DISTRIBUTION OF SULPHUR IN WHEAT

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TABLE OF CONTENTS

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INTRODUCTION

A marked improvement in the baking strength of flour from wheat grown in the grey wooded soils area has been observed following the application of a sulphur fertilizer. At Breton, where the soil is deficient in sulphur, differences in baking strength were caused by differences in protein quality. Sulphur content of Breton-grown wheat has been shown to be a better measure of quality than is crude protein.

This observed improvement in baking strength following the application of sulphur fertilizer at Breton was investigated by Rigby (17). He found a significant relationship between loaf volume and the sulphur in wheat, flour, and gluten. The crude protein was not an adequate indicator of baking strength and, in two of the three years, there was no relationship between the crude protein and the sulphur in wheat.

To study these relationships more closely the following investigations were planned:

A study of the uptake of sulphur by the wheat plant when increasing amounts were made available;

A further study of varietal differences in sulphur content of wheat;

A study of the cystine and methionine content of gluten, the relation of these acids to baking quality, and to the total sulphur and nitrogen content of wheat.

LITERATURE REVIEW

Increases in baking strength of flour from wheats following the application of a sulphur-bearing fertilizer as compared with those receiving no sulphur were demonstrated by Wyatt, Newton, and Ignatieff (28).

Peters (16), working with the same material, demonstrated that the application of sulphur-bearing fertilizers to wheat resulted in a higher sulphur content in the wheat kernel.

Rigby (17), also working with Breton material, has shown that the sulphur in wheat is a factor influencing the baking strength.

Harrow (11) lists four sulphur-containing amino acids, namely methionine, cystine, lanthionine, and djenkolic acid. He classifies cysteine as a reduced form of cystine, of importance in biological reactions because cystine and cysteine form an oxidation-reduction system.

The sulphur-containing amino acids have received

considerable attention in recent years. After Shohl (19) suggested that cystine in feed of sheep is a limiting factor in the production of wool, the cystine content of several grasses in Australia was determined.

Smith and Wang (20) have determined the cystine and methionine content of the leaves of four of the main fodder plants in Australia: Trifolium repens, Dactylis glomerata, Lolium perenne, and Phleum pratense.

Wood and Barrien (27) and Barrien and Wood (4) studied the sulphur uptake and cystine content of Sudan grass. Though the leaf and seed proteins differ considerably, their work is significant in relation to the studies reported in this paper. This was the first time that sulphur was considered as a factor in determining the quality of a plant as an animal food. The following results were obtained: an increased supply of sulphate did not increase the content of cystine or of protein sulphur. An increase in NH_3 increased the content of cystine and protein sulphur as well as the amino and protein nitrogen, but decreased the sulphate sulphur content. The authors state that NH_3 acts as a limiting factor in the formation of protein sulphur from sulphate.

Rigby (17) determined sulphur content of wheat receiving different fertilizer treatments and found no correlation between sulphur deposition and nitrogen deposition.

Thomas et al (24) were the first to report in any

detail the distribution of sulphur in the wheat plant. They supplied radioactive sulphur in the nutrient solution as $Na_2S^*O_4$, and by fumigation with S^*O_2 in an air-tight greenhouse. The radioautographs showed the radioactive sulphur to be distributed about equally among the different portions of the wheat kernel. There was little if any difference in the sulphur distribution resulting from treatment with S^*O_2 as compared with $Na_2S^*O_4$. The embryo and the bran, however, represent only about 15 per cent of the mass of the kernel, so that most of the sulphur was in the endosperm.

These workers determined the total sulphur content of the wheat kernel and the sulphur distribution as: labile or sulphide sulphur, acid soluble organic sulphur, acid insoluble organic sulphur, and inorganic sulphur. The labile or cystine sulphur was determined by alkaline hydrolysis. The acid soluble fraction contained the methionine.

Thomas and Hendricks (23) demonstrated that cystine was the principal source of labile sulphur. When using alkaline hydrolysis, 66 to 78% of the cystine sulphur was evolved as hydrogen sulphide, 6 to 19% as sulphur dioxide, and 4 to 9% was oxidized to sulphate. It is therefore practically impossible to determine from the data of Thomas, Hendricks, Collier, and Hill (24) the cystine and methionine contents of the wheat kernel. Further, no attempt was made to determine the relationship between any of the sulphur fractions and the quality of the wheat.

Greaves and Bracken (9) found no inorganic sulphur in the wheat they tested. They found a highly significant correlation between the total sulphur and total nitrogen of the wheat. The range of sulphur content was 0.15% to 0.22% and the correlation coefficient between sulphur and nitrogen was .81.

Gubler and Greaves (10) determined the cystine content of spring and winter wheats of Utah. In spring wheats the range was from .346 to .405 per cent, with an average of .372; and for winter wheats the range was from .338 to .385 per cent, with an average of .361. In general, variations in cystine content were associated with variations in sulphur and nitrogen content. The percentage difference between the lowest and highest sulphur value was 46%; between cystine values, 20%; and between nitrogen values, 31%. The average cystine sulphur content was 54.4% of the total sulphur, the range being from 47.1 to 66%. It was also observed that, in general, as the total sulphur and cystine content increasedthere was a decrease in the percentage of the total sulphur represented by cystine. This was also observed by Wood (26) in his study of leaf proteins. The correlation coefficient for cystine with total sulphur was .83; and for cystine with nitrogen, .75.

Csonka (5) determined the cystine content of Marquis, Tenmarq, and Fulhio wheats as .27, .17, and .15, respectively. The corresponding nitrogen contents were 3.31, 2.58, and 2.34%. The cystine to nitrogen ratios were 81, 65, and 67 mgm.

cystine per gm. of nitrogen.

Rigby (17) studied the effect of sulphur on the quality of wheat. The material he used was grown at Breton. The correlation coefficients were as follows:

Statistic	1941	1942
r_{vp}	.116	.553*
r_{vw}	.808**	.548*
r_{vg}	.742**	.027
r_{pw}	.337	.086
r_{cv}		.557*
r_{cg}		.640*
r_{cw}		.749**
$r_{vw.p}$	.906**	.602*
$R_{v.wp}$	.907**	.747**

v - loaf volume, cc.
p - protein, %
g - sulphur in gluten, %
c - cystine, %
w - sulphur in wheat, %
* - significant beyond 5% point
** - significant beyond 1% point.

In this material Rigby found that protein content was not an adequate indicator of baking strength. The sulphur content of the wheat was positively and significantly correlated with loaf volume. The gluten contained most of the sulphur in wheat and larger amounts of sulphur were present in glutens from strong flours. Sulphur appeared to modify the physical properties of the gluten by increasing its elastic, cohesive, and extensible characteristics. The cystine content of the gluten was closely related to loaf volume, to sulphur in gluten, and especially to sulphur in

wheat. The sulphur-nitrogen ratio in the wheat was not constant.

Wood (26) states that cystine and methionine are the only sulphur-containing amino acids in the protein molecule. This is supported by the experimental results of Baernstein (1).

Cysteine is highly reactive because the hydrogen of its sulphydryl group (SH) is readily surrendered to oxidizing agents. In recent years the S-H compounds have received considerable attention. Sulphydryl groups may be present in proteins in at least two forms. A sulphydryl group is said to be free or reactive when the native protein gives a positive reaction to nitroprusside test and is bound or non-reactive when the protein must be denatured to liberate the S-H group before a positive reaction can be obtained. Meyer and Working (15) say that there is no conclusive evidence of the presence of either reactive or non-reactive sulphydryl groups in the proteins of wheat flour, and also that gluten gave negative reactions before and after denaturation for both free and non-reactive sulphydryl groups.

Glutathione is a polypeptide of glutamic acid, glycine, and cysteine. Thus it too contains a sulphydryl group. Glutathione in wheat occurs chiefly in the germ. Sullivan, Howe, and Schmalz (22) say that no more than traces are found in commercial patent flours.

Baker, Parker, and Mize (3) suggest that the amount of glutathione present in flour depends on the amount of germ

milled into the flour. They found that water extracts of different flours contained varying amounts of S-H per unit of protein.

There are no references in the literature reviewed reporting the presence of lanthionine or djenkolic acid in wheat or its products. Lanthionine has been isolated from wool and djenkolic acid from the djenkol bean.

MATERIAL

Series I - Wheat Quality Test

In 1944 field plot experiments were set up at Fallis, Sundre, and Warburg. Fertilizers were applied to a series of seven plots replicated three times. In each series the application of ammonium sulphate was increased progressively from zero. The nitrogen application was kept constant by applying correspondingly diminishing amounts of ammonium nitrate. In 1945 the experiment was repeated at Fallis and Warburg. Red Bobs wheat was used throughout. Table I gives the rates of application for the two fertilizers used.

TABLE I

Rates of fertilizer applied in the wheat quality tests

Treatment	Fertilizer applied in grams per 18-ft. row	
	NH_4NO_3	$(NH_4)_2SO_4$
1	0	0
2	8.2	0
3	7.8	0.7
4	7.3	1.4
5	6.2	3.6
6	4.0	7.2
7	0.0	14.4

Series II - Plant Breeders' Varieties Test, 1944

Marquis, Thatcher, Red Bobs, a hybrid C.T.149 (Regent x Thatcher), and Mida (C.T.807) were grown from seed supplied by the Rust Research Laboratory, Winnipeg. These varieties were part of the Plant Breeders' Varieties test in 1944.

