PHYSICO-CHEMICAL CHANGES IN GLUTEN ACCOMPANYING AGING

James Donald McCaig

Department of Field Crops

University of Alberta

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INTRODUCTION

The development of new varieties of spring wheat, adapted to the prairie provinces, has progressed rapidly in recent years. Before a new variety can be distributed, it is essential that careful consideration be given to its quality characteristics.

The term "quality" is a relative one, and is significant only when used in connection with the purpose to which the product concerned is to be put. Hard red spring wheat of high quality possesses the characteristics necessary to produce large loaves of good texture, color, and flavor. The quality characteristic with which the present investigation is chiefly concerned is that of inherent baking strength, which is determined by the amount and properties of the gluten in the flour. Variation in the amount of gluten and its properties may be associated with variations in soil and climatic conditions.

Within the province of Alberta the same variety of wheat may vary in protein content from about 8% to 20%. Also marked variation in gluten properties has been demonstrated. Similar differences between varieties grown on the same field are frequently apparent. Two sources of variability in gluten content and properties are therefore well recognized. These are environmental and genetical. There is evidence that the environmental factor is more potent in affecting gluten content, while genetical influence has the more pronounced effect on gluten properties.

As an illustration of genetical differences in gluten quality, reference may be made to a confidential report issued by the Associate Committee on Grain Research (1939). Three newly developed rust resistant wheats were submitted to overseas institutions with a view to determining the suitability of these varieties for the baking industry. The consensus of opinion indicated that there were differences in gluten characteristics between the three varieties. These differences were exposed by means of fermentation tests. C.T. 118 exhibited increasing strength with dough development, the characteristically inherent strength enabling it to withstand long periods of fermentation. The strength, however, would not be available to the baker unless he modified his customary baking procedure. Thatcher, possessing a tougher, shorter gluten, reached maximum dough development in a relatively

short fermentation period. C.T. 118 differed so markedly from typical Manitoba wheat in gluten characteristics that it was discarded. The baking procedure used by commercial concerns would not develop the strength of this variety to its maximum. Therefore to maintain the necessary uniformity, which would give the best results with present baking procedures, C.T. 118 was excluded from commercial grades.

The environmental factors markedly affect gluten quantity. Their influence on the keeping properties of flour has also been observed (1). Wheat from relatively infertile areas of the province produces flour which deteriorates rapidly, whereas wheat obtained from more productive areas yields flour which retains its quality for comparatively long periods of storage. Quality changes accompanying aging of wheat flour have been studied in this laboratory (20). The tendency has been to follow the aging process by means of chemical analysis, but many of the results obtained with these methods cannot be interpreted with our present knowledge of fundamental principles. It was therefore necessary to study the problem by other methods. Methods involving measurements of changes in physical properties have been adapted to follow the changes accompanying aging. The results of a few experiments of a chemical nature are also included in this paper.

LITERATURE REVIEW

In 1907 Thomas B. Osborne published the results of an investigation on the wheat proteins (14). On a basis of solubility in water, alcohol, dilute acids, alkalis and neutral salt solutions, five distinct protein substances were recognized. As a result of this work, the identity of two gluten proteins was considered to be established. The gluten mass, consisting of about 80% of the total protein of wheat, was made up of glutenin and gliadin in approximately equal proportions.

Gliadin, distinguished by its solubility in 70% alcohol, was considered the most important constituent of gluten, imparting the necessary qualities for bread making. The glutenin, as extracted from the residue of the alcohol extraction with potassium hydroxide, had an ultimate elemental composition similar to gliadin, but differed from that substance in its insolubility in alcohol, its physical properties, and differences in the products of hydrolysis.

The view that gluten is made up of gliadin and glutenin, which impart to that mass its physical properties (14), is not as widely accepted today as formerly. Sorenson (21) has postulated a theory of proteins as "reversible-dissociable component systems". Most soluble proteins are not made up of individual

chemical compounds, but rather a series of components or complexes. Within each component the atoms are linked by main valencies, and the various components are comparatively loosely knit by residual valencies which each component is assumed to possess. The component linkages must be comparatively weak, and the application of various salt concentrations, hydrogen ion changes, alcohol concentrations and temperatures, gives rise to reversible dissociation of the component systems involved. When such alterations in the composition of the solution occur as render the formation of a component system insoluble or sparingly soluble under new conditions, such a system will tend to precipitate. Thus Sorenson regards solubility differences as being due to the heterogeneous chemical nature of the protein micelles. Gortner (19) maintains essentially the same viewpoint, but differs in that he attributes solubility differences to the physical heterogeneity of the protein micelle. Since the individuality of glutenin and gliadin has been questioned (3, 13, 17), it might be possible that Sorenson's conception could be extended to include the protein of gluten.

Since the publication of Osborne's researches, the individuality of his separations has been questioned. Gortner and his associates peptized the gluten complex with various solutions of inorganic salts, and were unable to classify the peptized portions as a definable substance.

Blish states that gliadin, as prepared by Osborne, is not a simple unaltered homogeneous substance but a derived protein. Sandstedt and Blish (17) and McCalla and Rose (13) have indicated that Sorenson's concepts might be applied to gluten proteins. The first mentioned authors distinguish three main component groups; the latter have not been able to distinguish special groups, and conclude that gluten is a single protein complex which may be separated into numerous fractions differing both in physical and chemical properties. Blish has arrived at his conclusions from fractional solubility studies, whereas McCalla and Rose used fractional precipitation methods. Blish has failed to demonstrate any systematic and progressive chemical and physical differences in successive fractions.

The previous work in this laboratory, dealing with the fractionation of gluten and the chemical and physical variations of the fractions, might be considered a background for this work, which is a physical study rather than a chemical one. A brief summary of this previous work is presented.

Critical studies on the extent of hydrolysis of gluten proteins dispersed in various solvents, including dilute alkali and acid, and the neutral solvents urea and sodium salicylate, established the superiority of sodium salicylate as a dispersing agent (6). Using sodium

salicylate, definite evidence of change in physical properties with aging was demonstrated (20). The dispersibility of poor or deteriorated flour was not complete, whereas fresh undeteriorated flour dispersed in approximately three hours. McCalla and Rose (13) dispersed gluten and precipitated successive fractions from the dispersion with increasing concentrations of magnesium sulphate. These fractions (excluding the 10-15% which was most soluble) contained successively more amide and less arginine nitrogen. When the fractions were re-dispersed and precipitated as a whole, gluten was recovered. They concluded from this work that the most soluble 10-15% of gluten is possibly a distinct protein substance, but that the remainder is a single protein complex which can be separated into many fractions differing systematically and progressively in physical and chemical properties.

Blish (3) recognized only three component groups using fractional solubility methods, and criticized the above hypothesis of a protein complex. Spencer and McCalla (22), employing the same principle, were able to obtain results agreeing very well with those obtained using the precipitation method. They attributed Blish's inability to obtain differences in physical and chemical properties to the lack of penetration of sodium salicylate and to the successive removal of portions of the most soluble 10-15%, which, being portions of an individual

substance, naturally would not exhibit chemical differences.

Lipoids are known to have an influence on the quality and keeping properties of flour (12, 23). Investigation here has indicated their importance in stored flour (20). It was shown that gluten owes its properties, to some extent, to the presence of the more insoluble lipoids. Alcohol extraction resulted in a condition similar to deterioration; the addition of ground germ largely restored gluten to its original condition. Evidently the more insoluble germ lipoids were the effective agents. The wetting of flour with alcohol, and its subsequent evaporation without the removal of extracted products soluble in alcohol, also resulted in a condition akin to deterioration. Thus the lipoids of flour are important constituents, probably forming a protein-lipoid complex which is altered by the action of alcohol.

The previous studies of gluten and flour, conducted in this laboratory, provide fairly conclusive evidence that gluten proteins are of a heterogeneous nature. However, little work has been done on the physical behaviour of gluten. Considerable work has been done in other laboratories, however.

