THE EFFECT OF HIGH VOLTAGE CATHODE RAY IONIZING RADIATION ON THE CHEMICAL PROPERTIES OF WHEAT FLOUR

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Two varieties of wheat, a high- and low-protein wheat, were irradiated with cathode rays at doses ranging from 2.5×10^4 to 6×10^6 rep. A Buhler experimental mill was used for milling the wheat into flour of approximately 60 per cent extraction. Quantitative determinations of protein, carbonyl and sulfhydryl compounds were made on each sample of flour. Samples of control flour were also irradiated to see if irradiation after milling produced different effects on the quantities of protein, carbonyl and sulfhydryl compounds in flour than irradiation before milling.

The protein contents of the irradiated high- and low-protein wheat were found to be 1[illegible].2 and 8.1 per cent respectively. The results indicate that total protein in wheat is not changed by ionizing radiation with doses as high as 6×10^6 rep.

In general, the concentration of carbonyl compounds in both varieties of wheat irrespective of the time of irradiation (before or after milling) increased with increasing irradiation except in the low-protein wheat where a decrease of water-extracted carbonyl was observed at 2.5×10^4 rep followed by a gradual increase. Carbonyls obtained by aqueous extraction might be derived from either proteins or carbohydrates and thereby become extraction indicators for fats in wheat are affected by ionizing radiation. The observations showed that flour irradiated after milling have higher carbonyl values than wheat irradiated before milling.

No statistically significant difference was observed in the sulfhydryl compounds of the two varieties of irradiated wheat even at radiation levels as high as 6×10^6 rep. However, in flour irradiated after milling a decrease in sulfhydryl compounds was observed at the radiation level 6×10^5 rep. The decrease in the sulfhydryl groups in irradiated flour at 6×10^5 rep suggests that sulfhydryl compounds may have been oxidized to form disulfide linkages. They might also have been lost as volatile compounds such as hydrogen sulfide.

It is evident that carbonyl and sulfhydryl compounds are more subject to quantitative alterations in irradiated flour than in flour milled from irradiated wheat. Therefore it seems that the study presented herein is accounting for the irradiation of wheat before milling. It is doubtful that it would ever be practicable to irradiate flour.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TABLES - CONTINUED

LIST OF TABLES - APPENDIX (Continued)

LIST OF ILLUSTRATIONS - CONTINUED

INTRODUCTION

In recent years many studies have been made with ionizing radiation on foods, with a view to its possible application as a preservation process.

It has been shown that ionizing radiation of sufficient dosage can prevent the great loss of stored grains and grain products caused by insect infestation and fungal growth. Besides being capable of sterilization, tenderizing and disinfecting cereal products, it is apparent that ionizing radiation affects certain chemical and physical properties of wheat protein, and certain side effects can occur which cause changes in the flavor, color and texture of products baked from this wheat.

Nicholas et al. (17) and Trounell, Berlin and Nelson (17) both reported that off-flavors in baked products made from irradiated flour could be detected at a dose of 5×10^4 rep and over. Trounell et al. (17) also found that cakes were considered to have a different flavor when exposed to a dosage level of 1×10^5 rep and 1.5×10^5 rep. In dry cake mixes, Thomas et al. (17) found that an off-flavor was produced at dosages higher than 5×10^5 rep. Lewis (7) observed that irradiation treatment of flour could be detected in biscuits at dosage levels of 5×10^4 rep. The results of the taste panel scores for color

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*rep = roentgen equivalent physical and is equivalent to the absorption of 93 ergs per gram of material of unit density.

and baking characteristics of biscuits made with flour treated with 1×10^5 rep or less were not significantly different from the control. At 5×10^5 rep marked changes in odor and color of the biscuits were noted. However, Milner and Yen (42) reported that wheat irradiated with doses as high as 1×10^6 rep had no easily detectable changes in flavor. Since some of these investigations were on irradiated flour and some on irradiated wheat, a comparative study on milled irradiated wheat versus irradiated flour might explain the conflicting reports.

Few studies of changes in chemical composition of irradiated wheat flour have been done. Milner and Yen (42) and Lee (34) all reported an increase in maltose value of irradiated flour with increasing radiation dosages. Milner and Yen (42) also studied the treatment of wheat with ionizing radiation and found no changes in fat acidity, protein solubility, or fluorescence of acid extracts of grain. Mai, Finney and Milner (37) observed that damage to the starch of flour milled from irradiated wheat was indicated by an increase in dextrin and a decrease in the chain length, a decrease in total starch content and an increase in reducing sugars.

It is known that sulfhydryl compounds, particularly cysteine and glutathione, are among the most radiosensitive of all organic compounds. Doty and Luther (17) found that sulfur compounds are the cause of some undesirable odors in irradiated meat and suggested that the off-odors are formed from some water-soluble compounds.

Luther et al. (11) also indicated that carbonyl compounds are formed when meat or meat products are subjected to irradiation for

sterilization, and observed that the carbonyl compounds increased with increasing irradiation.

Therefore, it seemed desirable to investigate the possibility of similar changes in chemical composition occurring upon irradiation of wheat. The study reported herein, was made to determine whether there is an effect in the amount of carbonyl and sulfhydryl compounds with increasing dosage levels of radiation and to see if irradiation before milling produces different effects on the quantities of carbonyl and sulfhydryl compounds in flour than irradiation after milling.

Types of Ionizing Radiation

Ever since the discovery of X-rays by Roentgen in 1895, penetrating ionizing radiations have been widely investigated, particularly in medicine where tumor cells are killed by radiation.

The use of ionizing radiation for sterilization of foods has received great attention only during the last fifteen years. Although there are many types of ionizing radiation, only a few can be applied to food. The types of ionizing radiation that seem to be promising for food preservation are high energy cathode rays, beta rays, soft X-rays from suitable generators, and penetrating gamma-rays from radioactive materials. There are other types of ionizing radiation which cannot be applied to foods due to the fact that they bring about nuclear transformations. They are the heavy particle radiations, alpha rays, neutrons, protons, deuterons and fission fragments, which can combine with stable nucleus to form radioactive atoms.

Nature of the Cathode Ray

Cathode rays consist of a beam of negatively charged electrons accelerated by an electrostatic or electro-magnetic field. The electrons are physically identical to the negatively charged beta rays emitted by radioisotopes. Cathode rays can be made monoenergetic when accelerated by a constant potential (55).

It was pointed out by Trump and Van de Graaf (54) that in cathode ray irradiation, electrons are directly projected into the

material. When a charged particle passes through matter it loses energy by collision with atomic electrons and causes the excitation or ionization of thousands of atoms in its path. These primary electrons distribute the energy of the cathode ray stream through the volume of the absorber. These authors also pointed out that in an absorber the chemical changes are brought about primarily by the ionization and to a lesser extent by the excitation of atoms. The dissociation of complex molecules generally occurs at these high energy states. The ionizing energy imparted to the absorber is so direct that profound chemical changes may often occur with negligible gross heating effects. The temperature rise resulting from doses that are able to kill cancer cells is about 0.01° C, while complete sterilization of bacteria can be accomplished with ionization doses resulting in a 2° C rise.

Proctor and Goldblith (24) indicated that approximately 75 per cent of the electron beam energy from the cathode-ray production can be utilized. According to Pohl (24) full utilization of the beam energy is prevented by non-uniform distribution in a wide, divergent beam and by depth-dose considerations. With a stationary beam the efficiency in the irradiation from one side is 75 per cent while, with a scanning beam, the efficiency in irradiation from two sides can rise up to 83 per cent. Proctor and Goldblith (74) pointed out that consideration should be given to this type of radiation for food preservation, because although the penetration into matter is less deep, than that of X-rays of corresponding voltage, the penetration range is of sufficient magnitude to food preservation. The biological and chemical effects of cathode rays on matter are a stand to be the same as those of X-rays, and neither cathode rays nor X-rays produce radioactivity except at voltages in excess of 21 million electron volts. It has been shown that the density of a material is a factor in the penetra-

tion of electrons, the container to be selected should be made of material of as low a density and as thin as possible.

Dunn et al. (2) showed that the dose required to destroy bacteria with cathode rays appears to be similar to that necessary with X-rays. Since the available output in roentgens per minute of cathode rays is much greater than when using the same equipment for X-rays, it is possible to obtain the result with cathode rays much more rapidly than with X-rays.

Units of Dosage

According to Trum, Wright and Pierce (3) cathode ray dosage is best expressed in equivalent roentgens for biological studies, but expressed in ergs and joules for physical studies. In radiation chemistry, the most common unit of dosage is the unit of ionization which is the roentgen in the case of X-rays and the roentgen equivalent physical (rep) in the case of cathode rays.

The unit rep is an unofficial unit which has never been precisely defined and has been interpreted differently by various authors. One rep is generally defined as being equivalent to 93 ergs per gram of tissue or water and 83 ergs per gram in air. Another unit, rad, which is the official unit is more commonly used now. It represents an energy absorption of 100 ergs per gram of the material.

Theory of Radiation

It has been pointed out by many investigators that when foods are subjected to ionizing radiation, chemical changes may be produced in the foods. As a result of these chemical changes, certain side reactions

can occur which cause changes in the flavor, color, odor and texture of the food. Two theories, "direct" and "indirect," have been postulated as possible explanations of these chemical changes.

In the direct effect, the molecule is said to be ionized directly by the radiation. It varies considerably with the nature of the molecule concerned. A free radical is usually one of the products of the cleavage and this free radical may result in a complex series of secondary reactions. The direct action of irradiation on organic molecules causes them to undergo degradation, denaturation, or depolymerization. Chemical changes resulting from irradiation of whole foods, cereals and fats could be due to a direct effect.

A great deal of study in support of indirect effects has been done in recent years. It is now generally believed that most of the chemical effects of radiation are brought about by the action of free radicals produced by the radiation decomposition of water. Hart (39) found that within a time interval of 10^{-10} seconds after the passage of ionizing particle through water, an equal number of H and OH ions were formed.

The main initial chemical reaction upon irradiation of water molecules has been demonstrated by various authors (1, 2, 13, 22, 34, 35) and can be summarized as follows:

$$H_2O \longrightarrow H + OH$$

$$2\,H_2O \longrightarrow H_2 + 2\,OH_2$$

Before the radicals have time to separate by diffusion or in the absence of any solute with which they can react, they can combine with each other and reform water. This back reaction is a rapid chain reaction which can be shown by the steps:

$$H + O_2 \longrightarrow HO_2$$

$$H + H \longrightarrow H_2$$

When both hydrogen and oxygen are present in a solution the only reaction that occurs is the formation of H_2O_2 with hydrogen and oxygen disappearing in equimolar amounts.

An effect similar to the ionizing radiation of water was shown in a study by Hart (3) on the reactions of formic acid solutions under gamma radiation. Formic acid decomposed to CO_2 and H_2 in solution. CO_2 and H_2 were formed through the addition of H_2O to HCOOH (formic acid).

Lea et al. (5) found that when enzymes or viruses were irradiated dry or in concentrated solution the inactivation was direct and was due to ionization produced inside the virus particle or enzyme molecules. The inactivation was mostly indirect at lower concentrations due to ionization of the water.

Similar results have been found in studies of the effects of ionizing radiation on the living cell. When cells were exposed in the frozen state to ionizing radiation, they proved to be remarkably insensitive, most likely because in the frozen state the diffusion rate of free radicals was slowed down. This has also been found true with foods. Decker and Proctor (6) pointed out that in contrast to the indirect effect, the denaturing, depolymerizing, or denaturing direct action of ionizing radiation was found to continue in spite of the frozen state of tissue during irradiation. This results in appreciable organic tissue destruction. They also pointed out that the direct ionizing action of irradiation accounts for damage and destruction to bacteria and was independent of temperature range, since it caused ionization in the cores and circumcores of the microorganisms, which

in turn prevented reproduction or gave lethal mutations. Thus, it seems evident that both types of radiation-induced chemical changes could occur during the radiation preservation of a food.

Effects on protein and amino acids

The effects of ionizing radiation on the physical and chemical properties of proteins have been extensively studied and it is known that both direct and indirect effects are involved.

It is believed that the most obvious effects of exposing protein solutions to radiation is the denaturation of the protein. Two distinct steps occur in this denaturation, one is that the protein molecule becomes a new chemical entity and the other is the formation of a visible coagulum.

Stetton and Tendel (35) have studied the effects of radiation from a quartz mercury vapor arc upon a series of twelve purified proteins. As a result, all the proteins produced the same characteristic odor and all tended to assume a yellowish tint. The temperature at which the proteins coagulated when heated was lowered. These changes appeared to be independent of the presence of water or air in the environment of the proteins.

Arnow (5, 6) observed that in irradiated protein solutions the actual coagulation process occured only if the pH of the protein solution was close to that of the isoelectric point. The addition of small quantities of acid or alkali protected against flocculation. It was observed that the total nitrogen of the protein solutions remained the same before and after irradiation, regardless of the pH at which the solutions were irradiated. The ultra violet absorption spectrum

was changed quantitatively by the irradiation and increased at or below the isoelectric point.

Luesberg and Greholt (89, 90, 91) found that upon irradiation with ultra violet light at pH 6.2, hemocyanin was split into half-molecules. Prolonged irradiation caused denaturation, finally resulting in complete precipitation. The weakest bond in the molecule of hemocyanin was the holding of the two halves together. An increase in the number of ionized groups was necessary in order to split the half-molecules further by ultraviolet light.

Rikov and Krounskya (101) studied the influence of irradiation on the viscosity of gelatin. They pointed out that the effect of the irradiation on gelatin was not only on the degree of dispersion, but also the molecules of the gelatin were split to a certain extent.

Koenig and Berringer (46) subjected dry protein to X-ray irradiation and found that the amount of insoluble material increased with radiation.

Sandiger, Krejci and Kraemer (82), Carroll, Mitchell and Callahan (14) and Barron and Finkelstein (10) all observed an increase in the absorption spectrum when aqueous serum albumin was exposed to radiation. The increase was more marked around 2800 Å (280 mμ).

Singer, Lewis and Schweigert (93) exposed pure pyocyanin extracts of meat to gamma-ray radiation and found that there was an increase in damage with increasing purity. Inconsistent results were obtained in crude extracts, including the formation of a green compound, and evidence of oxidation and reduction reactions. It was believed that the green compound was porphyrin which had been altered by irradiation.

In the radiation sterilization of foods the inactivation of enzymes by ionising radiation is very important. Unless the enzyme

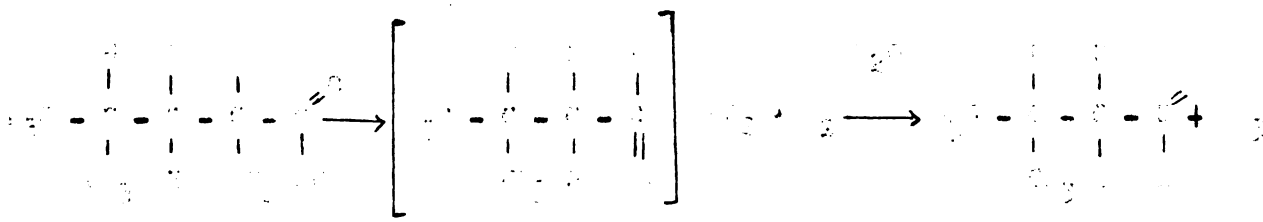
systems that may catalyze undesirable changes during extended storage are also inactivated, radiation cannot be applied to foods. Doty and Winter (26) pointed out that proteolytic enzymes were more resistant to ionizing radiation than microorganisms. At dosages of 1.6×10^6 rep about 50 per cent of the proteinase activity of beef muscle extractable at pH 7.6 was reduced upon exposure to gamma radiation. Proteinase activity at a dosage of 5×10^5 rep was a little reduced. This was indicated by the liberation of tyrosine from casein substrate. The reduction of the amount of tyrosine extractable from beef by irradiation suggested that this amino acid was changed by irradiation.

