CHANGES IN CHEMICAL AND PHYSICAL PROPERTIES OF FLOUR FROM WHEAT GROWN ON THE BLACK AND GRAY SOILS OF ALBERTA

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

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INTRODUCTION

The quality and keeping properties of flour milled from the 1932 and 1933 crops of wheat grown on the black soil at Edmonton and on the gray soil at Fallis have been found to differ markedly (1). Baking tests carried out one month after milling showed that the Edmonton grown samples produced the larger loaves, the differences in the average results being largely accounted for by the differences in protein content of the samples from the two places. Baking tests carried out ten months after milling showed that the Edmonton samples had maintained their original quality while the Fallis samples, stored under the same conditions, had deteriorated markedly. The results also showed that there were differences in the keeping properties of flour milled from different varieties of wheat.

Considerable work has been done on the changes taking place during the aging of flour, but as far as

the writer knows no results comparable to those obtained with the Edmonton and Fallis flours have been reported. It is known that definite changes in the chemical and physical properties of flour take place during the aging process. It seemed probable that the differences in behavior of the Edmonton and Fallis flours would be manifested by differences in the rates at which these changes took place.

The results of the investigations reported in this paper deal with the changes taking place in the chemical and physical properties of the Edmonton and Fallis flours. The results of these determinations suggested a number of miscellaneous experiments, the results of which might aid in explaining the general processes involved in the aging of flour. Several such experiments were carried out using flour which was available in large quantities, since the amount of Edmonton and Fallis flours remaining after the second baking test was small.

REVIEW

There is general agreement among bakers, millers and chemists that flour improves in baking quality during storage. This improvement is rapid at first and continues until a maximum is reached after which deterioration

begins. The time required to reach maximum improvement or noticeable deterioration depends on several factors such as, the soundness of wheat from which the flour is milled, the grade of flour, the moisture content of the flour, and the temperature and relative humidity at which the flour is stored.

High grade flour milled from sound wheat and stored under proper conditions shows no appreciable signs of deterioration for several years (3,6,34,35). It is believed that the processes causing improvement and deterioration of flour during storage are the same, deterioration being merely a result of the process going beyond the point of maximum improvement. High temperatures result in a rapid improvement in flour quality but shorten the time necessary for deterioration to become noticeable (3,34). Low grade flour deteriorates more rapidly than high grade, the difference being most noticeable when such flour is stored at high temperatures (23,35). Flour of high moisture content deteriorates more rapidly than flour from the same sample at a lower moisture content. Flour should therefore be stored in an atmosphere of low relative humidity or in a sealed container (6,35).

Considerable research on problems directly or indirectly related to the study of flour improvement and deterioration has been carried on during recent years, and

has served to indicate some of the factors involved. A comprehensive review of all the literature pertaining to this subject is beyond the scope of this paper and is not attempted. Representative papers dealing with phases of the work covered in the present study are discussed.

Acidity

It is generally agreed that the acidity of flour, measured either as titrable acidity or hydrogen ion concentration, increases progressively during storage. Greaves and Hirst (16) found, however, that the hydrogen ion concentration of flour decreased during the first two years of storage. They explained the decrease as being due to protein cleavage, the split-products not only neutralizing the acids produced but combining with the acid present at the time of storage. Their method for determining hydrogen ion concentration was not given, and this makes it difficult to evaluate the results. The fact that these pH values were so much higher than those obtained by most other workers necessitates that they be substantiated before they can be unquestionably accepted.

It is usually agreed that the increase in acidity of flour is due mainly to the break down of lipoids and that the amino and phosphoric acids contribute

only a small part (16,19). The process by which the fatty acids are formed from the lipoids is, however, not so well known. Kozmin (22) believes that they are formed by hydrolysis, that no oxygen is required and that temperature is the most important factor. There is, however, no reason to believe that oxygen is not an important factor in the break down of lipoids, since it has been shown that in the break down of free fats, oxygen is important.

There seems to be no general agreement as to the value of acidity as a measure of flour deterioration. The Greek government has imposed acidity limits on various grades of imported flours, but cereal chemists are not agreed that this is justifiable (7,9,24).

Flour Absorption

Many workers have found that the water absorption of flour increases with time of storage (16,34,35). The reason for this increase is not definitely known, but it is probably associated with the changes undergone by the proteins in the aging process. It has been suggested that this increase in absorption is due to the increase in acidity of the flour during storage. It is known that dilute acids cause an increase in the imbibitional properties of gluten. Kling, Froidevaux and Dubois (21)

believe that the protein particles are covered with an adsorbed layer of lipoids and the liberation of fatty acids causes a lowering of the interfacial tension between the water and the protein particles. This would increase the imbibitional properties of the proteins. Bailey (3), however, does not believe that increased acidity will account for all of the increase in absorption and suggests that some modification of the colloidal properties of the proteins, due to factors other than acidity, must take place. Kent-Jones (20) found that heat treated flours showed increased absorption due to a greater aggregation of the protein particles.

Lipoids

The importance of lipoids, as a factor in bringing about improvement or deterioration during the storage of flour, has received considerable attention in recent years. The term lipoid is a general one including a wide variety of substances. The nature of many of these substances makes it highly probable that they are adsorbed to other constituents in flour. It is therefore only with great difficulty that all of the lipoids are removed. This is no doubt one of the factors which has retarded the understanding of the part played by these substances. Such a solvent as hot alcohol which effects

a fairly complete removal of the lipoids denatures the proteins, thus making the flour useless for many studies. Ether, the common solvent used, extracts only part of the lipoids. This is probably the main reason for the disagreement commonly found in the results of various workers.

Many theories have been advanced as to the role played by lipoids in determining quality and keeping properties of flour. The summary of the work will of necessity be rather disconnected since it is difficult to relate all the results obtained.

Sullivan and Near (32) have suggested that the lipoid-protein ratio is an important index of flour quality. There is little evidence to substantiate this although the addition of germ to flour, which would increase the lipoid-protein ratio, is known to lower the baking quality (12,28). It is also known that the lower grades of flour are higher in lipoids than the better grades. The addition of various fats and oils also affect the quality of the flour, some beneficially and some deleteriously (10,12,28).

