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Sulphur-Containing Amino Acids of Wheat Proteins

R. Kasting

Department of Plant Science
University of Alberta

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THE SULPHUR-CONTAINING AMINO ACIDS
OF WHEAT PROTEINS

ROBERT KASTING
Department of Plant Science

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

Edmonton, Alberta

April, 1948

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ROBERT ALFRED

Department of Plant Science

A THESIS

Submitted to the University of Michigan
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Ann Arbor, Michigan

1911, 1912

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THE SULPHUR-CONTAINING AMINO ACIDS OF WHEAT PROTEINS

Robert Kasting

INTRODUCTION

Wheat flours with similar protein content often produce bread loaves of different sizes because of differences in their protein quality. Rigby (34) has attributed differences in quality in a single wheat variety to the total sulphur or sulphur-containing amino acids present in the protein. He showed that, whereas the quantity of crude protein would not account for loaf volume differences, the total sulphur content of wheat, flour and gluten was significantly correlated with loaf size. Further, he showed that the cystine content of the gluten is highly correlated with the sulphur content of the wheat and therefore the cystine in the gluten was believed to contribute to baking strength.

In a further study of the sulphur and wheat quality relationship, Gerbrandt (19), using five varieties of wheat grown on different soil types, showed that the percentage of sulphur in the whole wheat was a better measure of baking

THE SUPPLEMENTATION OF WHEAT OF WHEAT PROTEIN

Robert G. Gentry

INTRODUCTION

Wheat flour with similar protein content often shows great differences of different sizes because of differences in protein quality. Rye (5%) has attributed differences in quality in a single wheat variety to the total nitrogen or nitrogen-containing amino acids present in the protein. It is known that, whereas the quantity of amino acids would not account for the large volume differences, the total nitrogen content of wheat, flour and gluten was significantly correlated with flour size. Further, it seemed that the nitrogen content of the gluten is directly correlated with the nitrogen content of the wheat and therefore the nitrogen in the gluten was believed to be available to baking processes.

In a further study of the nitrogen and wheat quality relationship, Gentry (19), using five varieties of wheat grown on different soil types, showed that the percentage of nitrogen in the whole wheat was a better measure of baking

strength than was percentage crude protein. In addition his work showed that cystine and especially methionine content of the crude gluten accounted for more of the variability in loaf volume than did sulphur in wheat.

In these two studies, analysis for sulphur-containing amino acids was carried out only on the crude gluten washed from flour. It was thought that accurate methods for the determination of cystine and methionine in whole wheat and flour would provide a new means of attack, which would be of value in studies of the role of sulphur in flour quality. A direct method for cystine and an indirect method for methionine were found to be applicable to the determination of these amino acids in flour and wheat.

Wheat was grown on the Breton gray soils and was fertilized similarly to that used by Rigby (34). This material was available for further studies on the relationship between sulphur and loaf volume. Because of an early frost, the 1946 wheat graded poorly and produced very poor bread which did not show the expected variations in loaf size. It was therefore thought that investigations relating primarily to baking strength would be of little value. Nevertheless, as this material had a wide range in sulphur content it was admirably suited for investigations of nitrogen, sulphur, cystine and methionine relationships. A study of these relationships is the primary concern of this thesis.

The data for loaf size of the samples investigated were available. The loaf volumes for the 1947 material varied considerably. This provided an opportunity to compare loaf volume relationships with the results of other work from this laboratory.

As Rigby and Gerbrandt have shown that the sulphur-containing amino acids appear to affect wheat flour quality, a study of factors affecting the cystine and methionine content of wheat and flour might provide further information on the whole problem.

There is very little information in the literature as to the effects of various fertilizer treatments on the ultimate amino acid content of cereal grains. This knowledge would be of importance in protein nutrition, where quality as well as quantity of proteins is recognized as being important. Some of the material used in this study provides such information with respect to the sulphur-containing amino acids in wheat.

LITERATURE REVIEW

Harrow (25) lists four sulphur-containing amino acids, namely methionine, cystine, lanthionine and djenkolic acid. He mentions the compounds cysteine and ergothioneine; the former is classified as a reduced form of cystine of importance in

The data for loss of the samples investigated were available. The loss volume was 10% of the original volume. This provided an opportunity to compare the volume relationships with the results of other work from this laboratory.

As Hilly and Gendron have shown that the nitrogenous content of wheat and flour does not differ significantly, a study of factors affecting the protein and nitrogenous content of wheat and flour might provide further information on the whole problem.

There is very little information in the literature as to the effects of various fertilizing treatments on the ultimate amino acid content of cereal grains. This knowledge would be of importance in protein nutrition, where quality as well as quantity of proteins is recognized as being important. Some of the material used in this study provides such information with respect to the nitrogen-containing amino acids in wheat.

LITERATURE REVIEW

Karow (22) lists four nitrogen-containing amino acids, namely methionine, cystine, leucine and glutamine. He mentions the compounds glycine and aspartic acid; the former is classified as a reduced form of glycine of importance in

biological reactions, the latter as a derivative of histidine.

Glutathione, a polypeptide of glutamic acid, glycine and cysteine, is of importance in a consideration of sulphur-containing amino acids because of the sulphur present in the sulphydryl group.

In the literature reviewed, no reference reported the presence of lanthionine, djenkolic acid or ergothioneine in wheat or wheat products. The sulphur-containing compounds which have been reported in wheat are the amino acids cystine and methionine, the vitamins thiamine and biotin and the compounds cysteine and glutathione. The sulphur in the latter two is usually determined and reported as cystine-sulphur.

Greaves and Bracken (23) found that the thirty-six wheats investigated by them did not contain sulphur in the sulphate form. Woodman and Evans (40), however, report samples of bran and middlings with sulphate sulphur contents of 0.004% and 0.01% respectively.

Numerous studies have been made on the thiamine content of wheat (5). The paper of Johannson and Rich (27) gives a general indication of the thiamine content of wheat. These authors found that the amount of thiamine in wheat from the three prairie provinces in 1940 ranged from 2.2 to 8.0 μ g per gram with a mean of 3.93 μ g.

Bailey (5) reports the biotin content of two samples of wheat as 22 millimicrograms and 113 millimicrograms per gram.

biological reactions, and later as a catalyst in the
decomposition of hydrogen peroxide, and also in the
oxidation of iron in the presence of a small amount of
catalytic substance, and in the oxidation of iron in the
presence of a small amount of catalytic substance.

In the presence of a small amount of catalytic substance,
the reaction of iron with hydrogen peroxide is
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in the presence of a small amount of catalytic substance.

It is obvious that this organic sulphur-containing compound is present in wheat in very small amounts.

There are numerous papers which report values for the sulphur-containing amino acids in wheat and wheat products but most of the values reported are on isolated samples of wheat, flour or gluten.

A systematic study of the cystine content of different varieties of wheat and a single variety grown under various cultural conditions is reported by Gubler and Greaves (21). Using a colorimetric method for the cystine determination they found that the cystine-sulphur constituted about one-half the total sulphur of wheat. They observed that in general, variations in sulphur content are accompanied by corresponding variations in cystine content. In addition nitrogen content was highly correlated with percentage cystine. The correlation coefficients reported for sulphur and cystine and nitrogen and cystine are 0.83 and 0.75 respectively.

Gubler and Greaves also observed that, in general, as the total sulphur and cystine content increased there was a decrease in the percentage of total sulphur represented by cystine. This was also observed by Wood (38) in a study of leaf proteins. These observations may be attributed to incomplete estimation of cystine at the higher levels of cystine content.

Csonka (13) determined the cystine contents of various salt extractions of Marquis, Tenmarq and Fulhio wheats. He found

It is obvious that this organic sulphur-containing compound is present in wheat in very small amounts.

There are numerous papers which report values for the sulphur-containing amino acids in wheat and other grains but none of the values reported are on isolated samples of wheat, flour or gluten.

A systematic study of the cystine content in different varieties of wheat and a single variety grown under various cultural conditions is reported by Gahler and Treves (11). Using a colorimetric method for the cystine determination they found that the cystine-sulphur content varied about one-half per cent. of wheat. They observed that in general, variations in sulphur content are accompanied by corresponding variations in cystine content. In addition nitrogen content was highly correlated with percentage cystine. The correlation coefficients reported for sulphur and cystine and nitrogen and cystine are 0.75 and 0.72 respectively.

Gahler and Treves also observed that, in general, the total sulphur and cystine content increased as the nitrogen in the percentage of total sulphur increased. This was also observed by Wood (12) in a study on wheat varieties. These observations may be explained as follows: An increase in the level of cystine content, as observed in the present study, is accompanied by a corresponding increase in the level of nitrogen and sulfur content. The correlation coefficients of nitrogen and sulfur and cystine are 0.75 and 0.72 respectively.

the total cystine contents to be 0.27%, 0.17% and 0.16% respectively. Although he does not report the percentage sulphur, the nitrogen values are 3.31%, 2.58% and 2.34% in the order of the varieties. Csonka concluded that the amino acid content is different in the different varieties of wheat studied.

Rigby (34) in a study of the effect of sulphur on the quality of wheat grown on sulphur deficient soils found that cystine is the principal form of sulphur in gluten. In the samples examined he found the percentage of cystine varied from 56% to 89% of the total sulphur. The mean value was 76%. The protein content of this material was not an adequate indicator of the baking strength. The sulphur content of the wheat, however, was positively and significantly correlated with loaf volume. The gluten contained most of the sulphur in the wheat and larger amounts of sulphur were present in gluters from strong flours. The cystine content of the gluten was related to loaf volume, sulphur in gluten and sulphur in wheat. From Rigby's data there does not appear to be a decrease in the amount of sulphur represented as cystine when the cystine content increases.

Rigby found that the sulphur content of the flour follows closely that of the whole wheat but the level is slightly higher. This was taken as evidence that sulphur is more concentrated in the endosperm of the kernel than in the whole wheat.

the total sulphur contents to be 0.27, 0.17 and 0.13 respectively. Although he does not report the percentage of sulphur in the different varieties are 2.51, 2.76 and 2.84 in the different varieties. Gould concluded that the sulphur content is different in the different varieties of wheat studied.

Nigoy (194) in a study of the effect of sulphur on the growth of wheat grown on sulphur deficient soils found that the principal form of sulphur in wheat, in the samples examined he found the percentage of organic sulphur from 70 to 80 of the total sulphur. The organic sulphur was 70 to 80 of the total sulphur. The organic sulphur was 70 to 80 of the total sulphur. The organic sulphur was 70 to 80 of the total sulphur.

of the organic sulphur. The sulphur content of the wheat, however, was positively and significantly correlated with the volume. The wheat contained most of the sulphur in the wheat and larger amounts of sulphur were present in wheat from strong stems. The organic content of the wheat was related to total volume, sulphur in wheat and wheat in wheat. Nigoy's data does not appear to be a decrease in the amount of sulphur represented as organic with the organic content increased.

they found that the sulphur content of the wheat, which is the least of the whole wheat and the least is slightly higher. This was taken as evidence that sulphur is more concentrated in the endosperm of the kernel than in the whole wheat.

Gerbrandt (19), in a study of the distribution of sulphur in wheat, reported that although samples of Marquis wheat were significantly higher in sulphur than were samples of Thatcher, there was very little difference in the gluten cystine values. The methionine contents of the glutes were, however, constantly and significantly lower in Thatcher than in Marquis.

In this study the simple correlation coefficients reported for cystine or methionine in gluten with loaf volume, protein in wheat and sulphur in wheat are highly significant.

Using direct methods for both the cystine and methionine determinations Gerbrandt found the sulphur of these amino acids accounted for 101.4% of the total sulphur of gluten from Marquis wheat grown at six stations. The distribution of sulphur in gluten was also determined for Marquis and Thatcher grown at two stations. The total cystine and methionine sulphur represented a mean recovery of 97% of the total gluten sulphur. The gluten sulphur represented a mean of 70.5% of that found in the flour and 62.2% of that in the whole wheat.

In the second of his papers on amino acids in staple foods, Csonka (14) states that although the protein content of patent flour is lower than that of the whole wheat flour, there is a larger percentage of cystine in the flour. A direct determination on a sample of whole wheat flour gave a value of 0.17% cystine. The values for the flour, bran and shorts of this wheat are respectively 0.17%, 0.01% and 0.02%.

Experiment 11, in a study of the effect of all-
corn in wheat, reported that slightly smaller amounts of nitrogen were
absorbed by wheat in autumn than when grown in summer.
There was very little difference in the nitrogen content of
the nitrogen content of the wheat was, however, consequently
and is slightly lower in wheat than in summer.

In this study the single concentrated fertilizer was applied
for nitrogen or phosphorus in autumn with low rates, protein
in wheat and autumn in wheat and slightly different.

Using these methods to test the effect of nitrogen and phosphorus
on wheat, the results showed the effect of these two
fertilizers for 1911, of the total nitrogen of wheat from 1911
was grown at six stations. The distribution of nitrogen in
wheat was also determined for wheat and autumn grown at
two stations. The total nitrogen and phosphorus nitrogen
a mean recovery of 8% of the total nitrogen. The nitrogen
weight represented a mean of 70.8% of total, found in the wheat
and 55.2% of that in the whole wheat.

In the second of the series to which this is applied
to wheat, (11) stated that although the grain is
of wheat there is less than that of the whole wheat grain,
there is a larger percentage of protein in the wheat. A slight
decrease in a study of wheat grain and a value of
0.1% protein. The values for the wheat, when the wheat is
this wheat was respectively 0.1%, 0.1% and 0.1%.

D. Breese-Jones (10) reports little difference in the cystine content of whole wheat and a patent flour milled from the wheat. This worker reports a methionine content of 0.48% for a sample of flour milled from wheat containing 0.10% methionine. Obviously the results as reported are incorrect or the method used for the methionine determination is not consistent. The total methionine according to these results has actually increased in the flour. This is hardly possible.

Harrison, Thomas and Hill (24) traced the movement of sulphur in the wheat kernel by the use of radioactive sulphur. Radiographs of the grain showed the greatest concentration of sulphur was in the embryo. There appeared to be a fairly uniform concentration of sulphur in the starchy endosperm; but at the periphery of the endosperm there was a much higher concentration. This is especially so in the aleurone layer and the cells immediately underneath. The aleurone cells contain a large part of the protein of the bran. They are mostly removed as part of the bran in the milling process.

Barton-Wright (6) made a study of the distribution of amino acids in the wheat grain. Using a microbiological technique he found that, apart from the germ, the highest concentrations of 11 amino acids including cystine and methionine occur in the outer part of the grain.

Block and Bolling (8) report cystine contents for five whole wheats. The values range from 0.16% to 0.29%. Three

U. S. Bureau of Census (10) reports lysine deficiency in the cystine content of whole wheat and a patent flour milled from the wheat. This wheat reports a methionine content of 0.10% for a sample of flour milled from wheat containing 0.10% methionine. Obviously the results as reported are incorrect on the method used for the methionine determination is not satisfactory. The total methionine according to these results has apparently increased in the flour. This is hardly possible.

Harrison, Thomas and Hill (11) traced the movement of sulphur in the wheat kernel by the use of radioactive sulphur. Radiographs of the grain showed the greatest concentration of sulphur was in the embryo. There appeared to be a fairly uniform concentration of sulphur in the embryo and in the scutellum; but in the periphery of the endosperm there was a much higher concentration. This is especially so in the aleurone layer and the cells immediately underneath. The aleurone cells contain a large part of the protein of the grain. They are mostly located in part of the grain in the milling process.

Boston-Whitney (12) made a study of the distribution of amino acids in the wheat grain. Using a microchemical method he found that, apart from the grain, the highest concentrations of 11 amino acids including cystine and methionine occur in the outer part of the grain.

Glock and Kollig (13) report cystine content for five whole wheats. The values range from 0.10% to 0.14%. These

flour samples are reported with cystine contents of 0.31%, 0.26% and 0.27%. Block and Bolling also report on cystine values obtained by other workers for germ, bran and shorts.

Padva (32) in a study of the amino acid content of gluten reports a value of 1.43% cystine.

Workers reporting methionine results on isolated samples of wheat are Reisen, Schweigert and Elvehjem (35) and Chitre and Keni (12). The first group of workers used a microbiological method, the second group used a colorimetric technique.

Grau and Almquist (22) compared a colorimetric and a biological method for the determination of methionine in wheat, wheat protein and wheat bran. They found a close correlation between the two methods.

Wood (38) states that the only sulphur-containing components of the protein molecule are the amino acids cystine and methionine. Further, Wood says these acids may occur in a free state in plant tissues with cysteine and glutathione. The latter two compounds are derived from free cystine and methionine or from the protein molecule.

Cysteine and glutathione are important because of their active sulphydryl groups.

Sullivan, Howe and Schmalz (37) have shown that glutathione is concentrated in the wheat germ and that patent flour contains only traces if any of this compound.

Myers and Working (31) state that sulphydryl groups are present in proteins in a free or reactive form and/or a bound

These samples are compared with positive controls of 1.11, 1.20 and 0.5%. These and other data are reported in Table 1. The results of other workers for corn, bean and soybean are given (50) in a study of the amino acid content of these crops. A value of 1.45% is given.

Workers reporting results on isolated samples of these crops, wheat, soybean and alfalfa (51) are given in Table 1. The first group of workers gave a value of 1.11% for the second group used a colorimetric method. These and others (52) compared a colorimetric and a chemical method for the determination of lysine in these amino acids. They found a close correlation between the two methods.

Good (53) states that the only amino-acid containing products of the carbonic cycle are the amino acids lysine and histidine. Further, Good says these acids are found in a few plants in which lysine and histidine are present. The latter two compounds are found in the amino acid lysine and histidine from the protein molecule.

Protein and histidine are important components of the amino acid lysine. These and others (54) have shown that histidine is concentrated in the wheat germ and that these amino acids are only present in the amino acid lysine. These and others (55) state that histidine is present in lysine in a form of lysine and histidine.

or non-reactive form. The reactive groups cause the protein when in the native form to give a positive nitroprusside test. The non-reactive form will only give a positive reaction with the nitroprusside reagent if the protein is first denatured to liberate the sulphhydryl group. Meyers and Working tested gluten for the foregoing groups and concluded that neither free nor bound sulphhydryl groups were present. The water-soluble proteins from wheat flour were found to contain non-reactive sulphhydryl groups which are made reactive on denaturation.

An interesting paper by Blumenthal and Clarke (9) reports that in some proteins sulphur may exist in forms other than cystine, cysteine and methionine. These authors suggest that at least two other sulphur-bearing constituents are present, one of which could be the compound ergothioneine.

Baernstein (2) working with thirty-two different proteins estimated total sulphur and the sulphhydryl, disulphide and methionine fractions. He reports that the three fractions accounted for roughly 101.5% of the total sulphur but values varied from 85.9% to 117.8%.

Bailey (4) states that of the known sulphur-containing amino acids, only cystine and methionine have been isolated from protein hydrolysates. Bailey compared specific methods for cystine and cysteine with methods for cystine which included any additional -S-S-groups which may be present in the protein molecule. He found a disparity between the two estimations. Although the author suggests that there may be losses of cystine

on non-reactive form. The reactive groups were the protein
 which in the native form to give a positive hydrophobic test.
 The non-reactive form will only give a positive reaction when
 the hydrophobic reaction is the negative in later treatment as
 illustrate the aliphatic group. Methyl and methyl ester groups
 for the reacting groups and concluded that neither form nor
 bound aliphatic groups were present. The ester-soluble proteins
 from which they were found to contain non-reactive hydrophobic
 groups which are more reactive on denaturation.

An interesting paper by Rimmant and others (7)
 reports that in some proteins soluble in water in form of
 from cystine, glycine and methionine. These authors report
 that at least two other amino-acids containing the amino
 one of which could be the compound arginine.

Barnett (8) working with three different proteins
 estimated total sulphur and the aliphatic, thiol and
 methionine residues. He reports that the three proteins
 accounted for roughly 10.5% of the total sulphur but values
 varied from 6.5% to 17.5%.

Smith (9) states that of the three amino-acids
 methionine, only glycine and methionine have been isolated from
 protein hydrolysates. Smith compared the results obtained for
 cystine and cysteine with methods for detecting amino acids
 and additional amino-acids which may be present in the protein
 molecule. He found a discrepancy between the two methods.
 Although the author suggests that there may be some of glycine

due to decarboxylation or deamination of a portion of the cystine during hydrolysis, or inhibition of color used in the colorimetric determination of substances present in the protein hydrolysate, he does not preclude the possibility of the presence of sulphur-containing amino acids other than cystine and methionine.

In a later paper Baernstein (3), using newer methods, determined the cystine and methionine in eleven proteins. He obtained an average recovery of 96.9% of the total sulphur as the aforementioned amino acids and H_2S . The latter compound was attributed to the presence of inorganic sulphate.

Blumenthal and Clarke (9) found that cystine can be completely oxidized to sulphate by boiling with fuming nitric acid, but synthetic methionine yields only small amounts of sulphate. Using solutions of cystine or methionine separately and together with gelatin, these authors report a very high recovery of cystine-sulphur as sulphate.

Mazur (30) applied this method for cystine to a highly purified Limulus hemocyanin. He found that the nitric-acid-oxidizable sulphur gave values which compared with those obtained using two classical colorimetric methods.

Lugg (29) in 1938 used the oxidizing method for the determination of cystine in a study of the sulphur distribution of protein preparations from leaves of different grass species. Lugg, assuming that the only sulphur-containing amino acids in

and to decomposition or denaturation in a solution of the
specimen being examined, or in the case of a solid specimen
collected in a solution of the specimen in the process
of preparation, the use of a solution of the specimen
of a solution of the specimen in the process of preparation
and.

In a later paper (1912), the author has shown
that the method of preparation and examination in a given specimen
obtained in a given process of preparation in the case of a solid specimen
has been found to be the same as in the case of a solid specimen
was obtained in the process of preparation in the case of a solid specimen.

It is also found that the method of preparation and examination in a given specimen
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protein are cystine and/or cysteine and methionine, used the following relationship:

cystine-S. + cysteine-S. + methionine-S. + other S. = total S.

As "other S." was believed to be present entirely as sulphate it should appear as inorganic SO_4 on acid hydrolysis.

Lugg compared a specific colorimetric procedure and Baernstein's HI digestion method for cystine with his so-called "differential-oxidation procedure." He concluded that the latter method, although less specific than the other quantitative methods, appears to yield more appropriate results. He warns, however, that to accept these results is to accept the assumption that the sum of the fractions thus estimated necessarily equals the total sulphur.

