New Approach to Water-Soluble Carbohydrate Determination as a Tool for Evaluation of Plant Cell Wall Degrading Enzymes

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Studies were conducted to develop a rapid method for the determination of total water-soluble carbohydrates in feedstuffs. Conditions required to result in the satisfactory hydrolysis of plant carbohydrates using HCl without substantial decomposition of constituent sugars were established. The method was validated by recovery studies utilizing mixtures of pure carbohydrates and by comparison of results with those determined by individual analysis of soluble polysaccharides in canola samples. In addition, the total water-soluble carbohydrate content of a number of different feedstuffs was determined, and the application of the method in the evaluation of the efficacy of various commercially available carbohydrase-like enzyme preparations on the hydrolysis of canola meal cell wall polysaccharides was demonstrated.

INTRODUCTION

In recent years there has been a concerted effort to improve the nutritive worth of feedstuffs by using exogenous enzymes. It is well documented that most fungal and microbial enzyme preparations effectively degrade the viscous polysaccharides in barley, oats, and rye and thereby eliminate or reduce the antinutritive effects, particularly for the young chicken (Campbell and Bedford, 1992). The effect is achieved by the cleavage of relatively few linkages, albeit enough to result in the loss of the gel-forming properties of the soluble β-glucan in barley and the arabinoxylan in rye.

It has been suggested by Theander et al. (1989) that additional improvement in the nutritive value of cereals could be realized by the application of exogenous enzymes in the hydrolysis or solubilization of cell wall polysaccharides. In this regard, the effect would be the reduction or elimination of the encapsulating influence of the cell wall to enhance the availability of protein and starch and possibly to improve the utilization of nonstarch polysaccharides. Considering the complexity of the cell wall structure and the low water solubility of cell wall polysaccharides, it would appear that this latter application of exogenous enzymes is a much more complex issue than that for the viscous polysaccharides. Enzymes capable of hydrolyzing a wide array of complex linkages would be necessary, and the monitoring of the potency of various enzyme preparations would necessitate the analysis of a number of different compounds.

The extent of cell wall hydrolysis could be determined by monitoring the increase in total water-soluble carbohydrate content in samples, but the numerous methods available for the determination of simple sugars, oligosaccharides, and starch and nonstarch polysaccharides are usually specific for only one class of compound. Consequently, a method was developed for the comprehensive measurement of total water-soluble carbohydrates in feedstuffs. The development of the method and its application in the evaluation of exogenous enzyme preparations used to hydrolyze the carbohydrates in canola are outlined.

MATERIALS

Pure standards of monosaccharides, sucrose, oligosaccharides, inulin, arabinogalactan, wheat starch, and the enzymes pancreatin (4 × U.S.P.), α-amylase, and pullulanase were purchased from Sigma (St. Louis, MO). Pronase was obtained from Boehringer Mannheim (Laval, PQ). Feedstuff samples used in the study were purchased from a local feed company (Feed-Rite Ltd., Winnipeg, MB), and the commercial enzyme preparations were obtained from a number of companies: Energex, Pectinex Ultra SP, Bio-Feed Plus, Celluclast 1.5 L, and Bio-Feed Pro from Novo Industri A/S (Bagsvaerd, Denmark); Rohament CA, Rohament 7053, and Corolase PN from Rohm Tech Inc. (Malden, MA); Avizyme SX from Finfeeds International (Marlborough, U.K.); Cellulase TL-1000 and Multizyme II from Enzyme Development Corp. (New York). Canola seed samples were provided by several research groups: P. McVetty, University of Manitoba (Winnipeg, Canada), B. Uppstrom, Svalof AB (Svalov, Sweden); and K. Downey, Agriculture Canada (Saskatoon, Canada).