The five varieties were grown in replicated rod-row plots at Athabaska, Beaverlodge, Bon Accord, Fallis, Sundre, Warburg, and at Edmonton on fallow and stubble. Composites of varieties except Red Bobs were also received from the Rust Research Laboratory, Winnipeg.

Series III - Selected Varieties, 1945

Marquis, Thatcher, and Red Bobs were grown at Athabaska, Bon Accord, Edmonton, Fallis, and Warburg. These varieties were part of the U. C. C. hard spring wheat test.

METHODS

Nitrogen and Total Protein

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, with mercuric oxide as catalyst, except where the micro-Kjeldahl method is specified. In the latter method ammonia was distilled into a 2% boric acid solution. The ammonia was titrated with N/100 HCl, using a mixed indicator of methyl red and methylene blue (18, 21). The protein was calculated as nitrogen x 5.7, and results were reported on a 13.5% moisture basis.

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.

Sulphur

The A.O.A.C. (14) method, using nickel crucibles, open fusion with sodium-carbonate and sodium-peroxide as modified by Rigby (17) was used for sulphur determination.

Statistical Analysis

All statistical analyses carried out in this study follow the methods described by Goulden (7).

Gluten Washing

The solution suggested by Dill and Alsberg (6) was used, namely, 4% mono-phosphate adjusted to pH 6.8 by the addition of sodium di-phosphate. Using this as a stock solution, further dilution was made in the proportion of one part stock solution to forty parts of unboiled distilled water. Washing time was standardized at ten minutes for a 30-gram sample.

Weights of the dry gluten were obtained after drying in vacuo at 98° C. for 24 hours.

Methionine and Cystine in Gluten

Duplicate samples of about 4 grams each of gluten

were hydrolyzed for 20 hours with 6 N HCl. These hydrolyzates were concentrated to near dryness three times in vacuo with additions of about 10 ml. of distilled water between concentrations. The humin was filtered from each hydrolyzate and washed with boiling water. The filtrates were made up to 100 ml. Fifty ml. were used for the methionine determination. The balance was made up to 100 ml. for the cystine determination. Determinations were made on duplicate hydrolyzates. Results for cystine and methionine were calculated as mgm. per gm. of nitrogen and as mgm. in gluten representing 100 gm. of flour.

Cystine

A combination of the methods of Graff, Maculla, and Graff (8) and Zittle and O'dell (29) was found to be satisfactory. Zittle and O'dell (29), working with bull spermatozoa, found that when they determined the nitrogen content of the cuprous mercaptide precipitate they obtained high results for the cystine content. They attributed the high nitrogen values to the presence of purines. They therefore determined the sulphur content of the cuprous mercaptide precipitate with a Parr bomb. Bailey (2) gives the purine content of wheat flour as 0.015%. It was therefore decided to test this method on gluten. The results obtained indicate that nitrogen determinations on the cuprous mercaptide precipitates were satisfactory.

The procedure for the determination of cystine was as follows:

Triplicate 25-ml. samples were pipetted into conical tipped centrifuge tubes. The samples were heated to boiling over wire gauze, excess cuprous oxide (prepared as suggested by Zittle and O'dell (29)) was added dropwise. One minute after the addition of the last of the cuprous oxide, 0.5 ml. of saturated sodium acetate solution was added. During the heating and addition of the cuprous oxide and the sodium acetate the solutions were stirred mechanically. After forty minutes the samples were centrifuged at 2500 r.p.m. for 10 minutes. The cuprous mercaptide precipitates were washed four times with dilute citrate acetate buffer with stirring and centrifuging before each decantation.

The nitrogen content of the precipitates was then determined by micro-kjeldahl as outlined by Graff, Maculla and Graff (8).

To test this procedure determinations were carried out on pure solutions of cystine, on samples of gluten, and on samples of gluten to which cystine had been added. The following results were obtained:

1. Determination of cystine in solution by determining the nitrogen content on aliquots containing 10 mgm. of cystine by weight:

Aliquot	Cystine found, mgm.
1	9.51
2	9.49
Mean	9.50

2. Determination of cystine in pure solution by cuprous oxide precipitation on aliquots containing 10 mgm. of cystine by weight:

Aliquot	Cystine found, mgm.
1	9.44
2	9.40
Mean	9.42

$$\% \text{ cystine recovered} = \frac{9.42}{9.50} \times 100 = 99.2\%$$

3. To test whether cystine can be recovered quantitatively when added to gluten, the following experiment was conducted. All determinations were done in triplicate.

Gluten, gm.	Cystine added, mgm.	Cystine found, mgm.	Cystine, %
.956	nil	20.38	2.13
.980	20	40.90	

40.90 - 20.87 = 20.03 mgm.

Per cent recovery of added cystine was 100.2

It therefore appears that the purines, if present in gluten, do not interfere with the determination of cystine by nitrogen analysis on the cuprous mercaptide precipitates.

Methionine

The literature was reviewed with the object of finding a method that could be carried out with the available equipment. The method reported by Lavine (12), based on the

reversible reaction of methionine with iodine was tested. The procedure for casein as reported by Lavine (12) was used with the following modifications: carboraffin was not available so activated charcoal was used instead; the normality of the sodium thiosulphate used was about .01 N.

To test this procedure, determinations for methionine were carried out on pure solutions of methionine, on samples of gluten and on gluten to which methionine had been added.

Lavine (12) states that iodine should be present in excess between 25 to 50%. This was tested and it was found that for best results it should be present in excess between 35 and 50%.

1. Determination of methionine in pure solution by determining the nitrogen content. 20.6 mgm. of methionine were made up to 25 ml.; theoretically each 10 cc. aliquot contained 8.24 mgm. methionine.

Aliquot	Methionine found, mgm.
1	7.96
2	7.98
Mean	7.97

$\frac{7.97}{8.24}$ = 96.8% pure.

2. Experiment to determine the excess iodine necessary for the periodide titration:

Excess iodine, %	Methionine, as % of theoretical
27	92.3
31	95.3
35	99.6
41	100.1
46	100.7
48	101.0
54	101.8

3. Recovery of methionine when added to gluten before hydrolysis. Gluten was washed from three flours: (a) Robin Hood family patent; (b) Marquis; and (c) Thatcher. Both Marquis and Thatcher were grown in the experimental plots at Edmonton. Hydrolysis time was 15 hours for gluten from the Robin Hood family flour. For the glutens from both Marquis and Thatcher flours the hydrolysis time was 20 hours.

TABLE II

Recovery of methionine

Gluten source	Gluten, gm.	Methionine Added, mgm.	Found mgm.	Found %	Recovered, mgm.	Recovery, %
Robin Hood						
A	2.294	nil	25.4	1.150		
B	1.854	18.4	39.2		17.9	97
Marquis						
A	4.027	nil	36.8	.915		
B	4.130	nil	38.1	.923		
			Mean	.919		
C	1.958	17.6	35.2		17.2	97
Thatcher						
A	4.015	nil	34.9	.869		
B	3.993	nil	34.7	.869		
C	1.682	17.7	31.6		17.0	96
D	1.496	24.1	36.6		23.6	98
					Mean recovery	97%

RESULTS

Series I - Wheat Quality Test

The first experiments were carried out on the Quality Test material for 1944. Protein and sulphur content

were determined on the whole wheat. All samples were baked using the malt-phosphate-bromate formula. The results are presented in Table III.

Similar determinations were carried out on the 1945 Quality Test and the results are shown included in Table III. The plots at Sundre were snowed under and the test was therefore not harvested.

The protein content was not affected by fertilizer treatment. The 1944 samples all had uniformly low baking strength, the range in loaf volume for any one station being very small. Nearly all differences from the mean are within the experimental error. There are therefore no quality differences evident. Only at Warburg in 1945 is there any indication of a trend in sulphur content resulting from increased sulphur application. The samples that received the larger amounts of sulphur fertilizer have higher loaf volumes.

TABLE III

Wheat quality test results

Treatment	1944 Protein, %	1944 Sulphur, %	1944 Loaf volume, cc.	1945 Protein, %	1945 Sulphur, %	1945 Loaf volume, cc.
Fallis						
1	9.8	0.142	550	13.6	0.176	660
2	9.7	0.141	525	14.1	0.174	693
3	9.6	0.130	510	13.7	0.167	688
4	9.5	0.148	495	13.3	0.161	645
5	9.4	0.143	495	13.7	0.176	755
6	9.4	0.142	500	13.7	0.177	625
7	9.4	0.149	470	14.0	0.179	693
Mean	9.5	0.142	506	13.7	0.173	680
Warburg						
1	9.9	0.124	475	15.3	0.145	635
2	10.4	0.121	460	14.8	0.142	635
3	9.6	0.106	470	15.0	0.154	673
4	9.5	0.121	460	15.4	0.145	655
5	10.0	0.126	480	15.7	0.174	635
6	9.9	0.128	480	15.9	0.196	788
7	9.9	0.128	450	15.5	0.191	823
Mean	9.9	0.122	468	15.4	0.164	692
Sundre						
1	7.8	0.120	450			
2	8.4	0.123	428			
3	8.4	0.126	480			
4	8.2	0.125	455			
5	8.1	0.122	488			
6	8.2	0.122	480			
7	8.4	0.124	485			
Mean	8.2	0.123	469			

Series II - Plant Breeders' Varieties Test, 1944

The protein and loaf volume data for the varieties Marquis, Thatcher, Red Bobs, Mida, and C.T.149, are plotted in Figure 1. The correlation coefficients are given in Table IX.