Proctor and Whitis (73) attempted to determine the amino acid composition of irradiated haddock muscles. They found no measurable loss in amino acids after irradiation with dosages up to 5.7×10^6 rep of cathode rays. The greatest loss was 7% per cent of the tryptophan at 5.7×10^6 rep which they thought might be within the experimental error of the method. However, they concluded that most likely there was a small destruction of essential amino acids in this type of biological material.

Individual amino acids have been widely studied due to the belief that such reactions would explain the problem of protein degradation. Since many proteins when irradiated produced different characteristic irradiation odors, many investigators in an attempt to locate the sources of the odors, irradiated individual amino acids. It was found that odors were much stronger in irradiated solutions of amino acids than in irradiated dry amino acids.

Kelley and Lawton (1) in their studies on the problems of using high-voltage electrons for sterilization pointed out that the

sulfur-containing amino acids, cystine, cysteine and methionine and leucine were found to have the most an important color at any given dose level. Isovaleraldehyde was been isolated as the 2, 4-dinitrophenylhydrazine from irradiated leucine. Isovaleraldehyde was believed to result from the deaminization and oxidation of leucine. The suggested mechanism is:



Only carbon dioxide and hydrogen were found in the absence of water and no ammonia or nitrogen and little color was attributable. According to the authors, the dose ruling all the amino acids by irradiation and then it increased upon the addition of water to form isovaleraldehyde and ammonia. Isobutyric acid was also found to be produced from irradiated cysteine isolation but the doses were not nearly so pronounced.

Sulfhydryl compounds are among the most radiosensitive of all organic compounds. Mitchell, Lamb and Moore (26) have used cysteine, glutathione, and cysteamine as protective agents to prevent hydrogen sulfide and mercaptan formation which contribute odor to irradiated foods. The authors concluded that the blocking of sulfhydryl groups by specific chemical reactions was more effective than by the addition of free radical acceptors.

Doty and Lehmer (27) found that irradiated meat had some undesirable odors from sulfur compounds. In order to locate the source of some of the off-odors, beef samples were fractionated. The results

indicated that the odors originated from some water soluble constituents. Experiments of Pirosch and Doty (9) showed that in yeast-irradiated meat, free hydrogen sulfide was probably not present. They suggested that hydrogen sulfide was lost from the meat slurry or solution by oxidation.

Werner and Pickett (7) observed the reactivity of the sulfhydryl enzymes, cysteine plus formaldehyde polymerase and urease, when irradiated in dilute solutions with small doses of alpha rays from Po, beta rays from P^{32}, and gamma rays from Co. From inhibition of glutathione, partial reactivity of the enzyme was obtained. Inactivations were due to the oxidation of the sulfhydryl groups.

Stenstrom and Gunsalus (8) working with yeast cells (7) did not find liberation of hydrogen sulfide from cysteine when exposed to radiation, while on the other hand Proctor and Goldblith (7) observed the liberation of hydrogen sulfide when cysteine was exposed to high voltage cathode rays. It was suggested that the liberation of hydrogen sulfide indicated that cysteine was decomposed at the disulfide linkage upon irradiation. According to Proctor and Goldblith (7) the different results obtained by Gunsalus and Gavies (8) were due to the lower radiation dose used by the latter.

Gerschman, Gold and Pitcher (3) observed that irradiation of cysteine solutions caused a slow decrease of part of the cysteine which was accompanied by the formation of hydrogen peroxide. The extent of the latter reversible reaction varied with the dose and maxima and minima were found at nearly the same positions on the dose curve as with cysteine. In a deaerated solution there was no formation of hydrogen peroxide, but the amount of cysteine destruction was not altered appreciably. It was shown that there was no reduction to cystine and no formation of sulfide or sulfite.

The liberation of hydrogen sulfide by γ-irradiation from cysteine and glutathione was observed by Sakurai (22). It was found that cysteine solutions after γ-irradiation yielded H_2S and the yield increased as the concentration of cysteine increased. The H_2S yield was a maximum at pH 6. Cysteine was not destroyed. Glutathione gave similar yields of H_2S, but the curve of yield versus pH had a double maximum. Miller (23) also irradiated solutions of cysteine by γ-irradiation with cobalt-60 and found that the color of I_2 was noticeable during the irradiation. Another radiation study of cysteine solutions was studied by Fricker et al. (24). The disappearance of the thiol group and the formation of hydrogen peroxide and H_2S in solutions irradiated with a dose of 7×10^4 roentgens was found. The formation of I_2 indicated that the disappearance of the thiol group might be explained as:

$$\text{R-SH} \longrightarrow \text{R-S}\cdot \longrightarrow \text{R-S-S-R}$$

$$\text{R-SH} \longrightarrow \text{R-S}\cdot \longrightarrow \text{higher oxidation products}$$

At the end of the exposure, the amount of I_2 present in the solutions was not sufficient to account for the change in thiol concentration. Thiol loss first increased to a minimum around pH 4, then rose to a maximum near pH 6. The authors suggested that the variation was associated with the presence of oxygen rather than with changes in the amphoteric character of the amino acid or with ionization of the thiol groups, since the reaction showed little dependence on pH in deaerated solutions. Sulfide formation was highly influenced by the pH. The peroxide formation was reduced to a low level by the removal of air, and it was considerably greater in the presence of cysteine than in pure water.

Gurr, Samuel and Thomas (25) showed that one of the first observable effects of the irradiation of wet lecithin is the formation of

[illegible] with the accompanying rat insulin, followed by the renewal of the [illegible], leaving a homogeneous solution.

[illegible] (24) exposed some simple amino acids in dilute aqueous solutions to [illegible]. As a result, an oxidative deamination [illegible] and the corresponding [illegible] similar to those obtained from the same substances in vitro by the action of OH ion produced chemically was observed.

Lenstein and Weinert (25) found that when tyrosine in very aqueous solution was exposed to neutron radiation, tyrosine was changed in regard to the phenol groups. The amount changed was proportional to the dose of radiation absorbed and varied only slightly with the concentration. High dilution resulted in a small decrease in the efficiency of the radiation.

Proctor and Goutia (26) exposed trp, tyrosine, phenylalanine and cystine in aqueous solution to high voltage cathode rays from 1 x 10^5 to 1 x 10^6 r.u. in different concentration. All amino acids were decomposed upon irradiation. The dilute solutions were relatively more affected by a given dosation than were the more concentrated solutions. It was shown that the irradiation affected changes in the amino acids through free radicals. In 1953, Proctor and Goutia (27) made another irradiation study on the same three aromatic amino acids and also on L-histidine monohydrochloride monohydrate. Histidine was found to be deaminated to the greatest extent. The order of deamination was as follows: histidine>cystine> [illegible]ine>tyrosine>tryptophan. No significant change was noted in the pH of the solutions of phenylalanine and tryptophan upon irradiation with increasingly greater doses of cathode rays. But a slight drop was noted in the pH of a solution of tyrosine in which the tyrosine was from 2.0% to 1.0%. In the ultraviolet absorption spectra on the three aromatic amino acids it was

indicated that the high voltage cathode rays are capable of splitting the benzene ring. This observation was confirmed by actually exposing benzene to a cathode ray dose of 1.5 x 10^6 rep and it was clearly shown that the selective absorption curve characteristic of benzene disappeared. The above-mentioned authors subjected histidine monohydrochloride solutions to cathode-ray irradiation and reported that ammonia nitrogen evolved upon irradiation of histidine, was contributed both by the nitrogen in the imidazole ring and the alpha amino group. Evidence of rupture of the imidazole ring of histidine was determined by the color developed on treating histidine with p-diazobenzene-sulphonic acid.

In general, the breakdown of irradiated alpha amino acids consists of an oxidative deamination with intermediate production of alpha keto acids. Johnson, Pelchet and Weiss (13) investigated the formation of alpha keto acids from alpha amino acids by the action of H_2O_2 and Fe^{++} ions on a number of alpha amino acids. They found that irradiation in the presence of oxygen results in the formation of both alpha keto acids and aldehydes. From irradiation of glycine solutions, pyruvic acid was formed and upon irradiation in vacuo the yield was considerably accelerated. This suggests that the oxygen present in the irradiated solution plays a very important part in the mechanism of pyruvate production. The yield of NH_3 was found to be dependently upon pH, giving a minimum value in the region of the isoelectric point. Pyruvic acid formation did not depend so strongly on pH.

Dale and Davies (11) found that the ionic yield of the deamination of glycine upon irradiation rose gradually with its concentration. If the NH_2 was in the alpha position of the amino acids, ammonia was liberated, since the formation of NH_3 from lysine monohydrochloride was not increased when compared with glycine or alanine in equimolar solution. It was observed that glycine anhydride only gave traces of NH_3, and that the linkage in this case did not contribute to the NH_3

[illegible]. In an attempt to explain the radiolytic yield with glycine, these authors suggested a possible recombination of radicals occurring with deamination even at highest concentrations of glycine, due to low affinity of glycine to radicals. The mechanism of the chain reaction might be suggested as:

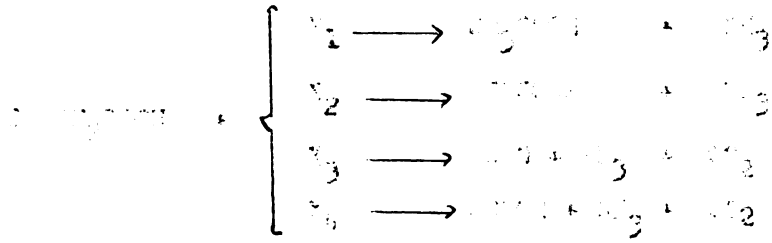
[illegible]

The reformed hydroxyl radical can then collide with another glycine molecule and so carry on the chain.

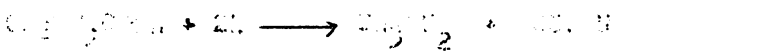
Garrison and Weiss [7] studied the action of X-rays on glycine, alanine, and serine in aqueous solutions under the presence of oxygen, in a vacuum, in argon, at different pH value, and at different concentrations. It was found that deamination occurred and that ammonia and other products were formed. The yield of NH_3 was found to depend, not only on the concentration of the amino acid and on the total dose given, but also on the [illegible]. Glycine was found to yield very small quantities of formaldehyde, alanine very small quantities of acetaldehyde, and serine relatively large quantities of glycolaldehyde and glyoxal. It was reported that in the case of glycine, not only the OH radicals, but also the H atoms attack the acceptor, both leading to the formation of ammonia. The other was apparently an aldehyde when the attack was by OH or HO_2, but was not an aldehyde when the attack was by H atoms.

Haxell, Saturen and Weiss [8] observed the action of X- and γ-rays on oxygen-free aqueous solutions of glycine and reported that nine products were formed among which eight are identified as primary products. They were: ammonia, glyoxalic acid, formaldehyde, acetic acid, formic acid, carbon dioxide and hydrogen. The secondary

product was identified as hydrogen peroxide. To account for the observed products the authors postulated five reactions. Four of the reactions were indirect reactions utilizing free radicals formed as the result of energy absorption by the water. The reactions are as follows:

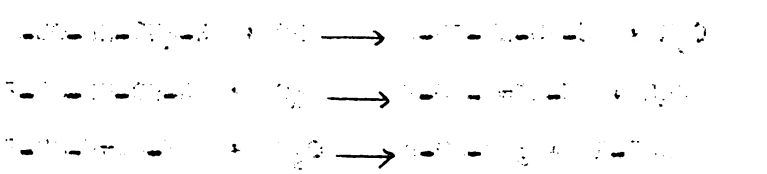


The indirect reaction utilizing energy absorbed by the glycine was given as:



Therefore it is apparent that from the studies reviewed irradiation of free amino acids can result in an alteration of sulfur linkages, and the formation of free ammonia and aldehydes.

Ducko and Garrison (6) made a study to determine if, through the indirect action of radiation on the peptide chain, primary amino and carboxyl terminal linkages are formed at the place of cleavage. Diglycine solutions were irradiated in the presence of oxygen and it was observed that primary amines and aldehydes were formed presumably through the intermediate formation and subsequent hydrolysis of the N-alkylamino acid. The reaction may be represented as:



The authors suggested that the reactions involved the radiation-induced cleavage of the peptide chain.

In studies of the production of carbonyl compounds during irradiation of meat and meat fats, Minor et al. (11) observed that carbonyl compounds of irradiated meat increased with increasing dose.

Rose et al. (-) irradiated cystine, cystine, and methionine, pepsin, and a number of sulfhydryl proteins and analyzed for products of radiolysis. Cysteine, cystine and glutathione gave hydrogen sulfide. For methionine, both ethyl mercaptan and methanethiol were found. Only traces of hydrogen sulfide were found in irradiated sulfhydryl proteins and general radiolytic cleavage was observed. Formation of amines and carbonyls suggested that some radiolytic cleavage of proteins was at the peptide bonds.

The effect of irradiation on the intact protein may be different from that on free amino acids. The few studies that have been done on chemical changes occurring upon irradiation of proteins indicate a less extensive alteration of amino acids than they are contained in the polypeptide.

Effects on Fats

Some of the alterations in quality of irradiated foods appear to originate from oxidation of lipids.

Lea and Moore (7) observed the action of cathode rays on drying oils and linseed oil and found that the chemical composition was changed. The changes in chemical composition were noted by an increase in the molecular weight and a decrease in iodine number. The oils were gravitationally thickened. The color increased progressively to the time of irradiation investigated. A reduction in the drying time of oils was also observed. These changes were probably due to the irradiant destruction of the pigments and antioxidants by oxidized radicals of the oil, or to an initiation of polymerization by the introduction of unsaturated free radicals.

Mukherjee (6) demonstrated that peroxides were formed during and after irradiation in the presence of oxygen. He observed that when butter fat was subjected to irradiation in the presence of oxygen, rancidity developed and was proportional to the intensity of the radiation source. Off flavors were not noted in the complete absence of oxygen.

Larson and Nelson (35, 37) studied some after-effects in fats irradiated with high energy electrons and X-rays. Ion natural fats were exposed to irradiation, a number of the effects were similar to air oxidation. Besides the immediate effects of irradiation, butter fats when irradiated in the presence of air and water at 37° C exhibited two types of chemical effects over a period of days following irradiation. The similar after-effect was the autoxidation chain reaction which developed much more rapidly than before irradiation. This was suggested to be due to the destruction of the natural antioxidants present in fat, mainly tocopherols, which protect the unirradiated fat. In addition, peroxides showed a maximum development at -40° C to -30° C which was thought to be due to the reaction between oxygen and reactive groups which were stable at these temperatures but become unstable with increasing temperatures and soft rind of the fat.