The water-soluble xylan used in the study was isolated from oat xylan (Sigma) following solubilization in hot dimethyl sulfoxide–water (1:3 v/v). The precipitate formed with 80% ethanol was dissolved in water and freeze-dried. The same procedure was used to isolate soluble β-glucan from barley β-glucan (Sigma). To obtain canola water-soluble nonstarch polysaccharides, canola meal was incubated with Tris-HCl buffer (0.1 M, pH 7.5), sodium azide (0.05%), and Pronase enzyme (1000:1 w/w) for 10 h, under continuous shaking. The isolate was centrifuged, and the precipitate formed with 80% ethanol was redissolved in hot sodium acetate buffer (0.1 M, pH 5.2) and then subjected to starch hydrolysis (Englyst et al., 1982). The residual pellet obtained after a subsequent 80% ethanol precipitation and centrifugation was dissolved in warm water and freeze-dried. Flax mucilage was isolated from flax meal using the identical procedure.

Other reagents used in the current study were of analytical grade.

METHOD DEVELOPMENT

Analytical Procedures Used in the Development of the Method. Nonstarch polysaccharides were determined by gas–liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedures described by Englyst and Cummings (1984) with minor modifications (Slominski and Campbell, 1990). Sucrose, raffinose, and stachyose were determined as described by Slominski and Campbell (1991) utilizing extraction in 80% ethanol, derivatization with pyridine/*N*-methyl-*N*-TMS-trifluoroacetamide/trimethylchlorosilane (100:50:10 v/v), and gas chromatographic separation on a glass column (1.2 m × 2 mm i.d.) packed with 2% OV-7 on Chromosorb W(HP) with helium gas at a flow rate of 40 mL min^{-1}. The oven temperature was programmed from 170 to 320 °C at 6 °C min^{-1}. Injection port and detector temperatures were 280 and 320 °C, respectively. The response factors were determined relative to that of *myo*-inositol (internal standard). Fructose, galactose,

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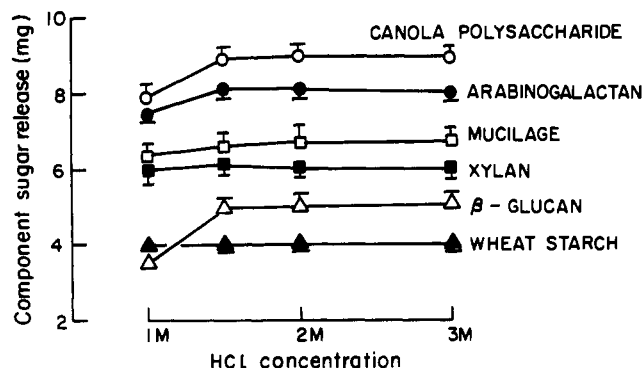

Figure 1. Release of component sugars on hydrolysis of water-soluble preparations of cell wall polysaccharides and wheat starch with various concentrations of hydrochloric acid. To facilitate gas chromatographic quantification of component sugars, various concentrations of polysaccharide standards were subjected to acid hydrolysis. Percent recoveries of polysaccharides on hydrolysis with 1.5 M HCl were as follows (mean ±SD): canola polysaccharide, 93.4 ± 2.3; arabinogalactan, 98.5 ± 1.4; flax mucilage, 89.6 ± 1.5; xylan, 103.4 ± 1.6; β-glucan, 95.3 ± 1.1; wheat starch, 100.3 ± 0.5.

Table I. Recovery of Monosaccharides following Incubation with 1.5 M HCl at 100 °C for 1 h

monosaccharide	recovery, %	monosaccharide	recovery, %
rhamnose	95.3 ± 2.8[a]	galactose	96.1 ± 1.2
arabinose	95.6 ± 2.8	glucose	97.7 ± 1.2
xylose	94.6 ± 1.4		
mannose	94.7 ± 1.2	av	95.7

[a] Mean ± SD.

