Carbohydrate Polymers 24 (1994) 123–132
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0144-8617/94/$07.00

A rapid semi-automated method for the determination of total and water-extractable pentosans in wheat flours

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(Received 24 September 1993; revised version received 20 January 1994; accepted 24 January 1994)

A rapid, semi-automated method for the determination of total and water-extractable pentosans in wheat flours is described. Total pentosans are solubilized by hot dilute acid within 10 min, and water-extractable pentosans by aqueous treatment within 15 min. The solubilization/extraction steps have been optimized with respect to accuracy (weighing of large amounts of sample), rapidity and yield of pentosans. The extracts are analyzed for pentose content by an automated adaptation of the phloroglucinol method of Douglas, on a continuous flow analyzer. The circuit allows the continuous generation of unstable phloroglucinol/acid reagent. The rate of analysis has been fixed at 40 samples h^{-1}. The pentosan content of 15 flour samples (total and water-extractable pentosans, in duplicate extractions), that is, 60 colorimetric determinations, may be carried out in one day by a single trained operator. The method is rapid, sensitive and reproducible (CV% < 3) and correlates well with other methods of pentosan determination. No interference has been found from the major components of wheat flours (starch, gluten). On the other hand, the presence of high levels of some sugars, possibly encountered in cereal-based products, can result in an underestimation (fructose, sucrose) or an overestimation (uronic acids) of the pentosan concentration.

INTRODUCTION

The non-starch polysaccharides (NSP) of wheat and rye endosperm, the pentosans, are composed mostly of arabinoxylans, which are made up of the pentose monosaccharides xylose and arabinose. These polysaccharides are found in minor amounts in flours (2–3% of a wheat flour, 5% of a rye flour) in both a water-extractable and unextractable form (Meuser & Suckow, 1986). Considerable attention has been paid to pentosans because they influence markedly the technological quality of flours, mainly because of their high affinity for water (Bushuk, 1966; Kulp, 1968; Jelaca & Hlynka, 1971; 1972; Kim & D'Appolonia, 1977; Michniewicz *et al.*, 1992). For example, the properties of doughs and baked products are strongly affected by their pentosan content and the form in which they occur (Cawley, 1964; Casier *et al.*, 1973; Hoseney, 1984; Shogren *et al.*, 1987; Graybosch *et al.*, 1993; Roels *et al.*, 1993; Rouau *et al.*, 1993).

The determination of the pentosan content of flours, doughs or baked products is of interest both for research and for routine analysis of the flours supplied to industry. Several methods are used concurrently for the determination of NSP in cereal flours or cereal-based products:

(i) In the determination of dietary fiber, sometimes referred to as enzymatic-gravimetric methods, NSP are isolated by removal of starch and protein from the sample and then the residue is weighed. These methods are not adapted to the determination of pentosans in flours because of the very low concentration of the latter, and the lack of specificity of the method.
(ii) In the chromatographic methods, samples are hydrolyzed into their monomeric constituents and the pentosans are quantified as the sum of arabinose and xylose that are separated by liquid or gas chromatography. These methods are

precise and specific and discriminate between arabinose and xylose, but the preparation procedure is often complex and time-consuming; it does not allow numerous samples to be analyzed daily.

(iii) In the colorimetric methods, a colored complex is formed with sugar derivatives obtained by hot concentrated acid treatment of the sample; the intensity of the color is proportional to the concentration of pentoses. The use of these methods needs the handling of hot concentrated acids; only a global value is given for pentosans without information on their constitution; interferences can also occur from non-pentosan components.

The full or partial automatization of a method can allow the routine analysis of large sets of samples, and solve problems of poor reproducibility often encountered with manual methods. Colorimetric methods are more suitable for the rapid determination of pentoses and are easier to automate than chromatographic methods. In the colorimetric methods for sugar determination, the conversion of pentosan into furfural by hot acid treatment is followed by a color reaction with chromogens. Methods using distillation of furfural and reaction with aniline-acetate (AACC, 1962; Cerning & Guilbot, 1973) are time-consuming and require special equipment. The orcinol-hydrochloric acid methods are subject to interferences by hexoses. Hashimoto *et al.* (1987) developed an orcinol-HCl method allowing for a distinction between water-soluble, enzyme-extractable and total pentosans. The interference of hexoses was limited by removal of glucose by yeast fermentation. The method was improved by Delcour *et al.* (1989) by using the absorbance reading at two wavelengths in order to eliminate overestimation of pentoses due to the presence of hexoses. Alternatively, the simple phloroglucinol method (Dische & Borenfreund, 1957) was also used for the determination of pentosans in white wheat flours (Cracknell & Moye, 1970; Douglas, 1981). The interference from hexoses was also eliminated by reading absorbance at two wavelengths. The method of Douglas was adapted by Bell (1985) for application to a wider range of cereal products of higher fiber content, by introducing a short prehydrolysis step using 0·5 M acid. This author reported that an operator can analyze up to 20 samples (10 duplicates) in one day. Although the procedure was simple and gave results which correlated well with other methods of pentosans or NSP determination, problems of reagent stability and fading of color could cause lack of reproducibility unless thorough care is taken by a competent and trained operator. We describe and discuss here a method of pentosan determination in flours, flour extracts or other products, suitable for the analysis of large sets of samples. A procedure of extraction of total and water-extractable pentosans was established with the aim of optimal simplicity, rapidity and yield in pentoses. The pentose content of extracts was determined by a modification of the phloroglucinol method, using a continuous flow analyzer.