Wood and Hardy discussed the amphoteric nature of gluten proteins (24), and their conclusions with little modification are accepted today. However, their attempt to explain the strength of flour on a basis of the presence of electrolytes is not.

Gortner and his co-workers demonstrated that the properties of gluten were not dependent on the presence of electrolytes, but that there were inherent differences in the properties of gluten from different wheats regardless of the quantity and variety of electrolytes present. Their conclusions were reached as a result of studies involving the swelling of gluten in acid-salt solution (10) and viscosity studies (11, 18). They concluded that "the acid and salt contents of flours are not responsible for the differences between strong and weak flours, and the difference is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties such as pertain to emulsoids, and that of a colloidal gel in which these properties are much less marked."

The principles underlying the previously mentioned work (10, 11, 18), also apply to the patented process of Berliner and Koopman (2) and Ruemele (16). The former workers utilize the variability in swelling of glutens in weak acids to classify flours as weak or strong, while the

latter uses variability in viscosity of gluten dispersions in lactic acid. Others (9) have attempted to apply Berliner and Koopman's technique to measurements of flour quality. However, the results were not closely related to quality measurements derived from the baking test.

The work of Bungenberg de Jong (4, 5) on the behaviour of glutenin and gliadin mixtures at various hydrogen ion concentrations has a direct bearing on the present study. His glutenin and gliadin preparations cannot be considered as distinct individual compounds, but the significance of the work is hardly affected by this fact. He observed (4) that the properties of mixtures were not the same as the properties of the two components in a solution between certain pH ranges. This deviation from the additive law indicated an interaction between components. Presumably the pH range was between the iso-electric points of the glutenin and gliadin preparations. This interaction suggested that charges on the particles must be the cause. Between the two iso-electric points, the one component, gliadin, would be positively, and the other, glutenin, would be negatively charged. At pH values outside the range of iso-electric points the components present would possess the same charge, either both negative or both positive. His components were dissolved in 0.001 N sodium hydroxide and phosphate buffers. The pH values, where interaction

occurred, were between 5.3 and 6.6. Within this range mixing tended to neutralize the charges. The mixture exhibited the greatest turbidity at different pH levels depending on the proportions of the two preparations used. It was concluded that an interaction between the two preparations in the indicated pH range occurred, and complete separation of components in this range was not to be expected.

If gluten is considered as a component system (13), Bungenberg de Jong's preparations must be regarded as two main component groups, the iso-electric point of each being the point at which the negative charges of some of the components just balance the positive charges of the remainder. If his two preparations were contained in proportions originally occurring in gluten, the iso-electric point, if it can be termed such, would be the point of balance of total positive and total negative charges. Bungenberg de Jong places this point at approximately pH 6.0.

In a previous study (20) the water absorbing capacity of deteriorated gluten was observed to be lower than that of gluten from a fresh undeteriorated flour between pH 4 and 7.5. Similar changes were also observed in various glutens between pH 6.8 and 7.5. No explanation was given for this behaviour, but studies have been continued and the work is reported in this paper.

MATERIALS AND METHODS

Materials

Factors which indicate the quality of flour from hard red spring wheat include protein content and loaf volume. Gluten quality cannot easily be correlated with such direct measurements, but it can be judged partially by the appearance and texture of the gluten. Data concerning the material used and other information significant to the present study are presented in Table I. Since a discussion of particular comparisons is dealt with throughout the paper, these data are not elaborated at this point. All flours with the exception of No. 4 were experimentally milled long patents, and were unbleached. Sealed containers were used for storage of flours in the laboratory, and the moisture content varied from 10-12%.

A supply of wheat harvested in 1936 was stored in a bin at 20°C. and samples from this were milled in the springs of 1937, 1938 and 1939. For comparison between fresh and aged flour, a sample from the 1937 milling was stored in a sealed container for two years. Another portion of the same flour was stored in a desiccator for one month at 40°C. over saturated ammonium sulphate. At the end of this period the flour had attained a moisture content of

TABLE I

Data pertaining to flours used in aging studies

Flour No.	Variety	Time from milling mo.	Protein in flour %	Acidity %++	Wet gluten+ %	Quality of wet gluten	Dry gluten+ %	Loaf volume** cc.
1	Garnet	6	8.9	0.83	20.5	Very poor	8.0	480
2	Garnet	6	13.5	0.55	41.6	Fairly good	14.6	668
3	Mixed	54	13.8	0.75	41.2	Very poor	16.0	430
4	Soft wheat*	--	6.9	0.29	18.9	Soft	6.4	410
5	Garnet	30	9.7	0.83	33.6	Very poor	11.7	340
6	Garnet	18	10.9	0.64	30.8	Poor	11.8	435
7	Garnet	6	8.9	0.65	25.2	Poor	9.6	439
8	Garnet	6	13.4	0.49	40.7	Fairly good	14.8	657
9	Red Bobs	6	8.5	0.31	25.2	Fairly good	9.2	564
10	Red Bobs	6	14.3	0.43	47.0	Good	16.2	874
11	Reward	2	15.0	0.27	55.0	Excellent	18.4	985

* A commercial cake flour.

** Malt-phosphate-bromate formula.

\+ Obtained at pH 6.8.

++ Expressed as C_{18} fatty acids.

20%, and showed the characteristics of a deteriorated flour. To prevent growth of moulds and bacteria, the desiccator was rinsed with toluene and, as a further precaution, a small open container of that substance was placed in the desiccator.

Methods

Washing Glutens at Various pH Levels.

In the preparation of gluten balls the procedure of Dill and Alsberg (7) was followed. Washing solutions were prepared using 0.1% phosphate buffers, the range being from pH 3.7 to 8.5. Sufficient flour was weighed out to yield 2.5 grams of moist gluten. A dough ball was made by mixing the flour with a minimum amount of washing solution. This ball was kneaded between the fingers while a constant stream of the particular solution involved bathed the mass. This procedure continued for 3.5 minutes, at the end of which time the washed gluten was immersed in the solution for one hour, when it was removed, weighed and dried "in vacuo" at 98°C. for 24 hours. The buffer solution used in forming the dough in the first operation was used in subsequent operations of kneading and immersion.

Gluten Swelling in 0.1 N Acetic Acid.

The method of Gortner and Doherty (10) formed the basis for the measurement of the swelling of gluten in 0.1 N acetic acid tests. Individual gluten balls rather than discs cut from gluten were used, because it was found that the majority of the glutens could not be flattened out to cut satisfactory discs. All samples were washed at pH 6.8. The washed glutens were placed in a series of beakers containing 0.1 N acetic acid. At regular intervals triplicate samples were removed, drained of excess solution on porcelain filters and weighed. The acid concentration of 0.1 N was used because preliminary tests showed that gluten from the poor flours had a much higher hydration capacity than the type of gluten studied by Gortner and Doherty (10), and the use of more dilute acid resulted in prolonged swelling time.

Berliner and Koopman Method.

The method of Berliner and Koopman (2), as outlined by Fisher and Halton (9), was tried on two flours. One gram portions of moist gluten were broken up into small pieces, and placed in florence flasks containing 25 cc. of lactic acid. The neck of the flask had been previously calibrated in cubic centimeters. The

flasks were inverted at intervals of one-half hour for a period of five hours, and the volume occupied by the gluten mass noted.

Acidity.

Acidity was determined by the Greek method (8) using tincture of curcuma as an indicator. The results are expressed as C_{18} fatty acids.

Dispersion of Gluten.

Gluten from 10 grams of flour was dispersed in 8% sodium salicylate. To facilitate penetration, mechanical stirring was done twice in the 24-hour period of dispersion. At the end of 24 hours the dispersion was centrifuged at 2500 R.P.M. for 20 minutes, and the liquid decanted into vessels of appropriate size.

Precipitation of Dispersed Proteins.