Table II. Percentage Decomposition of Fructose on Hydrolysis of Fructose-Containing Carbohydrates with 1.5 M HCl at 100 °C for 1 h

carbohydrate	fructose decomposed, %
sucrose	54.7 ± 2.3
raffinose	56.8 ± 1.9
inulin	57.5 ± 3.1

and glucose were determined by gas–liquid chromatography as alditol acetates according to the method for non-starch polysaccharides (Englyst and Cummings, 1984). For starch analysis samples were hydrolyzed enzymatically [α-amylase (EC 3.2.1.1; Termamyl 120L, Novo Industri A/S) and amyloglucosidase (EC 3.2.1.3; Boehringer Mannheim)] and the released glucose was determined by the hexokinase–glucose-6-phosphate dehydrogenase method (Boehringer Mannheim).

Hydrolysis of Water-Soluble Carbohydrates. Several different types of soluble carbohydrate were used in a study to investigate the optimum conditions required to accomplish complete hydrolysis and to minimize component sugar losses. Known amounts of selected water-soluble polysaccharides were subjected to hydrolysis at 100 °C for 1 h using various concentrations of HCl. The release of component sugars, as determined by gas-liquid chromatography, is shown in Figure 1. Hydrolysis was maximal at 1.5 M HCl, and consequently this concentration was chosen for further study.

The potential effect of hydrochloric acid on the sugar components of various plant polysaccharides was investigated by subjecting known amounts of selected monosaccharides (Table I) and fructose-containing carbohydrates (sucrose, raffinose, and inulin) to hydrolysis in 1.5 M HCl at 100 °C for 1 h. As shown in Table I, a high recovery was evident for monosaccharides studied, and consequently it can be inferred that minimal loss of constituent sugars in plant polysaccharides would occur with hydrolysis in 1.5 M HCl. Furthermore, the data indicate that, because of the similar loss for all monosaccharides, a correction could be made by subjecting the glucose standard to the hydrolysis conditions employed in sample preparation.

Further studies were conducted with the fructose-containing carbohydrates utilizing milder (<1.5 M HCl) hydrolysis conditions because substantial decomposition of fructose occurred with 1.5 M HCl (Table II). Known quantities of fructose, sucrose, raffinose, and inulin were subjected to hydrolysis with 0.25, 0.5, and 1.0 M HCl at 100 °C for 1 h. In this study, 0.5 M HCl was shown to be effective for the hydrolysis of the fructose-containing carbohydrate samples, but it was also apparent that decomposition of fructose occurred at this HCl concentration (Figure 2). However, it appeared from the data for samples hydrolyzed at 0.5 and 1.0 M HCl that the loss of fructose due to destruction during hydrolysis was balanced by the continuous release of fructose from hydrolyzed carbohydrates. Consequently, a constant yield of fructose was obtained for sucrose, inulin and, to a lesser extent, raffinose.

Ethanol precipitation was used to separate the carbohydrates to be hydrolyzed with 1.5 M HCl (i.e., starch and nonstarch polysaccharides) from those requiring milder hydrolysis conditions (i.e., sucrose, raffinose, and stachyose). The buffer used in the extraction step prior to ethanol precipitation was found to be critical in preliminary studies. A phosphate buffer (0.1 M, pH 7.0) resulted in substantial precipitation of raffinose (15%) and stachyose (44%) when ethanol was added, whereas a Tris-

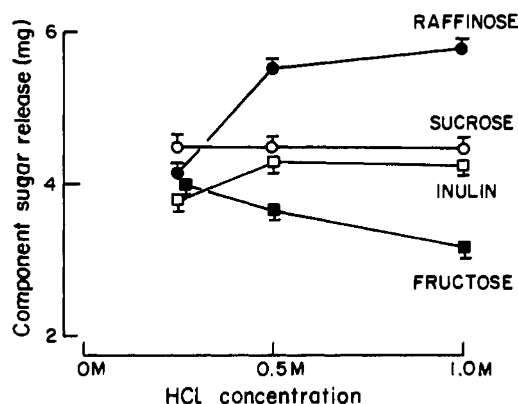

Figure 2. Release of component sugars vs decomposition of fructose on hydrolysis of fructose-containing carbohydrates with hydrochloric acid. Percent recoveries of carbohydrates on hydrolysis with 0.5 M HCl were as follows (mean ± SD): raffinose, 86.6 ± 1.2; sucrose, 94.6 ± 2.1; inulin, 96.3 ± 2.8; fructose, 91.2 ± 1.3.

