EFFECT OF LOW-DOSE GAMMA IRRADIATION ON BREADMAKING QUALITY OF WHEAT

by

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INTRODUCTION

Irradiation is a useful method of food preservation. Radioactive elements such as Co^{60} (formed during the operation of nuclear reactors) emit high energy gamma rays that penetrate food material during irradiation and act as preservation or disinfestation agents.

Irradiation is effective in prolonging the storage life of many perishable foods. It also delays ripening of fruits and inhibits the sprouting of vegetables. However, the effective use of irradiation is for disinfestation of cereal grains.

The production of cereals in the world is around 750 million tons per year, wheat contributes about 1/3 of the total. Before being consumed most wheat is stored; and during storage it is subject to damage by insects. Grain storage losses are especially heavy in tropical countries where insects breed and spread rapidly because of hot and damp climates. In India, 5% of the total wheat and wheat products are consumed by insects and about 25% become so heavily contaminated that they are unfit for human consumption (1).

Irradiation of wheat (20-25 Krads) controls the growth and development of all species of insects infesting wheat (2). Irradiation of wheat is more costly than fumigation, but is also more effective. Irradiated wheat has been shown to be nutritionally wholesome (3).

Most of the studies on irradiation of wheat deal with high doses of irradiation, much more than that required for disinfestation. There is a controversy amongst the reports whether the baking qualities of wheat after irradiation are improved or not, at low dose levels. It is difficult to draw any conclusion based on those reports since they have used different methods of baking and different dose levels (4-9).

The purpose of this project was to study the effect of low dose irradiation on rheological and baking properties of wheat, and to determine the fraction or fractions responsible for changes if any in those properties.

LITERATURE REVIEW

Historical Background

Radioactivity due to cosmic rays and naturally occurring radioactive substances has existed in the earth's crust and in the atmosphere since the beginning of time. However, its presence has been known to man for only the last 70 years. Shortly after radium was discovered, the deadly effects of its irradiation was harnessed by scientists to improve the health and welfare of mankind, for example in treating cancer.

Food preservation by irradiation was reported as early as 1909 (10). However, the first report of the Atomic Energy Commission in 1947 is considered a pioneer in this field of food preservation by irradiation.

The use of irradiation (20,000 to 50,000 rads) for disinfestation of wheat and wheat products was cleared by the FDA on 15 August, 1963 (1). The use of irradiation for food preservation has been promoted to utilize waste fission products formed in the uranium fuel elements of nuclear reactors.

Other types of irradiation such as sound waves, above the audible range (50,000 to 100,000 cycles per second) have been used to kill organisms, however, this was not feasible on a large scale. High frequency radiowaves, known as dielectric heating in nonconducting bodies, has also been reported as useful in inhibiting the growth of insects.

Recently many sources of irradiation have been developed, either by linear accelerator or from radioactive decay of various elements (11). The use of electron accelerators to control insect population, is limited because the method is effective only for thin layers of material or to irradiate just the surface of thick material.

Disinfestation of Wheat

Insects responsible for the losses in grains during storage include the following:

1) Rice weevil (*Sitophilus oryzae*)
2) Granary weevil (*Sitophilus granarius*)
3) Lesser grain borer (*Rhizopertha dominica*)
4) Angoumois grain moth (*Sitobroga cerealella*)
5) Flat grain beetle (*Laemophloeus pusillus*)
6) Saw-toothed grain beetle (*Oryzaephilus surinamensis*)
7) Yellow meal worm (*Tenebsio molitar*)
8) Bean weevil (*Acanthoscelides obtectus*)
9) Confused flour beetle (*Tribolium confusum*)
10) Indian meal moth (*Plodia interpunctella*)
11) Figmoth (*Cadra cautella*)
12) Khapra-beetle (*Trogoderma granarium*)

It is generally accepted that an irradiation exposure of 20-25 Krads controls the growth and development of all those species (2).

Powers (12) has discussed the effect of irradiation on those insects and has observed the following factors affected by irradiation: 1) lethality, 2) development, 3) reproduction, 4) longevity, 5) physiological phenomenan, and 6) genetic factors.

Another effective method to control the infestation in grains is fumigation. Fumigation is commonly used in many countries. Commonly used fumigants include, methyl bromide, ethylene dichloride, carbon tetrachloride and carbon disulfide.

There are many reports comparing irradiation and fumigation. Cornwell (13) has discussed the advantages and disadvantages of both the methods. Fumigation has some limitations. It kills insects immediately but some of the adult species (especially lesser grain borer, figmoth, and khapra-beetle) are somewhat resistant, and this can lead to reinfestation. Whereas irradiation causes reproductive sterilization of almost all insects. Fumigants may not adequately penetrate all the grains, on the other hand, irradiation penetration delivers a uniform rate and thus results in 100% control of the infestation. However, irradiated grain is subject to reinfestation from outside sources.

Fumigants may leave harmful residues, whereas at low dose levels irradiation is a safe and harmless method. In applying fumigation human error can be involved. Since irradiation is automated, such errors are minimized. However, use of irradiation on a commercial basis is still a problem. Initial installation of an irradiation source is costly, and this has held back the commercial use of irradiation.

Powers (12) has shown that use of linear accelerator for disinfestation costs about 37 cents per ton of grain. Cornwell (13) has estimated that Co^{60} costs range from about 12-36 cents/ton, the wide range taking into account the maximum output of irradiation and its utilization rate. An estimated cost of 4 cents/ton for fumigation is given by Nair and Brownell (1).

The FDA has approved 50 Krads dose of radiation of wheat and wheat products, however, the actual lethal dose is considered to be 18 Krads for disinfestation of wheat (1). Arvindakshan and Vakil (3) have shown that wheat or wheat products irradiated at 20 Krads or ten times higher dose (200 Krads) is nutritionally wholesome. They conducted long term feeding experiments on rats (up to 4 generations).

Effects of Irradiation of Wheat Proteins

Wheat grain contains from 8-15% protein (14). The protein content of flour is generally about 1% less than that of the wheat from which it was milled. About 80% of the flour protein is gluten. The unique properties of bread dough result basically from the properties of gluten. A dough is formed by mixing water and wheat flour, upon hydration and mixing gluten swells and forms a three dimensional network held together by hydrogen and disulfide bonds (15). The formation of the network, which retains the gas produced by yeast fermentation, forms the cell structure in bread (16). The quantity and quality of the gluten affects the characteristics of the resultant bread. Gluten contributes cohesiveness and elasticity to dough.

The properties of gluten are complex, it is composed of two protein fractions, glutenin and gliadin. Gliadins are relatively low molecular weight proteins (40,000), in comparison with the high molecular weight proteins (up to 3,000,000) of the glutenin fraction (17). Those two fractions also differ in structure and amino acid composition. Glutenin has a loosely folded structure held together by intermolecular disulfide bonds. In contrast, gliadin has tightly folded structure with intra-molecular disulfide bonds. The exact nature and function of those two fractions is not clear. Glutenin is thought to be responsible for the elasticity of dough. Gliadin is thought to be important in dough extensibility (18).

Doguchi (19) has worked extensively on the effect of gamma irradiation on wheat gluten. Most of his studies are with high doses of gamma irradiation, much above that needed for disinfestation of wheat. At a 10 Mrads level, irradiated gluten had lost all its properties of elasticity and extensibility. He found a significant decrease in cysteine content when gluten was irradiated with more than 1 Mrad (1000 Krads). Starch gel electrophoresis showed a loss of some bands at and above 6 Mrads dose level. Sephadex G-100 chromatography using aluminum-lactate, lactic acid buffer, pH 3.1, revealed that at 10 Mrads dose level the area under the curve of the second peak (denoted as gliadin) was lower than unirradiated or irradiated up to 3 Mrads dose level. Irradiation of gluten at high dose levels (at and above 6 Mrads), disrupted the proteins and this was attributed to random polymerization, possibly due to its effect on disulfide bonds. Doguchi suggested that irradiation at 10 Mrads dose level changed the molecular configuration of wheat gluten.

The sedimentation value of irradiated wheat (100-600 Krads) decreased linearly with the dose (20). Fifield et al (5) also reported decreases in sedimentation values of irradiated wheat. The total nitrogen content, total ash, and moisture content were not affected by irradiation.

