Table II. Recoveries of Glyphosate from Fortified Water Samples after Cleanup and Derivatization

| glyphosate, μg | concn | recoveries, %, of glyphosate[a] | |
|---|---|---|---|
| | | dist water | river water |
| 1000 | 4 ppm | 89.5 | 92.6 |
| 400 | 80 ppb | 80.0 | |
| 100 | 20 ppb | 102.2 | 98.0 |
| 50 | 10 ppb | det[b] | det |

[a] Average values of at least two determinations. [b] det signifies detectable.

Table III. Recoveries of Glyphosate from Three Fortified Soils

| sample | glyphosate | |
|---|---|---|
| | added, ppm | recoveries, % |
| Houston clay loam | 200 (2)[a] | 18.99 ± 1.15[b] |
| | 100 (2) | 25.85 ± 0.60 |
| | 50 (2) | det[c] |
| Sassafras sandy loam | 200 (4) | 55.33 ± 1.35 |
| | 50 (4) | 43.40 ± 1.10 |
| | 25 (4) | 35.79 ± 3.25 |
| | 5 (4) | det |
| Iuka silt loam | 200 (4) | 52.70 ± 2.19 |
| | 50 (2) | 39.55 ± 0.45 |
| | 25 (2) | 30.69 ± 2.45 |
| | 5 (2) | det |

[a] Number of determinations. [b] Averages and standard deviations. [c] det signifies detectable.

These results (not shown) demonstrated that analytical samples containing the glyphosate-FMOCCl derivative cannot be concentrated simply by boiling off the water. No satisfactory procedure using heat was developed for concentrating samples containing derivatized glyphosate in this study.

The data in Table II show the recoveries (percent) of glyphosate from fortified water samples after a single column cleanup and HPLC analysis of derivatized glyphosate. The results were similar for the distilled and river water samples. The minimum level of detection was 10 ppb in these water samples. It required ca. 2 h for a complete water analysis.

The recoveries (percent) of glyphosate from fortified Sassafras, Iuka, and Houston soils are shown in Table III. The lowest recovery of glyphosate was found on the Houston, which was the soil that possessed the highest clay content. This low recovery is attributed to adsorption of glyphosate by soil components. In an earlier study, Sprankle et al. (1975) showed that glyphoshate readily adsorbed to various clay fractions and organic matter from soils. The higher recoveries of glyphosate from the Sassafras and Iuka soils presumably are due to the lower rates of adsorption by these soils. The minimum levels of detection were 5 ppm on the Sassafras and Iuka soils and 50 ppm on the Houston. A complete soil analysis required ca. 3 h. In conclusion, it has been demonstrated that this HPLC method is reproducible and sensitive for the determination of glyphosate in soil and water samples. In comparison to other reported methods, this method is simpler, faster, and specific for glyphosate.

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**Registry No.** Glyphosate, 1071-83-6; water, 7732-18-5.

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# Determination of Reducing Sugars, Sucrose, and Inulin in Chicory Root by High-Performance Liquid Chromatography

Alistair W. Wight* and Pieter J. van Niekerk

A high-performance liquid chromatographic method for determination of reducing sugars, sucrose, and inulin in both freeze-dried and roasted chicory root is described. Reducing sugars are determined directly after postcolumn reaction with tetrazolium blue, and sucrose is determined indirectly as glucose after hydrolysis by β-fructosidase. Inulin is determined indirectly as fructose and glucose formed as a result of mild acid hydrolysis. The method is reasonably rapid and provides a more complete analysis than existing methods, and determinations can be made with a satisfactory degree of accuracy and precision.

Relatively little information on analysis of carbohydrates in chicory is available in the literature. Free fructose and glucose have been determined by enzymatic methods (Promayon et al., 1976; Blanc, 1978) while thin-layer chromatography (TLC) has been used to determine fructose, glucose, and sucrose (Bachman and Zegota, 1974). Chubey and Dorell (1978) determined fructose and glucose in two chicory cultivars harvested at different stages during two successive seasons.

Most of the methods described in the literature have limitations for routine analysis, however. TLC methods are rather time consuming and accurate quantitation is

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difficult, while enzymatic methods may provide incomplete information on sugar composition if sugars other than fructose, glucose, and sucrose are present.

High-performance liquid chromatography (HPLC) offers the advantage over enzymatic methods that a separate determination need not be made for each individual sugar present in the sample. The low sensitivity and lack of selectivity of most commercially available detectors have, however, limited the application of HPLC methods. So that these difficulties could be overcome, a detection system utilizing postcolumn reaction of reducing sugars with tetrazolium blue has been used (Wight and van Niekerk, 1983). This detection system provides sensitivity comparable with that of enzymatic methods while also providing information on less common sugars which may be present in small quantities in plant material.