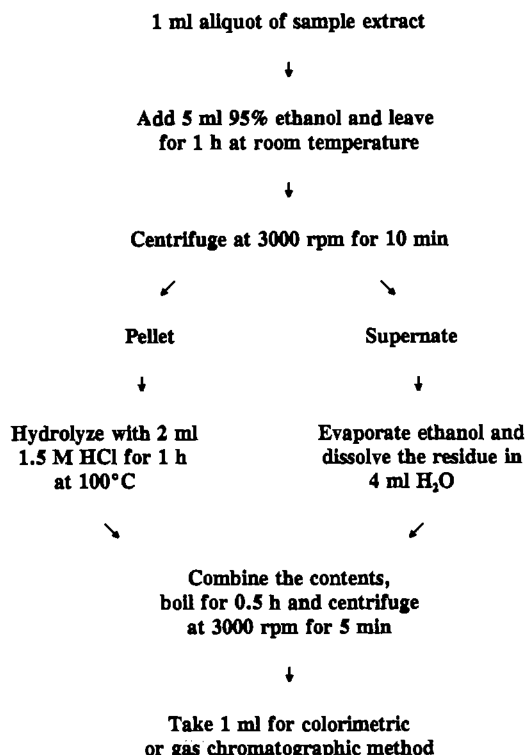

Figure 3. Procedure used for water-soluble carbohydrate determination in samples of feedstuffs and canola meal hydrolysates.

Table III. Mixtures of Pure Carbohydrates Used in Recovery Studies

sample	carbohydrate, mg						% of dry matter
	starch	arabinogalactan	sucrose	raffinose	glucose	fructose	
A	800	100	25	50	12.5	12.5	90.8
B	150	150	400	250	25	25	94.3
C	50	750	50	100	25	25	93.3
D	400	400	100	50	25	25	92.3
E	50	50	400	400	50	50	93.1

HCl buffer (0.1 M, pH 7.5) did not cause any precipitation. Consequently the Tris-HCl buffer was chosen for use.

Total Water-Soluble Carbohydrate Determination. Carbohydrates were determined by utilizing the hydrolysis conditions developed in the current study. The analytical scheme is summarized in Figure 3 and is described in detail below.

One hundred milligrams of samples containing 40–70% carbohydrates (i.e., high-starch products) or 200 mg for samples containing 10–30% carbohydrates was weighed into 10-mL test tubes. Three milliliters of dimethyl sulfoxide (DMSO) was then added, and the tubes were placed in a boiling water bath. The samples were mixed well for at least 5 min to ensure proper starch solubilization and were kept for an additional 20 min at 100 °C. The DMSO extract was not allowed to cool before 7 mL of warm water (60 °C) was added. The samples were mixed well and boiled for 1 h. Following centrifugation at 3000 rpm for 10 min, 1 mL of clear extract was transferred into a separate test tube (tube A). Five milliliters of 95% ethanol was then added, and the contents were mixed and allowed to stand for 1 h at room temperature. Following centrifugation, the supernatant liquid was transferred to another test tube (tube B) which was then placed on a hot plate (60–70 °C), and the ethanol was evaporated under a stream of air. The dry residue was dissolved in 4 mL of water. Two milliliters of 1.5 M HCl was added to tube A (pellet), and the contents were hydrolyzed for 1 h at 100 °C. The hydrolysate was then quantitatively transferred from tube B to tube A, and the hydrolysis was continued at 100 °C for 0.5 h. Following centrifugation at 3000 rpm for 5 min, 1 mL of supernatant was taken for colorimetric or gas–liquid chromatographic determination of total soluble carbohydrates.

