THE ROLE OF SULFHYDRYL GROUPS IN FLOUR

by

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INTRODUCTION

The role and significance of reactive sulfhydryl groups in wheat flour continues to intrigue cereal chemists in a great number of research centers in the world (Anderson, 1961; Bushuk and Hlynka, 1962; Frater et al., 1961; Sokol and Mecham, 1960; Sullivan, 1954).

The study of the mechanism of action of reducing agents and oxidants on rheological properties of dough has been concerned primarily with the action of the free thiol containing cysteine and glutathione and the maturing agents bromate, persulfate and iodate. A great amount of useful information has been obtained more recently by use of specific sulfhydryl-blocking reagents, N-ethylmaleimide and p-chloromercuribenzoate. The availability of thiol containing compounds comparable in molecular weight to flour proteins such as thiolated gelatin prepared according to the procedure of Benesch and Benesch (1958a) or relatively simple technics for the production of thiolated flour present a new tool for the study of the effect of sulfhydryl groups on dough properties.

The present study deals with the effect of thiolated gelatin, varying in molecular weight and sulfhydryl content, on the rheological properties of wheat doughs. In view of the large molecular size of the thiolated proteins and its possible association with wheat gluten, the effect of oxidants on reversibility of thiogel-induced changes in flour doughs has been studied.

REVIEW OF LITERATURE

Wheat Proteins

Wheat proteins are generally considered to be unique among cereals in their ability to form, on addition of water, a coherent mass called gluten. Wheat gluten is considered the most important component responsible for the unique properties of the wheat-flour-dough.

During the preparation of a dough from flour and water, hydration of the protein to form gluten occurs as the dough is mixed, the gluten particles uniting to form a continuous structure. This gluten structure is the main factor determining bread quality. The amount of protein is important but also is its quality. Extensive work has been done in attempt to elucidate the problem of what determines quality in wheat protein and how it can be measured.

The rubbery mass of crude gluten is usually obtained from flour by kneading a dough in water to wash away non-proteinaceous constituents, and it consistently contains about two-thirds by weight of water and one-third dry matter. The dry solids contain 75 to 85 per cent protein and 5 to 10 per cent lipids, with occluded carbohydrates and minerals.

The proteinaceous portion of the gluten is not homogeneous and for many years has been regarded as a mixture of approximately equal parts of gliadin and glutein. These two moieties are obtained by arbitrary separation employing solvents according to the classical methods of Osborne (1907). The terms gliadin and glutenin do not designate definite chemical substances but are almost universally retained for the sake of convenience. Gliadin refers to the portion of gluten soluble in 70-80 per cent aqueous alcohol,

and glutenin to the remainder, which is insoluble in neutral solvents but soluble in acidic or alkaline solvents.

According to Taylor and Cluskey (1962) the glutenin component of wheat behaves as a flexible, randomly coiled polyelectrolyte. It is the major contributor to the rheological properties of wheat gluten in solution because of its high viscosity, and it is probably the major factor in the viscoelastic properties of wet gluten.

In addition to gluten, wheat contains soluble proteins. Studies of soluble proteins have been limited despite their role in flour utilization and technology. Some of the soluble proteins are enzymes, others may substantially modify gluten behavior, even if they are not considered as structural moieties in the framework of the bread dough. It has been shown by Finney (1943) and Pence et al., (1951) that for maximum baking performance, the water soluble proteins are required.

Pence et al., (1956) have shown that even with Waring blendor washed glutens, 60 per cent of the albumins and 50 per cent of the globulins are still retained in gluten.

Albumins are known to be important to flour quality in that they are needed in reconstituted doughs, yet relatively little is known of the possible function of globulins.

Amino Acid Composition

The possibility that variations in gluten qualities might be due to differences in amino acid composition was first examined by Blish (1916) and Cross and Swain (1924). They failed to show any convincing evidence of differences in amino acid make-up among the various preparations of gliadin

and glutenin.

Pence et al., (1950) reported analysis of 17 flours milled from different varieties and types of wheat with a wide range of protein content and baking behavior, and found no essential differences in the amino acid composition.

Some workers have studied the amino acids, cystine and cysteine, as related to the importance of cystine disulfide bonds in imparting the desirable physical properties of gluten, and the sulfhydryl group of cysteine because of its relation to the improving action of certain oxidizing agents on dough properties. Miller et al., (1950) found no significant differences in the glutamic acid, lysine, cystine, and methionine composition of several wheat varieties grown under different environmental conditions. However, there was a significant difference (0.1% level) in the methionine and cystine content of samples grown under different environmental conditions during a single crop year. There was a significant difference in per cent cystine for samples grown during two crop years. Wheat with the most cystine required longest mixing time for optimum dough development. Miller et al., (1950) suggested that there may be a relationship between per cent cystine and dough mixing time as influenced by environment.

The cystine content of protein preparations from 23 wheat flours ranged from 2.2 to 4.5 per cent as determined pollarographically by Wöstmann (1950). The cystine content of the flour was positively correlated with physical properties of salt-water doughs. Miller et al., and Wöstmann suggested a possible correlation between cystine percentage in wheat protein and its quality.

McDermott and Pace (1957, 1960) studied extensively the amino acid

composition of wheat proteins. They fractionated gliadin and determined the amino acid composition of different fractions and found slight differences in the content of certain amino acids.

The main features of the amino acid composition of wheat gluten proteins are: 1) the high content of glutamic acid, 2) the relatively high proline content, 3) the relatively low content of basic amino acids, and 4) the high amide-N content, apparently correlated to the content of glutamic and aspartic acid (Pace, 1959).

Wheat protein varying considerably in their bread baking potentialities show minor difference in the amino acid content. From fractionation studies, the "insoluble" fraction appears to be broadly similar in different glutens. As this fraction accounts for most of the protein, its composition will dominate the amino acid "picture" of the whole protein. There has been no evidence, as yet, that one type of flour or gluten may contain a new or unusual amino acid.

Measurement of Gluten Quality

The simplest means of studying differences in protein quality is by examination of the washed out gluten, obtained by kneading a dough under a stream of water to wash away the starch. The gluten so obtained can vary markedly in its physical character, it may be soft and semifluid, or elastic resembling soft rubber, or tough and granular. The quantity and quality of gluten can be evaluated subjectively by an experienced operator, or objectively with any one of several instruments or by physico-chemical tests.

Physico-Chemical Tests

In view of the effect of numerous factors on the colloidal character of wheat gluten, a consideration of gluten quality is not complete without taking into account the composition and conditions of the assay. Wood (1907) found that gluten suspended in dilute acid increased in hydration and swelled, and that salts depressed the hydration of gluten in acid. Gortner and Doherty (1918) performed one of the earliest extensive investigations on the imbibitional properties of flour proteins. They concluded that moist glutens from flours of varying quality were hydrated to the same extent, but that gluten from weak flour had a much slower rate of hydration. Dilute solutions of lactic, acetic, and phosphoric acids caused gluten to imbibe water strongly and solutions of stronger acids diminished imbibition only slightly.

Although Lüers and Ostwald (1919) were the first to determine the viscosity of flour suspensions, Sharp and Gortner (1923) were the first to employ the viscosity of acidified flour-in-water suspensions as an easy, rapid, and accurate method to investigate the comparative imbitional properties of flour proteins.

In contrast to Gortner and Doherty (1918) most investigators believe that glutens from different wheats exhibit variation in their hydration capacity. Viscosity values have been useful in evaluating soft wheat flour (Bayfield, 1934); however, their utility in predicting hard wheat flour quality has been questionable (Blish and Sandstedt, 1925; Gortner, 1924; Bayfield, 1932; Rich, 1932). Factors that affect the test include temperature, amount of electrolyte present, quantity of protein, granulation, and degree of hydration of the flour constituents.

Finney and Yamazaki (1946) reported that viscosity was a linear function

of protein quantity, but that the regression of viscosity on protein content was different for each variety. Furthermore, viscosity did not evaluate properly the loaf volume potential of wheat varieties.

Berliner & Koopman (1929) also studied the swelling of gluten in acid and, by keeping the conditions constant showed that there are intrinsic differences in glutens from different wheats. Gluten from a weak flour when immersed in lactic acid imbibes water at a slower rate than a strong gluten but changes more rapidly from the gel to the sol condition. A strong gluten swells to a marked extent and shows little tendency to disperse for some time.

Finney and Yamazaki (1953) developed an alkaline viscosity test using sodium bicarbonate instead of lactic acid for evaluating soft wheat flours. Another test which also depends on the swelling of flour protein in acid is the sedimentation test of Zeleny (1947), in which, flour is suspended in dilute lactic acid, allowed to settle and the volume of sediment measured after a given time. This volume depends largely on the protein content of the flour but samples with the same protein content can have different sedimentation values indicating differences in the swelling capacities of their protein. Specific sedimentation, or the sedimentation value divided by protein percentage, has been considered a useful measure of gluten quality.

Physical Tests

Many devices have been built to test quality of gluten (Miller and Johnson, 1954). Some instruments have been designed to measure expansion of washed gluten, heated under empirical conditions (the Boland's aleurometer and Liebermann's device). Even modifications of these machines left errors

of measurement too great to be disregarded. The Auermann's elastometer, the Baker-Parker-Miaze device, Krtinsky's elastocope and fortiscope have been used to test recovery of gluten from compression. Only the fortiscope (Bailey, 1940) gave data correlated with baking quality and water absorption.

Extension of crude gluten has been tested by means of many instruments. These include the Baker-Parker-Mize device, Barbade's "aleurographe", the Brabender glutograph, the Hlynka-Anderson stretchometer, the James gluten tester, and many others (Bailey, 1940).

