A Specific Method for the Determination of Pentosans in Cereals and Cereal Products[1]

J. CERNING and A. GUILBOT, Station de Biochimie et Physico-Chimie des Cereales de l'I.N.R.A. au C.E.R.D.I.A. 91305, Massy, France

ABSTRACT

A method for the determination of total pentosans in cereals and cereal products is described. The pentosans are converted into furfural by hydrochloric acid and separated by a steam distillation. Compared with classic distillation techniques the apparatus developed by Duffau has several advantages: The hydrolysis of the pentosans into pentoses, their transformation into furfural, and the distillation take place without direct heating of the reaction medium. Furthermore, the total acid volume and its concentration are maintained constant during the whole operation. The colorimetric determination of furfural is carried out by means of the aniline reaction. The difficulty presented by the great instability of the aniline furfural color has been resolved by the use of a buffer which leads to a stabilization of the color reaction for at least 45 min. Furfural can be determined specifically in the presence of large amounts of hydroxy methyl furfural (HMF). Uronic acids, such as glucuronic acid, do not interfere up to a concentration of 10% of total pentosans. The method is quite simple and can be employed within a wide range of concentrations. Specificity, precision, and reproducibility are equal, if not superior, to current techniques.

The precision and reproducibility of the determination of pentosans in cereals depend on a number of factors, the first one being a reliable distillation method for the conversion of pentosans into furfural and the second one being the selectivity of the color reaction of furfural and the stability of the colored solution. Sensitivity and stability of the reagents used, the extent to which the calibration curve obeys Beer's law, and the number of manipulations necessary for the preparation of the colored solution are also important.

A considerable number of color reactions for furfural such as primary aromatic amines or certain types of phenols have been proposed (1,2,3,4). Many of them are not specific and give similar reactions with other aldehydes, such as 5-hydroxy methyl furfural (5-HMF) present in distillates. Because aniline appeared to give the most selective reaction with furfural we chose this reagent (5). While the standard AACC method 52-11 for pentosans is based on the same reagents, the color reaction is not stabilized and the recommended DUBOSCQ Colorimeter has been out of use for almost 15 years in most laboratories.

The aim of this study is to describe the procedure followed in our laboratory and to outline possible applications of the method in a cereal laboratory.

MATERIALS AND METHODS

Principle

The principle of the method is the hydrolysis of pentosans, the dehydration of the pentoses into furfural by 4.15N hydrochloric acid, and finally the distillation and colorimetric determination of the latter by aniline acetate.

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Apparatus

The distillation apparatus was developed by Duffau (6). Manufactured by SEVAL, 21, rue des Ecouffes, 75004 Paris, France (Fig. 1), this apparatus has