EXPERIMENTAL

Flours and mill streams

Wheat cultivars Baroudeur, Fandango, Fortal, Genial, Camp Remy and Soissons, grown in France with three levels of nitrogen supplementation (standard, −80, +80) and harvested in 1991, were collected and milled into experimental flours by the Institut Technique des Céréales et Fourrages (ITCF). These samples were used for the comparison between the semi-automated colorimetric method and the gas chromatography method for pentosan determination. Experimental flours and mill streams (B1, B2, B3, B4, C1, C2, C3, C4, remoulage blanc, remoulage brun) from the cultivars Apollo, Thésée and Soissons were provided by the Centre de Recherches sur les Pulvérulents de Créteil (CRPC, Grands Moulins de Paris). These samples were used for the determination of the reproducibility of the semi-automated colorimetric method.

Reagents

Phloroglucinol (1,3,5-Trihydroxybenzene) was purchased from Fluka Chemie AG, Buchs, Switzerland. Anhydrous ethyl alcohol RS for HPLC was obtained from Carlo Erba Reagenti, Milano, Italy. Glacial acetic acid (100%) pro analysis, fuming hydrochloric acid 37% pro analysis and sulphuric acid 95–97% pro analysis were obtained from Merck, Darmstadt, Germany. Other acids (orthophosphoric acid, citric acid, formic acid, propionic acid, trichloracetic acid) and sugars (xylose, arabinose, glucose, fructose, saccharose, galacturonic acid, glucuronic acid) of analytical grade were from Sigma Chemicals, St Louis, USA.

Instrumentation

An Alliance Instruments Evolution II auto-analyzer (Alliance Instruments, Cergy-Pontoise, France) was used. It was equipped with a fixed-speed peristaltic pump, two thermostats containing glass coils (1·5 mm i.d., 8 ml), a double wavelength spectrophotometer (550 nm/510 nm) fitted with a 1 cm light path flow cell. The spectrophotometer was connected through a data acquisition card to a computer equipped with Alliance Instruments analysis software package.

Tubing was purchased from Bioblock Scientific, IllKirch, France. Pharmed flexible tubing (Norton

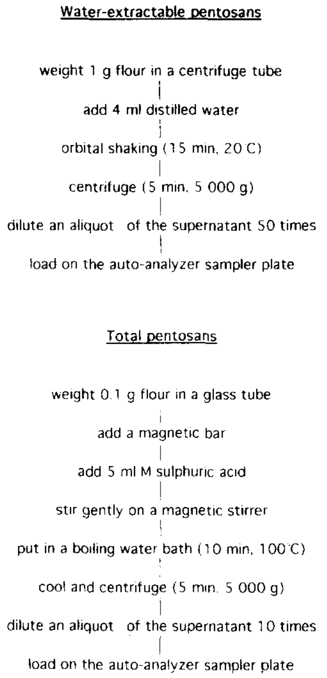

Fig. 1. Semi-automated colorimetric determination of wheat flour pentosans: manual extraction of water-extractable and total pentosans.

Company, USA) of appropriate internal diameter was used for acid reagent and phloroglucinol/glucose reagent pumping and Tygon tubing (Norton Company, USA) for sample and air pumping. Teflon connect tubes were obtained from Du Pont de Nemours, USA and Pharmed.

Procedure for determination of pentosans

The method consists of two stages: (1) the extraction of the total and water-extractable pentosans from the flour; (2) the colorimetric determination of the pentosan content of the extracts using a continuous flow auto-analyzer. The extraction procedure is summarized in Fig. 1.

Extraction of pentosans

Total pentosans. Flour (100 mg) was weighed in a pyrex glass tube (10 ml) fitted with a screw cap and containing a small magnetic bar. 1 M sulphuric acid (5 ml) was added and the flour gently dispersed for 30 s, using a magnetic stirrer. The tube was boiled for 10 min in a water bath, cooled in water, and then centrifuged for 5 min at 5 000 × *g*. An aliquot of the clear supernatant was diluted 10 times and loaded onto the sampler of the auto-analyzer.

Water-extractable pentosans. Flour (1 g) was weighed in a centrifuge tube (10 ml). Distilled water (4 ml) was added and the content of the tube homogenized by vigorous vortex agitation. The tube was then placed on an orbital shaker at 40 rev min^{-1} and agitated for 15 min at 20°C. It was subsequently centrifuged for 5 min at 5 000 × *g*. An aliquot of the clear supernatant was diluted 50 times and loaded onto the sampler of the auto-analyzer.

Colorimetric determination of pentoses

The experimental arrangement and flow rates are given in Fig. 2. The reagent mixture was created continuously on the auto-analyzer by mixing a solution A (110 ml glacial acetic acid, 2 ml fuming hydrochloric acid), segmented by air bubbling with a solution B (1 g phloroglucinol dissolved in 5 ml absolute ethanol plus 17·5 mg glucose dissolved in 1 ml distilled water) in the ratio 1:0·0463. The reagent was homogenized in two glass coils