Rheological Properties of Dough

The use of rheological methods has yielded valuable information concerning the relationship between physical properties of a dough and its baking quality.

The protein fraction of wheat flour is primarily responsible or is of fundamental importance in the formation of dough and its rheological behavior. It is well known that other constituents of the flour, i.e., lipids and carbohydrates are involved in governing rheological properties of wheat dough. Additionally, factors such as ionic strength can affect swelling and coagulation properties. The consensus seems to be, however, that the elastic properties of a flour-water dough are determined, other things being equal, by the total amount of wheat protein present and by the quality of the protein.

Although the quality of a protein present is a major factor in determining flour strength, flours of similar total protein content may differ in strength due to qualitative differences in the protein component, and probably due to differences in molecular structure of the protein molecules or to differences in intermolecular structure such as differences in degree of crosslinking.

It is evident that dough is a complex system, the physical properties

of which are modified by the many mechanical and physical actions which operate when it is processed into bread or other products. Formation of the continuous gluten network in dough is viewed as resulting from appropriate uncoiling and orientation of the protein in flour particles and establishment between neighboring particles of covalent disulfide linkages which are stable at baking temperatures. Axford and Elton (1960) suggested that in mechanical development of dough, bond breakage could arise as a result of the strain on protein molecules subjected to mechanical shearing forces. Such rupture of linkages would create free radicals which could subsequently recombine to form new disulfide bonds, or react and combine to produce various cross-linkages directly, or on subsequent oxidation aided by atmospheric oxygen, bromate or iodate. The formation of an expandable, three dimensional network would thus be effected by a re-orientation of protein chains.

For testing strength of flours numerous mechanical dough testing instruments have been deviced. Instruments such as the Brabender farinograph, Swanson and Working recording dough mixer, the Brabender extensograph, the Chopin alveograph, Halton's extensometer, etc., compare certain rheological properties of various doughs, while keeping another rheological property constant.

Brabender Farinograph. It is a physical-dough testing instrument of the dynamic type. It is essentially a recording dough-mixer that measures the plasticity and mobility of the dough which is being subjected to a prolonged, relatively gentle, mixing action, at a constant temperature. The resistance the dough offers to the mixing blades during mixing is transmitted to a dynamometer, which is connected to a lever and scale system and to a

pen which traces a curve on a kymograph chart.

The farinograph has found its widest use in determining flour absorption (Merritt and Bailey, 1939; Merritt and Stamberg, 1941; Hlynka, 1960). One objection to using the farinograph to determine absorption has been that consistency determined on the instrument is not always the desirable consistency at the end of fermentation. Some doughs tighten while others slacken during fermentation.

Munz and Brabender (1941) stated that water absorption capacity, general strength, shortness, and mixing sensitivity could be deduced from farinograms. Geddes et al., (1940) and Schiller et al., (1946) concluded that the farinograph is an important supplement to other control tests but is not itself adequate to evaluate strength.

Aitken and Geddes (1939) found both dough development time and absorption increased with each increment in protein content in flours enriched with protein gluten. Moore and Herman (1942) studied the effect of ingredients and variations in manipulation on the farinograph curve. They modified the method of evaluating farinograph curves and checked these curves under varying conditions. Johnson and Swanson (1946) found that the main factors that determine the valorimeter value of a flour were the length of time required to mix the flour to minimum mobility, and the descending slope of the curve. Johnson et al., (1946a) found that the valorimeter value indicated strength of flour because it was influenced by protein content. Many workers contend that the larger the valorimeter value, the stronger the flour.

Geddes et al., (1940) studied the relation between the normal farinograph and baking quality of Canadian spring wheat. They concluded that the utility of the farinograms appeared to be largely one of providing accessory information

on such properties as absorption, optimum mixing time, and mixing tolerance.

The farinograph has been used extensively in studies of the effects that some moieties such as detergents, proteolytic enzymes, reducing agents, oxidizing agents, and sulfhydryl-blocking agents have on doughs into which these compounds have been incorporated.

Extensograph. The physical properties of the gluten complex of a dough are changing continuously, the rate of change being contingent upon the treatment accorded to the dough, its age, the proportion of water used for its preparation and other variables. The brabender extensograph has been used to measure dough extensibility and resistance to extension in doughs after resting for suitable intervals of time. Essentially, the extensograph records resistance to stretching offered by a cylinder of either rested or non rested doughs. The resistance of the dough to stretching is automatically plotted against the distance it is stretched to give the extensograph curve. The height and shape of the curve give important information regarding the changes in behavior of dough with time. Munz and Brabender (1940) indicated that a study of the rate, direction and magnitude of change in extensibility and resistance to extension after a time of rest would be a valuable aid in classifying flours for specific uses.

Aitken et al., (1944) found a significant positive relationship between extensogram length, height, and protein content. Farinograph development time was positively correlated with extensogram height. Johnson et al., (1946b) found that extensibility, resistance to extension, and extensogram area were each correlated with protein content, farinogram mixing time, valorimeter value, mixogram area and height.

The extensograph has been used extensively for the study of the effect

of oxidants on dough in the structural relaxation. Structural relaxation has been used to designate the relatively slow changes that take place in the physical properties of resting doughs subsequent to mixing or mechanical manipulation.

Sulfhydryl Groups in Flour

The probable role of the sulfhydryl radical in dough chemistry has attracted the attention of many cereal chemists. The main premise has been that the sulfhydryl radical is potentially capable of undergoing a cross-linking reaction to form disulfide bridges between protein chains. Such a reaction would then be expected to have an important effect on the rheological properties of dough. In fact, it has been suggested as a chemical basis of the action of improving agents.

The sulfhydryl groups in wheat flour have been studied for a long time. The presence of these groups in wheat and flour was first recognized by Sullivan et al., (1936). These workers isolated glutathione from wheat germ, but reported that patent flours contained no free glutathione as indicated by the nitroprusside test. Balls and Hale (1940) showed that a positive nitroprusside test from petroleum ether extracts of patent flour suggested that sulfhydryl groups might be present in flour lipoprotein. They also isolated a water soluble sulfur containing polypeptide from the petroleum ether extract.

Sullivan et al., (1936) found that a solution of 2,6-dichlorophenolindophenol was decolorized when boiled in the presence of gluten. They interpreted this as indicating a reduction of the reagent by sulfhydryl groups. The results of these workers pointed to the need for a study of the

sulfhydryl groups of the protein and other fractions of wheat flour.

Myers and Working (1944) used the ferricyanide method of Anson (1941) and found no reactive sulfhydryl groups in gluten protein or in the water soluble proteins. They showed, however, that the water soluble proteins contained unreactive sulfhydryl groups which were liberated by denaturation with heat, guanidine hydrochloride or urea and Duponol. Baker et al., (1944) studied the action of oxidants on sulfhydryl compounds in doughs. They used the modified Anson method and found that the total decrease in sulfhydryl which resulted from the flour treatments was equivalent to approximately 100 ppm glutathione.

Nordin and Spencer (1952) modified the method of Hellerman et al., (1941) for the determination of sulfhydryl groups in certain proteins. Holme and Spencer (1952) also studied this method in studies on the effect of oxidizing agents (potassium bromate and ammonium persulfate) on sulfhydryl groups from different flour fractions, and found that the oxidants caused no apparent reduction in the amount of reactive sulfhydryl groups.

Matsumoto (1954, 1955) studied the effect of oxidizing agents on sulfhydryl groups in gluten using the method of Benesch and Benesch (1948). The apparatus of Hata for amperometric titration was employed with slight modification. The reaction mechanism of the oxidizing agents was closely related to the sulfhydryl content in the gluten dispersion. DeLange and Hintzer (1955) made polarographic determinations of the combined cystine and cysteine content of the gluten and soluble proteins.

The first efforts to estimate sulfhydryl groups in whole flour were those of Kong et al., (1957). They compared three methods: the iodosobenzoate titration, amperometric titration with mercuric chloride, and amperometric

titration with silver nitrate. Amperometric titrations at a rotating platinum electrode with either mercuric chloride or silver nitrate appeared preferable to o-iodosobenzoate titration, since the former were easily reproducible and more precise. Kong et al., (1957) solubilised the flour proteins with trypsin. Although the enzymatic digestion procedure appeared to be satisfactory with respect to the recovery of sulfhydryl groups from bovine serum albumin, digested in the absence and presence of flour, later experience showed that this was not true with flour proteins (Sokol et al., 1959).

Kong et al., (1957); Bloksma (1959) and Matsumoto and Hlynka (1959) made amperometric titrations of sulfhydryl groups in flour using different titration media. Bloksma found that variations in the results were due to differences between the media employed. These differences may refer to the type and amount of denaturing agent, the type and pH of the buffer, the temperature and the reagent used.

There is no consensus concerning the distribution of sulfhydryl groups in various flour components. The water and salt soluble fractions of flour seem to be rich in sulfhydryls. There are, however, conflicting results on the presence of sulfhydryl containing compounds in the so-called gluten proteins and in lipids. One of the chief obstacles encountered in the investigation of sulfhydryl groups in gluten has been the insolubility of gluten in the presence of salts. Matsumoto and Shimoda (1955) titrated intact gluten in a medium containing potassium chloride by using urea to prevent precipitation. Kong et al., (1957) solubilized gluten by digestion with trypsin. Schaefer et al., (1959) used a salt-free titration medium to overcome the solubility problem. The latter workers determined gluten

sulfhydryl groups by potentiometric titration with iodine in dilue acetic acid.