The yield of washed gluten after irradiation was studied by Sosedov and Vakar (21). They reported that at 3 Mrads dose level, the percentage recovery of gluten was low. Irradiation of low moisture gluten had a direct effect on the proteins, apparently a disaggregation of protein at higher doses of irradiation. Whereas, at higher moisture levels the indirect effect of irradiation causes aggregation of proteins due to oxidation.

Effect of Irradiation on Wheat Lipids

Wheat contains about 2-4% total lipids (22). The lipids interact with other constituents in flour and modify the behavior of flour in processing or baking (23). The free lipids (which constitute about 60% of the total wheat lipids) are defined as those extractable by petroleum ether or similar nonpolar solvents. The remaining bound lipids (40% of total wheat lipids, which are mostly polar) can be extracted with more polar solvents such as water-saturated butanol.

When reconstituted with defatted flour, free polar lipids increase the loaf volume substantially (22). Nonpolar lipids either by themselves or with bound polar lipids in their native state have no effect on loaf volume of bread, baked with shortening (24).

Free polar lipids can be bound to the gliadin fraction of gluten by hydrophilic bonds and to glutenin by hydrophobic bonds (24). The complex of gluten and lipids, which may be responsible for the retention of the gas in a dough, is thought to be as gliadin-glutenin protein units bound together by polar lipids. The flour lipids may also influence crumb firmness and other crumb properties (25).

Lai et al (20) reported no significant changes in fatty acid content at 10^6 rep (essentially equal to a rad) dose levels. However, Tipples and Norris (26) have found that linoleic and linolenic acid content of wheat was decreased at 10^7 rads. Unesterified fatty acids were reported to be more susceptible to irradiation damage than esterified fatty acids. The peroxide value was slightly higher in irradiated than unirradiated wheat. Carotenoids and certain antioxidants were found to decrease with increasing doses of irradiation.

The same authors further fractionated the wheat lipids by silicic acid column chromatography and found a decrease in triglycerides, galactolipids, phospholipids and an increase in free sterols, mono and diglycerides at 10^7 rads dose level of irradiated wheat. Chung et al (27) showed that the ratio between polar and nonpolar lipid fractions in irradiated wheat decreased at the 10^6 rep dose level.

Effect of Irradiation on Flour Enzymes and Vitamins

There are many enzymes present in wheat flour, of particular interest are the amylases and proteolytic enzymes. There are two types of amylases, also known as diastatic enzymes, α and β (28). They act together on starch and convert it into maltose. Starch is not utilized by yeast to produce CO_2, however, maltose can be used by yeast. Thus, for optimum gas production and good loaf volume, maltose is necessary, if no other sources of fermentable sugar are in the formula.

Proteases break down proteins into peptides. Prouty (29) has postulated that they may be helpful in forming a good network of gluten.

Linko and Milner (30) found no difference in the enzymatic activity of dehydrogenases, amylases, proteases and decarboxylases in wheat irradiated at 1 Mrad. They also reported that the increase in maltose values of irradiated wheat was due to increased susceptibility of starch.

Ananthaswamy et al (31) reported similar results. They found even low doses (20-200 Krads) of irradiation increased the maltose value. However, α and β amylase activities were unaltered. The sensitivity of starch to amylolysis increased with increasing doses of irradiation. They observed that the starch from moist seeds was more susceptible to irradiation than dry seeds.

Kennedy (32) found no difference in vitamin content of wheat irradiated at 20 Krads dose level whereas at 200 Krads dose level niacin, pantothenic acid, biotin were about 10% less. Poisson et al (33) reported about 20-35% less thiamine in irradiated wheat at 500 Krads dose level.

Changes in Physical Properties Due to Irradiation of Wheat

The most common use of wheat is to make bread. Any physico-chemical change in wheat flour will affect its performance during baking.

Fifield et al (5) found that 175 Krads dose had no influence on milling properties of wheat; however, the sedimentation value decreased with increasing levels of irradiation. The ash and protein content were unchanged and water absorption capacity increased during storage of irradiated wheat.

Milner (34) reported that the amylograph peak and sedimentation value were lower for irradiated wheat. The dough development time and extensibility decreased due to irradiation. Milner et al (8) found the viability of seed was destroyed at 625 Krads dose level.

Lee (6) reported that at and above 100 Krads level the hydration capacity (absorption) and the dough development time decreased, and tolerance to mixing increased slightly. There also was an increase in reducing sugar content and decrease in sedimentation values. He also found that wheat irradiated at 250 Krads dose or greater, had higher maltose values, the gassing power increased, and the recovery of gluten was lowered.

Effect of Irradiation on Baking Qualities of Wheat

Using a lean, straight dough formula several workers (6, 20, 34) reported an increase in bread loaf volume at lower dose levels of irradiation. However, Lee (6) reported lower loaf volume at 250 Krads dose level and above, using a rich formula.

Lai et al (20) using no malt and sugar in the baking formula noted an increase in loaf volume at low dose level, presumably due to increased susceptibility of starch to diastase enzyme. However, at higher doses the baking quality deteriorated.

Webb et al (35) studied the quality of cakes and biscuits made with irradiated flour (23-93 Krads) and found no significant difference. Brownell et al (4) reported no significant changes in bread or cake quality up to 50 Krads dose level. However, Miller et al (9) found that cake quality deteriorated when the flour was irradiated at 10^6 rads.

Fifield et al (5) using a rich, straight dough formula found no changes in baking qualities up to 175 Krads dose. There was no significant difference in organoleptic evaluation of breads prepared with irradiated and unirradiated wheat flour, at that level.

Nicholas (36) studied the flavor changes in bread due to irradiation at 500 and 1000 Krads dose using a triangle test. There was no significant difference in bread flavor up to 500 Krads; however, at the higher dose level, the changes in flavor were significant. He also noted an increase in loaf volume up to 500 Krads dose but not at higher dose level.

Miller et al (37) showed that bread and cake made with irradiated wheat flour (at 200 Krads and above) were distinguishable and less palatable than unirradiated flour. A musty or moldy odor made the products less palatable.

MATERIALS AND METHODS

Wheat Samples

Two hard winter wheat varieties were used for irradiation in this study. Tascosa, which had a medium mixing time, and Shawnee which had a medium-long mixing time. Both samples had a good loaf volume potential.

A Regional Baking Standard (RBS) flour was used in the study. It was a composite flour from many varieties harvested at many locations throughout the Southern and Central Great Plains of the United States in 1969. It had a good loaf volume potential and a medium mixing time.

Irradiation of Wheat

Two pounds whole wheat was packed in polyethylene bags, the bags were sealed, and irradiated in Co^{60} gamma experimental cell, at the Department of Nuclear Engineering, KSU. The dose rate was 1920 rads per minute. Samples of each variety of wheat were irradiated at 20, 200, and 1000 Krads dose levels. A sample of unirradiated wheat served as a control. After irradiation the samples were stored for 3 weeks, and milled on an Allis experimental mill.

Analytical Methods

Protein (N x 5.7) moisture, ash, amylograms, farinograms, and sedimentation test were determined as described in Cereal Laboratory Methods (38). Mixograms were obtained on a 10 g. mixograph as described by Finney et al (39). The samples were baked by a rich, straight dough procedure as described by Finney (40-42). For reconstitution studies (43) a microbaking procedure (10 g. flour) was used. Standard deviation for the duplicate of loaf volumes were 25 cc and 1.75 cc for the 100 g. and 10 g. methods, respectively.

Fractionation of Flour

The fractionation scheme shown in Figure 1 was used to fractionate flour into gluten, pH 6.1 insoluble, pH 6.1 solubles, water solubles, starch, glutenin and gliadin fractions from each sample. All the fractions were lyophilized, ground, and stored at -18°C until further use. The protein and moisture contents were determined using methods described by AACC (38).

Flour was extracted with petroleum ether (bp 30-60°C) for 18 hours in a Soxhlet apparatus to obtain total free lipids.