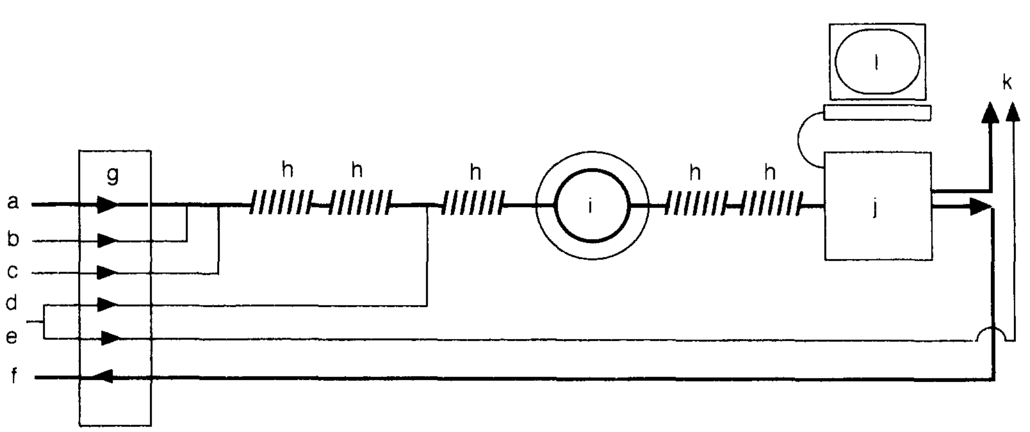

Fig. 2. Scheme of the circuit fitted to an auto-analyzer for the determination of pentosan by an automated phloroglucinol method: (a) glacial acetic acid (110 ml) + fuming hydrochloric acid (2 ml); Pharmed tube grey/grey (0·95 ml min^{-1}); (b) air bubbling; Tygon tube black/black; (c) phloroglucinol (1 g) in absolute ethanol (5 ml) + glucose (17·5 mg) in distilled water (1 ml); Pharmed tube orange/blue (0·044 ml min^{-1}); (d) sample; Tygon tube orange/blue (0·037 ml min^{-1}); (e) sample accelerator; Tygon tube white/white (0·25 ml min^{-1}); (f) flow cell way-out; Pharmed tube orange/orange (0·45 ml min^{-1}); (g) peristaltic pump; (h) glass coil (3 ml); (i) thermostat (96°C), glass coil (8 ml × 2); (j) double wavelength spectrophotometer (510 nm/550 nm); (k) waste; (l) computer.

(1·5 mm i.d., 2 × 3 ml). The sample was pumped from the sampling plate and injected in the reagent flow (ratio 1:0·0372); sample and reagent were mixed in a glass coil (1·5 mm i.d., 3 ml) and the mixture was circulated in two heating baths regulated at 96°C, in two glass coils (1·5 mm i.d., 2 × 8 ml). The mixture was then cooled in two glass coils (1·5 mm i.d., 2 × 3 ml) and passed through the flow cell of the spectrophotometer where the difference of absorbance between 550 nm and 510 nm was determined. The signal was fed to a computer equipped with a specific software analysis system.

Standards of D-xylose (0–50 μg/ml) made up in distilled water were measured, and allowed the construction of a calibration curve for the quantification of pentosans in solution. The overall retention time of a sample in the flow was 9 min. The analysis rate was fixed at 40 samples liter^{-1}, with a sample pumping time of 0·5 min and a rinsing time of 1 min.

Calculation

The percent of pentosans was given by

$$P\% = (A_{550\,\mathrm{nm}-510\,\mathrm{nm}} \cdot S \cdot D \cdot V \cdot 0{\cdot}88 \cdot 100)/F$$

where $A_{550\,\mathrm{nm}-510\,\mathrm{nm}}$ is the difference of absorbance between 550 nm and 510 nm; S is the slope of the xylose calibration plots; D is the dilution factor (50); V is the volume of extract; 0·88 is the polymerization factor, and F is the mass of flour (dry matter).

The content of water-unextractable pentosans can be deduced by subtracting the value of water-extractable pentosans from the value of total pentosans.

Gas–liquid chromatography method

The pentosans were also quantified as the sum of anhydro-xylose and anhydro-arabinose, determined by gas–liquid chromatographic analysis (DB 225 capillary column), of their alditol acetates obtained after sulphuric acid hydrolysis (1 M, 100°C, 2 h), of flour samples (total pentosans) or flour water extracts (water-extractable pentosans) (Blakeney *et al.*, 1983); inositol was used as an internal standard.

Statistical analysis

Results were treated by multifactor analysis of variance using a Stat-ITCF computer package.

RESULTS AND DISCUSSION

Extraction of pentosans from flour

Water-extractable pentosans

Different conditions of aqueous extraction of the flour were examined (Table 1). A solid:liquid ratio of 1:4 was chosen because it yielded sufficiently concentrated solutions for most of the current experiments on wheat flour water-extractable pentosans (gel permeation chromatography, capillary viscometry, gel formation, etc). At 4°C, the amount of extractable pentosans increased with agitation time in water. There was a 10% increase between 10 min and 20 min of extraction, then the rate of increase slowed down. When the extract was boiled for 5 min, cooled and centrifuged to remove the coagulated material (essentially protein), the pentosan content did not vary much. It appeared therefore that a step of protein elimination was not necessary for the determination of the flour water-extractable pentosans. This was confirmed by further experiments on the colorimetric response of protein (see below). NaCl (0·2 M), which is often used as extractant, solvent or eluent for chromatography, extracted slightly more pentosan than pure water. The water extraction of various amounts of flour was tested at 20°C, for 20 min, using the same solid:liquid ratio. No differences were found between the diverse samples. It is thus possible to use either large amounts (5 g) or small amounts (0·5 g) of flour, depending on the availability of sample and whether it is required as an appreciable quantity of material for other experiments after determining the pentosan content. For routine analysis, the use of 0·5 g of flour was found convenient. The amounts of pentosan extracted with water were slightly different at 4°C and 20°C. Although normal extraction should be carried out at 20°C, extraction with cold water (4°C) could minimize the activation of intrinsic or added enzymes in flours, which could act on pentosans and modify the extractable/unextractable ratio. For normal flours, the aqueous extracts must be diluted 50 times to fall in the convenient range of linearity for the colorimetric method. The extraction scheme is given in Fig. 1.

