DETERMINATION OF PENTOSANS OF WHEAT AND FLOUR AND
THEIR RELATION TO MINERAL MATTER

by

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INTRODUCTION

The quantitative determination of polysaccharides and investigations concerning their function in plant and animal life have increased in importance noticeably with the development of emphasis upon industrial utilization. Hemi-celluloses, particularly the pentosans, are widely distributed in most plant materials and are used by industry as the starting material for the preparation of the compound, furfuraldehyde. Furfuraldehyde is formed by the stepwise hydrolysis of pentosans to the corresponding pentose sugars with the subsequent loss of water. The principal uses of furfuraldehyde commercially are as a solvent in petroleum processing and as a constituent of plastics. The quantitative estimation of pentosans will undoubtedly become more important as new uses for this aldehyde are developed. For example, the brewing industry now uses pentosan values as an estimate of the available fermentable matter in grain.

Pentose sugars, as such, are rarely found in plants but their corresponding anhydrides occur in abundance. Xylans, anhydrides of xylose, are found in oat hulls, corn cobs, wheat bran, straw and wood. Arabans, anhydrides of arabinose, are found in minute quantities in cereal grains. Because of their association with cellulose it is believed that pentoses are utilised in formation of cell wall tissue. Bernardini and Gallucio (1913) imply that the function of pentoses in germinating seeds is to assist the cellulose in the formation of

plant tissue. Ravenna and Montonari (1912) suggest that the metabolism of these pentose sugars is similar to that of fructose and sucrose in plants. Bailey (1936) indicates that pentoses may be associated with lignin formation in wood.

The effect of the presence of pentosan in wheat flour is not clearly defined. It is almost certain it does not undergo changes analogous to starchy materials during baking operations due to its inactivity toward yeast. Baker, Parker and Mize (1943), in studying the gelling properties of soluble pentosans with action toward oxidizing agents, indicate a possible role in baking. In addition, the insoluble pentosans, which are found in proportion to the surface area of small starch granule, may account for some of the characteristic actions of enzymes on starch.

The qualitative and quantitative determinations of pentosans and pentoses have always been difficult, largely due to the similarity of many of their reactions to those of hexose sugars. Emil Fischer (1884), with his discovery of phenylhydrazine, elucidated the relationship of isometric pentoses. Quantitative estimation was more difficult because no distinctive reaction at ordinary temperatures was known. Pentosans and pentoses are unique in that heating in the presence of acid forms the volatile aldehyde, furfural.

It was Tollens (1902) who developed the characteristic reaction of furfural with phloroglucinol (1, 3, 5-trihydroxybenzene) to form the phloroglucinide precipitate. By use of controlled conditions and correction factors, the phloroglucinol

precipitate was found to correspond to calculated amounts of furfural, pentose and pentosan. Tollens' work is now considered classic in its field.

The purpose of this investigation was to find a method suitable for routine determinations of pentosans of wheat and flour, and to investigate the possibility that pentosan content is correlated with the mineral matter or ash value. Also, approaches to quantitative estimation of pentosans were studied. The ultimate end has been additional information concerning the distribution of pentosan in wheat and flour and the possible use of this information as a flour grading factor.

The estimation of pentosan from a particular source involves considerable adaptation of existing methods and standardization of procedure. The hydrolysis of pentosans is not quantitative in the production of furfural. A large number of factors influence the accuracy of this test, but the relative importance of some factors may be minimized under certain conditions. A few of the more important considerations are worthy of mention.

Determinations involving the production of furfural from pentosans or pentose sugars usually require distillation with 12 per cent hydrochloric acid, or equivalent strength of other acids, at a fixed rate (30 ml per ten minutes) with the addition of more acid to the flask to replace the amount distilled. This usually is accomplished by means of a graduated separatory funnel connected to the flask. Distillation is continued until 250 ml to 400 ml of furfural in acid is collected. The

time of distillation depends upon temperature and amount collected. Methods of collecting the distillate also vary the analysis, furfural being classed as a volatile liquid.

Cooke (1943) in his observation of the rate of the Krober distillation indicates that the production of furfural follows a hyperbolic function of milliliters distilled. The equation of the line is: $X = 0.00133t + 8.27 - 390/t$, where $X =$ per cent pentosan and $t = $ ml of distillate. Hallsworth (1941) corroborates this equation with his work on feeding stuffs, indicating that approximately 90 per cent of pentose or pentosan converted to furfural is found in the first 200 ml collected. The remainder is collected in the last 100 ml or more. His work also condenses the other factors affecting conversion to the aldehyde, namely, local charring, formation of methylfurfural from hexoses, and the different conversion rate of xylose and arabinose. Figure 1 shows the approximate rate of pentosan conversion at various values of collected distillate.

Pallada et al. (1914) point out that errors are caused by hexose sugars, particularly levulose. It is interesting to note his suggested use of barbituric acid at this early date. Recently, the thiobarbituric acid precipitation of furfural was accepted as an official method for cereal determinations. Cunningham and Dorée (1914) also list compounds which may affect the furfural value, and Oshima and Konds (1918) outlined methods of methylfurfural detection. It is now generally believed that these compounds are formed slowly and alter the results negligibly.
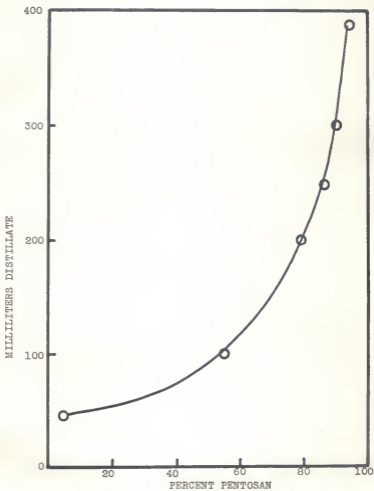
Fig. 1. The approximate amount of pentosan converted
as represented by milliliters of distillate
collected.

Van Haarst and Olivier (1914) have shown that l-arabinose is converted to furfural more slowly than l-xylose. In the usual combination of pentoses in cereal grains, xylan predominates. The rate of hydrolysis and yield of furfural will depend on the proportions of the sugars in the pentosan complex. Because of this, there are as many conversion factors as there are variations in hydrolysis conditions of the different compounds.

Most pentosan determinations depend upon the estimation of furfural. Conversion factors are then used to obtain pentose and polysaccharide equivalents. Many of the contributions to elucidating pentosan determinations resulted from wood products investigations. European chemists added greatly to pentose sugar studies.

Pinoff and Oude (1913) developed a spectroscopic method for pentoses in the presence of other sugars. Steenberger (1916) contributed greatly to clarifying and correcting the phloroglucinol method. Testoni (1917) initiated colorimetric determinations based on arabinose. Baker and Hulton (1916) made use of the reducing power of furfural by employing Fehling solution. Menaul and Dowell (1919) utilized phenylhydrazine as a precipitating agent. Macheleidt (1922) used the bisulphite fixation and determined excess sulphite with iodine. Powell and Whittacker (1924) and Pervier and Gortner (1923) were accredited with initial work employing the bromine addition reaction. Noll and Belz (1931) investigated the use of hydroxylamine-hydrochloride in determination of distillate.

This method calls for neutralization of the acid and reacting with reagent liberating hydrogen chloride into solution. Rosenberger, Rabinovich and Frenkel (1932) added to the bromine reaction. Suminokura (1931) devised a microcolorimetric method. Oshyman (1934) added variations to the existing Fehling solution method. Van Neenen (1936) and Bailey (1936) did considerable work in establishing the thiobarbituric acid precipitation method. The conclusive works of Hughes and Acree (1934) in the use of controlled bromination of furfural is particularly noteworthy. This method is easily adapted to quick routine analysis. The contributions of Vernon and Metzner (1941) enable the use of this reaction in daylight, adding to its facility as a control method.