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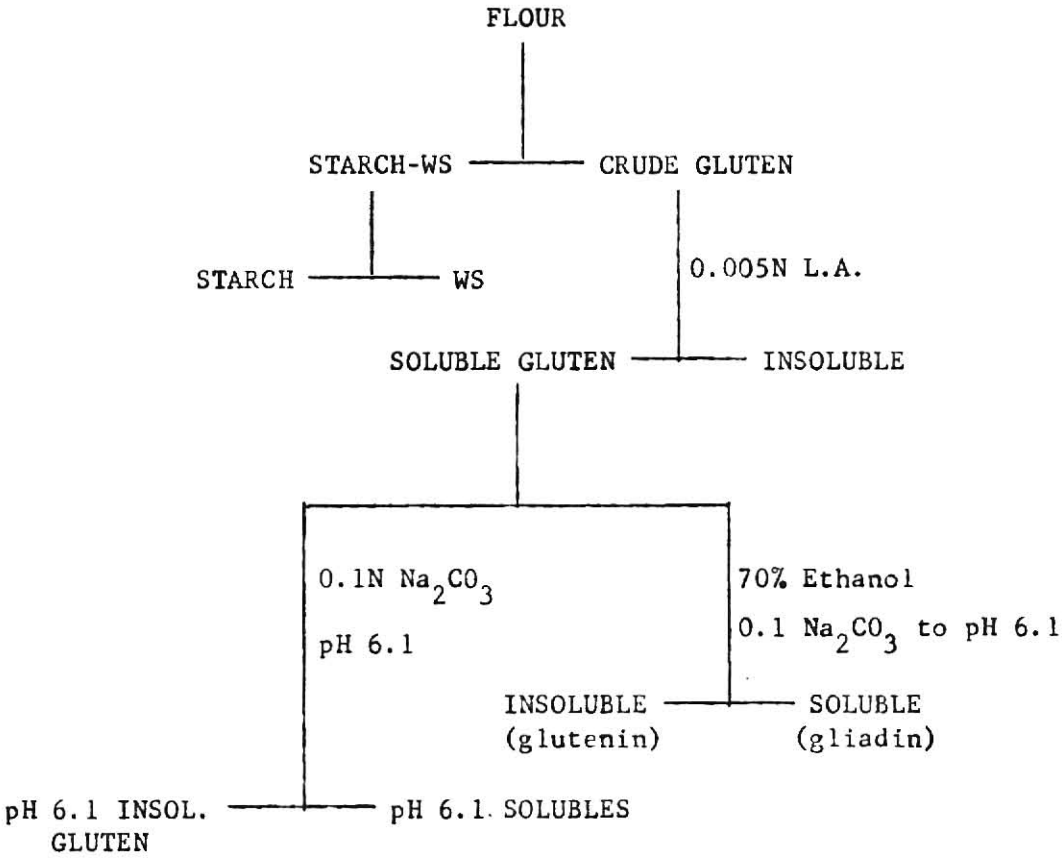

Fig. 1. Fractionation scheme for flour.

Reconstitution of Flour

Certain fractions were reconstituted and baked into bread. All flours were reconstituted to 10 g. (14% moisture basis) and to the protein content of the original flour.

Additionally gluten, starch, water solubles and total free lipid fractions from irradiated (1000 Krads) and control samples were interchanged. Thereby, the particular fraction or fractions, if any, damaged by irradiation and affecting the baking performance of the sample could be determined.

Starch Gel Electrophoresis

Starch gel electrophoresis was performed as described by Woychik et al (44). Starch gels were prepared with acid hydrolyzed starch and a lactic acid-aluminum lactate 0.017 M, pH 3.2 buffer system containing 3 M urea.

Protein fractions isolated from control and irradiated wheat samples were applied to the starch gel (50 μl of protein solution containing about 2 mg. protein). The electrophoresis was run in a vertical gel with the protein migrating downwards, and with a constant current (30 mA for the 12 x 32 gels).

The sliced gel was soaked in aqueous 0.1% amido black 10B dye overnight. Excess dye was washed out with distilled water. The electrophoretic patterns were then photographed.

RESULTS AND DISCUSSION

Effect of Irradiation on Rheological Properties of Dough

The physical properties of flours milled from irradiated wheat were determined with the farinograph, amylograph, and mixograph. The data obtained with the control flour (0 Krad) and flours milled from irradiated wheat with 20 to 1000 Krads dose level of gamma irradiation are shown in Table 1 and Figure 2.

Gelatinization viscosity or the amylogram peak decreased as the dose of irradiation increased. At the 20 Krads dose level, the drop in viscosity was more for Shawnee than for Tascosa wheat. At the 200 Krads dose, the viscosity was lowered considerably in both varieties, however, it was still within the range that is required for good performance of flour in baking (14). Similar results have been reported by other workers (6, 34).

Amylograph peak height has been widely used to estimate the amylase activity and the extent of damaged starch in flour. Ananthaswamy et al (45) reported no difference in amylase activity in irradiated flour compared to that of unirradiated flour. However, the susceptibility of starch to amylolysis was increased as the dose of irradiation increased, thus leading to a lower amylogram peak.

In an effort to explain the increased susceptibility of the starch to enzymatic action, scanning electron micro graphs (Fig. 3 and 4) of cut kernels of control and 1000 Krads Tascosa wheat were examined. No differences in the appearance of the starch from the two samples were noted.

Table 1. Amylogram and farinogram data for flours milled from Tascosa and Shawnee wheat irradiated at 0, 20, 200, and 1000 Krads.

Samples	Gelatinization Viscosity	Water Absorption	Development Time	Dough Stability
	B.U.	%	min.	min.
Tascosa control (0 Krad)	1000	60.6	4.5	4.5
" 20 Krads	960	60.8	4.5	4.0
" 200 Krads	670	61.3	4.8	2.5
" 1000 Krads	480	62.8	3.0	1.5
Shawnee control (0 Krad)	800	63.4	8.0	3.7
" 20 Krads	530	63.1	8.3	2.3
" 200 Krads	480	63.0	8.5	1.5
" 1000 Krads	140	64.8	8.5	1.5

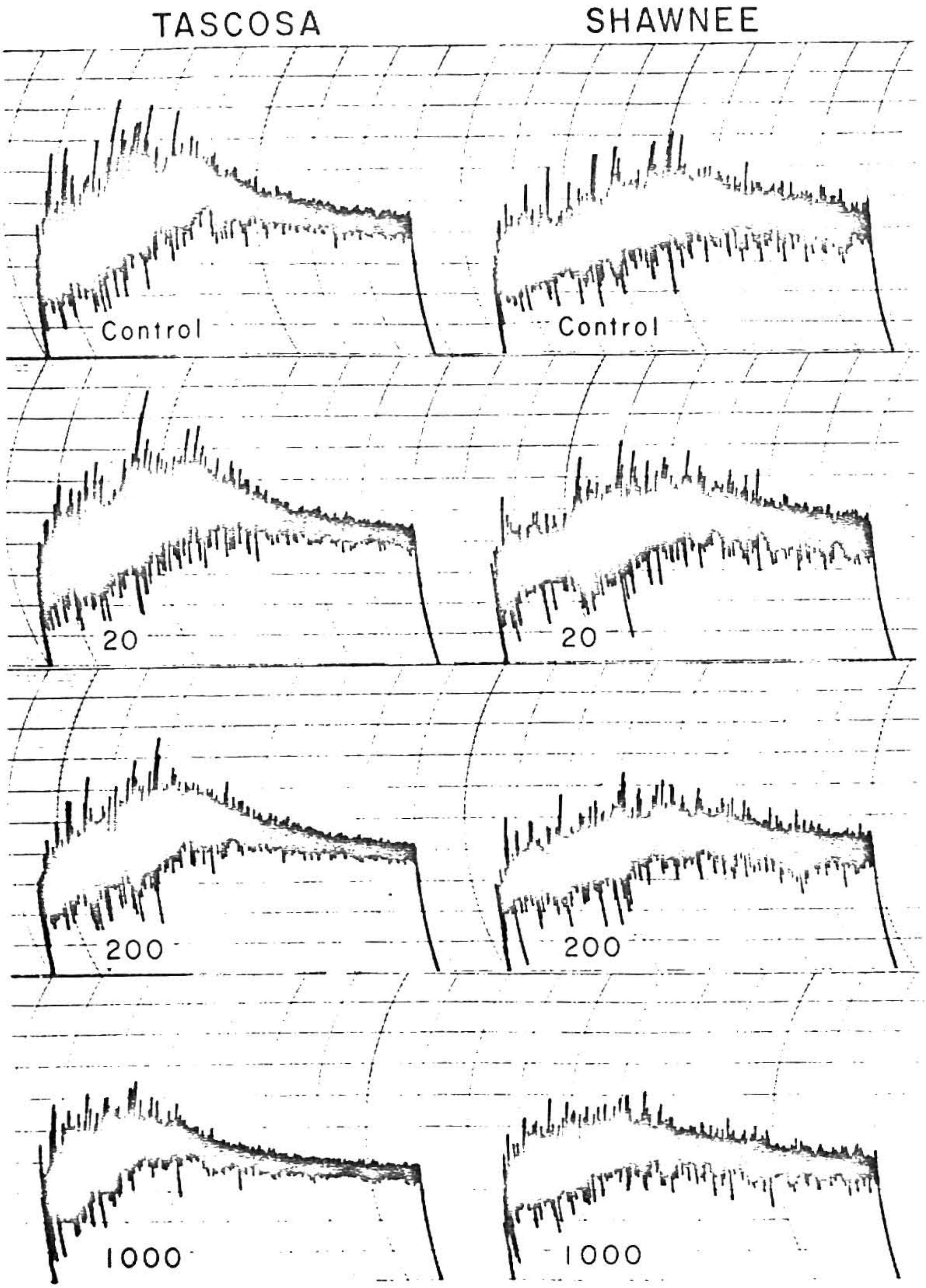

Fig. 2. Mixograms of flours milled from Tascosa and Shawnee wheats irradiated at 0, 20, 200, and 1000 Krads of γ-irradiation.