Iodometric methods have been criticized because of 1) possible over-oxidation of sulfhydryl groups beyond the disulfide stage, and 2) possible reaction of the reagent with other functional groups. The values obtained by Schaefer et al., (1959) were consistently higher than those obtained by other methods. Literature values for the sulfhydryl content of gluten vary widely. Ferrometric methods give values from 0.4 to 31.1 meq. sulfhydryl per g. protein (Baker, et al., 1944). The highest values, 23 to 50 meq. per g., were obtained pollarographically (DeLange, 1955). DeLange admits, however, that the values may be high because of possible reduction of disulfide bonds to sulfhydryl groups, in the ammoniacal-cobalt medium used. Matsumoto and Shimoda (1955) employing the amperometric method and urea as antiprecipitating agent of gluten, found 1.6 to 2.0 meq. per g. in a medium of dilute acetic acid.

Sullivan et al., (1961) used p-chloromercuribenzoate (PCMB) to protect the sulfhydryl groups from oxidation during measurement of the total as well as the water-soluble sulfhydryl groups of flour. Although PCMB reacts specifically with free sulfhydryl groups, these groups can still be measured in the presence of PCMB by the amperometric titration with mercuric chloride. Mercuric chloride apparently displaces the PCMB due to the stronger affinity it has for the sulfhydryl groups. Sullivan et al., (1961a) found that the sulfhydryl content per g. of flour was about equally divided between the water soluble and the gluten proteins. No sulfhydryl was found in the lipid or starch fraction of flour.

Bushuk (1961) described a method for the determination of accessible

sulfhydryl groups in flour-water doughs. The method was based on the rapid reaction of protein sulfhydryl groups with iodate ions, with application of an amperometric titration. He obtained reasonable agreement with calculated and experimental values of sulfhydryl for doughs which contained added glutathione or thiolated gelatin, and no reaction with iodate was obtained when sulfhydryl groups were blocked with N-ethylmaleimide (NEMI). Increases in sulfhydryl groups were obtained for doughs prepared from flours of decreasing particle size, doughs subjected to prolonged mixing, and doughs treated with guanidine.

Pomeranz and Shellenberger (1961) applied the method of Barnett and Seligman (1952) for histochemical visualization of protein-bound sulfhydryl groups in animal tissue cells, to localize sulfhydryl groups in wheat kernel. They found that the major sites of sulfhydryl groups were the aleurone layer and the germ. The histochemical method was specific and inhibited by oxidizing or sulfhydryl-blocking agents.

Despite the importance of the sulfhydryl-disulfide groups in flour and dough, the progress in this study has been hindered by limitations of the analytical methods available. A principal obstacle has been the low level at which sulfhydryl groups occur along in flour and the consequently analytical difficulties in tracing changes in sulfhydryl content.

Oxidizing, Reducing, and Sulfhydryl Blocking Reagents

The marked effects of oxidizing and reducing agents on the physical properties of the dough and gluten cannot yet be fully explained. Several mechanisms may be involved, including direct action with the gluten proteins and indirect action through other flour components such as soluble protein

and lipids. Sulfhydryl groups have been found associated with the effect that oxidizing, reducing and -SH blocking agents have on dough properties, which is in keeping with the possibility that they may have a role in flour oxidation (Sullivan, 1954; Pence et al., 1956).

Effect of Oxidizing Agents on Dough Properties

Minute amounts of oxidizing agents, such as potassium bromate, potassium iodate, ammonium persulfate, ascorbic acid, etc., are known to have the capability of improving the bread-making quality of flour. There has been much research and considerable speculation concerning the way in which potassium bromate and other oxidants cause the beneficial dough and bread effects. Rheological and baking tests have shown that the mechanisms of reactions in which these oxidants are involved during the improvement of dough properties much be diverse.

Jørgensen (1936) suggested that potassium bromate inhibits proteases which attack the native proteins, and thereby diminish the breakdown of the proteins of the dough during fermentation. Some chemists agreed with this theory, while work of others has revealed facts which do not support Jørgensen's theory.

Olcott et al., (1943) showed that reducing agents produce their characteristic effect in the complete absence of any protease, and concluded that the primary effect of reducing agents on gluten is primarily a chemical one and that enzyme activation is of secondary, if of any, importance. Reducing agents tend to soften dough and make it pliable; oxidation has an opposite effect of tightening the dough. The two effects thus counteract each other.

Baker et al., (1942) stated that softening of yeastless dough is a

physical change resulting from rearrangements of the dough components. They doubted that proteolysis plays more than a small part in reactions of dough made from sound flour, and attributed the action of bromate largely to modification of the physical properties of the gluten through action on the sulfhydryl containing moieties. Additional evidence against the proteolytic theory of bromate action in dough was presented by Howe and Glick (1946). Proteolytic activity of straight grade flour was not inhibited by bromate when used in concentrations covering the range employed in commercial practice (up to 0.01%). Freilich and Frey (1947) found that the protease content of American patent and baker's grade flours is so minute that the protease theory must assume a minor role in explaining oxidation effects.

Smith and Andrews (1952) used the extensograph to evaluate the effects of different oxidizing treatments. Unleavened and leavened doughs were mixed in different atmospheres and subjected to varying rest periods before being stretched. The addition of maturing agents, increased mixing, increased fermentation times; all tended to increase elasticity and decrease extensibility of doughs. They concluded that the effect of dough manipulation coupled with time from mixing the dough could not be adequately explained by the proteolytic theory of the action of oxidizing agents in dough.

Dempster et al., (1952, 1953) studied the kinetics of the bromate reaction and found that bromate produced changes in dough properties which were opposite in trend to those that occurred in unbromated doughs, i.e., structural relaxation rates increased and steady state loads decreased in unbromated doughs whereas the reverse occurred in bromated doughs. They attributed the behavior of dough to a three-dimensional or network structure, the cross links of which are labile bonds between groups of adjacent molecules. Bromate

was said to progressively create potentially reactive groups in certain molecules in the dough. These molecules interact to form additional non-labile cross-linkages between adjacent molecules only when brought together while the dough is worked. They confirmed that changes in the physical properties of dough brought about by bromate occur only when dough is shaped or otherwise manipulated after the time of reaction.

Hlynka et al., (1953) showed that the loss of bromate in dough as a measure of its oxidizing action could be attributed to at least three factors: 1) activity of the yeast, 2) reducing substances in flour, and 3) endogenous microbial activity of flour. They could not identify the mechanism of decomposition of bromate in dough with the mechanism of improver action.

Dempster et al., (1955) studied the influence of temperature on structural relaxation in bromated and unbromated doughs mixed in nitrogen and found that at a given temperature, structural relaxation in unbromated doughs tended to become more rapid with increasing reaction time. This effect was greater the higher the temperature. When bromate was present, structural relaxation at any one temperature became progressively slower with increasing reaction time. The change in dough properties increased with increased bromate level. These effects were enhanced by elevated temperatures.

In a study on the effects of bromate and iodate, Sullivan and co-workers (1940) noted that iodate showed its effect more quickly than bromate. Although iodate and bromate ions have essentially the same chemical properties, the rates at which they affect physical dough properties differ widely. The iodate effect is evident in a dough taken from the mixer, iodate reacting almost entirely during mixing, so that its effect is perceptible immediately.

Bromate requires a longer reaction time to show its effect. Sullivan et al., (1940) and Holme and Spencer (1952) presented data to show that improvers in general did not produce equivalent quantitative response in the baking test, and that less iodate than bromate was required to produce the same effect. The effect of iodate on extensograms was reported by Merritt and Bailey (1945), and by Smith and Andrews (1952).

Results of the study of Dempster et al., (1956) suggested that the improving effect of various reagents, i.e., bromate and iodate may not arise from a single specific chemical reaction. Rather each improver may cause a different reaction, each of which produces changes in the rheological behavior of dough. The addition of bromate to dough produced changes in the relaxation constants that differed widely from those produced by iodate and which emphasized again the time-dependence of the bromate reaction.

Suggestions have been made that improver action of oxidizing agents may be due to the formation of disulfide-cross linkages from oxidation of sulfhydryl groups of the gluten (Sullivan, 1954), or the inhibition of interchange reactions in the protein network between disulfide bonds and sulfhydryl groups presumably through the oxidative destruction of sulfhydryls (Goldstein, 1957; Frater et al., 1960).

Bushuk and Hlynka (1960) suggested that iodate and bromate react with the same group (s) in flour, except that iodate reacts much faster. Bushuk and Hlynka (1962) showed that for normal (short) mixing times iodate gave the improver effect over a wide range of concentrations.

The large differences in sensitivity to remixing and in recovery between doughs with different improvers has demonstrated that the improvers act in different ways (Bloksma and Hlynka, 1960). The addition of thiol-blocking

agent NEMI or the oxidant iodate gave doughs showing response, whereas bromate and oxygen are associated with high sensitivity and poor recovery. The behavior of the potassium iodate and NEMI seems to be in favor of the hypothesis that flour improvement is due to disappearance of sulfhydryl groups rather than to the formation of disulfide cross-links, as suggested by Goldstein (1957).

Effect of Sulfhydryl-Blocking Reagents on Dough Properties

It is known that both the soluble and the insoluble wheat protein fractions contain thiol and disulfide groups. There is evidence that these groups are involved in the formation and mixing properties of dough, and in the effects observed when flour or dough is treated with oxidizing agents. More direct evidence comes from the effects which are observed when the sulfhydryl groups are blocked by highly specific reagents such as NEMI and PCMB. These reagents when added to doughs in amounts equivalent to the sulfhydryl content of the flour cause great changes in dough consistencies.

