

INFRARED STUDIES OF WHEAT GLUTEN

by

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## INTRODUCTION AND REVIEW OF LITERATURE

It is generally accepted that the baking quality of wheat flour is related to the quality as well as the quantity of wheat gluten. The quantity of gluten present can easily be determined by Kjeldahl analysis. Various methods have been tried to determine the gluten quality. One such test, which is based on physical properties of the gluten, is the sedimentation test developed by Zeleny (10). Although this does give valuable information concerning the macrophysical properties of the gluten, more information must be obtained as to its microphysical and microchemical properties.

Attempts have been made to correlate baking quality with amino acid content. Pence, et al., (4) investigated glutens washed from 17 flours of widely different types and showed that there was no significant differences in amino acid content. In the investigations of Köstmann (9) it was found that loaf volume was positively correlated with the polarographically determined cystine content of wheat glutens. The correlation coefficient between the cystine content of a flour protein and its extensogram area was +.82, and between the total cystine content of flour and its extensogram area was +.92.

Sullivan, et al., (7) found that the sulphur content affected the quality of dough. They reported that oxidizing and reducing agents affected the sulphur linkages of gluten proteins.

Since small differences of molecular structure could be determined by the infrared spectrum of a substance, Dueker (2) made an attempt to correlate baking quality with the infrared spectra of the glutens. Spectra were measured employing a nujol mull technique and differences were observed for different glutens, but the method was not sufficiently reproducible that

any conclusions could be made.

Work performed by Suh (6) indicated that spectra made by a potassium bromide pellet method were more reproducible than those made by the mull technique.

The present study was originated to refine the potassium bromide pellet technique and investigate other methods of obtaining spectra which could be correlated with baking quality.

#### MATERIALS AND METHODS

Studies were made on glutens extracted from three different flours. These were Nebred, C. I. 12711 and Durum. The baking quality of these flours was determined by Johnson and Miller (3) and reported as follows in Table 1.

Table 1. The baking quality of flours studied.

Flour	Loaf volume cc	Over-all quality score
Nebred	2975	90
C.I. 12711	2750	78
Durum	2000	60

Spectra were run on a Perkin-Elmer, model 112 recording spectrometer, which has been modified to record per cent transmission directly. The spectral range from 2.5 to 14 microns was covered. All spectra used were run at constant slit width to reduce error from the adjustable slit drive.

All extractions were performed by Byron S. Miller and John S. Johnson



of the Milling Department, Kansas State College.

In the potassium bromide pellet technique employed by Suh (6), considerable difficulty in reproducibility was encountered due to variations in the amount of radiation scattered from the beam.

In order to decrease the amount of scattered radiation, various extraction techniques were employed to produce pellets which would be clearer to infrared radiation. The most successful of these was an extraction with a 40 per cent solution of ethanol in water. Gluten from 20.00 grams of flour was extracted with 50.0 cc of 40 per cent ethanol at 40° C. The solution was centrifuged at 50,000 g and the protein content determined by Kjeldahl analysis. Concentrations were adjusted to either five milligrams per cc or ten milligrams per cc.

Potassium bromide pellets were made using these solutions of known concentration. By using a 100 microliter pipette,  $(1.00 \pm 0.03)$  milligrams of protein was added to 500 milligrams of powdered optically pure potassium bromide in an agate mortar and pestle. This mixture was dried by blowing dry air over it while it was placed under a heat lamp. Then the sample was ground to insure uniform dispersion of the gluten. The sample was transferred to a die, evacuated for ten minutes to remove any water or ethanol vapors, and then pressed for five minutes under a pressure of approximately 60,000 pounds per square inch. Under this pressure potassium bromide forms a solid solution with the gluten suspended in it. The pellets were  $\frac{1}{2}$  inch in diameter and 1.5 millimeters thick.

The 40 per cent ethanol solution extractions were repeated three times and three pellets were made of each extraction, so that nine spectra were run for each flour.