The decanted solution was treated with calculated volumes of magnesium sulphate. This solution was stored at 10°C. over night. Centrifuging and decantation as used in the original dispersion were repeated.

Nitrogen Analysis.

Precipitates from the fractionations were hydrolysed in 20% hydrochloric acid for 22 hours. This was followed by filtering and washing, the filtrate being collected in a 100 cc. volumetric flask. Aliquots for the determination of total and amide nitrogen were taken. The total nitrogen determination was made by the Kjeldahl method, using mercuric oxide as a catalyst. Amide nitrogen was determined by the aeration method of Plimmer and Rosedale (15), except that magnesium oxide was used instead of calcium oxide.

RESULTS

Part I. Physical

Preliminary Experiments.

Preliminary experiments involved a study of the effect of the pH of washing solutions on the water content of gluten, and also on the swelling of gluten in 0.1 N acetic acid. The glutens were obtained from flours exhibiting various degrees of quality, the criterion of quality being the baking test. The 1937 results were checked in 1938 with a second series of flours. Both

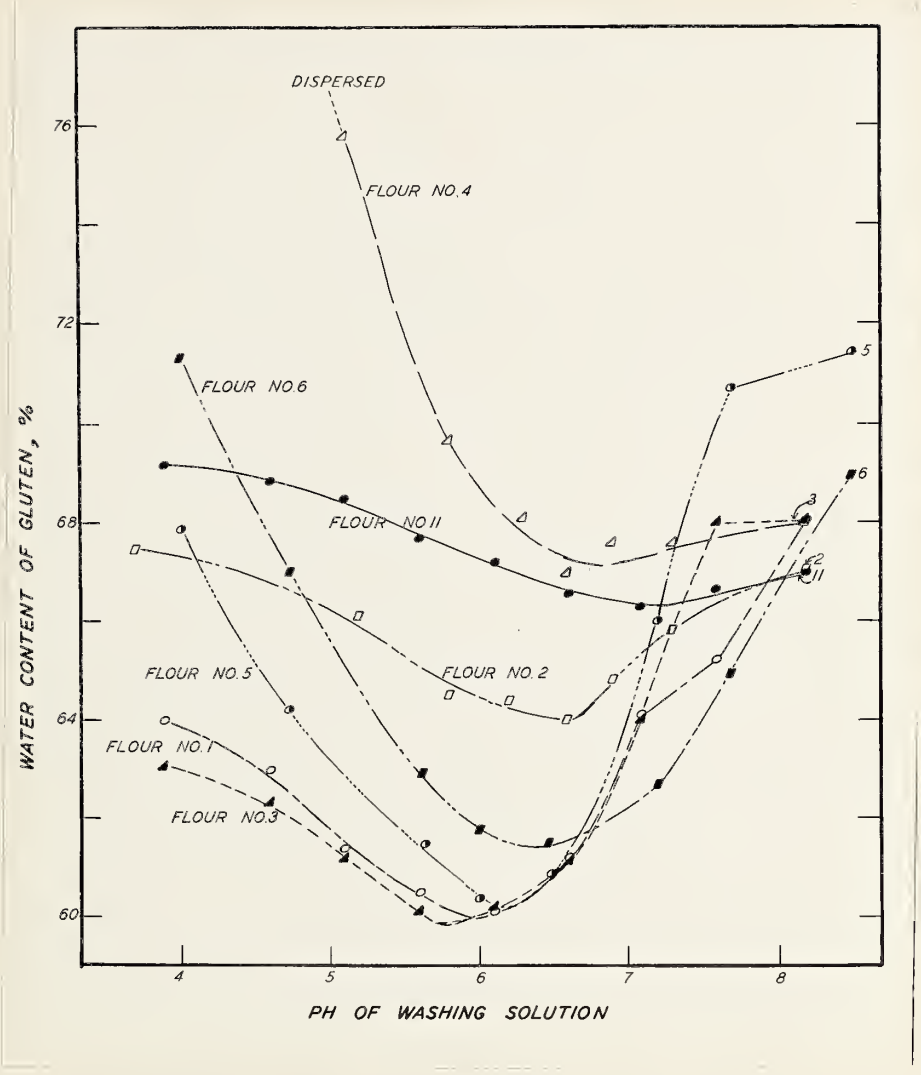

Figure 1

The effect of pH on the hydration capacity of various glutens

years' results are presented and discussed together, and are shown in Figure 1.

The results are comparable to those presented by Sinclair and McCalla (20), but are much more comprehensive. Two main conclusions may be drawn from this experiment. The water-holding capacity of glutens from different flours, and the pH at which the glutens exhibit a minimum water-holding capacity, decrease with the quality of the gluten. The quality data are presented in Table I. The remarks on quality of wet gluten are determined by the appearance, texture and feel; these factors give good indications of gluten quality. The gluten from soft wheat flour (No. 4) had the highest water-holding capacity of any of the glutens; however, it lacked elasticity, being very soft and extensible. The gluten from flour No. 11, a typical high grade spring wheat type, possessed excellent elasticity, remaining firm and extensible at all pH's of the washing solution. The glutens from flours Nos. 1, 3 and 5 were coarse, open and short, the term "short" indicating a lack of elasticity. Those from Nos. 2 and 6 were intermediate in quality characteristics, No. 2 being decidedly superior to No. 6. The weakness exhibited by flour No. 4 is decidedly different from that of Nos. 1, 3 and 5; the physical nature of this gluten is entirely unlike that of hard wheat gluten, possessing a slackness or fluidity not apparent in the gluten from the other flours.

Results of gluten swelling experiments are illustrated in Figure 2. The results are expressed as increases in percentage water in the gluten. These increases present an entirely different picture, since the poor quality glutens have a much higher hydration capacity in 0.1 N acetic acid. The poor glutens did not disperse in 2.5 hours, while the flour from No. 11 dispersed in less than 2 hours. At the end of 2.5 hours gluten No. 1 contained only 9% dry matter, but still formed a coherent mass which could be handled. In contrast to this, No. 11 dispersed while still containing almost 20% dry matter. The relation between flours Nos. 4 and 11 is in accord with the results of Gortner and Doherty (10).

The great hydration capacity of the poor quality gluten has not, as far as is known, been reported before. The gluten from deteriorated flour disperses less readily in sodium salicylate than does the gluten of a fresh high quality flour (20). The results indicate that this is also true in acid solutions, and that poor quality freshly milled flour yields gluten similar to that of deteriorated flour, although the hydration capacity seems to be even greater at a low pH (approximately 3.0).

Effect of Acid and Salt Concentration on Gluten Swelling.

The fact that differences in the concentration of acids and salts present in gluten do not determine

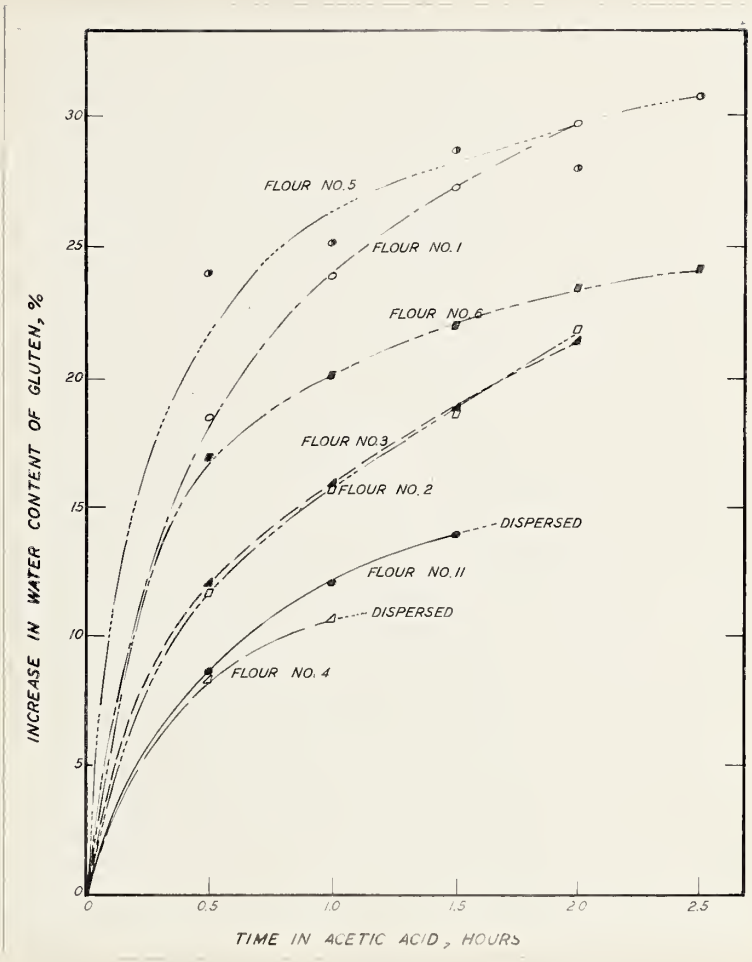

Figure 2

The swelling of glutens in 0.1 N acetic acid at intervals throughout 2.5 hour periods