Bromination is now well established as an accurate and rapid method that is widely used in control laboratories. Expensive equipment is not necessary and the total time of analysis is less than three hours.

MATERIALS AND METHODS

Initial studies were made of the phloroglucinal method as described in Cereal Laboratory Methods (Fourth edition, 1941) and the method of Noll and Belz (1931). The phloroglucinol precipitation proved too time-consuming for the number of analyses to be made, and the hydroxylamine-hydrochloride titration method lacked the flexibility necessary. In the initial work on flour the procedure of Vernon and Metzner (1941) was utilized. The method was later modified as follows:

The distillation apparatus consisted of a 500 ml boiling flask with two-hole stopper; a bent tube directed to the inside wall of the flask was connected to a separatory funnel graduated in 30 ml intervals. This allows acid to be periodically introduced. A Kjeldahl, connecting bulb, Iowa State type, linked the flask to an ordinary condenser. The distillate passed through a small filter and collected in a 500 ml glass stoppered Erlenmeyer flask, which had been marked in 30 ml graduations.

A weighed sample was introduced along with some glass beads, to prevent bumping, and heated with 125 ml of 12 per cent hydrochloric acid. Heat sources were 300 watt Goldfisch units connected by pairs in parallel circuit giving duplicate heating rates of 30 ml per 10 minutes and the minimum of local charring. Distillation was continued until 360 ml of distillate was collected, 30 ml acid portions being added to the boiling flask as equivalent amount collected. It is desirable to have all connections of ground glass because the acid forms an unsaturated

compound from the rubber which introduced an error. The error, as noted by Hughes and Acree (1938) amounts to 0.32 ml titration after boiling two grams of rubber under the conditions outlined in the method.

The flasks were closed and placed in an ice bath. When the furfural solution is at 0°-2° C, 25 ml of bromide-bromate solution (three grams of $KBrO_3$-50 grams KBr per liter) is introduced with a fast delivery pipette and the flask closed quickly. The reaction is allowed to continue for exactly four minutes. At the end of elapsed time, 10 ml of 10 per cent KI solution is added, closing the flask and shaking gently.

The solution is then titrated rapidly with 0.1 N sodium thiosulfate to a yellow color, starch indicator is added, and titration continued to colorless end point. A blank determination is made in the same manner. The following equation was used in calculating the amount of pentosan. Milliliters of 0.1 N. sodium thiosulfate (blank minus titration value) x 0.0082 equals grams of pentosan present in sample. Unless otherwise indicated, pentosans were determined by this method, and all determinations were made in duplicate.

Separation of bran layers was made, with small variations, by the method of Shetlar, Rankin, Lyman and France (1947). This method consisted of removing individual bran layers from the wheat kernel, and provided in this case a source of material for the study of the location of the pentosans and mineral matter in the bran. Ten varieties of hard red winter wheat were processed and analysed. The samples were prepared by cleaning

and scouring and processing through a wheat "thinning" device. This machine (Plate I) is a rotating slotted sieve. Holes are 0.076 inch wide and 0.75 inch long, retaining only whole kernels.

The epidermis was removed by suspending 100 grams of wheat in water and stirring at reduced speed in a Waring type blender equipped with a rubber paddle. This layer was collected by decanting through a silk cloth. After air drying the wheat, the cross layer was removed by soaking over night in a nearly saturated solution of alcoholic sodium hydroxide. The liquid was poured off and 95 per cent alcohol added. Short, gentle stirring removed the cross layer. More alcohol was added and the stirring repeated for about three minutes; this removed the testa layer, leaving the wheat de-branned to the hyaline layer. The wheat was placed on a funnel and washed with two per cent hydrochloric acid, rinsed with water and air dried.

The wheat so treated was tempered to 15.5 per cent moisture and milled on a Buhler experimental mill. This produced endosperm and hyaline-aleurone fractions. The hyaline-aleurone fraction contained endosperm and analyses were corrected on a starch basis. The epidermis and cross layer fractions were virtually pure. The testa composite contained amounts of both epidermis and cross layer material. Corrections for the amount of entire kernel were made as follows.

A small sample of testa composite was placed on a Levy counting chamber and the length and width of the particles in the marked area were recorded. The dimensions thus obtained were multiplied by the relative thickness factor of each layer.

EXPLANATION OF PLATE I

The wheat testing device used in pre-treatment
to eliminate small and broken kernels.

PLATE I

THIN WHEAT TESTER

TIMER

MOTOR

GEAR DRIVE
120 RPM

METAL REEL

LID

DRAWER

.076 x 3/4 in. OPENINGS
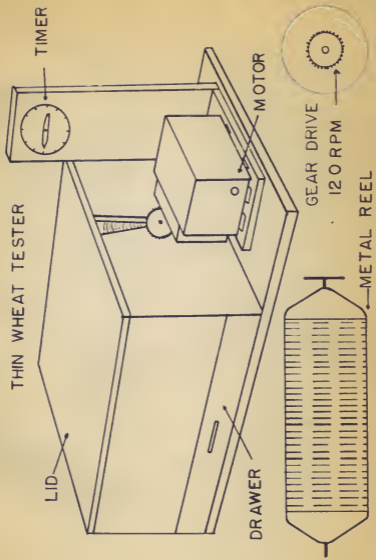
The volumes were then calculated as part of total volume. This procedure was repeated several times for each variety and the mean relative amounts of each portion added to the mean of the epidermis and cross layer values recorded. This correction was accurate enough for application to the amount each portion represented of the kernel but was not applied to ash and pentosan analysis. All analyses on testa were recorded as composite.

Extractions were repeated on each variety until enough material had been obtained for analysis. This amounted to about ten extractions for each variety. The epidermis, hyaline-aleurone and endosperm were ashed according to the method recommended in Cereal Laboratory Methods (Fifth edition, 1947). The cross layer and testa portions required special treatment. Ash of these two was obtained at 600°C with pre-treatment of glycerol-alcohol as described in Cereal Laboratory Methods (Fourth edition, 1941). The ash from cross layer, testa and endosperm samples was saved for sodium analysis. Quantitative sodium determinations were made by the spectrographic method of Morris, Pascoe and Alexander (1945) using 10 milligram samples. The sodium determined, calculated as sodium oxide, was deducted from the ash value.

Starch was determined in endosperm and hyaline-aleurone composite by the polarimetric method described in Cereal Laboratory Methods (Fifth edition, 1947). Calculation of pure hyaline-aleurone fraction was made on a starch basis and corrections applied to ash and pentosan.

EXPERIMENTAL

Investigations concerning ash-pentosan relation were start-
ed by analysis of patent grade flours according to the method
of Vernon and Metzner (1941). A ground glass jointed apparatus
was used; the distillate was divided into aliquots and assay
made by the bromate method. Samples of flour were chosen with
sufficient range of ash, without regard for varietal source or
chemical constituent values other than ash. Table 1 lists the
data of these tests and Fig. 2 illustrates the regression line.
The significant value of $r = +0.848$ indicates that such an ap-
proach would be worthy of further study.

Reviewing literature on pentosan values for flour, it ap-
pears that these values are slightly higher than generally ac-
cepted. Jacobs and Rask (1920) reported 2.95 to 3.20 per cent
pentosan on a 13 per cent moisture base. The upper limit of
their values is near the mean of this study. Local charring,
which was noticed, may be the cause of this difference. Since
aliquots and the use of singular equipment made such determin-
ations laboriously slow, further studies were made with equip-
ment previously described. The use of electric heaters eliminat-
ed local charring and permitted duplicate samples. The entire
distillate was used, avoiding dilution and aliquot errors.
Hughes and Acree (1938) point out that the use of rubber stoppers
caused an average error in the titration of only 0.04 to 0.06 ml
of 0.1 N sodium thiosulphate. Interviews with commercial lab-
oratories substantiated the use of such equipment.

