes no

FORM III
ELECTROPHORESIS EXPERIMENT

Date _____ Operator _____ Plate No. _____

I SAMPLE

Type _____ No. _____ Diluted with Buffer _____

Description _____ Conc. in Buffer _____

II BUFFER

Composition _____ pH _____ u _____

III CONDUCTIVITY (mhos)

Buffer _____ Solution _____ Average _____

IV CELLS (cross-sectional area)

Ascending _____ ; _____ Descending _____ ; _____

V ELECTROPHORESIS

Initial boundary Ascending _____ Descending _____

Scanning boundary " Light _____ Dark _____ " Light _____ Dark _____

Time	Milliamps.
Initial _____	_____
_____	_____
_____	_____
_____	_____
Final _____	_____

Average _____ Potent. Grad. _____ v/cm. Migration _____

Plate type _____ Gear ratio _____ Aperture _____ Filter _____

CONDUCTANCE

Sample	R-selector Switch	Ratio Slidewire Reading	R-cell

Table 12. Odor scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	4	4	4	4	4
R. A.	7	6	5	5	3
S. S.	6	6	6	6	5
			II		
B. S.	5	6	4	5	5
R. A.	7	3	3	4	3
S. S.	6	2	4	3	4
			III		
B. S.	5	5	4	5	-
R. A.	6	5	4	4	3
S. S.	3	5	4	5	5
			IV		
B. S.	5	5	-	5	5
R. A.	6	4	5	6	6
S. S.	5	4	3	5	3
Mean	5.4	4.6	4.2	4.8	4.2
S.D.	1.16	1.24	0.84	0.83	1.05

Table 13. Color of the crust scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	6	5	4	4	2
R. A.	4	6	6	5	6
S. S.	5	5	5	4	7
			II		
B. S.	5	6	5	4	6
R. A.	7	7	6	6	5
S. S.	4	4	4	3	3
			III		
B. S.	5	6	5	4	-
R. A.	6	7	4	5	5
S. S.	3	4	4	5	4
			IV		
B. S.	6	6	-	6	6
R. A.	7	4	6	5	6
S. S.	3	4	3	4	5
Mean	5.1	5.3	4.8	4.6	5.0
S.D.	1.38	1.15	0.96	0.90	1.41

Table 14. Color of the crumb scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	7	6	5	6	1
R. A.	6	6	6	6	3
S. S.	6	5	5	5	4
			II		
B. S.	7	7	7	5	4
R. A.	7	4	4	4	2
S. S.	6	6	6	5	4
			III		
B. S.	6	6	5	5	-
R. A.	6	6	5	4	2
S. S.	6	6	6	5	4
			IV		
B. S.	6	7	-	4	4
R. A.	6	6	6	6	4
S. S.	5	6	5	6	4
Mean	6.2	5.9	5.5	5.1	3.2
S.D.	0.57	0.79	0.80	0.79	1.05

Table 15. Flavor scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	4	4	4	4	3
R. A.	5	5	4	5	1
S. S.	6	5	5	5	3
			II		
B. S.	6	6	5	4	5
R. A.	5	6	6	5	3
S. S.	4	5	6	6	5
			III		
B. S.	5	5	4	4	-
R. A.	5	4	4	4	2
S. S.	4	5	4	6	6
			IV		
B. S.	4	5	-	5	5
R. A.	7	4	4	5	5
S. S.	5	6	4	3	5
Mean	5.0	5.0	4.5	4.7	3.9
S.D.	0.95	0.73	0.80	0.89	1.50

Table 16. Tenderness scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	5	5	4	6	3
R. A.	2	4	4	6	6
S. S.	6	6	5	5	6
			II		
B. S.	6	5	6	5	6
R. A.	3	5	6	6	3
S. S.	5	5	6	6	5
			III		
B. S.	6	6	5	4	-
R. A.	5	3	3	4	3
S. S.	3	4	4	7	6
			IV		
B. S.	7	6	-	5	4
R. A.	5	4	5	5	4
S. S.	5	5	5	5	5
Mean	4.8	4.8	4.8	5.3	4.6
S.D.	1.49	1.15	1.15	0.89	1.24