For the colorimetric measurement of carbohydrates, the procedure of Dahlqvist (1962) as recommended by Englyst and Hudson (1987) was used. To 1 mL of hydrolysate and the blank sample (1 mL of 0.5 M HCl) were added 0.5 mL of glucose solution (0.5 mg/mL) and 0.5 mL of 0.97 M NaOH. The contents were mixed in a vortex before addition of 2 mL of dinitrosalicylate solution which was prepared 2 days before use by dissolving 10 g of 3,5-dinitrosalicylic acid, 16 g of NaOH, and 300 g of sodium/potassium tartrate in 1 L of deionized water. The contents were then mixed, boiled for 10 min, and cooled to room temperature. Following addition of 20 mL of distilled water, the absorbance was read at 530 nm against the blank sample. The concentration of total sugars was calculated from the standard curve obtained by subjecting known amounts of glucose solution (0.5, 1.0, 1.5, and 2.0 mg per 1 mL of 0.5 M HCl) to the same treatment as the test sample.

Alternatively to the colorimetric procedure, total carbohydrates were determined by gas–liquid chromatography of component neutral sugars as alditol acetates. This method was initiated following the DMSO treatment in the extraction step of sample preparation. Ten milligrams of inositol (internal standard) was added, and the extraction and hydrolysis with HCl were performed as described above. One milliliter of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated according to the method for nonstarch polysaccharides (Englyst and Cummings, 1984). The hydrolysates were analyzed for uronic acids using the method of Scott (1979) as modified by Englyst and Cummings (1984).

RECOVERY AND METHOD EVALUATION STUDIES

A series of standards were prepared by mixing together various proportions of pure carbohydrates (Table III) to mimic the carbohydrate profiles in commonly used feedstuffs. These samples were used in recovery studies in which known quantities of the carbohydrate standards were subjected to the proposed two-step hydrolysis procedure described above. The contents of total carbohydrates were then determined by both colorimetry and gas–liquid chromatography analyses (Table IV). Six samples of canola meal, for which data on the individual carbohydrate content were available (Slominski and Campbell, 1991), were analyzed using the proposed procedure as a further evaluation of the method (Table V). In addition, several selected samples (Table VI) of feedstuffs were analyzed for total water-soluble carbohydrate content.

Table IV. Percentage Recovery of Carbohydrates As Determined by Gas–Liquid Chromatography (GLC) and Colorimetry

sample[a]	GLC	colorimetry
A	103.2 ± 2.9[b]	105.9 ± 1.1
B	100.2 ± 2.9	102.2 ± 2.6
C	106.1 ± 2.2	104.0 ± 0.9
D	105.1 ± 1.7	104.5 ± 0.6
E	99.0 ± 1.9	100.4 ± 2.3

[a] See Table III for carbohydrate identifications. [b] Mean ± SD; no correction factors were used to convert constituent sugar values to total carbohydrate values.

APPLICATION OF THE PROPOSED METHOD FOR CARBOHYDRASE-LIKE ENZYME EVALUATION

A study was conducted to determine the usefulness of the proposed methodology as an in vitro assay to evaluate the efficacy of enzyme preparations containing carbohydrase-like activities. The evaluation involved the incubation of an enzyme preparation with a carbohydrate source and the determination of the total water-soluble carbohydrate content of the incubation medium with and without added enzyme. Activity was expressed as milligrams of total soluble carbohydrate per milligram of enzyme. In the current study, canola meal was used as the carbohydrate source and various enzyme preparations (Table VII) available commercially as dietary additives were evaluated. In the assay, 10 g of canola meal was incubated for 5 h, under continuous mixing, with 50 mg of enzyme preparation in 200 mL of Tris-HCl buffer (0.1 M, pH 7.5) at 40 °C.