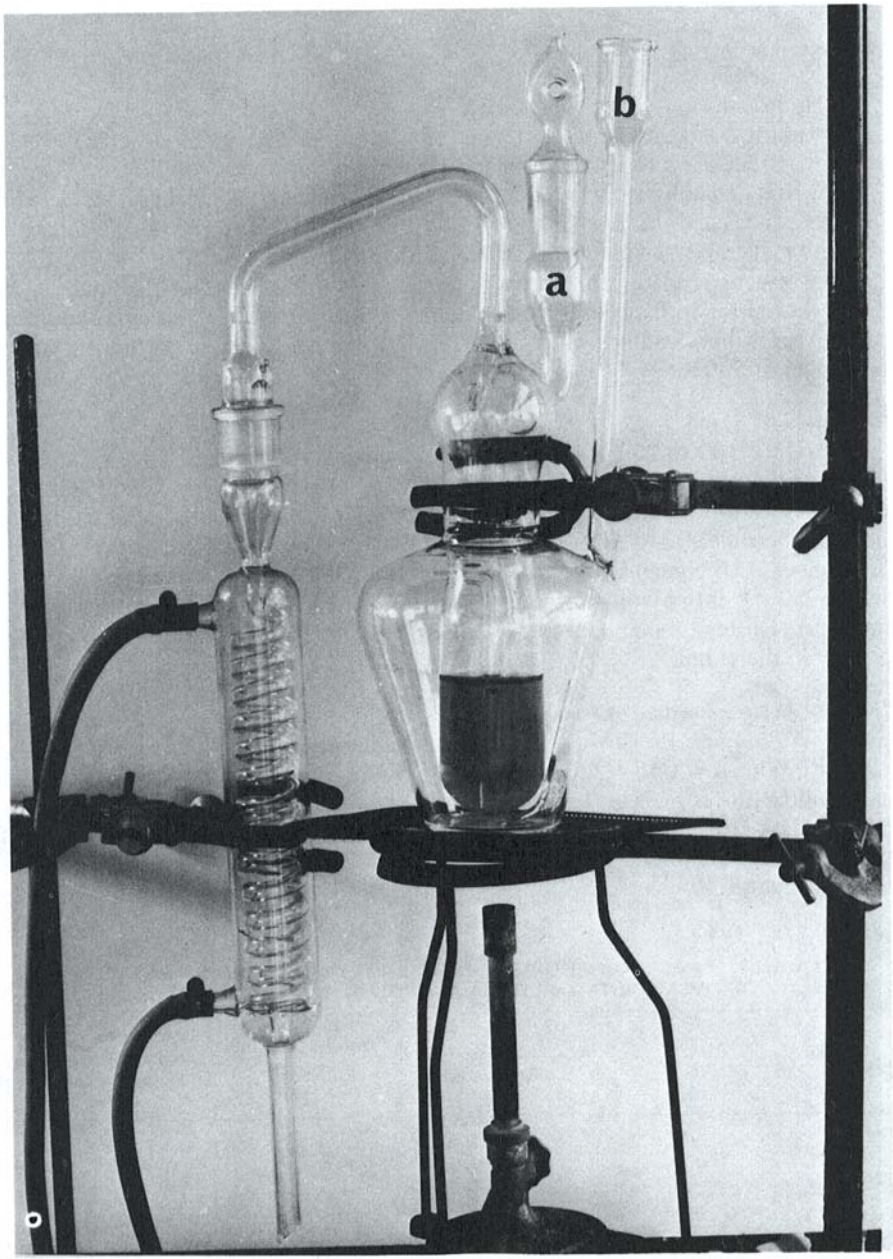

Fig. 1. Apparatus for steam distillation according to Duffau (6).

several advantages. Hydrolysis of the pentosans into pentoses, their conversion into furfural, and the distillation occur without direct heating of the reaction medium. Also, the volume of the reaction medium and its acid concentration are maintained constant during the whole operation.

Reagents

1. Xylose Merck.
2. Hydrochloric acid, d-1.06, 4.15N.
3. Aniline solution: Dissolve 10 ml. of freshly distilled aniline (Merck) in 95% ethanol and make up to a volume of 1,000 ml. with the same alcohol.
4. Buffer: Dissolve 40 g. anhydrous ammonium acetate, 2.7 g. stannous chloride ($SnCl_2 \cdot 2\ H_2O$) and 4.2 g. stannic chloride ($SnCl_4 \cdot 5\ H_2O$) in 133 ml. glacial acetic acid and make up to a volume of 240 ml. with distilled water.

The reagent is made up of two volumes of solution 3 and one volume of the buffer just prior to use. Only freshly prepared analytical-grade reagents should be used. Absorbance readings of the developed color are taken at the transmittance maximum of 530 nm.

Experimental

Introduce 100 to 250 mg. of a sample, which will yield at least 2 mg. of furfural in the total distillate, into part *a* of the distillation apparatus (Fig. 1). Add 30 ml. of the HCl solution into the same part so as to wash down particles adhering to the sides of the tube. Insert glass stopper, introduce 300 ml. of the same acid into part *b*, connect with condenser, and heat rather gently first and then regulate so as to distill 250 ml. into a volumetric flask within 1¼ to 1½ hr. Pass distillate through a small paper filter to eliminate fatty acids which arise from acid hydrolysis if fat is present in the sample.