Table 17. Lightness scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	5	5	5	4	4
R. A.	4	5	5	5	5
S. S.	6	4	5	5	6
			II		
B. S.	4	6	5	4	7
R. A.	5	6	6	5	2
S. S.	5	5	5	5	3
			III		
B. S.	4	5	5	6	-
R. A.	4	4	4	5	3
S. S.	3	4	5	6	5
			IV		
B. S.	5	6	-	6	5
R. A.	5	5	5	5	3
S. S.	6	6	5	5	4
Mean	4.7	5.1	5.0	5.1	4.3
S.D.	0.89	0.79	0.42	0.67	1.44

Table 18. Texture scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	7	5	6	4	5
R. A.	5	5	4	3	3
S. S.	6	6	7	6	4
			II		
B. S.	5	6	6	5	6
R. A.	5	6	5	2	2
S. S.	6	5	5	6	5
			III		
B. S.	6	5	6	6	-
R. A.	3	4	5	5	4
S. S.	6	5	5	6	5
			IV		
B. S.	4	4	-	5	5
R. A.	6	4	6	5	4
S. S.	5	4	4	5	5
Mean	5.3	4.9	5.4	4.8	4.4
S.D.	1.07	0.79	0.90	1.27	1.08

Table 19. Moisture scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	7	6	7	6	5
R. A.	4	5	5	6	3
S. S.	6	6	5	5	6
			II		
B. S.	4	5	5	6	7
R. A.	5	4	4	3	2
S. S.	5	6	6	6	6
			III		
B. S.	6	5	5	4	-
R. A.	4	2	5	5	5
S. S.	5	5	4	5	6
			IV		
B. S.	6	6	-	4	3
R. A.	6	4	5	5	3
S. S.	4	5	5	5	5
Mean	5.2	4.9	5.2	5.0	4.7
S.D.	1.03	1.17	0.84	0.95	1.56

Table 20. General eating quality scores for biscuits made with irradiated flour.

Judge	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
B. S.	5	5	5	5	3
R. A.	4	5	5	5	2
S. S.	6	5	5	5	4
			II		
B. S.	-	-	-	5	6
R. A.	5	5	5	4	2
S. S.	5	4	5	6	5
			III		
B. S.	5	5	4	4	-
R. A.	4	4	4	4	3
S. S.	3	4	5	6	6
			IV		
B. S.	5	6	-	5	5
R. A.	6	4	5	5	4
S. S.	5	6	4	4	5
Mean	4.8	4.8	4.7	4.8	4.1
S.D.	0.84	0.72	0.49	0.72	1.40

Table 21. Compressibility of the dough made with irradiated flour and tartrate baking powder (1/10 mm.).

Triplicate samples	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
			I		
1	96.0	97.0	111.0	105.0	90.0
2	100.0	87.0	105.5	106.0	106.0
3	92.0	89.0	94.0	91.5	86.5
			II		
1	105.0	108.0	104.0	85.5	73.0
2	107.5	96.0	105.5	94.5	104.5
3	103.0	99.0	105.0	105.0	92.0
			III		
1	92.5	102.0	98.5	100.0	91.5
2	102.5	110.0	104.5	90.0	86.5
3	105.5	103.5	101.5	99.0	82.0
			IV		
1	88.0	95.0	95.5	99.5	83.5
2	96.0	104.0	98.5	96.5	94.5
3	99.5	90.0	99.5	101.0	99.5
			V		
1	106.5	103.5	80.0	93.0	91.0
2	96.5	92.0	87.5	95.5	93.5
3	108.0	100.5	89.0	104.5	100.0
			VI		
1	95.0	84.5	91.5	90.0	83.5
2	89.5	85.0	86.0	94.0	85.5
3	90.5	88.5	87.5	92.5	81.0
Mean	98.5	96.4	96.9	96.8	90.2
S.D.	6.55	7.94	8.53	6.01	8.60

Table 22. Elasticity of the dough made with irradiated flour and tartrate baking powder (1/10 mm.)