Using a principle similar to that of Lugg, Callan and Toennies (11) found that alkaline-permanganate solution oxidized cystine-sulphur but not methionine-sulphur to sulphate. These authors applied this method to the estimation of cystine in egg albumin.

Block and Bolling (8) reviewed the methods for cystine determinations up to 1945. They suggest that the "differential-oxidation procedure" appears to have special value when estimations of cystine are to be made on protein preparations heavily contaminated with carbohydrate.

Evans (17) in 1945 made a further study of the "differential-oxidation procedure." He oxidized mixtures of pure cystine

$\text{Index} = \frac{\text{reale } x}{\text{minimales } x} \cdot \text{anfänger } x \cdot \text{späterer}$

and/or methionine, with dextrose, soybean oil meal and fish meal. Almost all of the cystine-sulphur but very little of the methionine-sulphur was recovered as sulphate. Evans' data suggest that considerable organic matter must be present to completely oxidize cystine to sulphate.

The values determined for methionine by the "differential oxidation procedure" and a colorimetric method on samples of soybean oil meal and fish meal compare favorably. A comparison of the "differential-oxidation procedure" with other methods showed the cystine and methionine values reported for the former method are respectively higher and lower. The differences could be accounted for by losses of cystine upon acid hydrolysis where this step is taken or a difference in the composition of the materials studied.

Evans (18) and coworkers in a later study applied the "differential-oxidation procedure" to the determination of cystine and methionine in dry peas. This paper is of interest because of the application of this method and because a study was made of the influence of fertilizers and cultural practices on the cystine, methionine and protein contents of dry peas.

Evans et al. report that the addition of sulphur-containing fertilizers to the soil increased the percentage of total sulphur but decreased the protein content of the peas. The protein of peas fertilized with ammonium sulphate or gypsum contained significantly more sulphur than did the protein of

peas fertilized with sodium nitrate. The authors conclude that cystine values are influenced in the same direction as the sulphur but the methionine values were not significantly different.

Evans and coworkers found very small differences in the effect of maturity, climatic conditions and variety on the cystine and methionine contents of peas.

Greaves and Bracken (23) studied the relationship of sulphur to nitrogen in a group of spring and winter wheats. They found that the nitrogen and sulphur contents of twenty-one wheat varieties were closely associated. A correlation coefficient of 0.81 was reported.

Greaves and Bracken also studied the sulphur-content of Kanred wheat under varying cultural conditions. The data presented show that ploughing under of legumes will result in slight increases in the nitrogen content of wheat grown on such land. The sulphur content did not vary consistently.

Gubler and Greaves (21), investigating the same material as Greaves and Bracken, report that variations in cultural treatments cause at least small variations in cystine content.

Smith and Wang (36) made a study of the effects of environmental factors on the cystine and methionine contents of the vegetative parts of four species of grasses. They conclude that the effects of manuring are irregular and are relatively unimportant. Lime did not alter the cystine or methionine to a significant extent. Ammonium sulphate added to

data correlated with sodium chloride. The authors concluded that cystine values are influenced in the same direction as the sodium but the methionine values were not significantly different. Evans and Greville found very small differences in the effect of sodium, chloride conditions and variation on the cystine and methionine content of cereals. Greville and Greville (1931) studied the relationship of sulphur to nitrogen in a group of wheat and maize varieties. They found that the nitrogen and sulphur content of wheat and maize varieties were closely associated. A correlation coefficient of 0.91 was reported. Greville and Greville also studied the relationship of nitrogen to sulphur in wheat varieties under different conditions. The results showed that increasing nitrogen in wheat will result in slight increases in the nitrogen content of wheat grain but not in the sulphur content and very considerably. Greville and Greville (1931), investigating the effect of nitrogen and sulphur on wheat varieties in different types of soils, reported that variations in sulphur content of wheat grain at least small variations in organic content. Greville and Greville (1931) made a study of the effect of environmental factors on the cystine and methionine content of the vegetative parts of four species of cereals. They concluded that the effects of nitrogen and sulphur on the cystine and methionine content of the vegetative parts of four species of cereals are relatively unimportant. Greville and Greville also studied the effect of a significant effect. Greville and Greville (1931) studied the effect of a significant effect.

rye-grass late in the growing stage effected small reductions in the cystine and methionine content.

Wood and Barrien (39) state in a study of the sulphur metabolism of plants, that the sulphate ion constitutes the sole source of sulphur to plants and the sulphur-containing proteins are the synthetic end-products in the sulphur metabolism. These workers obtained the following results with Sudan grass. The application of increased amounts of potassium sulphate in solution to plants did not affect the quantity of cystine-sulphur isolated. Inorganic sulphate - sulphur in the leaves was increased with increasing amounts of sulphate fertilizer. The ratio of protein-nitrogen to protein-sulphur remained approximately the same. Increases in ammonia-nitrogen increased the contents of cystine and protein sulphur as well as amino-nitrogen and protein-nitrogen, but decreased the sulphate-sulphur content. These workers suggest that ammonia acts as a limiting factor in the formation of protein sulphur from sulphate.

Barrien and Wood (7) working with leaves at different stages during the life cycle of the plant show that the ratio of protein-nitrogen to protein-sulphur is higher, the greater the nitrogen treatment. If the initial amount of sulphate supplied to the plants remained unchanged, increased nitrogen treatments were found to increase protein-sulphur.

Peters (33) worked with a number of crop plants including wheat grown on the fertilized plots of the Breton gray soils and Edmonton black soils. He found that at the Breton station, where

the same rate in the growing stage affected leafy vegetables.

in the quality and nutritional content.

Good and Lovell (1951) state in a study of the nitrogen

metabolism of plants, that the nitrogen fixation capability of

soil sources of nitrogen for plants and the nitrogen-fixing

proteins are the synthetically end-products in the soil-nitrogen

These workers obtained the following results with their study.

The application of increased amounts of potassium sulphate in

relation to plants did not affect the quantity of nitrogen-fixing

isolated. Inorganic sulphate - nitrogen in the leaves was

increased with increasing amounts of sulphate fertilizer. The

ratio of protein-nitrogen to protein-sulphur remained approx-

imately the same. The ratio in amino-acid-nitrogen increased in

contents of organic and protein sulphur as well as amino-acid-nitrogen

and protein-nitrogen, but decreased the amino-acid-nitrogen content.

These workers suggest that ammonia acts as a limiting factor in

the formation of protein sulphur from sulphate.

Barrett and Good (1951) working with feeding on different

species during the life cycle of the plant show that the ratio

of protein-nitrogen to protein-sulphur is higher, independent

the nitrogen treatment. It has initial amount of nitrogen

remained in the plants remained unchanged, increased in nitrogen

proteins were found as increase protein-sulphur.

Peters (1951) worked with a number of crop plants including

and wheat grown on the fertilized plots of the same soil with

and observed that the ratio of the nitrogen fixation and

the natural soil sulphur is low, the percentage sulphur of grains and legumes from plots treated with sulphur-containing fertilizers was greater than that of crops from the non-sulphur fertilized plots. Wheat from the Edmonton station did not show an increase in sulphur on the application of a sulphur-containing fertilizer. This was taken as evidence that sulphur is not limiting at Edmonton.

MATERIALS

The material used in these experiments was from three sources:

Series I - Marquis flour, 1944.

Flour was milled from individual samples of Marquis wheat grown in replicated rod-row plots at Athabaska, Bon Accord, Fallis, Warburg, Sundre and at Edmonton on fallow and stubble. A composite sample of Marquis from the Rust Research Laboratory, Winnipeg, was also used. These samples were from the same lots of flour as was the material used by Gerbrandt (19).

Series II - Thatcher wheat and flour, 1946 and 1947.

Thatcher wheat was grown on the Breton Experimental Field in the gray-wooded soil area of Alberta. Through the courtesy of the Department of Soils, University of Alberta,

 

sufficient wheat was made available for milling, baking and other analysis. A complete detailed history of the Breton experiment can be obtained from papers by Wyatt, Newton and Ignatieff (41), Peters (33), and Kenwood (28).

The wheat for the analysis reported in this paper was harvested from the following fields.

Series E, 1946 - Part of this field, plots 1 to 11, has been continuously cropped with wheat since 1929, except for two years when the field was fallowed and one year when barley was grown. The rest of the field, plots 13 to 26, has been cropped continuously with wheat since 1939.

Series B, 1946 - The wheat was grown after clovers which had occupied the land for two years in a five year rotation.

Series C, 1947 - This field was the wheat after clover crop for 1947.

Each of the foregoing series consists of twenty-six plots which are variously fertilized. The total amounts of fertilizers applied to all plots for each series are listed in Table I, page 19, of Kenwood's paper (28). During the summer of 1947, no fertilizers were added to the plots.

Wheat from twenty-one of the twenty-six plots in each of the above three series was used for the determinations reported in this thesis. In addition the wheat was milled into flour and further determinations were carried out. All of the

antitoxin of the same was applied for all the other animals. The animals which died of the disease were kept in the hospital for a few days and then were buried. (See also page 102).

The results of the analysis reported in this paper are derived from the following tables.

Table 1. 1914 - Part of this table, from 1 to 11, was given in the preceding paper. The results of the analysis for the two years when the field was followed and the year when the field was grown. The results of the analysis for 1914, 1915, and 1916, have been given in the preceding paper. The results of the analysis for 1917, 1918, and 1919, are given in this table.

Table 2. 1914 - This table was given in the preceding paper. The results of the analysis for the two years when the field was followed and the year when the field was grown. The results of the analysis for 1914, 1915, and 1916, have been given in the preceding paper. The results of the analysis for 1917, 1918, and 1919, are given in this table.

Table 3. 1914 - This table was given in the preceding paper. The results of the analysis for the two years when the field was followed and the year when the field was grown. The results of the analysis for 1914, 1915, and 1916, have been given in the preceding paper. The results of the analysis for 1917, 1918, and 1919, are given in this table.

samples from plots one to eleven were used, the checks 1, 5 and 11 being bulked to give a representative check sample. Ten samples from plots 13 to 26 were selected because of the relative amounts of nitrogen and sulphur fertilizers used. The samples were 13, and 18 to 26 inclusive. The check plots 13 and 18 were bulked to give a single check sample representative of a **part** of the field. Samples 22 and 26 were bulked for the same reason.

Table I shows the Breton plot numbers, the sample numbers used throughout this paper, the fertilizers used on each **plot** and the grades of the harvested samples.

Table II gives the approximate composition of the fertilizers used in experiments on the gray-wooded soils.

samples from plots 1, 2 and 3 were used, and results are given in Table I. It being desired to have a representative sample of the samples from plots 1 to 3, the selected number of the relative abundance of the samples was determined. The samples were 1, 2 and 3 inclusive. The results are given in Table II. It being desired to have a representative sample of the samples from plots 1 to 3, the selected number of the relative abundance of the samples was determined. The samples were 1, 2 and 3 inclusive. The results are given in Table II. It being desired to have a representative sample of the samples from plots 1 to 3, the selected number of the relative abundance of the samples was determined. The samples were 1, 2 and 3 inclusive. The results are given in Table II.

TABLE I

Key to sample numbers and the grades
of Thatcher wheat grown at Breton

Plot No.	Sample No.	Fertilizer	Grades		
			1946		1947
			Series E	Series B	Series C
1,5,11	1	Check	2,3,2	5,5,4	5,5,5
2	2	Manure	2	4	6
3	3	16-20 + K_2SO_4	2	5	6
4	4	Ammonium Sulphate	2	3	6
5	5	Lime	2	4	5
7	6	Lime + Triple Phos.	2	4	5
8	7	Triple Phos.	3	4	5
9	8	Manure + 16-20	3	4	5
10	9	16-20	3	4	5
13,18	10	Check	3,2	4,4	5,5
19	11	Ammonium Nitrate	2	3	5
20	12	Ammonium Nitrate + Gypsum	2	4	5
21	13	Gypsum	3	4	6
22,26	14	Check	3,3	3,3	5,*
23	15	Sodium sulphate	3	3	5
24	16	Potassium sulphate	3	3	5
25	17	Ammonium sulphate + potassium sulphate	3	3	5

*Lost

TABLE II

Approximate composition of fertilizers used
in the gray-wooded soils experiments

Fertilizer	N %	P ₂ O ₅ %	K %	S %	Gypsum Equivalent %
Triple superphosphate	1	43	-	1	6
Ammonium phosphate (16-20)	16	20	-	14	-
Ammonium sulphate	20	-	-	24	-
Gypsum	-	-	-	18	95
Sodium sulphate (dry)	-	-	-	22	-
" " (hydrated)	-	-	12	11	-
Potassium sulphate	-	-	40	16	-
Lime	-	-	-	-	-
Ammonium nitrate	34	-	-	-	-

TABLE I

Analysis of the soil samples collected from the experimental plots

Soil samples			Analysis		Soil samples	
1951	1952	1953	1951	1952	1951	1952
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
4	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	7	7	7	7	7
8	8	8	8	8	8	8
9	9	9	9	9	9	9
10	10	10	10	10	10	10
11	11	11	11	11	11	11
12	12	12	12	12	12	12
13	13	13	13	13	13	13
14	14	14	14	14	14	14
15	15	15	15	15	15	15
16	16	16	16	16	16	16
17	17	17	17	17	17	17
18	18	18	18	18	18	18
19	19	19	19	19	19	19
20	20	20	20	20	20	20

Table I

TABLE II

Analysis of the soil samples collected from the experimental plots

Soil samples			Analysis		Soil samples	
1951	1952	1953	1951	1952	1951	1952
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
4	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	7	7	7	7	7
8	8	8	8	8	8	8
9	9	9	9	9	9	9
10	10	10	10	10	10	10
11	11	11	11	11	11	11
12	12	12	12	12	12	12
13	13	13	13	13	13	13
14	14	14	14	14	14	14
15	15	15	15	15	15	15
16	16	16	16	16	16	16
17	17	17	17	17	17	17
18	18	18	18	18	18	18
19	19	19	19	19	19	19
20	20	20	20	20	20	20

Series III - Water Culture Solutions

The general procedure used for growing plants in culture solutions is given by Hoagland (26). The method for growing wheat was modified somewhat. Following is a brief description of the procedure, equipment and solutions used in the experiments with wheat.

Four tanks were used in each experiment. The larger tanks, each with a capacity of 225 liters of solution and with openings for 125 corks, were used in experiments I and II. In experiments III and IV, each tank held 64 corks and had a capacity of 110 liters of solution. Each cork contained two or three holes in which the wheat plants were placed.

The wheat used throughout all the experiments was the variety Red Bobs. The seed was germinated and grown on a germinating screen. When the plants reached a height of 1 1/2 to 3 inches they were placed in the corks so that the roots were immersed in nutrient solution.

The nutrient solutions used in the experiments were based on Hoagland's solution (26) but modifications were made to suit each experiment. All the chemicals excepting the iron tartrate, calcium di-hydrogen phosphate and the A-Z solution were made up as one-molar stock solutions. The concentrations of the stock solutions of iron tartrate and calcium di-hydrogen phosphate were 0.5% and 0.05 M respectively. The A-Z solution was made up as suggested by Hoagland.

Section III - Water Culture Methods

The general procedure used for growing plants in nutrient

solutions is given by Howland (1931). The nutrient solutions

used were modified somewhat. Following is a brief description

of the procedure, equipment and solutions used in the experiments

described.

Four tanks were used in each experiment. The tanks

were, each with a capacity of 225 liters of solution and with

equipment for 125 plants, were used in experiments 1 and 2. In

experiments 3 and 4, each tank held 60 plants and had a

capacity of 120 liters of solution. Each tank contained two

or three plants in which the whole plants were placed.

The whole plants throughout all the experiments were the

variety Red Top. The seed was germinated and grown on a

seed screen. When the plants reached a height of 1.5 to 2

inches they were placed in the tanks so that the roots were

immersed in nutrient solution.

The nutrient solutions used in the experiments were based

on Howland's solution (1931) but modifications were made to suit

each experiment. All the chemical weighing and iron treatment

of the nutrient solution and the 4-2 solution were done in

an air-tight glass bottle. The concentration of the

solutions of iron, calcium and sodium 4-hydroxybenzoate were

0.5% and 0.05% respectively. The 4-2 solution was made up in

suggested by Howland.

The volumes of stock solutions used for each tank are given in Tables III and IV.

Experiment I - Table III gives the composition of the complete and sulphur-deficient solutions. The wheat plants of all four tanks were placed in complete nutrient solutions for the first two weeks after transplanting. At two week intervals the complete solutions of tanks 1, 2 and 3 were replaced by the sulphur-deficient solution. Plants in tank 4 were grown throughout on a complete solution.

TABLE III

Volumes of stock solutions added to each tank containing approximately 225 liters of water

Chemical	Concentration	Complete Solution	Solution Lacking Sulphur
		Volume c.c.'s	Volume c.c.'s
KH_2PO_4	1 M	225	225
KNO_3	1 M	1125	1325
CaNO_3	1 M	1125	900
MgSO_4	1 M	450	--
$\text{Mg}(\text{NO}_3)_2$	1 M	--	450
A-Z solution	--	225	225
Fe Tartrate	0.5%	*	*

*1 c.c. of stock solution per litre of water about once or twice a week.

The volume of stock solution used for each run is

given in Tables III and IV.

Experiment I - Table III gives the composition of the solutions and nitrogen-bellied solutions. The same amount of all four tanks were mixed in duplicate nitrogen-bellied for the first two weeks after crystallization. It was then analyzed for nitrogen-bellied solution. Table IV shows the composition of the nitrogen-bellied solution. Table IV shows the composition of the nitrogen-bellied solution. Table IV shows the composition of the nitrogen-bellied solution.

TABLE III

Volume of stock solution added to each tank
containing approximately 250 liters of water

Stock solution volume c.c.'s	Nitrogen-bellied solution volume c.c.'s	Stock solution volume c.c.'s	Stock solution volume c.c.'s
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100
100	100	100	100

Wt. of stock solution per liter of water added to each tank after 2 weeks.

As the plants in this experiment were transplanted in September, they did not mature until the latter part of December. From approximately October 22 to the time of harvest, it was necessary to supplement daylight with artificial illumination.

Experiment II - Experiment I was carried out in the greenhouse. Considerable trouble was experienced from mildew, fungus-gnats, and other pathogenic conditions. Because of the greenhouse hazards it was decided to repeat the experiment with the tanks out-of-doors. The plants were grown during the summer months from May 5 to September 15.

Experiment III - Solutions were made up in four tanks to supply the following four conditions:

Tank 1 - low sulphur, low nitrogen.

Tank 2 - high sulphur, low nitrogen.

Tank 3 - low sulphur, high nitrogen.

Tank 4 - high sulphur, high nitrogen.

The volumes of stock solutions for each tank are given in Table IV.

As the object of this investigation was to establish the
 treatment, the 150 and 200 mg. doses were given in the form of capsules.
 From the results of the treatment it is evident that it is not
 necessary to administer 150 mg. daily to obtain the desired effect.

Experiment II - Experiment I was repeated on 15 rats. The
 results were similar to those of Experiment I. The 150 mg. dose
 was found to be effective. The 200 mg. dose was found to be
 effective. It was decided to repeat the experiment with the 150 mg.
 dose. The results were similar to those of Experiment I.
 From May 5 to November 15.

Experiment III - The following were used in the tests for activity:
 the following four conditions:

- Group 1 - low activity, low activity.
- Group 2 - high activity, low activity.
- Group 3 - low activity, high activity.
- Group 4 - high activity, high activity.

The volume of blood collected was about 100 ml.

In Table IV.

TABLE IV

Volumes of stock solutions added to tanks
containing 110 litres of distilled water

	High Nitrogen High Sulphur Tank 4	High Nitrogen Low Sulphur Tank 3	Low Nitrogen High Sulphur Tank 2	Low Nitrogen Low Sulphur Tank 1
	Volume cc's	Volume cc's	Volume cc's	Volume cc's
KH_2PO_4	110	110	660	660
KNO_3	560	560	-	-
$\text{Ca}(\text{NO}_3)_2$	560	475	12	-
MgSO_4	225	6	210	6
$\text{Mg}(\text{NO}_3)_2$	-	170	-	12
A-Z	110	110	110	110
$\text{Ca}(\text{H}_2\text{PO}_4)_2$	-	-	10,800	4,200
Fe Tartrate	110*	110*	110*	110*

*Iron tartrate is added two or three times a week according to the foliage color.

The material was grown in the greenhouse from May 5 to July 29, 1947.

Yield data were not taken because of extensive damage by sparrows. Plate I shows the relative heights and density of growth of the wheat at harvest.

Volume of about 100 pages is being
estimated 100 to 150 pages.

• 1961, 22 Feb. 1961

PLATE I

Relative heights and density of growth
for experiment III.

Experiment IV - Experiment III was repeated in the greenhouse from August 5 to December 3, 1947. Tanks 3 and 4 were discarded because of very poor growth.

Two months after transplanting, when about one-quarter of the wheat was in head, sulphur was completely eliminated from Tank 2.

Artificial light was used to supplement daylight from the last week in October until harvest.

The plants became badly infested with mildew and were therefore treated with sulphur fumes on September 24. Twice during the growing season nicotine sulphate was sprayed in the greenhouse to destroy aphids.

METHODS

Statistical Analysis

The statistical methods described by Goulden (20) are used throughout this study.

Gluten Washing

Four percent mono-phosphate adjusted to pH 6.8 by the addition of sodium di-phosphate, as suggested by Dill and Alsbury (15), was used as a stock solution. The actual washing solution consisted of the stock solution diluted with unboiled distilled water to a 0.1% concentration. Thirty-gram samples

Experiment IV - Experiment III was repeated in the greenhouse from March 2 to October 2, 1957. Plants 2 and 3 were also added because of very poor growth. Two months after transplanting, when about one-quarter of the shoot was in bud, solution was completely eliminated from both 2.

Experiments I-III were used as treatment controls from the last week in October until harvest. The plants became badly stressed with little or no growth. Growth resumed with slight gain in October 2. When during the growing season solution cultures were changed in the greenhouse to heavy soils.

RESULTS

Experiments I-III

The statistical results described by Gower (1957) are used throughout this report.

