A C T A A L I M E N T A R I A P O L O N I C A
Vol. XVI (XL), No. 3-4 1990

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COMPARISON OF METHODS OF DETERMINING PENTOSES AND HEXOSES IN LIGNOCELLULOSE RAW MATERIAL HYDROLYSATES. I. DETERMINATION OF PENTOSES AND HEXOSES IN MODEL SYSTEMS

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Key words: pentoses, hexoses, model systems, orcinol, o-toluidine.

Determinations of glucose and xylose occurring side by side in various proportions were performed with three colorimetric methods: two methods involing orcinol, and one employing o-toluidine. The comparison of results revealed that the best method of determining xylose error not exceeding 9% was the orcinol method with Glick's reagent. The o-toluidine method was found to be particularly effective in determinations of glucose and xylose side by side, especially when the proportion of the measured component is greater than of the other one.

Many studies are currently underway exploring possibilities of utilizing waste material in forestry and in processing wood and cellulose- and hemicellulose-rich non-arboreal plants to obtain glucose, protein, and, in more distant future, also ethanol by means of culturing fungi which use these materials as a source of carbon and energy. Enzymatic hydrolysis of cellulose and hemicelluloses of straw, sawdust and other such substrates produces hexoses and pentoses, the quantities of which are difficult to determine when they occur side by side. Most laboratories have sufficiently precise measurement apparatus, but the cost of such determinations is high, and sample preparation is time-consuming. In view of this, we decided to compare the various methods of determining pentoses and hexoses side by side in order to single out the method most useful in assaying the quantitative composition of lignocellulose raw materials and hydrolysates thereof.

METHODS

Pentoses and hexoses in a solution were determined with three colorimetric methods making use of the reaction of formation of 2-furaldehyde and 5-(hydroxymethyl)-2-furaldehyde during dehydration and cyclization of sugars

with strong acids. These compounds react with phenol derivatives forming color compounds different for pentoses and hexoses, and it is this that makes it possible to determine them side by side.

1. Method with orcinol [1]: two orcinol reagents in acid medium are prepared; one of them contains Fe^{+3} ions, and the 2-furaldehyde formed during the reaction produces a green complex with orcin in the presence of Fe salts; the other reagent gives a brown-green chromogen with 5-(hydroxymethyl)-2-furaldehyde.

The following reagents were used:

A. 0.2% orcinol solution in H_2SO_4 diluted with water in 1:2 weight ratio;

B. 0.2% orcinol solution in 20% HCl with 0.075% $FeCl_3$.

Procedure: 1-cm^3 samples containing 5-25 μg sugars were treated with 6 cm^3 of reagent A in a test tube, the components mixed, and sealed test tubes heated for 15 min in a boiling water bath. The samples were then cooled, and after 30 min absorbance was measured with a "Spekol" spectrophotometer at 425 nm wavelength. The procedure in the case of reagent B was similar, the only differences being that heating in the boiling water bath lasted 20 min, and that absorbance was measured at $\lambda = 660$ nm.

2. Method with orcinol [2] using just one reagent, proposed by Glick, containing Fe ions in addition to orcinol in 30% HCl. The reagent is composed of 6.75 g of ferric ammonium sulfate and 10 g of orcinol with distilled water to make up the volume of 250 cm^3. Prior to determination, 25 cm^3 of this solution was added to 415 cm^3 of concentrated HCl, and the mixture diluted with water to make up 500 cm^3. The procedure was as follows: 1.5 cm^3 of the solution was treated with 5 cm^3 of Glick's reagent, and the mixture heated for 20 min in a boiling water bath, and then cooled. Absorbance was measured with a spectrophotometer at wavelengths of 520 and 660 nm.

3. Method with o-toluidine [3]. The reagent used in this method is composed of 150 cm^3 of glacial acetic acid, 60 cm^3 of o-toluidine, 50 g of citric acid, 5 g of boric acid, 0.75 g of thiourea, and distilled water making up 500 cm^3. This reagent must be stored in an opaque bottle under refrigeration. The procedure employed was as follows: 0.5 cm^3 of the studied solution was treated with 4.0 cm^3 of the reagent described above. The obtained mixture was heated for 30 min in a boiling water bath and then cooled. Absorbance was measured at wavelengths of 385 and 630 nm.