the variability in the physical properties of that gluten has been established (10). Fisher and Halton (9) found that if 2% sodium chloride solution were used to wash out gluten, differences in swelling shown by the Berliner and Koopman method (2) disappeared. In order that our results might be comparable to those obtained by others, it was necessary to show that differences obtained using 0.1 N acetic acid could be obtained using more dilute acid and varying concentrations of salt. Experiments were carried out with flours Nos. 5 and 11 which represented extremes of quality. The results are presented in Table II. The glutens were washed in various concentrations of acid solution, and acid solution with added quantities of salt.

The greatest differentiation between the two glutens occurred at the highest acid concentration. However, the maximum swelling of the better gluten was in 0.1 N acetic acid. At all concentrations except 0.001 N the increased hydration capacity of flour No. 5 was evident. Increasing concentrations of salt decreased swelling of the glutens, but their relative positions as indicated in Figure 2 were maintained. The differences in the original properties of the gluten could not be due to salts present, since the gluten exhibiting the greatest swelling was decidedly higher in ash.

TABLE II

Effect of acid and salt concentration on hydration of gluten, one hour immersion

Effect of acid			Effect of salt		
Acid concentration Norm.	Increase in % water		Salt concentration %	Increase in % water	
	No. 5	No. 11		No. 5	No. 11
0.001	3.0	3.8	0	25.1	12.5
0.01	12.1	10.1	0.05	22.3	--
0.02	16.5	10.9	0.1	15.2	5.0
0.05	19.5	12.3	0.2	11.9	2.0
0.1	21.8	12.5	0.5	3.0	0.7
0.5	24.8	12.3	1.0	1.5	0.4
Ash in flour, %	0.67	0.40			
Ash in dry gluten, %	0.84	0.58			

It is concluded that the differences noted among the glutens (Figure 2) would be obtained if the method of Gortner and Doherty (10) had been followed exactly.

Berliner and Koopman Method.

The method of Berliner and Koopman (2) involves the same principles as does that of Gortner and Doherty (10). However, the method of measurement is different. Berliner and Koopman consider that a high swelling number indicates good quality gluten. Flours Nos. 5 and 11 were tested using this method. The results are presented in Table III.

TABLE III

Swelling numbers of gluten (Berliner and Koopman)

Time hr.	No. 5	No. 11
0		
0.5	11.8	4.1
1.0	17.6	5.1
1.5	23.7	7.7
2.0	27.1	7.8
2.5	30.0*	9.8
3.0	--	11.1
5.0	--	16.9

* Maximum possible to measure.

At the end of two hours the glutens from both flours had disintegrated to a considerable extent, but measurements were continued where possible. Observation indicates that the swelling number does not represent the real volume of the swollen gluten, as large amounts of water were entrapped among the pieces of gluten. The difference in the behaviour of the two glutens agrees with the other methods (10). According to the method of Berliner and Koopman, the gluten of sample No. 5 is superior in quality. This is incorrect. At the end of 22 hours the gluten from flour No. 11 had dispersed, but that from No. 5 was granular, the particles being fine and not settling readily, a behaviour similar to that of poor gluten in sodium salicylate.

Effect of Aging of an Individual Flour on Water Absorption of Gluten.

The results presented in Figures 1 and 2 indicate a marked difference in the physical properties of gluten from fresh and deteriorated flours. The flours, however, were obtained from various sections of the province, and wheats are known to differ in quality, a condition partially due to their origin.

Equally large differences, as exhibited by fresh and deteriorated flours, were also obtained with relatively fresh flours (Compare Nos. 1 and 11). Therefore, flour No. 11 was selected for a study, extending over a period of 24 months, to determine the changes which take place during aging. A portion of the flour was artificially aged and used in a similar study. No. 11 was selected because of its excellent quality, and because subsequent changes induced by aging could be more readily followed in this type. Uniformity was also assured, the sample having been grown on our own plots.

The results of gluten washing tests are given in Figure 3, and of swelling tests in Figure 4. The quantity of artificially aged flour was insufficient for the latter test.

Two years' storage of flour resulted in pronounced changes in the physical properties of the gluten; these changes resulted in a gluten similar to that obtained from any of the aged flours illustrated in Figures 1 and 2. Artificial aging for one month at 20% moisture and 40°C. had the same effect. As with the poor quality glutens of flours 1, 3 and 5, both the water-holding capacity and the pH at which minimum absorption occurs were reduced with aging. The glutens

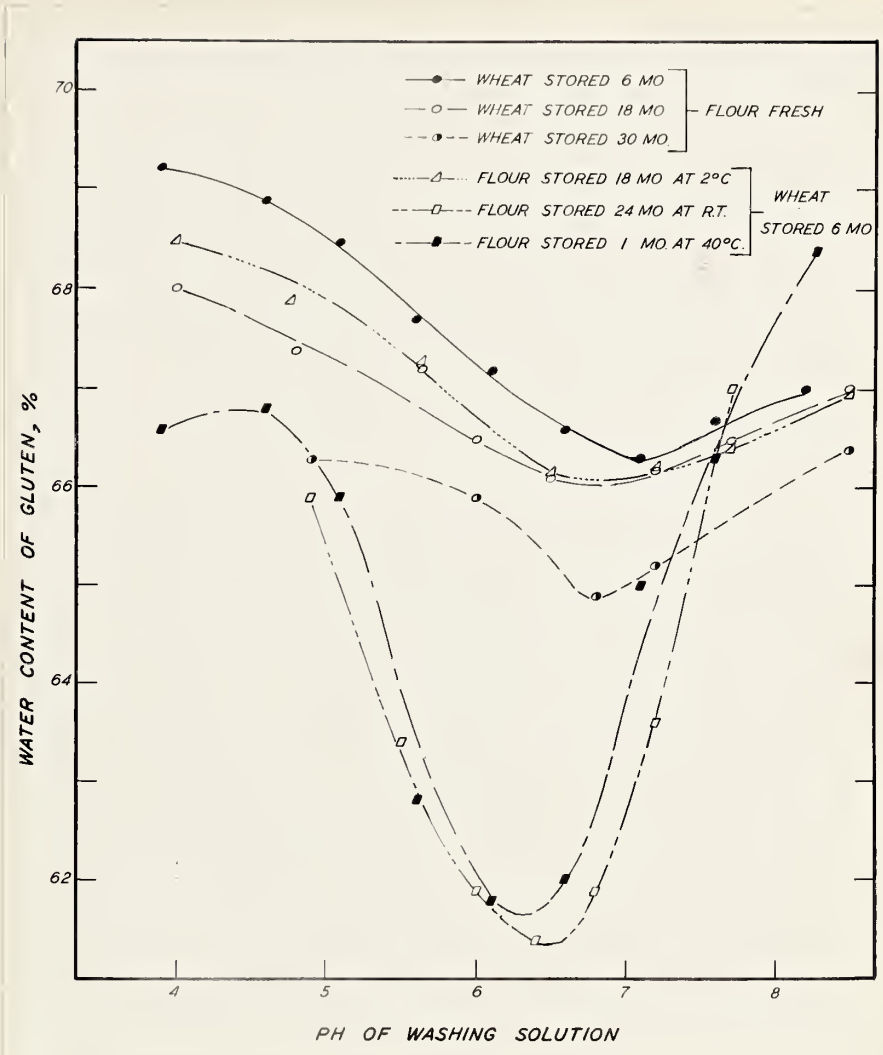

Figure 3

The effect of pH on the hydration capacity of gluten from an individual flour determined throughout a 24-month aging period