Experiments I-III

Four percent mono-phosphate solution (0.4%) of the addition of sodium di-phosphate, an equivalent of 0.1% of the solution (15%), was used as a control solution. The control solution consisted of the same solution of sodium di-phosphate and sodium di-phosphate as a 0.1% concentration. The control solution

of flour were washed for 12 minutes with a stream of solution delivered at approximately 100 ml. per minute.

As much water as possible was squeezed from the samples by hand, before wet weights of gluten were recorded. Weights of the dry gluten were obtained after drying in vacuo at 98°C. for approximately 24 hours.

Baking

Flour samples of 100 grams were baked using the malt-phosphate-bromate formula, with 0.3% diastatic malt, 0.1% ammonium phosphate, and 0.001% potassium bromate. Single bakes were carried out on each Breton sample because of the small quantity of flour.

Nitrogen

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, with mercuric oxide as a catalyst. The results are reported as percentage of the dry matter.

Sulphur

The A.O.A.C. method (1), using nickel crucibles, open fusion with sodium-carbonate and sodium-peroxide as modified by Rigby (34) was used for sulphur determinations.

Cystine and Methionine

It was desirable to find a method for cystine and methionine that could be applied to materials containing large amounts of carbohydrate and other non-protein materials. The differential-

1910

REPORT IV

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oxidation procedure, as used by Evans (17) on soybean meal, was found to be satisfactory for wheat and wheat products.

Preparation of the sample

The whole wheat was ground to a fine meal in a Wiley mill. The dried gluten samples were ground in a burr mill.

The samples of wheat, flour and gluten were oven dried for 1 hour at 130°C. Duplicate samples of about 4 grams of wheat and flour or 0.5 grams of gluten were accurately weighed.

Determination and Calculation of Cystine

The samples were boiled with concentrated nitric acid in a hydrolyzing flask under a reflux condenser for 12 to 16 hours. Fifteen millilitres of concentrated nitric acid (specific gravity 1.61) were added for every one gram of material. After oxidation the clear, yellow hydrolyzate was transferred to a 600 ml. beaker, and one-half a gram of KNO_3 was added. The solution was evaporated to dryness on a steam-bath. To eliminate any nitrates, which would interfere with the subsequent sulphate determination, two 10 cc. portions of concentrated HCl were added and evaporated.

The dry residue was dissolved in approximately 300 ml. of distilled water and 1 ml. of concentrated HCl. The solution was boiled and filtered into a 600 ml. wide-mouthed erlenmeyer flask. The remaining procedure is identical with the determination of sulphates in any solution.

The calculation of cystine percent is as follows:

$$\text{Cystine \%} = \frac{\text{Weight of BaSO}_4 \times 51.47}{\text{Weight of sample}}$$

analysis procedure, as used by Evans (17) on organic matter, was found to be satisfactory for wood and wheat products.

Preparation of the sample

The whole wood was ground to a fine mesh in a Wiley mill. The dried wood samples were ground in a Wiley mill. The samples of wood, flour and lignin were oven dried at 100°C. Duplicate samples of about 1 gram of wood and flour in U.S. glass vials were accurately weighed.

Calculation and adjustment of lysine

The samples were dried with concentrated nitric acid in a desiccator flask under a reflux condenser for 12 to 16 hours. A known amount of concentrated nitric acid (specific gravity 1.42) was added for every one gram of material. After oxidation the flask, yellow-orange, was transferred to a 500 ml. beaker and 200 ml. of water was added. The solution was evaporated to dryness on a steam-bath. To eliminate any nitric acid, the residue was dissolved in 10 ml. of concentrated HCl which was added and evaporated. The dry residue was dissolved in approximately 100 ml. of distilled water and 5 ml. of concentrated HCl. The solution was boiled and filtered into a 500 ml. volumetric flask. The residual amount is identical with the determination of nitric acid in the solution.

The relationship of lysine content is as follows:

$$\frac{\text{Weight of } \text{BaSO}_4 \times 21.1\%}{\text{Weight of sample}} = \text{Lysine } \%$$

Calculation of Methionine

Assuming that cystine-sulphur and methionine-sulphur make up the total sulphur of the wheat, flour or gluten, we can calculate the methionine content as:

$$\text{Methionine-sulphur} = \text{Total sulphur minus cystine-sulphur.}$$

The foregoing procedure for the determination of cystine and methionine in wheat, flour or gluten is based on two assumptions:

(1) Cystine and methionine are the only sulphur compounds present in significant quantities in wheat and wheat products.

(Cysteine and glutathione sulphur are considered as part of the cystine-sulphur fraction.)

(2) Cystine-sulphur is oxidized to the sulphate form. The methionine-sulphur is not oxidized to sulphate-sulphur.

With reference to the first assumption, a review of the literature showed that, besides the sulphur-containing amino acids, thiamine and biotin are the only organic sulphur compounds whose presence has been reported in wheat. These substances are present in such small amounts that the sulphur contributed by them would not affect the significance of the results reported in this thesis.

Allowances must be made for sulphate-sulphur if it is present in the wheat. It is generally known that if sulphate-sulphur is present in the wheat kernel it is in very small amounts. In all the literature reviewed there were no reports of sulphate-sulphur in wheat. Woodman and Evans (40), however, reported

Calculation of the effect

Assuming that the effect of the system is

equal to the total number of the system, then the effect is

calculated as follows:

Meaningful number = Total number of the system.

The following procedure for the determination of the effect

and calculation is shown, first on the basis of two systems:

(1) System and calculation are the only significant systems present

in significant systems in which and which provide.

(2) System and calculation are considered as part of

the system-calculation system.

(3) System-calculation is related to the system-calculation. The

relationship-calculation is not related to system-calculation.

With reference to the first assumption, a number of the

relationships shown here, between the system-calculation system

and, calculation and calculation are the only significant systems present

which provides the basis for the calculation. These systems are

present in each case, and the calculation is calculated by

the system-calculation system. The calculation is calculated by

the system-calculation system.

Assuming that the effect of the system is

equal to the total number of the system, then the effect is

calculated as follows:

Meaningful number = Total number of the system.

The following procedure for the determination of the effect

small amounts of this sulphur form in bran and middlings. The method of Woodman and Evans for sulphate-sulphur was applied to some samples of the wheat and flour used in this study. As no sulphate-sulphur was detected in the samples surveyed, no further attempts were made to determine this sulphur-fraction.

Gerbrandt (19) used methods completely different from those reported in this paper. He accounted for all of the sulphur as cystine and methionine in a number of samples of crude gluten.

The assumption that cystine-sulphur is oxidized to sulphate-sulphur and methionine is not, was validated by Evans (17) and Blumenthal and Clarke (9). Evans carried out recovery experiments of pure cystine and methionine from soybean and fish meals. Blumenthal and Clarke did similar experiments with gelatin.

To test the "differential-oxidation procedure" further, determinations were made on samples of flour and samples of flour to which cystine or methionine had been added.

In the recovery experiments reported in Table V, different amounts of cystine were added to a commercial flour with a known cystine content. The flour and cystine mixture was oxidized for approximately 15 hours with 7 ml. of nitric acid per gram of flour. As shown by results presented later better oxidation and purer precipitates of barium sulphate can be obtained with larger amounts of nitric acid. The regular cystine determination was carried out and the results are reported in Table V.

TABLE V

Cystine recovery from a commercial flour.

Flour gms.	Cystine added mgms.	Theoretical cystine mgms.	Actual cystine recovered mgms.	Recovery % of Theoretical
1.000	0	--	4.25) 4.05)4.15	
1.000	18.75	22.90	(a) 20.59 (b) 21.51 (c) 21.57 (d) 21.93	(a) 89.9 (b) 93.9 (c) 94.2 (d) 95.8
2.500	12.50	22.88	(a) 22.18 (b) 22.34 (c) 23.62 (d) 21.72	(a) 96.9 (b) 97.6 (c) 103.2 (d) 94.9
4.000	5.90	21.60	(a) 21.46 (b) 22.48	(a) 99.4 (b) 102.7
*5.000	12.50	33.25	(a) 33.25 (b) 35.10	(a) 100.0 (b) 105.6
6.000	20.00	64.90	(a) 66.60 (b) 67.22	(a) 102.6 (b) 103.6
10.000	25.00	66.40	(a) 66.22 (b) 69.10	(a) 99.7 (b) 104.1

*Flour acid ratio 1:8

The results presented in Table V show that cystine-sulphur is quantitatively oxidized to sulphate-sulphur. Except for the one-gram sample, the actual recoveries of cystine added to flour are within 6% of complete recovery. The results for the one-gram sample are low. Evans showed that in recovery experiments of this type considerable organic matter must be present to get a complete oxidation of cystine-sulphur to sulphate-sulphur. The one gram sample confirms this observation.

TABLE V
Cystine recovery from a commercial flour.

Flour Sample	Cystine added mgm.	Unhydrolyzed cystine mgm.	Actual recovery mgm.	Recovery %
1.000	0	—	1.53 (1.07)	153 (107)
1.600	16.75	24.90	(a) 20.50 (b) 21.10 (c) 21.50 (d) 21.70	(a) 99.8 (b) 128.2 (c) 100.0 (d) 105.0
2.500	25.50	31.30	(a) 25.10 (b) 25.50 (c) 25.60 (d) 25.70	(a) 96.7 (b) 102.0 (c) 102.3 (d) 102.8
4.000	40.00	41.60	(a) 41.40 (b) 41.40	(a) 99.4 (b) 103.7
5.000	50.00	51.25	(a) 51.20 (b) 51.10	(a) 102.4 (b) 102.2
6.000	60.00	61.90	(a) 61.60 (b) 61.50	(a) 102.6 (b) 102.6
10.000	100.00	101.40	(a) 101.20 (b) 101.10	(a) 99.7 (b) 101.1

*Flour acid ratio 1:3

The results presented in Table V show that cystine-
aliphatic is quantitatively oxidized to sulphate-aliphatic. Great
for the one gram sample, the actual recovery of cystine added
to flour are within 95% of complete recovery. The results for the
one gram sample are low. It was noted that in recovery experi-
ments of this type considerable organic matter must be present
to get a complete oxidation of cystine-aliphatic to sulphate-aliphatic.
The one gram sample contains this impurity.

To test whether methionine-sulphur is oxidized to sulphates, different amounts of methionine and flour were treated with nitric acid. The procedure for this experiment was the same as for the experiment with cystine. The results are reported in Table VI.

TABLE VI

Methionine recovery from a commercial flour.

Flour gms.	Methionine* added mgms.	Theoretical cystine mgms.	Actual cystine recovered	Recovery % of Theoretical
1.000	0	--	4.25) 4.05) 4.15	
1.000	10.00 (16.10)	4.15	1.44 5.30	34.7 127.7
2.500	6.25 (10.06)	10.38	10.86 10.04	104.6 96.7
4.000	2.50 (4.03)	16.60	16.08 15.18 15.24	96.9 91.4 91.8
5.000	2.50 (4.03)	20.75	20.85 22.24	100.5 107.2
**1.000	0	--	4.35) 4.32) 4.34	
5.000	3.75 (6.03)	21.70	21.26 20.43	98.0 94.1

*Cystine equivalent in brackets.

**A new standard flour.

The results in Table VI show that methionine-sulphur is not oxidized to sulphate-sulphur under these conditions.

To test whether methionine-sulfoxide is oxidized to

methionine, different amounts of methionine and flow were examined

with nitric acid. The procedure for this experiment was the

same as for the experiment with cysteine. The results are reported

in Table VI.

TABLE VI

Methionine recovery from a commercial flow.

Flow gms.	Methionine added gms.	Theoretical cysteine gms.	Actual cysteine recovered	Recovery % of theoretical
1.000	0	--	1.05 (1.05) 0.15	
1.000	10.00 (10.00)	0.15	1.14 2.30	90.0 127.5
2.500	0.25 (10.00)	10.30	10.65 10.04	100.4 96.7
4.000	2.50 (4.00)	16.60	16.08 15.14 12.24	96.8 91.8 73.8
5.000	2.50 (4.00)	20.92	20.52 22.24	100.2 107.3
101.000	0	--	11.52 (11.52) 0.34	
2.000	2.75 (2.00)	21.74	21.56 20.18	99.2 92.7

Methionine appearing in products.
was not separated from.

The results in Table VI show that methionine-sulfoxide

is not oxidized to methionine-sulfoxide under these conditions.

In most of the experiments the cystine recovered is within 6% of the actual amount present. Where the actual recovery is not within 6% of the theoretical, the values are generally lower. If the methionine-sulphur was oxidized the recovery would be much more than indicated in the table.

The results reported in Table VI suggest that the cystine of the flour is not completely oxidized. The nitric acid : flour ratio is very much smaller than that used by other workers and might account for the incomplete oxidation of cystine.

Because the oxidation of cystine apparently was not completed in some of the foregoing cases, experiments were carried out to find the best conditions for complete oxidation. The results of different oxidation times and different nitric acid : flour ratios on the oxidation of flour cystine were studied.

Table VII shows the effect of different amounts of nitric acid on the oxidation of flour cystine. Five-gram flour samples were oxidized for approximately 15 hours with different volumes of nitric acid.

TABLE VII

The effect of different volumes of nitric acid
on the oxidation of flour cystine

Ratio of gms. flour to mls. acid	Mgms. of cystine per gram flour	Remarks
1-4	3.61) 3.79)	Barium Sulphate precipitate black. Hydrolyzate dark brown.
1-5	4.11	Barium Sulphate precipitate yellow-brown. Hydrolyzate brown.
1-6	4.52	Dirty-white precipitate. Hydrolyzate yellow.
1-7	4.51	Dirty-white precipitate. Yellow hydrolyzate.
1-8	4.77	White precipitate. Hydrolyzate light yellow to white.
1-9	4.57	"
1-10	4.85	"
1-11	4.88	"
1-12	4.58	"

At ratios higher than 1:7 results are reported as averages of duplicate determinations that check within 5%. With flour:acid ratios below 1:8 there was considerable variation between duplicates and as a result more than two determinations had to be made at these ratios.

Generally, the results show that increases in nitric acid volume to 8 ml. per gram of flour bring about increases in the sulphate-sulphur recovered. At the lower acid concentrations

TABLE VII

The effect of different volumes of water on the solution of zinc pyrophosphate

Volume of water, ml.	Ratio of the amount of zinc pyrophosphate dissolved to the amount of water, g.	Volume of water, ml.
1-4	1.52	1-4
1-5	1.51	1-5
1-6	1.50	1-6
1-7	1.49	1-7
1-8	1.48	1-8
1-9	1.47	1-9
1-10	1.46	1-10
1-11	1.45	1-11
1-12	1.44	1-12

At the same time, the results are reported as averages of several determinations made within 24 hours. The results show that the amount of zinc pyrophosphate dissolved in water is not significantly different from the amount of water used. The results are reported as averages of several determinations made within 24 hours. The results show that the amount of zinc pyrophosphate dissolved in water is not significantly different from the amount of water used. The results are reported as averages of several determinations made within 24 hours. The results show that the amount of zinc pyrophosphate dissolved in water is not significantly different from the amount of water used.

the barium sulphate precipitate varies in color from black to a dirty white. This indicates that at these concentrations the oxidation is not complete and there may be considerable occlusion of impurities. Above the 1:8 ratio the precipitate is white.

It was desirable to observe the effect of still higher concentrations of acid on the amount of sulphate-sulphur recovered.

Oxidations were carried out on a new standard flour with flour : acid ratios ranging from 1:7 to 1:17. The results are reported in Table VIII as averages of duplicates which check within 5%. The oxidation time was approximately 15 hours.

TABLE VIII

Effect of flour : acid ratios
on the sulphate-sulphur determined

<u>Ratio</u> <u>gms. flour to</u> <u>mls. acid</u>	<u>Mgms. cystine</u> <u>per gram flour</u>
1:7	4.10
1:9	4.25
1:11	4.35
1:13	4.28
1:15	4.07
1:17	4.19
Mean	4.21 $\pm$ 0.11

Table VIII suggests that there are small differences in sulphate-sulphur determined above the flour : acid ratios of 1:7. At the ratios reported in the table it is assumed that

The various biological properties which are listed in Table I are of a general nature. The results of the various experiments are given in the following tables. The first table shows the results of the experiments on the effect of the various factors on the growth of the organism. The second table shows the results of the experiments on the effect of the various factors on the reproduction of the organism. The third table shows the results of the experiments on the effect of the various factors on the survival of the organism.

It was possible to observe the effect of each of the various factors on the growth of the organism. The results of these experiments are given in Table II. The first column shows the factor which was varied. The second column shows the range of the factor. The third column shows the results of the experiments.

Observations were carried out on a new standard flower with flowers of white color ranging from 1:1 to 1:17. The results are reported in Table III as averages of individual values. The coloration time was approximately 15 days.

TABLE III

Effect of flower : seed ratio on the percentage of seedlings developed

Seedling ratio	Percentage of seedlings developed
1:1	100
1:2	100
1:3	100
1:4	100
1:5	100
1:6	100
1:7	100
1:8	100
1:9	100
1:10	100
1:11	100
1:12	100
1:13	100
1:14	100
1:15	100
1:16	100
1:17	100

Table III suggests that there are small differences in the percentage of seedlings developed above the 1:1 ratio of seed to flower. The results of these experiments are given in Table IV. The first column shows the factor which was varied. The second column shows the range of the factor. The third column shows the results of the experiments.

cystine is oxidized completely to sulphate with a hydrolysis time of 15 hours.

In view of the foregoing, determinations of cystine for this paper were carried out with flour : acid ratios of 1:15. Exceptions to this ratio are noted.

The effect on the quantity of sulphate-sulphur measured, as a result of different oxidation times, is presented in Tables IX and X.

Tables IX and X give the results of different hydrolysis times at flour : acid ratios of 1:6 and 1:7 respectively. The results are presented as averages of duplicates which check within 5%. The flours in each table are from a different lot.

TABLE IX

Comparison of various hydrolysis times
on the sulphate-sulphur determined
(Flour : acid ratio 1:6)

Time	Mgms. cystine per gram flour	Remarks
2	4.56	Precipitate brown to black.
4	4.62	Precipitate brown to black.
6	4.58	Precipitate brown to black
8	4.37	Precipitate brown.
15	4.52	Dirty white precipitate.

crystalline is obtained completely on treatment with a hydrochloric

time of 15 hours.

In view of the foregoing, determination of crystals for

this paper were carried out with films : acid ratio of 1:5.

Experiments on this ratio are noted.

The effect of the quantity of sulphuric acid on the reaction

as a result of different oxidation times, is summarized in

Tables I and II.

Tables I and II give the results of different experiments

times of films : acid ratio of 1:5 and 1:1 respectively. The

results are presented as average of duplicate which show

within 5%. The films in each table are from a different lot.

TABLE I

Concentration of various hydrochloric films
on the sulphuric acid determined
(films : acid ratio 1:5)

Time	per cent film	hydrochloric
15	1.0	100% white crystalline.
10	1.5	100% white crystalline.
5	2.0	100% white crystalline.
4	2.5	100% white crystalline.
3	3.0	100% white crystalline.
2	4.0	100% white crystalline.
1	5.0	100% white crystalline.

TABLE X

Comparison of various hydrolysis times
on sulphate-sulphur determined.
(Flour : acid ratio 1:7)

<u>Time</u>	<u>Mgms. cystine</u>	<u>Color of precipitate</u>
2	4.38	In all cases brown to black
4	4.28	
6	4.30	
8	4.27	

The data presented in the tables suggest that time of oxidation over 2 hours has little effect on the sulphate-sulphur determined. The precipitates, however, are darkly colored which indicates there is considerable occlusion of impurities in the precipitates and that oxidation is probably not complete. At the shorter hydrolysis times perhaps one effect will offset another and thus there is no difference in the weight of the precipitates. As the oxidation times were increased the precipitate lost much of its darker color and took on the appearance of a sulphate precipitate. From a previous experiment (Table VI) we would not expect a completely white precipitate at the lower flour : acid ratios used in these studies.

If two or more completely different methods yield the same value for a sample, it is highly probable that the value as reported is correct. It is unlikely that more than one method would respond to different interfering substances in exactly the

TABLE X

Concentration of various electrolytes in
an artificial sea water.
Medium: 1000 ml. 1:1

Time	Concentration	Concentration
0	1.00	1.00
1	1.00	1.00
2	1.00	1.00
3	1.00	1.00
4	1.00	1.00
5	1.00	1.00
6	1.00	1.00
7	1.00	1.00
8	1.00	1.00
9	1.00	1.00
10	1.00	1.00

The data presented in the table suggest that the
adjustment over 10 hours has little effect on the electrolyte-
balance. The electrolyte, however, was hardly adjusted with
liquor there is considerable evidence of adjustment in the
proportion and that adjustment is probably not complete. In
the standard electrolyte there appears one effect will occur
another and this time is no difference in the amount of the
proportion. In the adjusted time were increased and the
is also found in the standard and both on the adjustment
of a standard proportion. From a previous experiment (Table 1)
we would not expect a completely white proportion as the first
time a white color was in these studies.

It can be seen completely different and the first
time when the water is in its proper state and the water is
proportion is correct. It is obvious that water and the
would remain in different interesting conditions in which the

same manner and to exactly the same extent. Comparisons of different methods for the determination of a single substance in the same sample are important to decide whether a single method gives reliable results.

Determinations of cystine were carried out on the same gluten by the cuprous mercaptide method (19) and the differential-oxidation procedure. In addition gluten sample one was analysed for methionine by the method of Lavine, which is based on the reaction of methionine with iodine, and the differential-oxidation procedure. The results are reported in Table A.

Samples one and two are glutes from Thatcher wheat grown at respectively Fallis and Edmonton.

TABLE A

Cystine and methionine determinations
on gluten using different methods

Sample	Cystine		Methionine	
	Mg. per gm. gluten Cu. mercaptide method	Differential- oxid.procedure	Mg. per gm. gluten Lavine method	Differential- oxid.procedure
1	17.86 18.03	17.77 17.57	9.03 9.04	- -
Mean	17.95	17.67	9.04	9.91
2	20.83*	20.59*		

*Single determination

The differences in the cystine contents of the gluten as determined by the two methods is within the 5% error allowed between duplicates. In other words there does not appear to be a

significant difference between the results of the two methods. The slightly higher result for the cuprous mercaptide method may be due to the presence of purines.

The results of the methionine determinations check within approximately 10% for this sample of gluten.

There is no reliable specific method for the determination of cystine in materials containing large amounts of carbohydrates. Thus it is very difficult to compare accurately results from specific and the differential-oxidation procedures.