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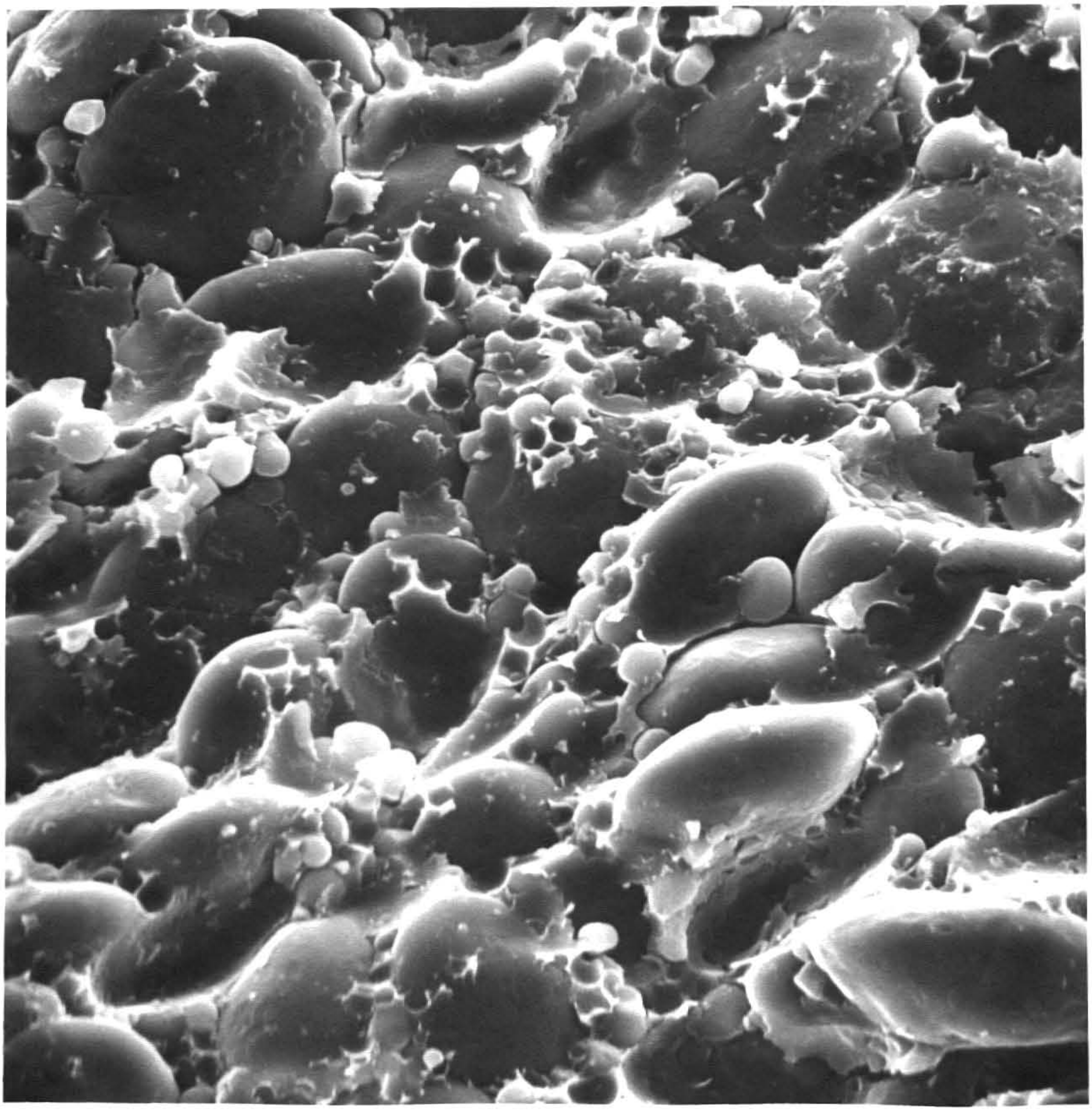

Fig. 3. Scanning electron micrograph of a cut surface of unirradiated Tascosa wheat (720X).

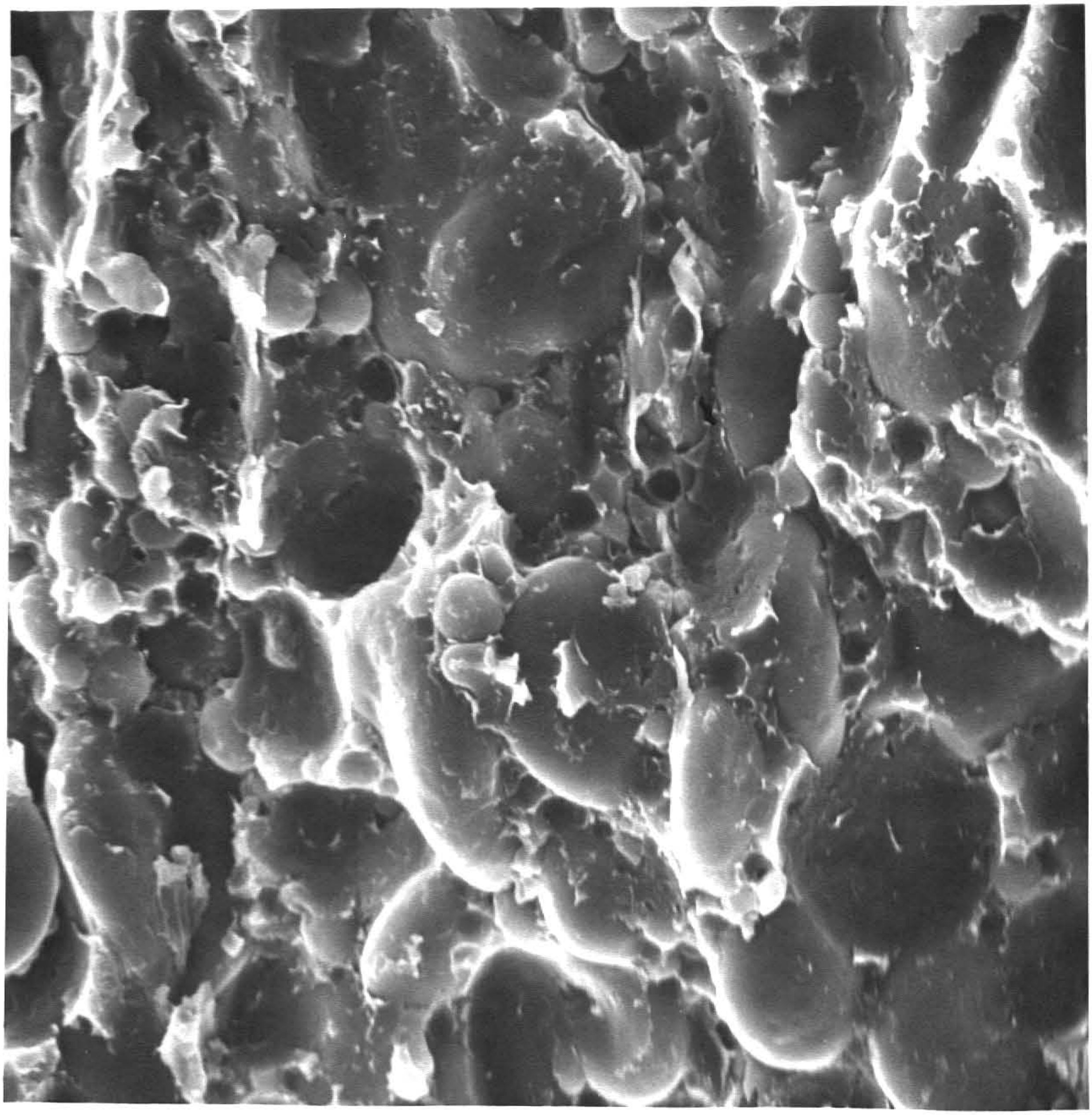

Fig. 4. Scanning electron micrograph of a cut surface of irradiated (1000 Krads) Tascosa wheat (820X).