Another extraction was used employing 0.1 N acetic acid. The same technique was used except for substituting acetic acid in place of ethanol. Concentrations were adjusted to two milligrams of protein per cc, and  $(0.20 \pm 0.006)$  milligrams protein was added to 500 milligrams potassium bromide to form a pellet.

Two extractions were made of each flour and three pellets were made of each extraction which gave six spectra for each flour.

## RESULTS AND DISCUSSION

### Spectra from 40 per cent Ethanol Extractions

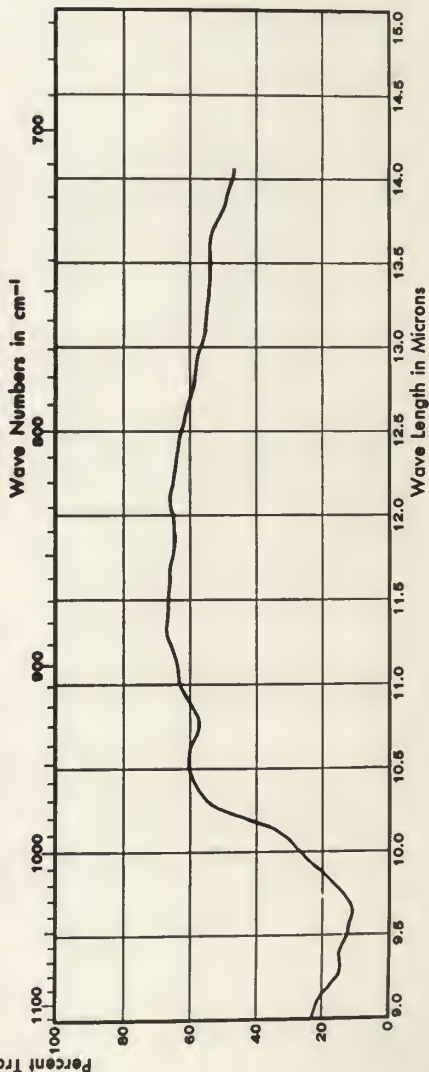
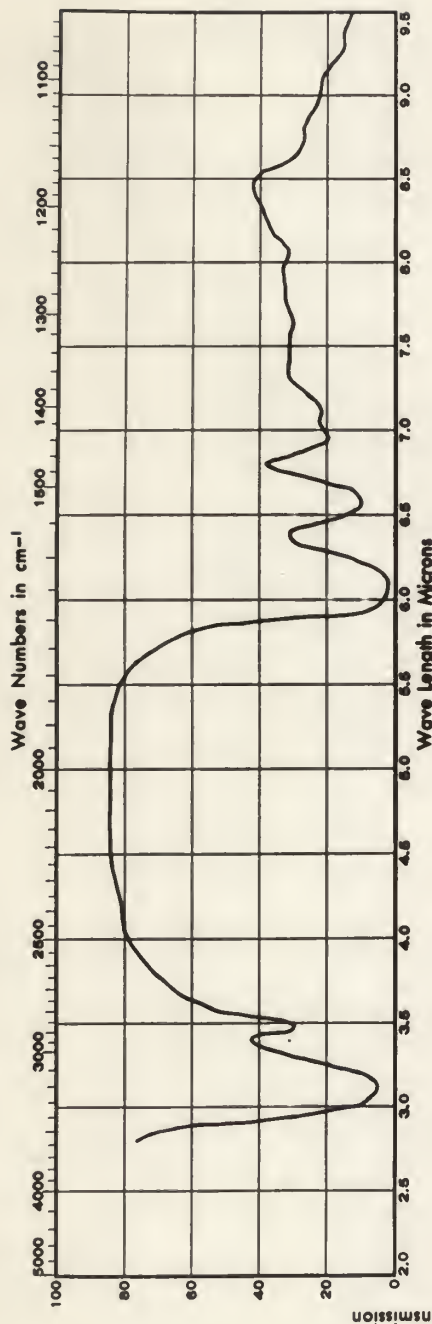
An average spectrum of one of the flours studied by utilizing the 40 per cent ethanol extraction is presented in Plate I. Principle adsorption bands and tentative assignments are listed in Table 2. These assignments were based on results reported by Dueker (2) and were consistent with results reported by Bellamy (1), Sutherland (8) and Randall, et al. (5).