Geddes (12) believes that the effect of heat treatment on a flour is associated mainly with the germ constituents. Kent-Jones (20), however, disagrees with this and maintains that the heat treatment of flour has a direct effect on the proteins. Since it is believed that lipoids affect the colloidal behavior of the proteins it

is possible that the two theories are not irreconcilable. Working (37) believes that phosphatides are the important constituents affecting the physical properties of gluten. He believes that the gluten forms a mesh work of strands with the phosphatides adsorbed on them and acting as a lubricant.

The amount of lipoids in flour is found to decrease during storage as a result of oxidation or hydrolytic processes (15,33). This decrease in lipoidal content may have some effect on the physical properties of proteins.

Physical Properties of Flour Proteins

It has been observed by many workers that as the aging of flour proceeds, changes in the physical properties of the proteins take place. The changes manifest themselves in the handling qualities of the gluten washed from the flour (3,22) and also in the baking properties (16,34). Kozmin (10) states the changes in physical properties of the gluten during the aging of flour are due to an accumulation of unsaturated fatty acids. These acids render the gluten less extensible and elastic and their removal restores the original quality of the gluten. Geoffroy (15) seems to agree with this explanation for he believes that the changes in the physical properties of

gluten are due mainly to the action of lipase on the glycerides to form fatty acids. As has already been mentioned, Working (36) suggests that the changes which cause the dispersion of the phosphatides bring about an increased lubrication of the gluten strands. This would result in changes in physical properties of the gluten. It is known that the removal of the lipoidal substances of flour (18,28) or the addition of fats and oils (10,12,37) cause changes in the physical properties of the gluten. Potel (26) believes that the reducing power of a flour gives an indication of the physical properties which can be expected of the dough. This was demonstrated by lowering the reducing power by heat, aging or addition of hydrogen peroxide which resulted in physical changes of the dough. Proteolytic enzymes may cause marked changes of the physical properties of the proteins of flour (5,27).

A brief review of the literature on the subject of the changes in flour during storage leads to the conclusion that there are many factors involved in the process. The evidence in favor of the lipoids being one of the most important factors bringing about the changes is becoming much stronger. It is for this reason that so much emphasis is placed on lipoids in the present study.

MATERIAL

1932 Series

The 1932 series of flour was experimentally milled from wheat of standard varieties and hybrids grown at Edmonton and Fallis. The flour was stored in the laboratory in cans with close fitting covers. Baking tests were carried out one and ten months after storage. After the second baking test such small quantities of individual samples remained that bulking was necessary in order that further studies might be carried on. Samples which had shown similar results during storage were mixed and placed in glass sealers with air-tight covers. The sealers were placed on shelves and subjected to the temperature variations of the laboratory.

1933 Series

The 1933 series of flour was experimentally milled in the fall of 1933 from wheat of standard varieties and hybrids grown at Edmonton and Fallis. Small quantities of the wheat were stored until May 1935 and the flour milled from this was used to compare with the flour milled in 1933. The storage of the flour was carried out in two types of containers, cotton sacks and sealers, fitted with

air-tight covers. The flour stored in the cotton sacks was exposed to the fluctuations in temperature and humidity of the laboratory, while that stored in the sealers was subjected to light and temperature changes. The laboratory in which the flour was stored was fairly well lighted and although it sometimes reached a fairly high temperature during the summer, it was seldom very cold during the winter. The humidity was in general low and the samples stored in sacks probably lost moisture fairly rapidly during the first part of the storage period. At the time of the second baking the moisture content of this flour was approximately 10% while that of the samples stored in sealers was approximately 13%.

Storage Experiment

The flours used in this experiment were milled from sound wheat of four standard varieties grown on the black soil. The three varieties, Garnet, Marquis and Reward were obtained from the Dominion Experimental Station at Lacombe and should, therefore, be fairly comparable. The Red Bobs sample was grown east of Edmonton and had a protein content slightly lower than that of Garnet. The flour was stored in the laboratory in cans with close fitting covers. The moisture content of the flour was approximately 12% and varied little during storage. The

conditions of storage in the laboratory were very similar to those which obtained during the storage of the 1932 series.

A high grade wheat germ obtained from a commercial mill was used in the experiments. It was finely ground in the experimental mill and most of the bran flakes removed by bolting. This germ was stored in a sealed container in the refrigerator at a temperature of 1°C.

METHODS

Baking

Unless otherwise stated all bread was baked using the bromate formula with one mgm. of potassium bromate per loaf (2). The use of the simple formula with the 1932 series and the malt-phosphate-bromate formula with the 1933 series gave results from which essentially the same conclusions were drawn as to the baking qualities of these flours. Furthermore, the results obtained by Geddes and McCalla (14) showed that wheat grown in Alberta yielded flour sufficiently high in diastatic activity to justify the use of the bromate formula, although increased loaf volumes were obtained when malt and phosphate were added to the formula.

The partial baking score was calculated in the manner described by Geddes, Malloch and Larmour (13) for the baking score except that the figure for loaf volume was excluded.

Protein Content

Nitrogen was determined by the Kjeldahl method using mercuric oxide as catalyst. Protein is reported as nitrogen x 5.7 and the results corrected to a 13.5% moisture basis.

Acidity

Acidity was determined by the Greek method (9) using tincture of curcuma as indicator. Results are given as the number of cc. of 0.02 normal sodium hydroxide to neutralize 10 cc. of the alcoholic extract.

Hydrogen ion Concentration

The hydrogen ion concentration, expressed in the tables as pH was determined using a type K potentiometer with the quinhydrone gold electrode.

Ten gm. samples of flour were each shaken with 50 cc. of distilled water. Most of the determinations were made on the resulting flour suspension which was maintained by a constant stirring device. Some determinations, however, were made on the decantate of the centrifuged flour and water suspensions.

Lipoids

The lipoids in the flour were extracted with ether, alcohol-ether and either a hot alcohol-ammonia solution or hot alcohol.

Ether Extraction.

Flour for the ether extraction was first dried in vacuum over concentrated sulphuric acid. A 10 gm. sample was then placed in a stoppered centrifuge tube with 50 cc. of diethyl ether and shaken at frequent intervals for two hours. The contents were then centrifuged and the ether extract decanted. The extracted flour was subsequently washed with two 25 cc. portions of ether and these extracts added to the original decantate. The total decantate was then evaporated to a small volume, placed in a tared vessel and dried to constant weight in an air oven at 60°C. Although this technique is somewhat different from that usually used, satisfactory results were obtained and the

amount of extract determined for the various flours agreed well with the results of other workers.