The proposed enzyme evaluation procedure was designed to measure enzyme activity by determining the yield of water-soluble carbohydrates following incubation and the two-step hydrolysis procedure. However, modification of the technique with a determination of total water-soluble carbohydrate content at each step of the hydrolysis procedure (i.e., ethanol precipitate and ethanol supernatant, see Figure 3) was done to allow an assessment of the extent of hydrolysis of polysaccharides during solubilization. In this regard, canola meal was incubated with and without selected individual and combined enzyme preparations (Table VIII). Total carbohydrate content was determined on both the ethanol-insoluble (precipitate) and the ethanol-soluble (supernatant) fractions.

Many commercial enzyme preparations contain protease enzymes, and the solubility of nonstarch polysaccharides may be influenced by amylase enzyme action on high-starch products; consequently, additional trials were conducted to test the effect of these parameters on the assay procedure. The potential effect of protease enzymes on the yield of total water-soluble carbohydrates in the assay procedure was assessed by determining the activity of enzyme preparations known to contain protease enzymes (Table IX). Canola meal was incubated with and without Corolase, Multizyme II, and Bio-Feed Pro enzyme preparations, and the total carbohydrate content was determined as described above. The influence of prior digestion with pancreatin on the yield of total water-soluble carbohydrates was also studied (Table X). Canola meal was incubated with and without pancreatin and combinations of pancreatin and carbohydrase-like enzyme

Table V. Total Water-Soluble Carbohydrate Content of Canola Samples Determined by the Proposed Procedure and Compared with the Summation of Individual Analyzed Values (Percent of Dry Matter)[a]

sample	sucrose	oligosaccharides	starch	NSP[b]	total water-soluble carbohydrates by summation	total water-soluble carbohydrates by proposed method
brown-seeded canola						
cv. Westar	7.7	3.4	2.3	1.4	14.8	15.7 ± 0.2[c]
cv. Legend	7.2	2.6	2.8	1.5	14.1	14.7 ± 0.1
cv. Kova	8.9	2.7	2.6	1.5	15.7	16.4 ± 0.2
yellow-seeded canola						
cv. Parkland	9.8	3.0	2.5	1.9	17.2	16.2 ± 0.1
cv. Colt	9.8	2.1	2.6	1.6	16.1	16.2 ± 0.2
cv. Horizon	9.4	2.5	2.6	2.3	16.8	16.5 ± 0.3

[a] See Slominski and Campbell (1991). [b] Nonstarch polysaccharides. [c] Mean ± SD.

Table VI. Total Water-Soluble Carbohydrate Content As Determined by Gas-Liquid Chromatography (GLC) and Colorimetry (Percent of Dry Matter)

sample	GLC	colorimetry
canola meal (brown)	13.7 ± 0.2[a]	14.7 ± 0.2
canola meal (yellow)	15.7 ± 0.2	16.5 ± 0.3
soybean (44%)	20.0 ± 0.3	19.5 ± 0.1
linseed (dark)	17.9 ± 0.3	18.1 ± 0.2
faba bean	44.0 ± 0.6	41.2 ± 0.4
sunflower	11.7 ± 0.1	12.8 ± 0.1
peas (yellow)	50.5 ± 0.5	49.6 ± 0.4
peas (green)	54.1 ± 0.4	53.6 ± 0.5
lentils	50.0 ± 0.4	51.9 ± 0.3
av	30.8	30.9

[a] Mean ± SD.

Table VII. Total Water-Soluble Carbohydrates of Canola Meal with and without Enzyme Treatment

enzyme	total water-soluble carbohydrates, % of dry matter	activity[a]
none	12.4 ± 0.2[b]	
Energex	14.6 ± 0.3	4.0
Pectinex Ultra SP	14.3 ± 0.1	3.4
Rohament CA	14.3 ± 0.1	3.4
Bio-Feed Plus	13.8 ± 0.2	2.5
Avizyme SX	13.8 ± 0.1	2.5
Rohament 7053	13.7 ± 0.0	2.3
Celluclast 1.5 L	13.4 ± 0.2	1.8
Cellulase TL-1000	13.3 ± 0.2	1.6

[a] mg of soluble carbohydrate per mg of enzyme. [b] Mean ± SD.