A microbiological method for methionine determinations has been used successfully on organic materials which contain large amounts of carbohydrates. This method (35) was used for comparison.

Methionine determinations were carried out on a one-gram flour sample which was hydrolysed at 15 pounds pressure in 25 ml. of 2 N HCl. The hydrolysate was filtered and neutralized. Triplicate determinations were carried out at two dilutions.

Methionine was also determined on the same flour using the differential-oxidation procedure. The results of the two methods are compared in Table B.

significant differences between the results of the two methods.

The slightly higher results for the gravimetric method

may be due to the presence of water.

The results of the gravimetric method are given

below approximately 100 for each sample of water.

There is no reliable method for the estimation

of oxygen in water containing large amounts of

hydrogen. Thus it is very difficult to compare accurately results

from gravimetric and the differential-oxidation processes.

A microchemical method for estimating hydrogen

has been used successfully on organic materials which contain

large amounts of carbonaceous matter. This method (19) was used for

comparison.

Microchemical determinations were carried out on a

few samples which were subjected to 15 minute treatment in 100

of 5 N HCl. The hydrolysis was filtered and neutralized.

Triphenyl phosphine oxide was added out of the solution.

Microchemical analysis was carried out on the same

one differential-oxidation process. The results of the two

methods are compared in Table 2.

TABLE B

Comparison of two methods for the
determination of methionine in flour

Microbiological Method		Differential oxidation procedure
Dilution 1:700	Dilution 1:1000	
<u>Methionine %</u>		<u>Methionine %</u>
0.228	0.251	0.275
0.236	0.244	0.261
0.233	0.265	
Mean 0.232	0.243	0.268

RESULTS

Series I - Marquis flour, 1944

Gerbrandt (19) has carried out analyses for the sulphur-containing amino acids in the gluten washed from these flours. He correlated the cystine and methionine contents of the gluten from 100 grams of flour with loaf volume. His conclusion was that both cystine and methionine so determined were better measures of baking quality than was crude protein in the whole wheat. Gerbrandt suggested that the correlations of the sulphur-containing amino acids with loaf volume were higher than those of crude protein and loaf volume because the former were measured on gluten while the latter is a measure of all nitrogen-containing

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121 10011 10011 10011 10011

compounds in the whole wheat. The quantity of gluten in a flour is usually directly related to loaf volume. For this reason the quantity of cystine or methionine in gluten from 100 grams of flour could be a better measure of loaf volume than the percentage nitrogen in the wheat. In other words the sulphur-containing amino acids of the gluten in 100 grams of flour are highly correlated with loaf volume because they are direct measures of gluten quantity.

As a method was available for the determination of cystine and methionine in flour it was of interest to compare with the previous work the correlations of loaf volume and the sulphur-containing amino acids in the complete flour.

The sulphur, cystine, methionine and nitrogen contents were determined on the flours and are presented in Table XI. The loaf volumes, which are the same as those used by Gerbrandt, are also included.

For the cystine determinations in this series, the samples were oxidized with 7 ml. of nitric acid per gram flour. Checks between duplicate samples were accepted when the values agreed within 5%.

The calculated correlation coefficients are given in Table XII.

compound in the whole sample. The quantity of liquid in the
flask is usually slightly related to the volume. For this
reason the quantity of oxygen or methane in the flask from
100 grams of liquid could be a better measure of total volume
than the percentage nitrogen in the sample. In other words
the nitrogen-containing volume ratio of the liquid to 100 grams
of flask and highly correlated with the volume ratio of the
liquid to the flask.

As a method was available for the determination of
oxygen and methane in the flask it was of interest to compare
with the nitrogen ratio the correlation of total volume and the
nitrogen-containing ratio in the flask. The
The nitrogen, oxygen, methane, and nitrogen contents
were determined on the flask and are presented in Table II.
The total volume, which was the same as the ratio of nitrogen,
are also included.

The correlation coefficients in this series are
samples were oxidized with 4 ml. of nitric acid and 100 ml.
Guthrie's reagent. The samples were subjected to the same
treatment as the other samples.

TABLE XI

Data - Marquis flour, 1944

Station	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume cc.
Athabaska	0.137	0.385	0.16	1.64	485
Bon Accord	0.170	0.419	0.27	2.47	578
Edmonton (fallow)	0.180	0.453	0.28	2.82	740
Edmonton (stubble)	0.194	0.507	0.27	2.98	857
Fallis	0.144	0.399	0.18	2.04	497
Sundre	0.113	0.305	0.15	1.69	490
Warburg	0.143	0.375	0.20	2.22	548
Composite	0.183	0.491	0.24	2.73	750

Table 1
Data - sample 1961

Year	1961	1962	1963	1964	1965
1961	1.00	1.00	1.00	1.00	1.00
1962	1.00	1.00	1.00	1.00	1.00
1963	1.00	1.00	1.00	1.00	1.00
1964	1.00	1.00	1.00	1.00	1.00
1965	1.00	1.00	1.00	1.00	1.00
1966	1.00	1.00	1.00	1.00	1.00
1967	1.00	1.00	1.00	1.00	1.00
1968	1.00	1.00	1.00	1.00	1.00
1969	1.00	1.00	1.00	1.00	1.00
1970	1.00	1.00	1.00	1.00	1.00
1971	1.00	1.00	1.00	1.00	1.00
1972	1.00	1.00	1.00	1.00	1.00

TABLE XII

Calculated statistics for Marquis flour, 1944.

r_{VC}	= 0.892**	r_{CS}	= 0.977**	b_{CS}	= 0.230%C/0.1% S.
$r_{VC.N}$	= 0.385	r_{CN}	= 0.888**	b_{MS}	= 0.17%M/0.1% S.
r_{VM}	= 0.69*	r_{MS}	= 0.79*		
$r_{VM.N}$	= -0.24	r_{MN}	= 0.80*		
r_{VS}	= 0.906**	r_{CM}	= 0.69*		
$r_{VS.N}$	= 0.164	r_{SN}	= 0.953**		
r_{VN}	= 0.932**	$r_{CS.M}$	= 0.942**		
$r_{VN.C}$	= 0.675*	$r_{CN.S}$	= -0.669*		
$r_{VN.M}$	= 0.876**	$r_{MS.N}$	= 0.14		
$r_{VN.S}$	= 0.515	$r_{MN.S}$	= 0.26		

V = loaf volume, cc's

N = nitrogen in flour, %

S = sulphur in flour, %

C = cystine in flour, %

M = methionine in flour, %

* Significant beyond 5% point.

** Significant beyond 1% point.

The simple and partial correlation coefficients r_{VN} , $r_{VN.S}$, r_{VS} and $r_{VS.N}$ show that the effect of nitrogen and sulphur contents of flour on loaf volume are apparently related. A relationship is expected because the simple correlation of sulphur and nitrogen is highly significant.

The simple correlations of sulphur and nitrogen content of wheat with loaf volume as described by Gerbrandt are highly significant. These correlations in conjunction with those determined in this study indicate that the sulphur and nitrogen contents of the flour are closely related to the sulphur and nitrogen contents of the wheat.

Nitrogen is a measure of the quantity of crude protein in flour. As the nitrogen and sulphur of the flour are highly significantly correlated there must be a relationship between the sulphur content and the quantity of protein in the flour. The measurement of the sulphur content of flour is therefore another measure of protein quantity and thus there is a high correlation between loaf-volume and sulphur content. However with the effect of nitrogen removed the correlation is not significant, indicating that the effect of sulphur on loaf volume is directly associated with the nitrogen effect.

The cystine, methionine and loaf volume statistics indicate that the quantity of sulphur-containing amino acids in flour is another measure of the quantity of protein in the flour. When the quantity of protein is kept constant there is no significant correlation of cystine or methionine with

The single and partial correlation coefficients

for "A", "B", and "C" show that the effect of

nitrogen and sulphur contents of flour on loaf volume are

apparently related. A relationship is expected because the

single correlation of sulphur and nitrogen is highly significant.

The single correlations of sulphur and nitrogen with

loaf volume are described as described or described as

highly significant. These correlations are consistent with

those determined in this study indicate that the sulphur and

nitrogen contents of the flour are closely related to loaf

volume and nitrogen contents of the wheat.

Nitrogen is a measure of the quantity of crude protein

in flour. As the nitrogen and sulphur of the flour are highly

significantly correlated there must be a relationship between

loaf volume content and the quantity of protein in the flour.

The measurement of the single content of flour is considered

another measure of protein quantity and also there is a high

correlation between loaf-volume and nitrogen content. However,

with the effect of nitrogen content on loaf volume is not

statistical, indicating that the effect of nitrogen on loaf

volume is directly associated with the nitrogen effect.

The pyridine, methionine and total amino acid

indicates that the quantity of sulphur-containing amino acids

in flour is another measure of the quantity of protein in the

flour. When the quantity of protein is high, the content of

is no significant correlation of protein on loaf volume.

loaf-volume. This relationship does not show up in Gerbrandt's study probably because his determinations of the amino acids were on crude gluten while the nitrogen was determined on the whole wheat.

That the cystine and methionine contents of the flour do not affect loaf volume is shown by the values for the partial coefficients $r_{NV.C}$ and $r_{NV.M}$. The nitrogen does affect the loaf volume.

Gerbrandt found that the quantity of cystine and methionine in the gluten in 100 grams of flour were factors which affected loaf volume. The conclusion from the present analysis is that loaf volume is more closely associated with protein content than with the content of the sulphur-containing amino acids in the flour.

As there are only eight samples in this experiment, the values of the simple and partial correlation coefficients indicate that the cystine content of flour is related to the total sulphur but not to the nitrogen content of the flour. The calculated regression line of cystine and sulphur is shown in Figure I,A. The regression coefficient is given in Table XII.

Gerbrandt found a highly significant correlation between the cystine content of gluten and the sulphur content of wheat. As the cystine and sulphur in flour are highly correlated and assuming the sulphur content of wheat and flour is closely

lost-volume. This relatively large loss was up to 10% of the total volume. The loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic.

That the gas is not perfectly elastic is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic. The loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic.

Therefore, the loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic. The loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic.

It is thus seen that the loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic. The loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic.

Therefore, the loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic. The loss of volume is due to the fact that the gas is not perfectly elastic and the loss of volume is due to the fact that the gas is not perfectly elastic.

related, a close correlation between the cystine contents of gluten, flour and wheat would be expected.

The methionine, sulphur and nitrogen correlations show that the effects of the nitrogen and sulphur contents of flour on the methionine content are apparently related. These data suggest that nitrogen and sulphur affect the methionine content of flour in much the same way as when taken together. Figure I,B gives the regression line of methionine and sulphur. The regression coefficient is given in Table XII.

The correlation coefficient of cystine and methionine suggests a relationship between the quantities of these amino acids in flour.

reflected, a slight correlation between the two groups of

figures, these two groups would be expected.

The relationship between the nitrogen containing

groups and the effects of the nitrogen and nitrogen compounds of

these on the nitrogen containing compounds is discussed in detail.

There is a slight correlation between the nitrogen and nitrogen

compounds of these in which the two groups are both present.

Figure 1, B, shows the correlation line of nitrogen and nitrogen.

The correlation coefficient is given in Table III.

The correlation coefficient of the two groups and nitrogen

compounds is also shown. The correlation of these two

groups is shown.

Series II - Thatcher wheat and flour, Breton, 1946 and 1947.

The material in this series is valuable for a study of factors affecting the sulphur and sulphur-containing amino acids because of the wide range in sulphur content.

Loaf volume relationships were determined only for the 1947 crop because, as was previously stated, there are very small volume differences in the 1946 crop. With a baking error of 60 cc. few plots in either Series E or B of the 1946 crop produced loaf volumes significantly different from the mean.

Table XIII and Figure II, A, B, C and D show the sulphur, cystine, methionine and nitrogen contents of wheat harvested from the continuous wheat plots of the Breton field. Included in the table are the loaf volumes and cystine-sulphur as a percentage of the total sulphur.

The analyses of variance for sulphur, cystine, methionine and nitrogen are given in Table XIV.

The variations in the nitrogen content of the wheat in the continuous rotation are very small. This is shown graphically in Figure II, C. The application of fertilizers had little affect on the nitrogen content of the wheat.

Series II - Residuals from the 1934, 1935, 1936 and 1937

The material in this series is available for a study

of factors affecting the nitrogen and sulphur content of

soils because of the wide range in nitrogen content.

Leaf volume relationships were determined only for the

1937 crop because, as was previously stated, there are very

small volume differences in the 1934 crop, with a maximum error

of 0.001, the limit in either Series I or II of the 1934 crop

proposed leaf volumes significantly different from the mean.

Table III and Figure II, B, C, and D show the

nitrogen, sulphur, and nitrogen content of these

residuals from the conditions where plots of the 1934 crop

included in the table are the leaf volume and nitrogen-sulphur

as a percentage of the total nitrogen.

The analysis of variance for nitrogen, sulphur, and

nitrogen and nitrogen are given in Table IV.

The variation in the nitrogen content of the soil

in the continuous rotation are very small. This is shown

graphically in Figure II, B. The application of fertilizers

had little effect on the nitrogen content of the soil.

TABLE XIII

Data - Thatcher wheat, Series E,
Breton, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	% Cystine- sulphur of total sulphur
1	0.132	0.349	0.18	1.86	418	71
2	0.113	0.358	0.08	2.09	475	84
3	0.125	0.378	0.11	1.77	445	81
4	0.126	0.388	0.10	1.76	428	82
5	0.130	0.315	0.21	1.85	418	65
6	0.127	0.372	0.13	1.82	435	78
7	0.127	0.355	0.15	2.03	435	74
8	0.146	0.404	0.18	2.19	465	74
9	0.146	0.448	0.12	1.94	475	82
10	0.126	0.385	0.11	2.15	435	82
11	0.118	0.338	0.13	2.07	428	76
12	0.173	0.518	0.16	2.15	475	81
13	0.162	0.525	0.10	2.06	455	87
14	0.136	0.398	0.14	1.93	408	78
15	0.162	0.495	0.14	2.03	505	82
16	0.135	0.391	0.14	1.81	445	78
17	0.157	0.454	0.17	2.02	465	77
Mean	0.139	0.404	0.14	1.97	448	78

Fig. II, A.

Fig. II, B.

Fertilizer effects on the cystine and sulphur content of Breton wheat, 1946.

Fertilizer effects on the nitrogen and methionine content of Breton wheat, 1946.

TABLE XIV

Analysis of Variance - Series E Wheat.

Variance due to	D.F.	Mean Square Nitrogen	Mean Square Sulphur	Mean Square Cystine	Mean Square Methionine
Treatment	16	0.03900**	0.0005884**	0.007839**	0.002243**
Error	17	0.00070	0.0000149	0.000045	0.000665
Total	33				

**Significant beyond the 1% point.

Minimum significant differences for treatment are:

0.008% sulphur.

0.05% nitrogen.

0.013% cystine.

0.05% methionine.

In most cases the application of sulphur fertilizers increases the sulphur content of the wheat. Usually the sulphur fertilized plots produce wheat significantly higher in cystine content than the non-fertilized plots. As would be expected the cystine content of the wheat is closely related to the sulphur content. As a rule the high sulphur wheats are highest in cystine content while the low sulphur wheats are lowest. The correlation coefficients show the close relationship between the cystine and sulphur contents of this wheat.

The calculated correlation and regression coefficients are presented in Table XV. The cystine, sulphur scatter is

plotted in Figure III, A.

The correlations and bar diagrams show that the cystine content of wheat is influenced more by the sulphur than the nitrogen content.

An average of 78 percent of the total sulphur in the wheat of this series is present as cystine-sulphur. There does not appear to be any relationship between the quality or quantity of fertilizers applied and the percentage of total sulphur represented as cystine-sulphur.

There are very small if any variations in the methionine contents of the various wheats from Series E. Check plot one is reported as containing 0.18 per cent methionine. This value appears relatively high. An error in either the cystine or sulphur determination would give such a result. Assuming either the cystine or the sulphur percentage as correct it is possible to obtain the alternate constituent from the regression line in Figure III, A or from the regression coefficient in Table XV. A corrected methionine content can be calculated as a difference between the cystine-sulphur and the total sulphur values so obtained. The corrected value for the check plot one is 0.15%. Using this value as a check, the wheat from the lime plot is significantly higher and the wheat from the manure plot significantly lower in methionine. The sulphur and cystine contents of these wheats suggest that the values are misrepresented due to errors in the determinations. When the values for methionine in these wheats are corrected as in the case of the check plot,

located in Figure III, A.

The correlation and the standard error of the estimate
content of acid is indicated by the regression line and the
standard error.

An average of 15 percent of the total nitrogen in the
acid of this series is present as cystine-sulfur. These acids
not appear to be any relationship between the quality or quantity
of cystine-sulfur applied and the percentage of total nitrogen
represented by cystine-sulfur.

There are very small differences in the relationship
between the various wheat lines. These lines are
is reported as containing 0.12 per cent methionine. This value
appears relatively high. An error in which the nitrogen is not
pure determination would give such a result. Assuming nitrogen to
cystine or the sulfur percentage as shown it is possible to
obtain the nitrogen content from the regression line in
Figure III, A or from the regression coefficient in Table IV.
A corrected methionine content can be calculated as a difference
between the cystine-sulfur and the total nitrogen values as
obtained. The corrected value for the wheat line is 0.12.
Using this value as a check, the error from the line plot is
approximately 0.01 and the error from the nitrogen value is approximately
0.01. The nitrogen and cystine-sulfur content of
these wheat lines suggest that the values are approximately the same
error in the determination. With the values for methionine
in wheat which are corrected as in the case of the wheat line,

there are no significant differences in the methionine of the wheat from Series E.

The results suggest that the methionine content of the continuous wheat is not affected by the applications of fertilizers.

The correlation coefficients r_{MS} and $r_{MS.N}$ suggest that the methionine in wheat is not influenced by the sulphur content. This is not what we would expect. In this particular series the methionine range is very small and a few values considerably out of line would upset the methionine-sulphur correlation.

The scatter diagram of methionine and sulphur in Figure III, B shows how a few values will destroy the correlation. When the values obviously out of line are corrected by the use of the cystine, total sulphur regression, the methionine content is directly related to the sulphur content of the wheat. There is no relationship between the methionine and nitrogen contents of wheat.

The percentages of the total sulphur present in wheat as methionine-sulphur can be obtained from Table XIII. Obviously the values are the differences between the cystine percentages and 100 percent.

TABLE XV

Regression and Correlation Coefficients, Series E,
Continuous Wheat, Breton, 1946.

r_{CS}	=	0.908**	b_{CS}	=	0.332%C/0.1%S.
$r_{CS.N}$	=	0.951**	b_{MS}	=	0.06%m./0.1%S.
r_{CN}	=	0.369			
$r_{CN.S}$	=	0.130			
r_{MS}	=	0.28			
$r_{MS.N}$	=	0.29			
r_{MN}	=	0.01			
$r_{MN.S}$	=	-0.09			
r_{CM}	=	-0.15			
r_{SN}	=	0.350			

C = % cystine in flour

S = % sulphur in flour

N = % nitrogen in flour

M = % methionine in flour

* Significant beyond the 5% point.

** Significant beyond the 1% point.

Figure III - A - Relation between cystine and sulphur.
Series E wheat, Breton, 1946.

Figure III, B - Relation between methionine and sulphur.
Series E wheat, Breton, 1946.

The sulphur, cystine, methionine and nitrogen contents of the wheat grown on Series B, Breton plots are presented in Table XVI and Figure II. The loaf volumes and the values for cystine-sulphur as a percentage of the total sulphur in the wheat are also presented in the table.

The analyses of variance for sulphur, cystine, methionine and nitrogen are given in Table XVII.

The calculated correlation and regression coefficients are presented in Table XVIII.

The loaf volumes of Series B exhibit a slightly greater range than those of Series E. No attempts were made to correlate loaf volume with the other results because the range of volumes is within the 60 cc. baking error of the mean.

The diagrams in figure II show that generally speaking wheat after clover has a greater range for each constituent determined than the continuous wheat.

The sulphur-containing fertilizers have decreased the nitrogen content of the wheat more significantly than the others. The lower nitrogen content of the grain can be attributed to increased yields resulting from the application of sulphur fertilizers (41). Table XVI and figure II, A show that the application of fertilizers containing significant amounts of sulphur have resulted in increases in the sulphur content of wheat. The increases in sulphur and decreases in nitrogen caused by the application of sulphur fertilizers explain the negative and significant simple correlation coefficient for sulphur and nitrogen.

The nitrogen, cystine, methionine and nitrogen contents of the wheat grown on Series I, Series II and Series III are given in Table XVI and Figure 11. The total volume and the values for cystine-sulphur as a percentage of the total sulphur in the wheat are also presented in the table.

The analyses of wheat for nitrogen, cystine, methionine and nitrogen are given in Table XVII.

The calculated correlation and regression coefficients are presented in Table XVIII.

The total volume of Series II exhibits a slightly greater range than those of Series I. No extreme values were observed in the total volume with the other results because the range of values is within the 50 cc. drying range of the mean.

The nitrogen in Series II was not uniformly distributed. Wheat after clover had a greater range for total nitrogen than the common wheat.

The sulphur-containing fertilizers have increased the nitrogen content of the wheat more significantly than the others. The lower nitrogen content of the wheat on Series I is due to increased yields resulting from the application of sulphur fertilizers (11). Table XVI and Figure 11 show that the application of fertilizers containing significant amounts of sulphur have resulted in increases in the sulphur content of wheat. The increases in sulphur and nitrogen in nitrogen caused by the application of sulphur fertilizers explain the negative and significant yields correlation coefficient for sulphur and nitrogen.

TABLE XVI

Data - Thatcher Wheat, Series B,
Breton, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	Cystine- sulphur as % of total sulphur
1	0.094	0.283	0.09	2.71	485	80.3
2	0.099	0.298	0.09	2.48	495	80.3
3	0.145	0.429	0.14	2.03	435	79.0
4	0.177	0.434	0.28	2.06	425	65.4
5	0.105	0.324	0.09	2.68	485	82.3
6	0.099	0.284	0.11	2.61	465	76.5
7	0.107	0.282	0.15	2.55	505	70.3
8	0.141	0.380	0.18	2.08	438	71.9
9	0.148	0.442	0.14	2.17	418	79.7
10	0.111	0.304	0.14	2.80	563	73.1
11	0.103	0.294	0.11	2.71	495	76.1
12	0.232	0.642	0.28	2.53	553	73.8
13	0.220	0.635	0.24	2.39	553	77.0
14	0.098	0.279	0.11	2.52	495	75.9
15	0.197	0.535	0.25	2.20	553	72.4
16	0.157	0.529	0.07	2.05	495	89.9
17	0.191	0.504	0.26	2.31	465	70.4
Mean	0.143	0.405	0.16	2.40	490	75.6

TABLE XVI

Calc - Thallium content, percent
 Thallium, ppm.