Table 1. Comparison of ash-pentosan values of some samples of patent flour.

Sample no.	Moisture per cent	Ash* per cent	Pentosan per cent wet	Pentosan per cent dry	Variety
238	14.0	0.35	2.67	3.10	Triumph
241	13.7	0.36	2.89	3.36	Triumph
243	13.5	0.39	2.98	3.46	Triumph
244	13.3	0.40	3.35	3.86	Triumph
Com. 1	12.2	0.40	3.05	3.47	Com. mix
249	11.5	0.41	3.46	3.91	Early Blackhull
Com. 2	10.8	0.41	3.09	3.46	Com. mix
239	12.8	0.43	3.31	3.80	Triumph
517	14.0	0.46	3.34	3.82	Comanche
501	15.0	0.48	3.46	4.06	Chiefkan
509	13.4	0.49	3.42	3.96	Kharkof
276	13.5	0.52	3.50	4.04	Red Chief
504	13.8	0.55	3.86	4.47	Martin-Tenmarq x Chiefkan

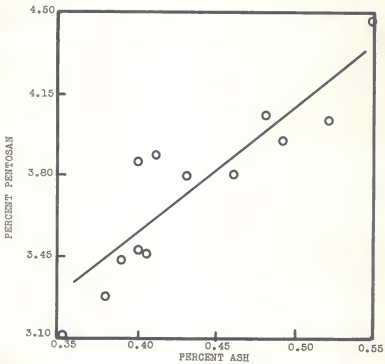* Reported on 14 per cent moisture basis.

Fig. 2. The relation of pentosan to ash in some patent flours.

In order to check the accuracy of the determinations under these conditions, comparisons were made with the phloroglucinol method. A sample of flour was subjected to the same distillation procedure and the furfural mixtures of same volume (360 ml) were determined by two methods. Also, distillations of less than the normal amount were made to fix the length of hydrolysis time. The bromine method (Table 2) gave results slightly higher than the mean of the phloroglucinol method. Volumes of 300 ml and 270 ml showed significant underestimations and lower precision due to comparatively higher production of furfural at these points.

Analyses were extended to samples of "clear" flour with ash values ranging from 0.60 to 1.20 per cent. Flour of this type is considered of poor quality, and contains greater amounts of branny material. This would indicate higher pentosan and ash values. Results show a slight rise in mean of pentosan, (Table 3 and Fig. 3) but a much lower correlation coefficient, $r = +0.138$. Later work corroborated these results.

Analysis of Mill Streams

The next step in this investigation consisted of the comparison of various flour streams. In this study, samples were taken from the 150-sack mill of Kansas State College during normal operation. The wheat was of the Pawnee variety milled at 16 per cent moisture. Table 4 shows results of mill streams ordinarily used in patent flour. Since the right and left portions

Table 2. Comparison of two methods of determination and study
         of arbitrary end point of hydrolysis.

| Moisture : | Ash | : Distillate : | Pentosan per cent: | | |
| per cent : | per cent : | ml. | : Wet : | Dry | : Average |

		Phloroglucinol method			
10.8	0.43	360	3.01	3.37	3.39
10.8	0.43	360	3.04	3.41	

		Bromine method			
					Difference
10.8	0.43	360	3.08	3.45	+ 0.08
10.8	0.43	360	3.09	3.48	+ 0.07
10.8	0.43	300	2.87	3.22	- 0.17
10.8	0.43	300	2.94	3.30	- 0.09
10.8	0.43	270	2.69	3.02	- 0.37

Table 3. Comparison of ash-pentosan values of some samples of "clear" flour.

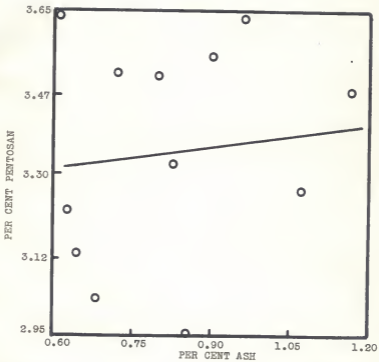| Sample no.	Moisture per cent	Ash* per cent	Pentosan per cent	
			Wet	Dry
197	12.8	0.61	3.17	3.64
177	12.2	0.63	2.82	3.21
192	11.9	0.65	2.76	3.13
189	11.6	0.68	2.69	3.04
191	11.0	0.72	3.13	3.52
241	12.1	0.80	3.10	3.53
203	11.7	0.82	2.94	3.33
131	11.6	0.86	2.62	2.96
205	11.7	0.90	3.14	3.56
203	11.8	0.96	3.22	3.65
212	11.8	1.07	2.88	3.27
246	11.1	1.16	3.04	3.42

* 14 per cent moisture basis.

Fig. 3.   Relation of pentosan to ash in some clear
flour samples.

Table 4. Comparison of ash-pentosan values of patent flour streams.

Stream		Moisture : per cent	Ash* : per cent	Pentosan per cent	
				Wet	Dry
Third break	R	13.5	0.44	2.24	2.58
	L	13.7	0.44	2.24	2.60
Sizing	R	13.4	0.32	2.29	2.64
	L	13.8	0.32	2.38	2.76
First middling	R	13.0	0.32	2.63	3.02
	L	13.3	0.34	2.45	2.82
Second middling	R	13.1	0.28	2.41	2.77
	L	12.8	0.29	2.46	2.82
Third middling	R	12.1	0.32	2.63	2.99
	L	12.4	0.32	2.61	2.98
Fourth middling	R	11.7	0.34	3.04	3.44
	L	11.6	0.37	3.10	3.50
Flour	R	12.2	0.40	3.05	3.47

* 14 per cent moisture.

of the streams were nearly identical, in actuality there are only seven values to which statistical analysis could be applied. Therefore a correlation coefficient is not practical. Interesting to note is the duplication of results on random sampling. There was little significant difference in pentosan values, except for fourth middling stream, which was notably higher. The overall analysis was in the range of other patent flours.

Another sampling was made at a later date of all the streams during the milling of the same variety of wheat under the same conditions. Results of this sampling (Table 5) show a low pentosan value of first break, with the following breaks converging around three per cent. The first three middlings streams of low ash show corresponding low pentosan content. Fourth and fifth middling streams have high values as noted previously (Table 4). The low grade streams of second tailings and reels show high ash and pentosan values. These observations are general and the calculated correlation is $r = + 0.398$; and though the poor grade streams are higher in pentosan, the relation to ash is only slightly significant (Fig. 4). Also, though the pentosan value of third break on Table 5 is higher than that of Table 4, the corresponding ash value was also higher. Analogous streams from both sampling sets are comparatively the same.

Relation of Particle Size to Pentosan

Another quality factor of flour was investigated for possible pentosan relation. Samples of flour fractions produced

Table 5. Comparison of ash-pentosan values of flour streams.

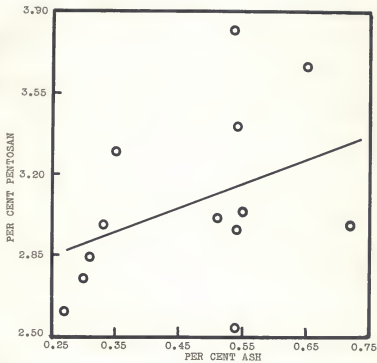| Stream	Moisture per cent	Ash* per cent	Pentosan per cent Wet	Pentosan per cent Dry
First break	13.8	0.54	2.17	2.52
Second break	13.0	0.55	2.68	3.08
Third break	13.6	0.51	2.63	3.04
Fourth break	13.0	0.72	2.61	2.99
Sizings	13.8	0.33	2.58	2.99
First middling	13.8	0.31	2.46	2.85
Second middling	13.0	0.27	2.27	2.61
Third middling	13.3	0.30	2.40	2.76
Fourth middling	11.8	0.35	2.92	3.31
Fifth middling	10.9	0.53	3.41	3.83
First tailing	12.4	0.54	2.56	2.92
Second tailing	11.0	0.55	3.04	3.41
First and second reels	11.0	0.65	3.26	3.66

* 14 per cent moisture.