Alcohol-ether Extraction.

The alcohol-ether extraction was similar to that described for the ether extraction except that a 1:1 mixture of diethyl ether and 95% ethyl alcohol was used as the solvent. The extract, after the alcohol and ether had been distilled off, was re-extracted with hot chloroform. The chloroform solution was placed in a tared vessel, evaporated to a small volume and dried to constant weight in an air oven at 100°C.

This method is a modification of the one used by Channon and Foster (8).

Sullivan and Near (31) used an alcohol-ether extraction method in which they first extracted with hot alcohol and then followed with an ether extraction, thus it is quite different from the method described above.

Hot Alcohol-ammonia Extraction.

The alcohol-ammonia extraction was made with 50 cc. of a 9:1 mixture of 95% ethyl alcohol and concentrated ammonium hydroxide added to 10 gm. of alcohol-ether extracted flour in a 100 cc. centrifuge tube. The extraction was carried out at 70°C. and repeated with a second volume of solvent. The extract was obtained by

centrifuging and decanting, and the decantate was treated in the same manner as that from the alcohol-ether extraction.

This method is a modification of the one used by Sullivan and Near (31) for determining the lipoids in gluten.

A number of determinations on various samples of flour gave irregular results with poor agreement among replicate determinations. Tests revealed that the alcohol-ammonia solution extracted a considerable quantity of protein from the flour and although the extracts were re-extracted with chloroform there still appeared to be contamination of the lipoids. Further investigations showed that hot 95% alcohol alone removed less protein and gave better agreement among replicate results. It was, therefore, used in all later work.

RESULTS

Baking

1932 Series.

The baking results obtained one and ten months after milling the samples in the 1932 series have been reported by Aamodt and McCalla (1). Neither the Edmonton nor the Fallis grown Supreme sample deteriorated during

ten months storage. Red Bobs and Early Triumph grown at Fallis deteriorated markedly during the same period although the Edmonton grown samples did not deteriorate.

TABLE I

Baking results of representative samples of the 1932 series after one and ten months storage

Variety	Origin	Protein	Loaf volume		Partial baking score	
			1 mo.	10 mo.	1 mo.	10 mo.
Supreme	Edmonton	14.2	705	744	53	52
	Fallis	11.0	618	630	50	48
Red Bobs	Edmonton	14.2	632	666	57	55
	Fallis	10.3	588	428	52	39
Early Triumph	Edmonton	14.4	702	650	54	54
	Fallis	10.5	571	498	54	44
S-28-2	Edmonton	15.3	673	484	52	49
	Fallis	10.8	544	503	50	41
Average of 15	Edmonton	14.9	694	700	54	54
	Fallis	11.5	617	503	53	41

S-28-2 grown at Edmonton showed marked deterioration as indicated by the decrease in loaf volume but only slight deterioration as indicated by the partial baking score, which summarizes the characteristics of the loaf other than volume. There was very little deterioration of the corresponding Fallis sample. The average of the results for the 15 varieties showed a wide difference between the Edmonton and Fallis samples. There was a marked decrease in loaf volume and partial baking score of the Fallis

samples after ten months storage while the Edmonton samples maintained their original quality. These results indicate that there were decided differences in the keeping properties of the flour from the different varieties of wheat.

The average baking results for nine varieties of the 1932 series are presented in Table II. The results obtained one and ten months after milling are the average of results for individual varieties. The results for two and three years after milling were obtained from baking tests carried out on the combined residues of the flour remaining after the second baking. The Edmonton sample had deteriorated little after two years storage. The partial baking score was actually higher than that for the two earlier tests, due mainly to an improvement in texture and crumb color. The Fallis sample had deteriorated more after ten months storage than the Edmonton sample had after three years. These results are definite proof that the rate of deterioration of the Fallis sample was much the greater.

TABLE II

Average baking results for nine varieties for the 1932 series

Time after milling	Loaf volume, cc.		Partial baking score	
	Edmonton	Fallis	Edmonton	Fallis
* 1 month	705	613	55	54
* 10 months	655	423	55	37
** 2 years	655	380	65	27
** 3 years	445	255	44	24

* Average of individual results.
** Average of the composite flour of the nine varieties.

1933 Series.

The baking results for the 1933 series stored by two methods are given in Table III. The results presented are for the varieties which were used in the later detailed studies, and the following discussion applies only to these samples. The results for the series as a whole have been discussed (1).

The Fallis samples, with the exception of I-28-60, all deteriorated after 12 months storage. Garnet and I-28-60 grown at Edmonton deteriorated considerably during the same storage period. As indicated by loaf volume, there was greater deterioration of the flour stored in sacks than of that stored in sealers. This was true for all varieties grown at both Edmonton and Fallis. The deterioration as indicated by partial baking score was not so evident, in fact there was little difference between the samples of the two series. Since the samples were all stored in the same laboratory, temperature effects should have been the same for both series, and the differences in loaf volume must have been brought about by differences in moisture, aeration and light.

Storage Experiment.

The effect of added germ on the loaf volume of

TABLE III

Baking results of samples of 1933 series used in detailed studies

Variety	Origin	Protein	Loaf volume, cc.			Partial baking score		
			Original	Stored in sacks	Stored in sealers	Original	Stored in sacks	Stored in sealers
Canus	Edmonton	15.1	697	607	720	55	53	51
	Fallis	10.9	623	446	541	44	37	36
Garnet	Edmonton	14.1	672	457	503	53	45	44
	Fallis	11.7	588	413	498	43	31	38
Huron	Edmonton	15.1	663	566	623	58	51	43
	Fallis	10.4	558	378	494	36	33	32
I-28-60	Edmonton	15.0	792	419	450	60	47	49
	Fallis	11.5	661	519	605	48	41	46
Red Bobs	Edmonton	14.7	694	627	687	53	57	52
	Fallis	10.6	632	440	602	45	37	44

stored flours is shown by the results presented in Table IV. This series was baked using the malt-phosphate-bromate formula (14). The addition of germ resulted in a decided decrease in loaf volume. The partial baking score, although the results are not given in the table, was also lowered about eight units on the average. The loaves were flat and had a coarse open texture and yellow crumb color. Other workers have reported the lowering in quality of flour as the result of the addition of germ. The absorption increased at approximately the same rate for all varieties during storage and seemed to have no relation to the stage of deterioration of the flour. The increase in absorption with lapse of time in storage has also been noted by other workers.