Arthur, Ingalls and Black part II observed an increase in the peroxide values during irradiation of lard fats and subsequent storage of the irradiated samples. Among the three components that were determined were peroxide values, carbonyl compounds and free fatty acids. Carbonyl compounds were found to have the lowest values, ranging from 0.1 per cent to 1.1 per cent for individual samples. At a dosage level of 3 or 4 x 10^6 rep it was shown that the three components determined could be increased in the irradiated lard and more fat when the oxygen content was minimized.

Ustrack et al. (7) observed the effect of high intensity electrons (1.5 x 10^6 rep) on vegetable and fish oils and found that the effect of irradiation depended largely on the composition of the individual oils. The radiation reactions were found to be polymerization, bond breakage and a variety of oxidative changes. In general, irradiation of vegetable oils at 1.5 x 10^7 rep caused only minor changes. There was an increase in the peroxide formation observed in fish oils, probably due to the destruction of the natural antioxidants. Irradiation of the anhydrous oils in the frozen state did not retard or diminish the radiation effects. This finding is in contrast to those on many foods that were found to benefit from irradiation in the frozen state.

Long and Proctor (9) observed the formation of monocarbonyl compounds from cotton seed, corn and olive oils when subjected to cathode ray irradiation at doses of 10^5 to 5 x 10^7 rep. When the dose was raised to 7.5 x 10^7 rep or higher the yield of monocarbonyl compounds dropped. The authors also reported that production of monocarbonyl compounds was not decreased when irradiation was carried out under low temperature, vacuum, or with addition of antioxidants.

Patton et al. (11) reported that when neat fats were subjected to gamma irradiation small amounts of carbonyl compounds were noted, but the carbonyl compounds increased with increasing dosages.

Ahrault et al. (10) studied the relationships between the chemical changes produced by irradiation of fats, and the undesirable flavors and odors that accompany them. The authors found that peroxides and carbonyl compounds were present in appreciable amounts upon irradiation. Flavor and odor changes were also observed, but they did not correlate with the carbonyl compounds and peroxides formed. The flavor and odor components were volatile compounds which reconstituted

only a fraction of the total carbonyl compounds produced. In the presence of air ozonic and nitrogen oxides may be formed when fats are irradiated, and the authors suggested that the off-flavors and odors might result from the action of these two components on fats. It was also observed that temperature sensitives had an effect on chemical and organoleptic changes during and after irradiation. Kitting and Incklaar (6) attempted to characterize and identify the volatile carbonyl compounds formed by the gamma-irradiation of lard. As a result the authors were able to identify seven saturated and two unsaturated aldehydes by means of spectrophotometric analysis and paper chromatography. The unsaturated aldehydes identified were acrolein and crotonal and the saturated aldehydes were propional, butonal, pentanal, hexanal, nonanal, decanal and 2, 4-undecadienal. It was observed that the presence of amino acids during irradiation affected the amount and type of carbonyls formed.

Effects on Carbohydrates

The effects of irradiation on carbohydrates has been less adequately dealt with in comparison to either proteins or lipids.

Hasten et al. (5), Hasen, Merrill and Lawton (4) and Hasegawa (3) all reported that cellulose was depolymerized upon exposure to irradiation. It was observed by Hasten et al. (5) and Hasen et al. (4) that at a dose of 10^8 rads cellulose became soluble in water. Many investigators have subjected lactose to ionizing irradiation and have observed extensive degradation. By means of chromatographic and isotope dilution methods, Phillips and Day (7) identified the main degradation products as glucose, tagatose, lactulose, galactosylerythrose, glycolic acid, glucuronic acid, glycerol, erythrose, and glycerolaldehyde.

In studies of the effects of cathode and gamma irradiation on some organic acid-carbohydrate systems, Wilkinson et al. (61) observed discoloration which was related to the magnitude of the dose. These authors also observed an increase in acidity and a shift in the wavelength of maximum ultraviolet absorption toward 260-265 mμ which is similar to the observation made by Proctor and Goldsmith (72) who exposed grape juice and acid treated sugar solutions to cathode rays and found a shift from absorption maximum at 280 mμ to a peak at 245 mμ. Increased viscosity of apple sauce and the loss of gelation in pectin gels was also noted upon irradiation.

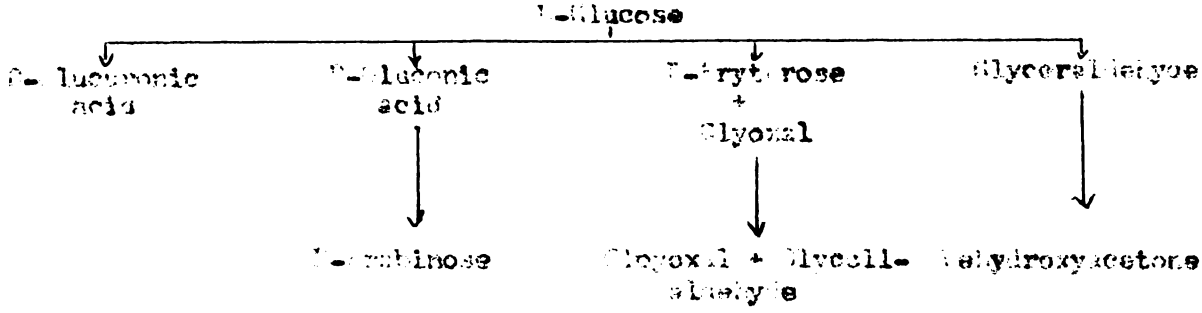
Hersten and Leight (32, 33) observed a decrease in the viscosity of sols made from γ-irradiated dry sugar and on the viscosity of sols made from soft γ-irradiated apple pectin powder.

Hortese et al. (30) demonstrated that gamma irradiation of 5 x 10^4 r degraded pectin powder of 9.6 per cent moisture content, while degradation of pectin in solution occurred at 3.8 x 10^3 r. Pectin solutions could be protected completely if irradiated in the presence of a high sugar concentration and the low pH required for jelly formation. No weakening was noted in jellies prepared with sucrose and irradiated up to 2 x 10^5 r. The solution viscosities of pectin isolated from irradiated jellies prepared with sucrose and from unirradiated jellies were similar.

Irradiation of three naturally occurring hexoses, D-glucose, D-galactose and D-mannose in dilute aqueous solution has been studied by Hilliers (29) who has pointed out that oxidation preferably attacked the primary alcohol group at carbon-6 in the sugar, forming a carboxyl group. By this reaction glucose was converted to glucuronic acid, galactose to galacturonic acid, but mannose was not converted into mann-

onic acid until quite appreciable concentrations had been built up in the solution. The presence of oxygen increased the rate of reaction and under such conditions hydrogen peroxide became one of the major products. Sugar alcohols were converted to aldehydes. The investigators also observed that the gas formed consisted mainly of hydrogen, carbon dioxide and a small amount of carbon monoxide.

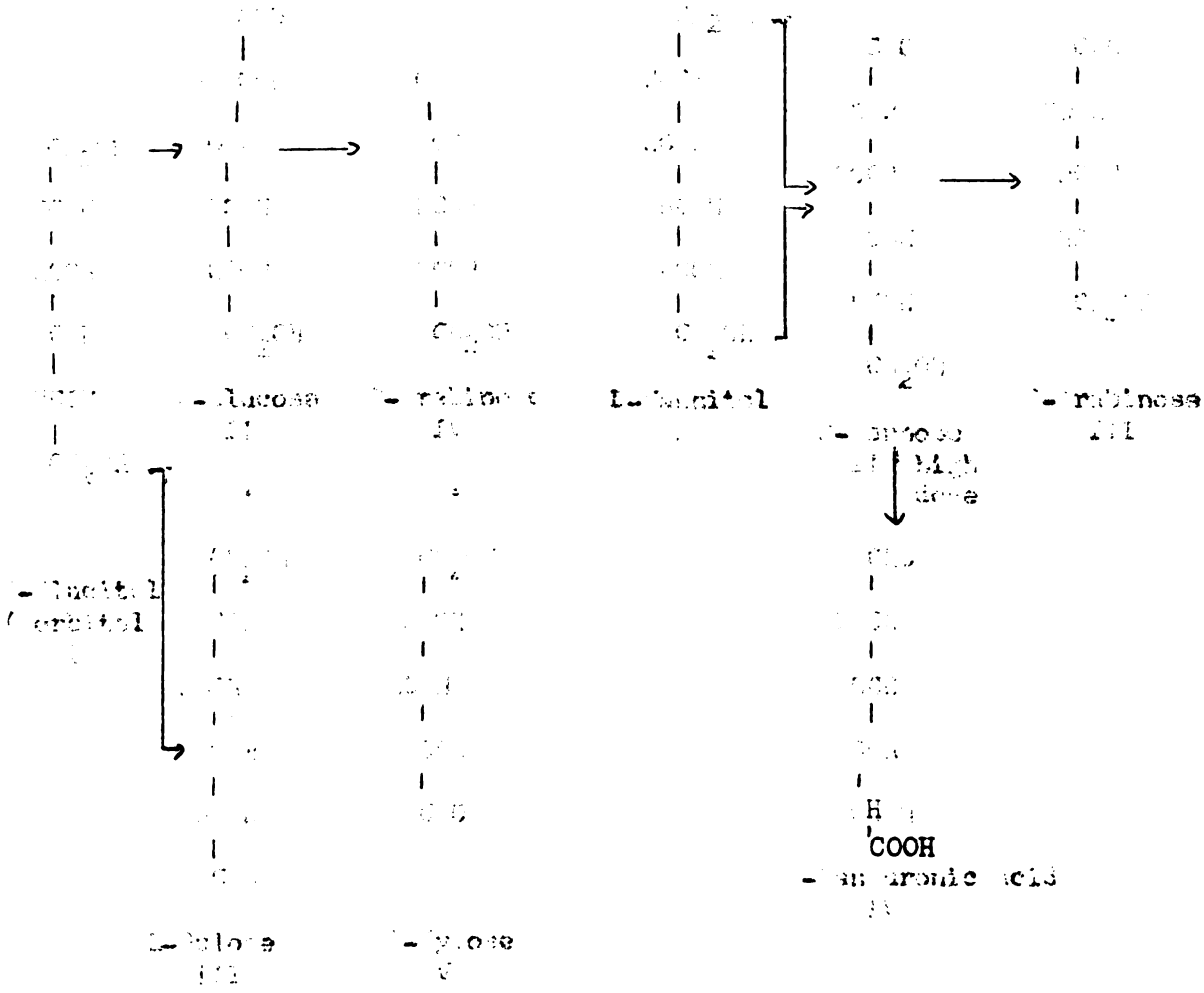
Phillips, Hoody and Mattok (44) observed the action of ionizing radiation on aqueous solutions of D-glucose and found that degradation occurred. The main steps of the degradation in the presence of oxygen could be shown by the following scheme as proposed by the authors:



Hydrogen peroxide was also formed. The irradiated solutions have an absorption maximum at 265 mμ and were related to dihydroxyacetone. The same authors, Hoody and Phillips (45), studied the action of ionizing radiation on solid carbohydrates, D-mannitol, sucrose, lactose, D-xylose, dextran, D-glucose, and D-fructose, and observed definite physical and chemical changes in several of the carbohydrates. The degradation of these irradiated carbohydrates was indicated by the change in melting point, optical rotation and acidity, and the characteristic absorption maximum between 270-300 mμ showed the introduction of keto groups into the molecule. Paper chromatograms by Phillips (46) showed that sucrose was hydrolyzed to give glucose and fructose. The main change in dextran was degradation rather than cross-linking since no significant molecular enlargement could be demonstrated. This confirms the observations made by Price,

ellary and Lawton (71) and Hicketts and Rose (72) also pointed out that degradation was a main change in irradiated dextran. D-fructose was found to be most susceptible to radiation damage and no additional compounds were detected in irradiated dextran, D-glucitol, and D-glucose. The authors suggested that the results support the view that degradation in solid carbohydrates was not as pronounced as that in solutions.

Clifford et al. (73) exposed samples of crystalline solid carbohydrates to irradiation by high voltage cathode rays. The irradiated samples were discolored and gave all fluoresced. A decrease in reducing sugars was observed when D-glucose and D-fructose were irradiated and under the same condition D-fructose was destroyed in a greater extent than D-glucose. When crystalline sucrose was irradiated at dosage levels of 1.3×10^6, 2.6×10^6 and 3.9×10^6 rep, hydrolysis occurred. Reducing groups were increased to a maximum at 2.6×10^6 rep and decreased at 3.9×10^6. Glucose and fructose were detected in the irradiation product of sucrose. D-glucitol (sorbitol) and D-mannitol in 50 per cent aqueous solutions were irradiated. D-glucitol, glucose, gulose, arabinose and xylose were identified as the irradiation products of D-glucitol. The irradiation products of D-mannitol, mannose, arabinose, and mannonic acid. This is in agreement with Phillips's observation (61). The authors postulated the following scheme for the reactions:



That glycolic [illegible] were very sensitive to ionizing radiation was indicated by the irradiation of the disaccharides, sucrose, maltose, and cellobiose, and trisaccharide raffinose. As the level of irradiation increased, the yields of the disaccharides and trisaccharides also increased.

From the results it is apparent that aldonates are formed upon irradiation of sugars and carbohydrates. Therefore it seems possible that similar reactions might occur upon irradiation of meat.

Irradiation of Wheat

Ionizing radiation is an effective means in insect disinfestation and sterilization of stored grain, flour, meals, and cereal products which suffer a tremendous loss annually. The required doses of gamma radiation for killing insects is much less than is necessary for sterilization of bacteria which requires 1 x 10^7 rep or more. Proctor and Goodwin (5) pointed out that a dose of 2.5 x 10^5 rep is sufficient for insect control in grain. It has been reported that at 2 x 10^4 rep flour insects and weevils would be destroyed and insect eggs would be made sterile. Besides the advantages of ionizing radiation there are certain problems which have become very important in the studies of wheat flour subjected to irradiation. The problems concerned are effects of irradiation on the solubility, physical and chemical properties of wheat protein.

Love, Linser and Tinney (37), Linser and Von (64) and Ali et al. (2) showed that there was a sharp maximum reduction of coagulation viscosities in flour milled from irradiated wheat. Irradiation of wheat caused doughs to lose strength, to have a shorter development time, and to become less extensible. Ali et al. (2) found that water absorption was increased with increasing dose up to 6 x 10^5 rep as shown by both farinograph and baking tests. This probably indicated a decrease of starch by irradiation. At 1 x 10^6 rep the absorption decreased, apparently because of an effect of radiation on some other constituents of wheat.

Ali et al. (2) observed that when wheat was treated with gamma radiation in doses up to 10^6 rep, small changes in the starch of flour milled from irradiated wheat were noted. It was in the sol

by a drop in gelatinization viscosity, an increase in maltrin and a decrease in the chain length, a decrease in actual starch content and an increase in reducing power. These workers postulated that this type of degradation of starch suggested that the polysaccharides of wheat were the fractions most seriously affected by irradiation.