The positions of bands and the appearance of spectra shown in Plate I vary a small amount from that reported by Dueker (2). These small differences are due to accuracy with which position of bands may be determined and the fact that spectra reported by Dueker (2) were from gluten which was in the solid state, while those made with potassium bromide pellets would be the spectra of gluten which is in solution.

Since some degree of success in correlating baking quality with sulphur content was achieved by Sullivan, et al., (7), and Dueker (2) showed that cystine possesses an adsorption band attributed to sulphur, it was hoped

#### EXPLANATION OF PLATE I

An average spectrum of gluten from Nebred flour extracted with 40 per cent ethanol solution. (Differences in spectra between samples were less than 3 per cent which could not be shown conveniently in graphical form. Differences are shown in Tables 3, 4, and 5.)



COMPOUND
GLUTEN FROM
NEBED FLOUR
SOURCE AND PURITY
40 % ETHANOL
EXTRACTION
STATE KBr PELLET
CONCENTRATION
1 TO 500
LABORATORY
K S C



Table 2. Tentative assignments of infra red adsorption bands found in gluten.

Wavelength of band in microns	:	Assignment
3.09	:	Amide N-H stretching
3.45	:	C-H stretching
6.08	:	C=O stretching
6.58	:	N-H bending
6.94	:	Unassigned
7.07	:	C-H bending
8.05	:	C-O stretching
8.63	:	C-H bending
9.33	:	C-OH stretching
10.74	:	Unassigned

Table 3. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from Nebred flour with 40 per cent ethanol in water.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	95.2	0.5
3.45	70.3	1.4
6.08	98.0	0.0
6.58	90.4	0.3
6.94	80.5	1.2
7.07	78.6	1.0
8.05	68.7	1.6
8.63	73.3	2.1
9.33	85.1	1.7
9.58	89.2	1.5
10.74	43.1	2.1

Table 4. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from the C.I. 12711 flour with 40 per cent ethanol in water.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	94.4	0.9
3.45	67.6	2.9
6.08	97.7	0.4
6.58	88.6	0.8
6.94	79.7	1.5
7.07	77.9	1.6
8.05	66.3	2.4
8.63	71.2	2.5
9.33	83.3	2.0
9.58	83.3	2.0
9.58	87.6	1.6
10.74	40.6	1.9

Table 5. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from Durum flour with 40 per cent ethanol in water.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	95.4	0.9
3.45	70.8	1.1
6.08	97.5	0.6
6.58	89.0	1.0
6.94	80.6	1.0
7.07	79.1	1.0
8.05	69.4	1.4
8.63	75.7	1.6
9.33	87.6	1.2
9.58	91.3	1.0
10.74	44.9	1.2



that the 40 per cent ethanol extraction would preferentially extract the portion of gluten which contained sulphur. Although cystine is soluble in ethanol, no adsorption band due to sulphur was observed. If sulphur were present, an adsorption band near four microns should be observed but no bands were observed in this region.

The average value of the per cent adsorption of each of the principle bands and the average deviation of adsorption of each of these bands for the nine spectra of the Nebred flour is given in Table 3. The same information for the C.I. 12711 flour is given in Table 4 and for the Durum flour in Table 5.