Mecham (1959) studied the effect of the above sulfhydryl-blocking reagents on the mixing characteristics of doughs. The time to maximum dough resistance was shortened, maximum dough resistance was increased somewhat, and dough breakdown rate was greatly increased. Such effects were noted with unbleached, bleached, and bromated flours, and with several levels of reagent. Mecham et al., (1960) suggested that the modification of dough mixing behavior by adding NEMI results from reaction with sulfhydryl groups in the less soluble portion of gluten, as indicated by the shape of mixing curves of doughs that contained various flour fractions treated with NEMI.

Goldstein (1957) used PCMB as blocking agent, and showed by means of

extensograms after a 135-minute rest period that when all available -SH groups were blocked, addition of agents such as ascorbic acid (probably in its oxidized form) and potassium bromate was without effect, and the effect of PCMB on the extensogram was similar to that of bromate or ascorbic acid.

Frater et al., (1960) obtained results which agreed with those of Mecham (1959) on the effect of iodate and NEMI on doughs. Both reagents alter the mixing properties of the dough initially in such way as to increase the resistance to mixing and later to lower it. The effects on the extensograms were even more marked, and these workers concluded that, at any given protein content of flour, the rheological properties of dough are directly related to the number of intermolecular disulfide bonds and the rate at which they can interchange with thiol groups. The action of improvers, both thiol-blocking and oxidizing agents, can be related to the strengthening of the dough through inhibition of disulfide exchange reactions. According to this mechanism the effect of thiol-blocking reagents on the physical properties of doughs as measured by the extensograph, is similar to that of fast oxidizing agents such as iodate. Both types of reagents decrease extensibility and increase resistance to extension. On the other hand, Sullivan et al., (1961) reported that NEMI and PCMB, when used in sufficient amounts to combine with sulfhydryl groups, shorten the mixing time of a flour, and eliminate the beneficial effect of maturing agents such as potassium bromate and potassium iodate. Extensograph and baking data showed that NEMI and PCMB increase extensibility and decrease resistance to extension as compared to the control.

Bushuk and Hlynka (1962) explained the discrepancy of the results cited above. They found that sulfhydryl-blocking reagents and common maturing

agents behave similarly or differently depending on the concentration of reagents and the severity of mixing. NEMI and iodate both gave the normal improver effect when used at comparable concentrations in dough mixed for 2.5 min. under nitrogen. In doughs mixed longer than 5 min. NEMI gave the reverse effect when it was added at a rate higher than the accessible sulfhydryl content of the dough. This reversal effect appears to be due to the breakdown of the dough by the mixing action and was probably caused only partly by the reagent. In doughs treated with iodate in excess of the sulfhydryl content, physical breakdown during mixing was partly inhibited by salt, so that the reverse effect could not be obtained by mixing up to 15 min. In salt-free, iodate-treated doughs, mixing for 15 min. the reverse effect could be obtained. From these results it seems to be clear that different results can be obtained depending on the atmosphere (nitrogen or air) in which the dough is mixed, salt-free or salt-doughs, and on the severity of mixing.

Effect of Reducing Agents on Dough Properties

Reducing agents produce an effect which is opposite to that of oxidizing agents on dough characteristics. Their action is primarily on the gluten proteins. Glutathione, thioglycolic acid, cysteine, and sodium sulfite cause a drop in the viscosity of gluten proteins and produce extreme extensibility, softness, and stickiness (Sullivan et al., 1940; Sullivan, 1954).

Olcott et al., (1943) showed that reducing agents caused marked changes in gluten in the absence of proteases. The effect was an immediate and marked decrease in viscosity. It was also observed that, depending upon the concentration of reducing agent used, the initial viscosity decrease was sometimes

followed by an increase in viscosity. Freilich and Frey (1944) reported that reducing agents, among other effects, shortened dough mixing time to a marked degree, and these effects were not eliminated by oxidizing agents even when present in excessive amounts or in amounts sufficient to overcome the loaf volume reduction due to glutathione. Merritt and Bailey (1945) reported that addition of bisulfite resulted in an increased extensibility and a decreased resistance to extension of doughs tested with the extensograph.

Hlynka (1949) reported the effect of bisulfite and other reducing agents and acetaldehyde on dough and gluten. He showed that the damage from bisulfite action to gluten could be reversed with acetaldehyde, although these compounds do not form an oxidation-reduction system. He also reported differences in action among bisulfite, cysteine, glutathione, and sodium sulfide. Consequently, Hlynka suggested that more than one reaction was involved. Glutathione action was not reversed by acetaldehyde. Hlynka interpreted his results in terms of the cleavage of a cross- linked network structure of dough.

Studies by Matsumoto et al., (1960) showed that addition of acetaldehyde which decreased the free sulfhydryl groups produced by bisulfite, was an evidence for the mode of interaction between acetaldehyde and thiols.

Bushuk and Hlynka (1961) studied the effect of reducing agents such as sodium bisulfite, sodium borohydride, thioglycolic acid, cysteine hydrochloride, reduced glutathione and thiolated gelatin on doughs, prior to the addition of potassium bromate, on the rate of bromate reaction. They reported that for each reagent, the increase in the reaction rate was proportional to the amount added. Increases in the rate were similar for the first 4 reagents,

slightly lower for bisulfite and much lower for borohydride. They also observed with regard to the handling properties of the dough, that all the reducing agents used, except thiolated gelatin, affected the physical properties of doughs to such extent that the doughs were difficult to handle in the usual manner. The doughs were slack, sticky, and highly extensible. As the bromate reaction proceeded, a definite decrease in stickiness, although not entirely comparable to the untreated dough was observed. The results were obtained after 5 hours of reaction time, and it was suggested that the time was insufficient to decrease the sulfhydryl content to the original level in the untreated dough. The effect of thiolated gelatin on the physical properties of dough was significantly different from that produced by other reducing agents. Doughs containing as much as 5 g. of thiolated gelatin (2.5 meq. -SH per g. of dough) did not feel different from control doughs. Two hypothetical explanations, both based on the high molecular weight of the thiolated gelatin used, were offered to explain the absence of a reducing effect of this reagent on dough. First it might be that the rate of the interchange reaction is negligible because of the high molecular weights of the components involved; the increase in the bromate reaction would then be result of the reaction of bromate ion with the sulfhydryls of thiolated gelatin. This later reaction, in aqueous solution of neutral pH, proceeds at about the same rate as the normal bromate reaction in dough. Second, it might be that the physical properties of the protein that results from the interchange are similar to the properties of the original protein, because the relative size of the molecules produced is not very different from the original size.

Hird and Yates (1961) studied oxidation of reduced proteins of gluten

with sodium borohydride, and oxidation of thiol groups of thiolated gelatin by means of iodate, bromate and persulfate. Oxidation of the high molecular moieties by iodate proceeded at a faster rate than oxidation by bromate and persulfate, which was in agreement with oxidation rates obtained with low molecular weight thiols. Disulfide has been shown to be the major product of oxidation, but there is the likelihood of the corresponding sulphinic acid and sulphonic acid being formed as happens with oxidation of cysteine. The increase in disulfide formation with concentration of thiolated gelatin suggests that the proximity of the thiol groups undergoing oxidation will determine the products formed.

Regarding the type of disulfide formed, Benesch and Benesch (1958b) showed that the oxidation by ferricyanide of thiolated gelatin at a concentration of 0.2 per cent produced intramolecular disulfide bonds, and at a concentration of 5 per cent the formation of intermolecular disulfide bonds caused gelling. Hird and Yates (1961) also obtained gel formation in oxidation by iodate of thiolated gelatin at concentrations of 5 per cent and 10 per cent.

The theory of Goldstein (1957), Mecham et al., (1959, 1960), and Frater and Hird's group (1960, 1961) that the flour proteins participate in sulfhydryl-disulfide interchange reactions has been studied also by McDermott and Pace (1961). These authors examined the effect of adding thiolated gelatin to flour-dough. The thiolated gelatin modified the load-extension curves, while the effect of essentially thiol-free gelatin was insignificant. Gluten was then washed from the doughs and the content of hydroxyproline determined. As there is about 15 per cent of this amino acid in gelatin and none in normal gluten, its presence indicated an "associated" gluten of flour

protein and gelatin. A significant portion of thiolated gelatin became firmly associated with the gluten. This degree of binding was not observed with gelatin and sulfhydryl compounds of low molecular weight. These workers concluded that there is not enough evidence at present to decide whether this was due to an interchange reaction leading to the formation of mixed flour protein-gelatin disulfides or whether it was due to association of thiolated gelatin by other mechanisms, for example, by hydrogen bonding, or formation of insoluble disulfide-gelatin.

The purpose of this work was to investigate the differences in the effect of thiolated gelatin, varying in molecular weight and thiol content and compare these effects with that produced by glutathione on rheological properties on doughs and baking potentialities. The effect of oxidants on reversibility of Thiogel and glutathione-induced changes in flour-doughs was also studied.

MATERIALS AND METHODS

The flour samples used in this study were 2 untreated patent flours commercially milled from Hard Red Winter wheats and 4 flours obtained from wheats experimentally milled on a Miag "Multomat" mill. These samples were obtained from Hard Red Winter, Soft Red Winter, Northern Spring, and Durum wheat.

Composition and characteristics of the commercial flours.

	Protein (Nx5.7)(%)	Ash (%)	Farinograph absorption (%)
Sample "A"	11.9	0.43	64.9
Sample "B"	14.4	0.39	62.4

Composition and characteristics of the experimentally milled flours.