Sample No.	Thallium %	Calcium %	Thallium ppm	Calcium ppm
1	0.001	0.001	1.0	1.0
2	0.001	0.001	1.0	1.0
3	0.001	0.001	1.0	1.0
4	0.001	0.001	1.0	1.0
5	0.001	0.001	1.0	1.0
6	0.001	0.001	1.0	1.0
7	0.001	0.001	1.0	1.0
8	0.001	0.001	1.0	1.0
9	0.001	0.001	1.0	1.0
10	0.001	0.001	1.0	1.0
11	0.001	0.001	1.0	1.0
12	0.001	0.001	1.0	1.0
13	0.001	0.001	1.0	1.0
14	0.001	0.001	1.0	1.0
15	0.001	0.001	1.0	1.0
16	0.001	0.001	1.0	1.0
17	0.001	0.001	1.0	1.0
18	0.001	0.001	1.0	1.0
19	0.001	0.001	1.0	1.0
Mean	0.001	0.001	1.0	1.0

TABLE XVII

Analysis of Variance, Series B,
Breton Wheat, 1946.

Variance due to	D.F.	Sulphur Mean Square	Cystine Mean Square	Methionine Mean Square	Nitrogen Mean Square
Treatment	16	0.004265**	0.031931**	0.01085**	0.14379**
Error	17	0.0000186	0.0000982	0.0009824	0.00151
Total	33				

**Significant beyond the 1% point.

Minimum significant differences for treatment are: 0.009% sulphur,
0.020% cystine,
0.06% methionine,
0.08% nitrogen.

TABLE XVIII

Regression and Correlation Coefficients, Series B,
Wheat after Clover, 1946.

$$r_{CS.} = 0.968**$$

$$r_{MS.N} = 0.83**$$

$$b_{CS} = 0.265\%C/0.1\%S.$$

$$r_{CS.N} = 0.957**$$

$$r_{MN} = -0.34$$

$$b_{MS} = 0.13\%M/0.1\%S.$$

$$r_{CN} = -0.510*$$

$$r_{MN.S} = 0.18$$

$$r_{CN.S} = -0.138$$

$$r_{MC} = 0.69**$$

$$r_{MS} = 0.85**$$

$$r_{SN} = -0.496*$$

*Significant beyond 5% point
**Significant beyond 1% point.

C = % cystine in flour

M = % methionine in flour

S = % sulphur in flour

N = % nitrogen in flour

TABLE XVII
Analysis of Variance, Series A,
Proton Beam, 1950.

Source of Variation	D.F.	Mean Square	Variance due to
Treatment	10	0.001255	
Error	17	0.000016	
Total	27		

Significant beyond the 1% point.

Minimum significant differences for treatment are: 0.0001 subject,
0.0001 crystal,
0.0001 material,
0.0001 nitrogen.

TABLE XVIII
Regression and Correlation Coefficients, Series B,
West after Oliver, 1950.

$r_{12} = 0.998$	$r_{23} = 0.998$	$r_{13} = 0.998$
$r_{14} = 0.997$	$r_{24} = 0.997$	$r_{14} = 0.997$
$r_{15} = 0.996$	$r_{25} = 0.996$	$r_{15} = 0.996$
$r_{16} = 0.995$	$r_{26} = 0.995$	$r_{16} = 0.995$
$r_{17} = 0.994$	$r_{27} = 0.994$	$r_{17} = 0.994$
$r_{18} = 0.993$	$r_{28} = 0.993$	$r_{18} = 0.993$

Significant beyond 5% point.
Significant beyond 1% point.
0 = 1 subject in flow
0 = 1 material in flow
0 = 1 crystal in flow
0 = 1 nitrogen in flow

The effect of the fertilizers on the cystine content of the wheat is similar to the effect on the sulphur content. This situation is similar to that observed in the wheat after wheat series. The regression line for cystine and sulphur is plotted in figure IV,A. As in the wheat after wheat series, the cystine content of Series B wheat is not related to the nitrogen content but is closely associated with the sulphur content. The application to wheat of fertilizers containing large amounts of sulphur results in large and significant increases in cystine and sulphur contents. The fertilizer treatments decrease the nitrogen content of wheat but this apparently has no effect on the cystine content.

Approximately 70 to 80 percent of the sulphur in Series B wheat is present as cystine-sulphur. This is a similar proportion to that in the Series E wheat. There is no apparent relationship between fertilizer treatment and the amount of sulphur present in cystine.

Generally the application of sulphur-containing fertilizers has resulted in increases in the methionine content of the wheat. The regression line figure IV, B shows that the methionine and sulphur contents of wheat are closely related. Nitrogen variations in the wheat have no influence on the methionine content.

The simple correlation coefficient r_{MC} is positive and highly significant. This suggests that in the wheat proteins of Series B the methionine and cystine contents are related.

The effect of the treatment on the yield of wheat is similar to the effect on the nitrogen content. This relation is similar to that observed in the wheat after

these studies. The regression lines for cystine and sulphur is shown in Figure 10, A. As in the wheat after wheat studies,

the positive content of cystine is not related to the nitrogen content but is closely associated with the nitrogen

content. The regression to wheat of fertilizers containing

large amounts of sulphur results in large and significant

changes in cystine and nitrogen contents. The fertilizer studies showed however the nitrogen content of wheat and this experiment

has no effect on the cystine content.

Approximately 70 to 80 percent of the sulphur in

Series 2 wheat is present as cystine-sulphur. This is a slight

proportion to that in the Series 1 wheat. There is no significant

relationship between fertilizer treatment and the amount of

sulphur present in cystine.

Generally the application of sulphur-treatment for

nitrogen was studied in Series 1 in the wheat after wheat

of the wheat. The regression line in Figure 10, B shows that the

relationship and sulphur contents of wheat are closely related.

Nitrogen variations in the wheat have no influence on the

relationship content.

The single correlation coefficient of A is positive and

highly significant. This suggests that in the wheat studies

of Series 1 the relationship and nitrogen contents and nitrogen

Figure IV, A - Cystine:sulphur relationship,
Breton wheat, Series B.

Figure IV, B - Methionine:sulphur relationship,
Breton wheat, Series B.

The analyses of flours from Series E and B were carried out to determine whether the sulphur, cystine, methionine and nitrogen relationships in flour are the same as in wheat.

The sulphur, cystine, methionine and nitrogen contents of the flours from Series E wheat are presented in Table XIX. The percentages as reported are slightly lower in the flour than in the wheat. There is a close relationship between these constituents in wheat and flour. This can be readily seen from the correlation coefficients in Table XXI. The wheat samples with the higher sulphur, cystine, methionine and nitrogen values produce flour higher in these constituents. The lower values for the wheat and flour are also related.

The analyses of variance for the data are given in Table XX. Fertilizer treatments have resulted in significant differences for the constituents determined.

The correlation and regression coefficients for Series E are presented in Table XXI. The regression lines for cystine and sulphur and methionine and sulphur are plotted in figure V, A and B. These data show that the cystine and methionine contents of flour, as of wheat, are determined by sulphur rather than nitrogen content of the flour.

The cystine-sulphur as a percentage of the total sulphur in the flour is included in Table XIX.

The significant simple correlation coefficient, r_{MC} suggests that the methionine and cystine in the flour are related. This was not observed in Series E wheat.

TABLE XIX

Data - Flour from Thatcher Wheat,
Series E, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-sulphur as % of total sulphur
1	0.114	0.317	0.14	1.79	74
2	0.120	0.322	0.16	1.98	72
3	0.128	0.329	0.19	1.65	69
4	0.116	0.341	0.12	1.59	78
5	0.122	0.337	0.15	1.84	74
6	0.116	0.299	0.17	1.84	69
7	0.114	0.325	0.13	1.82	76
8	0.144	0.404	0.17	2.04	75
9	0.143	0.382	0.19	2.05	71
10	0.129	0.365	0.15	1.94	76
11	0.109	0.320	0.11	1.99	78
12	0.161	0.448	0.19	2.04	74
13	0.160	0.441	0.19	1.98	74
14	0.131	0.352	0.17	1.93	72
15	0.163	0.429	0.23	1.95	70
16	0.127	0.376	0.12	1.74	79
17	0.123	0.367	0.12	1.79	80
Mean	0.131	0.362	0.16	1.88	74

TABLE XX

Analysis of Variance, Series E flour,
Breton, 1946.

Variance due to	D.F.	Mean Squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.000603**	0.004203**	0.00225*	0.03864**
Error	17	0.0000153	0.0000512	0.000712	0.000529
Total	33				

* Significant beyond 5% point.

** Significant beyond 1% point.

The minimum significant differences for treatment are:

0.008% sulphur

0.014% cystine

0.05% methionine

0.05% nitrogen.

Table 1

Analysis of Variance, Series A, 1954
 Boston, 1954

Source of Variation	Sum of Squares	df	Mean Square	F	Prob > F
Treatment	0.000000	1	0.000000	0.00	0.9999
Error	0.000000	1	0.000000	0.00	0.9999
Total	0.000000	2			

* Significant beyond 5% point.
 ** Significant beyond 1% point.

The minimum significant differences for treatment are:

- 0.0000 nitrogen
- 0.0000 phosphorus
- 0.0000 potassium
- 0.0000 calcium

TABLE XXI

Calculated statistics for Series E flour, 1946.

r_{CS}	= 0.946**	r_{MC}	= 0.56*
$r_{CS.N}$	= 0.930**	r_{SN}	= 0.530*
$r_{CN.}$	= 0.483	r_{WN}	= 0.604**
$r_{CN.S}$	= -0.066	r_{SH}	= 0.845**
r_{MS}	= 0.80**	b_{CS}	= 0.246%C./0.1%S.
$r_{MS.N}$	= 0.75**	b_{CM}	= 0.08%M./0.1%S.
r_{MN}	= 0.41		
$r_{MN.S}$	= -0.20		

* Significant beyond 5% point.

** Significant beyond 1% point.

S = % sulphur in flour

N = % nitrogen in flour

C = % cystine in flour

M = % methionine in flour

W = % nitrogen in wheat

H = % sulphur in wheat.

TABLE 241

Calculated statistics for series of 1000, 1966.

$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$
$\bar{X} = 0.000$	$\bar{Y} = 0.000$	$\bar{Z} = 0.000$	$\bar{W} = 0.000$	$\bar{V} = 0.000$

* Significant beyond 5% point.
** Significant beyond 1% point.

- 1 = 1 nitrogen in wheat
- 2 = 2 nitrogen in wheat
- 3 = 3 nitrogen in wheat
- 4 = 4 nitrogen in wheat
- 5 = 5 nitrogen in wheat
- 6 = 6 nitrogen in wheat
- 7 = 7 nitrogen in wheat
- 8 = 8 nitrogen in wheat
- 9 = 9 nitrogen in wheat
- 10 = 10 nitrogen in wheat

Series E flour, Breton, 1946

Figure V, A - Cystine:sulphur relationship.

Figure V, B - Methionine:sulphur relationship.

The sulphur, cystine, methionine and nitrogen contents of the flour milled from Series B wheat are presented in Table XXII. The cystine-sulphur as a percentage of the total sulphur is included in the table.

Generally there is a close relationship between the quantities of determined constituents in the flour and wheat. This is shown by the correlation coefficients in Table XXIV. The sulphur and cystine contents are generally smaller in the flour than in the wheat. The methionine values for flour, on the other hand, are not consistent. They are sometimes higher and sometimes lower than the values for wheat. Nitrogen determinations were not carried out on all of the Series B flour because of insufficient material. There is, however, a trend towards a lower nitrogen content in the flour than in the wheat.

The results of the simple analyses of variance on the data are presented in Table XXIII. The fertilizer treatments have a significant effect on the quantities of sulphur, cystine, methionine and nitrogen in the flour.

The correlation and regression coefficients for Series B are presented in Table XXIV. The regression lines for cystine and sulphur and methionine and sulphur are plotted in Figure VI, A and B. The relationships between sulphur, cystine, methionine and nitrogen are similar to those observed in Series E flour. The sulphur-containing amino acid content of flour is determined by sulphur rather than nitrogen content of flour.

The sulphur, nitrogen, and chlorine contents of the flour milled from Series B wheat are presented in Table XXII. The sulphur-sulphate is a by-product of the flour sulphur is included in the results.

Generally there is a close relationship between the quantities of different constituents in the flour and wheat. This is shown by the correlation coefficients in Table XXIII. The sulphur and nitrogen contents are generally similar in the flour when in the wheat. The relationship between the flour and the wheat, and not consistent. They are sometimes higher and sometimes lower than the values for wheat. Different correlations were not described and on all of the Series B flour fractions of identical material. There is, however, a trend towards a lower nitrogen content in the flour than in the wheat.

The results of the simple analysis of variance on the data are presented in Table XXIV. The fertilizer treatments have a significant effect on the quantities of sulphur, chlorine, nitrogen and nitrogen in the flour.

The correlation and regression coefficients for Series B are presented in Table XXV. The regression lines for sulphur and nitrogen and chlorine and sulphur are plotted in Figure VI, A and B. The relationships between sulphur, chlorine, nitrogen and nitrogen are similar to those observed in Series B flour. The sulphur-sulphate minus acid content of flour is determined by sulphur content when nitrogen content of flour.

TABLE XXII

Data - Flour from Thatcher Wheat,
Series B, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-Sulphur as % of Total Sulphur.
1	0.105	0.326	0.09	-	83
2	0.084	0.266	0.06	2.37	85
3	0.151	0.419	0.18	-	74
4	0.159	0.342	0.32	-	57
5	0.118	0.210	0.28	2.55*	48
6	0.083	0.273	0.05	2.52	88
7	0.097	0.284	0.10	2.39	78
8	0.133	0.361	0.17	2.06	72
9	0.144	0.436	0.13	2.05	81
10	0.102	0.269	0.14	2.66*	70
11	0.107	0.273	0.16	-	68
12	0.214	0.571	0.29	2.51	71
13	0.220	0.608	0.32	2.43	74
14	0.115	0.385	0.06	2.54*	89
15	0.190	0.488	0.28	2.21	69
16	0.145	0.493	0.13	2.13	82
17	0.184	0.530	0.20	2.32*	77
Mean	0.138	0.381	0.18	2.36	74

*Single determination

TABLE III

Tests - from Lowry method, 1951,
Gibbs & W. J. W.

Sample No.	Aspartic Acid	Glutamic Acid	Alanine	Valine	Leucine
1	0.107	0.107	0.107	0.107	0.107
2	0.107	0.107	0.107	0.107	0.107
3	0.107	0.107	0.107	0.107	0.107
4	0.107	0.107	0.107	0.107	0.107
5	0.107	0.107	0.107	0.107	0.107
6	0.107	0.107	0.107	0.107	0.107
7	0.107	0.107	0.107	0.107	0.107
8	0.107	0.107	0.107	0.107	0.107
9	0.107	0.107	0.107	0.107	0.107
10	0.107	0.107	0.107	0.107	0.107
11	0.107	0.107	0.107	0.107	0.107
12	0.107	0.107	0.107	0.107	0.107
13	0.107	0.107	0.107	0.107	0.107
14	0.107	0.107	0.107	0.107	0.107
15	0.107	0.107	0.107	0.107	0.107
16	0.107	0.107	0.107	0.107	0.107
17	0.107	0.107	0.107	0.107	0.107
18	0.107	0.107	0.107	0.107	0.107
19	0.107	0.107	0.107	0.107	0.107
20	0.107	0.107	0.107	0.107	0.107
21	0.107	0.107	0.107	0.107	0.107
22	0.107	0.107	0.107	0.107	0.107
23	0.107	0.107	0.107	0.107	0.107
24	0.107	0.107	0.107	0.107	0.107
25	0.107	0.107	0.107	0.107	0.107
26	0.107	0.107	0.107	0.107	0.107
27	0.107	0.107	0.107	0.107	0.107
28	0.107	0.107	0.107	0.107	0.107
29	0.107	0.107	0.107	0.107	0.107
30	0.107	0.107	0.107	0.107	0.107
31	0.107	0.107	0.107	0.107	0.107
32	0.107	0.107	0.107	0.107	0.107
33	0.107	0.107	0.107	0.107	0.107
34	0.107	0.107	0.107	0.107	0.107
35	0.107	0.107	0.107	0.107	0.107
36	0.107	0.107	0.107	0.107	0.107
37	0.107	0.107	0.107	0.107	0.107
38	0.107	0.107	0.107	0.107	0.107
39	0.107	0.107	0.107	0.107	0.107
40	0.107	0.107	0.107	0.107	0.107
41	0.107	0.107	0.107	0.107	0.107
42	0.107	0.107	0.107	0.107	0.107
43	0.107	0.107	0.107	0.107	0.107
44	0.107	0.107	0.107	0.107	0.107
45	0.107	0.107	0.107	0.107	0.107
46	0.107	0.107	0.107	0.107	0.107
47	0.107	0.107	0.107	0.107	0.107
48	0.107	0.107	0.107	0.107	0.107
49	0.107	0.107	0.107	0.107	0.107
50	0.107	0.107	0.107	0.107	0.107
51	0.107	0.107	0.107	0.107	0.107
52	0.107	0.107	0.107	0.107	0.107
53	0.107	0.107	0.107	0.107	0.107
54	0.107	0.107	0.107	0.107	0.107
55	0.107	0.107	0.107	0.107	0.107
56	0.107	0.107	0.107	0.107	0.107
57	0.107	0.107	0.107	0.107	0.107
58	0.107	0.107	0.107	0.107	0.107
59	0.107	0.107	0.107	0.107	0.107
60	0.107	0.107	0.107	0.107	0.107
61	0.107	0.107	0.107	0.107	0.107
62	0.107	0.107	0.107	0.107	0.107
63	0.107	0.107	0.107	0.107	0.107
64	0.107	0.107	0.107	0.107	0.107
65	0.107	0.107	0.107	0.107	0.107
66	0.107	0.107	0.107	0.107	0.107
67	0.107	0.107	0.107	0.107	0.107
68	0.107	0.107	0.107	0.107	0.107
69	0.107	0.107	0.107	0.107	0.107
70	0.107	0.107	0.107	0.107	0.107
71	0.107	0.107	0.107	0.107	0.107
72	0.107	0.107	0.107	0.107	0.107
73	0.107	0.107	0.107	0.107	0.107
74	0.107	0.107	0.107	0.107	0.107
75	0.107	0.107	0.107	0.107	0.107
76	0.107	0.107	0.107	0.107	0.107
77	0.107	0.107	0.107	0.107	0.107
78	0.107	0.107	0.107	0.107	0.107
79	0.107	0.107	0.107	0.107	0.107
80	0.107	0.107	0.107	0.107	0.107
81	0.107	0.107	0.107	0.107	0.107
82	0.107	0.107	0.107	0.107	0.107
83	0.107	0.107	0.107	0.107	0.107
84	0.107	0.107	0.107	0.107	0.107
85	0.107	0.107	0.107	0.107	0.107
86	0.107	0.107	0.107	0.107	0.107
87	0.107	0.107	0.107	0.107	0.107
88	0.107	0.107	0.107	0.107	0.107
89	0.107	0.107	0.107	0.107	0.107
90	0.107	0.107	0.107	0.107	0.107
91	0.107	0.107	0.107	0.107	0.107
92	0.107	0.107	0.107	0.107	0.107
93	0.107	0.107	0.107	0.107	0.107
94	0.107	0.107	0.107	0.107	0.107
95	0.107	0.107	0.107	0.107	0.107
96	0.107	0.107	0.107	0.107	0.107
97	0.107	0.107	0.107	0.107	0.107
98	0.107	0.107	0.107	0.107	0.107
99	0.107	0.107	0.107	0.107	0.107
100	0.107	0.107	0.107	0.107	0.107

* Sample determination

TABLE XXIII

Analysis of Variance
Flour from Thatcher Wheat, Series B, 1946.

Variance due to	D.F.	Mean Squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.003754**	0.02786**	0.01628**	0.06971**
Error	17	0.0000241	0.0000549	0.00136	0.000556
Total	33				

**Significant beyond 1% point.

The minimum significant differences for treatment are:

0.010% sulphur

0.015% cystine

0.07% methionine

0.05% nitrogen.

TABLE VIII

Analysis of Variance
From Two-Treatment Test, Series B, 1934.

Source of Variation	D.F.	Sum of Squares	Mean Square	Variance due to
Treatment	1	0.000000	0.000000	Treatment
Error	14	0.000000	0.000000	Error
Total	15	0.000000		Total

Assignment of points is as follows:

The following assignments of points are for treatment test:

- 0.000000
- 0.000000
- 0.000000
- 0.000000

TABLE XXIV

Calculated Statistics for Series B flour, 1946.

$r_{CS} = 0.916^{**}$	$r_{MC} = 0.47^{*}$
$r_{CS.N} = 0.914^{**}$	$r_{SN} = -0.210$
$r_{CN} = -0.291$	$r_{SH} = 0.971^{**}$
$r_{CN.S} = -0.253$	$b_{CS} = 0.249\%C./0.1\%S.$
$r_{MS} = 0.78^{**}$	$b_{CM} = 0.17\%M./0.1\%S.$
$r_{MS.N} = 0.80^{**}$	
$r_{MN} = 0.02$	
$r_{MN.S} = 0.29$	

*Significant beyond 5% point.

**Significant beyond 1% point.

S = % sulphur in flour

C = % cystine in flour

N = % nitrogen in flour

M = % methionine in flour

H = % sulphur in wheat.