The water absorption of flours milled from unirradiated wheat was 60.6% for Tascosa and 63.4% for Shawnee. At lower levels of irradiation the farinograph water absorption did not change (Table 1). However, at 1000 Krads dose level, the absorption of flour milled from Tascosa wheat was 62.8% and from Shawnee 64.8%. The increased water absorption might also be indicative of higher starch damage with higher levels of gamma irradiation. Fifield et al (5) also reported an increase in water absorption of flour due to irradiation.

The farinograph dough stability, the time (in minutes) at which the dough consistency was at 500 B.U. (Brabender Units), was reduced as the dose of irradiation increased. The decrease was more for flour milled from Tascosa than from Shawnee wheat. Dough development time decreased for flour milled from Tascosa at 1000 Krads dose but at lower doses for both wheats it was not affected.

Mixograms (Fig. 2) of the same samples showed a similar decrease in dough stability; however, the mixing time decreased with increased doses for both varieties.

Sedimentation value has been considered an indication of gluten strength. The sedimentation value decreased at higher doses of irradiation (Table 2). However, at low dose levels (20 Krads) there was no significant difference in sedimentation values from that of the control flours. Those data indicate that the gluten strength was impaired at higher doses of irradiation. A decrease in sedimentation values of irradiated wheat at low doses (20-500 Krads) was also observed by other workers (20, 34).

Table 2. Analytical values (14% moisture basis) for flours milled from wheat irradiated at 0, 20, 200, and 1000 Krads dose levels.

Samples		Moisture	Ash	Protein	Sedimentation Value
		%	%	%	
Tascosa	control (0 Krad)	13.0	0.34	12.2	42.9
"	20 Krads	12.4	0.37	12.3	42.4
"	200 Krads	12.3	0.36	12.3	38.9
"	1000 Krads	11.8	0.34	12.3	37.2
Shawnee	control (0 Krad)	12.4	0.37	11.5	50.7
"	20 Krads	12.3	0.37	11.6	50.0
"	200 Krads	12.0	0.37	11.5	44.8
"	1000 Krads	12.0	0.38	11.5	44.4

In general, the physical dough tests indicated that at 200 and 1000 Krads doses of irradiation, the rheological properties of flours milled from treated wheat were adversely affected, however, at 20 Krads dose the changes in rheological properties were not significant.

Effect of Irradiation on Baking Properties of Flour Milled from Irradiated Wheat

The loaf volume of bread baked from flour milled from irradiated wheat decreased as the dose of irradiation increased (Table 3). The rate of loaf volume decrease was greater for Tascosa than for Shawnee. At 20 Krads the loaf volume of both varieties was not significantly different from that of the control, however, at 200 Krads the Tascosa sample had a slightly lower loaf volume and a poorer crumb grain than that of the control. The optimum mixing time decreased as the dose of irradiation increased in samples from both wheat varieties, especially at the 1000 Krads dose level. The bake mixing times were similar to those obtained by the mixograph.

Flour milled from high dose levels of irradiated wheat (1000 Krads) had slightly lower baking absorption and higher bromate requirements than that of the control and low dose level samples. The crumb grain of the loaves was satisfactory at 20 Krads dose, however, at higher doses it was questionable. Breads prepared with flour milled from 1000 Krads irradiated wheat had a dark crust and crumb color and a peculiar flavor. Lee (6) and Lai et al (20) have shown that loaf volume deteriorates at higher dose levels (100-1000 Krads). Others (8, 36, 20) have reported an increase in loaf volume at dose levels of 50 to 125 Krads when using

Table 3. Baking data (100 grams) for flour milled from Tascosa and Shawnee wheat irradiated with 0, 20, 200, and 1000 Krads γ-irradiation.

Treatment	Mixing Time	Baking Absorption	$KBrO_3$ Requirement	Loaf Volume	Crumb Grain [a]
	min.	%	ppm	cc	
Tascosa control (0 Krad)	$3\frac{3}{4}$	62.7	5	1008	S
" 20 Krads	$3\frac{3}{4}$	61.2	5	1005	S
" 200 Krads	$3\frac{1}{4}$	63.4	10	983	Q-S
" 1000 Krads	$2\frac{1}{2}$	60.8	15	903	Q
Shawnee control (0 Krad)	$4\frac{3}{8}$	64.7	0	955	S
" 20 Krads	$4\frac{1}{4}$	65.9	0	963	S
" 200 Krads	$3\frac{3}{4}$	66.0	5	963	S
" 1000 Krads	$2\frac{3}{4}$	63.5	10	898	Q

a/ S = satisfactory, Q-S = questionable to satisfactory, and Q = questionable.

a formula without malt and sugar. At extremely high dose rates (3 to 10 Mrads) Finney et al (46) found the loaf volume deteriorates regardless of the formula used.

The formulation used in baking is of critical importance when comparing the baking results reported in one paper to that in another. For example, a formula without sugar or with an insufficient amount of sugar might show an increase in loaf volume with irradiated wheat flour at lower dose levels, because of the fermentable sugar produced by enzymatic action on damaged starch. With the two hard winter wheat varieties (Tascosa and Shawnee) used in this study, the decrease in loaf volume, at the same dose level of radiation, was different. Tascosa was affected more than Shawnee at higher dose levels of irradiation. This may be due to the fact that Shawnee has a longer mixing requirement than Tascosa. Previous work in Hard Wheat Quality laboratory has shown that the tolerance of wheat flour dough which requires less time for optimum mixing is usually less to any severe treatment. The work reported here supports that conclusion.

The baking absorptions and farinograph absorptions were quite different. Farinograph showed an increase in water absorption as the dose of irradiation increased. However, baking absorption showed just the reverse. Actually the farinograph may not give an accurate estimation of the baking absorption of flour (47). In baking other ingredients are used in addition to flour (which is the only one in the farinograph).

The flour milled from irradiated wheat required more bromate than that of the control. That finding may be due to the shortening of the mixing time. Generally, as the mixing time decreases, the bromate requirement increases (14). In the irradiated samples, mixing requirement decreased as the dose of radiation increased and therefore resulted in an increased bromate requirement.

Fractionation and Reconstitution Studies on Flour Milled from Irradiated Wheat

To determine what fraction or fractions of wheat flour were affected by irradiation, certain samples were fractionated, reconstituted (analytical data of fractions, Table 4), and baked on a micro scale. Data obtained by reconstituting the flours and interchanging the water solubles, starch, and gluten fractions from certain of the flours are shown in Tables 5 and 6.

Mixing time for the reconstituted flours decreased compared to that of the control flours. Similar results have been noted in previous reconstitution studies (48). The bromate requirement for reconstituted samples was the same as that of original flours. The loaf volumes for reconstitutes of RBS flour and flours milled from Tascosa wheat, control and irradiated, were the same as that of the original flours. For Shawnee, loaf volumes of reconstituted samples were slightly lower than that of the original unfractionated flours. However, these differences were not significant. Thus the procedure used in fractionation and reconstitution of the flours did not affect the baking performance of the samples.