The varieties showed differences in keeping properties as indicated by the baking results. The loaf volume of Reward was higher after 13 months storage than at the time of the original bake. Red Bobs deteriorated little in the same period, while Marquis and Garnet deteriorated decidedly. These results substantiate those obtained with the Edmonton and Fallis flours with respect to the differences in the keeping properties of flour from different varieties of wheat. Aamodt and McCalla (1) found that the keeping properties of the flour from Reward were better than those of the flour from most of the other standard varieties. The results for Reward are not

TABLE IV

Effect of added germ on stored flours

Variety	Protein	Sample	Loaf volume, cc.				Absorption, %			
			Time after milling, mo.				Time after milling, mo.			
			0	1	4	13	0	1	4	13
Reward	14.9	Control	811	888	950	875	65	70	70	77
		3% germ added	686	739	764	758	65	70	70	76
Red Bobs	12.1	Control	716	675	797	682	66	72	73	76
		3% germ added	608	626	642	610	67	72	73	77
Marquis	13.3	Control	754	752	762	652	70	72	73	77
		3% germ added	627	668	659	556	70	72	73	77
Garnet	12.3	Control	634	619	637	494	67	71	73	75
		3% germ added	569	557	607	498	67	71	73	76

presented in Tables I and III as there was insufficient wheat and flour to use in any of the later studies.

The addition of germ to the flour did not seem to affect the rate of deterioration. This was indicated by the fact that the spread between the loaf volume of the control and that of the sample with added germ did not increase during storage. Added germ would of necessity increase the lipoid content of the flour, but these results indicate that such lipoids are not important in bringing about deterioration of the flour. If lipoids are important in flour deterioration those from the endosperm rather than those from the germ must be instrumental in causing changes. The amount of lipoids resulting from germ contamination in a patent flour must be small compared with the actual lipoid content of the flour. It seems unlikely that differences in low and high grade flours are due to differences in the amount of lipoids resulting from contamination of the flours by germ constituents.

Acidity

1932 Series.

The results of acidity and pH determinations of flour from the 1932 series after ten months storage are

presented in Table V. A comparison of the acidity with the baking results given in Table I indicates that there was a definite relation between the acidity and the stage of deterioration of the flour. Samples of Supreme had not deteriorated and had low acidities; Red Bobs and Early Triumph grown at Fallis and S-28-2 grown at Edmonton had deteriorated markedly and had high acidities. Within varieties the relation between acidity and deterioration was even more marked, the deteriorated sample always having a much higher acidity.

TABLE V

Acidity and pH of flour from 1932 series after ten months storage

Variety	Origin	Acidity	pH*
Supreme	Edmonton	1.86	5.60
	Fallis	1.61	5.70
Red Bobs	Edmonton	2.18	5.75
	Fallis	3.18	5.65
Early Triumph	Edmonton	1.26	6.00
	Fallis	3.18	5.70
S-28-2	Edmonton	3.05	5.80
	Fallis	1.82	5.95

* pH's determined on suspensions.

There was no evident relation between pH and deterioration among varieties, but within a variety the

deteriorated sample had the lower pH. The range of pH values for the different flours was small so the determination of pH as a measure of the degree of deterioration of a flour is of little value. Buffer curves for a number of these flours were plotted but no great differences were found.

Within a variety the sample with the higher acidity had the lower pH. Differences in acidity, however, were so much greater than those in pH, that acidity must be considered the better indicator of deterioration.

1933 Series.

The results for acidities and pH values of the flours from the 1933 series one and 16 months after milling are given in Table VI. The relation between the acidity of the flour and the degree of deterioration was not so marked in this as in the 1932 series.

The acidity of the flour stored in sealers was decidedly higher than that of the flour stored in sacks. It is probable that this was due to differences in hydrolytic or oxidative processes caused by differences in light, aeration or moisture content of the flour in the two series. The lack of agreement between the baking and acidity results for flours stored under various conditions leads to the conclusion that acidity is a poor measure of

the degree of deterioration. This is the opinion of many investigators working on the aging processes of flour. It has also been observed in the study on the keeping properties of fats and oils, that acidity is a poor measure of rancidity.

TABLE VI

Acidities and pH's of flours from 1933 series, one and 16 months after milling

Variety	Origin	Acidity 0.02N NaOH, cc.			pH		
		1 mo.	16 mo.		1 mo.	16 mo.	
			Stored in sacks	Stored in sealers		Stored in sacks	Stored in sealers
Canus	Edmonton	1.04	1.44	1.41	*5.80	*5.88	**5.81
	Fallis	1.10	1.83	2.47	5.77	5.78	5.75
Garnet	Edmonton	1.00	2.38	3.57	5.78	5.92	5.72
	Fallis	--	1.90	2.23	--	5.89	5.90
Huron	Edmonton	1.20	1.33	1.88	5.95	5.87	5.80
	Fallis	0.91	2.37	2.00	6.00	5.79	5.95
I-28-60	Edmonton	1.14	3.18	3.49	5.88	5.53	5.69
	Fallis	1.04	1.59	3.54	5.95	5.85	5.71
Red Bobs	Edmonton	1.06	1.69	1.69	5.89	5.80	5.79
	Fallis	1.13	1.96	1.55	6.25	5.75	5.89

* pH determined on flour extract.
** pH determined on flour suspension.

The pH results presented in this table were obtained by two methods, and it is impossible to draw definite conclusions regarding the relation between pH and deterioration. It would appear, however, that pH is of

little value as a measure of the deterioration processes, since there is little agreement between the pH value and the degree of deterioration either among the varieties or within a variety.

There is also poor agreement between the acidity and the pH value of a flour as shown from these results. This is contradictory to the results presented in Table V, but it seems quite possible that the increases in acidity in the flours of the 1933 series were not necessarily of the same nature as the increases in acidity of those of the 1932 series.

Storage Experiment.

The results indicating the effect of added germ on the acidity of stored flours are presented in Table VII. A comparison of these with the baking results given in Table IV shows that there was a definite relation between the rate of increase in acidity and the keeping properties of the flour. Reward with the best keeping properties showed the least increase in acidity, while Garnet with the poorest keeping properties showed the greatest increase. The apparent relation between acidity and keeping properties does not necessarily mean that acidity was the cause of the deterioration. It is much more likely that it was an accompanying rather than a causal effect. The addition of 3% germ to the flour did

not appear to accelerate the rate of increase in acidity. This agrees with the baking results presented in Table IV. The increase in acidity due to added germ could have played no part in the resulting reduction of loaf volume. This reduction must have been brought about by some other constituent of the germ.