Triplicate samples	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
		I			
1	19.0	20.0	19.5	19.0	16.5
2	23.0	25.0	23.5	18.5	21.5
3	27.0	26.0	15.5	22.0	20.5
		II			
1	17.5	15.5	15.5	15.5	10.5
2	16.0	12.0	13.5	21.5	20.0
3	21.5	12.0	8.5	18.5	20.5
		III			
1	21.0	20.0	17.5	15.0	16.0
2	14.0	17.0	16.0	15.0	15.0
3	17.5	16.5	18.0	17.5	18.5
		IV			
1	12.5	21.5	24.5	22.0	18.0
2	25.0	16.0	15.0	15.0	19.5
3	20.5	16.0	21.0	17.5	16.5
		V			
1	24.0	20.0	12.5	20.0	13.0
2	20.0	10.5	17.5	9.0	11.5
3	20.0	15.5	14.0	17.0	14.0
		VI			
1	19.5	25.0	18.5	16.5	6.0
2	11.0	20.5	16.0	18.5	9.0
3	9.0	19.0	14.5	17.0	8.5
Mean	18.8	18.2	16.7	17.5	14.9
S.D.	4.85	4.50	3.93	3.11	4.64

Table 23. Volume of the biscuits made with irradiated flour and tartrate baking powder (1/10 mm.).

5 Biscuit unit	Level of irradiation - rep				
	Control	20,000	50,000	100,000	500,000
		I			
1	250	250	255	250	255
		II			
1	275	275	275	285	280
		III			
1	285	285	280	280	255
		IV			
1	300	300	290	280	275
		V			
1	280	255	280	280	265
		VI			
1	285	285	315	275	275
Mean	279	275	282	275	268
S.D.	16.6	19.2	19.7	12.6	10.8

Table 24. Analysis of variance for odor scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	71.93		
Treatments	4	11.93	2.982	2.68*
Judges	2	0.91	0.455	0.41
Error	53	59.09	1.115	

*Significant at the .05 level.

Table 25. Analysis of variance for color of crust scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	80.85		
Treatments	4	4.10	1.025	1.01
Judges	2	22.80	11.400	11.20**
Error	53	53.95	1.018	

**Significant at the .01 level.

Table 26. Analysis of variance for color of crumb scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	100.98		
Treatments	4	64.23	16.058	24.29**
Judges	2	1.73	0.865	1.31
Error	53	35.02	0.661	

**Significant at the .01 level.

Table 27. Analysis of variance for flavor scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	66.18		
Treatments	4	9.60	2.400	2.35
Judges	2	2.43	1.215	1.19
Error	53	54.15	1.022	

Table 28. Analysis of variance for tenderness scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	72.93		
Treatments	4	3.76	0.940	0.86
Judges	2	11.43	5.715	5.25**
Error	53	57.74	1.089	

**Significant at the .01 level.

Table 29. Analysis of variance for lightness scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	50.33		
Treatments	4	5.16	1.290	1.61
Judges	2	2.63	1.315	1.64
Error	53	42.54	0.803	

Table 30. Analysis of variance for texture scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	66.98		
Treatments	4	7.90	1.975	1.98
Judges	2	6.13	3.065	3.07
Error	53	52.95	0.999	

Table 31. Analysis of variance for moisture scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	72.98		
Treatments	4	2.06	0.515	0.50
Judges	2	16.23	8.115	7.86**
Error	53	54.69	1.032	

**Significant at the .01 level.

Table 32. Analysis of variance for general eating quality scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	59	164.61		
Treatments	4	4.00	1.000	0.34
Judges	2	5.23	2.615	0.89
Error	53	155.38	2.932	

Table 33. Analysis of variance for compressibility of the dough scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	89	5,652.08		
Treatments	4	741.21	185.30	3.18*
Triplicates	2	67.37	33.68	0.58
Error	83	4,843.50	58.36	

*Significant at the .05 level.

Table 34. Analysis of variance for elasticity of the dough scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	89	1,703.49		
Treatments	4	166.51	41.63	2.27
Triplicates	2	12.24	6.12	0.33
Error	83	1,524.74	18.37	

Table 35. Analysis of variance for volume scores of biscuits.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	29	7,304		
Treatments	4	758	1895	0.724
Error	25	6,546	2615	

Table 36. Yields of crude gluten extracted from non-irradiated and irradiated all-purpose flour.