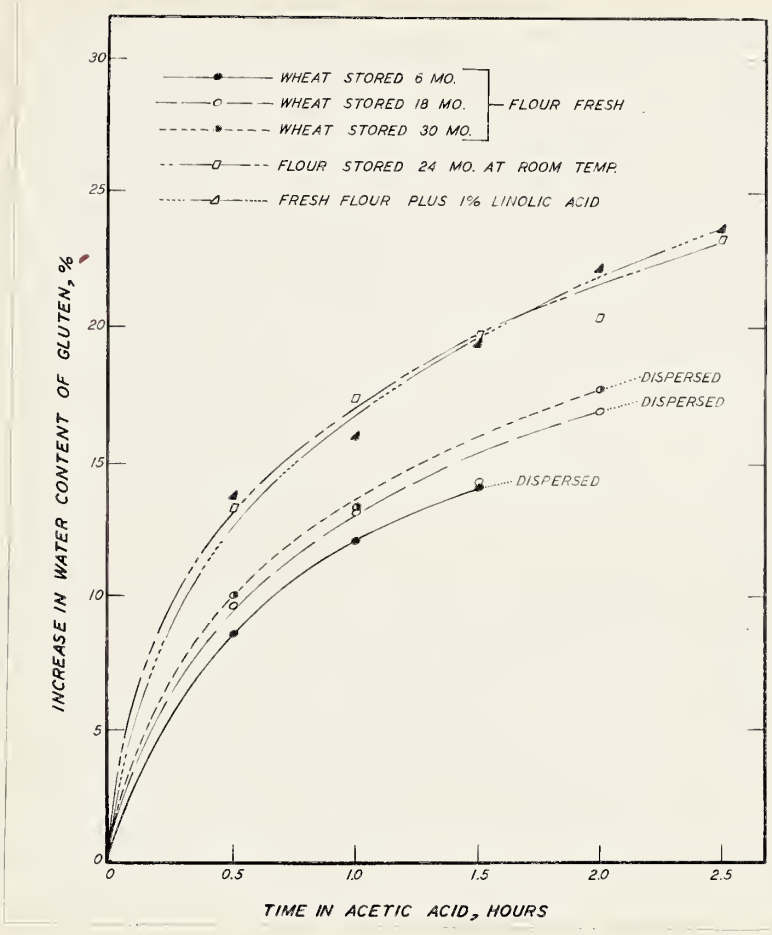

Figure 4

The effect of acetic acid on the swelling of gluten from an individual flour throughout intervals of 24 months

were coarse, open and short, lacking the extensibility of the original flour. The loaf volume of the bread baked from the naturally aged flour had decreased approximately 30% from that of the original.

The results also indicate that storage of the whole kernel resulted in gradual changes in the properties of the gluten. The rate of change was much slower than that observed in stored flour. A small amount of the flour from this wheat had been stored at 2°C. for 18 months. The flour at the end of this period was subjected to the washing procedure. The results (Figure 3) show that the change in gluten properties was small compared with the change which took place when the flour was stored at room temperature.

Effect of Variety and Origin of Wheat on Gluten.

The results contained in Figures 1 and 2 also showed that freshly milled samples of flour yielded glutens of widely differing quality. A great deal of the work carried on in this laboratory has been concerned with differences in quality of wheat produced on two of the three main types of soil found in Alberta, the black fertile loam, and the relatively infertile podsolic loam or gray soil. The former, typical of the Edmonton

district, is characterized by a thick black surface layer of loam, rich in organic material. The latter is typical of much of the northern and western portions of the province, being deficient in organic materials and badly leached. In general, the quality of wheat from the gray soil areas is poor, and that from the black loam good. This difference is apparent in variation in protein quantity, but protein quality differences have also been noted (1).

The results summarized in Figure 5 illustrate these differences. Flours 7 and 9 were milled from Garnet and Red Bobs wheat, respectively, grown on the podsolic loam, while flours 8 and 10 are from the same varieties grown on the black loam.

The results indicate differences not only in the gluten from wheat grown on the two soil areas, but also differences in gluten from the two varieties are apparent. These differences are in accord with baking results, except that the protein quantity factor has been removed.

Effect of Ether Extraction and Linoleic Acid on Gluten.

The results presented in Figure 4 suggested that changes in physical properties of gluten accompanying deterioration are similar to the changes

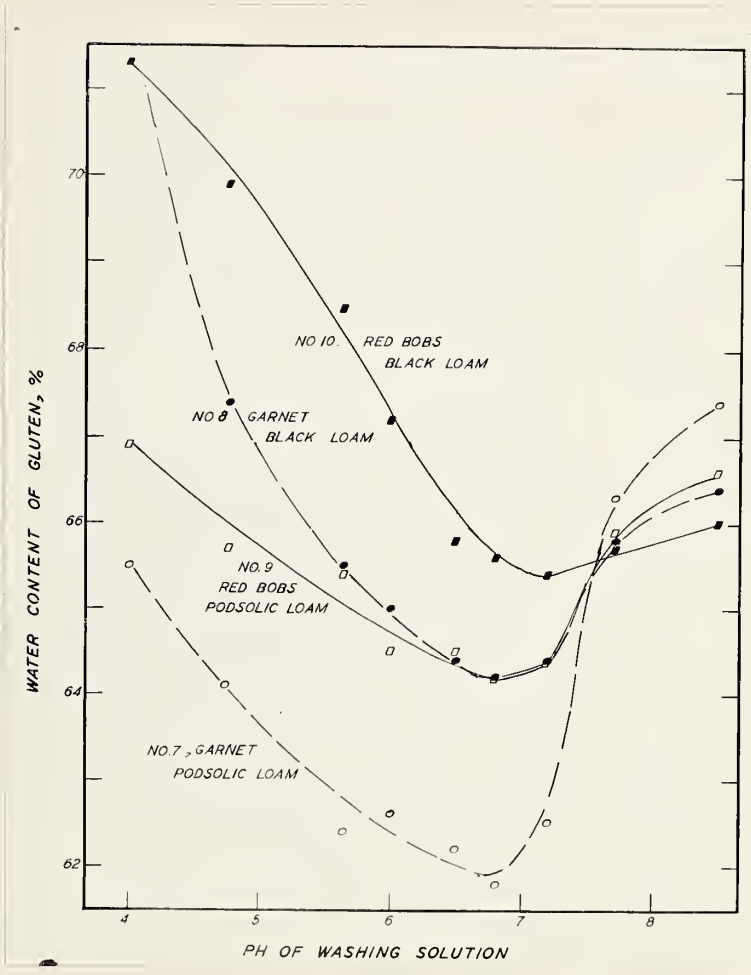

Figure 5

The effect of variety and origin of wheat on the hydration capacity of the gluten

resulting from the addition of linoleic acid. The effect of the addition of unsaturated fatty acids to flour has been investigated in this laboratory (20). On the introduction of fatty acids, a condition similar to deterioration resulted. An accumulation of these acids should be considered as a factor in the process of aging, but the breakdown of a complex between protein and the more insoluble lipoids was considered more important, at least to baking quality.