Table 4. Proximate analysis of different fractions of irradiated flours. a/

Samples	Starch		Crude Gluten		Water Solubles		6.1 Insol.Gluten		Glutenin		Gliadin	
	Protein	Moisture	Protein	Moisture	Protein	Moisture	Protein	Moisture	Protein	Moisture	Protein	Moisture
Tascosa control	--	10.6	59.1	1.5	24.7	3.9	78.8	3.9	77.6	6.5	65.2	5.9
" 20 Krads	--	12.8	59.1	1.6	22.7	5.2	79.2	2.6	79.0	7.0	66.6	6.5
" 200 Krads	--	10.7	61.0	2.8	25.3	5.3	74.9	1.1	77.0	8.2	66.0	8.9
" 1 Mrads	--	12.2	60.5	2.1	22.7	4.9	78.0	2.8	77.0	8.9	65.9	5.5
Shawnee control	--	11.2	60.8	2.6	22.7	5.0	77.2	1.7	75.8	6.8	65.7	6.6
" 20 Krads	--	10.3	60.5	2.2	22.6	4.6	76.1	2.5	78.0	8.6	65.8	5.4
" 200 Krads	--	9.9	60.8	2.6	22.7	5.0	78.6	3.7	74.7	5.2	65.5	4.9
" 1 Mrad	--	9.9	60.6	2.3	22.6	4.0	77.6	2.2	75.2	5.9	65.1	4.4

a/ Protein content is corrected on 14% moisture basis.

Table 5. Baking data (10 grams) for RBS, Tascosa and Shawnee control flours, flour milled from irradiated Tascosa and Shawnee wheats, and flours reconstituted from fractions of the above flours.

Samples and Treatment	Mixing Time	$KBrO_3$ Requirement	Loaf Volume
	min.	ppm	cc
RBS flour (F)	$3\frac{5}{8}$	20	80
RBS reconstitute (R)	$3\frac{1}{4}$	20	80
Tascosa (F)	$3\frac{3}{8}$	10	74
Tascosa (R)	$3\frac{1}{4}$	10	74
Tascosa 20 Krads (F)	3	10	75
Tascosa 20 Krads (R)	3	10	74
Tascosa 200 Krads (F)	3	10	76
Tascosa 200 Krads (R)	3	20	75
Tascosa 1000 Krads (F)	$2\frac{3}{8}$	20	71
Tascosa 1000 Krads (R)	$2\frac{7}{8}$	20	70
Shawnee (F)	4	0	80
Shawnee (R)	$3\frac{5}{8}$	0	77
Shawnee 20 Krads (F)	$3\frac{7}{8}$	0	75
Shawnee 20 Krads (R)	$3\frac{3}{4}$	0	74
Shawnee 200 Krads (F)	$3\frac{1}{2}$	10	75
Shawnee 200 Krads (R)	$3\frac{5}{8}$	10	74
Shawnee 1000 Krads (F)	$2\frac{1}{2}$	10	75
Shawnee 1000 Krads (R)	3	10	72

Table 6. Baking data for reconstituted Tascosa flours (10 grams).

Source of the Fractions			Mixing	$KBrO_3$	Loaf
Gluten	Starch	Water Solubles	Time	Requirement	Volume
			min.	ppm	cc
C[a]	I[b]	C	$3\frac{1}{4}$	10	73
C	C	I	$3\frac{1}{8}$	10	71
I	C	I	$2\frac{5}{8}$	20	67
I	I	C	3	20	66
C	I	I	$2\frac{7}{8}$	10	73
I	C	C	$2\frac{7}{8}$	20	69
C	C	C	$3\frac{1}{4}$	10	74
I	I	I	$2\frac{7}{8}$	20	70

a/ C = control - (unirradiated).

b/ I = irradiated at 1000 Krads dose level.

Reconstitution of the gluten and water soluble fractions from the control (unirradiated) flour with the starch fraction from irradiated flour (Tascosa 1000 Krads) produced a loaf volume not significantly different than that from the original flour (Table 6). This indicates that the starch fraction from the flour milled from irradiated Tascosa wheat was equal in baking quality to the starch fraction from the control flour. When the water soluble fraction from irradiated wheat was reconstituted with the starch and gluten fractions from flour milled from control wheat, the loaf volume was comparable to the control flour. Thus, the water soluble fraction was not responsible for the lower loaf volume of the irradiated sample.

However, when the gluten from flour milled from Tascosa wheat irradiated at 1000 Krads dose was reconstituted and baked with the water soluble and starch fraction from control flour, the loaf volume was significantly lower and comparable to the completely reconstituted irradiated sample. This indicates that the lower loaf volume of the irradiated sample was due to the gluten fraction.

All reconstitutes containing the gluten fraction from irradiated wheat gave loaf volumes comparable to the irradiated sample irregardless of the source of the starch and water soluble fraction. Thus, it appears that the loss of loaf volume potential due to irradiation at 1000 Krads dose level of Tascosa wheat resulted from damage to the gluten fraction.

Doguchi (19) has extensively studied the effect of irradiation at high dose levels (10 Mrads) on gluten, however, all his studies were concerned with biochemical properties of gluten. He did not correlate those results with baking performance.

Starch Gel Electrophoresis

The protein fractions obtained from control wheat flours and flours milled from irradiated wheat are shown in Figure 1. The electrophoretic patterns of crude gluten, pH 6.1 insoluble gluten and water solubles obtained from certain irradiated and control samples are shown in Figures 5, 6, and 7. All the fractions obtained from Tascosa, control and irradiated at 1000 Krads, samples are shown in Figures 8 and 9. No differences due to irradiation are noted in any of the fractions.

Although the changes in baking properties of flours milled from irradiated wheats, especially at 1000 Krads dose level, were prominent, and reconstitution studies indicated that the gluten fraction was responsible for the lower loaf volume potential of the irradiated samples, no differences were seen in the starch gel electrophoretic patterns of the gluten fraction. Chemical degradation of protein chains can be seen on starch gel electrophoretic patterns. Therefore, at 1000 Krads dose level there apparently was not any significant chemical degradation. Configurational changes in the gluten protein could also be responsible for the lowered loaf volume potential. Since the starch gel electrophoresis buffer contains 3.0 M urea, which breaks hydrogen bonds and thus alters the configuration of the protein, changes in configuration would not be seen in starch gel electrophoretic patterns.

Doguchi (19) has shown that gluten was severely affected at and above 3 Mrads dose levels. He also did not report any change in the electrophoretic pattern of gluten irradiated at 1 Mrad dose levels. At 6 Mrads and above several fast moving bands disappeared. Doguchi has

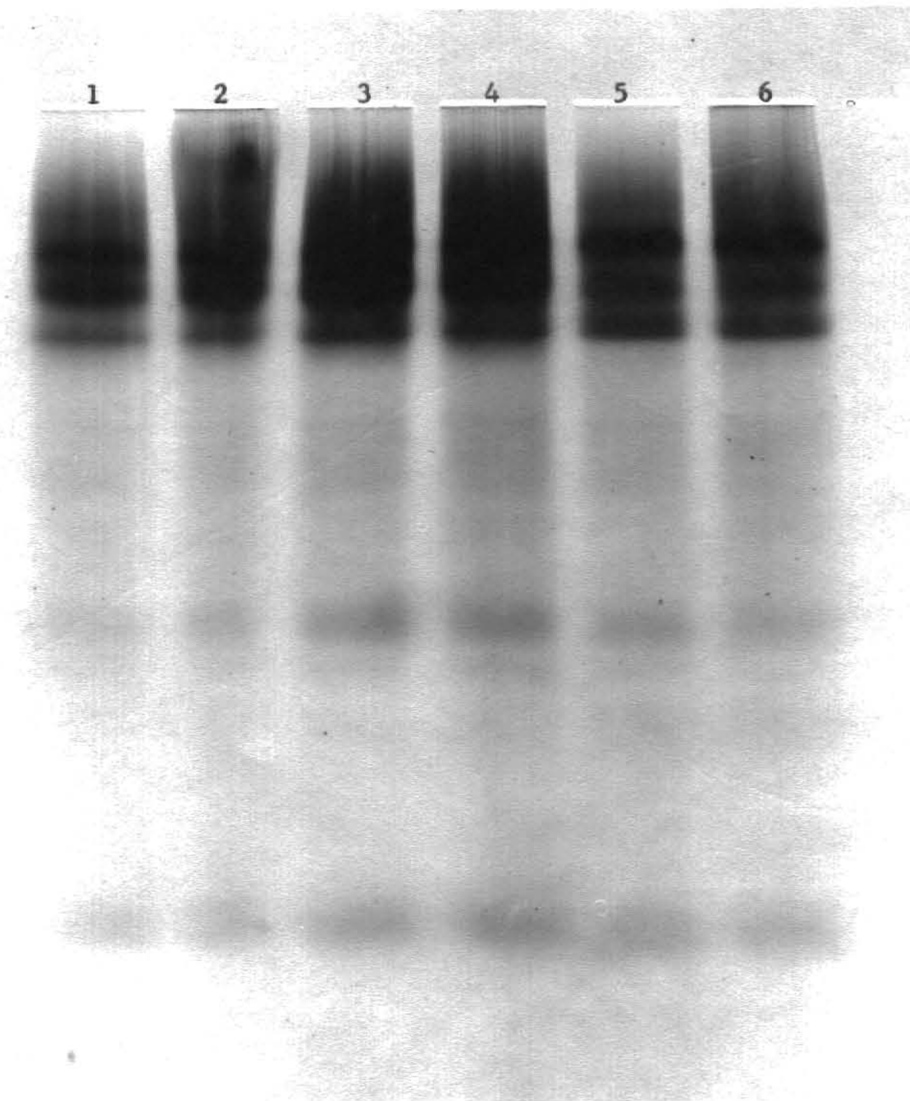

Fig. 5. Starch gel electrophoretic patterns of gluten; (1) Tascosa, 0 Krad, (2) Tascosa, 20 Krads, (3) Tascosa, 200 Krads, (4) Tascosa, 1000 Krads, (5) Shawnee, 0 Krad, and (6) Shawnee, 1000 Krads.