Table VIII. Examples of Water-Soluble Carbohydrate Distribution following Incubation of Canola Meal with Selected Enzyme Preparations (Percent of Dry Matter)

enzyme	80% ethanol precipitate	80% ethanol solubles	total
none	1.3 ± 0.06[a]	10.6 ± 0.0	11.9 ± 0.1
Energex	1.3 ± 0.04	12.8 ± 0.3	14.1 ± 0.3
Energex + Celluclast	1.4 ± 0.05	13.6 ± 0.2	15.0 ± 0.2
Pectinex U-SP	1.4 ± 0.03	13.0 ± 0.2	14.4 ± 0.1
Pectinex U-SP + Celluclast	1.6 ± 0.03	13.5 ± 0.1	15.1 ± 0.2

[a] Mean ± SD.

preparations (Energex and Pectinex Ultra SP). Total carbohydrate content was then determined in the various incubations to measure enzyme activity. In addition, the incubations were carried out at pH 5.2 (0.1 M acetate buffer) and pH 7.5 (0.1 M Tris-HCl buffer) to study the influence of pH on the assay procedure.

RESULTS AND DISCUSSION

Carbohydrate Recovery by the Proposed Procedure. Satisfactory recovery values were obtained for the mixtures of carbohydrate standards that were subjected to the two-step hydrolysis procedure (Table IV). In addition, both methods of analysis of component sugars (colorimetry and gas-liquid chromatography) gave the same high recovery values. In general, sugar yields from the polysaccharide-rich mixtures (i.e., samples A, C, and D) were higher than those for the mixtures containing substantial quantities of sucrose, raffinose, or free fructose (samples B and E). The differential in recovery between the two groups was most likely a consequence of the difference in molecular size between the respective mixtures of carbohydrates since no correction factors were used to correct constituent sugar values to total carbohydrate values. In this regard, such correction factors are difficult to establish for feedstuff samples, and consequently it is suggested that no correction factors be used in the proposed procedure. Any error caused by this omission would be offset, somewhat, by sugar losses (particularly fructose) and for incomplete hydrolysis with diluted HCl (0.5 M).

Total Water-Soluble Carbohydrate Content of Feedstuffs. The total water-soluble carbohydrate content as determined by the proposed procedure is given in Table V and compared with the summation of the soluble carbohydrate contents analyzed individually by specific methods. Similar results were obtained by both procedures, lending credence to the proposed method. Although the samples were similar in carbohydrate content, there were some differences among the samples with regard to sucrose and water-soluble NSP contents. Further validation of the method, however, is needed using samples of a more varied carbohydrate composition. In this regard, the data given in Table VI show the results for the total water-soluble carbohydrate content of various feedstuff samples. Values for the individual carbohydrates were not available for these samples, so no validation of the method could be done, but the data indicate good agreement for all samples tested between the determination of total carbohydrate content using either colorimetry or gas-liquid chromatography. Individual constituent sugar profiles were available from the gas-liquid chromatography analyses, but identification of specific carbohydrates in the various samples was inconclusive. Interpretation of the sugar profiles was difficult because several of the constituent monosaccharides (notably glucose) may originate from several sources (i.e., sucrose, oligosaccharides, and starch and nonstarch polysaccharides), and fructose appeared to be nonstereospecifically reduced to two isomeric peaks that were present on chromatograms at retention times of mannose and glucose (Figure 4). In addition, the hydrolysis of high-starch products resulted in the formation of two peaks. One of them was identified as 2-deoxyglucose (Figure 4). The origin of these peaks is uncertain and requires further study.

Evaluation of Carbohydrase-like Enzymes. The total water-soluble carbohydrate content of canola meal incubated with various enzyme preparations is shown in Table VII. In all of the samples tested a minor increase in the soluble carbohydrate fraction occurred with in-

Table IX. Solubilization of Polysaccharides on Incubation of Canola Meal with Protease Enzymes at pH 7.5

enzyme	total water-soluble carbohydrates, % of dry matter
none	12.3 ± 0.1[a]
Corolase	15.2 ± 0.2
Multizyme II	14.4 ± 0.2
Bio-Feed Pro	13.8 ± 0.1

[a] Mean ± SD.