For Marquis, Thatcher, and Red Bobs the correlations for protein with loaf volume are highly significant, while for Mida and C.T.149 they are just significant. In a previous study, an analysis of variance was carried out on combined data, which also included Newthatch and C.T.141, to test the linearity of the regression. The "F" value was significant beyond the 5% point, indicating a non-linear regression.

The test for heterogeneity of regression coefficients for protein on loaf volume indicated that there were no significant differences between the regression coefficients.

The analyses of variance on protein and loaf volume are given in Tables IV and V, respectively. Only Mida is significantly higher, while only Red Bobs is significantly lower than Marquis in protein content. All varieties are significantly lower than Marquis in loaf volume.

The above results indicate that Thatcher behaved abnormally in 1944. That is, it produced a loaf volume lower than expected for its protein content. The regression coefficient is not significantly higher than for Marquis

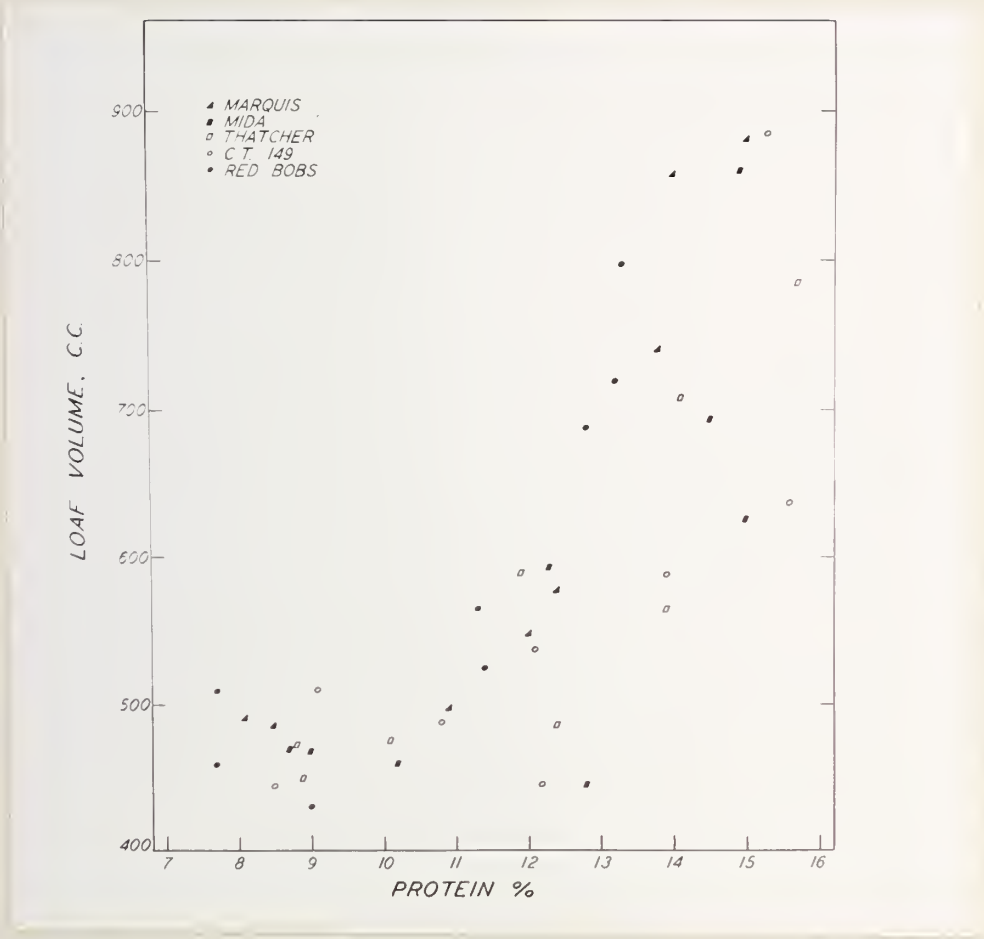

Figure 1

Scatter diagram showing the relation between protein and loaf volume, Series II

TABLE IV

Protein content of wheat, %

Station	Marquis	Thatcher	Red Bobs	Mida	C.T.149	Mean
Athabaska	8.5	8.9	7.7	9.0	8.5	8.5
Beaverlodge	15.0	15.7	13.3	14.9	15.3	14.8
Bon Accord	12.4	11.9	11.3	12.3	12.1	12.0
Edmonton (F)	13.8	14.1	12.8	14.5	14.6	14.0
Edmonton (S)	14.0	13.9	13.2	15.0	13.9	14.0
Fallis	10.5	10.1	9.0	10.2	10.8	10.1
Sundre	8.1	8.8	7.7	8.7	9.1	8.5
Warburg	12.0	12.4	11.4	12.8	12.2	12.2
Mean	11.8	12.0	10.8	12.2	12.1	

Analysis of variance

Variance due to	D.F.	Mean square	F	1% point
Variety	4	2.467	21.08	4.07
Station	7	31.077	265.62	3.36
Error	28	0.117		
Total	39			

Minimum significant difference for variety = 0.3%
Only Mida and C.T.149 are significantly higher than Marquis
Only Red Bobs is significantly lower than Marquis

Minimum significant difference for station = 0.4%
Only Beaverlodge is significantly higher than Edmonton
All other stations are significantly lower than Edmonton.

TABLE V

Loaf volume of wheat, cc.

Station	Marquis	Thatcher	Red Bobs	Mida	C.T.149	Mean
Athabaska	485	450	457	468	445	461
Beaverlodge	882	785	798	860	885	842
Bon Accord	578	590	565	593	538	573
Edmonton (F)	740	708	688	693	637	693
Edmonton (S)	857	615	720	625	589	681
Fallis	497	475	430	460	487	470
Sundre	490	472	510	470	510	490
Warburg	548	485	525	445	445	489
Mean	635	572	587	577	567	

Analysis of variance

Variance due to	D.F.	Mean square	F	5% point	1% point
Variety	4	5965	3.03	2.71	4.07
Station	7	95576	48.56	2.36	3.36
Error	28	1968			
Total	39				

Minimum significant difference for variety = 44 cc.
All varieties are significantly lower than Marquis.

Minimum significant difference for station= 57 cc.
Only Beaverlodge is significantly higher than Edmonton (F)
Athabaska, Bon Accord, Fallis, Sundre, and Warburg are significantly lower than Edmonton (F).

and the whole regression line is below the line for Marquis. In a six-year survey of Alberta-grown wheats, McCalla (13) found Thatcher to be consistently higher in protein and loaf volume than Marquis; also its regression coefficient was significantly higher. Since the whole regression line lay above the line for Marquis, the steeper slope did not indicate poorer quality at any protein level.

Sulphur determinations were made on the whole wheat. The individual values for sulphur content in the 1944 material are presented in Table VI.

A simple analysis of variance was carried out on the sulphur content of the five varieties (Table VI). The results show Marquis to be significantly higher than all other varieties and Red Bobs to be significantly lower. The largest differences were between stations. Edmonton after fallow and after stubble, and Beaverlodge, did not differ significantly from each other. All other stations are significantly lower in sulphur content than Edmonton after fallow.

Thatcher has a higher protein content and a significantly lower loaf volume and sulphur content than Marquis. Mida and C.T.149 have significantly higher protein content but significantly lower loaf volume and sulphur content than Marquis. Red Bobs is significantly lower in protein, loaf volume, and sulphur content, than Marquis.

TABLE VI

Sulphur in wheat, %

Station	Marquis	Thatcher	Red Bobs	Mida	C.T.149	Mean
Athabaska	.158	.150	.131	.146	.133	.144
Beaverlodge	.206	.188	.181	.180	.180	.187
Bon Accord	.170	.168	.150	.162	.166	.163
Edmonton (F)	.207	.186	.162	.189	.184	.186
Edmonton (S)	.215	.190	.177	.199	.191	.194
Fallis	.167	.151	.132	.142	.146	.148
Sundre	.152	.137	.111	.129	.141	.134
Warburg	.182	.161	.143	.142	.148	.155
Mean	.182	.166	.148	.161	.161	

Analysis of variance

Variance due to	D.F.	Mean square	F	1% point
Variety	4	.001200	24.49	4.07
Station	7	.002569	52.35	3.36
Error	28	.000049		
Total	39			

Minimum significant difference for variety = 0.007%
Marquis is significantly higher than all other varieties
Red Bobs is significantly lower than all other varieties.

Minimum significant difference for station = 0.009%
Athabaska, Bon Accord, Fallis, Sundre, and Warburg are significantly lower than Edmonton (F), (S), and Beaverlodge.

The regression lines for loaf volume on sulphur content are plotted in Figure 2. There are no significant differences between the regression coefficients for the five varieties as is shown by the results of an analysis of variance given in Table VII.