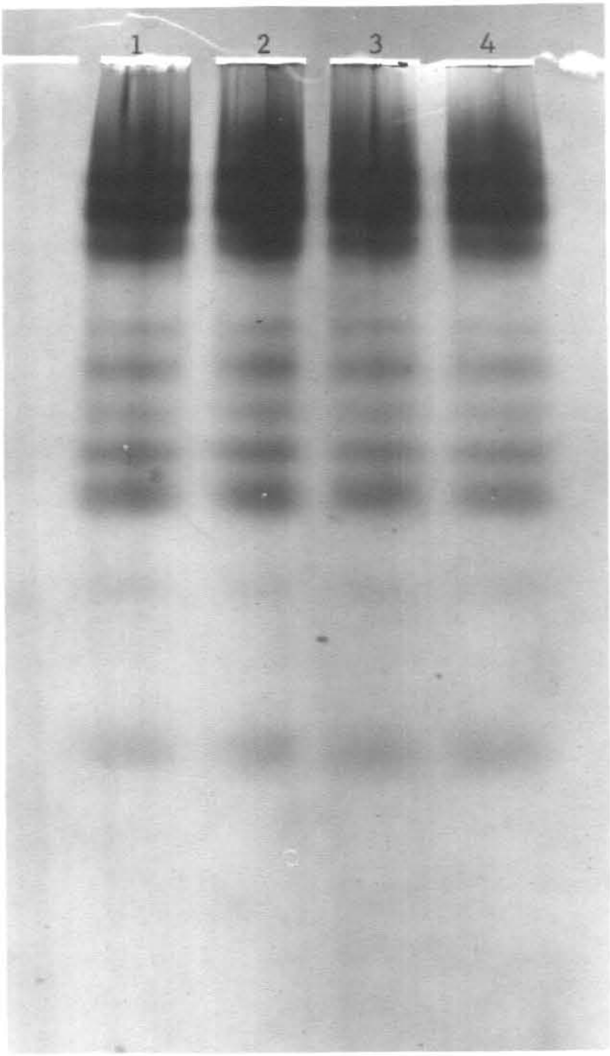

Fig. 6. Starch gel electrophoretic patterns of pH 6.1 insoluble gluten from Shawnee (1) 0 Krad, (2) 20 Krads, (3) 200 Krads, (4) 1000 Krads.

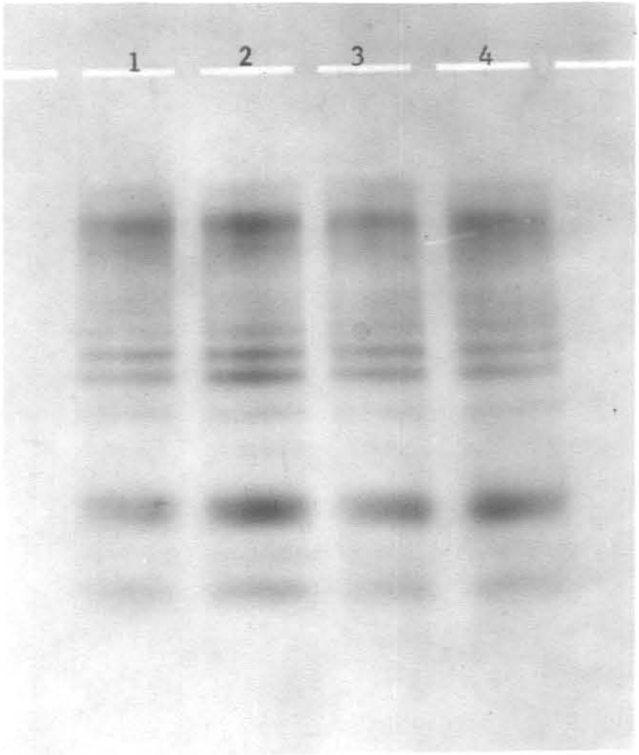

Fig. 7. Starch gel electrophoretic patterns of water solubles from Tascosa (1) 0 Krad, (2) 20 Krads, (3) 200 Krads, (4) 1000 Krads.

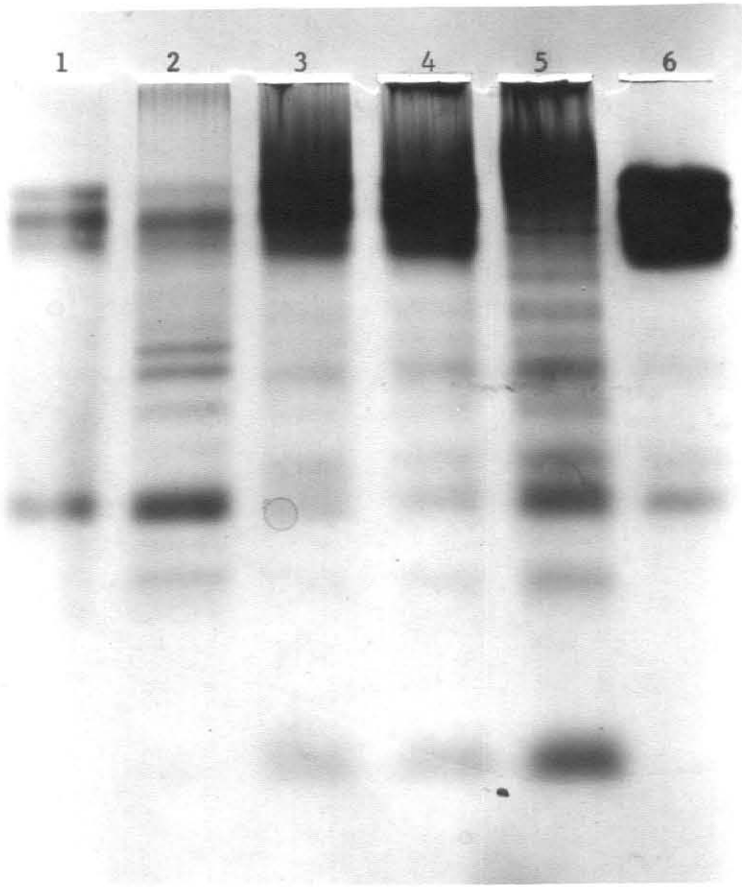

Fig. 8. Starch gel electrophoretic patterns of fractions from unirradiated Tascosa (1) pH 6.1 soluble, (2) water solubles, (3) pH 6.1 insoluble, (4) gluten, (5) glutenin, (6) gliadin.