Fig. 4. Relation of pentosan to ash in straight grade flour streams.

by the Ro-Tap sieve method of Wichser et al. (1947) were procured. These samples were of a flour milled from hard red winter wheat, 0.47 per cent ash on 14 per cent moisture basis. Comparison of ash-pentosan values showed no particular relation (Table 6), nor was there any striking relation of pentosan to particle size (Fig. 5), except for the high value of the 88-105 micron fraction. Below this size range there was little or no evidence of branny material. The trend below 53-61 micron size was a general falling pentosan value with decreasing particle size.

Baker, Parker and Mize (1943) in their study of small starch particles, found a high pentosan content associated with small granules.

In this investigation an effort has been made to study the smaller size fraction using a sifting method. It is assumed that when a substance of heterogenous particle size is sifted, the smaller particles are forced through the openings first. This assumption, coupled with the contention of Baker, Parker and Mize (1943) that pentosans are associated with small starch granules, might be expected to show that the first material through a 0-38 micron sieve has a higher pentosan value than the overs on the sieve.

Sifting was done for 0.5 minute and the "thrus" collected. Disturbing the sieve as little as possible, sifting was continued another 0.5 minute and the stock collected. Thereafter at each additional minute interval the "thrus" were collected. This was continued for a total of five minutes. The process was repeated until sufficient material was accumulated for analysis.

Table 6. Comparison of ash-pentosan values with particle
size fractions of a particular hard red winter
wheat flour.

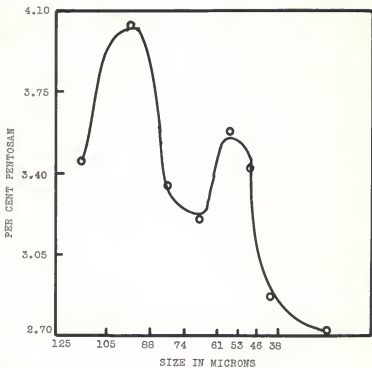| Size range microns	Moisture* per cent	Ash* per cent	Pentosan per cent	
			Wet	Dry
Flour	10.6	0.47	3.02	3.36
105-125	9.5	0.36	3.12	3.45
88-105	8.7	0.37	3.70	4.05
74-38	8.9	0.37	3.06	3.36
61-74	9.2	0.38	2.91	3.20
53-61	9.2	0.41	3.19	3.52
46-53	9.4	0.41	3.12	3.44
38-46	8.7	0.53	2.60	2.85
0-38	10.2	0.51	2.45	2.73

* 14 per cent moisture basis.

Fig. 5. Comparison of pentosan value to particle size.

Table 7 and Fig. 6 show the highest pentosan value at two minutes of sifting. The values converged around the value for the 0-38 micron size (Table 6). The constant decrease with time indicates that the smaller particles are higher in pentosan content.

Table 7. Relation of pentosan value to sifting
          time through a 400 wire, 0-38 micron
          sieve.

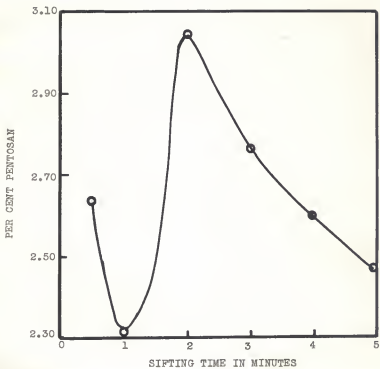| Sifting time : minutes | Moisture : per cent | Pentosan per cent | |
		Wet	Dry
0.5	10.1	2.36	2.65
1	10.3	2.08	2.32
2	10.7	2.72	3.05
3	10.2	2.46	2.75
4	10.2	2.32	2.59
5	10.4	2.22	2.47

Fig. 6. Relation of pentosan to sifting time of a
flour through a 400 wire, 0-38 micron sieve.

A Study of Individual Bran Layers and Endosperm

The technique of Shetlar et al. (1947) offers a convenient method for obtaining component parts of the wheat kernel and provides a means of studying varietal characteristics. The object of these separations was to gather sufficient material to expand the ash-pentosan studies beyond observations on flour and whole wheat.

The separation procedure was applied to 10 varieties of hard red winter wheat from the 1947 crop. The varieties studied were: 1. Tenmarq; 2. Wichita; 3. Comanche; 4. Pawnee; 5. Early Blackhull; 6. Kawvale; 7. Westar; 8. Triumph; 9. Red Chief, and 10. Chiefkan x Oro-Tenmarq. In the tables the varieties are referred to by number only.

The percentages of kernel represented by each layer separated are listed in Table 8. Table 9 represents the per cent of layers expressed as part of total bran. The figures for each variety are the mean of approximately 10 extractions, and are in agreement with those of Shetlar and associates, with some variation because of the greater number of varieties studied.

In processing, varietal differences were noted as to ease of separation. Though these observations are not pertinent to chemical studies, they are of interest as to physical characteristics. The cross layer of Early Blackhull removed easily, but the separation of testa layer was difficult and extra stirring was needed. This was also true of Red Chief and Chiefkan x Oro-

Table 8. Percentage distribution of individual bran layers
of some wheat samples, expressed as per cent of
kernel.

| Sample no. | Per cent | | | | |
	Epidermis	Cross layer	Testa	Hyaline aleurone	Bran
1	3.41	0.58	0.38	10.42	14.79
2	3.14	0.66	0.20	10.23	14.23
3	3.51	0.68	0.32	—	—
4	3.39	0.82	0.44	9.68	14.33
5	3.31	1.51	0.45	9.62	14.89
6	2.75	0.83	0.49	9.13	13.20
7	2.63	0.63	0.33	9.56	13.15
8	2.94	0.87	0.61	10.08	14.50
9	3.10	0.92	0.62	8.58	13.20
10	3.29	0.53	0.21	—	—

Table 9. The distribution of individual bran layers calcu-
lated as per cent of total bran.

Sample no.	Per cent			
	Epidermis	Cross layer	Testa	Hyaline-aleurone
1	23.06	3.92	2.59	70.45
2	22.07	4.64	1.41	71.89
3	-	-	-	-
4	23.66	5.72	3.07	67.55
5	22.77	10.15	3.02	64.61
6	20.83	6.29	3.71	72.42
7	20.00	4.79	2.51	72.70
8	20.28	6.00	4.21	69.51
9	23.45	6.96	4.69	64.90
10	-	-	-	-
Mean of samples	22.02	6.06	3.15	69.25

Tenmarq, to a lesser degree. Triumph was particularly difficult, an additional 16 hours of soaking in sodium hydroxide-alcohol solution being necessary in order that the testa layer could be separated with stirring. Samples of Kawvale and Westar needed careful handling during cross layer separation to prevent testa contamination. Other varieties separated without difficulty. Plates II and III are photomicrographs of separated material showing the cell structure by which individual layers are identified.

Analyses were made of the original wheat and of some samples after removal of epidermis, cross layer and testa (Table 10). The correlation of original wheat ash to pentosan was + 0.252, (Fig. 7). The pentosan values were in agreement with those reported for wheat.

The epidermis, after air drying, was analysed for ash and pentosan constituents. These findings for the air dried material are listed in Table 11. The range of ash is from 0.90 per cent to 1.45 per cent with the mean at 1.04 per cent. Pentosan range is from 43.56 per cent to a high of 48.40 per cent. The pentosan value is higher than previous reports, but recheck of samples showed no change of original data. There is a significant correlation of + 0.626 for ash-pentosan values of the epidermal material (Fig. 8). The ash values for Early Blackhull and Kawvale were particularly high. These data indicate a possible answer to the low correlation in clear flour. The ash value of the epidermis is only slightly higher than ash of clear flour, but the pentosan value is exceedingly high in comparison.