TABLE VII

Test of homogeneity of varietal regression coefficients by analysis of residual variance

Variance due to	D.F.	Mean square	F	5% point
Differences among varietal regression coefficients	38			
Deviations from individual varietal regressions	34	9359		
Difference	4	5442	0.58	2.62

There is a close relationship between sulphur and protein content for all varieties. The partial coefficient $r_{vs.p}$ for all varieties is less than the simple correlation coefficient r_{vs}, indicating that the elimination of the effect of protein causes a decrease in the apparent effect of sulphur on loaf volume. The partial coefficient $r_{vp.s}$ is less than the simple correlation coefficient r_{vp} indicating that the elimination of the effect of sulphur caused a decrease in the apparent effect of protein on loaf volume. The effects of protein and sulphur content on loaf volume are apparently related. This is to be expected when the correlation of

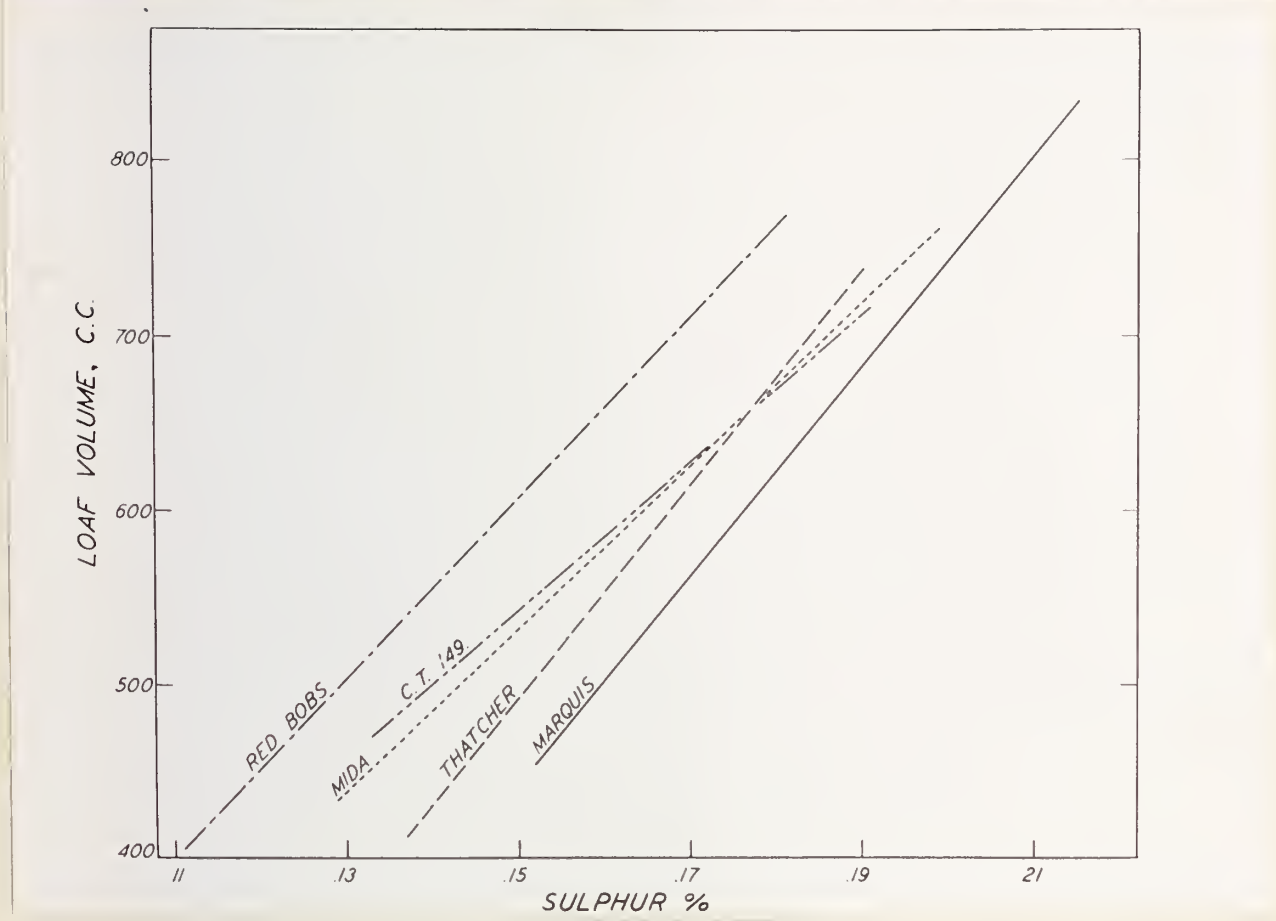

Figure 2

Relation between loaf volume and sulphur in wheat, Series II

protein with sulphur content is highly significant for all varieties.

The multiple correlation coefficient $R_{v.ps}$ for each of Marquis, Thatcher, and Red Bobs, was highly significant. This multiple coefficient was not, however, significantly higher than either the simple r_{vs} or r_{vp}. This indicates that, for these samples, that part of the variability in loaf volume accounted for by variability in sulphur content is closely associated with the variability in protein. In other words, the protein and sulphur measured the same variability in different ways.

The simple correlations with loaf volume for Mida and C.T.149 are all just significant, while the multiple correlations are not significant. Mida is the only variety having the relationship between loaf volume and sulphur in wheat lower than the relationship between loaf volume and protein.

The sulphur nitrogen ratios were calculated and found to vary considerably. The data are presented in Table VIII. In Figure 3, sulphur in wheat is plotted against protein content. For a given amount of protein Marquis has more sulphur than any other variety. Thatcher ranks second in this respect, while there is little if any difference among Red Bobs, Mida, and C.T.149.

TABLE VIII

Sulphur-nitrogen ratios - 1944

Station	Marquis	Thatcher	Red Bobs	Mida	C.T.149
Athabaska	.0913	.0833	.0834	.0798	.0773
Beaverlodge	.0675	.0589	.0670	.0596	.0582
Bon Accord	.0677	.0697	.0652	.0648	.0675
Edmonton (F)	.0739	.0650	.0625	.0643	.0622
Edmonton (S)	.0760	.0676	.0660	.0655	.0677
Fallis	.0784	.0740	.0721	.0686	.0664
Sundre	.0921	.0765	.0707	.0728	.0767
Warburg	.0749	.0639	.0616	.0548	.0600
Composite	.0775	.0672	--	.0664	.0594

TABLE IX

Correlation coefficients

Statistic	Marquis	Thatcher	Red Bobs	Mida	C.T.149
r_{vp}	.888**	.826**	.868**	.767*	.776*
r_{vs}	.914**	.835**	.915**	.790*	.672*
r_{ps}	.894**	.943**	.962**	.881**	.899**
$r_{vs.p}$	.472	.298	.588	.376	-.008
$r_{vp.s}$	.396	.213	-.109	.024	.531
$R_{v.ps}$	.927**	.843*	.999**	.790	.778

v = loaf volume, cc.
p = protein, %
s = sulphur in wheat, %
* - significant beyond 5% point
** - significant beyond 1% point

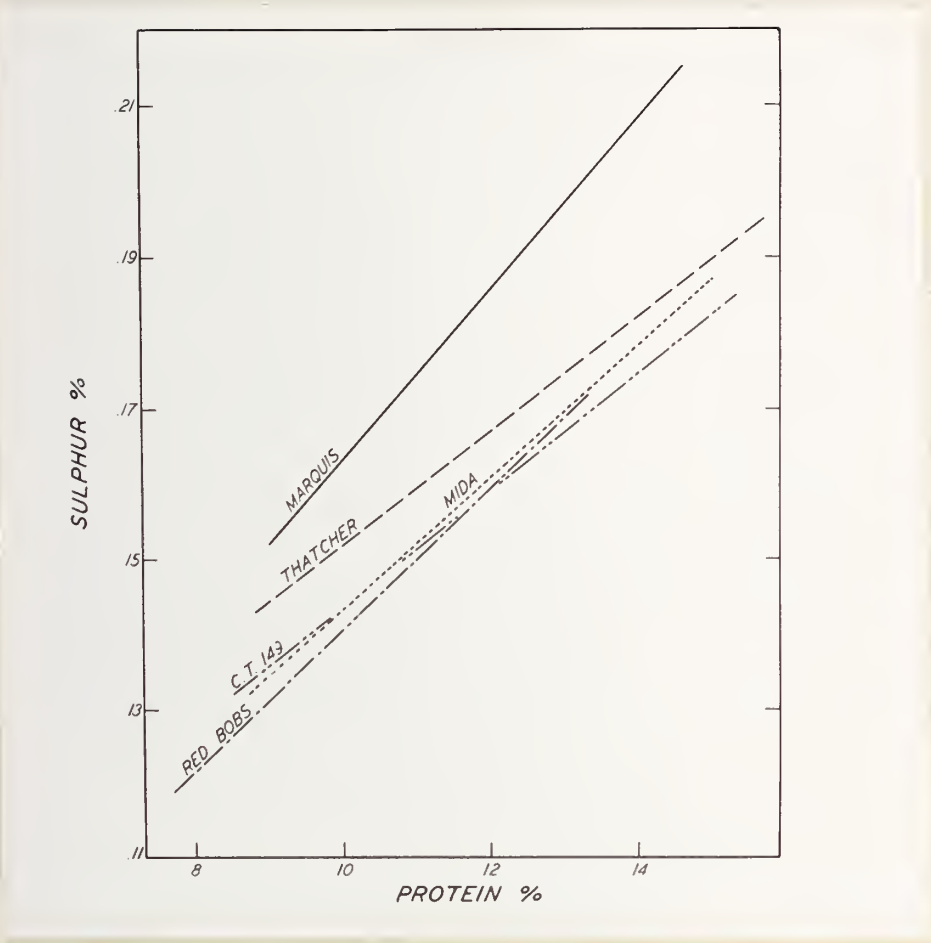

Figure 3

Relation between protein and sulphur in wheat, Series II

Methionine and cystine were determined on samples of crude gluten prepared from weighed samples of each flour. From the values obtained yields for 100 grams of flour were then calculated. These values permit correlation with quality, since loaf volumes were determined on 100 grams of flour.