As may be seen from Tables 3, 4, and 5, differences do exist between the spectra of the flours which were studied. Unfortunately, however, these differences are smaller than the average deviation of the spectra studied. Small variations in the amount of gluten present in a pellet could cause variations in the spectra. The ratios of the intensities of principle bands should be nearly independent of the amount of gluten present in a pellet so by taking ratios of the principle bands in each spectra, it was hoped that the variations between spectra of the same gluten could be reduced. The average ratio of the 6.58 micron band to the 3.09 micron band showed differences between glutens which could be correlated with baking quality. The ratio of these two bands was 0.956, 0.938, and 0.934 for the glutens Nebred, C.I. 12711 and Durum respectively. The average deviation of each ratio was 0.009, 0.005, and 0.008 for the flours Nebred, C.I. 12711 and Durum respectively. Although some correlation does exist, variations between spectra of the same flour are too large for the correlation to be significant. Other ratios showed a slight correlation

but again variations between spectra of the same flour were too large for the correlation to be significant.

The variations between spectra of the same sample were caused by two factors, variations in amount of protein placed in the radiation beam and variations in the amount of radiation scattered from the beam. The ratios of band intensities should reduce the variations which are caused by either of these two factors. Since no appreciable decrease in the variation between spectra for a given gluten was observed for ratios of bands as compared to the adsorption, the variation between spectra must be attributed to some other factor.

#### Spectra from 0.1 N Acetic Acid Extractions

Studies of gluten spectra performed by Suh (6) indicated that spectra from 0.1 N acetic acid extractions showed greater differences in spectra from different glutes than the differences observed for the 40 per cent ethanol extraction. In Suh's work much greater variations in the amount of scattering was observed. An attempt to reduce the amount of scattering was made, using the improved potassium bromide pellet technique.

Pellets produced by adding 1.00 milligram of protein were found to be rather opaque and the scattering from such pellets was rather high. In an attempt to reduce the amount of scattering pellets were made using 0.20 milligram of protein.

An average spectrum of one of the glutes studied is presented in Plate II. Principle bands were the same as those found for the spectra from the 40 per cent ethanol extractions, except that another band appeared at 8.97 microns and the band at 10.74 microns decreased in intensity. The

same band assignments as those made for the 40 per cent ethanol extraction given in Table 2 were made for the spectra of the 0.1 N acetic acid extraction. The additional band at 8.97 microns is unassigned.

The average value and the average deviation of the per cent adsorption of each of the principle bands of the spectra of the Nebred flour is given in Table 6. The same information is given in Table 7 for C.I. 12711 in Table 8 for Durum.

Differences in spectra of the glutens studied do exist but the variation in the spectra of the same gluten are much larger than for the spectra of the 40 per cent ethanol extraction. This may be seen by comparing the data presented in Tables 3, 4, and 5 with the data presented in Tables 6, 7, and 8. Ratios of band intensities were taken to reduce errors arising from variations of the amount of gluten in the pellets, but variations in the spectra of a given sample were so great that no significant correlation could be found between spectral differences and baking quality.

#### EXPLANATION OF PLATE II

An average spectrum of gluten from Nebred flour extracted with 0.1 N acetic acid. (Differences in spectra between samples were less than 3 per cent which could not be shown conveniently in graphical form. Differences are shown in Tables 6, 7, and 8.)