Colorimetric Determination of Furfural

Pipet 5 ml. of the distillate or the distillate diluted in HCl, with a furfural concentration of 400 to 1,200 p.p.m., into a 50-ml. volumetric flask. Fill to volume with aniline-buffer reagent. Let the rose color develop for exactly 45 min. at 25°C., protect flask from sunlight, and then read absorbance at 530 nm. The furfural of the sample is determined with a calibration curve, and the results are expressed in terms of xylose.

TABLE I. PRECISION AND REPRODUCIBILITY OBTAINED BY HOT-ACID TREATMENT AND DISTILLATION OF PURE XYLOSE[a]

Xylose (Theoretical) mg.	Xylose (Recovered) mg.	Difference between Mean Experimental and Theoretical Values %
80	79 ± 0.5	1.25
120	122 ± 0.5	1.70
160	163 ± 1.0	1.90

[a]Mean of three determinations.

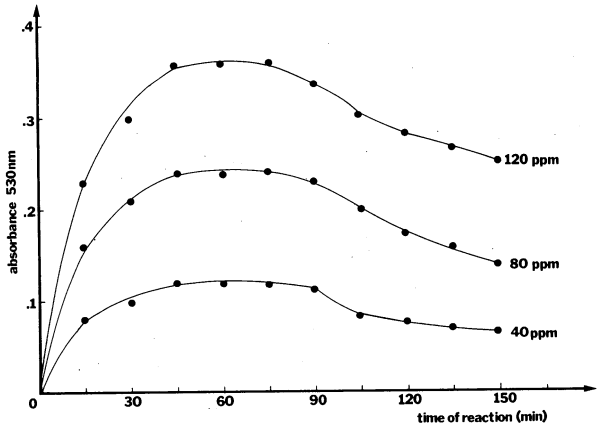

Fig. 2. Reaction of furfural and the aniline acetate reagent at 25°C. as a function of time.

Calibration Curve

Distill 100 mg. of xylose with HCl solution, dilute 1, 2, and 3 ml., respectively, of the distillate in a 50-ml. volumetric flask and make up to a volume of 50 ml. with the same HCl solution. Pipet 5 ml. of each of these dilutions into another volumetric 50-ml. flask which constitutes the solution for the calibration curve of 40, 80, and 120 p.p.m. Make colorimetric determination as described above.

The calibration curve established with three dilutions of the same distillate of pure xylose is the same as that traced with the absorbance readings of three distillates of different quantities of xylose. The following discussion will clarify our experimental conditions.

RESULTS AND DISCUSSION

Yield of Furfural from Pure Xylose

The yield of furfural in the steam distillation has been verified by the phoroglucin method of the AOAC (2). This reaction is not very specific when applied to distillates from complex material (interference such as 5-HMF derivatives of hexoses), but it can very well serve as a control for the distillates of pure xylose. Table I illustrates that the furfural yield of varying quantities of xylose using the Duffau reactor is quite satisfactory.

Reaction of Furfural and Aniline Acetate

The most important drawback of the furfural-aniline reaction is the formation of a very fugacious color which makes precise absorbance readings difficult, if not impossible. With the use of a buffer solution containing stannous and stannic chloride, the color develops slowly and stabilizes during at least 30 min. (between 45 and 75 min.), as illustrated in Fig. 2.

Stability of the Distillates

The determination of furfural in the distillates as a function of storage time at 4°C. shows that furfural, contrary to what has been indicated in the literature (6), is stable for at least 5 days. This facilitates the application of the method in routine

TABLE II. STABILITY OF FURFURAL DURING A SECOND DISTILLATION[a]

Furfural (Introduced) mg.	Furfural (Recovered) mg.	Difference between Mean Experimental and Theoretical Values %
49.6	51.0 ± 0.5	2.8
99.8	97.0 ± 0.6	2.8
149.4	149.0 ± 0.5	0.3

[a]Mean of two determinations.