In order to compare the various methods, we performed our experiments with solutions of sugars of analytical grade, and solutions of various sugars mixed in various proportions. In these solutions we determined the contents of pentoses and hexoses with the studied three methods. There were ten repetitions for each combination. The results were calculated by the determinants method using absorbance values specific for the series of standard solutions, and then analysed statistically.

RESULTS AND DISCUSSION

The sensitivity of the considered methods varied. As can be seen in Table 1, the most sensitive method was the second orcinol one: absorbance in the case of the solution containing 1 g xylose and 1 g glucose per dm^3 measured with the "Spekol" spectrophotometer was 380 for xylose and 196 for glucose at 425 nm; at 660 nm the figures were 70 and just 1.7, respectively. The orcinol method with Glick's reagent proved to be 30 times less sensitive for glucose than for xylose at

Table 1. Sensitivity of the considered methods

Method	Wavelength (nm)	Specific absorbance ($c = g/dm^3$)	
		xylose	glucose
o-toluidine	630	5.4	17.0
	385	36.0	25.0
orcinol	660	70.0	1.7
	425	380.0	196.0
orcinol with Glick's reagent	660	234.0	7.8
	520	31.0	10.4

$\lambda = 660$ nm. The o-toluidine method was the least sensitive of all with regard to pentose, but more sensitive in the case of hexose: at $\lambda = 630$ nm it indicated a three times higher specific absorbance for glucose than for xylose.

The results of xylose determination with the orcinol method (Tables 2 and 5)

Table 2. Results of determinations by the orcinol method of xylose and glucose occuring in various proportions in a solution

Solution component	Amount introducent (μg)	Amount determined ($\bar{\mu g}$)	SD	V	Error (%)
xylose	10.0	9.8	0.3	2.9	-2
1 glucose	10.0	7.4	0.8	11.2	-26
xylose	5.0	5.4	0.2	4.0	+8
2 glucose	10.0	7.8	0.6	9.8	-22
xylose	5.0	5.7	0.2	4.6	+14
3 glucose	20.0	19.8	0.7	3.9	-1
xylose	2.5	2.8	0.4	11.1	+12
4 glucose	22.5	20.2	0.7	4.0	-10
xylose	10.0	9.2	0.2	1.3	-8
5 glucose	5.0	3.8	1.2	46.9	-25
xylose	20.0	18.6	0.5	2.9	-7
6 glucose	5.0	3.8	0.9	43.8	-24
xylose	15.0	14.2	0.3	2.6	-5
7 glucose	2.5	2.2	0.6	25.3	-13

Table 3. Results of xylose and glucose determinations by the orcinol method with Glick's reagent

Solution component	Amount introducent (μg)	Amount determined ($\bar{\mu}$g)	SD	V	Error (%)
xylose	5.0	4.8	0.1	2.9	-4
1 glucose	5.0	13.8	1.4	10.8	+175
xylose	5.0	5.2	0.2	2.8	+4
2 glucose	12.5	30.4	1.0	1.1	+143
xylose	25.0	27.0	0.4	2.0	+8
3 glucose	12.5	32.9	2.8	8.9	+163
xylose	7.5	7.2	0.3	4.7	-4
4 glucose	2.5	5.8	2.5	44.4	+133
xylose	50.0	45.6	1.4	3.2	-9
5 glucose	12.5	35.3	5.6	16.6	+182
xylose	25.0	22.9	0.7	3.5	-8
6 glucose	5.0	11.4	3.1	28.2	+128
xylose	75.0	69.9	3.9	5.9	−7
7 glucose	12.5	32.0	5.8	14.6	+156