## PLATE II

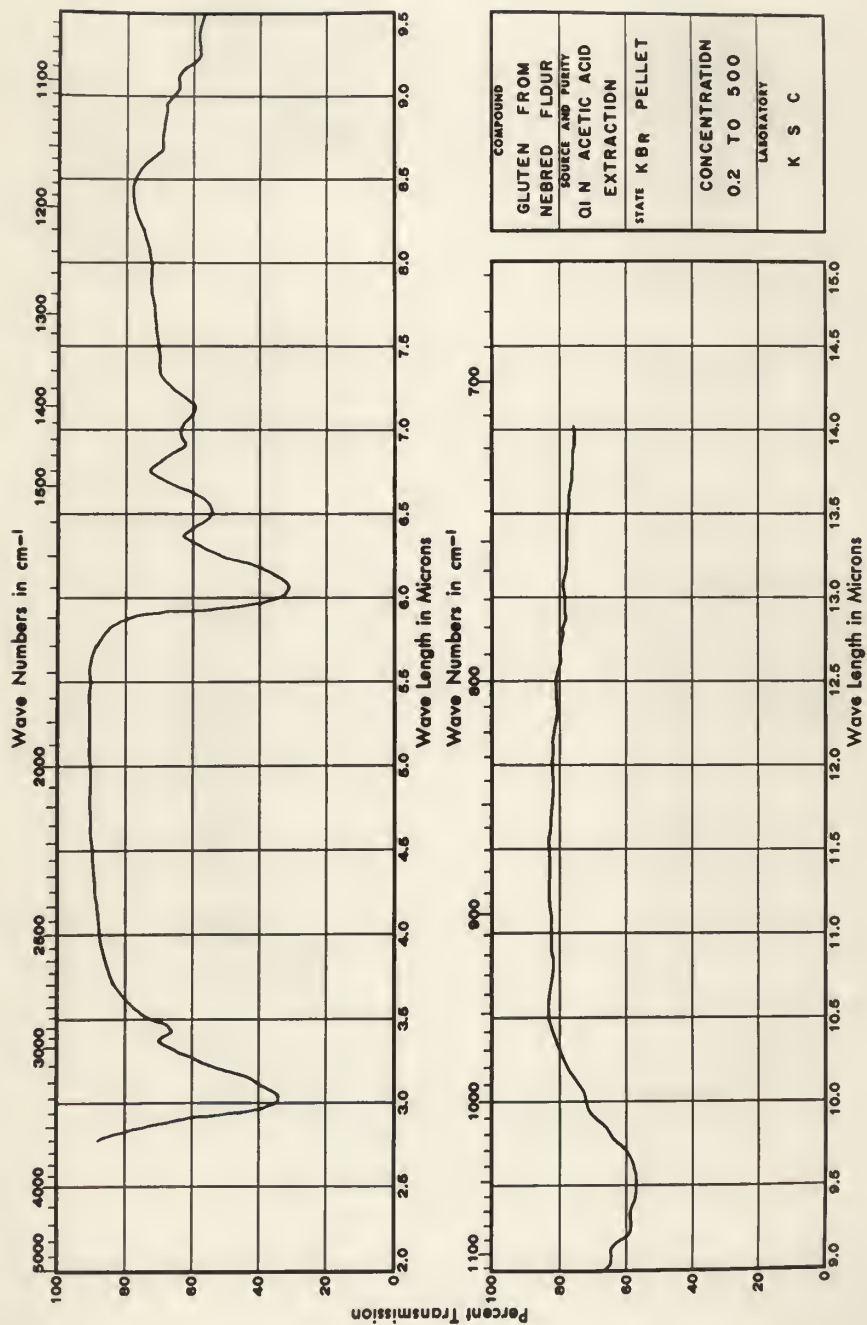


Table 6. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from the Nebred flour with 0.1 N acetic acid.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	65.7	1.1
3.45	34.0	2.0
6.08	69.8	0.6
6.58	45.3	0.6
6.94	37.7	1.2
7.07	40.5	2.5
8.63	30.6	0.6
8.97	35.6	1.6
9.33	41.1	1.4
9.58	42.8	0.8

Table 7. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from C.I. 12711 flour with 0.1 N acetic acid.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	63.3	4.1
3.45	33.9	1.3
6.08	71.0	1.8
6.58	47.4	3.3
6.94	38.5	2.7
7.07	40.7	1.7
8.63	30.8	3.6
8.97	35.3	2.2
9.33	40.9	2.2
9.58	43.4	2.4

Table 8. Average values and average deviations of the per cent adsorption of the principle bands in the spectrum of gluten extracted from Durum with 0.1 N acetic acid.