Total pentosans

The determination of pentosans by continuous flow analysis requires that they be initially solubilized. Water-unextractable pentosans belong to the hemicellulosic class of polysaccharides and are mostly extractable by alkali, such as sodium hydroxide, potassium hydroxide (Mares & Stone, 1973; Brillouet & Mercier, 1981; Fincher & Stone, 1986) or barium hydroxide (Gruppen *et al.*, 1991). Arabinoxylans that have been extracted by alkali become generally water-soluble. They can therefore be diluted with water. The use of alkali with flours, however, leads to the swelling and the partial solubilization of starch that makes it difficult to recover a clear extract, unless a large amount of liquid is employed. On the other hand, total pentosans can be solubilized also by partial acid hydrolysis, in mild conditions. Bell (1985) described a prehydrolysis step using 0·5 M sulphuric acid at 100°C for 30 min, before the colorimetric determination of pentose by the phloroglucinol method. We have investigated the optimal conditions of wheat flour pentosan solubilization,

Table 1. Influence of extraction conditions on the determination of the water-extractable pentosan content of a flour

Extraction time	4°C, 1 g 4 ml^{-1}			20°C, Distilled water			
	Distilled water	Boiled and centrifuged	NaCl (0·2 M)	5 g 20 ml^{-1}	1 g 4 ml^{-1}	0·5 g 2 ml^{-1}	Boiled and centrifuged
10 min	0·50	—	—	—	—	—	—
15 min	0·53	—	—	—	—	—	—
20 min	0·55	0·54	0·57	0·57	0·57	0·57	0·58
30 min	0·56	—	—	—	—	—	—

[a] Percent of flour dry matter, means of replicates ($n = 4$).

with the aim of getting a maximum pentose recovery with as short a pretreatment time as possible.

The total pentosan content of a flour (reference value) was determined by gas chromatography of alditol acetates after 1 M sulphuric acid hydrolysis (100°C, 2 h). The total pentosan content of this flour was 2·05%. An acid extraction step was developed as follows: flour (0·1 g) was mixed with various acids (5 ml), at various concentrations, for increased periods of time at 100°C, then the mixture was centrifuged, the supernatant was neutralized and analyzed for its pentosan content by the gas chromatography method. The use of hydrochloric acid and acetic acid, either in a mixture similar to the reagent solution of the Douglas method (glacial acetic acid:fuming hydrochloric acid, 110:2) or as separate diluted aqueous solutions (1 M, 2 M) was not satisfactory due to hazardous handling and underestimated pentosan values (not shown).

Sulphuric acid was found to be more convenient. The results of these experiments are shown in Fig. 3. With 0·25 M concentration, the reference value of the pentosan content of the flour was not reached even under prolonged hydrolysis time. Only 85% of the pentosan passed into solution in these conditions. When 0·5 M sulphuric acid was used, all of the pentosan could be found in the extract after 30 min of treatment, which is consistent with the results of Bell (1985). However, the maximum was obtained in only 10 min with a 1 M concentration. When short extraction times are more closely examined, it appears that after only 5 min of treatment, the maximum pentosan value is obtained with 1 M sulphuric acid, For lower and higher concentrations, all the pentosans were not recovered because of incomplete extraction or pentose degradation. Similar results were obtained when the pentoses were determined by the Douglas procedure after sulphuric acid extraction. In this case, the acid extract must be diluted 10 times before determination. It was not necessary to neutralize the samples before applying the phloroglucinol method. In some cases, the pentosan values were lower after sodium hydroxide neutralization than with a diluted crude acid extract. This may be due to a partial precipitation of arabinoxylans in neutral solutions. The acid extracts remained stable over a period of time of 3 h. For longer storage times, it is advisable to refrigerate the extracts. The final preferred pentosan extraction procedure is summarized in Fig. 1.

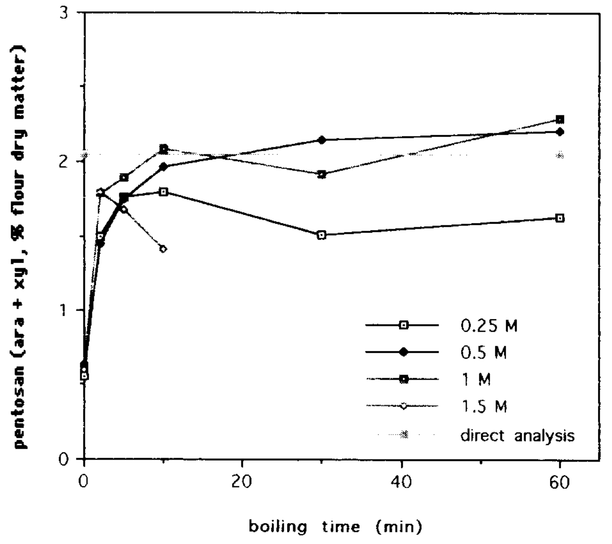

Fig. 3. Extraction of total pentosan from flour by hot dilute acid treatment: effect of acid concentration and boiling time on the yield of pentoses.

