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Determination of Soluble and Insoluble Pentosans in Wheat Flour

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Summary: A method is given for the determination of soluble and insoluble pentosans in wheat flour. The method involves a modified hydrochloric acid distillation procedure followed by furfural colorimetric measurement. Arabinose produced the same colour as xylose but gave lower recoveries. L-Arabinose was found by paper chromatography to be present in substantial quantity in relation to D-xylose in the Queensland wheat flour.

Introduction

Although there is much published work on the effects[1] of soluble and insoluble pentosans on the baking quality of what flour, there is no work reported where the soluble and the insoluble pentosans have been separated and measured on a routine basis. A possible explanation for this is the lack of a suitable method for measuring pentosans in flour and wheat.

A number of furfural distillation methods[2] have been reported for pentosan estimation. The first available reference[3] seems to have appeared as early as 1891 but the Cerning and Guilbot[4] method using the Daffau[5] apparatus seemed to be the most reliable. When this method was applied in this laboratory some difficulties were experienced. These difficulties were overcome and the method modified.

In most of the distillation methods D-xylose and L-arabinose were generally assumed the give the same amount of furfural. The rate of conversion of the L-arabinose to furfural was found to be slower than the rate of D-xylose conversion. The intention of this work was to produce a rapid and routine method with good reproducibility for measuring pentosans in flour and wheat.

Experimental

2.1. Flour samples:

The 6 flours used in the study were obtained from 6 different wheat varieties grown in Queenslard and were milled on a Buhler experimental mill.

2.2. Method of extraction:

At room temperature 22°C: A flour sample (500mg) was mixed with water (20ml) in a SORVAL blender at high speed for 5 minutes. During this process the mixture was maintained at room temperature by immersion in a water bath. The mixture was then centrifuged until the supernatant liquid was clear. The clear solution was regarded as the soluble fraction and the residue as the water-insoluble fraction. At 40°C, the same procedure as above was followed except that the water in the mixer and in the water bath was at 40±2°C instead of room temperature.

2.3. Enzyme deactivation:

The flour sample (2.0g) was boiled with ethanol (50ml) for 30 minutes. Supernatant ethanol was decanted and the remaining ethanol was removed at 40°C on a rotary evaporator under vacuum.

2.4. Method of measuring pentosants:

2.4.1. Reagent

i) HCl: 4.2 ± .05N

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ii) Aniline: 1% soln. of freshly distilled aniline in 95% ethanol.

iii) Buffer: Anhydrous ammonium acetate (40g), stannous chloride $SnCl_2.2H_2O$ (2.7g) and stannic chloride $SnCl_4$ $5H_2O$ (4.2g) were dissolved in glacial acetic acid (133ml) and made to volume (240ml) with distilled water. The colour reagent[6] was prepared by mixing reagents (ii) & (iii) (2:1 by volume).

2.4.2. Apparatus

The original Daffau apparatus[5] was constructed all in one piece. In the present design ground glass joints were incorporated so that it was easy to clean. It consisted of two detachable partsan outer chamber of 1300 ml capacity and a tube of 90 ml capacity to fit inside it. The still head was also separate. Cerning[7] seemed to have difficulties with the apparatus.

2.4.3. Distillation

In the case of both the flour and the water-insoluble fraction, the sample (200-500mg) was transferred into the inner tube with hydrochloric acid. (4.2 N) to a final volume of 35-40ml. In the case of the water-soluble fraction, the extract (20ml) was transferred into the inner tube, and hydrochloric acid (14.6 ml of 10 N) was added to make the hydrochloric acid 4.2 N.

Hydrochloric acid (350 ml of 4.2 N) was added to the outer chamber. The apparatus was placed on an asbestos gauze and boiling commenced after about 10 minutes. The time of distillation was taken from when the first drop distilled over. The distillate (ca 250 ml) was collected in a 250 ml volumetric flask and made to volume with hydrochloric acid (4.2 N).

2.2.4. Colorimetric determination

The distillate (5ml) was pipetted into a 50ml volumetric flask which was then made to volume with the colour reagent. The rose colour produced by the furfural and aniline reagent developed fully at room temperature $(21.0\pm0.5)^{o}C$ after 45 minutes and remained stable up to 2 hours. The colour was always developed at room temperature A xylose standard distillate was run each time as a check.

Aniline-furfural gave a spectrum having a peak at 19400cm^{-1} (516nm). Absorbance was recorded on a UNICAM SP 800 spectrophotometer which was calibrated for wavelength accuracy using a didymium filter.

2.5. Calibration curve with xylose:

A calibration curve was made by distilling xylose (100, 50 and 25 mg) and the distillate (250ml) was diluted (3 to 50 ml) with hydrochloric acid (4.2N). The diluted solution (5 ml) was used to produce the colour in a 50 ml flask. This gave solutions of 120, 60 and 30 μg in the final solution, respectively. The 60 μg solution gave an absorbance of 0.27 in 10nm glass cells. Beer's Las was obeyed up to 120 μg.