Samples of flours Nos. 3 and 11 were extracted with ether (20), and the glutens from these washed in solutions of various pH values. Linoleic acid was added to other samples of ether extracted flour, compensating for the fatty acids removed by extraction. The results are presented in Figure 6.

Ether extraction increased the water-holding capacity of gluten from flour No. 11 at all pH values and of gluten from No. 3 at all values below 6.5. The improvement in water-holding capacity is attributed to the removal of fatty acids by extraction. The gluten from flour No. 3 was still coarse, open and short, an indication that extraction had not removed all the effects of aging. The flour originally was one of excellent quality, giving a smooth elastic gluten quite comparable to that from No. 11. The addition of linoleic acid to

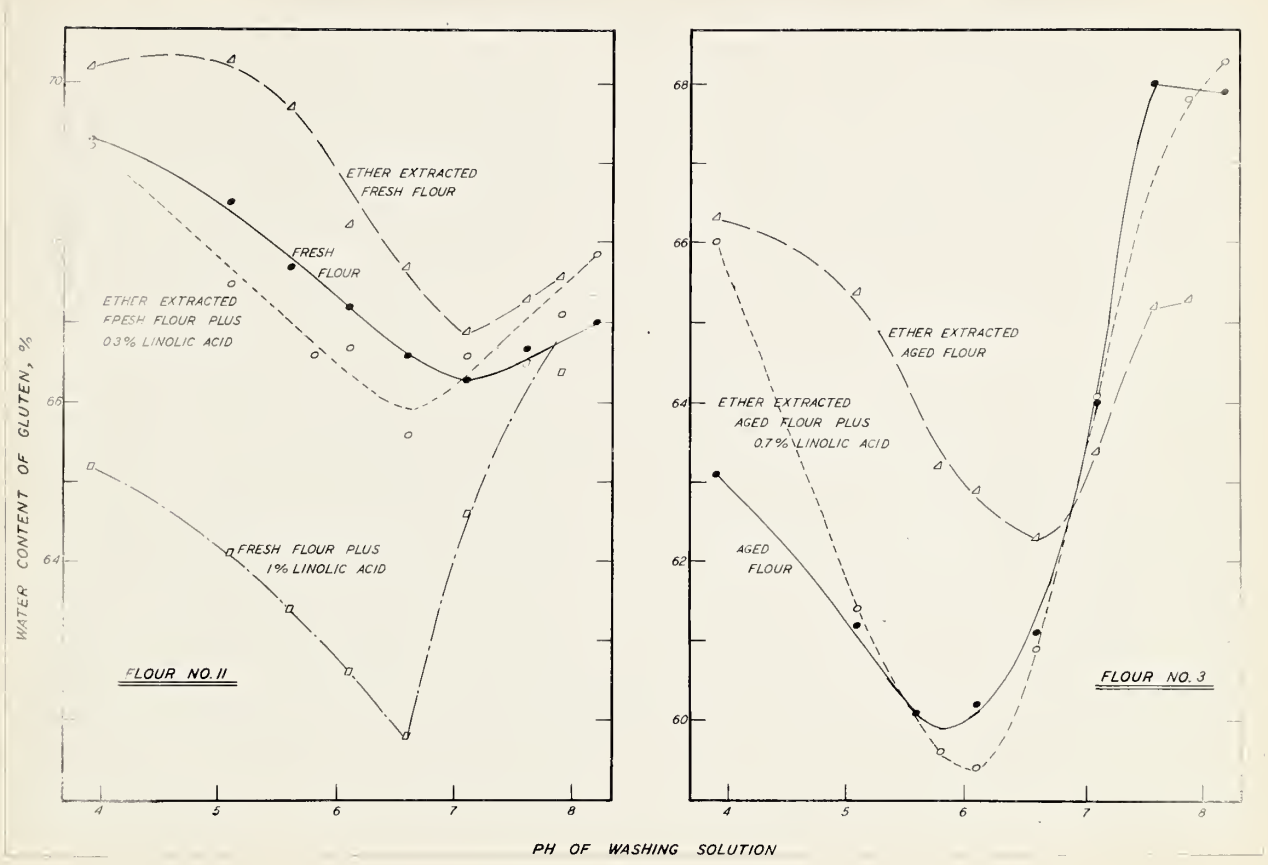

Figure 6

The effect of the addition of linoleic acid to flour on the hydration capacity of the gluten

compensate for extracted acids made the gluten from extracted flour comparable to that of gluten from unextracted flour.

Previous workers (20) believe that there are at least two factors which determine the quality of gluten from deteriorated flour, namely, the presence of a protein-lipoid complex, and the accumulation of fatty acids. The results partially substantiate this hypothesis, in that the removal or addition of fatty acids to flour will affect gluten quality. The accumulation of naturally occurring fatty acids during aging is presumed to result from the breakdown of the protein-lipoid complex. The competition of the acids with the more insoluble lipoidal substances largely determines the type of gluten complex formed on washing. If the fatty acids are extracted, gluten quality is improved, but unless important lipoids are available good quality gluten cannot be obtained.

Part II. Chemical

The nature and extent of deterioration or aging of flour markedly affects the physical properties of the gluten. The alterations in physical properties are accompanied by chemical changes (20). An attempt

has been made to follow these chemical changes by an analysis of fractions using the methods of McCalla and Rose (13).

The Effect of pH of Sodium Salicylate on the Fractionation of Gluten Proteins and on the Amide Nitrogen of These Fractions.

Bungenberg de Jong (4) suggested that complex formations resulting from the interaction of his prepared components might make separation impossible. The sodium salicylate solutions used in this laboratory had a pH value close to 6.0, the region in which Bungenberg de Jong (4) considers complex formation to be at a maximum. To justify the continued use of the fractionation methods employed in this laboratory, it was necessary to fractionate gluten proteins at other pH values. If Bungenberg de Jong's suggestion was correct, conclusions based on results obtained at pH 6 would not be substantiated by those obtained at higher or lower pH values.

A quantity of sodium salicylate (8%) was prepared, and portions of this adjusted to pH 5.3, 6.3 and 7.2 by the addition of sodium hydroxide or salicylic acid. Gluten was obtained from flour No. 11.

Preparation of gluten and the subsequent operations of dispersion, precipitation and nitrogen analysis were carried out as outlined in the methods.

The results are presented in Table IV. The difference in behaviour of the three dispersions at the second fraction is quite marked, and even the fourth fraction from pH 5.4 salicylate solution precipitated more readily than the other two. From Bungenberg de Jong's work it might have been expected that precipitation would occur most readily at pH 6.3. While pH certainly affects the fractionation of the gluten proteins, the evidence does not indicate a more ready separation of components at pH 5 or 7 than between these values. The real test of this suggestion, as applied to fractionation from sodium salicylate solution, lies in the analysis of the fractions. If separation of components is not complete at pH 6.3, but is complete at lower or higher pH values, then the amide values for the various fractions would not fall on a single curve; but determinations obtained from the pH 6.3 fractions would be different from the others. The percentage amide nitrogen determinations are presented in Figure 7. There are no significant differences in distribution of amide nitrogen in the fractions from these three dispersions, since the amide nitrogen content is determined by the position of the fraction in the

TABLE IV

Effect of pH of sodium salicylate solution on the fractionation of gluten protein and the composition of the fractions

Concentration of magnesium sulphate, % of saturation	Gluten nitrogen precipitated			Mid-point of fractions			Amide nitrogen, % of total nitrogen of fraction		
	pH 5.4	pH 6.3	pH 7.2	pH 5.4	pH 6.3	pH 7.2	pH 5.4	pH 6.3	pH 7.2
0	8.6	8.5	6.6	4.3	4.2	3.3	13.6	14.8	12.5
1.5	28.0	17.7	11.4	17.7	13.1	9.0	19.0	17.6	16.1
6.0	62.8	55.9	55.9	45.4	36.8	34.0	24.3	24.4	23.2
20.0	92.8	87.9	89.6	77.8	71.9	73.2	26.0	25.9	25.8