Colorimetric automated determination of the pentose content of the extracts

Analytical circuit

Tubing material. The reagent of the Douglas method is mostly composed of glacial acetic acid, with small amounts of hydrochloric acid, an aqueous solution of glucose and an ethanolic solution of phloroglucinol. Preliminary experiments showed that when the peristaltic pump tubes were made of conventional acid-resistant material they did not resist the reagent for very long. After a few hours of use, they softened and distended with subsequent changes in the flow rate. Different tests with other conventional material also gave unsatisfactory results: PVC tubes, solvent-resistant tubes and silicone tubes had short life times, were permeable to acid vapors and gave varying flow rates. These problems were supposed to be due to the volatile and solvent properties of acetic acid. Attempts to substitute other acids for acetic acid were unsuccessful. Neither mineral acids (hydrochloric acid, sulphuric acid, orthophosphoric acid) nor organic acids (citric acid, formic acid, propionic acid, trichloracetic acid) at different concentrations allowed the formation of a colored complex absorbing at the right wavelengths. Finally, the highly resistant Pharmed tubes were used for pumping glacial acetic acid-based reagent. Experiments showed that these tubes can work continuously with glacial acetic acid/hydrochloric acid over a period of 150 h without significant changes in flow rates. However, in the case of daily use, with flow interruption and rinsing of the circuit, acid pumping Pharmed tubes should be replaced every week. Connecting tubes of the circuit were in Pharmed or in Teflon. After use, the circuit was rinsed with acetic acid then emptied.

Analytical scheme

The scheme of the analytical circuit is given in Fig. 2. In the first experiments, the complete Douglas reagent, which was made up by first mixing the acetic and hydrochloric acids, and then adding the ethanolic solution of phloroglucinol and the aqueous solution of glucose, was used. The values of pentose concentration obtained with this decreased continuously over a rather short period of time. This was attributed to the instability of the Douglas reagent due to prolonged exposure of glucose and phloroglucinol to concentrated acids. Indeed, Bell (1985) reported that the reagent was stable 1·5 h after pentose values started to decrease. It appeared that dividing the Douglas reagent into two components that were used immediately after mixing gave more reproducible results. The first half-reagent was composed of glacial acetic acid (110 ml) and fuming hydrochloric acid (2 ml); the second half-reagent was composed of a mixture of phloroglucinol (1 g) dissolved in absolute ethanol (5 ml) and glucose (17·5 mg) dissolved in water (1 ml). Both half-reagents are stable at ambient temperature and can be kept for at least two weeks at 4°C. In order to maintain a ratio between acids and ethanol/water half-reagents close to that described by Douglas, and taking into account the commercially available tubes, an assembly comprising a Pharmed tube (grey/grey) for acid pumping (flow rate: 0·95 ml min^{-1}) and the smallest Pharmed tube (orange/blue) for ethanol/water pumping (flow rate: 0·044 ml min^{-1}) was mounted. The ratio between both half-reagents was 1:0·046. This differed from the ratio in the original Douglas method (1:0·053), but nevertheless the reaction developed satisfactorily, as shown below.

Samples were pumped through a Tygon tube (orange/blue, flow rate: 0·037 ml min^{-1}) and mixed with the reagent in a coil (3 ml). The mixture was thereafter moved through a heating bath thermostated at 96°C for approximately 5·5 min (25 min at 100°C in the original Douglas procedure). The colored solution then passed through the flow cell (1 ml) of a double wavelength (550 nm/510 nm) spectrophotometer connected to a computer. The pentose concentration was calculated from the peak heights.

Colorimetric responses

Dische and Borenfreund (1957), and subsequently Douglas (1981), have demonstrated that the phloroglucinol method was specific for pentoses in the presence of hexoses. Indeed, the colored furfural/phloroglucinol complex derived from pentoses exhibited a maximum of absorbance at 552 nm but absorbed poorly at 510 nm, whereas the hydroxy-methyl-furfural/phloroglucinol complex derived from hexoses had the same absorbance at 552 nm and 510 nm. Therefore, the difference of absorbance between 552 nm (or more practically 550 nm) and 510 nm allows the specific quantification of pentoses.

The response of different sugars to the automated colorimetric method at various concentrations was investigated. The results appear in Table 2. The response of xylose was linear up to 100 μg ml^{-1} (Fig. 4). Bell (1985) found a good linearity of xylose response up to 150 μg ml^{-1}, with a slightly modified Douglas method. In this case, the boiling time of the reagent mixture was 25 min instead of 5·5 min for the automated method. The difference in the domain of linearity probably originated from these different reaction times, although the volumetric ratio between the reagent and the sample was also different. Xylose and arabinose gave similar pentose values. Xylose was chosen for the

Table 2. Response of various sugars to the phloroglucinol method (expressed as xylose equivalent, μg ml^{-1})

Xylose	Arabinose	Glucose	Fructose	Saccharose	Glucuronic acid	Galacturonic acid
10·0	9·8	—	—	—	0·3	0·9
20·0	19·0	—	—	—	—	—
25·0	—	0	—	—	0·7	2·4
30·0	28·1	—	—	—	—	—
40·0	38·0	—	—	—	—	—
50·0	48·0	0	—	—	1·7	5·0
100·0	—	—	−0·8	—	3·3	10·5
250·0	—	—	−5·1	−2·6	—	—
500·0	—	0	−10·4	−5·2	17·0	52·3

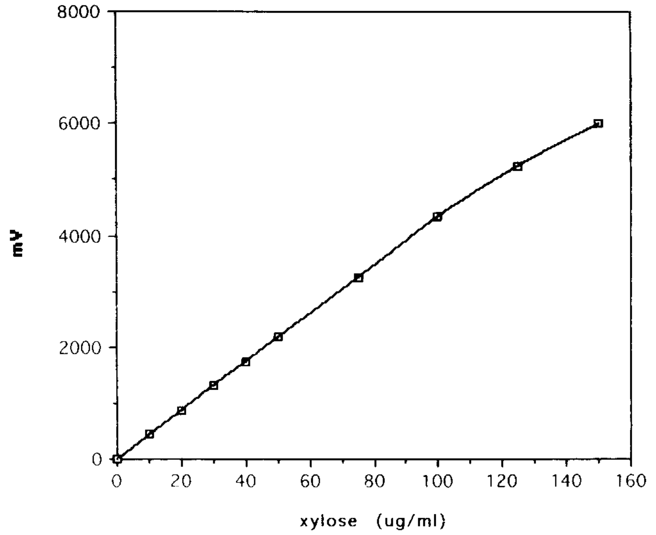

Fig. 4. Typical calibration curve obtained with xylose standards.

construction of the calibration curve and the estimation of the pentose content of extracts.