The concentrations as given by Cerning[4] appear to be in error. Instead of 40, 80 and 120 p.p.m. it should be 40, 80 and 120 μg in the final solution (50ml).

2.6. Effect of glucose on the recovery:

Up to 427mg of glucose was added to xylose and the reaction carried out to see the effect.

2.7. Hydrolysis and chromatography:

The flour (100 mg) was hydrolysed[8] with sulphuric acid (15ml of 1 N) on a boiling water bath. Aliquots were taken after 1, 2 and 4 h, nuetralised with barium carbonate and centrifuged. The clear hydrolysate was chromatographed in ethyl acetate: pyridine: water (10:4:3) on Whatman paper No. 1 with the decending technique for 16h. p-Anisidine-HCl in butan-1-ol was used as the spray reagent. The chromatogram was heated at 105°C for 5 minutes for colour development.

Results

All the results on flour are reported on a 14% moisture basis and are expressed as percent xylose.

3.1 Effect of glucose on recovery:

When glucose was added to the distillation flask no effect on xylose recovery was observed as shown in table-1.

Table 1. Effect of glucose on the recovery.

Sugar distilled	Sugars recovered	% recovery
Xylose 2.1 mg + glucose 172.1 mg.	2.0 mg	95.2%
Xylose 2.9 mg + glucose 201.1 mg.	2.9 mg	100%
Xylose 5.2 mg + glucose 427.2 mg.	5.0 mg	96.2%
Glucose 100 – 500 mg.	None	–

Table 2. Dis-tillation and recovery of L-arabinose.

Distillation	% Recovery (calculated from xylose calibration graph)
A. Preliminary results:	
1. Arabinose (100mg) distilled to 250ml.	70.8
distilled to next 100ml.	12.4
distilled to next 100ml.	4.6
2. Arabinose (100mg) distilled to 250ml.	76.7
distilled to next 100ml.	5.4
distilled to next 100ml.	2.9
B. Arabinose distilled on smaller scale:	
1. Arabinose (4mg) distilled to 250ml.	61.3
distilled to next 100ml.	18.5
2. Arabinose (2mg) distilled to 250ml.	61.3
distilled to next 100ml.	18.5

3.2 Arabinose reaction:

When arabinose was reacted in the same way as xylose, recoveries were not the same as for xylose. The results are summarized in table-2.

3.3. Hydrolysis of flour:

The one hour hydrolysis of flour showed the presence of D-xylose, L-arabinose and D-glucose identical in R_f and colour with the reference spots when examined by paper chromatography.

The two and four hour hydrolysis reactions showed the presence of a large quantity of D-glucose, D-xylose and L-arabinose, identical with the reference spots in R_f and colour. By visual observation the ratio of D-xylose to L-arabinose was 3:2.

3.4. Total, soluble and insolube pentosans:

The total pentosans (A) were estimated firstly on the total flour: then the soluble (B) were separated from th insolubles (C) and pentosans were determined on each of the fractions. It was found that the two (B + C) gave a total (A') which was close to the total (A) as shown in table-3.

Table 3. Soluble, insoluble and total pentonans.

Sample No.	Soluble B%	Insoluble C%	Total A% (B+C)	Total A% (by analysis
1	0.53	1.03	1.56	1.61
2	0.56	1.09	1.65	1.42
3	0.55	1.04	1.59	1.41
4	0.50	1.11	1.61	1.41
5	0.35	1.10	1.45	1.35
6	0.62	1.16	1.78	1.64

Table 4. Insoluble pentosans.

Sample No.	1	2	3	4	5	6	7
Non-Deactivated enzyme at R.T.	1.03	1.09	1.04	1.11	1.10	1.16	1.09
Deactivated enzyme at L.R.T.	1.28	1.16	1.26	1.18	1.19	1.16	1.21
Deactivated enzyme at 40°C	1.20	1.04	1.07	1.02	1.06	1.06	1.08
Non-Deactivated enzyme at 40°C	0.98	0.94	0.98	1.00	1.00	0.93	0.97

L.S.D. 5% .07 1% .09 R.T. = Room temperature.

3.5. Insoluble pentosans:

The insoluble pentosan content of the flours was measured after extraction of soluble pentosans by four methods:

i) Extraction at room temperature without deactivation of enzymes.

ii) Extraction at room temperature after deactivation of enzymes.

iii) Extraction at 40°C after deactivation of enzymes.

iv) Extraction at 40°C without deactivation of enzymes.

The results are presented in table-4.