TABLE VII

Effect of added germ on the acidity of stored flour

Variety	Treatment at time of milling	Time after milling in mo.		
		1	4	13
Reward	Control	0.99	1.26	1.54
	3% germ added	1.17	1.50	1.82
Red Bobs	Control	0.87	1.41	1.97
	3% germ added	1.05	1.66	2.07
Marquis	Control	1.02	1.73	2.56
	3% germ added	1.20	1.87	2.39
Garnet	Control	1.12	2.05	2.32
	3% germ added	1.30	2.28	2.79

Lipoids

1933 Series.

The study of the lipoids in flour was not begun until the supply of flour from the 1932 series was exhausted. The lipoid content of fresh and stored flours of the 1933 series are given in Table VIII. There was

little difference in the amount of ether extract from the various samples of flour, but a decided decrease in ether extract took place with deterioration. There was a decrease in the alcohol-ether extract of all but one of the stored samples, deteriorated or non-deteriorated. The results for the hot alcohol-ammonia extracts are not presented in the tables since little confidence could be placed on their accuracy. The results, however, indicated a decrease in the amount of extract after storage. The general decrease in lipoidal content of the stored flours suggests that a breakdown of the lipoids occurred and that some of the products of decomposition were rendered insoluble in the solvents used.

TABLE VIII

Lipoid content of representative fresh and stored flours, 1933 series

Variety	Origin	Ether extract		Alcohol-ether extract	
		Fresh	Stored	Fresh	Stored
Huron	Edmonton	1.10	1.18	1.50	1.44
	Fallis	1.18	0.92	1.60	1.34
Red Bobs	Edmonton	1.14	1.16	1.60	1.34
	Fallis	1.16	0.90	1.74	1.26
Canus	Fallis	1.26	0.88	1.54	1.54
Garnet	Edmonton	1.10	0.98	1.52	1.36
I-28-60	Edmonton	1.16	0.88	1.22	1.00

The lipoid-protein ratios for the representative fresh and stored flours of the 1933 series are presented in Table IX. The higher ratios were obtained for the Fallis samples. This was to be expected from the results presented in Tables III and VIII which showed that the Fallis samples were definitely lower in protein but little different in lipoid content. Sullivan and Near (31) have suggested the lipoid-protein ratio as an index of quality; the higher the ratio the poorer the quality.

TABLE IX

Lipoid-protein ratio in representative fresh and stored flours of the 1933 series

Variety	Origin	Ether extract/protein		Alcohol-ether extract/protein	
		Fresh	Stored	Fresh	Stored
Huron	Edmonton	0.073	0.078	0.102	0.100
	Fallis	0.113	0.089	0.156	0.132
Red Bobs	Edmonton	0.078	0.079	0.118	0.098
	Fallis	0.109	0.085	0.167	0.121
Canus	Fallis	0.116	0.081	0.150	0.150
Garnet	Edmonton	0.078	0.070	0.112	0.100
I-28-60	Edmonton	0.077	0.059	0.083	0.068

The results indicate that the protein content gives all the information regarding quality that might be obtained from the lipoid-protein ratio. The lipoid-protein ratios of the deteriorated were noticeably lower than those of

the non-deteriorated stored samples, and this suggests that as flour ages the lower the ratio the poorer the quality. This indicates that the lipoid-protein ratio can be taken as neither an index of quality nor a measure of deterioration.

Physical Properties of Gluten

1932 Series.

The results of the studies on the properties of wet gluten in relation to the acidity of flour are presented in Table X. There appears to be a direct relation between the moisture content of the gluten and the quality; the lower the moisture content the poorer the quality. This indicates that as deterioration proceeds the imbibitional capacity of the gluten proteins decreases. The results also show that in general flour with a high acidity yielded gluten of low moisture content and poor quality. The results presented in Table VIII indicated that the lipoids decreased during the aging of the flour. It is possible that the breakdown of lipoids during deterioration causes not only an increase in the acidity but also a decrease in the imbibitional capacity of the proteins.

TABLE X

Properties of wet gluten in relation to the acidity of flour, 1932 series

Variety	Origin	Acidity	Moisture content, %	Gluten quality
Early Triumph	Edmonton	1.26	66.2	Excellent
Supreme	Edmonton	1.86	66.5	Excellent
Supreme	Fallis	1.61	65.1	Good
Red Bobs	Edmonton	2.18	64.9	Good
Early Triumph	Fallis	2.80	62.4	Poor
S-28-2	Fallis	1.82	62.1	Poor
S-28-2	Edmonton	3.05	61.2	Poor
Red Bobs	Fallis	3.18	59.7	Very poor

The results of the effect of pH of the wash water on the moisture content of gluten from Early Triumph and Red Bobs flours from the 1932 series are given in Table XI. These flours were chosen because they yielded glutens of decidedly different quality as shown in Table X. Sorenson's phosphate buffers of various pH values were used in mixing the dough and as wash water. The lowering of the pH of the wash water increased the moisture content of the washed gluten. This increase was less for the high than for the low quality gluten, probably indicating that under the conditions of the experiment the good quality was closer than was the poor

quality gluten to its maximum swelling capacity. The poor quality gluten obtained when wash water with a pH of 4.0 was used had a lower moisture content than the good gluten obtained with wash water at a pH of 6.8. Increasing the acidity of the wash water to a pH of 3.5 made it impossible to wash gluten from the poor quality flours.

TABLE XI

Effect of pH on the moisture content of gluten, 1932 series

Variety	Origin	pH, wash water	Moisture content, %
Early Triumph	Edmonton	4.0	67.6
"	"	5.0	67.7
"	"	5.5	67.0
"	"	6.0	67.0
"	"	6.8	66.4
Red Bobs	Fallis	4.0	65.5
"	"	5.0	63.6
"	"	5.5	63.0
"	"	6.0	61.8
"	"	6.8	59.7

These results indicated that the imbibitional properties of the gluten proteins were increased with the increase in acidity of the mixed dough and the wash water. This agrees with the results of other workers on the swelling of glutens in dilute acids, but is seemingly contradictory to the results given in Table X. From work on gluten it would be expected that flours with high acidity

would yield glutens with increased imbibitional capacity. This suggests that the effect of fatty acids may be quite different from that of other acids. It has already been suggested that the fatty acids accumulate as a result of the breakdown of lipoids, and that it is the breakdown of lipoids rather than the accumulation of the fatty acids which is directly responsible for the changes in the imbibitional capacity of the gluten.