Pentosans are normally found together with large amounts of glucans (mostly starch or starch-derived molecules) in flours that give high glucose levels in extracts. However there was no interference with the pentosan determination because the response of glucose remained at zero up to a concentration of 5000 μg ml^{-1}. Conversely, fructose, as already noted earlier by Dische and Borenfreund (1957), and saccharose, of which rather large amounts can be found in certain cereal-based products (biscuits, cakes or gingerbread), caused negative responses to the phloroglucinol reaction. The negative response was approximately 50 times less intense than the xylose response for fructose and 100 times for saccharose. This negative response affected the determination of xylose concentration: 500 μg of fructose decreased the xylose value by 30% and 500 μg of saccharose by 15%. This effect was linearly related to the concentration of fructose and saccharose (Fig. 5). However, below 40 μg of fructose and 75 μg of saccharose the lowering effect was no longer significant. In taking into account the dilution factor between the extraction and the colorimetric analysis, the values of pentosan content of products having more than 0·75–1% fructose or 1·5–2% saccharose in their composition will be underestimated. Correction factors, depending on the fructose and saccharose concentrations, should then be applied. The experiments in the present study have shown that the pentose (xylose) value must be increased to 1 μg/100 μg of sacharose present in the analyzed solution and 2 μg/100 μg of fructose present in the solution. Douglas (1981) reported that starch and maltose did not cause interference with the pentosan determination.

Dische and Borenfreund (1957) have shown also that uronic acids reacted positively to the phloroglucinol method. The experiments discussed here showed that the positive responses of glucuronic acid and galacturonic acid were 30 times less and 10 times less than xylose respectively. Although glucuronic acid, or its 4-O-methyl ether, can be present in wheat products (especially those rich in bran), or whole wheat flours, only traces are found normally in bread or biscuit flours. On the other hand, galacturonic acid, which is the main component of pectins, is largely present in dicots cell-walls but essentially absent in monocots. However, care must be taken if the colorimetric pentosan determination is to be applied to complex food, or to feed containing cereal flour added with fruit or root pulps, or Leguminosae meals that are rich in pectins.

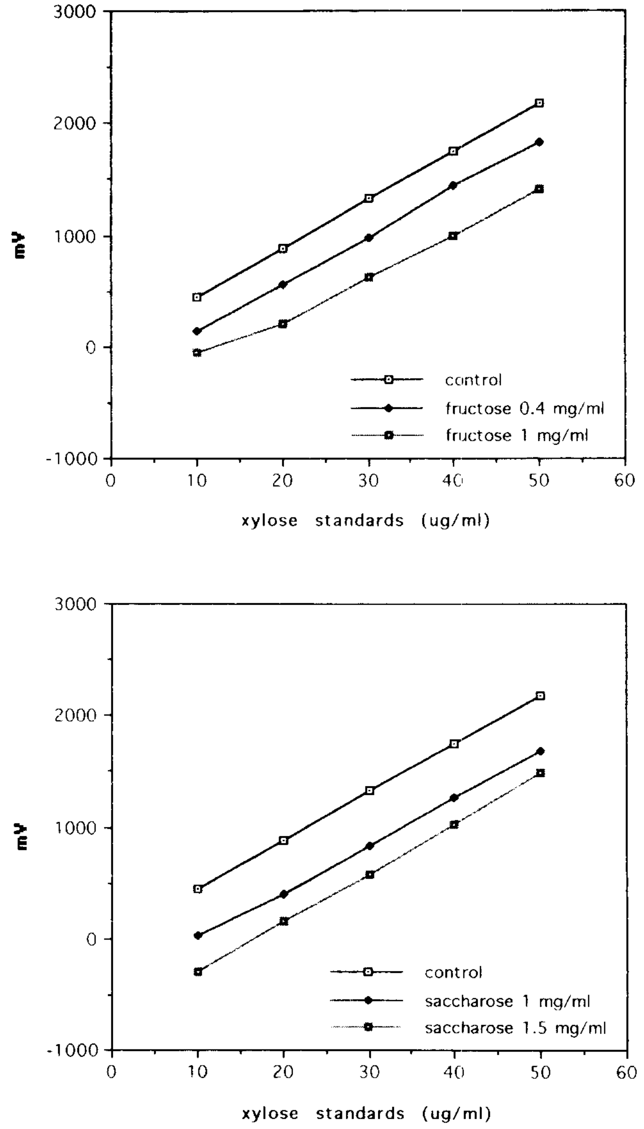

Fig. 5. Response of xylose standards to the automated phloroglucinol method in the presence of fructose and saccharose.

Proteins are also important components of flours and cereal-based food products. The response of bovine serum albumin was investigated: at a concentration of 100 μg ml^{-1} it gave no response when used alone; more-over, no effect was observed on the xylose response when bovine serum albumin was added to the sugar solution at different concentrations.