Ulmer and Yen (61), Wilson (62) and Lee (34) all obtained an increase in maltose value of irradiated flour with increasing radiation dosages. They suggested that gamma irradiation either activated the beta-amylase or rendered the starch more susceptible to the enzymatic hydrolysis.

Wilson and Yen (62), studying the treatment of wheat with ionizing radiation, found no changes in fat acidity, protein solubility, or flavor scores of acid extracts of grain.

Lee (34) found that there was a slight decrease in the recoveries of crude gluten from irradiated flour at dosage levels of 2.5 x 10^5 and 5 x 10^5 rep, while at a dose of 1 x 10^6 rep a marked decrease was observed. This suggested a marked change in protein upon irradiation at high doses and subsequent deterioration of baking quality. Lee (34) observed an increase in the yield of crude gluten when flour was exposed to doses of 1 x 10^6 rep or less and a decrease at doses of 3 x 10^6 rep or more.

Brownell, Grain and Cereals (12) and Brownell et al. (64) both reported that off flavors in bread products using irradiated flour could be detected at a dose of 5 x 10^5 rep. Brownell et al. (12) also found that cakes were considered to have a different flavor when exposed to a dosage level of 1 x 10^6 and 1.5 x 10^6 rep, particularly in the grits from which flour was milled.

Tilner and Yan (41) studied the treatment of wheat with gamma radiation at levels up to 1 x 10⁶ rep. The dosage levels they used in this experiment covered the range that required for the elimination of storage pupal and insects. Their observation is not in agreement with the above-mentioned studies. They showed that irradiated wheat with a dose as high as 1 x 10⁶ rep was found with no easily detectable changes in flavor.

In other cases, Thomas et al. (42) observed that off-odor was produced at dosages of 1 x 10⁵ rep or above. On subsequent storage of matters at 3 x 10⁴ and 5 x 10⁴ rep changes in odor and color also showed up. Lee (43) showed that the irradiation treatment of flour could be detected in biscuits at the recommended range for disinfestation 5 x 10⁴ rep. Taste panel scores for color, odor, and baking characteristics showed no significant differences from the control at dosage levels of 1 x 10⁵ rep or less. A marked change in odor and color of the biscuits was noted at 5 x 10⁵ rep.

It is apparent that off-flavors are detected in baked products upon irradiation. Therefore an investigation of chemical compounds such as carbonyl and sulfhydryl might explain the detected off-flavor. Furthermore, since some of the studies reported in the literature were on irradiated flour and some on irradiated wheat, a study on irradiated wheat versus irradiated flour might shed in part the lack of agreement among authors.

Irradiation and Irradiation of Wheat. Each of two varieties of wheat, a high-protein spring wheat and a low-protein soft red wheat, were divided into five-pound lots and each lot irradiated with cathode rays produced by a 1 million electron volt generator, resonant transformer type,* at one of the following dosages:

2.5×10^4 rep	1×10^5 rep
5×10^4 rep	1×10^6 rep
1×10^5 rep	5×10^6 rep
2×10^5 rep	6×10^6 rep
4×10^5 rep	

The wheat was irradiated at a depth of one kernel in thickness to obtain a complete penetration, since penetration is the limiting factor with cathode rays. A ten pound lot of each variety served as the unirradiated control. The irradiated samples and the non-irradiated control were milled into flour at the International Milling Company, Minneapolis, Minnesota. The wheat was tempered to a 15 per cent moisture content and allowed to stand for approximately 18 hours. Then the sample was run through a laboratory pilot experimental mill, at the rate of about five pounds in 12 minutes, to an average flour extraction of 72 per cent. The flour was collected and weighed, and the percentage yield calculated on a wheat input basis. The percentage yield for the high-protein flour was 73.4 per cent and that for the low-protein flour was 70.6 per cent **.

*Department of Agricultural Engineering, Michigan State University.

**See Appendix, Table 1.

Two-pound samples of the control flour were irradiated at levels of 2.5×10^4, 1×10^5, 4×10^5, 1×10^6, and 4×10^6 rep. The samples were rolled out to a depth of not more than 1/4 inches and then irradiated so that complete penetration of radiant energy could be effected.

Determination of Carbonyl Compounds. Quantitative estimations of carbonyl groups were made on each sample of flour. The colorimetric method of Smith and Hardy (9) for the determination of carbonyl compounds was made on acid-salt and benzene-extracted flour samples according to the extraction procedure of Watzer et al. (11) as follows:

Acid-Salt Extraction. A 25-gram sample of flour was mixed with 60 ml of 3 per cent metaphosphoric acid and 20 ml of distilled water for 2 minutes in a Waring blendor. Thirty grams of sodium chloride were then added and mixing resumed for 1 minute. The mixture was centrifuged for 25 minutes at 2500 r.p.m. Three ml of the supernatant was transferred to a test tube. To this was added 1 ml of saturated 2, 4-dinitrophenylhydrazine solution, which had been twice recrystallized from methanol, and 1 drop of concentrated hydrochloric acid. The tube was loosely stoppered and placed in a water bath at 50° C for 30 minutes. After cooling to room temperature, 5 ml of 10 per cent potassium hydroxide in ethanol were added. The solution was then filtered to remove the precipitate of sodium chloride. Optical density of the red color was determined on a Spectronic 505 spectrophotometer Model "L" at 480 mμ wavelength. A solution of 2-heptadecanone in methanol was used for the standard curve.

Benzene Extraction. A 15-gram sample of irradiated flour was mixed with 80 ml of benzene for 2 minutes in a Waring blendor. The mixture was centrifuged for 25 minutes at 2500 r.p.m. Three ml of the supernatant were transferred to a 25 ml volumetric flask. Then 3 ml of 4 per cent trichloroacetic acid in benzene and 3 ml of saturated

2, 4-dinitrophenylhydrazine solution in benzene were added. The mixture was kept in a water bath at 60° C for 30 minutes. After cooling to room temperature 5 ml of 4 per cent potassium hydroxide solution in ethyl alcohol were added and the resulting solution was brought to volume with ethyl alcohol. The solution was mixed, filtered, and the optical density of the red color was read on a Beckman spectrophotometer Model B at 420 mu wavelength. A solution of 4-heptadienyne in benzene was used for the standard curve.

All reagents used for both types of extraction were treated with 2, 4-dinitrophenylhydrazine to render them carbonyl-free according to the procedures outlined by Lappin and Clark (51).

Determination of Sulfhydryl Compounds. The procedure for the determination of sulfhydryl groups by silver-tris titration was based on the method for wheat flour according to Kerr, Sullivan and Cluskey (33). Two grams of flour was placed in a 100 ml beaker and combined with 3.0 ml 1N nitric acid, 4.0 ml of 1 tris (hydroxymethyl) aminomethane, and 0.3 ml of 1N potassium chloride. The beaker was placed in an ice bath at all times to prevent loss of sulfhydryls. The mixture was mixed with a magnetic stirrer. Thirty ml of 5M urea solution and 1 ml of 0.05% ethylenediamine tetraacetate were then added to give a final volume of 37.7 ml. Ethylenediamine tetraacetate due to its chelating action on the heavy metal ions prevented the loss of sulfhydryl compounds. The rotating platinum electrode was immersed in the titration mixture. Increments of 0.001 N silver nitrate solution were added at 1 minute intervals when the current was stabilized at a constant value. The titration mixture was stirred with the magnetic mixer for 20 seconds after each addition, and the current

value was recorded 30 seconds after mixing was discontinued. The readings of the current were plotted against the volume of silver nitrate added. The end-point was determined graphically by the intersection of the excess silver nitrate line and the base line. A typical titration curve for flour is presented in Figure 1.

The apparatus used was essentially the same as that described by Koltoff and Harris (17). The reference electrode was made up according to Kunsert, Lorig and Hansen (11) and had a potential of -0.10 volt versus the saturated calomel electrode. A Sargent manual polarograph Model III was used to indicate the current. The titration solution was added from a syringe micro burette.

Protein determination. The boric acid modification of the micro-Kjeldahl method (2) was used to determine the nitrogen content of the flour. The total protein was obtained by multiplying the total nitrogen by the factor 5.7. In cereals the nitrogen content is higher than 16 per cent and the factor 5.7 more nearly represents the ratio between the protein and the nitrogen.

Statistical analysis. Analysis of variance was made on results of both carbonyl and sulfhydryl groups. Where significant difference due to irradiation treatment was observed from the F test, the radiation means were compared with significant studentized ranges by Duncan's multiple range test (5).

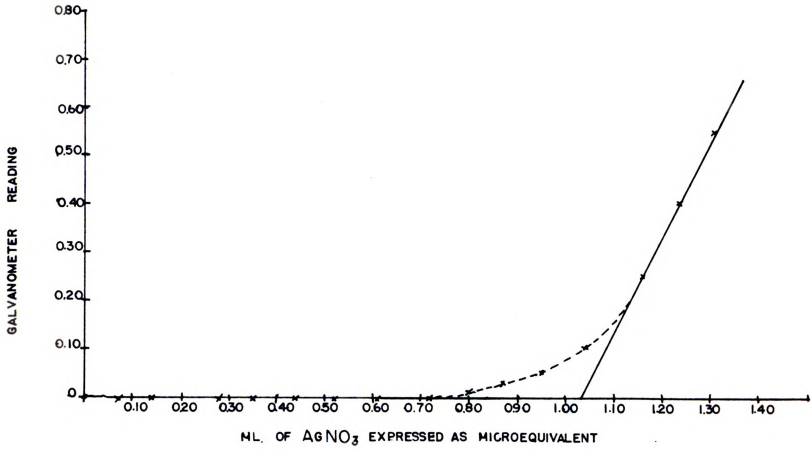


FIGURE I: A TYPICAL TITRATION CURVE FOR SULFHYDRYL IN FLOUR

Protein. The average protein content of the high-protein wheat was [illegible] per cent and that of the low-protein wheat was [illegible] per cent. No significant differences were observed in the protein content due to irradiation of either the high- or low-protein wheat. The results indicate that total protein in wheat is not changed by ionizing radiation [illegible] x 10^5 r.

Carbonyl Compounds. The average carbonyl values of flour milled from irradiated high- and low-protein wheat and extracted by cold-salt are given in Table 1. An overall increase of carbonyl compounds in both high- and low-protein wheat resulted from irradiation at the lowest dosage level used in this study, 1 x 10^4, 3 x 10^4 and 6 x 10^4 r. Doses above 2 x 10^5 r of the high-protein wheat produced statistically significant increases over the control, at the 1 per cent level of probability. However, significant increases were not found in the irradiated low-protein wheat at 4 x 10^5 r and lower.

Table 1

Carbonyl values for carbonyl compounds extracted by cold-salt from flour milled from irradiated wheat

Irradiation dose in r	Control	2.5x10^4	5x10^4	1.1x10^5	2x10^5	4x10^5	6x10^5	1x10^6	3x10^6	6x10^6
High protein	1.6	1.[illegible]	2.0	4.[illegible]	6.2[illegible]	3.[illegible]	3.[illegible]	[illegible].0	9.[illegible]	1[illegible].7
Low protein	2.0	1.[illegible]	1.[illegible]2	[illegible].[illegible]	2.[illegible]	3.[illegible]	4.[illegible]	5.[illegible]	6.[illegible]	[illegible].

* Expressed as moles x 10^{-5} per gram of flour.

[illegible] Table 1.

[illegible] were obtained in the carbonyl contents of the acid-salt extract of the high- and low-protein flour irradiated after milling. Table 4 shows the mean values of carbonyl contents at the highest level of irradiation, 5 x 10^5 rep, the content of carbonyl compounds in both types of flour was observed to be twice as high as that of the flour milled from irradiated wheat. In both the irradiated high- and low-protein flour, statistical analysis indicated significantly higher carbonyl values at 1 x 10^5 rep and over, at the 1 per cent level.

Table 4

Mean values for carbonyl compounds extracted by acid-salt from flour irradiated after milling

Irradiation dose in rep	Control	0.5x10^5	1x10^5	2x10^5	4x10^5	5x10^5
High protein	2.03*	2.61	3.10	3.75	4.75	5.74
Low protein	3.13	3.33	3.64	4.45	7.15	8.35

* Expressed as moles x10^{-5} per gram of flour.

The results of carbonyl determinations on the acid-salt extract of irradiated high-protein wheat and flour are presented graphically in Figure 2 and those of irradiated low-protein wheat and flour in Figure 3. These figures clearly show the higher carbonyl content produced by irradiation of the flour after milling as opposed to irradiation of the wheat before milling, especially in the case of the low-protein wheat.

Table 5 gives the mean values of carbonyl compounds extracted by benzene from the two varieties of irradiated wheat. Although in the irradiated low-protein wheat there was an apparent increase at the