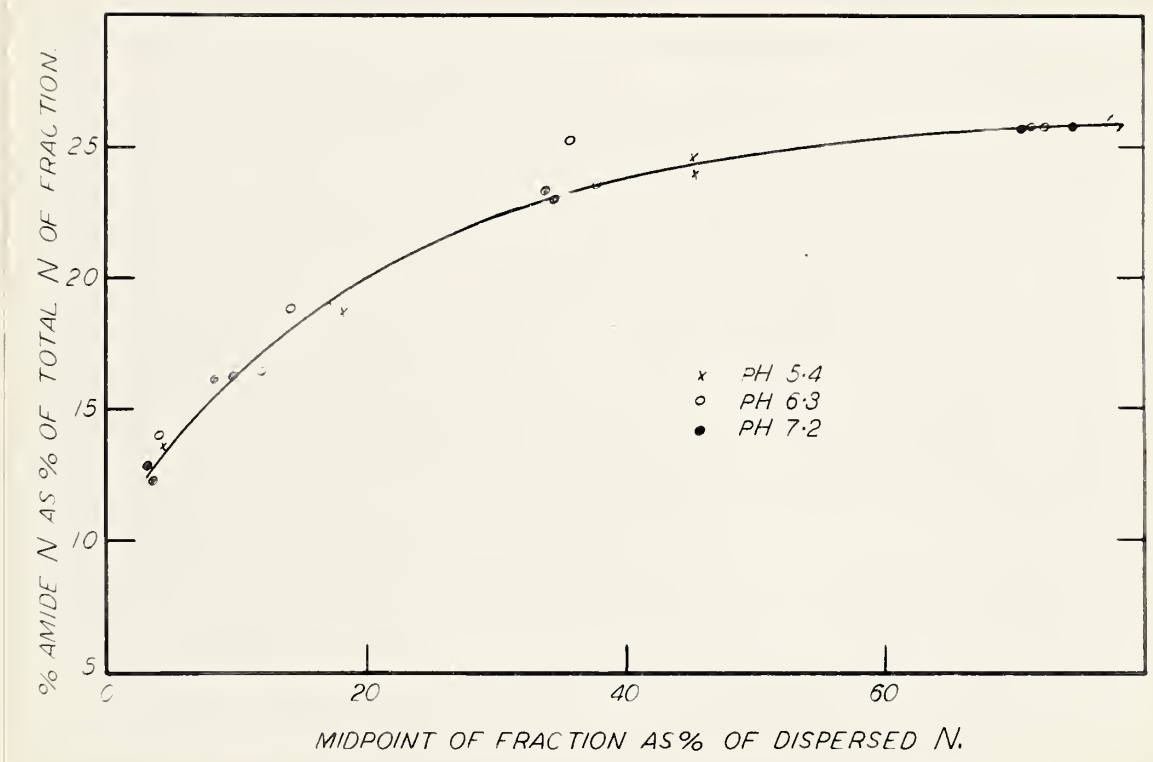

Figure 7

The effect of pH on the fractionation and amide nitrogen analysis of gluten

complex, and not by the concentration of magnesium sulphate used in the precipitation. It is therefore concluded that, within the range from pH 5 to 7, there is no essential difference in the separation of protein fractions.

The Dispersibility of Gluten as Affected by Quality.

Glutens were washed from all the flours included in Table I, and were dispersed in sodium salicylate. The non-dispersed fractions were centrifuged off and hydrolysed. Calculated amounts of magnesium sulphate were added to make the dispersed fraction 20% saturated. The resulting precipitate was washed and hydrolysed. Total and amide nitrogen were determined on the hydrolysates. The results of this experiment are presented in Table V.

The dispersibility of glutens from the flours varied from approximately 97% of the gluten protein washed from good quality flour, to as low as 58% of that from poor quality flour. There was a definite relationship between gluten quality and dispersibility (20). The glutens from flours Nos. 1 and 11 exhibit extremes of quality, the former, yielding a very poor gluten, had a non-dispersed fraction of 32.8% of the total nitrogen; the latter, giving excellent gluten, only 4.2%.

TABLE V

Dispersion and analysis of the resulting fractions of flour samples Nos. 1-11

Sample	Non-dispersed fraction			Dispersed fraction (precipitated with 20% magnesium sulphate)	
	% of total nitrogen	Mid-point of fraction	Amide nitrogen % of total nitrogen	Mid-point of fraction	Amide nitrogen % of total nitrogen
1	32.8	16.4	19.0	58.9	25.1
2	9.8	4.9	17.5	50.1	23.7
8	8.2	4.1	15.4	44.2	24.5
3	7.6	3.8	16.6	49.0	23.2
4	6.8	3.4	17.0	49.6	24.7
9	6.0	3.0	15.7	46.2	23.3
5	4.4	2.2	17.8	47.1	23.3
7	4.4	2.2	15.2	47.0	23.7
6	3.8	1.9	16.3	47.7	24.1
10	3.2	1.6	15.9	44.8	24.0
11	4.2	2.1	17.7	48.8	23.7

Other glutens, considered intermediates of these extremes, do not show such marked differences. The amide nitrogen percentages of the non-dispersed fractions vary between 15% and 19%, and in the dispersed fraction from 23% to 25%. These results may be considered as a verification of other work (20).

The Effect of Aging on the Fractionation of Gluten Proteins and the Composition of the Fractions.

Two flours were used in this experiment. Flour No. 11 was artificially aged for a period of 60 days. Samples were removed at intervals, and the gluten washed from these samples dispersed in 8% sodium salicylate. The dispersed and non-dispersed fractions were analysed for total and amide nitrogen. Flour No. 10 was artificially aged for 30 days. At the end of this period the gluten was washed from this flour and dispersed in sodium salicylate. Successive fractions were precipitated from the dispersion and analysed, as were those from gluten of flour No. 11. A sample of gluten from the fresh flour (No. 10) was also fractionated progressively with magnesium sulphate. The results are presented in Tables VI and VII. Aging leads to a decrease in the fractions dispersed in sodium salicylate in both cases. The data

TABLE VI

Effect of aging flour from Reward wheat on the fractionation of gluten protein and composition of the fractions

Time of aging* days	Non-dispersed fraction			Dispersed fraction			Total recovery of amide nitrogen mgm.
	% of total nitrogen	Mid-point of fraction %	Amide nitrogen % of total nitrogen	% of total nitrogen	Mid-point of fraction %	Amide nitrogen % of total nitrogen	
0	4.0	2.0	17.7	88.9	48.4	23.6	23.35
6	4.1	2.0	17.9	89.0	49.0	23.5	23.25
10	5.8	2.9	17.3	83.2	47.4	23.5	23.09
20	10.7	5.4	18.3	75.7	48.5	24.4	23.65
30	17.4	8.7	18.7	67.3	50.1	24.7	23.46
40	26.3	13.2	18.8	60.0	56.3	24.8	22.97
60	42.1	21.0	21.1	44.6	64.4	25.3	23.25

* Aged at 20% moisture content and 40°C. (Stored over saturated ammonium sulphate in a desiccator).