Table 4. Results of xylose and glucose determinations by the o-toluidine method

Solution component	Amount introducent (μg)	Amount determined ($\bar{\mu}$g)	SD	V	Error (%)
xylose	25.0	22.0	1.3	6.4	-12
1 glucose	25.0	25.7	1.3	5.1	+3
xylose	25.0	22.2	1.8	8.6	-11
2 glucose	50.0	53.3	1.3	2.7	+7
xylose	12.5	9.8	2.6	38.4	-22
3 glucose	50.0	51.5	3.0	4.4	+3
xylose	12.5	9.4	1.8	55.51	-23
4 glucose	75.0	77.2	1.0	1.1	+3
xylose	6.3	4.0	1.3	54.7	-36
5 glucose	56.7	57.4	1.3	2.0	+2
xylose	50.0	46.2	3.3	7.5	-8
6 glucose	25.0	27.2	2.2	10.6	+9
xylose	50.0	52.5	3.2	5.2	+5
7 glucose	12.5	15.4	3.5	23.7	+23
xylose	75.0	81.7	3.6	4.7	+9
8 glucose	12.5	16.0	2.5	12.4	+28
xylose	56.7	61.8	2.2	3.8 ·	+10
9 glucose	6.3	8.0	1.7	26.8	+29

at 1 : 1 xylose-to-glucose ratio were burdened with a small error not exceeding 2%. When glucose content in the solution was six or nine times greater than xylose content, the error increased to 15%, with the indicated values being higher than the actual ones. When the ratio of the two sugars was reversed, the error in pentose determination dropped from 8 to 2%, and the figures obtained were

lower than in reality. The error in xylose determination decreased with the increase of this sugar's content in the mixture, and the scatter of results ranged between 1.3 and 2.9 (Table 2 V).

The orcinol method with Glick's reagent (Table 3) also gave satisfactory results in determinations of xylose in the presence of glucose. When pentose content remained constant and hexose contents changed, the results of xylose determinations were similar. As the xylose-to-glucose ratio was increased, the results were burdened with errors of 4-9%. The high precision of xylose determination with this method is indicated by values of standard deviation and variability coefficient ranging from 2 to 6% (Table 3 V).

The o-toluidine method (Tables 4 and 5) gave greater errors in determinations of xylose in the presence of glucose than the methods discussed above. At constant amounts of xylose and increasing concentrations of glucose, the error increased from 11 to 36%, the latter figure being obtained when there was nine times more hexose than pentose in the studied solution. When this method was used to determine xylose in solutions containing various amounts of this sugar alongside a fixed amount of glucose, the measurement error did not exceed 10% (Table 5).

Table 5. Comparison of results of determination of xylose in the presence of glucose by the investigated methods

xylose: glucose	Method					
	o-toluidine		orcinol		orcinol with Glick's reagent	
	xylose	error (%)	xylose	error (%)	xylose	error (%)
1:1	8.8	-12.0	9.8	-2.0	9.6	-3.6
1:2	8.9	-11.0	10.8	+8.0	10.4	+4.0
1:4	7.8	-22.0	11.3	+13.2	10.7	+7.0
1:6	7.7	-23.0	11.5	+15.0	9.8	-2.0
1:9	6.2	-36.0	11.3	+13.2	10.6	+6.0
2:1	18.5	-7.5	18.3	-8.5	18.4	-8.0
4:1	42.1	+5.3	36.9	-7.8	36.5	-8.8
6:1	65.3	+8.8	57.0	-5.1	55.9	-6.8
9:1	98.6	+9.6	87.9	-2.3	86.5	-3.9

The situation was different when the investigated methods were used to determine glucose in the presence of xylose. In the case of the orcinol method (Tables 2 and 6), the results for glucose were burdened with errors of 10-27% with the exception of the 1 : 4 and 1 : 6 combinations in which the determined amount of the sugar was only 1% lower than the one actually introduced. It seems, however, that such a small error was accidental. The recovery percentage error in all the studied solutions had a negative value. Moreover, there was a considerable scatter of results, from 3.9 to 47%, regardless od the xylose-to-glucose proportion