1933 Series.

The quality of gluten from fresh flour and that stored in sacks of the 1933 series is qualitatively indicated in Table XII. There was little difference between the glutens of corresponding Edmonton and Fallis samples from freshly milled flour. The gluten from the stored flour, however, showed decided differences in quality. The properties of gluten from the non-deteriorated flour were unchanged, while those of the gluten from the deteriorated flour had altered markedly. The latter gluten was coarse, open, hard, inelastic and non-extensible. It has been found that such gluten is characteristic of all deteriorated flour.

Numerous theories have been advanced to account for the changes in the physical properties of gluten during storage and these will be discussed later in this paper. The results of the foregoing experiments indicate

TABLE XII

Quality of gluten from fresh and stored flours, 1933 series

Variety	Origin	Firmness		Elasticity		Extensibility	
		Fresh	Stored	Fresh	Stored	Fresh	Stored
Huron	Edmonton	Moderate	Moderate	Good	Good	Good	Fair
	Fallis	Moderate	Hard	Good	Poor	Good	Poor
Red Bobs	Edmonton	Firm	Firm	Very good	Good	Very good	Good
	Fallis	Firm	Hard	Very good	Poor	Very good	Poor
Canus	Fallis	Firm	Hard	Good	Poor	Fair	Poor
Garnet	Edmonton	Firm	Hard	Good	Poor	Fair	Poor
I-28-60	Edmonton	Firm	Hard	Very good	Poor	Very good	Poor

that the changes are associated with increased acidity and decreased lipoidal content, but no definite conclusions as to the direct relation of these factors can be drawn.

Miscellaneous Results

The results presented in the following tables are from a number of preliminary experiments carried out with the object of obtaining more information on the processes involved in the deterioration of flour. It was expected that the information obtained from these experiments might aid in explaining the differences in keeping properties of the flours from the wheat grown at Edmonton and Fallis.

The results indicating the effect of the addition of various substances on the baking quality of an unbleached commercial patent flour are presented in Table XIII. The addition of 5% germ to the flower lowered the loaf volume and partial baking score of the baked bread, an effect already discussed. Neither ether nor hot alcohol extraction of the germ removed its deleterious effect. This indicates that some material not soluble in these solvents, possibly the protein, was responsible for the lowering of the quality of the flour. The baking results were not significantly altered by either the

ether or the hot alcohol extract of the germ. Neither were the baking results affected by similar extracts of a deteriorated or non-deteriorated flour. The bread baked from the ether extracted flour was lower than the control in loaf volume, but higher in partial baking score. Previous experiments with ether extracted flour are in agreement with this result.

TABLE XIII

The effect of the addition of various substances on the baking quality of an unbleached commercial patent flour

Additions	Loaf volume cc.	Partial baking score
Control	750	49
5 gm. germ	682	41
5 gm. of ether extracted germ	680	42
5 gm. of hot alcohol extracted germ	655	41
Ether extract of 5 gm. germ	710	48
Hot alcohol extract of 5 gm. germ	718	47
Ether extract of 100 gm. flour	800	51
Ether extract of 100 gm. deteriorated flour	735	53
Hot alcohol extract of 100 gm. flour	780	51
Hot alcohol extract of 100 gm. deteriorated flour	788	51
Ether extracted flour, nothing added	660	55

The results of extraction by various solvents on the gluten of flour from Marquis wheat are presented in Table XIV. Ether extracted flour yielded a gluten which was little different from the control in physical properties, although it was perhaps a little firmer and less extensible. The glutens from the alcohol-ether or

TABLE XIV

The effect of extraction by various solvents on the gluten of flour from Marquis wheat

Treatment	Gluten quality			Dispersion in 10% Na Sal.
	Firmness	Elasticity	Extensibility	
Control	Firm	Very good	Very good	Complete
Ether extracted	Firm	Very good	Good	Complete
Alcohol-ether extracted	Hard	Fair	Poor	Incomplete
Alcohol extracted	Hard	Very poor	Very poor	Incomplete
Control, 10% germ added	Soft	Poor	Very good	Complete
Ether-extracted, 10% germ added	Moderate	Good	Very good	Complete
Alcohol-ether extracted, 10% germ added	Firm	Fairly good	Fair	Complete
Alcohol extracted, 10% germ added	Firm	Fair	Fair	Complete

alcohol extracted flour, however, were markedly different in handling properties. They were hard and non-extensible, resembling gluten from badly deteriorated flour. The addition of germ to the flours improved the quality of the gluten but did not restore all of the original properties. It is recognized that the addition of germ would not replace substances of the same nature as those removed by the solvents used, so the original properties could not be expected to be entirely restored. The results obtained indicate, however, that lipoidal substances are important in determining the physical properties of the gluten.

In previous experiments attempts were made to disperse the glutens obtained from deteriorated and non-deteriorated flour. The method of dispersion employed was similar to that described by Rose and Cook (30) who used 10% sodium salicylate as the solvent. This reagent effects complete dispersion of good quality wet gluten in approximately 3 hours. The gluten from the deteriorated flour had not completely dispersed after 5 hr. of frequent shaking in the solvent. It broke up readily into fine particles which settled to the bottom of the comparatively transparent non-viscous solution. The gluten from the non-deteriorated flour was completely dispersed in approximately 2 hr. and produced a viscous semi-opaque solution with no visible particles of gluten present. When germ was added to the deteriorated flour the gluten

could be completely dispersed and when added to the non-deteriorated flour the gluten could be dispersed in approximately 0.5 hr.

It was assumed from these results that lipoids might be the factor affecting the solubility of the gluten, so dispersion of the glutens from the extracted flours was attempted. Qualitative results only are reported because of the difficulty in effecting a complete separation of the very fine particles of gluten from the rather viscous solution. The glutens from the control and the ether extracted flour could be completely dispersed. If germ were added to these flours the dispersion of the gluten was effected in a much shorter time. This indicates that some constituent of the germ, presumably the lipoids, affected the solubility of the gluten. The alcohol-ether and alcohol extracted flours yielded glutens which could not be completely dispersed in the solvent. If germ were added to these flours, the glutens could then be completely dispersed. It is believed by many workers that alcohol denatures the gluten proteins, but if this is so, it is difficult to explain why the addition of germ to the extracted flour restores the solubility of the gluten. The alcohol-ether and alcohol extractions probably removed fairly completely the lipoidal substances of the flour, and it seems

likely that the lipoids of the germ restored the original solubility of the gluten.