Replications	Weight - gm. Irradiation dosage - rep				
	Control	50,000	100,000	250,000	500,000
1	10.0	10.3	9.9	9.9	11.4
2	9.0	10.5	11.7	11.2	11.3
3	8.9	10.5	9.8	10.6	11.2
4	9.0	10.3	10.4	10.4	11.2
5	9.8	9.8	10.0	10.0	10.1
6	9.7	9.5	9.6	10.1	10.3
Mean	9.4	10.1	10.2	10.4	10.9
S.D.	0.50	0.40	0.78	0.48	0.55

	Irradiation dosage - rep				
	Control	300,000	1,000,000	3,000,000	10,000,000
1	9.8	10.4	10.3	0.9	0.0
2	10.3	11.0	10.4	1.0	0.0
3	9.4	10.6	10.7	0.3	0.0
4	9.4	10.1	10.5	0.2	0.0
5	9.6	11.7	10.4	0.6	0.0
6	9.4	10.2	10.3	0.4	0.0
Mean	9.6	10.7	10.5	0.6	0.0
S.D.	0.37	0.61	0.16	0.32	0.00

Table 37. Volumes of baked gluten balls made with non-irradiated and irradiated all-purpose flour.

Replications	Volume - cc.				
	Irradiation dosage - rep				
	Control	50,000	100,000	250,000	500,000
1	114	103	91	59	82
2	78	98	86	88	80
3	83	109	91	87	82
4	82	73	88	78	80
5	83	84	84	88	88
6	62	77	107	102	86
Mean	83.7	90.7	91.2	83.7	83.0
S.D.	16.9	6.1	8.3	14.3	3.3

	Irradiation dosage - rep				
	Control	300,000	1,000,000	3,000,000	10,000,000
1	95	83	121	7	0
2	92	82	82	10	0
3	84	109	100	3	0
4	93	77	89	3	0
5	80	78	100	2	0
6	91	68	82	3	0
Mean	89.2	82.8	95.7	4.7	0.0
S.D.	5.8	13.9	14.8	3.1	

Table 38. Analysis of variance for yields of crude gluten extracted from non-irradiated and irradiated all-purpose flour.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	53	519.03		
Treatments	8	508.24	63.53	264.71**
Error	45	10.79	0.24	

**Significant at the .01 level.

Table 39. Analysis of variance for volumes of baked gluten balls made with non-irradiated and irradiated all-purpose flour.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	53	43,770.83		
Treatments	8	37,561.66	4,695.21	34.03**
Error	45	6,209.17	137.98	

**Significant at the .01 level.

Table 40. Protein concentrations of acetic acid dispersions of flour after centrifugation and dialysis for 72 hours against citric acid-disodium phosphate buffer, pH 2.2.

Irradiation dosage - rep	Protein concentrations of solutions				
	Triplicate samples				
	1	2	3	Mean	S.D.
	%	%	%	%	
None-control	0.89	0.73	0.79	0.80	0.081
300,000	0.80	0.82	0.87	0.83	0.036
1,000,000	0.82	0.79	0.84	0.82	0.025
3,000,000	0.79	0.74	0.82	0.78	0.040
10,000,000	0.81	0.74	0.83	0.79	0.047

Table 41. Analysis of variance for protein concentrations of acetic acid dispersions of flour.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Total	14	0.0288		
Treatments	4	0.0041	0.00102	.415
Error	10	0.0247	0.00247	

Table 42. Distribution of protein among the various electrophoretic components of untreated and irradiated flour (ascending patterns).