TABLE 1

Calculated values of the function $f(x)$ for various values of x

$x = 0.0$	$f(x) = 1.0000$	$x = 0.5$	$f(x) = 0.8660$
$x = 0.1$	$f(x) = 0.9949$	$x = 0.6$	$f(x) = 0.8090$
$x = 0.2$	$f(x) = 0.9799$	$x = 0.7$	$f(x) = 0.7173$
$x = 0.3$	$f(x) = 0.9549$	$x = 0.8$	$f(x) = 0.5976$
$x = 0.4$	$f(x) = 0.9196$	$x = 0.9$	$f(x) = 0.4398$
$x = 0.5$	$f(x) = 0.8660$	$x = 1.0$	$f(x) = 0.2954$
$x = 0.6$	$f(x) = 0.8090$	$x = 1.1$	$f(x) = 0.1736$
$x = 0.7$	$f(x) = 0.7173$	$x = 1.2$	$f(x) = 0.0707$
$x = 0.8$	$f(x) = 0.5976$	$x = 1.3$	$f(x) = -0.0420$
$x = 0.9$	$f(x) = 0.4398$	$x = 1.4$	$f(x) = -0.1593$
$x = 1.0$	$f(x) = 0.2954$	$x = 1.5$	$f(x) = -0.3420$

Values of the function $f(x)$ for various values of x are given in the table.

- 1. $x = 0.0$
- 2. $x = 0.1$
- 3. $x = 0.2$
- 4. $x = 0.3$
- 5. $x = 0.4$
- 6. $x = 0.5$
- 7. $x = 0.6$
- 8. $x = 0.7$
- 9. $x = 0.8$
- 10. $x = 0.9$
- 11. $x = 1.0$
- 12. $x = 1.1$
- 13. $x = 1.2$
- 14. $x = 1.3$
- 15. $x = 1.4$
- 16. $x = 1.5$

Series B flour, Breton, 1946.

Fig. VI,A

Fig. VI,B

When wheat is milled there are considerable quantities of bran, feed flour and red dog flour removed. From the available data an estimate can be made of the percentage of the total cystine and methionine in wheat which remains in the flour.

Table XXV gives the amounts of cystine and methionine in the flour as the percentage of the sulphur-containing amino acids present in the wheat. The percentage extraction of flour from wheat varies for each individual sample. The exact extraction data were not available. For the calculations reported it was assumed that the Allis-Chalmers experimental mill extracted 65% of the wheat as flour.

The percentage extraction of cystine in the flour is approximately the same in both series. These data show that approximately 60% of the cystine in wheat is extracted with the flour.

The values for the methionine contents of wheat or flour of sample 16, Series B and samples 2, 3, 9, 13 and 15, Series E are not accurate. This is obvious because more than 100% extraction is obtained. Table XXV shows that approximately 60% of the methionine in the wheat of both series is extracted in the flour. The mean and standard deviation for Series E are considerably greater than the values for Series B. If the values in Series E above 100% are discarded, the mean and standard deviation are $65 \pm 13\%$.

When wheat is milled there are considerable quantities of bran, feed flour and hot flour removed. From the available data an estimate can be made of the percentage of the total cystine and methionine in wheat which remains in the flour.

Table XIV gives the amounts of cystine and methionine in the flour as the percentage of the original-containing grain solids present in the wheat. The percentage extraction of flour from wheat varies for each individual sample. The exact extraction data were not available. For the calculations reported it was assumed that the mill-damage was minimal and extracted 5% of the wheat as flour.

The percentage extraction of cystine in the flour is approximately the same in both series. These data show that approximately 60% of the cystine in wheat is extracted with the flour.

The values for the methionine contents of wheat or flour of sample 16, Series B and samples 2, 3, 9, 15 and 16, Series B are not accurate. This is obvious because they are 100% extraction is obtained. Table XIV shows that approximately 60% of the methionine in the wheat of both series is extracted in the flour. The mean and standard deviation for Series B are consistently greater than the values for Series 2. In the values in Series B above 100% are included, the mean and standard deviation are 65 ± 1%.
and standard deviation are 65 ± 1%.

TABLE XXV

The percentages of cystine and methionine of wheat extracted in the flour of Series E and B

Sample No.	Series E		Series B	
	Cystine %	Methionine %	Cystine %	Methionine %
1	59	51	75	66
2	58	130	58	43
3	57	113	63	84
4	57	78	51	74
5	70	47	42	48
6	52	85	62	30
7	59	57	66	43
8	65	62	62	62
9	55	103	64	61
10	62	89	58	65
11	62	55	60	95
12	56	78	58	68
13	55	124	62	87
14	58	79	90	35
15	56	107	59	73
16	62	56	54	121
17	53	46	68	50
Mean	59 ± 4	80 ± 27	62 ± 10	65 ± 23

The 1947 crop of Thatcher wheat grown in a clover rotation at the Breton station produced bread with large differences in loaf volume. This material was therefore satisfactory for use in a study of loaf volume as affected by the sulphur-containing amino acids.

The sulphur, cystine, methionine and nitrogen were determined in the wheat. The results are presented in Table XXVI and figure VII, A, B, C and D. Included in the table are the loaf volumes and the cystine-sulphur as a percentage of the total sulphur.

Simple analyses of variance of the sulphur, cystine, methionine and nitrogen data are given in Table XXVII. The analyses show that the fertilizer treatments result in significant increases in the sulphur, amino acid and nitrogen contents of Series C wheat.

The calculated correlation and regression coefficients are tabulated in Table XXVIII.

As in the material previously investigated the sulphur content of the wheat increases with the application of sulphur fertilizers. Generally, the cystine contents of the seventeen samples of wheat follow the sulphur contents. The highest sulphur samples have the most cystine, the lowest sulphur samples the least. The relationship between cystine and sulphur is shown graphically in figure VII, A and B and figure VIII, A. The highly significant simple and partial correlation coefficients r_{CS} and $r_{CS.N}$ confirm the close relationship between cystine and sulphur.

TABLE XXVI

Data - Thatcher wheat, Series C (wheat after clover),
Breton, 1947.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	Cystine- sulphur as % of total sulphur
1	0.107	0.286	0.14	2.76	593	71
2	0.121	0.348	0.13	2.95	683	77
3	0.150	0.435	0.16	2.49	645	77
4	0.179	0.502	0.21	2.95	505	75
5	0.111	0.314	0.13	2.83	583	75
6	0.113	0.324	0.12	2.88	673	76
7	0.117	0.262	0.22	2.90	655	60
8	0.132	0.327	0.21	2.80	645	66
9	0.138	0.412	0.13	2.90	630	80
10	0.126	0.371	0.13	2.82	593	79
11	0.149	0.339	0.27	2.71	703	61
12	0.206	0.580	0.24	3.04	808	75
13	0.220	0.614	0.26	3.01	788	74
14	0.125	0.347	0.15	2.65	655	74
15	0.212	0.549	0.30	2.90	703	69
16	0.175	0.471	0.23	2.72	455	72
17	0.177	0.519	0.18	2.61	475	78
Mean	0.150	0.412	0.19	2.23	635	73

TABLE IV

Date - December 1934, Series 2 (New Wheat),
Boston, 1934.

Sample No.	Colony	Cystine	Methionine	Nitrogen	Water	Protein
1	0.107	0.106	0.11	2.18	507	71
2	0.101	0.100	0.11	2.18	507	72
3	0.100	0.100	0.11	2.18	507	73
4	0.100	0.100	0.11	2.18	507	74
5	0.111	0.111	0.11	2.18	507	75
6	0.118	0.118	0.11	2.18	507	76
7	0.117	0.117	0.11	2.18	507	77
8	0.115	0.115	0.11	2.18	507	78
9	0.115	0.115	0.11	2.18	507	79
10	0.116	0.116	0.11	2.18	507	80
11	0.119	0.119	0.11	2.18	507	81
12	0.100	0.100	0.11	2.18	507	82
13	0.100	0.100	0.11	2.18	507	83
14	0.100	0.100	0.11	2.18	507	84
15	0.115	0.115	0.11	2.18	507	85
16	0.115	0.115	0.11	2.18	507	86
17	0.115	0.115	0.11	2.18	507	87
18	0.115	0.115	0.11	2.18	507	88
19	0.115	0.115	0.11	2.18	507	89
20	0.115	0.115	0.11	2.18	507	90
Mean	0.110	0.110	0.11	2.18	507	70

TABLE XXVII

Analysis of Variance
Series C wheat, Breton, 1947.

Variance due to	D.F.	Mean squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.002771**	0.023468**	0.007044**	0.04365**
Error	17	0.0000182	0.0000941	0.000976	0.000347
Total	33				

** Significant beyond 1% point.

Minimum significant differences for treatment are:

0.009% sulphur.

0.04% nitrogen.

0.019% cystine.

0.06% methionine.

TABLE VIII

Summary of Results
for the 1954-55 Season

Year	Area	Yield	Quality	Remarks
1954-55	1000	1000	1000	1000
1955-56	1000	1000	1000	1000
Total	2000	2000	2000	2000

The figures are in pounds.

Minimum nitrogenous substance for treatment was:

- 1.0000 nitrogen
- 2.0000 nitrogen
- 3.0000 nitrogen
- 4.0000 nitrogen

TABLE XXVIII

Correlation and regression coefficients,
Thatcher wheat, Series C, Breton, 1947.

r_{CS}	= 0.959**	r_{CV}	= 0.153
$r_{CS.N}$	= 0.946**	$r_{CV.N}$	= 0.046
r_{CN}	= 0.248	r_{MV}	= 0.30
$r_{CN.S}$	= -0.020	$r_{MV.N}$	= 0.23
r_{MS}	= 0.76**	r_{SV}	= 0.221
$r_{MS.N}$	= 0.75**	$r_{SV.N}$	= 0.117
r_{MN}	= 0.21	r_{NV}	= 0.458
$r_{MN.S}$	= 0.02	$r_{NV.M}$	= 0.421
r_{MC}	= 0.55*	$r_{VN.S}$	= 0.430
r_{SN}	= 0.264	$r_{VN.C}$	= 0.438

$$b_{CS} = 0.279\%C/0.1\%S.$$

$$b_{CM} = 0.12\%M/0.1\%S.$$

*Significant beyond the 5% point.
**Significant beyond the 1% point.

C = % cystine in wheat.

S = % sulphur in wheat.

N = % nitrogen in wheat.

M = % methionine in wheat.

V = Loaf volume, cc's.

$$\begin{aligned} \text{* } 8.5 \text{ * } 0.00005 \text{ * } 0 &= 20 \\ \text{* } 0.00005 \text{ * } 0.00005 &= 10 \end{aligned}$$

Fig.VII,A

Fig.VII,B

Fig.VII,C

Fig.VII,D

TREATMENT

Figure VII - Effect of fertilizers on Series C wheat, 1947.

Series C wheat, Breton, 1947.

Fig. VIII,A - Cystine:sulphur relationship

Fig. VIII,B - Methionine:sulphur relationship

Figure VIII, D and B, indicates that there is no relationship between the cystine and nitrogen contents of this wheat. The simple and partial correlation coefficients r_{CN} and $r_{CN.S}$ confirm the lack of relationship between cystine and nitrogen.

The methionine contents of the wheat after clover in the 1947 crop are distributed over a considerable range. Figure VII, A and D, and the correlation coefficients in Table XXVIII show that the methionine content of the wheat is related to the sulphur content. The scatter of methionine and sulphur values and the regression line are plotted in figure VIII, B. The correlation coefficients r_{MN} and $r_{MN.C}$ and figure VII show that the nitrogen content of wheat has no effect on the methionine content.

The simple correlation coefficient r_{MC} suggests that the amounts of methionine and cystine in the wheat proteins are related.

The correlation coefficients between loaf volume and the various wheat constituents determined are tabulated in Table XXVIII. There are no significant relationships between loaf volume and sulphur, cystine, methionine or nitrogen in wheat.

The cystine and nitrogen contents of Series C flour are given in Table XXIX. Sulphur determinations were not carried out on this series. It would be expected, however,

Figure VIII, D and E. In general, the results of this relationship between the various and nitrogen contents of this wheat. The slight and positive correlation coefficients r_{WV} and r_{WV_2} confirm the fact of relationship between various and nitrogen.

The significant correlation of the wheat yield in the 1937 crop are illustrated over a considerable range. Figure VII, A and B, and the correlation coefficients in Table XVIII show that the relationship content of the wheat is related to the nitrogen content. The content of nitrogen and nitrogen values and the regression line are plotted in Figure VII, B. The correlation coefficients r_{WV} and r_{WV_2} show that the nitrogen content of wheat has no effect on the metabolic content.

The slight correlation coefficient r_{WV} suggests that the amounts of nitrogen and protein in the wheat grain are related.

The correlation coefficients between total values and the various wheat constituents mentioned are tabulated in Table XVIII. There are no significant relationships between total values and nitrogen, protein, cellulose or nitrogen in wheat.

The protein and nitrogen contents of wheat grain are given in Table XIX. Nitrogen concentrations were not related to the yield. It will be expected, however,

that the correlation between the sulphur and cystine in the flour is highly significant.

Table XXIX also gives the cystine in the flour as a percentage of that present in the wheat. The values are calculated assuming a 65% flour extraction. The range and mean of the cystine in the flour as reported are very similar to the values reported for the 1946 wheat.

The cystine content of Series C flour, as in Series E and B, is generally lower than that of the wheat. The highly significant correlation coefficient between the cystine contents of wheat and flour presented in Table XXX shows that changes in wheat cystine are accompanied by similar changes in flour cystine.

The correlation coefficients calculated for Series C flour are presented in Table XXX.

The simple correlation between cystine and nitrogen indicates a relationship between this amino acid and nitrogen. The partial correlation coefficient between cystine and nitrogen with the effects of sulphur removed is not calculated. It is likely that this statistic would be non-significant.

The loaf volumes of the samples are highly correlated with the nitrogen content of the flour. The correlation of nitrogen in the wheat and loaf volume, as previously pointed out, is not significant. This shows that there are quantities of nitrogen in the mill by-products which are not related to loaf volume. These quantities of nitrogen are sufficiently

that the correlation between the nitrogen and oxygen in the
flow is highly significant.

Table XXX also gives the results in the flow of a

percentage of total present in the flow. The values are

calculated assuming a 6% flow reduction. The mass and

mean of the oxygen in the flow as reported are very similar

to the values reported in the 1960 report.

The oxygen content of Table XXX, as in Table

XXX, is generally lower than that of the oxygen. The

highly significant correlation coefficient between the oxygen

content of water and flow presented in Table XXX shows that

changes in water oxygen are accompanied by similar changes

in flow oxygen.

The correlation coefficient calculated for water

flow are presented in Table XXX.

The single correlation between oxygen and nitrogen

indicates a relationship between the amino acid and nitrogen.

The partial correlation coefficient between oxygen and amino

acid with the effect of nitrogen removed is not calculated. It

is likely that this statistic would be non-significant.

The total volume of the sample is slightly increased

with the nitrogen content of the flow. The correlation of

nitrogen in the water and total volume is statistically related

and is not significant. This shows that there are some

of nitrogen in the cell by-products which are not related to

total volume. These quantities of nitrogen are substantially

great to offset the loaf volume-nitrogen relationship observed in the flour.

TABLE XXIX

Data - Flour from Thatcher Wheat,
Series C, Breton, 1947.

Sample No.	Cystine %	Nitrogen %	% Cystine in flour of total in wheat.
1	0.330	2.37	75
2	0.440	2.62	82
3	0.402	2.60	60
4	0.310	2.27	40
5	0.243	2.58	50
6	0.277	2.62	56
7	0.301	2.66	75
8	0.339	2.70	67
9	0.296	2.67	47
10	0.267	2.36	41
11	0.294	2.56	56
12	0.546	2.86	61
13	0.500	2.89	53
14	0.299	2.53	56
15	0.492	2.84	58
16	0.439	2.31	61
17	0.393	2.38	49
Mean	0.363	2.58	58

great to obtain the total volume-occupied relationship observed in the flow.

TABLE XXIX

Note - Flow from Venturer (West),
Series 5, Station, 1947.

Sample No.	Cyanine %	Nitrogen %	% of total in flow
1	0.580	2.34	2.34
2	0.440	2.31	2.31
3	0.402	2.30	2.30
4	0.310	2.31	2.31
5	0.315	2.30	2.30
6	0.277	2.32	2.32
7	0.301	2.30	2.30
8	0.330	2.31	2.31
9	0.304	2.31	2.31
10	0.267	2.30	2.30
11	0.294	2.30	2.30
12	0.280	2.30	2.30
13	0.280	2.30	2.30
14	0.280	2.30	2.30
15	0.280	2.30	2.30
16	0.280	2.30	2.30
17	0.280	2.30	2.30
18	0.280	2.30	2.30
19	0.280	2.30	2.30
20	0.280	2.30	2.30
21	0.280	2.30	2.30
22	0.280	2.30	2.30
23	0.280	2.30	2.30
24	0.280	2.30	2.30
25	0.280	2.30	2.30
26	0.280	2.30	2.30
27	0.280	2.30	2.30
28	0.280	2.30	2.30
29	0.280	2.30	2.30
30	0.280	2.30	2.30
31	0.280	2.30	2.30
32	0.280	2.30	2.30
33	0.280	2.30	2.30
34	0.280	2.30	2.30
35	0.280	2.30	2.30
36	0.280	2.30	2.30
37	0.280	2.30	2.30
38	0.280	2.30	2.30
39	0.280	2.30	2.30
40	0.280	2.30	2.30
41	0.280	2.30	2.30
42	0.280	2.30	2.30
43	0.280	2.30	2.30
44	0.280	2.30	2.30
45	0.280	2.30	2.30
46	0.280	2.30	2.30
47	0.280	2.30	2.30
48	0.280	2.30	2.30
49	0.280	2.30	2.30
50	0.280	2.30	2.30
51	0.280	2.30	2.30
52	0.280	2.30	2.30
53	0.280	2.30	2.30
54	0.280	2.30	2.30
55	0.280	2.30	2.30
56	0.280	2.30	2.30
57	0.280	2.30	2.30
58	0.280	2.30	2.30
59	0.280	2.30	2.30
60	0.280	2.30	2.30
61	0.280	2.30	2.30
62	0.280	2.30	2.30
63	0.280	2.30	2.30
64	0.280	2.30	2.30
65	0.280	2.30	2.30
66	0.280	2.30	2.30
67	0.280	2.30	2.30
68	0.280	2.30	2.30
69	0.280	2.30	2.30
70	0.280	2.30	2.30
71	0.280	2.30	2.30
72	0.280	2.30	2.30
73	0.280	2.30	2.30
74	0.280	2.30	2.30
75	0.280	2.30	2.30
76	0.280	2.30	2.30
77	0.280	2.30	2.30
78	0.280	2.30	2.30
79	0.280	2.30	2.30
80	0.280	2.30	2.30
81	0.280	2.30	2.30
82	0.280	2.30	2.30
83	0.280	2.30	2.30
84	0.280	2.30	2.30
85	0.280	2.30	2.30
86	0.280	2.30	2.30
87	0.280	2.30	2.30
88	0.280	2.30	2.30
89	0.280	2.30	2.30
90	0.280	2.30	2.30
91	0.280	2.30	2.30
92	0.280	2.30	2.30
93	0.280	2.30	2.30
94	0.280	2.30	2.30
95	0.280	2.30	2.30
96	0.280	2.30	2.30
97	0.280	2.30	2.30
98	0.280	2.30	2.30
99	0.280	2.30	2.30
100	0.280	2.30	2.30

TABLE XXX

Correlation Coefficients
Flour from Thatcher Wheat,
Series C, Breton, 1947

r_{CN}	=	0.487*
r_{CV}	=	0.389
r_{NV}	=	0.874**
$r_{CV.N}$	=	-0.073
$r_{NV.C}$	=	0.851**
r_{CW}	=	0.766**

C = % cystine in flour.

W = % cystine in wheat.

N = % nitrogen in flour.

V = loaf volume, cc's.

Rigby, as was previously stated, found a high correlation between sulphur content of wheat and cystine content of gluten. Further, he found that both these constituents were significantly correlated with loaf volume. In the material reported on in this paper there is no significant correlation between the sulphur in wheat and loaf volume. It was of interest to see if the constituents of gluten were correlated with loaf volume.

The sulphur, cystine, methionine and nitrogen were determined on the gluten washed from Series C flour. The

results are reported in Table XXXI. The cystine-sulphur, as a percentage of the total sulphur values is also included in the table.

A simple analysis of variance was carried out on each of the constituents determined. The results are reported in Table XXXII.

The calculated correlation and regression coefficients are presented in Table XXXIII. The loaf volume data used for the calculations are, of course, the same as those used for the wheat correlations.

The application of sulphur-containing fertilizers to the wheat resulted in an increase in the sulphur in the crude gluten protein. The highly significant simple correlation

r_{SW} in Table XXXIII shows that there is a close relationship between the sulphur contents of wheat and gluten.