The results indicating the effect on gluten of fatty acids added to an unbleached commercial patent flour are given in Table XV. The addition of 1% stearic acid as a powder had little effect on the gluten, but when the acid was added in ether solution a decided effect was noted. The gluten obtained was hard and non-extensible and could not be completely dispersed in sodium salicylate. In these respects it resembled the gluten from a deteriorated flour. Stearic acid is insoluble in water and when added as a powder was probably carried away mechanically during the washing of the gluten. When added in ether solution the acid was in a form much more likely to be adsorbed and was probably not removed in the washing. The addition of 1% oleic acid to the flour yielded a gluten similar to that produced with the addition of stearic acid dissolved in ether. Previous experiments demonstrated that if the same quantity of oleic acid were added to the two flours differing in protein content, the effect would be less noticeable on the flour with the higher protein. If the quantity of oleic acid added was in proportion to the protein content the effect on the two flours was the same. The amount of oleic acid required to produce a

gluten of inferior quality raised the acidity of the flour to that characteristic of a deteriorated sample. This would indicate that the amount of acidity produced by a flour could cause changes in the physical properties of the gluten. The results of the effect of fatty acids on gluten agree with those of other workers, but the effect of stearic acid in ether solution has not been reported as far as the writer knows.

TABLE XV

Effect of added fatty acids on the gluten from an unbleached commercial patent flour

Treatment	Gluten quality			Dispersion in 10% sodium salicylate
	Firmness	Elasticity	Extensibility	
Control	Firm	Good	Fairly good	Complete
1% stearic acid	Firm	Good	Fairly good	Complete
*1% stearic acid dissolved in ether	Hard	Poor	Very poor	Incomplete
1% oleic acid	Hard	Poor	Poor	Incomplete

* Acid dissolved in ether and added to the flour with thorough mixing. Ether then allowed to evaporate.

The results of the effect of the addition of fatty acids on the loaf volume of the flour used in the gluten experiments are presented in Table XVI. The addition of the acids to the flour seems to have little

effect on the loaf volume of the baked bread. The lower volume of the loaves from the flour to which oleic acid had been added was probably due to insufficient water being added in the mixing of the dough. These loaves were equal in all other respects to the bread from the controls. Increasing the amount of acids to three times the concentration which gave decided effects on the gluten had little effect on the baking results and greater concentrations of these acids could not normally occur in flour, so there was no point in raising them further.

TABLE XVI

Loaf volume of an unbleached commercial patent flour as affected by the addition of fatty acids

Treatment	Amount of acid added		
	1 gm.	2 gm.	3 gm.
Control, nothing added	795	795	795
Ether wetted	802	802	802
Stearic acid	830	848	865
Stearic acid dissolved in ether	820	810	815
Stearic acid added to ether wetted flour	770	825	795
Oleic acid	745	735	735
Oleic acid dissolved in ether	725	732	700

It would be expected from the results on the effect of fatty acids on gluten, Table XIII, that the effect on the flour when baked would have been more marked. The conditions of the two experiments are,

however, entirely different. In baking the gluten is dispersed throughout the starch and other constituents of the flour in the dough; when washed the gluten is free from most of the constituents of the flour, and under these conditions the acids might exert a greater effect on it.

These results indicate that the physical properties of gluten may not be a good measure of the baking quality of flour. If this is true Kozmin's (22) conclusions on the effect of fatty acids in the aging of flour need to be substantiated by baking results before they could be accepted as proof that the accumulation of unsaturated fatty acids causes the change in quality.

Artificially Deteriorated Flour.

The amount of deteriorated flour for experimental purposes was very limited so attempts were made to bring about rapid deterioration of the fresh samples to provide sufficient material for study. Results obtained by other workers, and in the present study, indicate that deterioration was due mainly to enzymic processes. Increasing the moisture content of the flour and storing at raised temperatures should therefore hasten the processes involved. The moisture content of the flour was raised to approximately 20% by placing it over a saturated solution of ammonium sulphate in a sealed container.

The flour was then stored in the container at a temperature of approximately 40°C. Toluene was used to prevent the growth of bacteria and moulds. The flour treated in this way is referred to in this paper as artificially deteriorated flour.

The results comparing artificially deteriorated flour with that stored in sacks are presented in Table XVII. The artificially deteriorated flour was in general comparable to the flour deteriorated under laboratory conditions. This was indicated by an increase in acidity, a decrease in ether and alcohol-ether extract, changes in the physical properties of the gluten and the manner in which the gluten dispersed in sodium salicylate. The results of baking tests, however, showed that it was much lower in quality than even the most badly deteriorated flour obtained under the storage conditions obtaining in the laboratory.

The Fallis sample of flour showed a greater rate of change in chemical and physical properties than did the corresponding Edmonton sample. This agrees with the results for these flours stored under laboratory conditions. The increase in acidity of the Fallis sample, however, was less than that of the Edmonton sample which does not agree with the results obtained under ordinary storage conditions. This is a further

TABLE XVII

A comparison of artificially deteriorated flour from Red Bobs wheat with that stored in sacks

Sample	Origin	Acidity	Ether extract %	Alcohol-ether extract %	Gluten quality			Dispersion in 10% Na sal.
					Firmness	Elasticity	Extensibility	
Control	Edmonton	1.06	1.14	1.60	Firm	Very good	Very good	Complete
	Fallis	1.13	1.16	1.74	Firm	Very good	Very good	Complete
*Stored 2 dy.	Edmonton	1.43	--	--	Firm	Good	Good	-
	Fallis	1.28	--	--	Hard	Fair	Fair	-
*Stored 4 dy.	Edmonton	1.75	--	--	Firm	Fair	Fair	-
	Fallis	1.41	--	--	Hard	Poor	Poor	-
*Stored 6 dy.	Edmonton	2.56	0.84	1.56	Hard	Poor	Poor	Incomplete
	Fallis	2.23	0.80	1.26	Hard	Very poor	Very poor	Incomplete
**Stored 16 mo.	Edmonton	1.69	1.16	1.34	Firm	Good	Good	Complete
	Fallis	1.96	0.90	1.26	Hard	Poor	Poor	Incomplete

* Stored at 40°C. with a moisture content of 20%.