Discussion

When the Daffau apparatus was heated with a bunsen burner, the rate of distillation was slow (250ml/105 min). The rate of distillation was increased by putting a metal shield round the apparatus. Most distillations were than completed within 60-70 min.

During the distillation the volume of the liquid in the inner tube usually increased. It was found impossible to keep the volume constant. Looking at the relative standard deviation of the analytical results of flour samples (found to be 3.7% calculated[9] from results of 8 samples each analysed in duplicate), it seems unimportant to keep the inside volume constant as claimed by Cerning and Guilbot. It is difficult to seen how such an apparatus can be designed to keep the inside volume constant.

For measuring the furfural-aniline colour Bethge[2] used a Zeisa Elko II filter photometer, using an S53 filter which has maximum transmittance at 530 nm. Cerning did not mention the instrument but measured

the absorbance at 530 nm. We used a UNICAM SP 800 spectrophotometer to confirm that the colour has a peak at 516 nm in its spectrum.

Reproducible results were obtained on flour samples. To check the results further, soluble and insoluble fractions were separated and analysed separately. The results of the two fractions were added to give a total (A') which was sufficiently close to the total pentosan content (A) determined prior to its separation into two fractions (table 3). The error was probably caused by experimental difficultes in analysing the soluble fraction.

When the soluble fraction was stored in a refrigerator a cloudiness appeared, sometimes leading to slight precipitation. The insolubles could be frozen and analysed when convenient and so were easier to work with. The solubles could be calculated by the difference of total and insoluble pentonans.

The effect of temperature and enzymes on the extraction procedure was also studied using only the insoluble fractions. We used 40°C extraction to study the effect of higher temperature on the solublity of pentosans because at higher than 52°C starch[10] tends to swell and may interfere with the extraction of pentosans.

As evident from table 4,40°C extraction gives less insoluble pentosans than extraction at room temperature. The same is true for both the non-deactivated and deactivated flours. Because dough preparation is normally carried out at room temperature the extraction at room temperature is recommended.

Enzymes can also cause a difference in the solubility of pentosans. As seen in table 4 deactivation of enzymes in flour gives higher insoluble pentosans at room temperature extraction as well as at 40°C extraction compared with the non-deactivated flours. It is therefore, recommended to deactivate the enzymes in flour before pentosan analysis.

Under the same conditions of reaction as used for D-xylose and flours only 61-77% of L-arabinose was recovered (Table 2) in the first 250 ml distillate, whereas xylose and flour samples gave constant recoveries in the first 250 ml distillate. Further distillation of xylose and flours gave only traces of furfural and so no more distillation was necessary. Arabinose gave inconsistent results.

This prompted us to think that Queensland flour pentosans may be composed mainly of xylose and that arabinose may form only a very small portion of the pentosan structure. A flour sample when hydrolysed and chromatographed showed D-xylose and L-arabinose in the approximate ratio of 3:2. Although not enough work was done to establish accurately this ratio mainly because it was beyond the scope of this work, it nevertheless proved helpful in showing that a substantial quantity of arabinose is present in total flour pentosans.

Cerning and Guilbot found that ribose and arabinose gave less colour than xylose. The present work shows (Table 2) that with further distillation more furfural is distilled. This may be due to the furanose structure of arabinose and ribose and the possible resistance of such a structure to furfural conversion.

It is shown that wheat flour contains xylose and arabinose in the approximate ratio of 3:2. It is also shown that arabinose recoveries are low using the proposed method. Hence we must expect that pentosan analyses for flour will be low. It is concluded that since the results of flour analyses are reproducible the method can be used to estimate pentosans in flour. The results of analysis will not be total but since the method is reproducible it is quite adequate for correlative studies.

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References

1. D' Appolonia, B.L. *Baker's Dig.*, **45, (6) 20**, (1971).
2. Bethge, P.O. *Sevensk Paperstid.*, 67' 873, (1964).
3. Stone W.E. *Chemical News.* p. 206-207, (1981).
4. Cerming, J.; Guilbot, A. *Cereal Chem.* (1973), **50**, 176.
5. Daffau, A. *Bull. Soc. Chem. Biol.* 28, 837 (1946).
6. Bethge, P.O.; Eggers, J.H. *Sevensk Papperstid.*, **63**, 745, (1960).
7. Cerning, *J. Cereal Chem.*, **51**, 145 (1974).
8. Alam, M.; McIlroy, R.J. *J. Chem. Soc.*, (C) 1577, (1967).
9. Youden, W.J. *"Statistical Techniques for Collaborative Tests"*, (1969), 18. Washington, D.C., Assoc. of Official Analytical Chemists. Inc.
10. Leach, H.W. *Starch Chemistry and Technology*, (1965), I, 289, (Whistler, R.L.; Pascall, E.F.), Academic Press.