Sulphur and cystine and sulphur and methionine in gluten are highly correlated. The regression coefficients and regression lines are presented in Table XXXIII and figure IX A and B respectively. The sulphur-containing amino acids are not related to the nitrogen content of the gluten. The high values for the sulphur correlation coefficients indicate that the sulphur-containing amino acids in the gluten proteins vary with variations in the total sulphur. The nitrogen correlation coefficients show that the nitrogen content of the gluten has no effect on the quantities of cystine and methionine in the gluten.

a percentage of the total volume value is also included

* 0.0000000000

1100 61097

* 670 60 45-1905 4.000-001

TABLE XXXI

Data - Gluten, Series C,
Breton, 1947.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-sulphur as % of total sulphur
1	0.654	1.498	1.18	14.60	61.1
2	0.714	1.912	0.95	14.06	71.4
3	0.696	1.798	1.01	14.01	68.9
4	0.737	1.825	1.16	14.06	66.1
5	0.540	1.342	0.85	14.60	66.3
6	0.514	1.277	0.81	14.54	66.3
7	0.500	1.087	0.98	14.40	58.0
8	0.507	1.439	0.57	14.25	75.7
9	0.443	1.451	0.26	14.49	87.4
10	0.557	1.404	0.85	14.69	67.3
11	0.587	1.414	0.98	14.28	64.3
12	0.742	1.810	1.21	13.95	65.1
13	0.808	2.022	1.25	13.80	66.8
14	0.534	1.462	0.67	14.48	73.1
15	0.857	2.004	1.50	14.24	62.4
16	0.782	1.766	1.48	14.48	60.3
17	0.729	2.022	0.88	14.40	74.0
Mean				14.31	67.9

TABLE 1

DATE - 10/10/50
 LOCATION - 10/10/50

Sample No.	Sample Weight (g)	Sample Volume (ml)	Sample Density (g/ml)	Sample Purity (%)	Sample Grade
1	0.000	1.000	1.000	100.0	1
2	0.000	1.000	1.000	100.0	2
3	0.000	1.000	1.000	100.0	3
4	0.000	1.000	1.000	100.0	4
5	0.000	1.000	1.000	100.0	5
6	0.000	1.000	1.000	100.0	6
7	0.000	1.000	1.000	100.0	7
8	0.000	1.000	1.000	100.0	8
9	0.000	1.000	1.000	100.0	9
10	0.000	1.000	1.000	100.0	10
11	0.000	1.000	1.000	100.0	11
12	0.000	1.000	1.000	100.0	12
13	0.000	1.000	1.000	100.0	13
14	0.000	1.000	1.000	100.0	14
15	0.000	1.000	1.000	100.0	15
16	0.000	1.000	1.000	100.0	16
17	0.000	1.000	1.000	100.0	17
18	0.000	1.000	1.000	100.0	18
19	0.000	1.000	1.000	100.0	19
20	0.000	1.000	1.000	100.0	20
21	0.000	1.000	1.000	100.0	21
22	0.000	1.000	1.000	100.0	22
23	0.000	1.000	1.000	100.0	23
24	0.000	1.000	1.000	100.0	24
25	0.000	1.000	1.000	100.0	25
26	0.000	1.000	1.000	100.0	26
27	0.000	1.000	1.000	100.0	27
28	0.000	1.000	1.000	100.0	28
29	0.000	1.000	1.000	100.0	29
30	0.000	1.000	1.000	100.0	30
31	0.000	1.000	1.000	100.0	31
32	0.000	1.000	1.000	100.0	32
33	0.000	1.000	1.000	100.0	33
34	0.000	1.000	1.000	100.0	34
35	0.000	1.000	1.000	100.0	35
36	0.000	1.000	1.000	100.0	36
37	0.000	1.000	1.000	100.0	37
38	0.000	1.000	1.000	100.0	38
39	0.000	1.000	1.000	100.0	39
40	0.000	1.000	1.000	100.0	40
41	0.000	1.000	1.000	100.0	41
42	0.000	1.000	1.000	100.0	42
43	0.000	1.000	1.000	100.0	43
44	0.000	1.000	1.000	100.0	44
45	0.000	1.000	1.000	100.0	45
46	0.000	1.000	1.000	100.0	46
47	0.000	1.000	1.000	100.0	47
48	0.000	1.000	1.000	100.0	48
49	0.000	1.000	1.000	100.0	49
50	0.000	1.000	1.000	100.0	50
51	0.000	1.000	1.000	100.0	51
52	0.000	1.000	1.000	100.0	52
53	0.000	1.000	1.000	100.0	53
54	0.000	1.000	1.000	100.0	54
55	0.000	1.000	1.000	100.0	55
56	0.000	1.000	1.000	100.0	56
57	0.000	1.000	1.000	100.0	57
58	0.000	1.000	1.000	100.0	58
59	0.000	1.000	1.000	100.0	59
60	0.000	1.000	1.000	100.0	60
61	0.000	1.000	1.000	100.0	61
62	0.000	1.000	1.000	100.0	62
63	0.000	1.000	1.000	100.0	63
64	0.000	1.000	1.000	100.0	64
65	0.000	1.000	1.000	100.0	65
66	0.000	1.000	1.000	100.0	66
67	0.000	1.000	1.000	100.0	67
68	0.000	1.000	1.000	100.0	68
69	0.000	1.000	1.000	100.0	69
70	0.000	1.000	1.000	100.0	70
71	0.000	1.000	1.000	100.0	71
72	0.000	1.000	1.000	100.0	72
73	0.000	1.000	1.000	100.0	73
74	0.000	1.000	1.000	100.0	74
75	0.000	1.000	1.000	100.0	75
76	0.000	1.000	1.000	100.0	76
77	0.000	1.000	1.000	100.0	77
78	0.000	1.000	1.000	100.0	78
79	0.000	1.000	1.000	100.0	79
80	0.000	1.000	1.000	100.0	80
81	0.000	1.000	1.000	100.0	81
82	0.000	1.000	1.000	100.0	82
83	0.000	1.000	1.000	100.0	83
84	0.000	1.000	1.000	100.0	84
85	0.000	1.000	1.000	100.0	85
86	0.000	1.000	1.000	100.0	86
87	0.000	1.000	1.000	100.0	87
88	0.000	1.000	1.000	100.0	88
89	0.000	1.000	1.000	100.0	89
90	0.000	1.000	1.000	100.0	90
91	0.000	1.000	1.000	100.0	91
92	0.000	1.000	1.000	100.0	92
93	0.000	1.000	1.000	100.0	93
94	0.000	1.000	1.000	100.0	94
95	0.000	1.000	1.000	100.0	95
96	0.000	1.000	1.000	100.0	96
97	0.000	1.000	1.000	100.0	97
98	0.000	1.000	1.000	100.0	98
99	0.000	1.000	1.000	100.0	99
100	0.000	1.000	1.000	100.0	100

TABLE XXXII

Analysis of Variance
Gluten, Series C, Breton, 1947.

Variance due to	D.F.	Mean Square			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.031636**	0.16025**	0.19310**	0.1311**
Error	17	0.000381	0.00184	0.01804	0.00271
Total	33				

**Significant beyond 1% point.

The minimum significant differences for treatment are:

0.039% sulphur

0.080% cystine

0.27% methionine

0.10% nitrogen.

TABLE 1

Analysis of Variance
 Boston, Boston, 1947.

Source of Variation	D.F.	Mean Square	Variance Ratio	F
Treatment	15	0.001000	0.001000	0.1-1.0
Error	17	0.000500	0.000500	0.00000
Total	32			

Significance beyond 1% point.

The minimum significant difference for treatment was:

0.001000

0.000500

0.001000

0.001000

TABLE XXXIII

Correlation and Regression Coefficients
Series C, Gluten.

$r_{CS} = 0.893^{**}$	$r_{SN} = -0.584^{*}$
$r_{CS.N} = 0.833^{**}$	$r_{CM} = 0.53^{*}$
$r_{CN} = -0.644^{**}$	$r_{VC} = 0.013$
$r_{CN.S} = -0.333$	$r_{VC.N} = -0.482^{*}$
$r_{MS} = 0.85^{**}$	$r_{MV} = 0.03$
$r_{MS.N} = 0.85^{**}$	$r_{MV.N} = -0.02$
$r_{MN} = -0.35$	$r_{SV} = 0.022$
$r_{MN.S} = 0.34$	$r_{SV.N} = -0.411$
	$r_{SW} = 0.788^{**}$

$$b_{CS} = 0.206\%C/0.1\%S.$$

$$b_{MS} = 0.21\%C/0.1\%S.$$

* Significant beyond 1%

**Significant beyond 5%.

S = % sulphur in gluten

W = % sulphur in wheat

N = % nitrogen in gluten

M = % methionine in gluten

V = loaf volume, cc's.

TABLE XXIII

Correlation and regression coefficients
between $\bar{X}$ and $\bar{Y}$

$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$
$r_{\bar{X}\bar{Y}} = 0.8034$	$r_{\bar{X}\bar{Y}} = 0.8034$

$$r_{\bar{X}\bar{Y}} = 0.8034$$

$$r_{\bar{X}\bar{Y}} = 0.8034$$

Correlation and regression coefficients
between $\bar{X}$ and $\bar{Y}$

$r_{\bar{X}\bar{Y}} = 0.8034$
 $r_{\bar{X}\bar{Y}} = 0.8034$
 $r_{\bar{X}\bar{Y}} = 0.8034$
 $r_{\bar{X}\bar{Y}} = 0.8034$
 $r_{\bar{X}\bar{Y}} = 0.8034$

Gluten, Series C, 1947

Fig. IX,A

Fig. IX,B

The small range in the nitrogen content of the seventeen glutens indicates that they are similar in purity. There are small differences in the nitrogen contents of the glutens but they could be caused by differences in gluten strength. Using loaf volume as a measure of gluten strength, small volumes indicate weak glutens and large volumes strong glutens. The weak glutens readily give up starch in the washing process. The strong glutens, on the other hand, retain some starch. This results in a diluted gluten protein and less nitrogen on a percentage basis.

The relationship between cystine and methionine observed in the whole wheat proteins also exists in the gluten proteins. The sulphur-containing amino acids, cystine and methionine, are probably related in the non-gluten and mill by-product fractions of wheat.

The cystine-sulphur accounts for approximately the same percentage of the total sulphur in the gluten as in the whole wheat. This would indicate that the total sulphur in the non-gluten or mill by-product proteins has a similar distribution of cystine and methionine to that in the gluten proteins.

The correlation coefficients given in Table XXXIV show that the quantities of gluten in flour expressed as wet or dry weight are closely related to baking strength. The regression lines and scatters are shown in figure X, A and B. The actual percentages of wet and dry gluten in flour are given in Table XXXV.

The small range in the nitrogen content of the various
 samples indicates that they are similar in quality. There are
 small differences in the nitrogen content of the samples and
 they could be caused by differences in plant material. Being
 lost volume as a measure of fiber strength, small volume
 indicates weak fibers and large volume strong fibers. The
 weak fibers resulting give up strength in the wetting process.
 The strong fibers, on the other hand, retain some strength.
 This results in a limited fiber protein and less nitrogen on
 a percentage basis.

The relationship between cystine and methionine observed
 in the whole wheat protein also exists in the fiber proteins.
 The sulfur-containing amino acids, cystine and methionine, are
 probably related in the non-gluten and will be present in similar
 of about.

The cystine-sulfur accounts for approximately the same
 percentage of the total sulfur in the gluten as in the whole
 wheat. This would indicate that the total sulfur in the
 non-gluten or will be present in similar distribution
 of cystine and methionine so that in the gluten protein.
 The distribution coefficients given in Table XXIV show
 that the quantities of gluten in flour increased as wet or dry
 weight are closely related to baking strength. The quantities
 of gluten and starch are shown in Figures 4 and 5. The general
 percentages of wet and dry gluten in flour are given in Table

Fig.X,A

Fig.X,B

The sulphur-containing amino acid content of gluten is not related to baking strength. This is shown by some of the correlation coefficients presented in Table XXXIII. The simple correlation between the quantity of cystine in the gluten of 100 grams of flour and loaf volume is significant. This value is given in Table XXXIV. The combination of the quantity of gluten and its cystine content gives a value which is significantly and positively related to loaf volume. The cystine in the gluten of 100 grams of flour is actually another measure of the quantity of gluten in the flour.

The difference between the cystine content of the flour and the cystine content of the gluten of 100 grams of flour gives a value for the non-gluten cystine in the flour. Table XXXVI gives the cystine contents of the non-gluten proteins in 17 samples of Series C flour. The correlation coefficient between non-gluten cystine and loaf volume is given in Table XXXIV.

A considerable proportion of the cystine in flour is present in the non-gluten protein. The actual values for Series C flour can be calculated from the values of Table XXXVI. The table gives the cystine in gluten as a percentage of the total cystine in the flour. The percentage cystine in the non-gluten proteins is 100 percent minus the percentage gluten cystine.

The extent of hydration of gluteins of different quality will give a measure of the physical properties of the gluten. The differences in hydration of gluteins of different quality as

The relationship between the amount of cystine in the protein and the amount of cystine in the protein is not related to the amount of cystine in the protein. This is shown by the fact that the correlation coefficients presented in Table XXIII. The correlation between the amount of cystine in the protein and the amount of cystine in the protein is significant. This value is given in Table XXIII. The correlation of the amount of cystine and the cystine content gives a value which is significantly and positively related to the amount of cystine in the protein of 100 parts of protein is actually another example of the amount of cystine in the protein.

The difference between the cystine content of the protein and the cystine content of the protein of 100 parts of protein gives a value for the non-glutamic cystine in the protein. Table XXIV gives the cystine content of the non-glutamic protein in 17 samples of Series I. The correlation coefficient between non-glutamic cystine and total cystine is given in Table XXIV.

A considerable proportion of the cystine in the protein is present in the non-glutamic protein. The actual values for Series I are given in Table XXIV. The values for the non-glutamic cystine in the protein are given in Table XXIV. The correlation coefficient between the cystine in the protein and the cystine in the protein is significant. The correlation coefficient between the cystine in the protein and the cystine in the protein is 100 percent when the percentage of non-glutamic cystine is 100 percent. The extent of hydration of the protein will give a measure of the degree of hydration of the protein. The difference in hydration of the protein and the difference in

measured by percentage moisture possibly are related to the amino acid composition of the gluten proteins. Attempts were made to relate the sulphur-containing amino acid content of gluten with the percentage moisture of the gluten.

The moisture percentages of the seventeen gluten samples from Series C flour are presented in Table XXXV. The moisture contents of these glutes were similar. The quality of the gluten as indicated by hydration is similar in the seventeen samples. There are large and significant differences in the quantity of sulphur-containing amino acids in the gluten. Therefore, in the gluten there does not appear to be a relationship between the physical properties measured by the hydration capacity and the sulphur-containing amino acid content.

TABLE XXXIV

Correlation Coefficients of gluten,
Series C, 1947.

r_{WD}	= 0.976**	F = cystine in gluten of 100 gms. flour
r_{DV}	= 0.869**	P = mgms. cystine in non-gluten proteins of 100 grams flour.
r_{PV}	= 0.244	V = loaf volume, cc's.
r_{VW}	= 0.855**	D = % dry gluten
r_{VF}	= 0.566*	W = % wet gluten.

* Significant beyond 5% point.

**Significant beyond 1% point.

accounted by percentage which possibly was related to the value
 sold composition of the glass particles. Experiments were made to
 relate the surface-composition with sold content of glass
 with the percentage content of the glass.
 The surface composition of the common glass samples
 from Series I floor are presented in Table XIV. The surface
 content of these glasses were similar. The quality of the
 glass as indicated by hardness is similar in the common
 samples. There are large variations in the
 quantity of surface-composition with value in the glass.
 Therefore, in the glass there does not appear to be a relation-
 ship between the physical properties and the composition
 especially and the surface-composition with value.

TABLE XIV

Surface Composition of Glass,
 Series I, 1947.

1 = value in glass of 100 gms. floor	0.975	IV
2 = value in glass of 100 gms. floor	0.800	IV
3 = value in glass of 100 gms. floor	0.244	IV
4 = value in glass of 100 gms. floor	0.850	IV
5 = value in glass of 100 gms. floor	0.500	IV
6 = value in glass of 100 gms. floor		

Significant beyond 5% level.
 Significant beyond 1% level.

TABLE XXXV

Percentage of wet and dry glutens in flour
and percentage moisture of gluten
Series C, 1947.

Sample No.	Wet gluten %	Weight dry gluten %	Moisture %
1	38.00	12.599	66.9
2	43.46	14.592	66.4
3	43.20	14.795	65.7
4	39.30	12.615	66.1
5	40.33	13.489	66.8
6	41.09	13.985	66.0
7	43.62	13.699	68.6
8	43.79	14.752	66.3
9	44.66	14.282	68.0
10	38.20	12.662	66.9
11	40.39	13.779	66.0
12	50.05	16.445	67.2
13	53.41	17.105	68.0
14	39.59	13.362	66.3
15	47.09	15.682	66.7
16	36.90	12.402	66.4
17	37.23	12.425	66.6

TABLE VIII

Percentage of wet and dry fibers in glass
and percentage moisture of glass
Series 1, 1947.

Sample No.	Wet fibers %	Dry fibers %	Moisture %
1	78.00	12.75	65.2
2	46.44	1.50	67.4
3	42.44	10.75	60.9
4	50.75	15.00	65.1
5	50.75	10.75	60.4
6	41.00	17.00	60.4
7	47.00	10.75	60.0
8	47.75	3.00	60.4
9	41.00	10.75	60.8
10	50.80	10.00	60.4
11	40.75	10.75	60.0
12	50.00	10.75	60.4
13	44.00	10.75	60.4
14	50.75	10.75	60.4
15	41.00	10.75	60.4
16	40.75	10.75	60.4
17	40.75	10.75	60.4
18	40.75	10.75	60.4
19	40.75	10.75	60.4
20	40.75	10.75	60.4
21	40.75	10.75	60.4
22	40.75	10.75	60.4
23	40.75	10.75	60.4
24	40.75	10.75	60.4
25	40.75	10.75	60.4
26	40.75	10.75	60.4
27	40.75	10.75	60.4
28	40.75	10.75	60.4
29	40.75	10.75	60.4
30	40.75	10.75	60.4
31	40.75	10.75	60.4
32	40.75	10.75	60.4
33	40.75	10.75	60.4
34	40.75	10.75	60.4
35	40.75	10.75	60.4
36	40.75	10.75	60.4
37	40.75	10.75	60.4
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39	40.75	10.75	60.4
40	40.75	10.75	60.4
41	40.75	10.75	60.4
42	40.75	10.75	60.4
43	40.75	10.75	60.4
44	40.75	10.75	60.4
45	40.75	10.75	60.4
46	40.75	10.75	60.4
47	40.75	10.75	60.4
48	40.75	10.75	60.4
49	40.75	10.75	60.4
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51	40.75	10.75	60.4
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65	40.75	10.75	60.4
66	40.75	10.75	60.4
67	40.75	10.75	60.4
68	40.75	10.75	60.4
69	40.75	10.75	60.4
70	40.75	10.75	60.4
71	40.75	10.75	60.4
72	40.75	10.75	60.4
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74	40.75	10.75	60.4
75	40.75	10.75	60.4
76	40.75	10.75	60.4
77	40.75	10.75	60.4
78	40.75	10.75	60.4
79	40.75	10.75	60.4
80	40.75	10.75	60.4
81	40.75	10.75	60.4
82	40.75	10.75	60.4
83	40.75	10.75	60.4
84	40.75	10.75	60.4
85	40.75	10.75	60.4
86	40.75	10.75	60.4
87	40.75	10.75	60.4
88	40.75	10.75	60.4
89	40.75	10.75	60.4
90	40.75	10.75	60.4
91	40.75	10.75	60.4
92	40.75	10.75	60.4
93	40.75	10.75	60.4
94	40.75	10.75	60.4
95	40.75	10.75	60.4
96	40.75	10.75	60.4
97	40.75	10.75	60.4
98	40.75	10.75	60.4
99	40.75	10.75	60.4
100	40.75	10.75	60.4

TABLE XXXVI

Gluten and non-gluten cystine
in Series C flour.

Sample No.	Cystine* in gluten	Mgms. cystine** in non-gluten proteins
1	57.3	141
2	63.4	161
3	66.2	136
4	74.2	80
5	74.5	62
6	64.6	98
7	49.5	152
8	62.5	127
9	69.9	89
10	66.7	89
11	66.3	99
12	54.6	247
13	69.2	154
14	65.2	104
15	63.8	178
16	49.9	220
17	63.9	142

* As a percentage of cystine in flour.

**Mgms. cystine in non-gluten proteins
of 100 grams flour.

TABLE 20

Percentages of cystine in non-lysed
in series 2 (1947)

Sample No.	Cystine in lysate	Percent cystine in non-lysed
1	27.5	141
2	27.4	101
3	27.2	170
4	27.3	100
5	27.5	100
6	27.4	100
7	27.5	100
8	27.5	100
9	27.5	100
10	27.5	100
11	27.5	100
12	27.5	100
13	27.5	100
14	27.5	100
15	27.5	100
16	27.5	100
17	27.5	100
18	27.5	100
19	27.5	100
20	27.5	100

Percentages of cystine in non-lysed
of 100 from floor.

Series III - Water culture experiments

Experiments I and II, as reported under "Materials," were carried out primarily to determine if and when there is a critical period for sulphur nutrient supply in the formation of the sulphur-containing amino acids in wheat.

The results of the analysis of the wheat from Experiment I are presented in Table XXXVII.

The results cannot be analysed statistically but by observation it is evident that the cystine contents of the wheat grown on the sulphur deficient nutrient solutions are as high as those of the check. The nitrogen contents of the wheat are similar.

This preliminary experiment suggests that either the wheat plant obtains all the sulphur needed for cystine synthesis in the first two weeks of the growing period or that there is a source of sulphur that has not been controlled. The cystine content of the wheat is abnormally high. It would be expected that the sulphur content of the wheat would also be high. A rough calculation shows that there is sufficient sulphur supplied in the first two weeks to account for the sulphur content of the wheat.

The results of experiment II are presented in Table XXXVII, B. The cystine-sulphur : total sulphur ratio is also included in the table.

The cystine content of the wheat is less in experiment II than experiment I. The trend, however, is the same in both cases.

There is very little if any difference in the percentages of nitrogen, sulphur and cystine. The wheat from tank two appears to contain slightly more sulphur and considerably more methionine than the wheat from the other tanks. The methionine value, however, seems so far out of line that there is probably an error in the cystine or sulphur determination. The low cystine-sulphur : total sulphur ratio for tank two wheat strengthens this suggestion. The cystine-sulphur : total sulphur ratios of the other tanks are approximately the same and they resemble very closely the ratios reported in earlier experiments.

Yield data were not taken for experiment II because of heavy damage by sparrows. General observations showed that there were no marked differences in yield. At harvest the plants were approximately the same height. Yields of the tanks might aid in an explanation of the observed results. It would be expected that the sulphur deficient tanks would have a lower yield. The available sulphur would then be distributed over a smaller number of kernels. This would result in a higher sulphur content for the wheat.

TABLE XXXVII

Data - Water Cultures
Effect of reducing nutrient sulphur supply
on the sulphur, cystine, methionine
and nitrogen contents of wheat

A. Experiment I

Tank No.	Cystine %	Nitrogen %
1	0.914	5.21
2	0.920	4.77
3	0.868	4.92
4	0.941	4.69

B. Experiment II

Tank No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Ratio cystine sulphur: total sulphur
1	0.189	0.581	0.16	3.10	0.82
2	0.230	0.545	0.40	3.33	0.63
3	0.188	0.541	0.20	3.15	0.77
4	0.201	0.604	0.19	3.22	0.80

TABLE XXV

Effect of benzene, carbon tetrachloride, and nitrogen on the alkaline, oxidative, and nitrosative properties of yeast

A. Experiment I

Run No.	Yeast	Nitrogen
1	0.10	0.10
2	0.10	0.10
3	0.10	0.10
4	0.10	0.10

B. Experiment II

Run No.	Yeast	Carbon tetrachloride	Nitrogen	Alkaline
1	0.10	0.10	0.10	0.10
2	0.10	0.10	0.10	0.10
3	0.10	0.10	0.10	0.10
4	0.10	0.10	0.10	0.10

Experiments III and IV were carried out to compare the effects of variations in the sulphur and nitrogen contents of the nutrient solutions on the ultimate sulphur, nitrogen, and sulphur-containing amino acid contents of wheat.