Rate of analysis

The duration of analysis for a sample, from pumping onto the sampler plate to leaving the spectrophotometer, was 9 min. A rate of analysis of 40 samples h^{-1} with a pumping/rinsing ratio of 0·5/1 min was found to be the best compromise between speed of analysis and optimal separation of the peaks of two successive samples, whatever their concentration. Control solutions of xylose (for example 40 $\mu g\, ml^{-1}$) were regularly interspersed on the sampler plate to detect drift. Also, a diluted flour water extract that had been previously prepared, divided into aliquots and stored frozen, was inserted at the beginning of each set of analysis to monitor the reproducibility of the determination and ensure the validity of the results over consecutive days. The authors would recommend that limiting the number of samples to 40 after which new xylose standards are analyzed to construct a new calibration curve. This procedure results in the elimination of drift problems.

Application of the method to the determination of water-extractable and total pentosans in wheat flours

Number of determinations per day

The whole procedure of pentosans determination as described in the preceding sections have permitted the treatment of 15–20 samples of flour (total and water-extractable pentosans in duplicate extractions, or alternatively 30–40 samples for total and water-extractable pentosans in single extraction, or alternatively, 60–80 single determinations of either total or water-extractable flour pentosans) by a single operator. The limiting step was not the colorimetric measurement of pentoses, since the analysis rate was fixed at 40 samples h^{-1}. The extraction stages were more time-consuming. However, sets of 20 samples can be treated at the same time; water extraction and hot acid extraction can be started with a short lag time, then centrifuged and diluted. Finally, the weighing of the flour samples appears to be the limiting step for a great number of daily analyses. In typical laboratories, weighing of all samples for a week may be concentrated into one day; over 60 pentosans analyses can thus be carried out per day by experienced operators.

Comparison with the original Douglas procedure

A comparison of the results of total pentosan determination of three wheat flours by the direct conventional Douglas procedure, by the manual Douglas procedure following the acid extraction step, and by the semi-automated method described here, is given in Table 3. The mean pentosan values obtained by the three applications of the method were similar but the standard deviations were much higher with the direct Douglas procedure. In addition to the instability of the reagent and the fading of color (which were avoided with the use of the auto-analyzer) another source of variation with the Douglas procedure results from the weighing of very small amounts of flour samples (approximately 5 mg). In the method presented, this source of error was eliminated by the use of a 0·1 g sample (0·5 g or 1 g for water-extractable pentosans) which allowed accuracy in weighing. This can be seen by comparing the standard deviations of the direct Douglas procedure and the Douglas procedure after separate acid extraction (0·1 g starting material). The method described here gave results that are very consistent with those obtained by the original method of Douglas, but improve appreciably the precision of the determination due to standardization of the reagent and the reaction times and the possibility of starting from larger amounts of flour.

Correlation with other methods

Eighteen samples of flours (T 550 type) were used in this study. They were obtained by experimental milling of six pure wheat cultivars grown using three levels of nitrogen. The flours were concurrently analyzed for total and water-extractable pentosans by a gas–liquid chromatography method, and the semi-automated colorimetric method. The results appear in Fig. 6. The values of total pentosan as determined by gas–liquid chromatography ranged from 2·86%–1·75%, with a mean value of 2·22%. Water-extractable pentosans comprised between 0·76% and 0·36% (mean value: 0·52%). For determination of total and water-extractable pentosans, the results from both methods corre-

Table 3. Comparison of pentosan determination in three flour samples by three procedures using the phloroglucinol reaction

Flour samples	Conventional manual Douglas procedure		Acid extraction followed by manual Douglas procedure		Semi-automated method	
	Pentosan (%)	σ	Pentosan (%)	σ	Pentosan (%)	σ
Apollo	1·81	0·26	1·81	0·06	1·83	0·02
Soissons	1·68	0·25	1·68	0·06	1·68	0·02
Thésée	2·01	0·14	2·08	0·11	2·07	0·06