Inulin has been determined by acid hydrolysis of the polysaccharide followed by enzymatic determination of the fructose formed (Blanc, 1978; Gutmann and Bergmeyer, 1974). This procedure may be criticized on the grounds that sucrose (which may be present in appreciable quantities in fresh chicory) will also liberate fructose on acid hydrolysis and some error will result if a separate determination of sucrose is not made. Also, structural evidence indicates that inulin is not a simple fructan as was originally believed but contains a small proportion of glucose which is linked to the fructan chain as a nonreducing end group as in sucrose (Aspinall, 1970). Methods for determination of inulin based on hydrolysis should, therefore, take into account both fructose and glucose formation if a high degree of accuracy is required.

So that these objections to existing methods could be overcome, an HPLC method was developed for determination of inulin in addition to reducing sugars and sucrose. Details of the procedure used are given in the following sections.

## MATERIALS AND METHODS

**Chicory Samples.** A sample of fresh roots (cultivar Wixor) from the 1981 South African crop was used. Part of the sample was freeze-dried while the remainder was dried overnight at 70 °C and roasted (6 h at 150 °C followed by 20 min at 190 °C). The dried material was ground to pass a 25-mesh screen and used for analysis of free sugars and inulin.

**Inulin.** Inulin used for recovery experiments was obtained from Merck.

**Determination of Reducing Sugars and Sucrose.** The procedures used for extraction of sugars, sample preparation, and analysis by HPLC were described in detail in a previous publication (Wight and van Niekerk, 1983) and are described in outline here.

Sugars and inulin were extracted from the sample (approximately 5 g plus sufficient calcium carbonate to neutralize acidity) with hot water (80 mL, 80–90 °C, 1 h). Octanol (1–2 drops) was added to minimize frothing during extraction. The extract was cooled and made up to 100 mL, and part of the extract was clarified with neutral lead acetate, deionized, treated with ethanol (to 80% ethanol concentration), and filtered.

Reducing sugars were determined by direct HPLC analysis of the clarified filtrate. Sucrose was determined indirectly as glucose by hydrolyzing part of the hot water extract with β-fructosidase prior to clarification.

A plain silica column was used for chromatography, and acetonitrile–water (75:25) containing 0.01% HPLC amine modifier I (Aitzetmüller, 1978) was used as the mobile phase (flow rate 3 mL/min). Sugars were detected by absorbance at 550 nm after postcolumn reduction of tetrazolium blue at 85 °C in a stainless steel reactor.

**Determination of Inulin.** An aliquot of unclarified hot water extract (prepared as described in the previous section and containing approximately 50 mg of inulin) was transferred to a 100-mL volumetric flask. Hydrochloric acid (25 mL, 2.5%) was added and the mixture was incubated for 15 min at 70 °C. The hydrolysate was cooled to room temperature and neutralized with calcium carbonate (1 drop of octanol was added in order to minimize frothing). Saturated neutral lead acetate (sufficient to complete precipitation) was added, the solution was made up to volume, deionized, treated with ethanol (to 80% ethanol concentration), and filtered, and the filtrate was used for chromatography.

**Calculation of Inulin Content.** If the free glucose content of the sample = $a$ and the fructose content of the sample = $a_f$ and the total glucose content of the sample after treatment with β-fructosidase (i.e., free glucose plus glucose from hydrolyzed sucrose) = $b$, then the sucrose content ($S$) is given by

$$S = 1.90(b - a)$$

The factor 1.90 arises from the uptake of one molecule of water per molecule of sucrose hydrolyzed:

$$\underset{M_r\ 342}{\text{sucrose}} + H_2O \rightarrow \text{fructose} + \underset{M_r\ 180}{\text{glucose}}$$

The conversion factor (glucose → sucrose) is thus 342/180 = 1.90. It is assumed that no glucose is formed by hydrolysis of other oligosaccharides or of inulin. Some fructose is certainly formed by enzymatic hydrolysis of inulin from both fresh and roasted chicory. About 20% of the inulin in these samples was hydrolyzed to fructose under experimental conditions.

Inulin is readily hydrolyzed under mildly acidic conditions and is determined as fructose and glucose formed on hydrolysis. It is assumed for the purpose of this calculation that complete hydrolysis of inulin and sucrose to fructose and glucose occurs under experimental conditions, that no sugars are destroyed during hydrolysis, and that hydrolysis of other polysaccharides does not occur.