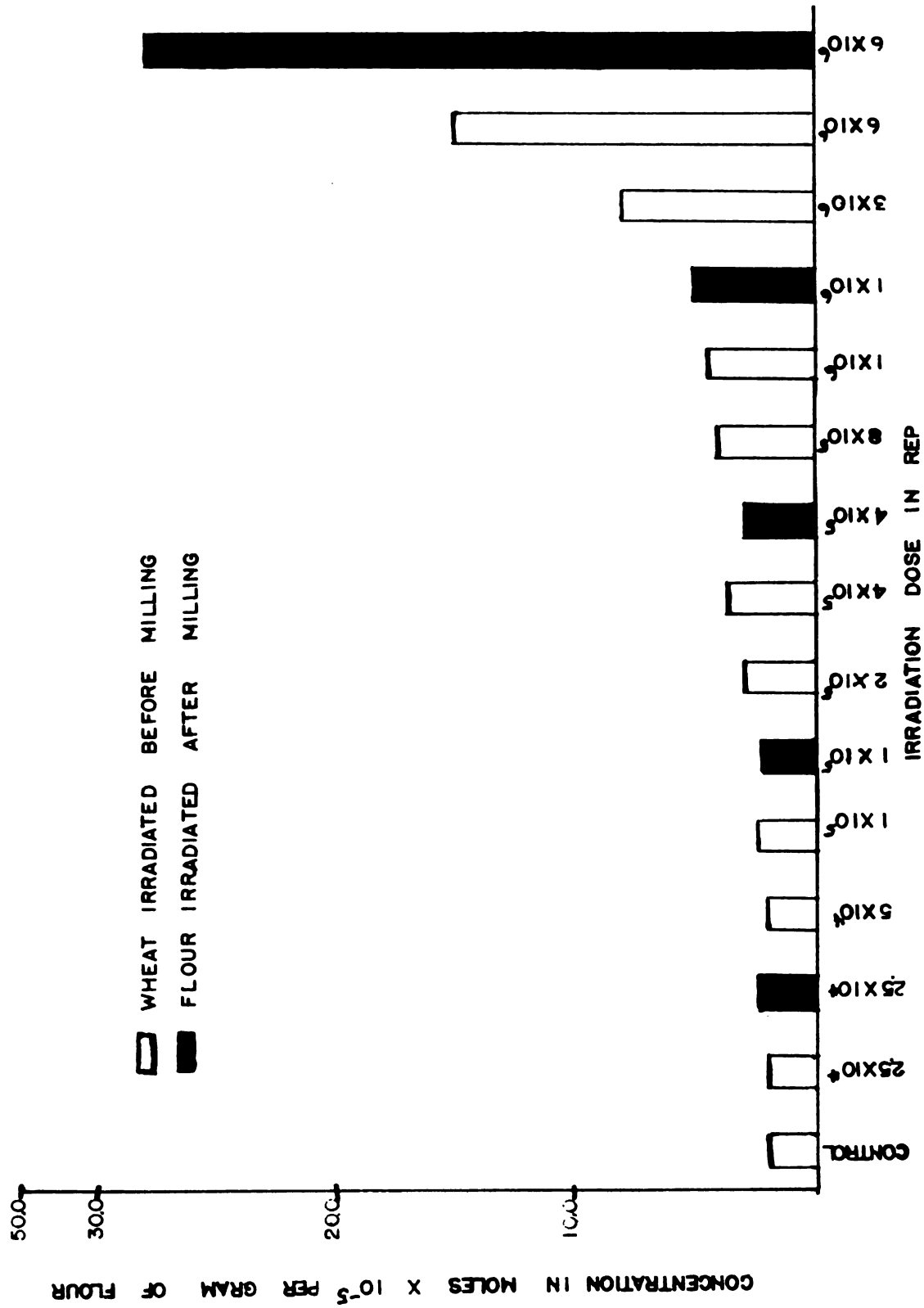


FIGURE 2: MEAN VALUES FOR CARBONYL EXTRACTED BY ACID-SALT FROM HIGH PROTEIN WHEAT

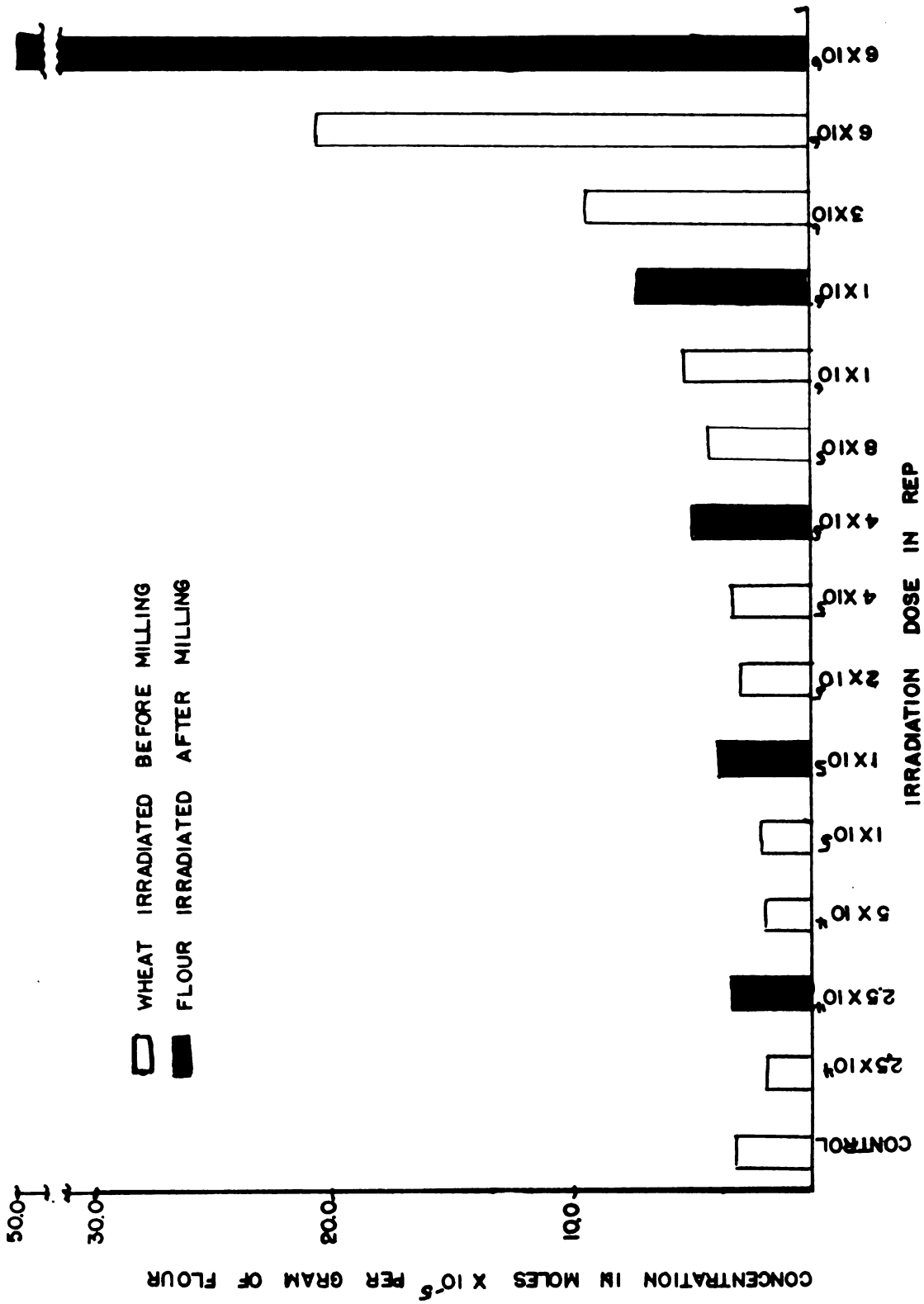


FIGURE 3: MEAN VALUES FOR CARBONYL EXTRACTED BY ACID-SALT FROM LOW PROTEIN WHEAT

highest level, 6×10^5 rads, no statistically significant differences were found among the values for the different radiation levels. The increase in the carbonyl content of the high-protein wheat irradiated at 2×10^5 rads and 6×10^5 rads was shown to be significant at the 1 per cent level.

Table 3

Mean values for carbonyl compounds extracted by benzene from flour milled from irradiated wheat

Irradiation dose in rads	Control	0.5×10^5	1×10^5	2×10^5	3×10^5	4×10^5	5×10^5	6×10^5	7×10^5	8×10^5
High protein	2.10*	2.11	2.22	2.26	2.30	2.37	2.41	2.38	2.35	2.31
Low protein	2.37	2.35	2.36	2.37	2.39	2.44	2.48	2.42	2.39	2.34

* Expressed as moles $\times 10^{-5}$ per gram of flour.

The mean carbonyl values of the benzene extract of irradiated high- and low-protein flour are given in Table 4. The results indicate a marked increase at the highest level of irradiation. For both types of flour the carbonyl values from 2.5×10^5 rads to 8×10^5 rads were found not significantly different from one another but all were significantly greater than the control at the 1 per cent level.

Table 4

Mean values for carbonyl compounds extracted by benzene from flour irradiated after milling

Irradiation dose in rads	Control	2.5×10^5	3×10^5	4×10^5	5×10^5	6×10^5	7×10^5	8×10^5
High protein	2.03*	3.00	3.05	3.01	3.04	3.03	3.03	3.03
Low protein	2.10	4.17	4.03	4.07	3.97	11.03	11.03	11.03

* Expressed as moles $\times 10^{-5}$ per gram of flour.

The results of carbonyl determinations on the benzene extract of irradiated high-protein wheat and flour are presented graphically in Figure 4 and those of irradiated low-protein wheat and flour in Figure 5.

All the determinations of the carbonyl compounds extracted by benzene in general indicate an increase with increasing levels of irradiation except in irradiated low-protein wheat where a decrease was observed at the lowest dosage level (8.5×10^5 rad) followed by a gradual increase (Figure 5). Except for this last observation, the results are in parallel with those of Ahmed et al. (1) who found that when meat or meat products were subjected to irradiation for sterilization at 2×10^5 to 2×10^6 rad, carbonyl compounds were formed and that their concentration increased with increasing irradiation.

Carbonyl compounds may be formed from the oxidative degradation of the various constituents of the wheat kernel (6, 7). Some of these carbonyl compounds may perhaps be derived by oxidative deamination of any free amino acids present in or produced by irradiation of the flour. Some of them may be derived from carbohydrates which is also a major constituent of flour. Upon irradiation of carbohydrates aldehydes are found as one of the irradiation products. Phillips (8), and Phillips, Lowy and Patrick (9) have reported that minor amounts of aldehydes are converted to aldehydes by irradiation.

The carbonyl compounds obtained by benzene extraction indicated that fat constituents in wheat are affected by ionizing radiation. The values obtained by benzene extraction are much lower in comparison to the values obtained by acid-alcohol extraction. This is probably because of the small amount of fat present in wheat. Hamdan (5)

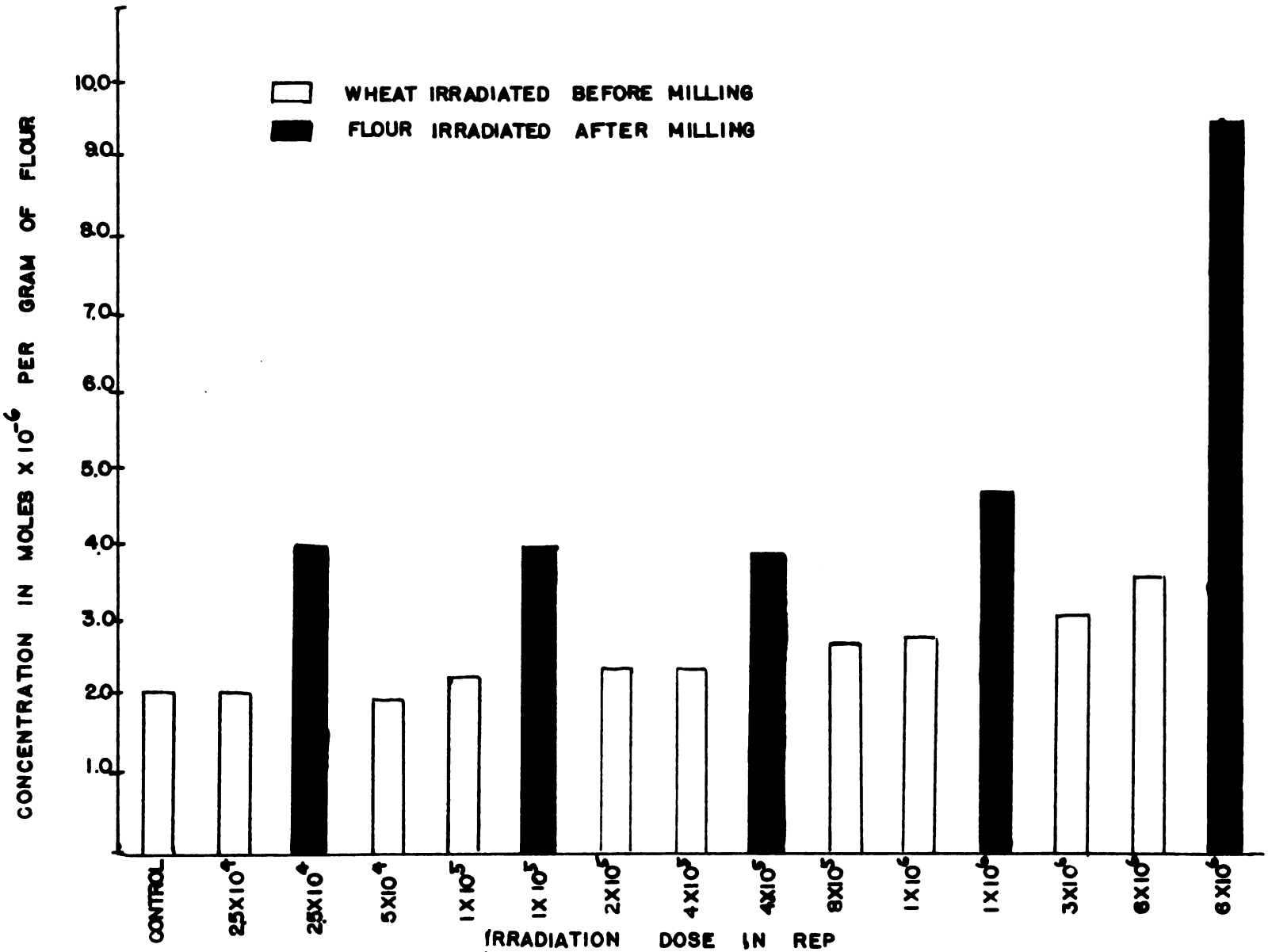


FIGURE 4: MEAN VALUES FOR CARBONYL EXTRACTED BY BENZENE FROM HIGH PROTEIN WHEAT

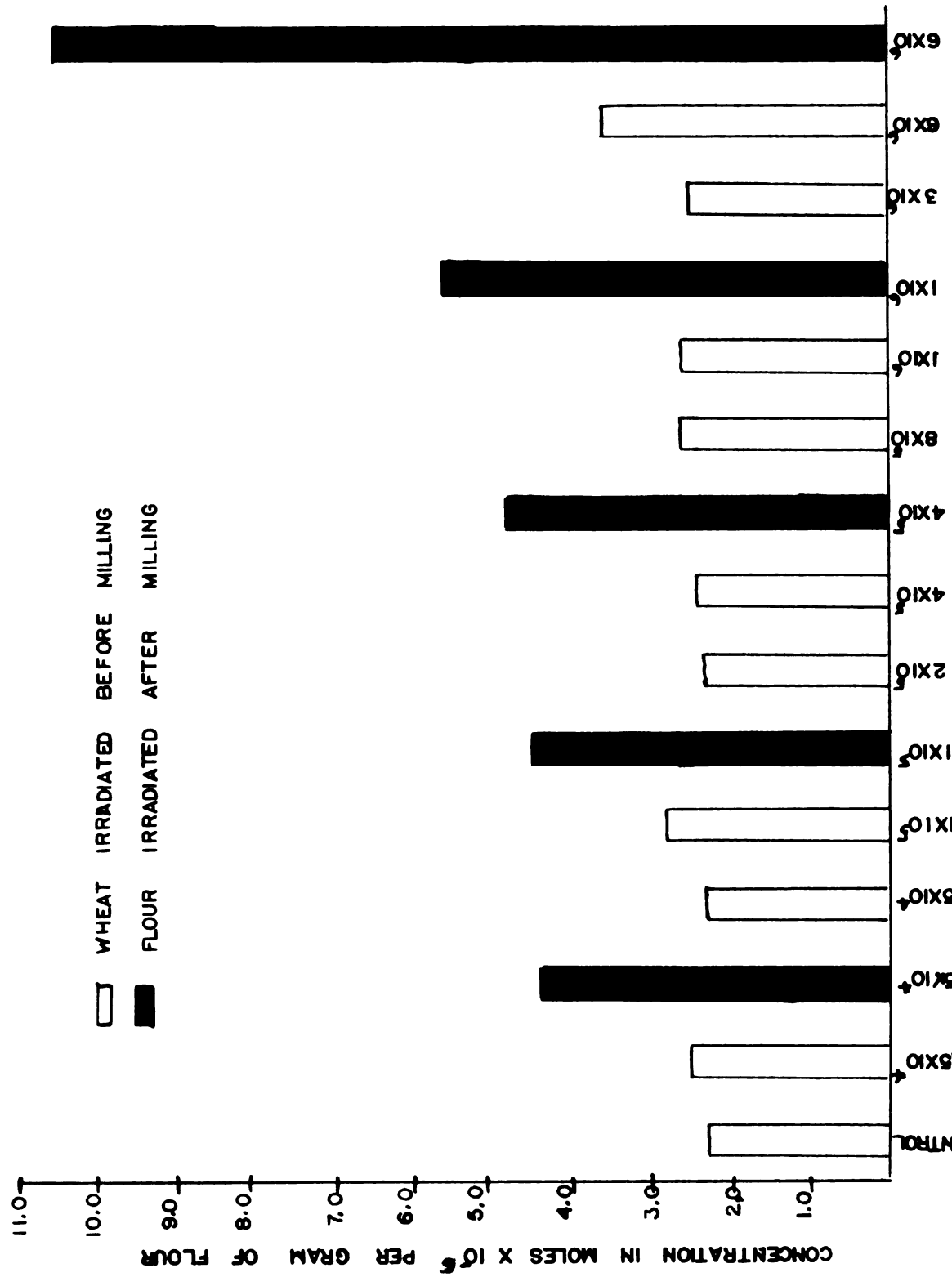


FIGURE 5: MEAN VALUES FOR CARBONYL EXTRACTED BY BENZENE FROM LOW PROTEIN WHEAT

who has studied short and flow cytometry. Further data [illegible] present in very small [illegible] are to be very important in this study [illegible] which flow cytometers in stores. [illegible], the effect of irradiation on [illegible] flow could be very important.