	Extraction (%)	Ash (%)	Protein (Nx5.7)(%)	Farinograph absorption (%)
Hard Red Winter	73.17	0.43	10.7	66.4
Soft Red Winter	70.92	0.36	9.1	56.0
Northern Spring	72.65	0.44	13.1	64.6
Durum	66.95	0.56	11.9	53.6

The thiolated gelatins were obtained from Schwarz Bio-Research, Inc., Mount Vernon, N. Y. The properties of these compounds were:

	Average molecular weight	Average -SH equivalents in 100,000 grams
Thiogel A	10,000	14.2
Thiogel B	100,000	13.3
Thiogel C	100,000	3.2

The gelatin was obtained from Difco Laboratories; the oxidizing, -SH blocking, and reducing agents employed were of analytical reagent grade and were purchased from Nutritional Biochemical Corporation, Cleveland, Ohio, or from Fisher Scientific Co., St. Louis, Missouri.

Methods: Moisture, protein, and ash contents of the flours were determined by the methods of the AACC (1957).

Farinograms were made by mixing 50 g. of the flour in a small stainless steel farinograph bowl (56 r.p.m. drive) with sufficient distilled water to give a maximum dough consistency centered around the 500 Brabender unit line. From the results obtained, the absorption, dough development time and valorimeter value were computed.

Absorption is the amount of water necessary to center the farinograph curve around the 500-B.U. line for a flour-water dough.

Dough development time is the time to the nearest half min. from the first addition of the water to the development of the dough's maximum consistency, minimum mobility, immediately before the first indication of weakening.

Valorimeter value is an empirical, single figure, quality score; it is based on the development time and tolerance to mixing and is derived from the farinogram by means of a special template supplied by the manufacturer of the farinograph. The valorimeter value is dependent upon two characteristics of the farinogram: the dough development time or peak time, and the rate at which the dough breaks down after the peak time.

Extensograms were obtained by mixing 300 g. of flour, 6 g. of salt, reducing agents added when used, and distilled water equal to the farinograph absorption (corrected for the salt and use of the large bowl). Doughs were mixed for one min., rested 5 min., and remixed until the curve was centered about the 500-B.U. line. All doughs were non-yeasted. In some cases, the samples were mixed in the Hobart mixer (indicated where applicable), employing an absorption as indicated by the farinogram, and constant mixing time of 5 minutes.

After the dough was removed from the Hobart mixer or from the farinograph bowl, two 150 g. portions were scaled, each rounded 20 times, molded, and placed in the dough holders. No dusting flour was used except in extreme cases of stickiness when a dough could not otherwise have been rounded or molded. The dough holders were placed in the extensograph cabinet maintained at 30°C. Curves were drawn for duplicate doughs after time intervals of

45, 90, and 135 min.. After the first and second stretch the doughs were rounded 20 times and remolded into dough cylinders. To test the effect of potassium bromate in the case that extensograms were done as cited above, at the end of the third stretch (135 min. rest period) the doughs were remixed for 3 min. at 28 r.p.m. in the farinograph bowl to incorporate the oxidant. The doughs were then molded and rested for 135 min. at 30°C in the extensograph cabinet and stretched.

In other cases (as indicated in appropriate places), the dough was removed from the farinograph bowl in a single piece, handled as little as possible, and placed in a dry clean fermentation bowl. A reaction time of 45 min. was given in the extensograph cabinet. After this period, the dough was remixed 3 min. at 28 r.p.m. to incorporate the oxidants or NEMI. Two 150 g. dough pieces were scaled rounded and molded, placed again in the extensograph cabinet for 135 min. and stretched.

For relaxation studies, two batches of dough from 300 g. of flour, 2 per cent salt, and additional reagents were prepared. The water and mixing time in each case were those required for optimum consistency. Six 150 g. dough pieces for following the relaxation process were scaled, balled and molded. After a 45 min. reaction period, one dough piece was stretched without remolding. The other dough pieces were remolded and stretched after time intervals of 0, 5, 15, 30, and 45 min. after molding.

The baking experiments were on a laboratory scale employing the straight-dough procedure.

The basic formula employed in bread-baking was:

Ingredients	Parts
Flour	100
Water	variable
Yeast	2
Sucrose	6
Malted wheat flour	0.5
Shortening	3
Sodium chloride	2
Reducing agents	as indicated

After mixing to optimum consistency, the doughs were fermented at 30° C. After 110 min. the doughs were punched, and 50 min. later punched again, divided, rested for 20 min., and molded. The doughs were proofed at 30° C for 45 min. and baked for 25 min. at 210° C.

RESULTS AND DISCUSSION

The effect of thiolated gelatin on farinogram characteristics of flours is shown in Table 1. Comparing the effect of the Thiogels with either the control or the gelatin-containing flours shows a small decrease in water absorption. No consistent effect on water absorption has been recorded among the three tested Thiogels themselves. It is significant, however, that the Thiogels caused a reduction in the dough development time as might be expected if these agents act as typical dough reducing agents. Thiogel B had the most profound effect and Thiogel C the least effect on decrease either dough development time or valorimeter value. In all samples was observed a slightly more pronounced effect of Thiogel B than of Thiogel A despite the lower sulfhydryl

Table 1. Effect of 0.5% Thiogel on farinograph characteristics of flours.

Reagent	-SH	Commercial H. R. W.			Experimental H. R. W.			Experimental S. R. W.			Experimental H. R. S.			Experimental Durum		
		Absorption	Dough Devel. Time	Valorimeter Value	Absorption	Dough Devel. Time	Valorimeter Value	Absorption	Dough Devel. Time	Valorimeter Value	Absorption	Dough Devel. Time	Valorimeter Value	Absorption	Dough Devel. Time	Valorimeter Value
	(meq./g.flour)	(%)	(min.)		(%)	(min.)		(%)	(min.)		(%)	(min.)		(%)	(min.)	
-	-	67.4	13.0	88	66.4	8.0	76	56.0	9.5	81	64.4	11.0	85	63.6	3.5	59
Gelatin	0	66.9	13.5	89	69.0	10.0	82	57.6	6.0	69	66.2	11.5	85	64.8	3.0	58
Thiogel A	0.71	66.0	7.5	75	65.0	5.0	66	55.8	5.5	68	64.4	7.0	73	62.4	3.0	55
Thiogel B	0.66	65.0	4.5	64	64.0	4.0	61	57.2	3.0	56	64.2	6.0	70	62.6	2.5	53
Thiogel C	0.16	65.0	10.0	82	66.0	6.0	69	57.2	5.5	71	65.0	9.5	81	63.0	3.0	56

content of Thiogel B. This effect might be due to the higher stability of the high-molecular Thiogel B during mixing.

The effect of thiolated gelatin as measured with the extensograph in experimentally milled flours is shown in Table 2. Durum wheat under these conditions could not be tested since the doughs obtained were impossible to handle, even when a short mixing time was given. Thiogel A has the more pronounced effect in increasing extensibility and reducing resistance to extension, consequently the reduction in area of the extensogram was very marked. The effect was similar in the 3 flours tested. Thiogel B and Thiogel C decreased resistance to extension but Thiogel B also decreased extensibility.

The data cited above cannot be compared with those summarized in Tables 3, 4, and 5, because the doughs were prepared under different conditions. The latter doughs were mixed in the farinograph bowl until maximum consistency. The extensograms obtained after 135 min. show that at the levels employed, the three Thiogels increased the extensibility of the tested doughs to an extent comparable to the action of glutathione. Glutathione had, however, a much more pronounced effect, normally associated with reducing agents, namely an increase in extensibility and reduction of resistance to extension.

The pronounced effect of Thiogels A and B on the decrease of resistance to extension was noted with all the flours. The effect of Thiogel C on dough characteristics was less consistent and in the case of Northern Spring wheat flour the resistance to extension after 45, 90, and 135 min. was actually increased. There was no consistent difference between the action of thiolated gelatins having a molecular weight of either 10,000 or 100,000 provided they did not differ in thiol content.

Addition of an excess of bromate to doughs which were previously treated

Table 2. Effect of 0.5% thiolated gelatin on experimentally milled flours.

Wheat flour[1]	Reagent	-SH (meq./g.)	EXTENSOGRAPH DATA AFTER A REST PERIOD OF 45 min. A	B	C	D	90 min. A	B	C	D	135 min. A	B	C	D
Hard Red Winter	-	-	25	160	320	104	23	200	430	124	24	250	540	160
	Gelatin	0	25	140	340	104	22	190	420	112	22	240	470	132
	Thiogel A	0.71	23	60	70	28	26	60	80	30	22	60	90	23
	Thiogel B	0.66	16	190	230	53	16	210	260	52	17	180	220	50
	Thiogel C	0.16	24	160	290	94	23	200	370	109	24	230	430	132
Hard Red Spring	-	-	24	240	540	154	25	290	610	200	25	300	700	230
	Gelatin	0	24	210	470	143	21	300	710	185	22	315	710	197
	Thiogel A	0.71	21	105	135	43	26	130	170	61	24	110	160	53
	Thiogel B	0.66	19	250	390	94	17	290	420	95	19	270	420	100
	Thiogel C	0.16	23	210	420	124	22	290	580	160	22	310	630	164
Soft Red Winter	-	-	22	220	460	131	20	260	420	129	19	310	580	135
	Gelatin	0	22	210	430	114	19	280	540	125	19	270	540	129
	Thiogel A	0.71	24	70	90	30	24	80	100	38	21	70	90	25
	Thiogel B	0.66	15	170	210	43	13	170	200	36	15	170	200	42
	Thiogel C	0.16	21	160	340	92	21	210	390	104	20	250	470	114

[1] All samples were mixed 5 min. in a Hobart mixer at high speed, at optimum absorption. Each sample contained 2% NaCl (flour basis).