TABLE III. COLOR REACTION OF DIFFERENT PENTOSES WITH ANILINE-ACETATE

Pentoses	Absorbance (at 530 nm.)		
	40 p.p.m.	80 p.p.m.	120 p.p.m.
Xylose	120	240	360
Ribose	90	180	265
Arabinose	80	155	235

use. We have even reintroduced distilled furfural into a second distillation without finding any destruction of furfural.

Table II illustrates the results of these experiments. As seen in all three cases, the furfural added to a second distillation is recovered with a mean reproducibility of about 2% with respect to the relative value.

Color Reaction of Pentoses Other than Xylose with Aniline Acetate

Under the same experimental conditions, equal quantities of arabinose or ribose produce a color whose absorbance is lower than that of xylose (Table III).

During our investigations of the carbohydrate composition of maturing cereals, we did not consider this important difference.

In fact, studies on nature and composition of pentosans in cereals have been conducted mostly on either the soluble or the insoluble fractions of these constituents, which had been previously isolated and purified from different parts

TABLE IV. INFLUENCE OF 5-HMF ON THE COLORIMETRIC DETERMINATION OF FURFURAL BY ANILINE ACETATE[a]

Distillation		Xylose (Recovered) mg.	Difference between Mean Experimental and Theoretical Values %
mg. Glucose	mg. Xylose		
250	2.5	2.48 ± 0.6	1.0
500	1.25	1.27 ± 0.5	1.5

[a]Mean of three determinations.

of the wheat kernel (flour, bran). Therefore, our knowledge of the exact proportions of the different pentose units in the cereal as a whole remain too fragmentary to permit making up a control mixture of pentoses, the quantities of which would precisely imitate those existing in the cereal. This applies to wheat and barley and even more to maize. Consequently we chose xylose, which is present in pentosans in the highest proportion. Analysis results expressed in terms of xylose are arbitrary, just as different sugars, for example, are expressed in terms of glucose.

However, in a comparative study of a series of samples of rather uniform origin (flour, wheat, bran) and with a known composition of pentosans, it is possible to establish the calibration curve with a mixture of xylose and arabinose, which comes close to that existing in the pentosan fraction of the cereal to be analyzed.

Influence of Hexose Derivatives on the Reaction

The interference of hexose derivatives such as 5-HMF on the colorimetric determination has been studied by addition of known glucose quantities to xylose before distillation.