If the total fructose content of the sample after acid hydrolysis (i.e., free fructose + fructose from hydrolyzed inulin and sucrose) = $c_f$, then the fructose content ($F$) resulting from hydrolysis of inulin is given by

$$F = c_f - (b - a) - a_f$$

Note that the fructose content from hydrolyzed sucrose = glucose content from hydrolyzed sucrose = $b - a$. This value cannot be obtained from the difference in fructose values before and after enzymatic hydrolysis because of hydrolysis of inulin. If the total glucose content of the sample after acid hydrolysis = $c$, then the glucose content ($G$) resulting from hydrolysis of inulin is given by

$$G = c - b$$

The inulin content of the sample ($I$) is then given by

$$I = 0.90(F + G)$$

The factor of 0.90 arises from the uptake of one molecule of water per fructose (or glucose) residue in the polysaccharide during hydrolysis.

## RESULTS AND DISCUSSION

**Evaluation of the Method.** Five subsamples of roasted chicory were subjected to extraction, preparation of extracts, and chromatographic analysis of reducing sugars, sucrose, and inulin. Duplicate determinations were made on each extract. The results, which are listed in Table I, were used to assess the precision of the method.

Table I. Analysis Data[a] (Roasted Chicory)

| extract no. | determination no. | fructose | glucose | sucrose | inulin |
|---|---|---|---|---|---|
| 1 | i | 2.28 | 0.80 | 1.56 | 19.21 |
| | ii | 2.31 | 0.84 | 1.43 | 19.74 |
| 2 | i | 2.05 | 0.94 | 1.44 | 20.42 |
| | ii | 2.09 | 0.96 | 1.29 | 20.88 |
| 3 | i | 2.38 | 0.87 | 1.44 | 19.85 |
| | ii | 2.40 | 0.88 | 1.46 | 20.09 |
| 4 | i | 2.29 | 0.77 | 1.65 | 18.76 |
| | ii | 2.25 | 0.81 | 1.71 | 19.34 |
| 5 | i | 2.24 | 0.85 | 1.48 | 19.85 |
| | ii | 2.30 | 0.83 | 1.58 | 20.87 |
| mean value | | 2.259 | 0.855 | 1.504 | 19.901 |
| CV1[b] | | 5.09 | 6.99 | 6.92 | 2.86 |
| CV2[c] | | 1.26 | 2.37 | 4.86 | 2.21 |
| CV3[d] | | 5.24 | 7.38 | 8.45 | 3.61 |

[a] Values listed are g/100 g of sample. Duplicate determinations for each extract were made on separate aliquots of extract which were processed as described in the text. [b] Coefficient of variation between subsamples. [c] Coefficient of variation within subsamples. [d] Total coefficient of variation.

Table II. Recovery Data for Inulin Added to Roasted Chicory Root before Extraction

| extract no. | determination no. | inulin found[a] | inulin added[a] | recovery, % | mean recovery, % |
|---|---|---|---|---|---|
| 1 | i | 19.50 | 18.55 | 105.1 | |
| | ii | 18.19 | 18.55 | 98.1 | 103.5 |
| 2 | i | 18.72 | 18.55 | 100.9 | |
| | ii | 20.36 | 18.55 | 109.7 | |

[a] Values listed are g of inulin (dry weight basis) per 100 g of sample. Duplicate determinations for each extract were made as per Table I.

Statistical calculations were based on a one-way components of variance model (Graybill, 1976). Coefficients of variation between subsamples and within subsamples and total variation applying to each determination were calculated and are listed in Table I. The variance between subsamples (i.e., variance resulting from sample inhomogeneity and/or extraction) was significant at the 99.5% confidence level in the case of fructose and glucose content and at the 90% confidence level in the case of sucrose and inulin content. Coefficients of variation within subsamples (i.e., due to sample preparation procedure after extraction and chromatography) were low except in the case of sucrose. The higher value for sucrose probably arises as a result of its determination by difference between two low values for glucose content.

The accuracy of the method was assessed on the basis of recovery of inulin added to two further subsamples of roasted chicory before extraction. Inulin recovery was determined on the basis of additional fructose and glucose formed on hydrolysis of the samples. The results are listed in Table II. These results, and also recovery data obtained previously for sugars added to similar samples (Wight and van Niekerk, 1983), indicate that losses do not occur during extraction and sample preparation.