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

with reducing agents shows (Tables 3, 4, 5, and 6) that the extent of modification and potential reversal of this modification by adding oxidants varies with the reducing agent used. Whereas the addition of glutathione (Tables 4 and 5) resulted in a most profound, irreversible change in rheological characteristics as assessed by the extensograph, the changes introduced by the thiolated gelatin could be partially reversed (Tables 4, 5, and 6). The extent of reversibility depended both on the type of flour employed and on the sulfhydryl content of the Thiogel added. It is feasible that the profound difference in action between the thiolated gelatins and glutathione is a result of the association of the former with the wheat gluten, as observed by McDermott and Pace (1961). Such an association would tend to confine the action of Thiogels to a limited number of sites on the wheat protein molecule.

The effect of different levels of bromate was studied on a commercially milled flour containing 0.5 per cent of Thiogel B; the data are summarized in Table 7. Extensogram characteristics of one control salt-dough and one gelatin-dough are compared with the doughs containing Thiogel and bromate. There was observed no measurable reversal action using 25, 50, and 100 per cent of bromate equivalent to the per cent of the sulfhydryl groups added in form of thiolated gelatin. Consequently, an equivalent level of 300 per cent bromate was used in subsequent studies.

A study of the effect of bromate, persulfate, iodate, and NEMI on Thiogel-treated doughs is shown in Table 8. The oxidants and the NEMI were used at a level equivalent to 300 per cent of added sulfhydryl groups. The reaction time of the reducing agents with the flours was 45 min., and that for the oxidants or NEMI was an additional period of 135 min.

Table 3. Effect of 0.5% thiolated gelatin on rheological properties of Hard Red Winter wheat flour and response to bromate.

Reagent	-SH (meq./g.)	EXTENSOGRAPH DATA: 45 min. A	45 min. B	45 min. C	45 min. D	90 min. A	90 min. B	90 min. C	90 min. D	135 min. A	135 min. B	135 min. C	135 min. D	135 min. after addition of $KBrO_3$[1] A	135 min. after addition of $KBrO_3$[1] B	135 min. after addition of $KBrO_3$[1] C	135 min. after addition of $KBrO_3$[1] D
-	-	21	280	540	148	15	490	890	162	13	600	940	141	15	440	610	111
Gelatin	0	21	215	440	115	12	570	850	118	12	630	920	115	14	450	670	110
Thiogel A	0.71	23	140	180	61	24	130	170	59	24	130	180	64	19	210	270	71
Thiogel B	0.66	23	220	390	117	21	200	260	79	20	200	260	74	20	200	310	82
Thiogel C	0.16	23	260	470	144	20	400	700	174	18	420	730	161	19	350	590	155

[1] $KBrO_3$ equivalent to 300% of added -SH groups.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

Table 4. Effect of 0.5% thiolated gelatin on rheological properties of Hard Red Spring Wheat flour and response to bromate.

Reagent	-SH	EXTENSOGRAPH DATA															
		45 min.				90 min.				135 min.				135 min. after addition of $KBrO_3$ [1]			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)																
-	-	20	280	580	143	12	560	980	123	12	660	980	141	15	460	770	145
Gelatin	0	22	260	580	151	13	600	980	141	13	650	960	139	16	500	850	153
Thiogel A	0.71	23	130	240	73	23	130	250	77	26	170	350	120	26	250	600	192
Thiogel B	0.66	21	230	390	104	22	240	470	130	22	250	460	133	21	270	530	140
Thiogel C	0.16	25	300	710	226	22	420	900	254	18	510	940	217	16	490	880	177
Glutathione	0.66	25	70	80	31	26	80	110	41	25	150	250	87	24	80	110	38

[1] $KBrO_3$ equivalent to 300% of added -SH groups.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

Table 5. Effect of 0.5% thiolated gelatin on rheological properties of Soft Red Winter wheat flour.

Reagent	-SH	EXTENSOGRAPH DATA															
		45 min.				90 min.				135 min.				135 min. after addition of $KBrO_3$[1]			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)																
-	-	23	150	310	87	13	420	630	93	12	540	720	96	17	300	520	109
Gelatin	0	18	250	450	107	15	360	620	112	13	430	690	108	17	300	500	106
Thiogel A	0.71	22	60	80	26	25	40	80	26	25	90	140	49	25	140	280	91
Thiogel B	0.66	18	140	180	45	20	110	140	37	20	100	130	38	20	130	190	51
Thiogel C	0.16	22	215	410	111	18	310	520	121	18	330	580	133	18	290	460	106
Glutathione	0.66	22	40	40	15	24	50	50	21	22	50	70	24	22	60	60	21

[1] $KBrO_3$ equivalent to 300% of added -SH groups.

A = length, cm.
B = height at 5 cm. B.U.
C = maximum height, B.U.
D = area, cm^2

Table 6. Effect of 0.5% thiolated gelatin on rheological properties of Durum wheat flour.

Reagent	-SH	EXTENSOGRAPH DATA															
		45 min.				90 min.				135 min.				135 min. after addition of $KBrO_3$[1]			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)																
-	-	20	130	160	48	18	190	240	57	18	210	280	64	21	130	160	46
Gelatin	0	19	130	150	36	18	180	240	57	17	220	290	64	22	130	160	47
Thiogel A	0.71	13	40	40	7	20	80	80	21	23	90	100	31	17	280	300	71

[1] $KBrO_3$ equivalent to 300% of added -SH groups.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

Table 7. Effect of different levels of $KBrO_3$ on untreated commercially milled flour (A) containing 0.5% Thiogel B.

Reagent	-SH	$KBrO_3$[1]	EXTENSOGRAPH DATA															
			45 min.				90 min.				135 min.				135 min. after addition of $KBrO_3$			
			A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)																	
-	-	-	20	330	640	160	13	600	980	140	11	720	980	120	15	480	790	137
Gelatin	0	-	20	320	720	172	14	580	980	157	12	680	980	134	14	420	670	116
Thiogel B	0.66	-	20	320	530	147	19	360	590	147	19	380	640	161	21	290	510	144
Thiogel B	0.66	25	20	290	470	122	19	320	510	133	21	300	520	140	21	290	520	144
Thiogel B	0.66	50	20	290	470	128	19	320	540	140	20	300	530	134	22	270	500	146
Thiogel B	0.66	100	19	310	500	124	19	320	530	133	21	270	470	133	21	270	510	143

[1] Equivalent to % of the thiol groups added.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

Table 8. Effect of oxidants and NEMI on rheological properties of a commercially milled flour (A), treated with reducing agents.

Reagent	-SH	Oxidant or NEMI	Oxidant level	FARINOGRAPH DATA: Absorption	FARINOGRAPH DATA: Dough Devel. Time	EXTENSOGRAPH DATA: A	B	C	D
	(meq./g.)		(mg./g.)		(min.)				
-	-	-	-	66.4	14.5	19	300	540	125
0.5% Gelatin	-	-	-	66.0	11.5	21	330	650	145
-	-	$KBrO_3$	0.333	66.4	15.5	18	310	550	121
-	-	$(NH_4)_2S_2O_8$	0.455	66.4	13.0	15	420	640	119
-	-	KIO_3	0.427	66.4	14.5	12	470	610	88
-	-	NEMI	0.250	66.4	14.5	12	480	620	74
Thiogel B	0.66	-	-	62.8	2.5	22	220	340	106
Thiogel B	0.66	$KBrO_3$	0.333	62.8		22	290	530	155
Thiogel B	0.66	$(NH_4)_2S_2O_8$	0.455	62.8		14	410	590	109
Thiogel B	0.66	KIO_3	0.427	62.8		9	690	800	90
Thiogel B	0.66	NEMI	0.250	62.8		10	530	650	82
Glutathione	0.66	-	-	65.2	4.0	24	110	200	66
Glutathione	0.66	$KBrO_3$	0.333	65.2		24	130	240	78
Glutathione	0.66	$(NH_4)_2S_2O_8$	0.455	65.2		25	190	370	128
Glutathione	0.66	KIO_3	0.427	65.2		19	260	340	83
Glutathione	0.66	NEMI	0.250	65.2		21	140	170	48

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area cm^2

The large excess of either bromate or persulfate (in the absence of reducing agents) had no deleterious effect on the doughs, probably due to remixing after the first rest period. In case of iodate and NEMI the remix did not eliminate the deleterious effect of excess of oxidant or sulfhydryl blocking reagent. Addition of oxidants to Thiogel-treated doughs reversed the effect of reductant on rheological properties of the dough, but no measurable effect had been observed when any of the oxidants or NEMI was added to glutathione-treated flour. The highest reversible response was obtained with bromate.

The effects of the Thiogels on the rheological properties of a commercially milled flour are shown in Table 9. The effects of oxidants on the reversal effect on doughs treated with reducing agents are shown in Table 10. The effect of the Thiogels depended on the level of sulfhydryl content. The increase in extensibility and decrease in resistance to extension was more marked in the case of Thiogel A. When oxidants were added to Thiogel-containing doughs, the effect of reducing agents was reversed, the reversal in glutathione-treated doughs was small. The addition of NEMI after glutathione gave a very sticky dough, impossible to handle. The highest response in the reversal action was obtained with bromate. The effect of iodate and NEMI was again very similar. The similarity of behavior of these two compounds has been discussed by Mecham (1959) in relation to mixing.