Table IV illustrates that no interference occurs even when the glucose

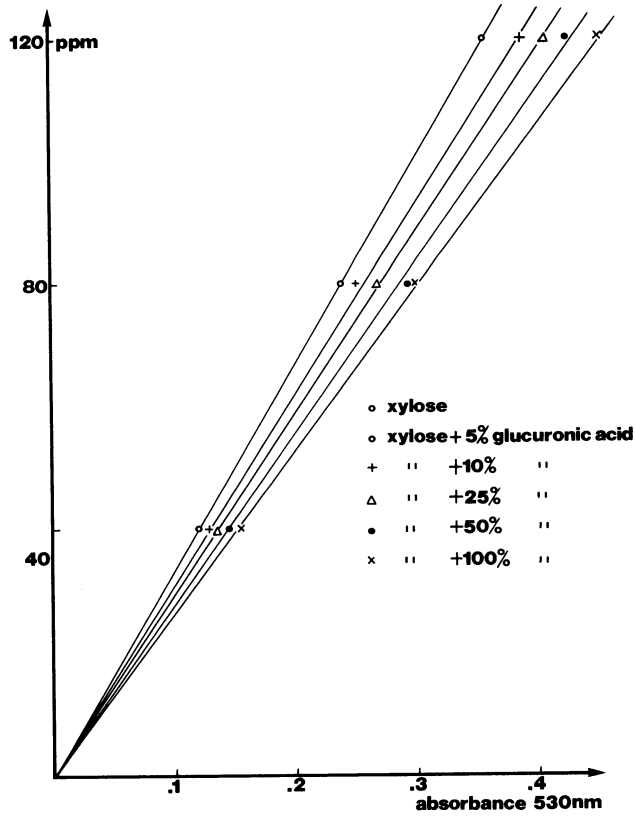

Fig. 3. Influence of glucuronic acid on the color reaction with the aniline acetate reagent.

TABLE V. APPLICATION OF THE PROPOSED METHOD TO WHEAT, MILLING PRODUCTS AND AIR-CLASSIFIED FLOUR FRACTIONS

Sample	Proportion of Wheat and Flour %	Pentosan Dry Matter Expressed as Xylose %				Balance[a] %	Difference %
		First Distillation[b]	Second Distillation[b]	Difference with Respect to Relative Value	Mean		
Flour	76.5	1.18	1.20	1.7	1.19	0.91	
Bran	16.4	26.20	26.50	1.1	26.35	4.32	
Shorts	5.5	20.15	20.45	1.5	20.30	1.12	
Red dog flour	1.6	8.36	8.46	1.2	8.41	1.35	
Wheat (Etoile de Choisy)	100	7.50	7.60	1.3	7.55	7.70	0.15
High-protein fine fraction	10.5	1.21	1.23	1.60	1.22	0.128	
Coarse fraction	46.8	1.50	1.52	1.3	1.51	0.707	
Medium fraction	42.7	0.83	0.842	1.4	0.84	0.359	
Flour	100	1.21	1.18	2.5	1.20	1.19	0.01

[a]Calculated with the proportion of wheat and flour and mean value of pentosan content.

[b]Mean value of two colorimetric determinations.

concentration is 400 times that of xylose. It is thus possible to determine the pentosans in starch by the proposed method.

Influence of Uronic Acids on the Reaction

The formation of furfural from hexuronic acids from which carbon dioxide is split off upon heating with mineral acids can be a source of error. The rest of the molecule is then converted into furfural.

From a first distillation of pure glucuronic acid it appeared that the distillate gave a slight color reaction with aniline acetate. We have therefore examined the interference systematically by adding increasing amounts of glucuronic acid to xylose before distillation.

The results of these experiments are summarized in Fig. 3 which shows that glucuronic acid does not interfere as long as the concentration is less than 10% with respect to xylose. At higher concentrations a correction factor has to be applied. Tests have been made with galacturonic acid. No interference takes place, as long as the concentration of this acid is less than 20% with respect to xylose. Such a concentration is not likely to occur in cereals and cereal products.

APPLICATION OF THE PROPOSED METHOD TO CEREAL PRODUCTS

Table V lists the results of different analyses of a wheat sample, milling fractions, and air-classified flours from an experimental milling procedure. The slight differences between duplicate analyses of these samples which vary markedly in pentosan contents indicate that the procedure can be conducted with precision. The mean reproducibility with respect to the relative value is 1.3%. Although not shown in this report, these values have been confirmed in our laboratory on at least 50 corn and wheat samples and 25 different flour types.

Since the proportion of the wheat kernel in each of the milling fractions and that of the parent flour in the air-classified fractions were known (Table V), the results could be checked by calculation of the balance. The very low difference of 0.15% and 0.01%, respectively, gives further evidence for the precision with which the proposed method can be applied.

SUMMARY

Since the quantities of free pentoses and those originating from nucleic acid derivatives remain small in cereals, pentosans in cereals can be determined by this technique with a reproducibility of 1.5 to 2% with respect to the relative value. The method is simple and rapid and can be employed within a wide range of pentosan concentrations. Specificity, precision, and reproducibility are equal, if not superior, to current techniques.

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