** Stored in sacks at room temperature with a moisture content of approximately 10%.

indication that acidity is not a good measure of the stage of deterioration, as has already been mentioned.

Although the work on artificially deteriorated flour was of a preliminary nature it indicates that the method might be a valuable aid in measuring the keeping properties of a flour. It may also be important in studying the changes taking place during storage as these could be brought about quickly. It would at least obviate the necessity of waiting long periods to obtain quantities of deteriorated flour as was the case under such conditions of storage as were used for the three series of flours discussed in the earlier sections of this paper.

DISCUSSION

Differences in the keeping properties of the flour from wheat grown on the black and gray soils have been definitely established. Associated with the differences in keeping properties are the differences in rate of change in chemical and physical properties of the flour. The rate of change was greatest in the flour with the poorest keeping properties, that is, the flour showing the most rapid deterioration. There is no reason to believe that the type of deterioration occurring

in these flours is any different from that usually encountered in prolonged storage experiments. Inherent differences in the different varieties of wheat grown on the two soils must be responsible for the differences in keeping properties of the flour.

The differences in protein content of the flour from Edmonton and Fallis grown wheat has been noted. These differences, however, will not account for the differences in the keeping properties, as it was shown that some flours with high protein content deteriorated as rapidly as other flours in which the protein content was low. The chemical composition of the proteins of different wheats is not likely to be associated with quality, as it has never been conclusively shown that proteins from widely different wheats vary in composition.

There was little difference in the lipoid content of the flour from the wheat of different varieties grown on various soils. The chemical composition of the lipoids was not determined, but differences such as color, viscosity and amount of solid material were observed. It is possible, therefore, that differences in the amount of sterols, phosphatides or other constituents of the lipoids do exist. Evidence that the lipoids affect the physical properties of protein has been presented. Differences in the lipoids might cause the differences in the rate of change in the physical

properties of gluten occurring during storage.

Investigations by other workers on the rancidity of fats and oils have shown that their keeping properties differ markedly. The differences are attributed to variations in the amounts of pro- and antioxygenic substances or to the amounts of such catalysts as iron and copper present in the fats and oils. It has been shown that wheat oils differ in their antioxidant properties and this may be an important factor in determining the rate of flour deterioration. Lecithin and sterols have also been suggested as antioxidant substances and these may influence the changes in lipoids of flour. It seems reasonable to assume that the different varieties of wheat grown on various soils differ in metabolism to the extent that the lipoids formed differ as to the ease or rapidity with which they decompose. Lipoids which decompose rapidly cause a greater rate of change in the physical properties of the protein. It also seems probable that differences in the amount of metals which act as catalysts might exist as a result of differences in nutritional conditions.

The results of the experiments discussed in this paper largely support the general idea of Working's theory (36), although the nature of the lipoid involved has not been determined. He believes that phosphatides are the important constituents in flour aging. The

dispersion of such phosphatides is increased by changes in their solubility in water due to slight oxidation, and their destruction is caused by excessive oxidation. As evidence that oxidation is important in flour aging he reports that flours bleached with oxidizing agents deteriorated more rapidly than unbleached flours.

It seems probable that changes in the lipoid protein complex are important in determining the changes which occur in flour during storage. Improvement in flour quality may be due to slight changes in the lipoids brought about in the early stages of oxidation while deterioration may be due to more excessive oxidation or hydrolytic processes. Such a theory will explain most of the results obtained in the various experiments reported in this paper and also aids in the reconciliation of seemingly contradictory results.

Results on the extraction of flour by various solvents showed that ether, which removes only the free or loosely combined lipoids, had little effect on the physical properties of the proteins. Hot alcohol, which removes the closely adsorbed lipoids, changes the physical properties of the gluten proteins. As further evidence that the lipoids in the complex are important, the addition of lipoids to flour does not affect the rate of deterioration. If free lipoids were important, their removal by ether or their addition to flour should

affect deterioration but no evidence for this was obtained.

Kozmin's claim (22) that the accumulation of the unsaturated fatty acids is the cause of the marked change in flour quality is contradictory to the above theory, but it has already been shown that the fatty acids affect the gluten properties, but do not affect the baking results. It was also shown that the saturated stearic acid if added to flour in solution rather than in the solid form caused marked changes in the gluten properties, but the effect was not noticeable in the baking results. Evidence has also been presented to show that the acidity of the flour is not an accurate measure of the degree of deterioration. It seems far more likely that the acidity is merely an accompanying effect and that the breakdown of the lipoids is the causal agent in deterioration.

There is no agreement among the various workers as to whether fatty acids arise by oxidation or hydrolytic processes. Kozmin states rather definitely that oxidation is not important in the process. She claims that oxidizing agents have no effect on the lipoids as ether extracted flours and untreated flours reacted to them in the same way. It has already been pointed out that the ether soluble constituents have little effect on the physical properties of the proteins, thus it does not seem that her conclusions are valid. It has

already been pointed out that the assumption that ether extracts all the lipoids leads to incorrect conclusions regarding the importance of lipoids in flour.

The evidence presented in the various experiments agrees with the lipoid-protein complex theory. It also gives a reasonable explanation for the differences in the keeping properties of the flours studied. It was unfortunate that the small amount of material limited the experiments which could be carried out with the flours from Edmonton and Fallis grown wheat. These experiments are being continued with a view to obtaining evidence of the validity of this theory.

SUMMARY OF RESULTS

Differences in the keeping properties of flour from different varieties of wheat grown on the two soils were established.

Titrable acidity and hydrogen ion concentration of the flours increased with the time of storage.

The lipoid content of the flours decreased during storage.

The solubility in sodium salicylate of the gluten from a deteriorated flour is much lower than that of the gluten from a non-deteriorated flour, and addition of lipoids increased the solubility.

The addition of fatty acids to a flour affects the physical properties of the gluten, but has no apparent effect on the baking properties of the flour.

The addition of wheat germ to flour increases neither the rate of deterioration nor the rate of increase in acidity.

Lipoids of the germ or the free or loosely combined lipoids of the flour have little effect on the baking quality.

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