Irradiation dosage - rep		Relative concentration - % Components					
		a	b	c	d	e	f
Control	1	16.9	2.8	10.8	9.9	51.3	8.3
	2	22.0	2.3	11.1	6.0	52.7	6.0
	3	19.8	2.8	10.8	8.7	51.4	6.6
300,000	1	23.1	3.0	8.9	9.5	49.4	6.1
	2	21.0	2.5	11.4	6.7	52.6	5.6
	3	13.0	2.2	12.2	8.8	54.9	8.9
1,000,000	1	23.1	2.3	7.6	10.8	50.8	5.3
	2	21.7	3.4	9.5	8.6	49.5	7.2
	3	21.2	3.0	8.5	9.3	50.2	7.8
3,000,000	1	28.9	2.6	8.3	9.8	45.4	4.8
	2	20.4	3.5	8.2	12.2	48.6	7.1
	3	24.6	2.6	6.4	11.3	49.7	5.3
10,000,000	1	38.2	3.8	-	-	54.0	4.1
	2	30.7	3.8	-	-	61.8	3.8
	3	36.9	2.7	-	-	56.8	3.6

Table 43. Electrophoretic mobilities of the protein components of triplicate samples of untreated and irradiated flour (ascending patterns).

Irradiation dosage - rep		Electrophoretic mobility $cm^2\ volt^{-1}\ sec.^{-1} \times 10^5$ Components				
		b	c	d	e	f
Control	1	2.19	2.86	3.47	4.52	9.20
	2	1.87	2.66	3.23	4.31	8.62
	3	1.82	2.77	3.13	4.52	9.32
300,000	1	1.97	2.68	3.24	4.16	8.60
	2	1.78	2.54	3.15	3.97	8.36
	3	1.93	2.72	3.37	4.51	8.81
1,000,000	1	2.08	2.96	3.56	4.52	9.41
	2	2.01	2.72	3.30	4.30	8.75
	3	1.88	2.67	3.32	4.33	8.81
3,000,000	1	1.97	2.95	3.44	4.36	8.86
	2	1.99	2.70	3.27	4.27	8.75
	3	1.93	2.75	3.27	4.30	8.76
10,000,000	1	1.65	-	-	3.81	8.77
	2	1.63	-	-	3.90	8.52
	3	1.68	-	-	3.80	8.72

EXPLANATION OF PLATE III

Triplicate electrophoretic patterns of wheat protein extracted directly from untreated all-purpose flour. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

PLATE III

Fig. 1

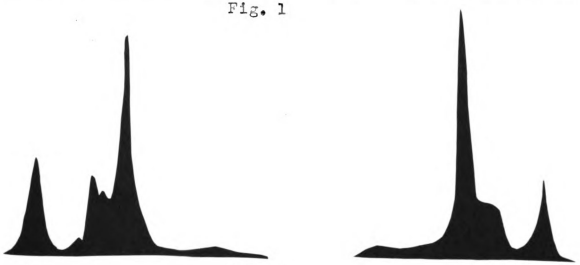


Fig. 2

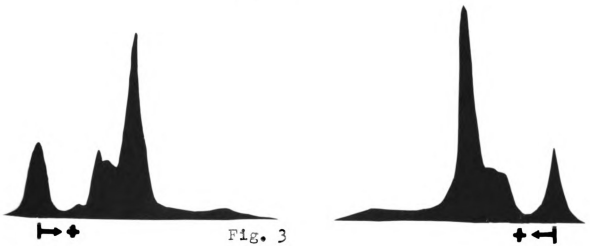


Fig. 3

EXPLANATION OF PLATE IV

Triplicate electrophoretic patterns of wheat protein extracted directly from flour treated with 300,000 rep ionizing radiation. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2; ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