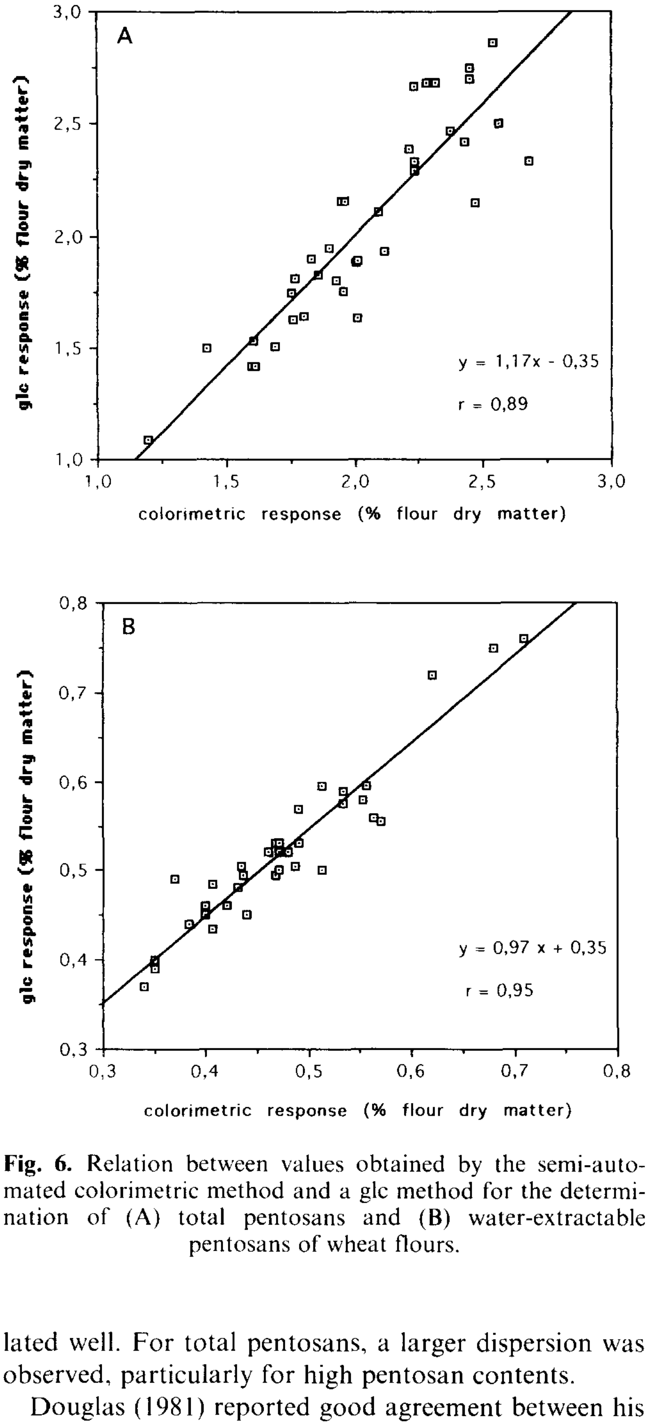

Fig. 6. Relation between values obtained by the semi-automated colorimetric method and a glc method for the determination of (A) total pentosans and (B) water-extractable pentosans of wheat flours.

lated well. For total pentosans, a larger dispersion was observed, particularly for high pentosan contents.

Douglas (1981) reported good agreement between his phloroglucinol method and the traditional Tollens method (Fraser *et al.*, 1956), especially in the range of low pentosan content. Bell (1985) also found excellent correlations between the dietary fiber content of wheat products determined by colorimetry (Southgate, 1976) or gas–liquid chromatography (Englyst *et al.*, 1982) and the pentose content determined by the phloroglucinol method after a sulphuric acid prehydrolysis step. This good agreement exists because the major part of wheat grain cell walls, particularly the endosperm, is composed of arabinoxylan, a polysaccharide made of pentose. For other cereals like barley and oats, where β-glucans contribute largely to endosperm cell wall composition, a less close relation would probably be obtained.

Reproducibility of the method and discrimination of flours
The reproducibility of the semi-automated method was tested by analyzing numerous wheat flours or wheat mill streams. Of the 66 samples analyzed in duplicate, the mean value of total pentosan was 2·16% and that of water-extractable pentosan was 0·63% (Table 4). When the colorimetric determination of only pentoses was taken into account, the coefficient of variation was less than 2%. It was less than 3% for the flour pentosan analysis using the complete procedure described in this paper. Similar or higher coefficients of variation are usually obtained with the gas–liquid chromatography method. However, to get such results the latter method needs great care from a trained operator. On the other hand, the semi-automated method did provide reliable results without particular care or extensive practical experience. The accuracy and reproducibility of the method enabled flours to be discriminated on the basis of their pentosan content. This is evidenced by the low values of least significant differences ($P < 0·05$) obtained. The method presented here appears therefore to be especially useful for routine analysis of flours.

CONCLUSION

A rapid method of pentosan determination in flours is presented, comprising two steps: first the solubilization of pentosans by hot dilute acid (total pentosans) or water (water-extractable pentosans) followed by the colorimetric determination of the solubilized pentose-

Table 4. Precision and reproducibility of the semi-automated method of pentosan determination ($N = 66$, $k = 2$)

	Colorimetric determination (mean value = 2·16%)	Total pentosan (acid solubilization + colorimetric determination) (mean value = 2·16%)	Water-extractable pentosan (water extraction + colorimetric determination) (mean value = 0·63%)
Standard error	0·03	0·1	0·02
Coefficient of variation	1·6%	2·6%	2·5%
Least significant difference	0·06	0·14	0·03

containing polymers by an adaptation of the phloroglucinol method of Douglas, using a continuous flow auto-analyzer. The extraction steps were optimized with respect to the accuracy of sampling (weighing), the rapidity of the procedure and the yield in pentoses. The assembly of a circuit for pentose analysis was made as simple as possible but required specific peristaltic pump tubes able to resist the destructive effect of concentrated acetic acid. Owing to the short reaction times involved, up to 40 sample extracts may be analyzed per hour for their pentosan content. The number of flour pentosan determinations per day comprise between 15 and 60. The proposed method exhibited excellent sensitivity and reproducibility and correlated well with other methods of pentosan determination, in particular a chromatographic method. When compared with the latter, the proposed method was easier to carry out and allowed larger sets of samples to be analyzed daily. On the other hand, no structural information (for example in arabinose:xylose ratio) may be drawn from the global value of pentosan obtained.

The method is especially convenient for routine determination of the pentosan content of wheat flours. The detection of pentoses is specific and remains unaffected by the presence of large amounts of starch or protein, the main components of flours. This method can also be applied to other pentosan-containing material, such as doughs (subsequent to drying and grinding), breads, biscuits and various cereal-based foods or feed. For samples containing cereal components rich in secondary cell-walls (bran), where pentosans are associated with cellulose and lignin, the acid solubilization step should be optimized. The colorimetric determination of pentosans was affected by the presence of fructose and sucrose (underestimation) and uronic acids (overestimation). Therefore, when complex samples are analyzed for pentosan content, knowledge of their gross composition is important to ensure more reliable results.

ACKNOWLEDGEMENTS

The technical assistance of M. Meyer (Alliance Instruments) is gratefully acknowledged.

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