Fig. 1. Photomicrograph of epidermis, approx-
imate magnification 225 x.

Fig. 2. Photomicrograph of cross layer cells,
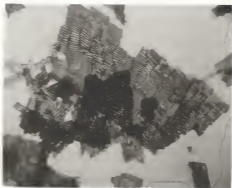approximate magnification 500 x.

PLATE II

Fig. 1.

Fig. 2.

EXPLANATION OF PLATE III

Fig. 3. Photomicrograph of testa composite show-
ing testa, cross layer and epidermis par-
ticles, approximate magnification 500 x.

Fig. 4. Photomicrographs of hyaline-aleurone com-
posite showing endosperm contamination,
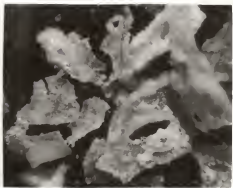approximate magnification 225 x.

PLATE III

Fig. 3.

Fig. 4.

Table 10. Comparison of ash and pentosan content of original
wheat samples, and ash content of wheat minus epi-
dermis, cross layer and testa.

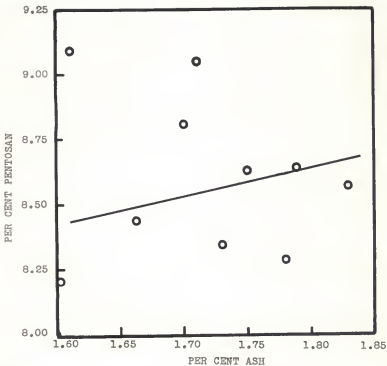| Sample no.	Moisture per cent	Ash* per cent	Pentosan per cent (dry)	De-branned wheat Moisture:	Ash*
1	11.5	1.75	8.64	11.6	1.73
2	11.7	1.73	8.35	12.8	1.89
3	11.7	1.71	9.06	-	-
4	11.6	1.60	8.21	12.5	1.23
5	11.8	1.63	8.57	12.2	1.22
6	11.8	1.78	8.29	13.0	1.27
7	11.6	1.62	8.00	13.2	1.25
8	11.9	1.66	8.44	11.5	1.24
9	11.6	1.61	9.11	13.9	1.23
10	12.0	1.70	8.61	11.8	1.25

* 14 per cent moisture basis.

Fig. 7. Relation of pentosan to ash in original wheat.

Table 11. Comparison of ash and pentosan content
of air-dried epidermis.

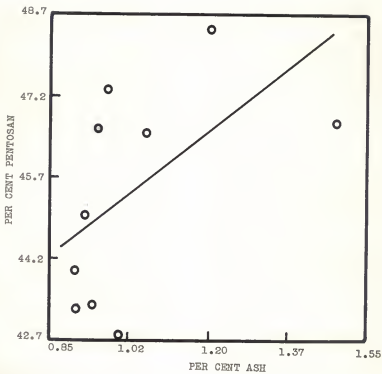| Sample no.	Ash per cent	Pentosan per cent
1	0.90	44.05
2	0.94	43.62
3	0.90	43.56
4	1.00	42.72
5	1.20	48.40
6	1.49	46.67
7	1.08	46.58
8	0.98	47.30
9	0.96	46.62
10	0.93	45.04
Mean	1.04	45.48

Fig. 8. The relation of pentosan to ash in epidermis material.

Small amounts of bran contamination to flour in the form of "beeswing" or epidermis would vary the pentosan value far in excess of the effect on ash. This also may be a reason for wide variation in clear flour pentosan values.

Cross layer samples were incinerated and weight of ash recorded. Ash samples of 10 mg were weighed and spectrographic quantitive analyses for sodium were made according to the procedure of Morris, Pascoe and Alexander (1945). Sodium correction, calculated as sodium oxide, was deducted from weight of ash and true ash calculated. This was necessary because of the large amount of sodium hydroxide absorbed during separation. From Table 12 it can be seen there is little indication of positive correlation. The ash values vary greatly from a high of 17.47 per cent for Tenmarq to 5.61 per cent low for Triumph. The values of samples one to four are much higher than others, and this factor appears in other analyses.

The testa layer composites containing some epidermis and cross layer cells (Plate III, Fig. 3), were analyzed in the same manner for ash and pentosan. The sodium correction was applied but no attempt was made to apply the microscopic technique values to determine by calculation the true ash and pentosan values of pure testa layer. Qualities, similar to cross layer, are apparent in the testa composite (Table 13). The variation of ash is not as extreme and samples one to four are higher again. There is no positive correlation; the ash values vary greatly in comparison to the fairly uniform pentosan values.

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Table 12. The per cent of ash, corrected for absorbed sodium hydroxide, and pentosan of cross layer samples.

Sample no.	Moisture: per cent	Ash : per cent	Sodium : mg./10 mg. ash	Ash* : per cent	Pentosan : per cent
1	12.2	23.15	1.72	17.47	27.67
2	11.7	16.75	1.38	13.40	30.93
3	10.8	14.08	1.33	11.13	33.38
4	10.3	12.78	1.34	9.80	35.95
5	11.1	10.08	1.43	8.45	39.20
6	10.1	9.75	1.37	7.64	37.38
7	10.2	7.97	1.32	6.33	39.10
8	9.4	7.16	1.27	5.61	43.35
9	10.2	7.87	1.44	6.06	38.20
10	9.1	8.17	1.43	6.25	38.95

* 14 per cent moisture basis.

Table 13. The per cent of ash, corrected for absorbed sodium hydroxide, and pentosan of testa composite samples.

Sample no.	Moisture: per cent	Ash : per cent	Sodium : mg./10 mg. ash	Ash* : per cent	Pentosan : per cent
1	11.9	18.88	1.24	15.46	32.25
2	12.2	19.71	1.34	15.74	30.03
3	12.3	20.62	1.36	16.51	28.04
4	11.2	15.75	1.14	12.93	32.29
5	11.2	13.95	1.43	10.85	36.00
6	11.4	13.12	1.35	10.63	33.12
7	11.0	11.41	1.18	10.34	37.31
8	10.6	10.48	1.16	8.52	37.24
9	10.8	10.61	1.39	8.33	35.22
10	10.5	10.42	1.21	8.48	36.06

* 14 per cent moisture basis.

To obtain samples of hyaline-aleurone layers, the de-branned wheat was tempered to 15.5 per cent moisture and milled on the Buhler experimental mill. The hyaline-aleurone fraction corresponds to bran scalp and endosperm to straight grade flour. No attempt was made to grind for a high flour yield. The reason for this was to give as good grade of flour as possible and keep possible bran contamination to a minimum. Since it is nearly impossible to obtain pure bran in this manner the amount of endosperm remaining with the hyaline-aleurone portion was not of importance. The amount of endosperm adherent to bran was calculated by starch analysis. Except for matter remaining in the crease of the wheat, the hyaline-aleurone will be contaminated by endosperm and some germ (Plate III, Fig. 4).

Ash, pentosan and starch were determined in the endosperm and hyaline-aleurone composite. Assuming the starch of the endosperm and endosperm contaminating the hyaline-aleurone are the same, the amount of endosperm correction for the hyaline-aleurone composite can be calculated since the pure hyaline-aleurone is void of starch. The ash and pentosan of the endosperm being known, and the true amount of hyaline-aleurone having been calculated, corrections for true ash and pentosan of the hyaline-aleurone can be calculated. The composites contained an average of 30 per cent endosperm contamination.

The corrected values of ash and pentosan agree with those reported in literature (Table 14). Though there is no positive correlation of ash-pentosan the values for both are rather constant. These layers constitute the major portion of the total

Table 14. Comparison of ash and pentosan values of
hyaline-aleurone bran layers.