The higher carbonyl values obtained from the irradiated flour as compared to the unirradiated control, may be due to the fact that the wheat kernel is denser than flour. It has been suggested that the density of a material is a factor in the production of electrostatic [illegible]. Hence, future research might be another factor that caused the higher carbonyl values in irradiated flour. The relative content of the flour used in this experiment is known to be slightly [illegible] than the [illegible] used. The effect on carbonyl content may be brought about by the action of the free radicals by the radiation decomposition of the water. These free radicals may result in a complex series of secondary reactions as has been shown by many studies in radiation chemistry.

Since the method of determining carbonyls in this study was only a crude determination in that only total carbonyls were evaluated, a more specific determination such as gas chromatography, could reveal the derivatives of these carbonyl compounds.

Sulfhydryl Compounds. The mean values of sulfhydryl compounds representing high- and low-protein wheat irradiated before milling, and flour irradiated after milling are given in Table 5 and 6 and are presented graphically in Figures 6 and 7. Either the high- or the low-protein flour milled from irradiated wheat showed any significant differences from the control even at the highest level of irradiation. This result indicates that sulfhydryl groups in dough are not affected by high-voltage cathode ray ionizing radiation with dosage levels as high as 4×10^{7} r.

Table 5

Mean values of sulfhydryl compounds in wheat irradiated before milling

Irradiation dose in rep	Control	2.5×10^{4}	5×10^{4}	1×10^{5}	2×10^{5}	4×10^{5}	7×10^{5}	1×10^{6}	2×10^{6}	4×10^{6}
High protein	0.50*	0.56	0.55	0.54	0.54	0.54	0.54	0.52	0.53	0.51
Low protein	0.40	0.37	0.37	0.37	0.38	0.37	0.41	0.43	0.44	0.41

*Expressed as micromoles per gram of flour.

Table 6

Mean values of sulfhydryl compounds in flour irradiated after milling

Irradiation dose in rep	Control	2.5×10^{4}	1×10^{5}	4×10^{5}	1×10^{6}	4×10^{6}
High protein	0.51*	0.50	0.51	0.52	0.49	0.47
Low protein	0.32	0.32	0.33	0.34	0.33	0.31

*Expressed as micromoles per gram of flour.

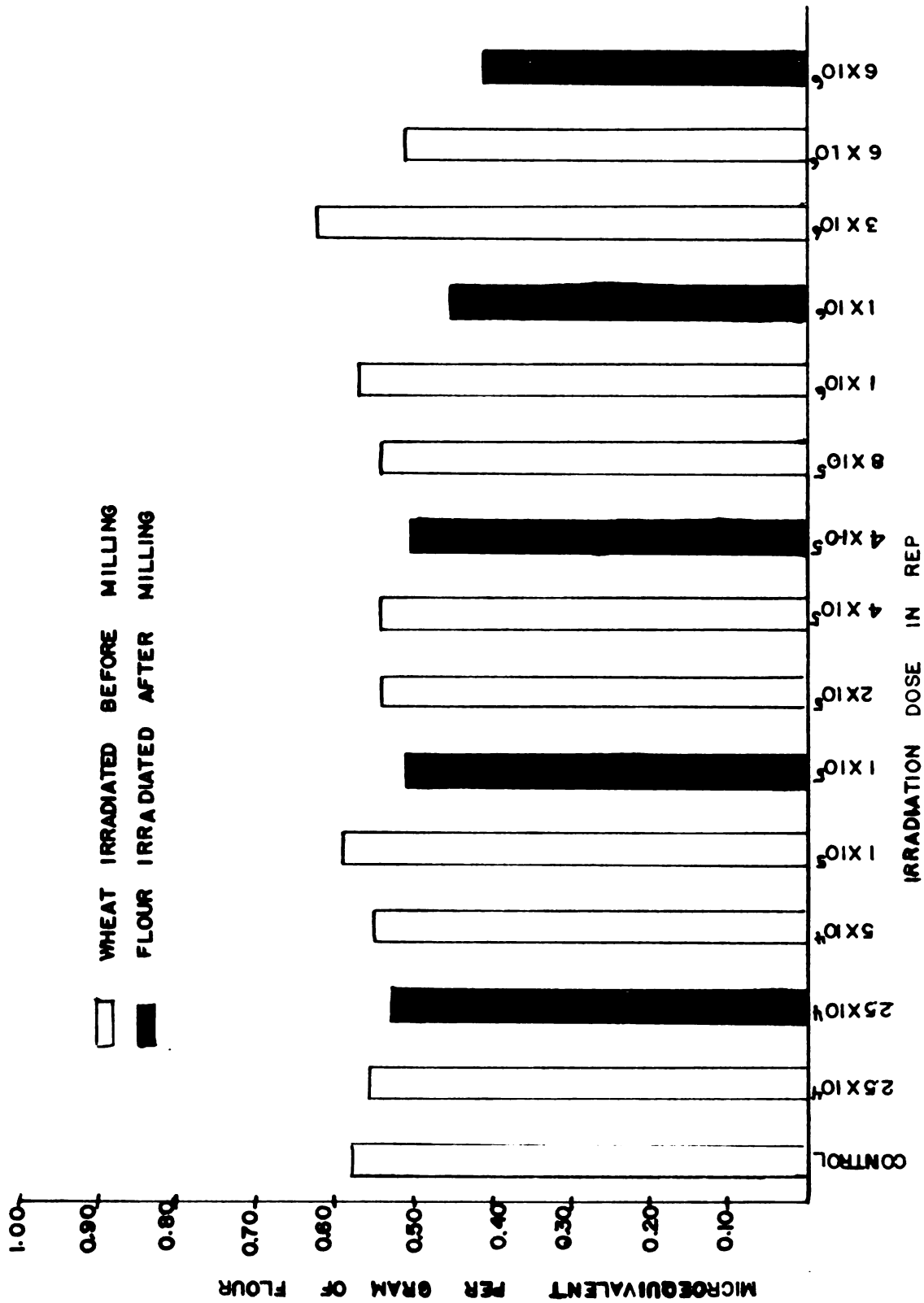


FIGURE 6: MEAN SULFHYDRYL VALUES OF HIGH PROTEIN WHEAT

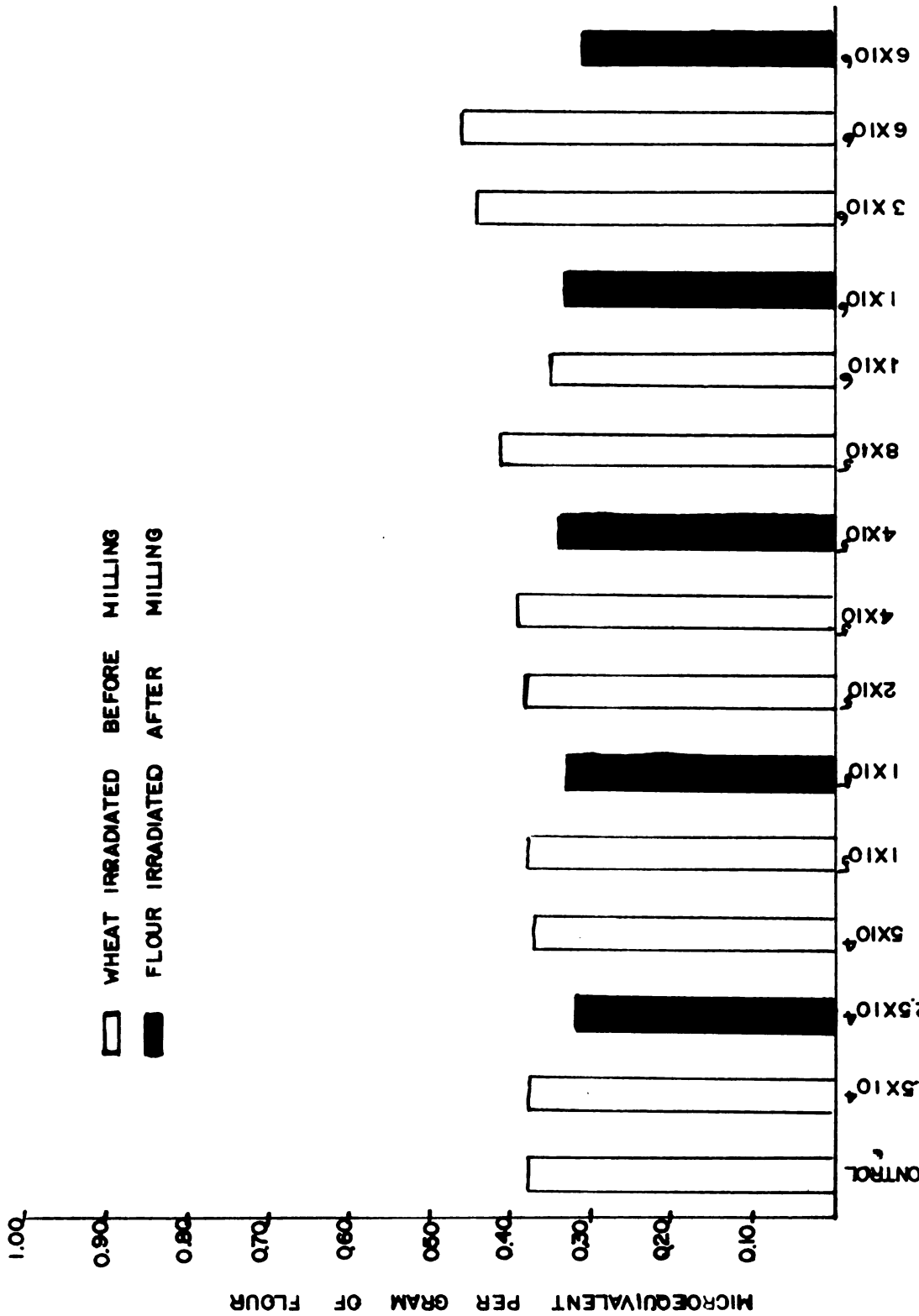


FIGURE 7: MEAN SULFHYDRYL VALUES OF LOW PROTEIN WHEAT

However, when the flour was irradiated after milling, a statistically significant decrease in sulfhydryl compounds occurred at the radiation level 6×10^6 rep. This again might be due to the fact that wheat kernel is denser than flour since it is known that penetration is a limiting factor in ionizing irradiation. The decrease in the sulfhydryl compounds in irradiated flour at 6×10^6 rep suggests that sulfhydryl groups may have been oxidized to form disulfide linkages (41). Also they could have been lost as volatile compounds such as hydrogen sulfide (7).

The results of this study suggest that the alterations in carbonyl and sulfhydryl compounds may account for some of the off-flavors and odors produced in baked products made from irradiated flour. It is apparent that the two chemical components have a different concentration in irradiated flour than in flour milled from irradiated wheat. These findings might account for the lack of agreement among various workers as to the dosage level at which variation in flavor and altered quality of baked products are detectable. It is apparent from this study that both the variety of wheat and the time of irradiation (before or after milling) are factors in determining the extent to which a certain dose of radiation will affect at least two of the chemical constituents. Therefore, studies on wheat before milling would be preferable in the future since it is doubtful that it would ever be practicable to irradiate flour.

Discussion

High-protein spring wheat and low-protein soft red wheat were irradiated with cobalt-60 rays at dosages ranging from 0.5 x 10^5 to 6 x 10^5 r. Non-irradiated samples served as controls. Another experiment will be used for milling the wheat into flour of approximately 72 per cent extraction. Quantitative determinations of protein, carbonyl and sulfhydryl components were made on each sample of flour. Samples of control flour were also irradiated to see if irradiation after milling produced different effects on the quantities of protein, carbonyl and sulfhydryl components in flour than irradiation before milling.

The results indicate that total protein in wheat or flour is not changed by cobalt-60 ionizing radiation with dosages as high as 6 x 10^5 r. The average protein content of high-protein wheat was 14.2 per cent and that of low-protein wheat was 8.1 per cent.

Both aqueous- and benzene-extracted carbonyl components increased with increasing irradiation except in the low-protein wheat where a decrease in aqueous-extracted carbonyls was observed at 0.5 x 10^5 r followed by a gradual increase. Some of these carbonyls obtained by aqueous extraction may have been derived from protein by the radiolytic cleavage of the peptide bonds or perhaps by oxidative deamination of any free amino acids present in the flour by irradiation. Also carbohydrate is also a major constituent of flour some of these may have been derived from it. The carbonyl components obtained by benzene extraction may indicate that fats in wheat are

affected by ionizing radiation. In general, a higher carbonyl value was obtained from the flour irradiated after milling as compared to the wheat irradiated before milling.

Sulfhydryl compounds of flour milled from irradiated wheat of the two varieties of wheat showed no significant differences from the control even at the highest level of irradiation. However, when the flour was irradiated after milling, a statistically significant decrease occurred at the radiation level 6×10^6 rep. The decrease might suggest that probably sulfhydryl groups may have been oxidized to form disulfide linkages. Or they could be lost as volatile compounds such as hydrogen sulfide.

The results might suggest that the different concentration of carbonyls and sulfhydryls observed in this study between the irradiated flour and flour milled from irradiated wheat, may be related to the off-flavors and odors produced in baked products made from irradiated flour. They also might explain the conflicting reports concerning the level of irradiation where off-flavors and altered quality of baked products are detectable.

It has been found that insect infestation can be controlled at about 2.5×10^4 rad. The two chemical compounds determined in the present study were found not affected at this level in wheat irradiated before milling. However, a significant difference was noted in the benzene-extracted carbonyls at 2.5×10^4 rep in the flour irradiated after milling. Therefore it would be desirable to apply irradiation to wheat before milling.

1. Allen, A. O. Radiation chemistry of aqueous solutions. J. Phys. and Colloid. Chem., 52, 479 (1948).

2. Allen, A. O. The yields of free H and OH in the irradiation of water. Rad. Res., 1, 85 (1954).

3. Olsen, C. W. The effects of high voltage cathode ray ionizing radiation on some of the physical and chemical properties of wheat flour protein. Unpublished M. S. thesis, 1952. Michigan State University.