TABLE VII

Effect of aging of flour from Red Bobs wheat on the fractionation of gluten protein and composition of the fractions

Concentration of magnesium sulphate % of saturation	Fresh flour			Flour aged 30 days		
	% of total nitrogen	Mid-point of fraction %	Amide nitrogen % of total nitrogen	% of total nitrogen	Mid-point of fraction %	Amide nitrogen % of total nitrogen
0	4.0	2.0	16.7	19.6	9.8	18.8
0	4.2	2.1	17.6	22.4	11.2	19.4
1.5	31.4	19.6	19.3	26.4	32.8	21.8
1.5	29.4	18.9	19.5	20.2	32.6	21.8
3.5	17.5	44.2	24.4	11.3	52.6	25.9
3.5	17.9	42.5	24.0	11.1	48.2	24.6
6.5	17.1	61.5	25.8	15.7	65.1	26.0
6.5	18.0	60.5	25.7	13.6	60.5	25.3
50	29.5	84.8	23.4	26.6	86.2	22.0
	30.1	85.5	23.6	26.7	80.7	20.7

presented in Table VI indicate that a decrease in dispersibility is accompanied by an increase in the amount of amide nitrogen in both the dispersed and non-dispersed fractions. In explanation of this observation, it is suggested that the portion of the originally dispersed fraction which fails to disperse with aging is lower in amide nitrogen than the original dispersible fraction, but higher than the original non-dispersible fraction. Therefore its withdrawal from and addition to the dispersed and non-dispersed fractions, respectively, leads to an increase in percentage amide nitrogen of both. Thus, fresh flour had a non-dispersible fraction representing 4% of the total nitrogen. As aging proceeded, the non-dispersible fraction increased to 42.1%. The percentage amide nitrogen increased in both the dispersed and non-dispersed fractions, the increases being from 17.7% to 21.1% and 23.6% to 25.3% in the non-dispersed and dispersed, respectively. Gluten from flour No. 10 exhibited a decrease in dispersibility, whereas that from fresh flour had a non-dispersed fraction of 4.0%. The same flour subjected to artificial aging had a non-dispersed fraction of about 20%. These data are in complete accord with previous results (20). It is observed that the results presented in Table VI show the effect of progressive aging, and those in Table VII

the effect on multiple fractionation of aged gluten as compared with fresh gluten. There seems to be no evidence of fundamental change in the chemistry of the gluten, but the differences found are attributable to changes in the physical properties. The total recovery of amide nitrogen, determined at various intervals during the aging process, indicates that there is no alteration of the protein mass as a whole.

DISCUSSION

The conception of gluten as a complex of protein and lipoids has been discussed by Sinclair and McCalla (20) and Blish (3). It was suggested by the first mentioned authors that the physical changes in gluten, which accompany aging, are due to two factors: first, the breakdown of the protein-lipoid complex; and, second, the accumulation of end products of which fatty acids were considered most important. Ether extraction of deteriorated flour resulted in a marked improvement, whereas alcohol extraction of fresh flour had a deleterious effect, decreasing the solubility in sodium salicylate of the gluten from that flour. The addition of wheat germ to the alcohol extracted flour

restored the solubility of its gluten. The same authors concluded that the insoluble lipoids as extracted with alcohol conferred on gluten the qualities of extensibility and elasticity. It was postulated that the lipoids formed an adsorption complex with gluten, and alcohol extraction is able to break that complex.

In the present study the addition of fatty acids to flour has been shown to have an effect similar to deterioration. Solubility in sodium salicylate of gluten washed from flour treated with linoleic acid decreases (unpublished data). If a particular type of lipoidal substance is necessary to produce high quality gluten, the following conclusions may be drawn from the results presented in this study. High quality flour produces coherent, extensible and elastic gluten because the essential lipoids are adsorbed on the protein of the gluten. Fatty acids added to a high quality flour compete with the lipoids for the adsorption bonds, and if they are present in sufficient quantity may replace lipoids to such an extent that the gluten loses much of its coherence and extensibility. As flour ages the essential lipoids break down, and fatty acids accumulate. As this process continues the flour will yield coarser, shorter, more open gluten. When fatty acids are extracted the quality of the gluten is improved, as the

fatty acids no longer compete with the remaining lipoids. The original quality of the gluten is not restored because the breakdown of the original lipoids has proceeded too far.

In this study another type of flour was encountered. This flour (No. 1) did not yield a good quality gluten even at the time of milling. There is a possibility that the lipoidal substances necessary to produce a high quality gluten complex were not originally present in the flour, that is, they had not been metabolized in the wheat plant.

These conclusions permit the acceptance of Bungenberg de Jong's hypothesis (4). If gluten is considered as a single colloidal complex made up of various components plus one or more lipoidal substances, this general hypothesis fits all the results obtained in the present study. A complex of proteins would have an iso-electric point at the point of balance between positive and negative charges of the components. The presence of lipoidal substances alters this point, its exact position being determined by the amounts and proportions of the various lipoids. The results indicate that in a high quality gluten this point, which probably should not be called an iso-electric point, except that the term seems to indicate a point of minimum swelling, is at or near pH 7.0. Alterations

taking place in lipoidal substances with aging result in this point shifting to a lower pH. From Bungenberg de Jong's results it seems probable that the protein complex alone would have its iso-electric point at approximately pH 6.0. The lowest point in the present study is about pH 6.0, which is probably only a coincidental agreement, since some fatty acids and lipoids were undoubtedly present in all of the flours used.

The results of the gluten swelling experiments in 0.1 N acetic acid carried out on these flours are determined, it is believed, by different factors, since pH 3.0 is well on the acid side of the iso-electric point of any of the components. This test gives an indication of the dispersibility of gluten rather than a measure of its hydration role. The most easily dispersed gluten exhibits the slowest swelling and the lowest hydration at which coherence is maintained.

The pH of the dispersion medium has a marked effect on the fractionation of gluten proteins. The distribution of amide nitrogen, however, is not affected. The amide nitrogen is determined by the position of the fraction in the complex and not by the concentration of magnesium sulphate used in the precipitation. Data obtained using sodium salicylate solutions of a pH 6.0 may therefore be regarded as

reliable, and there is no basis for an objection to the continued use of this solution in further studies.

Just as the pH of the dispersion medium affects the fractionation of the gluten proteins, so does aging affect this fractionation. There is a loss in solubility of specific fractions which is directly related to aging or deterioration. This effect, however, does not alter the chemical nature of specific fractions, since the results with glutens from flours Nos. 10 and 11 show that the amide nitrogen of specific fractions is unaltered by aging. The effect of aging may be best followed by using an individual flour, since this eliminates a major source of error, namely, the variations in original quality between different flours.

Little has been said with reference to the flour milled from soft wheat. There is no doubt that this flour differs in its gluten characteristics from that of a hard red spring wheat flour. Too little work has been done to define its properties, and another investigation would be necessary to compare different types of wheat flour. The quality of the flour samples used in this study varied enormously. The study of gluten reveals differences, but whether such methods as described here would be of any great value in differentiating flours of similar type and general quality remains to be seen.

SUMMARY OF RESULTS

1. The physical properties of gluten, as indicated by the water-holding capacity of the gluten between pH 4 and 7, are deleteriously affected by the aging of flour. As the quality of the gluten from different flours decreases, the water-holding capacity and the pH at which gluten exhibits minimum water-holding capacity also decrease.

2. Differences in physical properties of the gluten seem to be related to the lipoidal substances present in the flour.

3. The effects of aging may be partially removed by ether extraction of a deteriorated flour.

4. The addition of linoleic acid to freshly milled flour results in a condition similar to deterioration.

5. Swelling of gluten in 0.1 N acetic acid is not a good indication of gluten quality. Differences in the swelling properties of gluten could not be attributed to differences in the acids and salts naturally present in flour. It may be a measure of dispersibility of gluten, and thus could be used as a measure of deterioration as is sodium salicylate.

6. The quality of gluten depends to a considerable extent on the nature of the adsorbed lipoids. Relatively insoluble lipoids confer desirable characteristics on gluten, while their replacement by fatty acids is deleterious to gluten quality.

7. Separation of the components of gluten in the region where the hydration capacity of the gluten is at a minimum is possible.

8. Aging of flour results in a decrease in solubility of the gluten in sodium salicylate. Amide nitrogen distribution is probably altered due to the alterations in solubility which accompany the aging process.

9. There is no evidence that aging of the flour and its accompanying physical changes in the gluten has any effect on the chemical make-up of the gluten as a whole or of the specific fractions of the gluten.

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