Gluten was used for two reasons: First, Rigby (17) found that a highly significant correlation existed between sulphur content of gluten and loaf volume and that the sulphur was concentrated in the gluten. Second, during hydrolysis less humin is formed than when flour or whole wheat is hydrolyzed. In these determinations the hydrolyzates contained an average of 1% humin nitrogen. In the determination of methionine and cystine, checks between duplicate hydrolyzates were accepted when the duplicate values in mgm. of methionine or cystine per gram of nitrogen agreed within 5%. The nitrogen in the hydrolyzate was determined by the micro-kjeldahl method, using mercuric oxide as catalyst and three-hour digestion. The humin nitrogen was determined by the macro method. The complete analysis for cystine and methionine required about one week. The cystine and methionine were first determined in Thatcher. In this first series of determinations the standard error between duplicates for cystine was 2.3% and for methionine 1.6%. In the second series the results on Marquis were a little better, mainly because of more experience with the methods. The standard error between duplicates was: for cystine, 0.5%; and for methionine, 1.0%.

The cystine and methionine values for Marquis and Thatcher in 1944, along with loaf volume, protein, and sulphur in wheat, are presented in Table X.

There is not much difference between Marquis and Thatcher in cystine content. The methionine content is consistently and significantly lower in Thatcher than in Marquis. The range for cystine in Marquis is from 211 to 362 mgm., with a mean of 296 mgm. in gluten, representing 100 gm. of flour. In Thatcher the range is from 185 to 378 mgm. with a mean of 276 mgm. For methionine the ranges are: Marquis, 126 to 206 mgm., mean 165 mgm; Thatcher, 102 to 156 mgm., mean 125 mgm. All values are in mgm, representing gluten from 100 gm. of flour.

The cystine data for Marquis and Thatcher are plotted against loaf volume in Figure 4. The methionine data for both varieties are plotted against loaf volume in Figure 5. The calculated regression lines are plotted in all cases.

The correlation coefficients for cystine and methionine with loaf volume, protein and sulphur in wheat are presented in Table XI. All the simple and multiple coefficients are highly significant.

The partial coefficients, $r_{vc.p}$ and $r_{vm.p}$ for Thatcher are significant. It appears that the measurement of cystine, independent of protein, accounts for more of the variability in loaf volume than does the measurement of crude protein; also the measurement $r_{vm.p}$ takes into account nearly

TABLE X

Data - 1944

	Loaf volume, cc.	Protein in wheat, %	Sulphur in wheat, %	Cystine in gluten,* mgm.	Methionine in gluten,* mgm.
Athabaska					
Marquis	485	8.5	.158	211	128
Thatcher	450	8.9	.150	224	102
Beaverlodge					
Marquis	882	15.0	.206	362	206
Thatcher	785	15.7	.188	378	155
Bon Accord					
Marquis	578	12.4	.170	276	164
Thatcher	590	11.9	.168	265	125
Edmonton (after fallow)					
Marquis	740	13.8	.207	337	177
Thatcher	708	14.1	.186	302	144
Edmonton (after stubble)					
Marquis	857	14.0	.215	337	192
Thatcher	615	13.9	.190	290	133
Fallis					
Marquis	497	10.5	.167	215	126
Thatcher	475	10.1	.151	235	103
Sundre					
Marquis	490	8.1	.152	-	-
Thatcher	472	8.8	.137	185	104
Warburg					
Marquis	548	12.0	.182	279	154
Thatcher	485	12.4	.161	244	106
Composite					
Marquis	750	12.9	.203	348	187
Thatcher	827	13.4	.182	357	156

* From 100 gm. of flour.

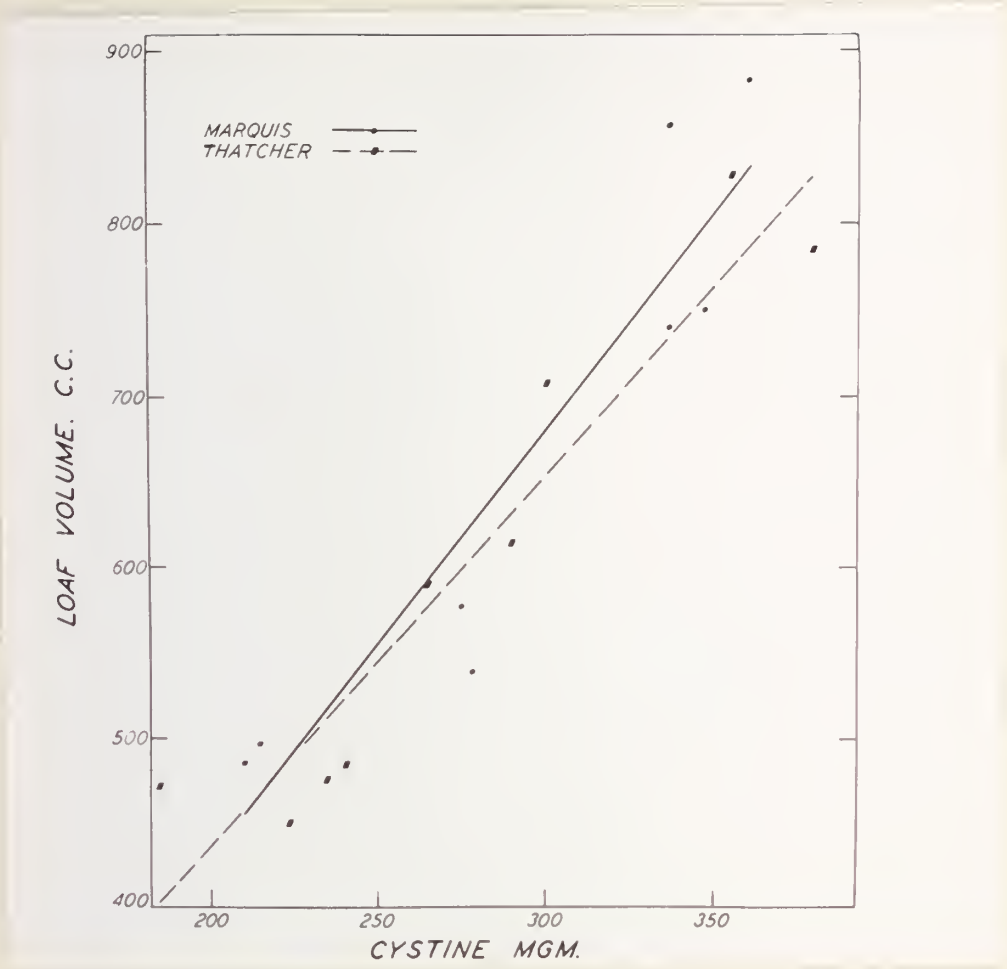

Figure 4

Relation between cystine in gluten per 100 grams of flour and loaf volume, Series II

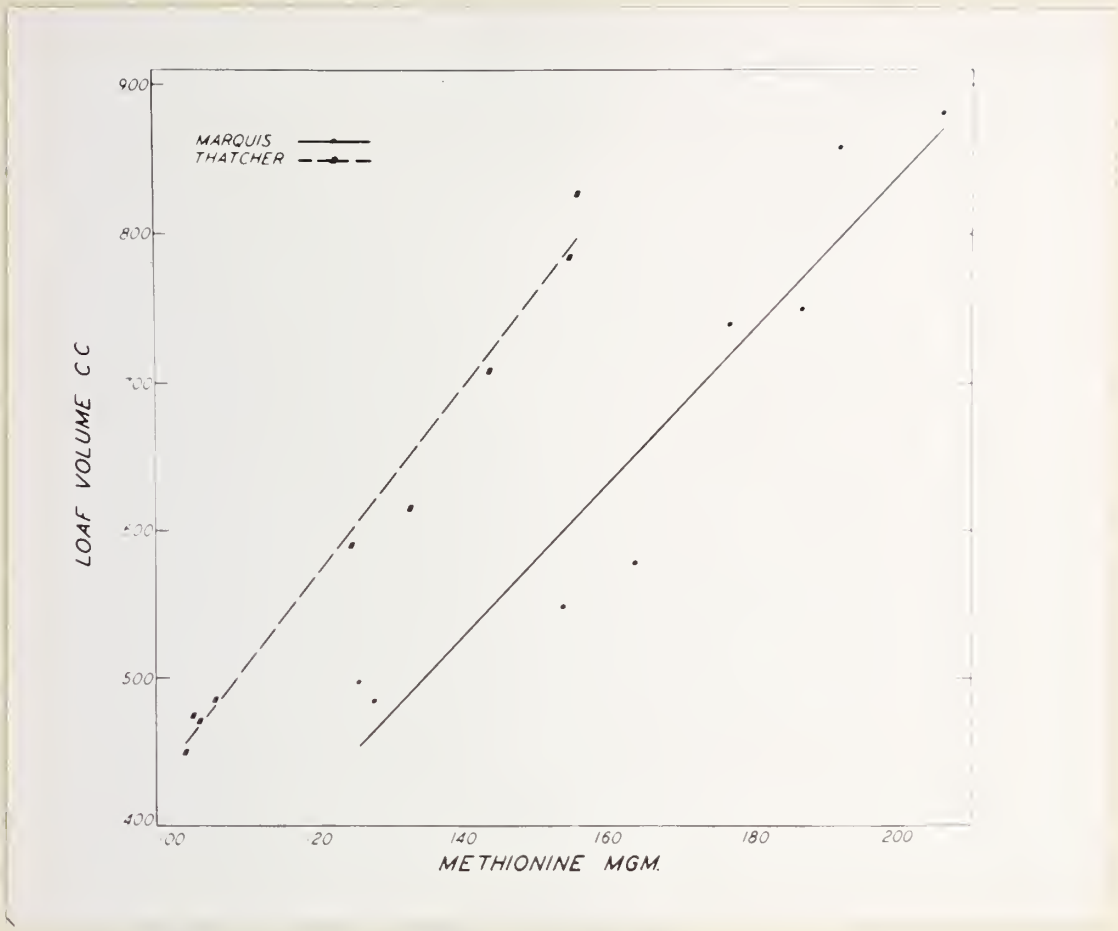

Figure 5

Relation between methionine, in gluten per 100 grams of flour, and loaf volume, Series II