4. Price, B. J., Daniel, I. S. and Henni, A. Damage to proteins and amino acids by ionizing radiation. Food Technol., 10(4), 187 (1956). Contract No. 19-, Mass. Institute of Technology.

5. Proctor, B. E. Physicochemical effects produced by the irradiation of crystalline egg albumin solutions with alpha particles. J. Biol. Chem., 11, 49 (1945).

6. Proctor, B. E. Effects produced by the irradiation of proteins and amino acids. Physiol. Rev., 17, 671 (1956).

7. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis, p. 405 (1955).

8. Störmer, F., Gordon, S., Proctor, B. and Weber, A. Effects of high intensity electron beams upon various vegetable and fish oils. Food Res., 17, 399 (1952).

9. Warren, J. J. S. and Weiss, J. Studies on the mechanism of action of ionizing radiations. II. Inhibition of sulfhydryl enzymes by alpha, beta and gamma rays. J. Gen. Physiol., 33, 515 (1950).

10. Warren, J. J. S. and Finkelstein. Studies on the mechanism of action of ionizing radiations. V. Effect of X-rays on some physicochemical properties of proteins. Arch. Biochem. Biophys., 41, 212 (1953).

11. Watzer, C. S., Rooney, C., Doty, D. M. and Quackenbush, F. W. Irradiation effects in meat: Formation of carbonyl compounds during irradiation of meat and meat fats. J. Agr. Food Chem., 5, 290 (1957).

12. Watson, J. S., Weber, C., Stein, J. M., Farrell, F. W. and Watson, J. M. Effect of gamma irradiation on cane sugar at high and low moisture levels. Food Technol., 1, 183 (1947).

13. Bellamy, W. D. and Paxton, H. J. Radiation sterilization. VII. Progress in high-voltage electrons for sterilization. Nucleonics, 14, (4), 98 (1956).

14. Breese, K. D., Lamy, H. A. and Tenesco, J. The sulfhydryl groups of crystalline proteins. J. Biol. Chem., 216, 613 (1955).

15. Butts, W. C. and Proctor, B. E. Effects of high-voltage cathode rays on aqueous solutions of histidine and hydroxylamine. Biochem. J., 63, 655 (1956).

16. Doepke, W. Hydrogen peroxide formation in water exposed to ionizing radiations. Brit. J. Rad., 29, 442 (1956).

17. Fronsell, B. W., Harlin, M. A. and Telandis, J. V. A preliminary study of the effect of gamma radiation on the baking quality of flours. Food Technol., 9, 601 (1955).

18. Farrell, W. J., Mitchell, P. D. and Fallaman, B. D. Polymerization of serum albumin by X-rays. Arch. Biochem. Biophys., 57, 232 (1955).

19. Filbert, J. A., Privett, O. S., Ivano, B. S., Nickell, C. D. and Lundberg, W. O. Effect of ionizing radiations on fatty acid esters. Industr. Engng. Chem., 47, 1113 (1955).

20. Hinton, R. H. A review of the evidence for the production of free radicals in water consequent on the absorption of ionizing radiations. Brit. J. Rad., 28, 321 (1955).

21. Hale, C. F. and Davies, J. V. Relationship between structure of nitrogenous compounds and their sensitivity to X-rays. Nature, 181, 44 (1958).

22. Hale, C. F. and Davies, J. V. The liberation of hydrogen sulfide by X-radiation from cysteine and glutathione. Biochem. J., 62, 159 (1956).

23. Hale, C. F., Meredith, W. J. and Weedon, B. C. L. Mode of action of ionizing radiations on aqueous solutions. Nature, 171, 640 (1953).

24. Hayburst, R. J., Stein, G. and Weiss, J. A theoretical survey of the radiation chemistry of water and aqueous solutions. Trans. Faraday Soc., 1, 42 (1954).

25. Discussions of the Faraday Society, Radiation Chemistry, No. 12, (1952).

26. Doty, P. M. and Mecter, W. H. Influence of gamma radiation on proteolytic enzyme activity of beef muscle. J. Agr. Food Chem., 2, 61 (1955).

27. Doty, P. M. and Mecter, W. H. Nature of antibacterial colors formed by gamma irradiation of beef. J. Agr. Food Chem., 3, 64 (1955).

4. [illegible] and Wright, C. I. The viscosity of solutions from [illegible]. J. Soc. Chem. Ind., 41, [illegible] (1922).

5. [illegible], [illegible], [illegible], [illegible] and Levin, L. [illegible] of ionizing radiations on pectin. Food Res., 2, [illegible] (1937).

6. [illegible], W. A. and Herrington, B. L. Irradiation ion effects of radiation. I. Effects of X-rays on fibrinogen as revealed by the ultracentrifuge and viscosity. Amer. Physiol. Chem., 21, 1[illegible] (1937).

7. Kolthoff, I. M. and Harris, W. E. Amperometric titration of [illegible] with silver nitrate - using the rotating platinum electrode. Ind. and Eng. Chem., Anal. Ed., 11, 1[illegible] (1939).

8. [illegible], L. A. Arnold, L. W. and Oberwalle, A. W. In vitro lability of [illegible] to ionizing radiations. J. Bact. and Ser., 14c, 25 (1927).

9. Lea, A. G., Ivory, L. W. and Milner, A. Treatment of wheat with ionizing radiations. II. Qualitative, physical and microbiological changes. Cereal Chem., 24, 5[illegible] (1947).

10. Lea, C. H. and Parker, H. W. The effects of ionizing radiations on lipins. I. The conjugated system in vegetable oils. J. Soc. Chem. Ind. (London), 22, [illegible] (1943).

11. Levin, L. A. and Clark, L. C. A colorimetric method for the determination of carbonyl compounds. Anal. Chem., 23, 541 (1951).

12. Loeton, A. J., Bellamy, A. J., Lamb, F. J., Bryant, W. J. and [illegible], A. The effects of high velocity of electrons on wood. Science, 113, 30 (1951).

13. Lea, C., Smith, F. A., Nelson, D. and Tomkins, R. Direct and indirect actions of radiation on viruses and enzymes. Parasitology, 36, 119 (1944).

14. Lee, C. C. The baking quality and maltose values of flour irradiated with [illegible] rays. Cereal Chem., 16, 73 (1939).

15. Levin, L. Irradiation sterilization. IX. Radiobiological safety in cathode ray sterilization. Nucleonics, 12, (4) [illegible] (1954).

16. Litton, G. F., Garr, W. F. and Claus, J. F. Protection of sulfhydryl groups against ionizing radiation. Science, 114, [illegible] (1951).

17. Lloyd, W. J., Milner, A. and Kinney, L. F. Treatment of wheat with ionizing radiation. I. Some effects of X-rays on gluten and gluten soils. Cereal Chem., 24, 55 (1947).

18. Lowe, J. F. and Moore, C. J. Action of cathode rays on drying oils. Industr. Engng. Chem., 15, 701 (1923).

59. [illegible], T. F. and [illegible], T. T. "Sulfides released from gamma-irradiated meat as estimated by condensation with N-methyl-p-phenylenediamine. J. Agr. Food Chem., 4, 891 (1956).

60. [illegible], J. F., [illegible], T. S. and [illegible], T. F. The effect of ionizing radiation on amino acids. I. The effect of X-rays on aqueous solutions of glycine. Rad. Res., 1, 57[illegible] (1954).

61. [illegible], J. L., Jr., and [illegible], T. T. Low temperature sterilization of organic tissue by high-voltage cathode-ray irradiation. Science, 114, 597 (1951).

62. Milner, M. and Yen, Y. C. Treatment of wheat with ionizing radiations. III. The effect on breadmaking and related properties. Cereal Technol., 10, 57[illegible] (1956).

63. Milner, M. Some effects of gamma-irradiation on the biochemical, storage and breadmaking properties of wheat. Cereal Sci. Today, 2, 10[illegible] (1957).

64. Moody, C. J. and Phillips, G. O. Action of ionizing radiations on solid carbohydrates. Chem. and Ind., p. 1067 (1956).

65. Mukherjee, T. Studies on rancidity of butterfat. VI. The action of light. J. Ind. Chem. Soc., 32, 5[illegible] (1955).

66. Naik-Kurudi, A. B., Lavington, C. A., Francis, F. J. and Peterson. Effects of cathode ray and gamma ray irradiation on some organic acid-carbohydrate systems. Food Research, 23, 611 (1958).

67. Nichols, L. C., Weirko, T. T., Jones, T. T., Hunt, L. T., Pflug, I. J. and Jones, T. A. Radiation-induced changes in bread flavor. Food Technol., 12, 32 (1958).

68. Phillips, G. O. Action of ionizing radiation on aqueous solutions of carbohydrates. Nature, 173, 1004 (1954).

69. Phillips, G. O., Moody, C. J. and Tuttle, E. L. Radiation chemistry of carbohydrates. Part I. Action of ionizing radiation on aqueous solutions of D-glucose. J. Chem. Soc., p. 3524 (1956).

70. Phillips, G. O. and Moody, C. J. Radiation chemistry of carbohydrates. Part II. Irradiation of aqueous solutions of dextran with gamma-radiation. J. Chem. Soc., p. 3904 (1958).

71. Price, T. T., Dellacy, T. T. and Lawton, T. J. Effect of high velocity electrons on dry dextran. J. Phys. Chem., 58, 921 (1954).

72. Proctor, B. E. and Goldblith, S. A. The effect of submicrowave cathode rays on the nonenzymatic browning reaction of dried fruits and on chemical compounds pertaining thereto. Science, 104, 527 (1946).

73. Proctor, B. E. and Goldin, A. R. Effect of high-voltage cathode rays on amino acids in fish muscle. Food Technol., 4, 351 (1950).

74. Proctor, B. E. and Goldblith, S. A. Food processing with ionizing radiation. Food Technol., 5, 376 (1951).

75. Proctor, B. E. and Goldin, A. R. Effects of high-voltage cathode rays on aqueous solutions of tryptophan, tyrosine, phenylalanine and cystine. Biochem. J., 51, 495 (1952).

76. Proctor, B. E. and Goldin, A. R. Loss of action of high-voltage cathode rays on aqueous solutions of amino acids. Biochem. J., 52, 1 (1952).

77. Proctor, B. E. and Goldblith, S. A. Radiation sterilization. VI. Relative merits of cathode ray and gamma irradiations. Nucleonics, 12 (5), 32 (1954).

78. Pickett, F. S. and Rose, F. T. The effect of gamma rays upon dextran. Chem. and Ind., p. 1174 (1954).

79. Robinson, R. E. Some fundamentals of radiation sterilization. Food Technol., 8, 191 (1954).

80. Rotherham, A., Ford, A. and Fletcher, J. L. Decomposition of cystine by irradiation of its aqueous solutions. Naturwissenschaften, 22, 494 (1952).

81. Saeman, J. F., Merrill, E. C. and Lawton, E. J. Effect of high-energy cathode rays on cellulose. Industr. Engng. Chem., 44, 2424 (1952).

82. Saniear, E. E., Projot, L. C. and Brocker, L. C. I. The effect of ultraviolet radiation and of soft x-rays on the sedimentation behavior and light absorption of purified human serum albumin. Biochem. J., 31, 1 (1937).

83. Sowul, A. F., Cochran, E. F. and Conca, J. A. Further studies on the determination of sulfhydryl groups in wheat flours. Cereal Chem., 34, 127 (1957).

84. Tribby, I., Davis, B. J. and Chenoweth, W. A. Radiation sterilization: Effect of irradiation on meat fats. J. Agr. Food Chem., 3, 953 (1955).

85. Tedrow, F. F. and Conrad, E. E. The effects of radiations from a quartz mercury vapor arc upon the reaction of proteins. U. S. J. Physiol., 27, 1 (1910).

86. Stein, G. and Weiss, J. Destruction of amino acids by γ-rays. Nature, 142, 1141 (1938).

87. Stein, G. and Weiss, J. Chemical actions of ionizing radiations on aqueous solution. Part III. The action of γ-rays on some amino acids. J. Chem. Soc., p. 3245 (1949).

88. Sumstrom, J. and Schwann, H. Effect of roentgen radiation on solutions of tyrosine and cystine. J. Biol. Chem., 74, 655 (1927).

89. Svedberg, T. The ultra-centrifuge and the study of high-molecular compounds. Nature, 133, 1051 (1934).

90. Svedberg, T. and Pedersen, K. Splitting of the hemocyanin molecule by ultra-violet light. Nature, 142, 1130 (1938).

91. Svedberg, T. and Pedersen, K. Splitting of protein molecules by ultra-violet light and alpha rays. Nature, 142, 998 (1938).

92. Swallow, A. J. The action of gamma radiation on aqueous solutions of cysteine solutions. J. Chem. Soc., p. 1994 (1952).

93. Swanson, C. O. Wheat and Flour Quality Burgess Publishing Co., Minneapolis, Minn., p. 20 (1938).

94. Trump, J. G. and Van de Graaf, R. J. Irradiation of biological materials by high energy roentgen rays and cathode rays. J. Appl. Phys., 19, 599 (1948).

95. Trump, J. G., Wright, L. W. and Clarke, F. H. Distribution of ionization in materials irradiated by two and three million-volt cathode rays. J. Appl. Phys., 21, 345 (1950).

96. Tuve, R. Possible sources of radiation for the irradiation of foodstuffs from symposium on "Preservation of food with ionizing radiations." Chem. and Eng., p. 330 (1950).

97. Whitcher, C. L., Rothrock, W. and Todd, L. Radiation chemistry of cysteine solutions. Nucleonics, 11 (9), 37 (1953).

98. Whitting, L. A. and Schweigert, B. S. A characterization of volatile carbonyl compounds isolated from beef fat subjected to gamma-radiation. J. Am. Oil Chemists' Soc., 35, 413 (1958).

99. Wilson, C. L., Hinkley, C. C., Wesley, C. C., Thom, M. T. and Nicholas, J. V. The effect of ionizing radiations on carbohydrates. Rad. Res., 10, 37 (1959).

100. Yeo, W. C., Silver, M. and Lee, C. T. Treatment of wheat with ionizing radiations. II. Effect on respiration and other indices of storage deterioration. Food Technol., 15, 411 (1961).

101. Zhukov, I. T. and Ushpuskaya, V. A. Influence of radon on the viscosity of gelatin solutions. J. Russ. Phys. Chem. Soc., 62, 511 (1930) (as abstracted in Chem. Abstr., 24, 4410 (1930)).