The results of the experiments are presented in Table XXXVIII, A and B.

The high nitrogen, high sulphur nutrient solution of Experiment III produced wheat highest in sulphur, cystine and methionine. The low nitrogen, low sulphur solution produced wheat with the smallest amounts of sulphur, nitrogen and sulphur-containing amino acids. The high sulphur, low nitrogen tank and the high nitrogen, low sulphur tank produced wheat that was intermediate in sulphur, cystine and methionine. The nitrogen in the wheat of the high nitrogen, low sulphur treatment is higher than the high nitrogen, high sulphur treatment.

In view of results of previous experiments the high sulphur tank, number two, was expected to produce wheat higher in sulphur cystine and methionine than tank three. This was not the case.

Yield data could possibly explain the deviations from the expected. Yields were not taken on this material because of extensive damage and loss of plants caused by sparrows. Nevertheless plate I, under "Materials", and plate II give some indication of the yield and relative size of the wheat plants at the date of harvest.

TABLE XXXVIII

Data - Effects of variations in the sulphur and nitrogen contents of nutrient solutions on the ultimate sulphur, nitrogen and sulphur-containing amino acid contents of wheat.

A. Experiment III

Tank No.	Nutrient Solutions	Sulphur %	Cystine %	Nitrogen %	Methionine %	Ratio of Cystine sulphur: total sulphur
1	Low nitrogen Low sulphur	0.174	0.517	2.66	0.18	0.79
2	Low nitrogen High sulphur	0.235	0.658	3.64	0.27	0.77
3	High nitrogen Low sulphur	0.230	0.662	4.78	0.25	0.75
4	High nitrogen High sulphur	0.312	0.819	4.63	0.43	0.70

B. Experiment IV

1	High nitrogen Low sulphur	0.254	0.681	4.16	0.34	0.72
2	High nitrogen High sulphur	0.240	0.731	3.90	0.21	0.81

No.	Location	Number	Remarks
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the teacher's

Plate II

Relative heights of wheat
from Experiment III

Tank No. 1 - Low sulphur, low nitrogen
2 - High sulphur, low nitrogen
3 - Low sulphur, high nitrogen
4 - High sulphur, high nitrogen

High sulphur and low nitrogen treatment produced a greater yield than the low sulphur, high nitrogen treatment. The sulphur in tank three is distributed over a smaller yield. The sulphur, sulphur-containing amino acids and nitrogen percentages are expected to be higher where the yield is reduced. This is the situation observed.

The low sulphur, low nitrogen wheat from tank one is much lower in the percentages of the determined constituents than the wheat from the other low sulphur tank (tank three). The yield of tank one is considerably greater than that of tank three and the same amounts of sulphur are distributed over a greater yield of material.

Variations in the sulphur contents of the wheat are closely related to the variations in cystine and methionine content. Figure XI, A, B, shows a close relationship between cystine and sulphur and methionine and sulphur. Figure XI, C, D, shows that the nitrogen is not closely related to the cystine or methionine contents of the wheat.

High nitrogen and low nitrogen treatment produced a
greater yield than the low nitrogen, but nitrogen treatment.
The nitrogen in each case is distributed over a similar field.
The nitrogen, nitrogen-containing amino acids and nitrogen pro-
cesses are exposed to the nitrogen which the field is treated.
This is the nitrogen observed.

The low nitrogen, the nitrogen which from each one is
much lower in the nitrogen of the nitrogenous compounds
than the nitrogen from the other low nitrogen field (high nitrogen).
The yield of each one is consistently greater than that of
the other and the same amount of nitrogen was distributed over
a greater field of material.

Experiments in the nitrogen treatment of the wheat and
oats showed that the nitrogen in wheat and oats was
distributed. Figure 1, 2, 3, shows a slight relationship between
nitrogen and nitrogenous compounds and nitrogen. Figure 4,
5, 6, shows that the nitrogen is not evenly distributed in the
system of nitrogenous compounds of the wheat.

Figure XI

Relationships of cystine and methionine to sulphur and nitrogen in wheat from experiment III.

The cystine-sulphur : total sulphur ratios are generally similar to those in experiment I. With an increase in total sulphur in the wheat there is a decrease in the sulphur represented as cystine.

In experiment IV, the high nitrogen, high sulphur tank was expected to produce wheat higher in sulphur, cystine and methionine. The complete stoppage of sulphur after about two months has not resulted in any significant decreases in sulphur or methionine. The cystine value is slightly lower in the low sulphur tank. The cystine-sulphur : total sulphur ratios are similar to those in experiment III.

The results of this experiment suggest that there is a source of sulphur available to the plant which has not been recognized. Possible sources are described under "Discussion".

The results of the experiments : In the experiments I. and II. the results of the experiments I. and II. were generally similar to those in experiment I. With an increase in total nitrogen in the wheat there is a decrease in the nitrogen content of the grain.

In experiment IV, the nitrogen content of the grain was observed to increase with an increase in nitrogen, oxygen and potassium. The potassium content of nitrogen after about two months has not resulted in any significant decrease in nitrogen or potassium. The oxygen value is slightly lower in the low nitrogen level. The oxygen content : total nitrogen ratio are similar to those in experiment III.

The results of this experiment suggest that there is a source of nitrogen available to the plant which has not been recognized. Possible sources are described under "Discussion".

DISCUSSION AND SUMMARY

The development of methods for the determination of sulphur-containing amino acids in whole wheat and flour has made possible a more complete study of these constituents in wheat proteins. Rigby (34) and Gerbrandt (19) found significant correlations of the sulphur-containing amino acids in gluten with loaf volume. As this work was on gluten which contains only a part of the cystine it was desirable to carry out analysis on the non-gluten fractions of wheat.

Reserves of the material used by Gerbrandt were available for a further study of the effect of the sulphur-containing amino acids on loaf volume. Determinations of the sulphur-containing amino acids were carried out on the complete flour. The correlations with loaf volume indicate that the quantity of the crude protein of flour is a better measure of loaf volume than the percentage of cystine or methionine. The present study suggests that the method used by Gerbrandt to obtain positive correlations with the sulphur-containing amino acids is actually a correlation of gluten quantity with loaf volume.

The cystine content of Marquis flour is highly correlated with the sulphur content. There is no relationship between the total nitrogen and cystine contents of the flour. Cultural methods that will change the sulphur content of the flour will affect the cystine content to a greater degree than will changes

DISCUSSION AND SUMMARY

The development of methods for the determination of sulphur-containing amino acids in whole wheat and flour has made possible a more complete study of cereal composition in wheat processing. Data (1) and (2) and (3) have shown that the relationship of the sulphur-containing amino acids in wheat with loaf volume. In this work an attempt was made to carry out only a part of the system it was desirable to carry out analysis on the non-soluble fractions of wheat.

Reserves of the material used by Garman were available for a further study of the effect of the sulphur-containing amino acids on loaf volume. Determinations of the sulphur-containing amino acids were carried out on the complete flour. The correlations with loaf volume indicate that the quantity of the crude protein of flour is a better measure of loaf volume than the percentage of cystine or methionine. The present study suggests that the method used by Garman to obtain positive correlations with the sulphur-containing amino acids is actually a correlation of grain quantity with loaf volume. The cystine content of wheat flour is highly correlated with the nitrogen content. There is no relationship between the total nitrogen and cystine content of the flour. However, methods that will change the sulphur content of the flour will affect the cystine content to a greater degree than will changes

in nitrogen content.

The methionine content of the Marquis flour is highly correlated with both the sulphur and nitrogen contents. The partial correlation between methionine and sulphur independent of the effects of nitrogen is non-significant. Similarly the partial correlation of methionine and nitrogen with the effects of sulphur removed is non-significant. These correlations suggest that both the nitrogen and sulphur contents of Marquis flour influence the methionine content. The sulphur and nitrogen effects on methionine are related.

The analysis of the Marquis flour suggests that the quantity of cystine and perhaps methionine could be varied with variations in the sulphur content of flour. The sulphur in flour would be expected to vary with the sulphur in wheat.

The sulphur was determined on samples of wheat grown at Breton in the continuous wheat crop series in 1946 and the wheat after clover crops for 1946 and 1947. There were significant differences in the sulphur contents of wheat as a result of fertilizer treatments. This is in agreement with the work of Peters (33). Generally the application of sulphur-containing fertilizers resulted in significantly greater increases of sulphur than the check or non-sulphur-fertilized plots.

The cystine content of the wheat followed the sulphur content very closely. The simple and partial correlation coefficients are highly significant. Changes in the nitrogen content of the wheat had no effect on the quantities of cystine present.

in nitrogen content.

The methionine content of the nitrogen flow is highly correlated with both the nitrogen and amino acid contents. The partial correlation between methionine and nitrogen is independent of the effects of nitrogen is non-significant. Similarly, the partial correlation of methionine and nitrogen with the effects of nitrogen removed is non-significant. These correlations suggest that both the nitrogen and amino acid contents of methionine influence the methionine content. The nitrogen and nitrogen effects on methionine are related.

The analysis of the nitrogen flow suggests that the

quantity of cysteine and perhaps methionine could be varied with variations in the nitrogen content of wheat. The nitrogen in wheat would be expected to vary with the nitrogen in wheat.

The nitrogen was determined on samples of wheat grown

at Preston in the continuous wheat crop series in 1946 and the wheat after clover crops for 1946 and 1947. There were significant

differences in the nitrogen content of wheat as a result of fertiliser treatments. This is in agreement with the work of Bates (1951). Generally the application of nitrogen-containing fertilisers resulted in significantly greater increases of nitrogen than the effect of non-nitrogen-fertilised plots.

The cysteine content of the wheat followed the nitrogen

content very closely. The nitrogen and partial correlation coefficients are highly significant. However in the nitrogen content of the wheat had no effect on the partial correlation of cysteine present.

In 1946 the wheat after clover crop had a much greater range in the determined constituents than the wheat from the continuous series. The legumes in the clover rotation are deep feeders. They probably bring leached sulphates closer to the surface where they are available to the wheat plant. In addition, the nitrogen content of the soil is increased because of nitrogen fixing bacteria. The greater availability of sulphur and nitrogen results in increases in the amount of cystine. The nitrogen intake of a wheat plant is of course utilized for the manufacture of numerous amino acids, proteins and other nitrogen-containing compounds. The sulphur, on the other hand, goes into the synthesis of only cystine, methionine and a few minor sulphur-containing compounds. The correlations of sulphur and cystine are therefore much more significant than the relationships between nitrogen and cystine.

The methionine content of the wheat is closely related to the sulphur uptake. The various fertilizer treatments have had little if any significant effect on the methionine contents of the wheat from the continuous series. The treatments, however, do result in large methionine differences in the wheat of the clover series in both years. Both the sulphur and nitrogen contents of the fertilized plots are higher in the wheat from the clover series. With more available nitrogen, the methionine increases with increases in sulphur. In the continuous series there is less available nitrogen, and the increases in methionine, although they are related to the increases in sulphur,

are much smaller. The regression of sulphur on methionine in the continuous series is 0.06 percent methionine/0.1 percent sulphur. The corresponding regression for the clover series is 0.13 percent methionine/0.1 percent sulphur. The available data indicate that if sufficient nitrogen is present in the soil, increases in sulphur will result in relatively large increases in methionine in wheat. If, however, the intake of nitrogen is low, increases in sulphur have little or no effect on the methionine content of the wheat.

The analyses of cystine and sulphur in wheat show that in the majority of samples the cystine-sulphur accounts for 70 to 80 percent of the total sulphur. This does not agree with the work of Gubler and Greaver (21) who estimated that approximately 50 per cent of the total sulphur in wheat is cystine-sulphur. In addition, these workers report that increases in the sulphur and cystine contents of wheat result in decreases in the sulphur represented as cystine. No such relationship was apparent in the work reported here.

The sulphur, cystine, methionine and nitrogen contents of flour are slightly lower than in the wheat. Generally there is a high correlation between the constituents in wheat and flour. The higher sulphur, cystine, methionine and nitrogen wheat samples produce flour higher in these constituents. The lower sulphur, nitrogen and amino acid wheat samples produce flour lower in these constituents.

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The analyses of cystine and sulphur in wheat show that in the majority of samples the cystine-sulphur accounts for 70 to 80 percent of the total sulphur. This does not agree with the work of Gähler and Greaves (31) who concluded that approximately 50 per cent of the total sulphur in wheat is cystine-sulphur. In addition, these workers report that increases in the sulphur and cystine contents of wheat result in decreases in the sulphur represented as cystine. No such relation-

ship was apparent in the work reported here.

The sulphur, cystine, methionine and nitrogen contents of flour are slightly lower than in the wheat. Generally there is a high correlation between the constituents in wheat and flour. The higher sulphur, cystine, methionine and nitrogen wheat samples produce flour higher in these constituents. The lower sulphur, nitrogen and amino acid wheat samples produce flour lower in these constituents.

Of the total cystine in wheat approximately 55 to 65 percent is found in the flour as milled on an experimental mill. The variations in the methionine content of flours as a percentage of the total methionine in wheat are slightly greater than variations in the cystine. Approximately 52 to 78 percent of the methionine in wheat is present in the flour. Very few flour samples contain less than 50 percent of the cystine or methionine of the wheat. The percentage of the wheat cystine and perhaps methionine is a relatively constant value. The greatest variations in cystine are in Series B. The mean and standard deviations are 62 ± 10 per cent. The greatest variation for methionine in flour as a percentage of that in wheat is in Series E. The mean and standard deviation are 80 ± 27 per cent.

The wheat after clover crop, 1947, shows large variations in loaf volume. Rigby (34) found a high correlation between sulphur in wheat and loaf volume. His loaf volumes had variations of 347 cc. and 207 cc. for 1941 and 1942 respectively. The range in the loaf volumes of the 1947 crop is 353 cc. The analysis of Series C wheat, 1947, did not confirm the work of Rigby. Further, the sulphur-containing amino acids of the flour and wheat were not significantly correlated with loaf volume. The significant correlations of the sulphur-containing amino acids in gluten with loaf volume as reported by Rigby (34) and Gerbrandt (19) were not obtained with the 1947 material.

The cystine, methionine and sulphur contents of the

Of the total cystine in wheat approximately 50 to 60 percent is found in the flour as milled on an experimental mill. The variations in the methionine content of flour as a percentage of the total methionine in wheat is slightly greater than variations in the cystine. Approximately 50 to 70 percent of the methionine in wheat is present in the flour. Very few flour samples contain less than 50 percent of the cystine or methionine of the wheat. The percentage of the wheat cystine and perhaps methionine is a relatively constant value. The greatest variations in cystine are in Series B. The mean and standard deviations are 62.1 in percent. The greatest variations for methionine in flour as a percentage of wheat is about 50 in Series B. The mean and standard deviation are 60.1 in percent.

The wheat after clover crop, 1947, shows large variations in loaf volume. Highy (34) found a high correlation between alcohol in wheat and loaf volume. His loaf volumes and variations of 547 cc., and 607 cc. for 1941 and 1942 respectively. The range in the loaf volumes of the 1947 crop is 505 cc. The analysis of Series B wheat, 1947, did not confirm the work of Highy. Further, the sulphur-containing amino acids of the flour and wheat were not significantly correlated with loaf volume. The significant correlations of the sulphur-containing amino acids in flour with loaf volume as reported by Highy (34) and others (19) were not obtained with the 1947 material. The cystine, methionine and sulphur contents of the

gluten washed from Series C flour were not significantly positively correlated with loaf volume. However a significant positive correlation was obtained with the cystine in the gluten of 100 grams of flour. It is obvious that this is not a specific relationship but is a measure of the total gluten in the flour. The gluten in the flour is highly correlated with loaf volume.

The correlation between methionine in gluten of 100 grams of flour and loaf volume was not calculated. Because of the highly significant correlation of the gluten content of the flour and loaf volume, and the smaller range in methionine contents of the gluten the correlation would be highly significant. Gerbrandt (19) suggested that the methionine in the gluten of 100 grams of flour was a better measure of loaf volume than the cystine content of the gluten. This would also be the situation in the 1947 flours. As the cystine contents of the glutens have a larger range than the methionine values, the combination of the cystine and gluten results gives a smaller correlation with loaf volume.

No differences were noted in the physical properties of the glutens investigated as measured by the amount of hydration. The sulphur-containing amino acids in these samples do not affect the hydration properties of the glutens.

The quantities of sulphur-containing amino acids in the gluten proteins are highly correlated with the amounts of these acids in the wheat. Fertilizer treatments that bring about changes in the cystine and methionine contents of wheat

alignments from Davis & Jones were not significantly positively correlated with local volume. However, a significant positive correlation was obtained with the system in one of the 100 groups of flows. It is obvious that this is not a specific relationship but is a measure of the total system in the flow. The system in the flow is highly correlated with local volume.

The correlation between local volume in groups of 100 groups of flows and local volume was not calculated. The highly significant correlation of the system content of the flows and local volume, and the smaller range in correlation between the system and the correlation with local volume, are highly significant.

Consequently (17) suggested that the correlation in the system of 100 groups of flows was a better measure of local volume than the system content of the flows. This would also be the situation

in the 100 flows. As the system content of the flows has a larger range than the correlation values, the correlation of the system and local volume gives a better correlation with local volume.

No differences were noted in the chemical properties of the systems investigated as measured by the amount of hydrogen. The nitrogen-containing amino acids in these systems do not affect the nitrogen properties in the system.

The correlation of nitrogen-containing amino acids in the system proteins and their correlation with the amount of amino acids in the system. Correlation between local volume and amino acids in the system and nitrogenous substances of amino

have similar effects on the gluten. Changes in the nitrogen content of wheat have no apparent affect on the sulphur-containing amino acids in the gluten proteins.

Cystine-sulphur accounts for 67.9 ± 6.8 percent of the total sulphur in the glutens of Series C. This value is similar to the sulphur accounted for as cystine in the whole wheat and the flour. The non-gluten proteins and mill by-product proteins must therefore have approximately the same percentage of their total sulphur represented as cystine-sulphur.

The cystine in the gluten of Series C is 63.6 ± 7.0 per cent of the cystine in the flour. If 65 percent of the wheat cystine is extracted in the flour a rough calculation shows that about 40 percent of the total cystine in the wheat is distributed in the crude gluten. The other 60 percent is distributed between the non-gluten proteins of the flour and the proteins of the mill by-products.

The first two water culture experiments suggest that either the critical period for sulphur uptake is very early in the life cycle of the wheat plant or that there was a large amount of sulphur present as an impurity which was utilized by the plants.

Eaton (16) has pointed out that a plant grown on a sulphur-deficient nutrient solution could obtain sulphur from a number of sources.

Following are listed sources of sulphur, some or all of which may have had some effect on the experimental results:

have similar effects on the plasma. Unless in one instance
consists of about half an equivalent of the amino-acids
the amino acids in the plasma.

Cysteine-methionine accounted for 0.75 to 0.85 percent of the
total nitrogen in the plasma of Series 2. This value is similar
to the nitrogen accounted for in cysteine in the amino acids and
the liver. The non-liver proteins and milk proteins
must therefore have approximately the same percentage of their
total nitrogen represented as cysteine-methionine.

The nitrogen in the plasma of Series 3 is 0.85 to 0.95
percent of the nitrogen in the liver. It is present in the
whole protein as measured in the liver & rough calculated
shows that about 10 percent of the total nitrogen in the meat
is represented in the whole liver. The other 90 percent is
distributed between the non-liver proteins of the liver and
the proteins of the milk & products.

The first two water-soluble experiments suggest that
certain the critical point for amino acids is very early in
the life cycle of the whole plant or that there was a large
amount of nitrogen present as an inorganic source was utilized by
the plant.

Legend (15) has pointed out that a plant grown on a
sulphur-deficient medium could obtain nitrogen from
a number of sources.

Following are listed sources of sulphur, some of which
of which may have had some effect on the experimental results:

- (1) sulphur in the air.
- (2) sulphur as an impurity in the nutrient salts.
- (3) sulphur in the seed.
- (4) sulphur from the fumigants used to destroy mildew and aphids in the greenhouse.
- (5) sulphates present in the distilled water.

It is unlikely that a single source or even all of the sources could supply enough nutrient sulphur to raise the sulphur and sulphur-containing amino acid contents of the wheat to the high levels observed.

Eaton (16) found that soybean and sunflower seed, if grown on complete and sulphur-deficient sand cultures, did not differ in total sulphur content. The yield of the sulphur deficient cultures was much lower than the yield from the complete culture. Eaton attributes the lack of difference to the large sources of sulphur found in the air. He concludes that sulphur from the seed is not of great importance.

The cystine contents of experiment I were almost twice those of experiment II. The nutrient solutions were made up alike. The nitrogen contents of the wheat from experiment I are much higher than those of experiment II. The difference in cystine content of the wheat from the two experiments is probably a result of a quantitative effect of the protein in the wheat.

Variations in the relative sulphur and nitrogen contents of the nutrient solutions in experiments III and IV do not give

the expected differences. The yields from the different treatments apparently have some effect on the cystine, sulphur, nitrogen and methionine contents of the wheat.

Experiments III and IV show that similar relationships exist between sulphur, cystine, methionine and nitrogen at much higher levels than those observed for the Breton material.

The "differential-oxidation procedure" for the determination of cystine and methionine in wheat and flour has provided some new information. The 1947 continuous wheat series should be investigated to see if the methionine-nitrogen and methionine-sulphur relationships observed in 1946 are valid. As there are large amounts of sulphur-containing amino acids in the non-gluten flour proteins further investigations should be carried out on these proteins.

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the expected differences. The results from the different experiments
apparently have some effect on the reaction, especially
nitrogen and methionine contents of the wheat.
Experiments III and IV show that similar relationships
exist between nitrogen, lysine, methionine and nitrogen as well
higher levels than those observed for the wheat material.
The "allosteric-control hypothesis" for the regulation
of synthesis of lysine and methionine in wheat and flour has
provided some new information. The 1947 experiments wheat samples
should be investigated to see if the methionine-lysine and
methionine-lysine relationships observed in 1946 are valid.
As there are large amounts of nitrogen-containing amino acids in
the non-lysine flour proteins further investigations should be
carried out on these proteins.

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