The rate of relaxation in doughs following the introduction of strains was studied in a flour treated with the three Thiogels and glutathione, and compared with either the control or the gelatin-containing flours.

Table 11 and Plate I show that balling and shaping introduced strains

Table 9. Effect of 0.5% thiolated gelatin on the rheological properties of a commercially milled flour (B).

Reagent	-SH	FARINOGRAPH		EXTENSOGRAPH DATA AFTER											
				45 min.				90 min.				135 min.			
		Absorption	Dough Devel. Time	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)	(%)	(min.)												
-	-	60.7	18.5	24	390	970	293	17	630	980	214	16	650	980	206
Gelatin	0	61.7	20.0	26	290	760	238	15	680	980	187	15	640	980	179
Thiogel A	0.71	59.3	4.0	28	200	390	142	28	210	460	162	28	220	490	176
Thiogel B	0.66	59.7	3.5	27	240	470	172	28	220	480	179	28	220	470	176
Thiogel C	0.16	60.9	21.5	21	450	980	250	14	720	980	180	15	840	980	188

A = length, cm.
B = height at cm., B.U.
C = maximum height, B.U.
D = area, cm^2

Table 10. Effect of oxidants and NEMI on rheological properties of a commercially milled flour (B), treated with reducing agents.

Reagent	-SH	EXTENSOGRAPH DATA															
		Controls				$KBrO_3$[1]				KIO_3[1]				NEMI[1]			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	(meq./g.)																
-	-	19	420	760	182	18	410	670	145	13	670	910	128	12	520	610	76
Gelatin	0	21	340	640	176	21	340	590	168	20	300	440	112	19	340	480	115
Thiogel A	0.71	27	160	380	132	28	240	620	218	12	630	770	99	15	450	610	107
Thiogel B	0.66	28	190	390	146	26	240	560	191	12	710	880	114	16	490	660	133
Thiogel C	0.16	19	360	670	161	16	400	630	127	11	660	780	97	11	620	740	84
Glutathione	0.66	23	100	130	39	25	130	240	75	21	280	380	102	-	-	-	-

[1]Equivalent to 300% of -SH content of Thiogel B.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height
D = area, cm^2

Table 11. Effect of reducing agents on the rate of relaxation of a commercially milled flour (A).

Reagent	-SH (meq./g.)	Measurement	EXTENSOGRAPH DATA Control	1	2	3	4	5
Control	-	A	17	8	9	11	11	12
		B	430	880	920	690	620	620
		C	580	980	980	980	980	980
		D	128	89	91	121	120	120
Gelatin	0	A	16	7	10	10	11	14
		B	380	980	890	880	680	580
		C	820	980	980	980	980	980
		D	150	85	112	117	122	149
Thiogel A	0.71	A	23	9	14	22	24	25
		B	210	860	530	310	260	230
		C	400	980	980	700	530	470
		D	123	100	162	196	174	159
Thiogel B	0.66	A	20	8	13	15	18	24
		B	310	980	650	460	380	340
		C	560	980	980	770	640	600
		D	147	88	154	140	151	157
Thiogel C	0.16	A	16	8	10	9	9	11
		B	500	960	960	960	860	840
		C	880	980	980	980	980	980
		D	168	88	121	104	102	126
Glutathione	0.66	A	27	9	11	17	24	25
		B	100	820	530	300	220	200
		C	240	980	930	560	430	380
		D	86	87	104	115	135	128

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

(Control was stretched 45 min. after molding; doughs 1, 2, 3, 4, and 5 were stretched immediately, 5, 15, 30, and 45 min., rest after molding at the end of the initial 45 min. rest period).

EXPLANATION OF PLATE I

Extensograms of doughs tested at varying time intervals after molding. Control was stretched after a 45 min. reaction period. The other dough pieces were remolded after the reaction time of 45 min. and stretched after time intervals of 0, 5, 15, 30 and 45 min.

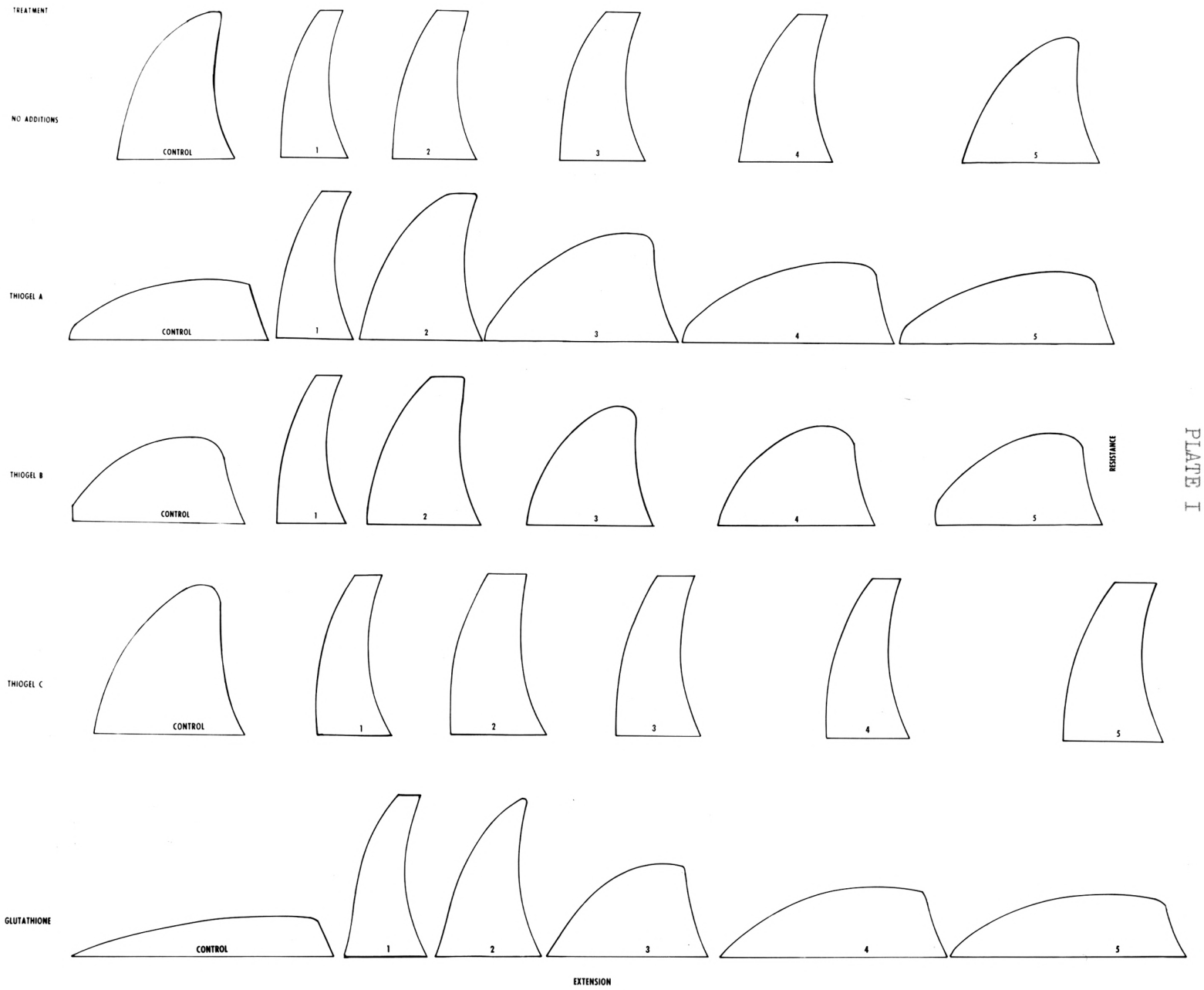
TREATMENT
NO ADDITIONS
CONTROL
1
2
3
4
5
THIOGEL A
CONTROL
1
2
3
4
5
THIOGEL B
CONTROL
1
2
3
4
5
RESISTANCE
THIOGEL C
CONTROL
1
2
3
4
5
GLUTATHIONE
CONTROL
1
2
3
4
5
EXTENSION

PLATE I

in dough that resulted in a dough markedly toughened and shortened. These strains relaxed only slowly in the control doughs. Addition of reducing agents did not prevent the buildup of strains in the dough after balling and shaping but allowed these strains to relax more and at a faster rate than in the control, especially in the case of glutathione. However, no relaxation was observed with Thiogel C during a period of 45 min. after the strains were introduced.

In Table 12 are summarized the data of the effect of bromate and NEMI on relaxation studies of the doughs treated with Thiogel B. Both bromate and NEMI decreased the rate of relaxation although to a different extent. NEMI decreased the rate of relaxation to a larger extent than did bromate, a slow oxidizing agent. Frater et al., (1961) suggested that the major effect of NEMI or of iodate was to stabilize the strained and/or oriented structure normally introduced into the dough previously, during the balling and shaping process. The rapid action of NEMI in the blocking of sulfhydryl groups does not permit the dough to relax like in the case of bromate that has a slow action.

The baking test was performed with a commercially milled flour ("B") treated with gelatin, the three Thiogels, and glutathione at different levels. The results are presented in Table 13. Thiogel A and B at the 0.5 per cent and 1 per cent level decreased the loaf volume and affected somewhat the other bread characteristics. Thiogel C seems to have under certain conditions a slight improvement effect.

Glutathione added at a comparable sulfhydryl level had a detrimental effect throughout the whole course of the baking process. The loaf volume was considerably decreased and the bread characteristics were affected

Table 12. Effect of $KBrO_3$ and NEMI on the rate of relaxation of a commercially milled flour (A), treated with 0.5 Thiogel B (0.66 meq. -SH/g. flour).