The mean value for glucose content of inulin present in the roasted chicory sample, as determined by the HPLC method, was 6.0% and the corresponding value for the

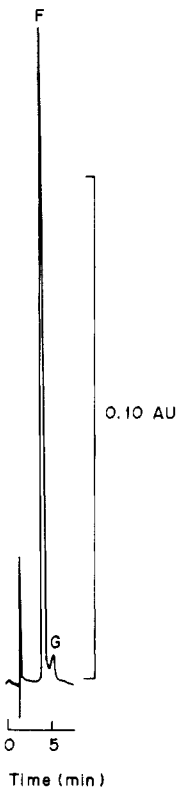


**Figure 1.** Chromatogram of an acid hydrolysate of an extract from roasted chicory root. The main component is fructose (F). The hydrolysate also contains a small quantity of glucose (G) but no detectable quantities of other sugars. Chromatographic conditions: column, LiChrosorb Si 60 (5 μm), 25 × 0.4 cm; mobile phase, acetonitrile–water (75:25) containing HPLC amine modifier I (0.01%); detection by postcolumn reaction with tetrazolium blue and measurement of absorbance at 550 nm. Detector range 0.128 AUFS.

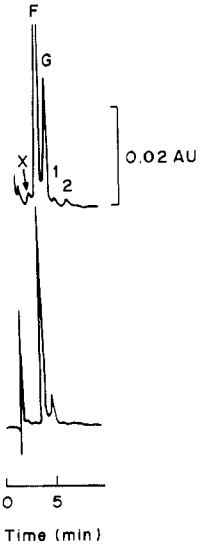


**Figure 2.** Chromatograms of reducing sugars in an extract from roasted chicory root. The extract contains fructose (F), glucose (G), and also traces of xylose (X) and two unidentified components (1 and 2) eluting after glucose. Chromatographic conditions as in Figure 1. Detector range 0.128 AUFS (lower chromatogram) and 0.032 AUFS (upper chromatogram).

added inulin was 6.4%. Hirst et al. (1950) obtained a similar value for the glucose content of dahlia inulin. The glucose content of 6% in chicory inulin probably represents an average value as other plant material contains fructose polymers having a range of molecular weights and a glucose

Table III. Analysis Data[a] for Freeze-Dried Chicory Sample

| determination no. | fructose | glucose | sucrose | inulin |
|---|---|---|---|---|
| i | 2.29 | 0.57 | 5.70 | 54.95 |
| ii | 2.15 | 0.57 | 5.70 | 56.10 |

[a] Values listed are g/100 g of sample.

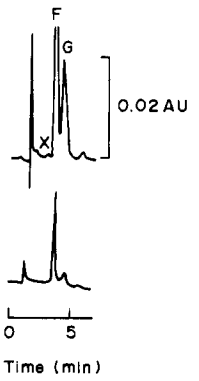


**Figure 3.** Chromatograms of reducing sugars in an extract from freeze-dried chicory root. In addition to fructose (F) and glucose (G), traces of xylose (X) and an unidentified component eluting after glucose appear in the upper chromatogram. Chromatographic conditions as in Figure 1. Detector range 0.128 AUFS (lower chromatogram) and 0.032 AUFS (upper chromatogram).

content of between 3% and 15% (Zittan, 1981).

Comparison of the chromatogram of the acid hydrolysate of the roasted chicory sample (Figure 1) with chromatograms of the free reducing sugars from this sample (Figure 2) indicates that only fructose and a small amount of glucose are formed as a result of acid hydrolysis. No other reducing sugars are formed. These results indicate that hydrolysis of carbohydrate material, other than inulin and sucrose, occurs to a negligible extent in chicory under experimental conditions.

A simplified version of the method could, of course, be applied if only reducing sugar content and/or total fructose content is required.

**Comparison of Fresh and Roasted Chicory.** Analysis data for the freeze-dried chicory sample are listed in Table III. The decrease in inulin content as a result of roasting is in agreement with the findings of other research workers (Blanc, 1978; Pozola and Cieslak, 1979). Considerable decrease in sucrose content also occurs as a result of roasting (Tables I and III) though little change in free fructose and glucose content was observed. Similar results were obtained for fresh and roasted chicory samples from other crops which were analyzed in this laboratory, though the reducing sugar content was more variable. As the source of these samples was unknown, definite conclusions could not be drawn regarding the effect of roasting on carbohydrate composition.

In addition to fructose and glucose, traces of xylose and unidentified components eluting after glucose were found in the hot water extracts of both the freeze-dried and the roasted chicory samples (Figures 2 and 3). While these are very minor components (ca. 0.1%) of this particular cultivar, they may occur in larger quantities in other cultivars and crops.

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**Registry No.** Fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; inulin, 9005-80-5.

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