TABLE XI

Cystine and methionine statistics

	Marquis	Thatcher
r_{vc}	.935**	.948**
r_{vm}	.957**	.992**
r_{pc}	.932**	.893**
r_{pm}	.938**	.868**
r_{cs}	.940**	.882**
r_{ms}	.899**	.886**
r_{cm}	.977**	.948**
$r_{vp.c}$	.132	-.147
$r_{vp.m}$	-.100	-.555
$r_{vc.p}$	.630*	.831**
$r_{vm.p}$	.763*	.982**
$R_{v.cp}$	.936**	.949**
$R_{v.mp}$	.996**	.994**

v = loaf volume, cc.
p = protein, %
s = sulphur in wheat, %
c = cystine (mgm.) in gluten per 100 gm of flour
m = methionine (mgm.) in gluten per 100 gm. of flour

* significant beyond 5% point
** significant beyond 1% point.

all of the factors affecting loaf volume. There is, of course, considerable overlapping in the effects of crude protein, cystine, and methionine on loaf volume, but these results suggest strongly that volume is more closely associated with the content of the individual amino acids than with the protein content. This may be because the cystine and methionine are measured on gluten, while the protein content includes all nitrogen-containing compounds.

The multiple $R_{v.cp}$ for Marquis is highly significant but is not significantly higher than either r_{vc} or r_{vp}. $R_{v.mp}$ is highly significant and the difference between $R_{v.mp}$ and either of r_{vp} or r_{vm} is significant beyond the 1% point.

The multiple $R_{v.cp}$ is highly significant, while the difference between $R_{v.cp}$ and r_{vc} is not significant and that between $R_{v.cp}$ and r_{vp} is significant beyond the 5% point. The difference between the highly significant $R_{v.mp}$ and r_{vm} is not significant, but between $R_{v.mp}$ and r_{vp} the difference is significant beyond the 1% point.

It is significant that the variability in methionine and protein combined accounts for 99% of the variability in the loaf volume of both Marquis and Thatcher in 1944.

The sulphur content of several samples of Marquis gluten was determined to compare the recovery of sulphur as found in methionine and cystine with the total sulphur in gluten. For this purpose the gluten sulphur in flour was calculated because the cystine and methionine results were

calculated for gluten representing 100 gm. of flour. Six samples gave a mean recovery of 101.4% (Table XII). The sulphur content of the gluten from the Edmonton (F) sample was probably too low. When this sample was omitted the mean recovery was 99.3%.

The mean values for methionine and cystine in mgm. per gram of nitrogen for Marquis and Thatcher at all stations in 1944 are presented in Table XIII. In general the lower quality samples have larger quantities of cystine and methionine per gram of nitrogen.

The methionine-cystine sulphur ratio for Marquis was consistently higher than for Thatcher (Table XIV). This ratio difference may be one of the reasons why Thatcher behaved abnormally in 1944, but the data are too meagre to justify any definite conclusion.

TABLE XII

Recovery of sulphur as methionine and cystine from gluten

	Gluten in flour, %	Sulphur in gluten, %	Gluten sulphur in flour, %	Total sulphur recovered, %	Recovery, %
Athabaska	8.8	.993	.874	.836	95.7
Bon Accord	13.3	.814	1.082	1.088	100.5
Fallis	10.1	.834	.843	.843	100.0
Warburg	13.0	.812	1.054	1.074	100.2
Edmonton (F)	14.8	.768	1.138	1.278	112.3
Composite	16.1	.826	1.328	1.327	99.9
				Mean recovery	101.4%

TABLE XIII

Methionine and cystine per gram nitrogen

	Marquis		Thatcher	
	Methionine, mgm.	Cystine, mgm.	Methionine, mgm.	Cystine, mgm.
1944				
Athabaska	104	170	68	179
Beaverlodge	82	145	61	148
Bon Accord	87	143	70	146
Edmonton (F)	84	158	64	135
Edmonton (S)	89	157	67	145
Fallis	95	162	72	166
Sundre	-	-	88	160
Warburg	86	157	62	137
Composite	88	154	72	163
1945				
Edmonton	73	141	78	163
Fallis	83	163	71	146

TABLE XIV

Methionine-cystine sulphur ratio

Station	Marquis	Thatcher
Athabaska	.49	.37
Beaverlodge	.46	.31
Bon Accord	.48	.38
Edmonton (F)	.42	.39
Edmonton (S)	.46	.36
Fallis	.47	.35
Sundre	-	.45
Warburg	.44	.35
Composite	.43	.35
Mean	.46	.37

Series III - Selected Varieties, 1945

The ranges for protein are very narrow in the 1945 material. The protein ranges are as follows: Marquis 12.7 to 15.9%, mean 15.1%; Thatcher 15.2 to 15.8%, mean 15.6%; Red Bobs 12.7 to 15.2%, mean 14.1%. The sulphur in wheat ranges are: Marquis .177 to .221%, mean .200%; Thatcher .184 to .212%, mean .199%; Red Bobs .176 to .206%, mean .197%.

The data for loaf volume, protein, and sulphur in Table XV are plotted in Figures 6 and 7. The correlations between loaf volume and protein and between loaf volume and sulphur are obviously not of a high order. They were not calculated because the population is so small.

The distribution of sulphur in gluten was determined for Marquis and Thatcher at Edmonton and Fallis. The total cystine and methionine sulphur found gave a mean recovery of 97% (Table XVI).

The amounts of methionine and cystine in gluten representing 100 grams of flour (Table XVII) are plotted in Figure 8. In 1945 only methionine appears to be related to loaf volume, but the ranges are small and the levels high.

The results for sulphur in wheat, flour, and gluten indicate that the highest concentration of sulphur occurs in the gluten from the lowest quality flour. This sulphur in the gluten represents a mean of 70.5% of that found in the flour and 62.2% of that found in the whole wheat (Table XVIII).

TABLE XV

Results for 1945

Variety	Loaf volume	Protein in wheat, %	Nitrogen in wheat, %	Sulphur in wheat, %	S/N ratio
Athabaska					
Marquis	865	14.9	3.03	.177	.584
Thatcher	803	15.5	3.15	.184	.584
Red Bobs	818	14.1	2.85	.200	.702
Bon Accord					
Marquis	783	15.4	3.12	.214	.687
Thatcher	770	15.7	3.19	.212	.665
Red Bobs	760	14.2	2.88	.203	.705
Edmonton					
Marquis	845	15.6	3.16	.207	.655
Thatcher	923	15.6	3.16	.210	.665
Red Bobs	813	14.3	2.90	.198	.683
Fallis					
Marquis	740	12.7	2.57	.182	.708
Thatcher	875	15.2	3.07	.193	.629
Red Bobs	730	12.7	2.58	.176	.683
Warburg					
Marquis	778	15.9	3.23	.221	.684
Thatcher	818	15.8	3.21	.198	.617
Red Bobs	828	15.2	3.06	.206	.673