Sample no.	Moisture per cent	Ash[e] per cent	Pentosan per cent (dry)
1	13.2	7.23	27.30
2	13.0	8.35	27.12
3	13.1	8.08	24.46
4	13.3	5.68	26.63
5	12.9	5.45	27.65
6	12.7	4.79	27.43
7	13.0	4.72	29.05
8	13.0	5.99	30.37
9	12.6	6.55	29.39
10	11.8	6.71	27.67

e 14 per cent moisture basis.

bran, approximately 69 per cent, and contribute the largest amount of ash and pentosan of entire kernel, approximately 40 and 30 per cent respectively. The position of these layers with respect to the endosperm indicates they are the major factor affecting ash and pentosan of flour. This is particularly true of the aleurone layer.

In the analysis of endosperm or flour it was noticed that the ash values were considerably higher than found in normally milled samples. Also, ashing difficulties were encountered similar to that experienced when the samples contained high sodium.

Though the de-branned wheat had been washed thoroughly with dilute acid and water to remove excess sodium deposites in the hyaline-aleurone fraction, it was possible the sodium had penetrated to the endosperm. Spectrographic analysis of flour ash confirmed this. Morris, Pascoe and Alexander (1945) report approximately 0.6 per cent of the ash of endosperm as sodium. The flour samples of this study contained from less than 5 per cent to more than 10 per cent of ash as sodium (Table 15). Therefore corrections were applied as on cross layer and testa. The corrected ash values were slightly higher than those of normally milled flours. Samples one through four were again the highest values. No positive correlation of ash-pentosan values was apparent, but one particular point was quite evident. The pentosan values for these flour samples were consistently lower than any previous pentosan determinations on flour in comparable ash range.

Table 15. Comparison of ash-pentosan values of flour from de-branned wheat.

Sample no.	Moisture: per cent	Ash per cent	Sodium per cent/10 mg. ash	Ashe per cent	Pentosan per cent Wet	Dry
1	13.3	0.73	1.03	0.62	2.47	2.85
2	13.1	0.80	1.08	0.67	2.34	2.69
3	13.0	0.88	1.03	0.75	2.24	2.57
4	13.0	0.82	1.01	0.70	2.06	2.37
5	13.0	0.68	0.92	0.78	2.56	2.94
6	13.2	0.70	0.87	0.61	2.40	2.76
7	13.2	0.64	0.83	0.54	2.42	2.79
8	13.2	0.60	0.72	0.55	2.16	2.49
9	12.6	0.61	0.71	0.59	2.41	2.76
10	13.1	0.60	0.49	0.57	2.52	2.90

e 14 per cent moisture basis.

In order to validate these observations, samples of the original wheat were cleaned, scoured and "thinned", tempered to 15.5 per cent moisture and milled on the Buhler experimental mill in the same manner as the de-branned wheat. Results of pentosan determinations on normally milled flour show the values to be higher than the flour of the de-branned wheat (Table 16). Again it was noted that the ash was highest in flour samples number one to four (Table 16). Comparison of Tables 15 and 16 indicates a constant loss of 0.4-0.5 per cent pentosan except for sample six.

At least two explanations might be offered. Milling properties of the de-branned wheat may be responsible for the difference. The second is the possible loss of soluble pentosans, which would disrupt the ash-pentosan correlation. Baker, Parker and Mize (1943) indicate approximately 0.06 gram of soluble pentosan in five grams of flour. On a percentage basis, this value is about twice the difference of values of the de-branned wheat flour and normally milled flour.

There was no evidence of a positive correlation in the de-branned wheat flour, but a correlation coefficient of +0.551 was evident in normally milled flours from the same wheat (Fig. 9). The relation is similar to Fig. 2, although not so pronounced. The increase of pentosan with increasing ash value was not so large as the relationship of Fig. 2. The mean pentosan value of the variety study was less, and the range was smaller.

Table 16. Comparison of ash-pentosan values of flour from untreated wheat.

Sample no.	Moisture per cent *	Ash* per cent	Pentosan per cent Wet	Dry
1	12.2	0.59	2.90	3.30
2	12.5	0.54	2.99	3.30
3	12.2	0.55	2.79	3.18
4	12.3	0.56	2.53	2.90
5	12.6	0.53	2.93	3.35
6	12.2	0.49	2.90	3.32
7	12.9	0.44	2.66	3.05
8	12.7	0.45	2.74	3.13
9	12.7	0.49	2.99	3.42
10	12.8	0.53	3.07	3.52

* 14 per cent moisture basis.
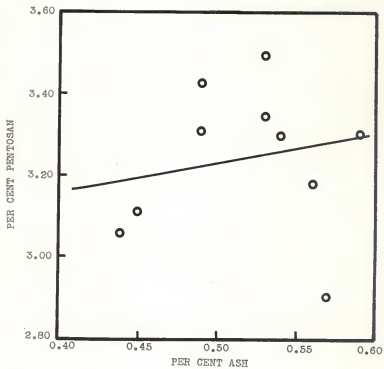
Fig. 9. Relation of pentosan to ash in flour of ten wheat varieties.

QUANTITATIVE ESTIMATION OF PENTOSES WITH NAPHTHORESORCINOL

A study was made of possible approaches to a direct estimation of pentose sugars and pentosans without the formation of furfural. Tollens (1908) reported a qualitative test for galacturonic acid using naphthoresorcinol as the color producing agent. Mandel and Neuberg (1908) extended the work and noted interference of pentose sugars. Neuberg and Kobel (1931) classified colors produced by several sugars in various solvents. Maughan, Evelyn and Browne (1938) determined galacturonic acid quantitatively with naphthoresorcinol using an ether-ethyl alcohol mixture as the solvent. Ratish and Bullows (1943) established the insolubility of the pentose polymer in ether-alcohol mixture. Messier (1945) made extensive galacturonic determinations with naphthoresorcinol. His procedure was adapted to this study. The method employed to determine pentose sugars is as follows.

To Pyrex test tubes were added one ml of sugar solution, one ml of 0.5 per cent aqueous naphthoresorcinol, 0.5 ml of distilled water and 2.5 ml of two normal hydrochloric acid. Tubes were then immersed in boiling water for 35 minutes. After cooling, two ml of 10 per cent sodium hydroxide were added and let stand 10 minutes. Twenty-five ml of solvent were added and tubes shaken and then centrifuged to remove water. Solvent was pipetted off and transmission read on suitable colorimeter at 550 mμ wave length.

Arabinose, c.p. pectin sugar and l-xylose (dextrorotatory) were used. Because the pentose-naphthoresorcinol polymers are

insoluble in liquids used for galacturonic acid analysis, solu-
bility tests for pentose polymer were necessary (Table 16). All
tubes contained 30 mg of arabinose, and tubes number one to seven
were handled as indicated in the method outlined. Tubes number
8 to 14 had a final addition of two ml of concentrated hydro-
chloric acid before addition of solvent. This step is recom-
mended for galacturonic acid. The elimination of acid addition
had no apparent effect on pentose polymer. Normal butyl alcohol,
iso-butyl alcohol and ethyl acetate were the best solvents.
Acetone was a good solvent but is miscible with water and there
is the possibility of interference by acetone-furfural conden-
sation. The same purple color was found in all solvents.

Absorption curves were determined with a Coleman spectro-
photometer using pure solvent as the blank. Plate IV illustrates
the absorption curve of xylose polymer in three solvents, and
Plate V the absorption curve of arabinose polymer with normal
butyl alcohol solvent. The wave band of maximum absorption is
500-600 mμ. This agrees with the findings of Messier (1945),
who recommended 500 mμ.

Solutions of various sugar strengths were prepared and con-
centration-absorption data plotted (Figs. 10 and 11). The dif-
ference between xylose and arabinose was not anticipated. Since
only four strengths of arabinose were used the upper part of the
curve is of doubtful value.