Table 1

Per cent [illegible] kill yield for the various varieties of wheat

Irradiation dose in rep	High protein	Low protein
Control	53.6	46.20
3.5×10^4	53.20	[illegible]
5×10^4	[illegible]	51.00
1×10^5	[illegible]	[illegible]
2×10^5	53.60	[illegible]
4×10^5	[illegible]	[illegible]
[illegible] $\times 10^5$	55.00	52.20
1×10^6	[illegible]	[illegible]
3×10^6	[illegible]	[illegible]
6×10^6	[illegible]	56.40

Table 2

Per cent of protein for the various samples of wheat and flour

Irradiation dose in rep	Wheat		Flour	
	High protein	Low protein	High protein	Low protein
Control	14.26	8.45		
2.5×10^4	14.11	7.85	13.94	8.26
5×10^4	14.12	7.87		
1×10^5	13.98	8.01	14.12	8.26
2×10^5	14.13	7.97		
4×10^5	13.94	8.03	14.28	8.31
8×10^5	14.13	7.96		
1×10^6	14.31	7.97	14.04	7.96
3×10^6	14.26	7.97		
6×10^6	14.09	8.04	13.94	8.31

Table 3

Carbonyl values of high protein flour milled from irradiated wheat extracted by acid-salt

No. of determination	Irradiation dose in roentgens									
	Control	2.5x10^{1}	5x10^{1}	1x10^{2}	2x10^{2}	4x10^{2}	7x10^{2}	1x10^{3}	3 x [illegible]	6x10^{3}
1	2.31*	2.47	2.84	2.96	3.24	4.10	4.72	5.16	[illegible]	15.16
2	2.13	2.36	2.36	2.46	3.12	3.41	4.30	4.34	9.43	15.74
3	1.93	1.78	1.96	2.08	2.70	3.15	4.07	4.45	9.39	13.99
4	1.12	1.54	1.46	1.70	2.39	2.96	3.12	3.46	6.34	13.44
5	1.79	2.04	1.92	2.34	2.74	3.91	4.04	4.80	9.17	15.97
6	1.64	1.13	1.94	2.04	2.70	3.25	3.80	4.36	8.50	14.26
Mean	1.90	1.89	2.09	2.26	2.74	3.49	3.95	4.40	8.47	14.75

* Moles x10^{-5} per gram of flour.

Table 4

Carbonyl values of low protein flour milled from irradiated wheat extracted by acid-salt

No. of determination	Irradiation dose in rep									
	Control	2.5x10⁴	5x10⁴	1x10⁵	2x10⁵	4x10⁵	7x10⁵	1x10⁶	3x10⁶	6x10⁶
1	2.9 *	1.9	1.92	1.96	2.51	2.[illegible]	3.66	4.70	8.78	10.[illegible]
2	2.[illegible]4	1.9[illegible]	1.[illegible]	1.73	2.[illegible]0	2.63	3.[illegible]4	4.36	7.41	11.[illegible]
3	[illegible].3[illegible]	1.1[illegible]	1.10	1.5[illegible]	[illegible].2[illegible]	[illegible].7[illegible]	3.[illegible]2	4.13	7.[illegible]4	12.97
4	3.31	1.[illegible]6	1.[illegible]	2.[illegible]9	3.10	3.6[illegible]	4.7[illegible]	[illegible].[illegible]	11.[illegible]4	17.1[illegible]
5	3.24	2.31	2.[illegible]	2.35	3.62	3.[illegible]6	5.35	5.72	1[illegible].1[illegible]	1[illegible].[illegible]4
6	[illegible].5[illegible]	1.[illegible]9	2.[illegible]4	2.91	3.44	3.3[illegible]	4.[illegible]8	[illegible].[illegible]6	[illegible].[illegible]0	[illegible]4.[illegible]
Mean	[illegible].[illegible]4	1.6[illegible]	1.7[illegible]	2.02	2.[illegible]8	3.[illegible]3	[illegible].1[illegible]	5.1[illegible]	[illegible].[illegible]8	2.[illegible]2

* [illegible] x10⁻⁴ mol./gm. of flour.

Table 5

Carbonyl values of high protein flour irradiated after milling; extracted by acid-salt

No. of determination	Irradiation dose in rep					
	Control	2.5×10^4	1×10^5	4×10^5	1×10^6	6×10^6
1	2.10*	2.22	1.93	2.92	5.2*	3*.06
2	2.02	1.73	1.*1	2.42	4.33	2*.**
3	1.**	2.29	2.46	2.92	4.79	25.**
Mean	2.06	2.0*	2.10	2.75	4.*5	**.0*

* Moles $x10^{-3}$ per gram of flour.

Table 6

Carbonyl values of low protein flour irradiated after milling extracted by acid-salt

No. of determination	Irradiation dose in rep					
	Control	2.5×10^{4}	1×10^{5}	5×10^{5}	1×10^{6}	4×10^{6}
1	3.97*	3.52	4.10	5.96	[illegible]	45.52
2	3.75	2.99	3.33	4.04	5.71	[illegible]
3	3.84	3.28	3.49	4.56	[illegible]	[illegible]
Mean	3.85	3.33	3.64	4.85	7.13	[illegible]

* Moles $\times 10^{-5}$ per gram of flour.

Table 7

Carbonyl values of high protein flour milled from irradiated wheat extracted by benzene

No. of Deter-mination	Irradiation dose in rep									
	Control	2.5×10^{4}	5×10^{4}	1×10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	1×10^{6}	3×10^{6}	6×10^{6}
1	2.36*	2.31	2.40	2.50	2.46	2.57	2.91	2.99	3.13	3.79
2	2.02	2.08	1.94	2.16	2.21	2.26	2.71	2.93	3.21	3.36
3	1.94	1.98	1.72	2.12	2.25	2.15	2.32	2.39	2.74	3.31
Mean	2.10	2.11	2.02	2.26	2.40	2.37	2.66	2.78	3.03	3.55

* oles $x10^{-5}$ per gram of flour.

Table 6

Carbonyl values of low protein flour milled from irradiated wheat extracted by benzene

No. of determination	Irradiation dose in rep									
	Control	2.5×10^{4}	5×10^{4}	1×10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	1×10^{6}	3×10^{6}	6×10^{6}
1	2.94*	4.29	3.73	3.31	3.13	3.92	2.98	2.04	2.41	4.53
2	1.53	1.42	1.61	1.[illegible]8	1.[illegible]3	2.04	1.84	2.15	1.92	2.65
3	3.06	3.24	2.95	3.12	2.5[illegible]	2.5[illegible]	2.0[illegible]	1.56	3.32	2.76
4	2.26	1.[illegible]	2.3[illegible]	3.7[illegible]	2.96	3.47	4.31	4.[illegible]	4.8[illegible]	6.2[illegible]
5	2.[illegible]0	1.[illegible]1	2.[illegible]0	1.[illegible]2	1.7[illegible]	2.[illegible]3	1.[illegible]5	1.[illegible]2	1.7[illegible]	2.[illegible]7
Mean	2.37	2.55	2.32	2.77	2.2[illegible]	2.[illegible]4	2.64	2.[illegible]3	2.50	3.64

* Moles $\times10^{-6}$ per gram of flour.

Table 9

Carbonyl values of high protein flour irradiated after milling extracted by benzene

No. of determination	Irradiation dose in rep					
	Control	2.5×10^{4}	1×10^{5}	4×10^{5}	1×10^{6}	6×10^{6}
1	1.42*	3.74	2.91	3.42	4.49	8.39
2	1.69	3.43	3.26	3.43	4.59	9.21
3	1.52	4.62	4.72	4.66	5.76	11.46
4	3.51	4.06	4.91	4.07	4.12	9.47
mean	2.03	3.96	3.95	3.91	4.74	9.53

* moles $\times10^{-6}$ per gram of flour

Table 10

Carbonyl values of low protein flour irradiated after milling, extracted by benzene

No. of determination	Irradiation dose in rep					
	Control	2.5×10^{4}	1×10^{5}	4×10^{5}	1×10^{6}	6×10^{6}
1	1.56*	4.42	4.60	4.94	6.04	10.14
2	1.60	5.24	5.06	5.52	6.11	11.92
3	1.71	4.44	4.86	4.73	5.26	11.47
4	3.73	3.78	3.56	3.92	4.53	10.52
Mean	2.14	4.47	4.54	4.78	5.54	11.02

* olds $x10^{-6}$ per gram of flour.

Table 11

Sulfhydryl values of high protein flour milled from irradiated wheat

No of determination	Irradiation dose in rep									
	Control	2.5×10^{6}	5×10^{6}	1×10^{7}	2×10^{7}	4×10^{7}	6×10^{7}	1×10^{8}	3×10^{8}	6×10^{8}
1	0.60*	0.55	0.52	0.56	0.52	0.55	0.53	0.55	0.60	0.52
2	0.56	0.55	0.56	0.60	0.49	0.52	0.51	0.59	0.64	0.51
3	0.54	0.59	0.51	0.60	0.60	0.54	0.57	0.57	0.64	0.51
Mean	0.57	0.56	0.55	0.59	0.54	0.54	0.54	0.57	0.63	0.51

* Microequivalent per gram of flour.

Table 12

Sulfhydryl values of low protein flour
milled from irradiated wheat

No. of determination	Irradiation dose in r									
	Control	2.5×10^{3}	5×10^{4}	1×10^{5}	2×10^{5}	4×10^{5}	8×10^{5}	1×10^{6}	3×10^{6}	6×10^{6}
1	0.39*	0.36	0.32	0.3[illegible]	0.38	0.41	0.40	0.36	0.44	0.43
2	0.41	0.39	0.40	0.3[illegible]	0.39	0.39	0.41	0.35	0.40	0.40
3	0.40	0.40	0.3[illegible]	0.3[illegible]	0.39	0.39	0.43	0.39	0.46	0.46
Mean	0.40	0.38	0.37	0.38	0.39	0.39	0.41	0.39	0.44	0.43

* Microequivalent per gram of flour.

Table 13

Sulfhydryl values of high protein flour irradiated after milling

No. of determination	Irradiation dose in rep					
	Control	2.5×10^4	1×10^5	4×10^5	1×10^6	6×10^6
1	0.56*	0.52	0.52	0.49	0.46	0.3[illegible]
2	0.59	0.53	0.53	0.51	0.46	0.43
3	0.60	0.53	0.49	0.49	0.43	0.[illegible]
Mean	0.58	0.53	0.51	0.50	0.45	0.42

* Across equivalent per gram of flour.

Table 14

Sulfhydryl values of low protein flour irradiated after milling

No. of determination	Irradiation dose in rep					
	Control	2.5×10^4	1×10^5	4×10^5	1×10^6	6×10^6
1	0.39*	0.33	0.33	0.33	0.34	0.31
2	0.36	0.33	0.33	0.33	0.33	0.29
3	0.36	0.31	0.33	0.35	0.33	0.33
Mean	0.37	0.32	0.33	0.34	0.33	0.31

* ieroequivalent per gram of flour.

Table 15a

Analysis of variance: F values

Carbonyl compounds

		Irradiation	Fumigation
High protein	Acid-salt extraction - Irrad. wheat	771.21**	18.46**
	Acid-salt extraction - Irrad. flour	534.82**	0.51
	Benzene extraction - Irrad. wheat	56.63**	50.51**
	Benzene extraction - Irrad. flour	46.72**	3.87*
Low protein	Acid-salt extraction - Irrad. wheat	171.87**	5.65*
	Acid-salt extraction - Irrad. flour	520.63**	0.3
	Benzene extraction - Irrad. wheat	1.56	11.05**
	Benzene extraction - Irrad. flour	65.69**	1.41

Table 15b

Sulfhydryl compounds

		Fumigation
High protein	Irrad. wheat	4.00*
	Irrad. flour	33.01**
Low protein	Irrad. wheat	6.20**
	Irrad. flour	7.06**

*Indicates statistical significance at the 5 per cent level.
**Indicates statistical significance at the 1 per cent level.

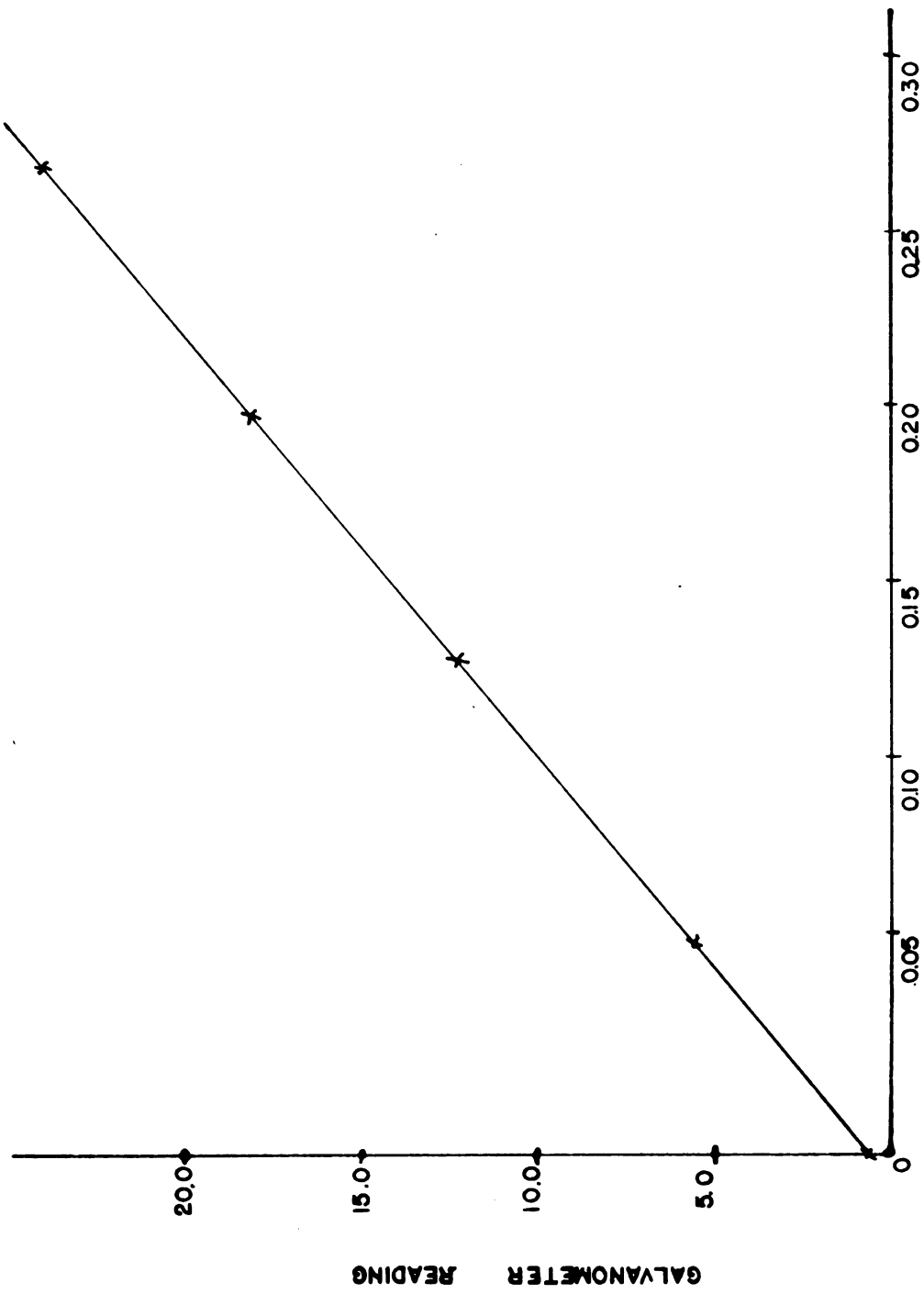


FIGURE 1: SILVER NITRATE CURVE FREE OF SULFHYDRYL COMPOUNDS