Reagent	Level of reagent[a]	Measurement	EXTENSOGRAPH DATA					
			Control	1	2	3	4	5
$KBrO_3$	12.5	A	18	8	10	10	11	15
		B	410	980	980	940	790	620
		C	760	980	980	980	980	980
		D	173	94	119	116	134	175
$KBrO_3$	100	A	18	8	9	12	12	13
		B	490	980	980	840	780	820
		C	810	980	980	980	940	940
		D	189	90	109	142	142	165
NEMI	100	A	12	7	9	8	9	10
		B	760	980	980	980	980	880
		C	980	980	980	980	980	980
		D	140	80	105	92	100	104

[a]Equivalent to % of the thiol groups added.

A = length, cm.
B = height at 5 cm., B.U.
C = maximum height, B.U.
D = area, cm^2

(Control was stretched 45 min. after molding; doughs 1, 2, 3, 4, and 5 were stretched immediately, 5, 15, 30, and 45 min. rest after molding at the end of the initial 45 min. rest period).

Table 13. Effect of reducing agents on baking characteristics of a commercially milled flour (B).

Reagent	Reagent Level	-SH	Loaf Volume	Crust Color	Symmetry	Break & Shred	Texture	Grain	Crumb Color
		(meq./g.)	(cc)						
Control	-	-	945	9	10	9	16	16	10
Gelatin	0.10	0	1005	9	10	10	17	15	10
	0.25	0	1015	9	10	9	17	14	10
	0.50	0	1005	9	9	8	18	15	10
	1.00	0	965	9	10	8	18	15	10
Thiogel A	0.10	0.14	975	9	9	8	17	15	10
	0.25	0.36	915	9	8	8	17	16	10
	0.50	0.71	810	9	8	7	16	16	10
	1.00	1.42	710	9	8	6	12	12	7
Thiogel B	0.10	0.13	915	9	8	7	16	16	10
	0.25	0.33	970	9	8	8	17	15	10
	0.50	0.66	875	9	8	7	17	16	10
	1.00	1.32	800	9	9	7	16	17	10
Thiogel C	0.10	0.03	995	9	8	7	17	17	10
	0.25	0.08	970	9	9	8	16	16	10
	0.50	0.16	1005	9	8	7	17	16	10
	1.00	0.32	985	9	8	7	18	16	10
Glutathione	0.00	0.13	910	9	8	7	17	17	10
	0.01	0.33	785	9	9	6	16	15	10
	0.02	0.66	650	9	9	4	14	13	7
	0.04	1.32	560	9	7	0	10	10	6

adversely. These data confirmed the results obtained in the study of rheological properties of the dough treated with the reducing agents.

Baking tests of the four experimentally milled flours containing the reducing agents are summarized in Table 14. The results obtained were similar to those obtained with the commercially milled flour. The effects of the reducing agents depended on the kind of flour employed. Whereas glutathione had a consistently highly detrimental effect, the thiolated gelatins varied in their effect on the different flours. High levels of Thiogel A or B decreased loaf volume and bread quality appreciably. Thiogel C, low in sulfhydryl content, had little if any, effect on flours milled from Hard Red Winter, Soft Red Winter, or Durum wheat. Both the commercially milled strong flour, and the experimentally milled flour from Northern Spring wheat seem to be improved slightly by the addition of Thiogel C. This effect is comparable to that observed on addition of very low levels of proteases and seems to be a mellowing effect of the "bucky" high-protein flour.

SUMMARY AND CONCLUSIONS

The addition of three thiolated gelatins varying in molecular weight and sulfhydryl content, resulted in a slight decrease in farinograph water absorption and in a pronounced reduction in dough development time and valorimeter value. Thiolated gelatins and glutathione increased the extensibility and decreased the elasticity and extensogram area of flours milled experimentally from 4 classes of wheat or of 2 commercially milled untreated flours. The extent of modification varied with the reducing agent and flour employed. Glutathione had consistently the most detrimental effect, the low

Table 14. Effect of reducing agents on baking characteristics of experimentally milled flours.

Flour	Reagent	-SH (meq./g.)	Loaf Volume (cc)	Crust Color	Symmetry	Break & Shred	Texture	Grain	Crumb Color
Hard Red Winter	-	-	780	7	9	8	16	15	10
	0.25% Gelatin	0	850	7	9	8	17	15	10
	Thiogel A	0.35	775	8	9	8	16	15	10
	Thiogel B	0.33	790	8	9	8	16	17	10
	Thiogel C	0.08	795	8	8	6	16	16	10
	Glutathione	0.33	705	8	8	6	15	14	10
Soft Red Winter	-	-	710	6	8	6	14	14	10
	0.25% Gelatin	0	750	7	8	7	15	15	10
	Thiogel A	0.35	700	7	7	6	14	14	10
	Thiogel B	0.33	690	6	8	6	14	14	10
	Thiogel C	0.08	725	7	8	7	15	14	10
	Glutathione	0.33	695	6	9	7	15	14	10
Northern Spring	-	-	900	9	8	7	16	16	10
	0.25% Gelatin	-	905	9	10	8	16	16	10
	Thiogel A	0.35	875	9	10	8	16	17	10
	Thiogel B	0.33	835	9	10	8	16	16	10
	Thiogel C	0.08	965	9	10	10	18	16	10
	Glutathione	0.33	810	9	10	8	17	16	10
Durum	-	-	675	9	9	6	14	14	7
	0.25% Gelatin	0	660	9	9	6	14	14	7
	Thiogel A	0.35	650	9	9	4	12	14	7
	Thiogel B	0.33	550	9	6	1	13	12	7
	Thiogel C	0.08	665	9	8	4	14	14	7
	Glutathione	0.33	550	9	6	1	10	10	7

sulfhydryl containing thiolated gelatin exerted a smaller effect than did the gelatins containing higher sulfhydryl levels. The rheological modifications induced by the reducing agents could be reversed by the addition of excess of oxidants or N-ethylmaleimide. The extent of reversibility depended on the extent of modification resulting from the action of the reducing agents. It was highest in the low thiol-containing gelatin, and lowest in the case of glutathione addition. The addition of an excess of bromate or persulfate was cancelled on remixing whereas no such effect was noted on addition of iodate or NEMI.

Glutathione and thiolated gelatins accelerated the rate of relaxation of strains introduced by mechanical treatment. Addition of NEMI to doughs containing thiolated gelatin cancelled the rate of relaxation completely, bromate reduced the relaxation rate to a smaller extent.

Glutathione and high levels of thiolated gelatin of high thiol content impaired substantially volume and characteristics of bread from the experimentally milled and from a commercially milled flour. Thiolated gelatin of low thiol content had in case of the commercially milled, high-protein flour and the flour milled experimentally from a Northern Red Spring wheat, a slight beneficial effect. This improvement seemed to be due to "mellowing" of the "bucky" dough.

SUGGESTIONS FOR FUTURE WORK

The purpose of this work was to investigate the differences in the effect of reducing agents varying in molecular weight and thiol content on rheological properties and bread baking potentialities of wheat doughs. The results obtained have suggested several additional areas for future

investigations.

It would seem highly desirable that the changes observed as a result of the addition of either glutathione or thiolated gelatin be followed by measurements of changes in thiol content of the treated flours, and separation (electrophoretically or with molecular sieves) of the products of reduction. Use of thiolated gelatins of a molecular weight comparable to that of wheat proteins seems preferable to a study in which the small molecular weight glutathione is employed. It seems, however, that additional and more useful information could be obtained by studying the effect of thiolated or reduced flour proteins from various wheat classes.

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THE ROLE OF SULFHYDRYL GROUPS IN FLOUR

by

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The purpose of this work was to investigate the effects of reducing agents varying in molecular weight and thiol content on rheological properties and bread baking potentialities of wheat doughs. In view of the large molecular size of the thiolated proteins and its possible association with wheat gluten, the effect of oxidants on reversibility of Thiogel-induced changes in flour doughs was studied.

The addition of three thiolated gelatins varying in molecular weight and -SH content, and glutathione, resulted in a slight decrease in farinograph water absorption and in a pronounced reduction in dough development time and valorimeter value. Thiolated gelatin and glutathione increased the extensibility and decreased the elasticity and extensogram area of the flours milled experimentally from 4 classes of wheat or of two commercially milled untreated flours. The extent of modification varied with the reducing agent and flour employed. Glutathione had consistently the most detrimental effect, the low -SH containing thiolated gelatin excerted a smaller effect than did the gelatins containing higher -SH levels. The rheological modifications induced by the reducing agents could be reversed by the addition of an excess of oxidants or N-ethylmaleimide. The extent of reversibility depended on the extent of modification resulting from the action of the reducing agents. It was highest in the low thiol containing gelatin, and lowest in the case of glutathione addition. The addition of an excess of bromate or persulfate (in the absence of reducing agents) was cancelled on remixing whereas no such effect was noted on addition of iodate or NEMI.

Glutathione and thiolated gelatin accelerated the rate of relaxation of strains introduced by mechanical treatment. Addition of NEMI to doughs containing thiolated gelatin cancelled the rate of relaxation completely, bromate reduced it to a smaller extent.

Glutathione and high levels of thiolated gelatin of high thiol content impaired substantially volume and characteristics of bread from experimentally milled and from a commercially milled flour. Thiolated gelatin of low -SH content had in case of the commercially milled, high-protein flour and the flour milled experimentally from a Northern Spring wheat, a slight beneficial effect. This improvement seemed to be due to "mellowing" of the "bucky" dough.