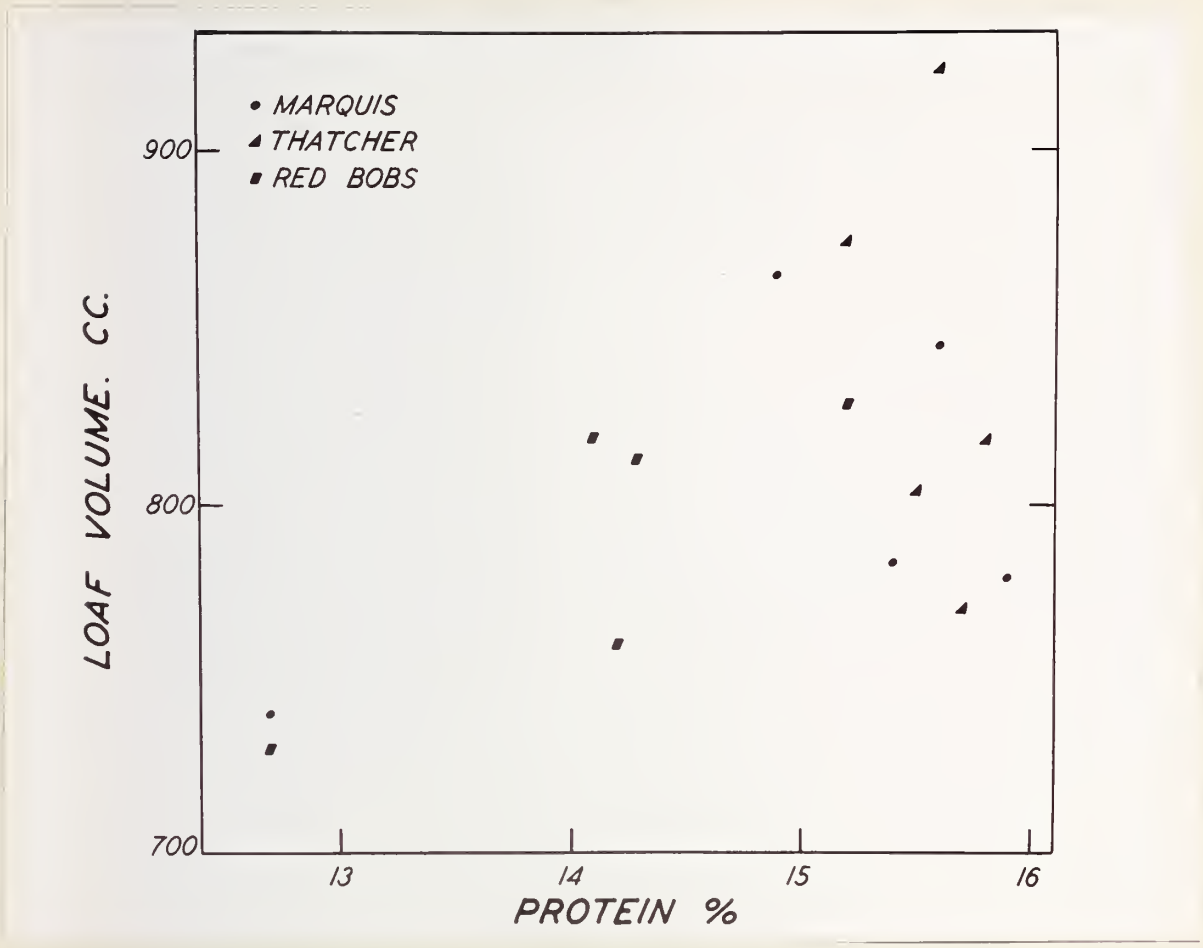

Figure 6

Relation between loaf volume and protein content, Series III

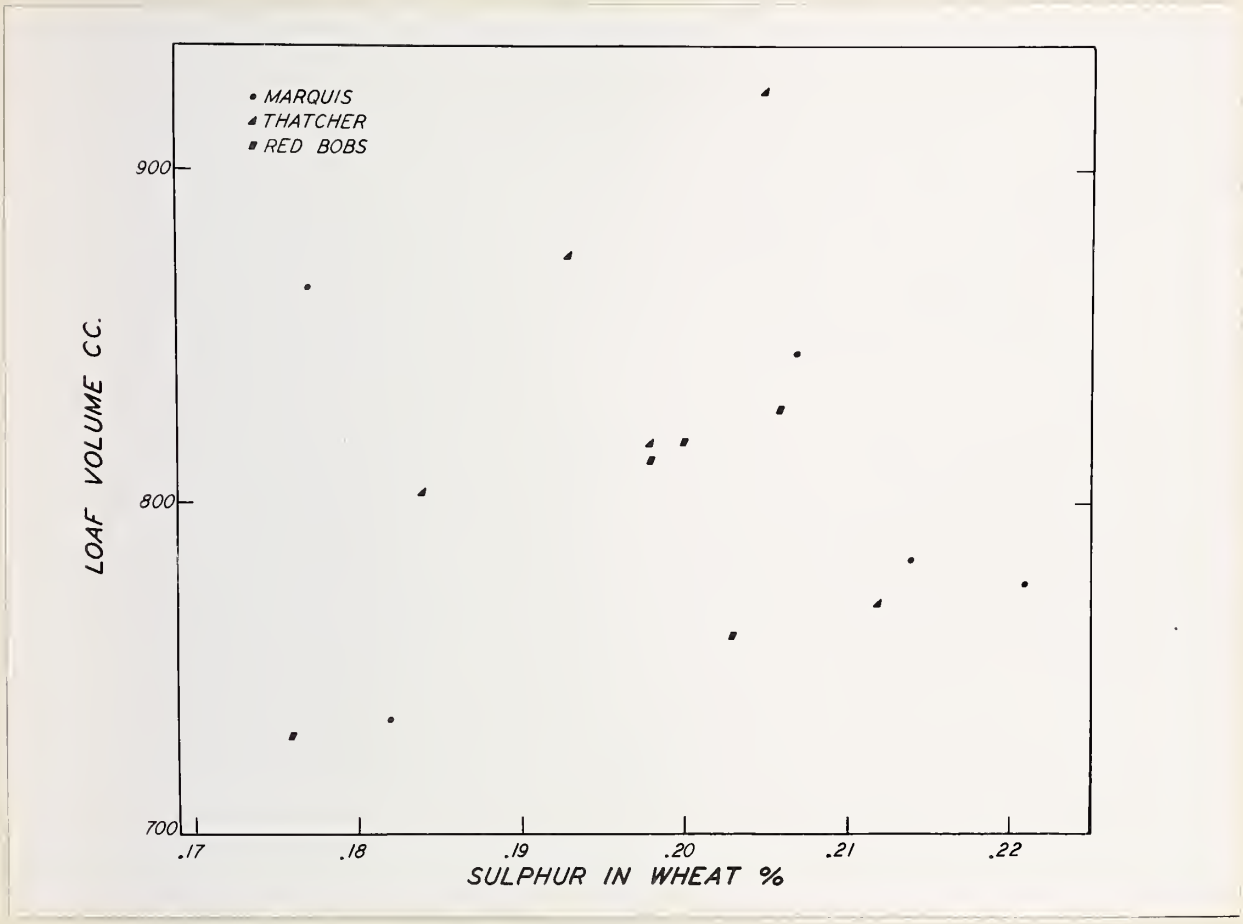

Figure 7

Relation between loaf volume and sulphur in wheat, Series III

TABLE XVI

Sulphur distribution in gluten
expressed in percent

	Edmonton		Fallis	
	Marquis	Thatcher	Marquis	Thatcher
Methionine in gluten	0.956	1.028	1.063	0.904
Methionine sulphur	0.215	0.220	0.240	0.204
Cystine in gluten	1.954	2.083	2.028	1.795
Cystine sulphur	0.521	0.555	0.539	0.477
Total sulphur recovered	0.736	0.775	0.779	0.681
Gluten sulphur	0.779	0.783	0.821	0.684
Recovery	94.7	99.0	94.8	99.6

Mean recovery - 97.0%

TABLE XVII

Cystine and methionine content, 1945

	Loaf volume, cc.	In gluten per 100 gm. flour	
		Methionine, mgm.	Cystine, mgm.
Edmonton			
Marquis	845	158	313
Thatcher	923	184	290
Fallis			
Marquis	740	152	373
Thatcher	875	170	266

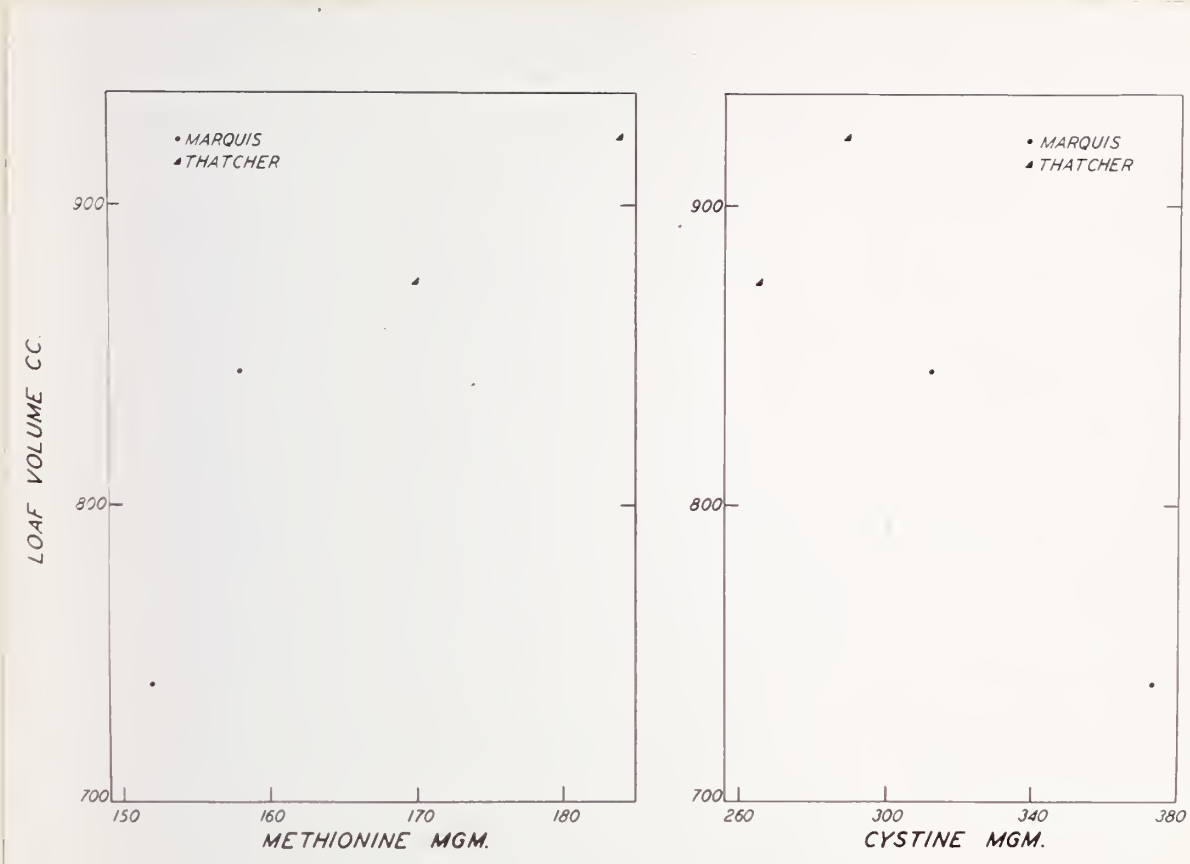

Figure 8

1. Relation between methionine, in gluten per 100 grams of flour, and loaf volume, Series III

2. Relation between cystine, in gluten per 100 grams of flour, and loaf volume, Series III

TABLE XVIII

Recovery of sulphur, expressed in percent

	Edmonton		Fallis	
	Marquis	Thatcher	Marquis	Thatcher
Gluten in flour	16.0	17.9	14.3	18.8
Sulphur in gluten	0.779	0.732	0.821	0.684
Gluten sulphur in flour	0.125	0.131	0.117	0.123
Sulphur in flour	0.178	0.186	0.177	0.163
Recovery from flour	70.0	70.5	66.2	75.5
Sulphur in wheat	0.207	0.210	0.182	0.193
Recovery from wheat	60.3	62.4	64.3	63.8

Mean recovery from flour - 70.5%
Mean recovery from wheat - 62.7%

The mean values for cystine and methionine in mgm. per gram of nitrogen are included with the 1945 results (Table XIII).