Table X. Effect of Pancreatin Addition on Solubilization of Polysaccharides following Incubation of Canola Meal with Selected Enzymes under Different pH Conditions

enzyme	water-soluble carbohydrates, % of dry matter	
	pH 5.2	pH 7.5
none	12.2 ± 0.2[a]	12.4 ± 0.0
Pancreatin	16.2 ± 0.4	16.3 ± 0.3
Pancreatin + Energex	17.8 ± 0.3	17.3 ± 0.1
Pancreatin + Pectinex Ultra SP	17.9 ± 0.2	16.6 ± 0.2

[a] Mean ± SD.

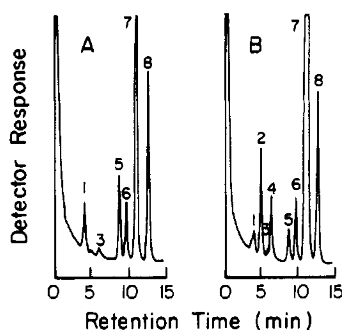

Figure 4. Monosaccharide profile of total water-soluble carbohydrates from (A) canola meal and (B) peas: 1, arabinose; 2, unknown; 3, xylose; 4, 2-deoxyglucose; 5, mannose and fructose isomer; 6, galactose; 7, glucose and fructose isomer; 8, *myo*-inositol (internal standard).

creasing activity levels (milligrams of soluble carbohydrate per milligram of enzyme) ranging from 1.6 to 4.0. Presumably the increase in total water-soluble carbohydrates that did occur was due to hydrolysis or solubilization of cell wall polysaccharides. The data in Table VIII indicate that the majority of the increase in total water-soluble carbohydrates was in the ethanol-soluble fraction and hence suggests low molecular weight carbohydrates. These data agree with the suggestion that carbohydrase enzymes exert their effects by a mechanism of surface peeling, which would result in the release of short-chain polysaccharides from the cell wall (Hotten, 1991). This process, however, would appear to be limited by the low water solubility of cell wall polysaccharides and possibly by a high degree of cell wall lignification. As shown in Table V, canola contains only a low content of water-soluble polysaccharides. In addition, canola meal as reported by Slominski and Campbell (1991) has a relatively high content of lignin, particularly brown-seeded canola which was the type of canola meal used in the current study. As shown in Table IX, incubation with enzyme preparations containing high proteolytic activity increased the total water-soluble carbohydrate content of canola meal. A similar effect was noted for pancreatin (Table X). These data indicate that care must be taken in the evaluation of the activity of various enzyme preparations as most commercial preparations are crude and contain a wide array of enzyme activities (Campbell and Bedford, 1992). In this regard, the activities of Energex and Pectinex Ultra SP were lower when incubation in pancreatin was used as the control than when no enzyme addition was the control (Table X vs Table VII). On the basis of these results it could be suggested that pancreatin (porcine pancrease) be included with an enzyme preparation in any evaluation of cell wall degrading enzymes. Furthermore, for high-starch products the amylase activity may become limiting and require fortification with α-amylase (Babinszky et al., 1990) and amyloglucosidase (Asp et al., 1983). Further research in this area is warranted.

As shown in Table X, the activities of Energex and Pectinex Ultra SP were higher at pH 5.2 than at pH 7.5. This is in agreement with the fact that many fungal enzyme preparations are known to have optimum activity at low pH. Despite this fact, it is recommended that evaluation assays be conducted at a pH of 7–7.5 since this more closely reflects the pH of the intestinal tract of animals where the enzyme is likely to exert its effect. The crop of the chicken may be an exception to this, but the residence time of food in this compartment of the intestinal tract is relatively short (Shires et al., 1987).

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