Wavelength of band in microns	Average per cent adsorption	Average deviation of per cent adsorption
3.09	64.0	3.2
3.45	33.3	4.7
6.08	68.3	3.7
6.58	43.5	4.2
6.94	36.8	3.8
7.07	40.3	5.1
8.63	28.6	3.1
8.97	34.1	4.6
9.33	39.1	4.4
9.58	41.3	3.3



## SUMMARY

Glutens which possess different baking qualities do exhibit differences in their spectra, however these differences are too small to have much significance due to the accuracy with which a given spectrum may be reproduced.

The two most apparent causes of variation between spectra for a given sample are (a) variation in the amount of sample material and (b) variation in the scattering due to the dispersion of the sample material in a potassium bromide pellet. The variation in ratios of per cent adsorption of adsorption bands should be relatively insensitive to variations caused by either of these factors. Since the ratios of the per cent adsorption of adsorption bands in the spectra of a given sample showed variations of about the same magnitude as the variations in per cent adsorption, it must be concluded that the major part of the variation between spectra of the same sample was due to some other factor, probably the variation is related to the micro surroundings of gluten particles in a pellet.

The per cent adsorption of different samples of gluten are remarkably constant for samples of widely different baking quality.

The functional groups responsible for the observed adsorptions are:  $\text{CH}$ ,  $\text{CH}_2$ ,  $\text{CH}_3$ ,  $\text{NH}$ ,  $\text{C=O}$ ,  $\text{C-OH}$ , and  $\text{C-O}$ . Hence one may conclude that the variations in baking quality of different flours are not due to gross differences in the amounts of the above functional groups. The baking quality may, however, be influenced by the presence of very small amounts of functional groups which do not show infrared adsorptions because of their small concentrations, or by intermolecular bonding which is not observed.

It is unlikely that any great improvement can be made in the techniques used in this study. Therefore, it seems unlikely that any test of baking quality can be obtained from the infrared spectra of wheat gluten which will depend on a knowledge of the per cent adsorption of adsorption bands to a greater accuracy than three per cent.

If the baking quality of a flour is related to the presence of one or several functional groups or amino acids of very slight concentration, then it may be possible to find some technique to increase the differences between spectra of glutens with different baking quality to a point where a reliable test for baking quality can be based on infrared spectra. However, if the baking quality is dependent solely on the intermolecular bonding between the protein molecules in gluten the preparation of samples for observation in the infrared may destroy the very thing one is trying to observe and no infrared technique will show any significant differences between glutens.

## ACKNOWLEDGMENTS

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The purpose of this research was to investigate the possibilities of correlating infrared spectral differences of wheat glutens with corresponding differences in baking quality of the glutens.

Gluten was extracted from three flours of different baking quality by washing with 40 per cent ethanol and 0.1 N acetic acid. Spectra were measured by a potassium bromide pellet technique. The method of adding a known amount of gluten to each pellet was greatly improved and the accuracy with which a given spectrum could be reproduced was considerably improved.

Tentative assignments were given to most of the adsorption bands found in the 2.5 to 14.0 micron region. Differences in the spectra of different glutens were found.

Although differences do exist in spectra of glutens possessing different baking quality, errors in reproducing the spectrum of a given sample make it improbable that a test for baking quality will be obtained from the infrared spectra of wheat gluten extracted in either ethanol solution or acetic acid solution.

It is unlikely that any great improvement can be made in the techniques used in this study. Therefore, it seems unlikely that any test of baking quality can be obtained from the infrared spectra of wheat gluten which will depend on a knowledge of the per cent adsorption of adsorption bands to a greater accuracy than three per cent.

If the baking quality of a flour is related to the presence of one or several functional groups or amino acids of very slight concentration, then it may be possible to find some technique to increase the differences between spectra of glutens with different baking quality to a point where

a reliable test for baking quality can be based on infrared spectra. However, if the baking quality is dependent solely on the intermolecular bonding between the protein molecules in gluten the preparation of samples for observation in the infrared may destroy the very thing one is trying to observe and no infrared technique will show any significant differences between glutens.