Table 6. Comparison of results of determination of glucose in the presence of xylose by the investigated methods

xylose: glucose	Method					
	o-toluidine		orcinol		orcinol with Glick's reagent	
	glucose	error (%)	glucose	error (%)	glucose	error (%)
1:1	10.3	+3.0	7.4	-26.0	27.5	+175
1:2	21.3	+6.5	14.6	-27.0	56.6	+183
1:4	41.2	+3.0	39.7	-1.0	93.4	+133
1:6	61.7	+2.8	59.5	-1.0	132.8	+121
1:9	92.1	+2.3	81.0	-10.0	155.9	+73
2:1	10.9	+9.0	7.5	-25.0	31.5	+215
4:1	12.3	+23.0	7.6	-24.0	28.3	+183
6:1	12.8	+28.0	8.7	-13.0	22.9	+129
9:1	12.9	+29.0	8.0	-20.0	23.4	+134

(Table 2 V). Only when this proportion was 1:4 and 1:9, the variability coefficients were 3.9 and 4.0 which might suggest that the method is applicable in cases of solutions rich in glucose and containing small amounts of xylose. In such conditions both sugars may be determined with considerable accuracy.

The orcinol method with Glick's reagent was virtually useless in determining glucose in the presence of xylose: the figures for this sugar were two-three times higher than the amount actually introduced into the studied solution (Tables 3 and 6).

In the case of the o-toluidine method, when xylose content was constant and glucose contents varied, the errors in hexose determinations ranged from 2 to 7% (Tables 4 and 6). When xylose amounts increased alongside constant amounts of glucose, the error in glucose determination increased from 9 to 30%. When there was nine times more glucose than xylose in the solution, the error in pentose determinations was 36%, and in glucose determinations it was 2%. When the proportion was reversed, the error for xylose was about 10%, and for glucose it increased to 30%. The reproducibility of results in glucose determinations with the o-toluidine method was good even when there was twice as much xylose than glucose in the mixture, this being evidenced by the values of standard deviation, variability coefficient, and error calculated from mean figures (about 10%) (Table 4). However, when the xylose-to-glucose ratio was higher than 2:1, the measurement precision declined, the variability coefficient increased to 27%, and the determined glucose concentration differed by 23-29% from the actual one.

Comparing the results of xylose determination with the three methods (Table 5), we can see that the smallest errors are received by the method with Glick's reagent: the error percentage calculated from the obtained results concerning xylose ranged from −8.8 to +6.0%. The respective figures for the orcinol method are −8.5 and +15%. For the least effective o-toluidine method, the error ranged

from −36% (1:9 xylose-to-glucose ratio) to +9.6% (9:1 xylose-to-glucose ratio).

Glucose may be determined in the presence of xylose with the greatest precision with the o-toluidine method when there is less xylose than glucose in the solution or when the amount of the former is not more than twice greater than the amount of the latter. When this is the case, the error ranges from +2.8 to 9.0%. At higher xylose-to-glucose proportions, the error increased considerably, even up to 29% (Table 6).

When glucose was determined by the orcinol method, satisfactory results were obtained when glucose content in the solution was four or six times greater than xylose content (error: 1%). The method with Glick's reagent cannot be used to determine glucose since it gives figures two or three times higher than the actual ones. A disadvantage of the orcinol method is that two reagents must be used to determine glucose and xylose side by side. In this respect, the o-toluidine method is more convenient since the contents of both these substances are determined in a single test (repeated several times).

Analysis of variance for one-way classification and Tukey's confidence intervals that for selected concentration ratios for which the recovery error in determinations of the two considered sugars was acceptable, the average glucose and xylose contents determined with the various methods differed significantly. In the case of xylose, the calculated mean values differed significantly in the case of the o-toluidine and orcinol methods, and in the case of the o-toluidine and the Glick reagent methods. As regards glucose, all three methods gave significantly different results.