A new set of standards was prepared with a greater number of
concentrations below 30 mg. The Klett Fluorimeter-colorimeter
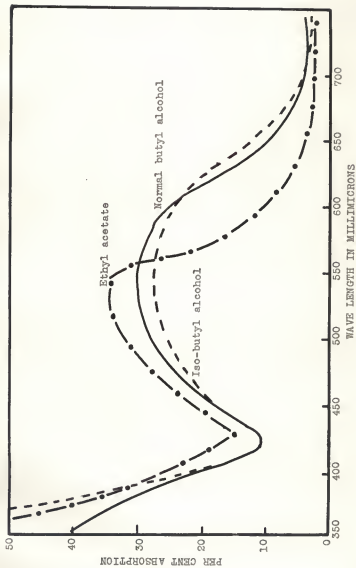was used with the 546 mμ wave band isolated. Figures 12 and 13

Table 16. Results of solubility tests of arabinose-naphthore-
           sorcinol polymer.

Without final acid			With final acid		
Tube no.	Solvent	Class	Tube no.	Solvent	Class
1	N-Butyl alcohol	V. Sol.	8	N-Butyl alcohol	V. Sol.
2	Iso-butyl alcohol	V. Sol.	9	Iso-butyl alcohol	V. Sol.
3	Toluene	Insol.	10	Toluene	Insol.
4	Benzene	Insol.	11	Benzene	Insol.
5	Carbon disulfide	Insol.	12	Carbon disulfide	Insol.
6	Ethyl acetate	V. Sol.	13	Ethyl acetate	V. Sol.
7	Acetone	V. Sol.	14	Acetone	V. Sol.

EXPLANATION OF PLATE VI

Absorption curves of the xylose-

naphthoresorcinol polymer in various solvents.

PLATE IV

Figure showing per cent absorption versus wave length in millimicrons for Ethyl acetate, Normal butyl alcohol, and Iso-butyl alcohol.

EXPLANATION OF PLATE V

The absorption curve of the arabinose-
naphthoresorcinol polymer in normal butyl
alcohol solvent.

PLATE V

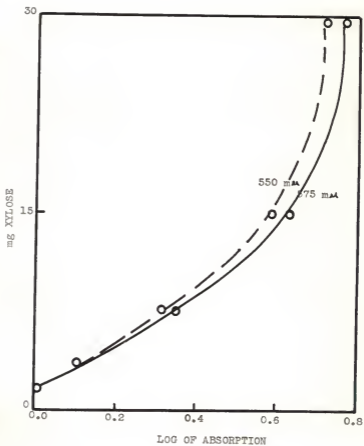WAVE LENGTH IN MILLIMICRONS
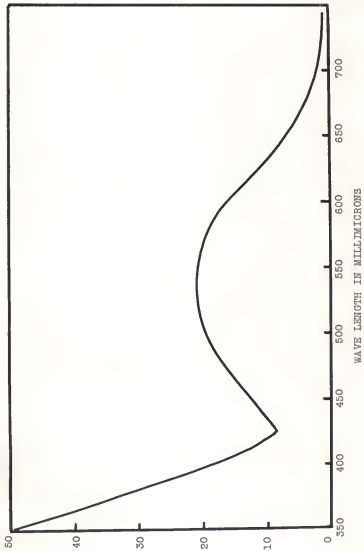
Fig. 10. Absorption-concentration curves of xylose-naphthoresorcinol polymer at two wave lengths.
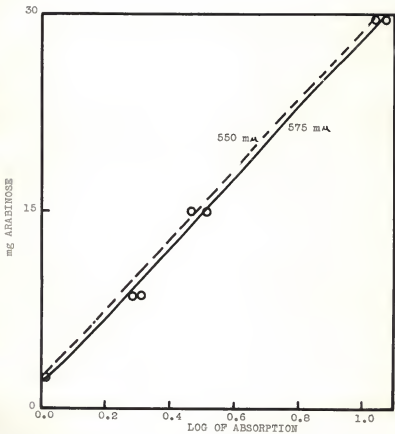
Fig. 11. Absorption-concentration curves of arabinose-naphthoresorcinol polymer at two wave lengths.
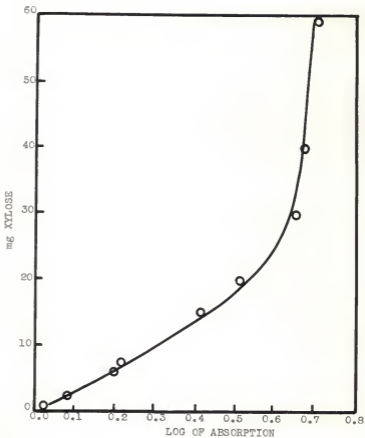
Fig. 12. Extended absorption-concentration curve of
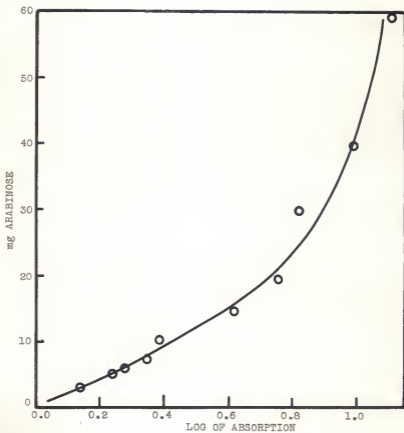xylose-naphthoresorcinol polymer.

Fig. 13. Extended absorption-concentration curve of arabinose-naphthoresorcinol polymer.

represent a more accurate working curve. The curve follows
Beer's law below 20 mg of either sugar. The deviation above this
value is attributed to lack of reagent. The polymer does not
form on a molecular weight basis and the practical limits of
naphthoresorcinol can only be determined by experiment.

The effect of hexose sugars is considered to be negligible.
Tests were made using a constant strength of xylose (10 mg) and
various concentrations of glucose (Table 17). The maximum error
was five per cent between extreme readings.

Application of Naphthoresorcinol Test to Flour

Hauser and Brunner (1922) indicate maximum yield of pentose
from xylans of bleached straw cellulose with 12 per cent hydro-
chloric acid in 5 to 10 minutes, and 30 minutes with 5 per cent
hydrochloric acid at 90°C. Testoni (1917) claimed complete
hydrolysis of pentosans of flour with 90 per cent acetic acid
and 10 per cent hydrochloric acid mixture. Keller (1940)
patented the use of 0.5 per cent solution of paratoluene sul-
fonic acid at two atmospheres pressure, yielding 85 per cent
hydrolysis of wheat straw xylans. Studies were made of hydro-
lysis conditions and polymerization of pentose and naphthoresor-
cinol in flour suspensions. A flour of known pentosan content
was used throughout these tests.

One gram of flour was placed in a 125 ml Erlenmeyer flask.
Five ml of water, 25 ml of two normal hydrochloric acid, and one
ml of 0.5 per cent aqueous naphthoresorcinol were added and the

Table 17. The effect of glucose upon the absorption readings of xylose-naphthoresorcinol color.

Tube no.	l-Xylose mg	Glucose mg	Logarithm of absorption	Absorption per cent
1	10	1.25	0.156	14.3
2	10	2.50	0.137	13.7
3	10	3.75	0.136	13.7
4	10	5.00	0.158	14.4
5	10	10.00	0.143	13.9
6	10	none	0.137	13.7

flask set in a water bath at 90°C. After hydrolysis (time varied)
the suspension was cooled and 20 ml of 10 per cent sodium hydro-
xide added and allowed to stand 10 minutes. The entire amount
was transferred to a centrifuge tube. The flask was washed with
25 ml of water and this added to the tube. The suspension was
centrifuged at 1800 r.p.m. for five minutes, supernatant liquid
decanted and 25 ml of normal butyl alcohol added. After shak-
ing, the material was centrifuged again, and solvent decanted.