PLATE IV

Fig. 1

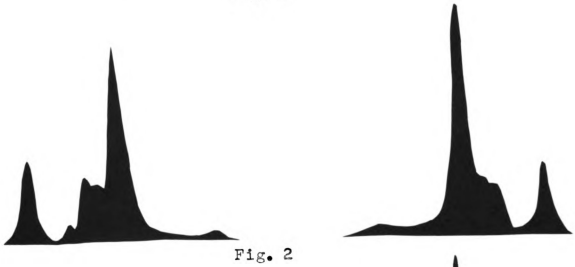


Fig. 2

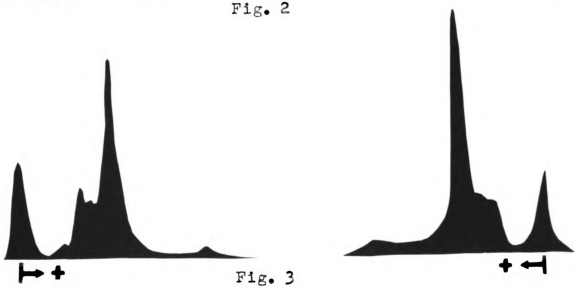


Fig. 3

EXPLANATION OF PLATE V

Triplicate electrophoretic patterns of wheat protein extracted directly from flour treated with 1,000,000 rep ionizing radiation. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

PLATE V

Fig. 1

Fig. 2

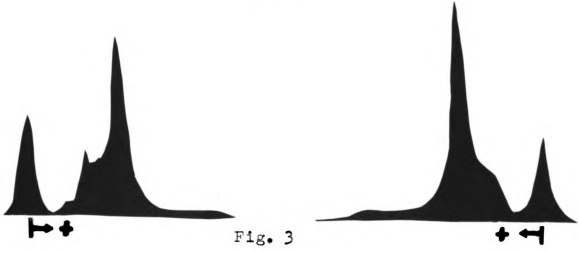


Fig. 3

EXPLANATION OF PLATE VI

Triplicate electrophoretic patterns of wheat protein extracted directly from flour treated with 3,000,000 rep ionizing radiation. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

PLATE VI

Fig. 1

Fig. 2

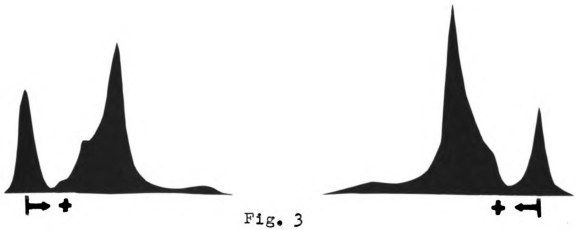


Fig. 3

EXPLANATION OF PLATE VII

Triplicate electrophoretic patterns of wheat protein extracted directly from flour treated with 10,000,000 rep ionizing radiation. Ascending boundary on left, descending on right. Citric acid-disodium phosphate buffer pH 2.2, ionic strength 0.024; field strength 6.80 volts/centimeter; temperature 2.1°C.; time 90 minutes.

PLATE VII

Fig. 1

Fig. 2

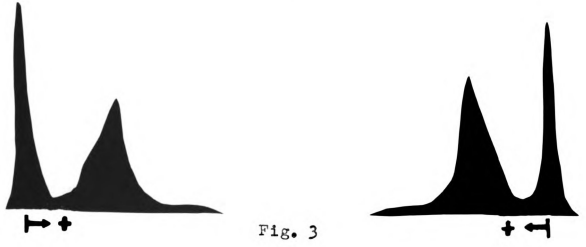


Fig. 3

VITA

Elsie Beth Alsup

candidate for the degree of

Doctor of Philosophy

Final Examination, October, 1959

Thesis: The Effects of High Voltage Cathode Ray Ionizing Radiation on Some of the Physical and Chemical Properties of Wheat Flour Protein

Outline of Studies

Major subject: Foods and Nutrition
Minor subject: Physiology

Biographical Items

Born, June 25, 1925, Kopperl, Texas

High School, Albuquerque High School, New Mexico

Undergraduate Studies, University of New Mexico, 1942-45

Graduate Studies, Ohio State University, 1948-1949, Michigan State University 1953-1954, 1956-1958

Experience: Home Economics Teacher, Belen (New Mexico) High School, 1945-1947, Raton (New Mexico) Jr. High School, 1947-1948; Graduate Research Assistant, Ohio State University, 1948-1949, Assistant Professor of Foods and Nutrition Research, South Dakota State College, 1949-1953, 1954-1956; Graduate Research Assistant, Michigan State University, 1956-1958; Associate Professor of Foods and Nutrition, Kansas State University, 1959-

Member of Kappa Omicron Phi, Pi Lambda Theta, and Sigma Delta Epsilon

ROOM USE ONLY.