An examination of the mean results of xylose determinations in solutions containing various proportions of xylose and glucose by the analysis of variance method of two way classification revealed no significant differences between the various determination methods in this respect. Such differences were found in glucose determinations by the o-toluidine and the Glick reagent methods, and determinations by the two orcinol methods; no significant differences were found between results obtained by the o-toluidine and orcinol methods.

Holtzapple and Humphrey [2] propose to determine pentoses in the presence of glucose by the orcinol method with Glick's reagent using a correction coefficient to calculate the content of the saccharide in question, thereby eliminating the interference of hexoses. This coefficient enabled the reduction of the error of determination from 37 to 5%. Our results are similar to those of Holtzapple and Humphrey although we did not use the factor they propose, calculating xylose and glucose contents from a system of equations.

Korniejczuk et al. [3] used the o-toluidine method to determine pentoses, hexoses and uronic acid in cellulose semi-finished products. Since their results in model mixtures of sugars were burdened with considerable errors, the authors used coefficients in their calculations which led to a ca. ten-fold reduction of these errors. The authors found that the magnitude of those coefficients depends on the ratio of the sugars in the mixture. Accordingly, in order to obtain correct results,

it is necessary to determine the various coefficients by analysing saccharides mixtures containing the individual sugars in such ratios as those occurring in the analysed raw materials.

To sum up briefly, it seems that the best results in determinations of xylose concentrations in mixtures with glucose are provided by the orcinol method with Glick's reagent, while the method most effective in determining glucose in mixtures with xylose and in determining the sum of both these saccharides is the one involving o-toluidine.

CONCLUSIONS

1. The orcinol method with Glick's reagent is recommended in determinations of xylose in solutions containing glucose.
2. The o-toluidine method is applicable in determinations of glucose in the presence of xylose, especially when the content of the former is equal or greater than the content of the latter.
3. Xylose and glucose content in a solution may be determined by the o-toluidine method but only when the two sugars are in 1:1, 1:2 or 2:1 proportion.

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Manuscript received: March 1989
Authors address: 20-934 Lublin, Akademicka 13

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PORÓWNANIE METOD OZNACZANIA PENTOZ I HEKSOZ W HYDROLIZATACH SUROWCÓW LIGNOCELULOZOWYCH. I. OZNACZANIE PENTOZ I HEKSOZ W UKŁADACH MODELOWYCH*)

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Streszczenie

Porównano wyniki oznaczania glukozy obok ksylozy w roztworach modelowych przygotowanych przez zmieszanie w różnych proporcjach ww. cukrów za pomocą trzech metod kolorymetrycznych, dwóch z orcinolem i jednej z o-toluidyną. Wyniki obliczono metodą wyznaczników oraz poddano je analizie statystycznej. Oznaczając zawartość ksylozy za pomocą podanych wyżej metod stwierdzono, że najmniejszy błąd (-8,8 do +6,0%) popełniono stosując metodę orcinolową z odczynnikiem Glicka, większy przy drugiej metodzie z orcinolem (-8,5 do +15%) i wreszcie z o-toluidyną, gdzie błąd odzysku wynosił od -36% przy stosunku ksylozy do

glukozy 1 : 9 do 9,6% przy proporcji odwrotnej. Glukozę natomiast oznaczyć można obok ksylozy z największą dokładnością metodą o-toluidynową, kiedy ilości ksylozy są mniejsze do glukozy lub tylko dwa razy większe (2,8 do 9%). Przy zastosowaniu metod orcinolowych błąd oznaczenia glukozy wzrastał; jedynie w przypadku proporcji ksylozy do glukozy od 1 : 4 do 1 : 9 można było oznaczyć heksozę metodą orcinolową II z błędem odzysku do 10%. W związku z tym do oznaczania glukozy i ksylozy obok siebie można polecić metodę o-toluidynową, zwłaszcza wówczas gdy stosunek oznaczanych składników wynosi 1 : 1, 1 : 2 i 2 : 1.

*) Pracę wykonano w ramach tematu CPBP nr 04.11/2.27