The supernatant liquid from the first centrifuge and the
solid residue of the alcohol extraction were combined and pento-
san determined by the phlorglucinol method. Table 18 shows
that only a small fraction of the total pentosan reacted as
pentose with naphthoresorcinol. The use of double strength naph-
thoresorcinol showed some increase in reaction during short hydro-
lysis. All alcohol extracts were too dark for colorimetric read-
ings. This was attributed to long heating of the polymer. The
same test was repeated varying the hydrolysis time and adding
reagent for a short constant time (Table 19). The amount of
recovered pentosan was less and the color produced in the solvent
was light enough for comparison. The color of the solution was
not violet as with the pure sugar, but a green-brown color.
Spectrographic absorption analysis indicated no special point of
maximum absorption. The presence of the flour during extraction
may have contributed an interfering factor.

To test this a flour suspension was hydrolyzed for 40 min-
utes and filtered through Filtercel and washed with hot water.
To this clear pentose extract the naphthoresorcinol was added and

Table 18. Comparison of pentosan recovery with variable hydrolysis time in the presence of naphthoresorcinol.

Hydrolysis time in hours	Per cent of total pentosan recovered (A)	Per cent reacted (100-A)
Five mg Naphthoresorcinol		
1	89.7	10.3
2	83.2	16.8
4	79.2	20.8
6	85.3	14.7
10 mg Naphthoresorcinol		
1	74.7	25.3
2	76.7	23.3
4	81.3	18.7
6	79.2	20.8

Table 19. Comparison of pentosan recovery with variable
hydrolysis time and constant reaction time with
naphthoresorcinol.

Hydrolysis time in minutes	Reaction time in minutes	Per cent pentosan recovered	Per cent reacted
		Five mg Naphthoresorcinol	
10	10	66.3	33.7
20	10	61.3	38.7
40	10	62.7	37.3
80	10	61.7	38.3
120	10	59.8	40.2
		10 mg Naphthoresorcinol	
10	10	62.7	37.3
20	10	63.0	37.0
40	10	59.0	41.0

heating continued for 10 minutes. The formation of the polymer was similar to the pure sugar but the color remained green-brown. High pentosan content flours gave darker extracts than the low pentosan flours, but precision was poor and no working curve could be determined.

Interfering compounds may be the cause of the color produced. It is also possible the polymer formed under these conditions is a molecule so large as to lose its color absorption qualities. Similar conditions can be produced by allowing the polymer to stand in sunlight or by heating for a long time.

DISCUSSION

The bromination method can be used for the determination of pentosans of flour and wheat. By standardizing the procedure and including the equipment and comparing the results with those obtained by official methods, the determination can be utilized for analysis of a large number of samples where precipitation methods would be too slow. Electric heating units should be used to insure constant temperature and to prevent local charring. For small samples and accurate studies, ground glass jointed equipment is recommended. The Hughes-Acree flask is convenient for addition of bromate and iodine solutions and prevents the loss of bromine.

Pentosan and ash were found to be significantly correlated in patent flours. In general, low ash flours are low on pentosan content. Above the ash range of approximately 0.60 per cent

the correlation is very small. Extreme variations of pentosan values exist in clear flours.

Flour stream analyses indicate that better grade flour streams are consistently low in pentosans, particularly flour from middling streams. Flour of tailing rolls and reels show high pentosan content. There is no apparent correlation of pentosan to granulation or the ash of particle size fractions in the flour studied. Micron ranges of 38-46 and 38-0 had significantly lower pentosan content than other fractions, while the ash was higher. There was some indication that the very small starch granules contain a large part of the pentosan of the 38-0 micron range in the flour analysed.

Individual bran layers were removed from 10 varieties of hard red winter wheat; the approximate percentage of the kernel represented by the layers removed was as follows: Epidermis, 3.1 per cent; cross layer, 0.8 per cent; hyaline-aleurone, 9.6 per cent; the endosperm constituted 86.1 per cent.

There was a marked cohesion between testa and hyaline layers of some samples. Triumph, Early Blackhull, and to a lesser degree, Red Chief, required particular care to remove the testa layer.

The hyaline-aleurone layers contributed the greatest part of the total ash and pentosan of the kernel. Great variations of ash values existed between different layers. Pentosans were more uniformly distributed throughout the bran layers, and the epidermis contained the greatest amount. Poor quality flour, due to bran contamination, would be more easily recognized by

the ash value than pentosan. The difference between endosperm ash and aleurone ash is greater than pentosan difference, but bran contamination by the epidermis could be recognized more readily by the pentosan value.

The results of this study indicated there is a varietal variation of individual layer ash value, but the pentosan values of all varieties were quite constant. Variation in ash was particularly noticeable in cross layer and testa layer. Correlation of ash and pentosan was found only in the epidermis and endosperm. The pentosan value can be used as an auxiliary test with ash to determine flour quality. This test would be particularly useful in corroborating high ash value due to bran contamination. Pentosan, along with starch, may be used to determine the point of yield loss in the mill.

Varietal differences were particularly noticeable in the hyaline-aleurone layers. Parallel ash values of the Buhler-milled samples and the hyaline-aleurone are apparent, samples six through eight are lowest. The ash values of the last five samples of both cross layer and testa were consistently lower.

The separation method used in this study could be used to study the distribution of mineral elements in the bran coats. The study of pentosans of flour warrants further investigations. Extraction of pentosans from flour and addition of increments might reveal the effect they have in baking; also the quantitative study during the baking process might indicate a function. Investigation of possible enzyme hydrolysis of pentosans would

be of commercial interest. Extension of the work of Horowitz-Vlassova and Novotelnov (1934) concerning the action of micro-organisms of pentosans of bran may answer some storage and wheat disease questions.

The study of pentose sugar determinations indicates that arabinose and xylose can be determined quantitatively by use of naphthoresorcinol reagent. Under the conditions described the effect of glucose was negligible in concentrations equal to the pentoses. The extent of polymerization appeared to be greater with arabinose as shown by higher absorption values than xylose under the same conditions. Tests show that approximately 20 mg of pentose sugar can be determined on a straight line relation with five mg of naphthoresorcinol.

The test was not applicable to flour under the conditions described. Lack of complete hydrolysis was apparent. Possible interfering compounds prevented the formation of the violet polymer that characterized the pure pentose.

SUMMARY

The adaption of a suitable method for numerous pentosan determinations of wheat and flour has been investigated. A study was made of the possible correlation of pentosan to mineral matter or ash. Consideration was given to different approaches to the determination of pentoses and pentosan. The results of this study demonstrate that:

1. The Hughes-Acree method for the determination of pentosans is adaptable to wheat and flour.

2. The coefficient of correlation for the patent flours analyzed was $r = +0.848$. Samples of clear flour showed a low correlation, $r = +0.138$.

3. Analyses of flour streams indicated no positive correlation. Streams of low ash value generally showed low pentosan content.

4. There was little relation of pentosan to ash in flour particle size fractions analyzed. Pentosan content decreased with particle size. There is indication that small starch particles contain a large amount of pentosan.

5. The study of individual bran layers demonstrated that the epidermis contains the highest percentage of pentosan. There appeared a positive correlation of $r = +0.626$ for pentosan and ash of this layer.

6. No correlation was evident in cross layer, testa, hyaline-aleurone layers or endosperm of the 10 samples obtained by the method outlined.

7. Flour produced by the Buhler mill of the same 10 samples had a pentosan-ash correlation of $r = +0.581$.

8. A method for determination of arabinose and xylose employing naphthoresorcinol is described. The method is restricted to the absence of hexuronic acids but is not affected by glucose in the amounts indicated.

9. Naphthoresorcinol was applied to hydrolyzed flour and flour extracts. Interfering color and incomplete hydrolysis under conditions described limited the usefulness of the test.

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