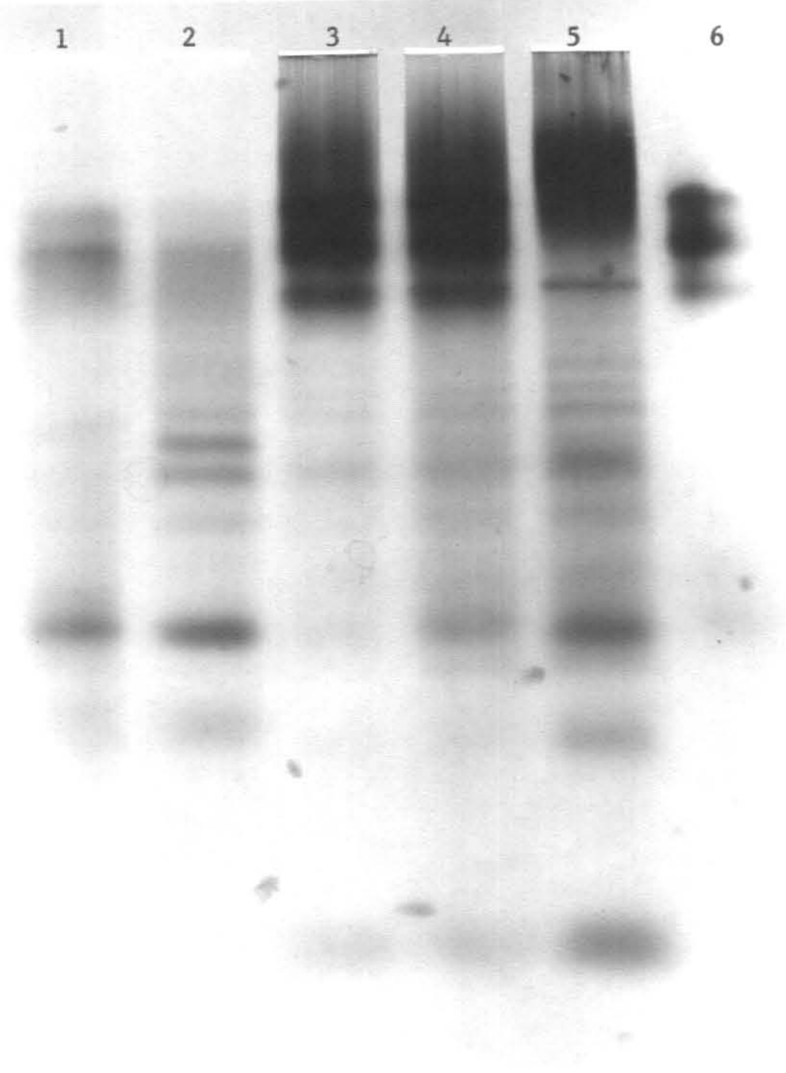

Fig. 9. Starch gel electrophoretic patterns of fractions from irradiated (1000 Krads) Tascosa (1) pH 6.1 soluble, (2) water solubles, (3) 6.1 insoluble, (4) gluten, (5) glutenin, (6) gliadin.

concluded that irradiation at 3 Mrads and above changed the configuration of gluten, especially the gliadin component of wheat protein. At 10 Mrads dose level, he has reported that gluten properties are almost completely destroyed, as determined by chemical and analytical methods. Gluten as an isolated gluten, was irradiated in his studies. Both the use of isolated gluten and high dose levels of irradiation may be responsible for his findings of severe damage in gluten due to irradiation.

Sosedov and Vakar (21) have shown that gluten quality deteriorates at 3 Mrads dose of irradiation in dry grain (7 - 14% moisture). The gluten was reported to be weaker, losing its resiliency and extensibility. However in damp grain (18 - 26% moisture) irradiation at the same dose did not show those changes, instead the gluten became strong, short, and crumbly.

The effect of irradiation was postulated to be disaggregation of proteins with dry grain. With higher moisture in the grain they postulated that irradiation had an indirect effect, and the products of water radiolysis strengthened protein due to oxidation.

Effect of Irradiation on the Baking Properties of Flour Lipids

The gluten fraction as isolated in this study, contains a major part of the wheat flour lipids. Those lipids, and particularly the total free lipids, have been shown (49) to be important in the baking performance of a flour. Therefore, it was possible that the lower loaf volume potential of the gluten fraction of flour from irradiated wheat was due to the lipids instead of the protein in that fraction. To check this possibility, the total free lipids were extracted from flour milled from Tascosa wheat irradiated at 1000 Krads, and those lipids reconstituted with defatted flours (both the irradiated and a standard flour, RBS). Baking data obtained by those reconstitutions are given in Table 7.

Both defatted flours showed lower loaf volume and slight decrease in mixing time. When the total free lipid (TFL) from the irradiated sample was reconstituted with control (RBS) defatted flour the loaf volume was comparable to the unfractionated RBS flour. However, when defatted flour prepared from irradiated (1000 Krads) Tascosa wheat was reconstituted with the TFL from irradiated sample an increase in loaf volume over that of the unfractionated Tascosa flour was noted.

This data indicates that the lipid fraction of the flour was not responsible for the decrease in loaf volume of flours milled from irradiated wheat (1000 Krads). The total free lipids from irradiated flour was comparable to the control lipids. Therefore, it appears that the decrease in loaf volume potential of irradiated wheat is indeed due to damage to the gluten proteins.

SUMMARY

Two hard winter wheats, Tascosa and Shawnee, were subjected to gamma irradiation (20, 200, and 1000 Krads) from a Co^{60} source. Physico chemical studies on flours milled from those wheat samples indicated no adverse effects on quality at the dose level required for disinfestation of wheat (20 Krads). As the irradiation dose

Table 7. Baking data for defatted Tascosa (1000 Krads) and RBS flours reconstituted (10 grams) with total free lipid (TFL).

Flour and Treatment	Mixing Time	$KBrO_3$ Requirement	Water	Loaf Volume
	min.	ppm	%	cc.
RBS	$3\frac{5}{8}$	20	67	80
RBS defatted (D)	$3\frac{3}{4}$	20	68	72
RBS defatted + TFL[a/]	$3\frac{1}{8}$	20	67	82
Tascosa (1000 Krads)	$2\frac{3}{8}$	20	66	71
Tascosa defatted (1000 Krads)	$2\frac{5}{8}$	20	64	68
Tascosa defatted (1000 Krads) + TFL	$2\frac{1}{2}$	20	66	76

a/ TFL = 0.8% total free lipid from Tascosa flour milled from wheat irradiated at 1000 Krads.

increased, the amylogram peak height and dough stability decreased. At 1000 Krads the mixing requirement was considerably shorter. Loaf volume decreased for samples irradiated at higher doses compared to control samples. That effect was greater for Tascosa than for Shawnee wheat.

Fractionation and reconstitution studies indicated that the gluten fraction was responsible for the lower loaf volume potential of flour milled from irradiated (1000 Krads) wheat. However, starch gel electrophoresis patterns of certain protein fractions did not show any change due to irradiation up to 1000 Krads. The gluten fraction contains considerable lipid material along with the protein, and that lipid is known to be important for the breadmaking performance of a flour. Therefore, a total free lipid sample was extracted from an irradiated sample (1000 Krads) and reconstituted with a standard defatted flour. Since the lipid fraction was normal in breadmaking performance, it appears that detrimental effect of gamma irradiation is an effect on the gluten proteins.

Considerable work indicates that the starch in irradiated wheat is more susceptible to enzymatic attack than that in nonirradiated wheat. In an effort to show the assumed starch damage, scanning electron micrographs of a cut surfaces of irradiated and unirradiated wheat kernels were examined. No differences were seen in starch granules from irradiated (1000 Krads) compared to that from control wheat kernels.

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EFFECT OF LOW-DOSE GAMMA IRRADIATION ON BREADMAKING QUALITY OF WHEAT

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Two hard winter wheats, Tascosa and Shawnee, were subjected to gamma irradiation (20, 200, and 1000 Krads) from a Co^{60} source. Physico chemical studies on flours milled from those wheat samples indicated no adverse effects on quality at the dose level required for disinfestation of wheat (20 Krads). As the irradiation dose increased, height of amylogram peak and dough stability decreased. At 1000 Krads the mixing requirement was reduced about 35%. Loaf volume was decreased for samples irradiated at 1000 Krads. That effect was greater for Tascosa than for Shawnee wheat.

Fractionation and reconstitution studies indicated that the gluten fraction was responsible for the impaired loaf volume potential of flour milled from irradiated (1000 Krads) wheat. However, starch gel electrophoresis patterns of certain protein fractions appeared essentially unaltered by irradiation up to 1000 Krads. The gluten protein fraction contains considerable lipid material, and that lipid is known to be important for the breadmaking performance of a flour. Therefore total free lipids extracted from an irradiated sample (1000 Krads) were reconstituted with RBS-70A defatted flour. Since the lipid fraction was normal in breadmaking performance, it appears that the detrimental effect of gamma irradiation was on the gluten proteins.

Since considerable work indicates that the starch in irradiated wheat is more susceptible to enzymatic attack than that in unirradiated wheat, scanning electron micrographs of cut surface of irradiated and unirradiated wheat samples were examined. No differences were seen in starch granules from irradiated (1000 Krads) compared to those from the control.