DISCUSSION AND SUMMARY

Rigby (17) found sulphur to be a factor affecting the baking strength of wheat. The material he used showed improved baking strength not explainable on the basis of protein content. This improved baking strength was, however, associated with the sulphur content of wheat, flour, and gluten. Since the material was grown on sulphur-deficient soil

the uptake of sulphur was greatly increased in the samples from plots receiving a sulphur-bearing fertilizer. The nitrogen content, however, appears to be uniformly low in the three years that wheat was studied.

To have a good range in sulphur content of wheat the quality test was grown at Fallis, Sundre, and Warburg. It is regrettable that this planting did not provide samples giving the expected differences.

The sulphur content of five varieties of wheat was determined and significant differences between varieties were found. This is in agreement with the work of Greaves and Bracken (9). The results also indicate that the sulphur-nitrogen ratio is not constant, which substantiates Rigby's work (17). In general, high sulphur-nitrogen ratios were found in samples of low baking strength. This, however, does not agree with Rigby's results. He found that high sulphur-nitrogen ratios were associated with high baking strength. The range for protein in all of the wheat samples studied by Rigby was 10.0 to 14.4%, while for sulphur in wheat it was 0.10 to 0.25%. In these samples, then, the range for sulphur was proportionately greater than for protein. Therefore, with increases in sulphur content increases in the sulphur-nitrogen ratios were obtained.

The material for this study was grown in different areas and nitrogen and sulphur were not always limiting. It appears that in the grey wooded areas sulphur was in shorter

supply than nitrogen. In 1944, the Plant Breeders' Varieties material all received the same fertilizer treatment in the field, so that differences in protein and sulphur content were due to varietal differences and to environmental differences at the various stations; while differences in the material used by Rigby were due only to the fertilizer treatment the plots received since Thatcher was used throughout. The protein range was 7.7 to 15.7%, which is nearly twice the range in the material used by Rigby; and the range for sulphur was 0.131 to 0.215%, which is about one-half of the range in the material used by Rigby.

The sulphur content was highly correlated with protein content in the 1944 material. In all varieties except C.T.149 the correlation coefficient for sulphur with loaf volume was higher than for protein with loaf volume. The multiple $R_{v.ps}$, however, was not significantly higher than any of the simple correlation coefficients.

The methionine and cystine in gluten from Marquis at eight stations and from Thatcher at nine stations were determined. The baking strength was correlated with the methionine and cystine in gluten. The relationships between methionine and loaf volume take into account more of the variability in loaf volume than do the relationships between protein and loaf volume or sulphur in wheat and loaf volume.

In Marquis, both of the partial correlation coefficients $r_{vc.p}$ and $r_{vm.p}$ account for more of the variability in loaf volume than does the simple r_{vp}. There is, therefore,

some overlapping in the measurement of the variability in loaf volume by $r_{vc.p}$ and $r_{vm.p}$.

The multiple correlation coefficient $R_{v.mp}$ accounts for 99% of the variability in the loaf volume in both Marquis and Thatcher, while $R_{v.cp}$ accounts for 90% and 87%. It appears then that the methionine in gluten is more directly related to the baking strength of the wheat flours examined than is cystine in gluten. The determinations of methionine and cystine in gluten from Thatcher and Marquis at Fallis and Edmonton in 1945 support the above hypothesis.

Significant information has been obtained by using crude gluten for the estimation of methionine and cystine, although analyses should be made on more of the 1945 material. The writer is of the opinion that a further investigation of methods for the accurate determination of the sulphur-containing amino acids in flour rather than in gluten would be of value in continued studies of the role of sulphur in flour quality. He also believes that the methionine and cystine content of the different flour proteins should be determined.

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REFERENCES

1. BAERNSTEIN, H.D. The sulphur distribution in proteins. Jour. Biol. Chem. 97:669-674. 1932.

2. BAILEY, C.H. The Constituents of Wheat and Wheat Products. Amer. Chem. Soc. Monograph Ser. Reinhold Pub. Corp. N.Y. 1944.

3. BAKER, J.C., PARKER, H.K. and MIZE, M.D. The action of oxidizing agents on sulphydryl compounds in dough. Cer. Chem. 21:97-107. 1944.

4. BARRIEN, B.S. and WOOD, J.G. Studies on the sulphur metabolism of plants. II. The effect of nitrogen supply on the amounts of protein sulphur, sulphate sulphur, and on the value of the ratio of protein nitrogen to protein sulphur in leaves at different stages during the life cycle of the plant. New Phyt. 38:257-264, 1939.

5. CSONKA, F.A. Amino acids in staple foods. I. Wheat (Triticum vulgare). Jour. Biol. Chem. 118:147-153. 1937.

6. DILL, D.B. and ALSBERG, C.L. Some critical considerations of the gluten washing problem. Cer. Chem. 1:222-246. 1924.

7. GOULDEN, C.H. Methods of Statistical Analysis. John Wiley & Sons Inc., N.Y. 1939.

8. GRAFF, S., MACULLA, E. and GRAFF, A.M. The composition of tissue proteins. IV. The estimation of cystine. Jour. Biol. Chem. 121:81-86. 1937.

9. GREAVES, J.E. and BRACKEN, A.F. The sulphur content of wheat. Cer. Chem. 14:578-581. 1937.

10. GUBLER, C.J. and GREAVES, J.E. Cystine content of wheat and its quantitative determination. Food Res. 7:405-413. 1942.

11. HARROW, B. Textbook of Biochemistry. 3rd ed. W.B. Saunders Co. Pa., U.S.A. 1943.

12. LAVINE, T.F. An iodometric determination of methionine. Jour. Biol. Chem. 151:281-297. 1943.

13. McCALLA, A.G. Varietal differences in the relation between protein content of wheat and loaf volume. Can. Jour. Res. C.18:111-121. 1940.

14. Methods of Analysis. A.O.A.C. 5th ed. Geo. Banta Pub. Co., Menasha, Wisconsin. 1940.

15. MYERS, H.S. and WORKING, E.B. The sulphydryl groups of wheat flour. Cer. Chem. 21:32-37. 1944.

16. PETERS, T.W. The effect of fertilizers on the sulphur content of soil and crop plants. M.Sc. Thesis, Dept. of Soils (unpublished). U. of Alta. 1942.

17. RIGBY, F.L. The effects of sulphur on the quality of wheat. M.Sc. Thesis, Dept. of Field Crops (unpublished). U. of Alta. 1944.

18. SANDIN, R.B. and STOVER, N.M. A rapid method for the determination of organic nitrogen in liquids. Can. Jour. Res. 2:264-266. 1930.

19. SHOHL, A.T. Mineral Metabolism. Monograph Ser. No. 82. Reinhold Pub. Corp. N.Y. 1939.

20. SMITH, A.M. and WANG, T. The cystine and methionine in certain species of grassland herbage. Bioch. Jour. 35:404-412. 1941.

21. STOVER, N.M. and SANDIN, R.B. Use of boric acid in micro-kjeldahl determination of nitrogen. Ind. & Eng. Chem. Analytical Ed. 3:240-242. 1931.

22. SULLIVAN, B., HOWE, M., and SCHMALZ, F.D. On the presence of glutathione in wheat germ. Cer. Chem. 13:665-669. 1936.

23. THOMAS, M.D. and HENDRICKS, R.H. Hydrolysis of cystine and fractionation of sulphur in plant tissues. Jour. Biol. Chem. 153:313-325. 1944.

24. THOMAS, M.D., HENDRICKS, R.H., COLLIER, T.R. and HILL, G.R. The utilization of sulfate and sulfur dioxide for the sulfur nutrition of alfalfa. Plant Phys. 18:345-371. 1943.

25. VICKERY, H.B. and WHITE, A. The use of cysteine cuprous mercaptide in the determination of cystine. Jour. Biol. Chem. 99:701-715. 1932.

26. WOOD, J.G. Metabolism of sulfur in plants. Chron. Bot. 7:14-19. 1942.

27. _____ and BARRIEN, B.S. Studies on the sulfur metabolism of plants. I. Preliminary investigations on the effects of different external concentrations of sulfate, ammonia, and cystine on the amounts of sulfur-containing compounds in leaves. New Phyt. 38: 125-149. 1939.

28. WYATT, F.A., NEWTON, J.D. and IGNATIEFF, V. Wooded soils and their management. Bul. 21. 3rd ed. Rev. 1941. U. of Alta.

29. ZITTLE, C.A. and O'DELL, R.A. The determination of cystine: The use of cuprous oxide for simultaneous reduction and precipitation of cystine as the cuprous mercaptide. Jour. Biol. Chem. 139:753-759. 1941.