# The Sugars of Safflower

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## Abstract

Examination of sugars in safflower hull and kernel revealed sucrose and raffinose to be predominent, with smaller amounts of D-glucose and D-fructose. Galactinol (1-O-α-D-galactopyranosyl-myoinositol) and other carbohydrate material which appear to contain uronic acids, fucose, glucose, fructose and arabinose, were also present.


## Introduction

Safflower (*Carthamus tinctorius* L.), a relatively new crop in the United States, is grown almost entirely for its oil content. The residue remaining after commercial extraction of oil from safflower seed has been shown in our laboratory (1,2) to have great potential as a source of animal feed or human diet supplement. A very thorough analysis of safflower seeds has been published (2), but only preliminary information on the free sugars (viz. sugars extractable with 70% aqueous ethanol) of the kernel were recorded. This report describes the isolation and identification of the free sugars and some glycosides of both the hull and kernel. Knowledge of these sugars will assist in development of the non-oil part of the seed as an important feedstuff.

## Materials and Methods

A commercial seed, preponderantly Gila variety, was used (3). The seeds were split and air-classified into hull and kernel fractions in an identical manner to the method previously described (2). The ratio of kernel to hull was 1:0.7.

Sugars, including uronic acids, were measured by the phenol-sulfuric acid method (4). In addition, because uronic acids could be selectively absorbed on Duolite A4 ($OH^-$) resin, we had indirect measure of uronic acids in mixtures. Compounds containing D-galactose were also measured with galactose oxidase, and D-glucose was also measured with glucose oxidase (both enzyme preparations were purchased from Worthington Biochemical Corporation as Galactostat and Glucostat, respectively). Myoinositol assay was carried out by the Wisconsin Alumni Foundation, Madison, Wisconsin.

Paper chromatography was carried out on Whatman No. 1 or 3 MM paper in the descending fashion in solvents: A, 1-butanol-pyridine-water (6:4:3 v/v) and B, ethyl acetate-pyridine-water saturated with boric acid (12:5:4 v/v). Thin layer chromatography (TLC) was carried out on silica gel plates (Brinkmann Instruments) in solvent 1-propanol-ethyl acetate-water (7:2:1 v/v). Sugars were detected on paper with alkaline silver nitrate after use of solvent A, or anisidine-HCl after use of solvent B. On TLC plates, sugars were detected with the anisaldehyde-sulfuric acid reagent.

Mild acid hydrolysis refers to hydrolysis in 0.05 HCl at 100 C for 30 min; strong acid hydrolysis refers to hydrolysis in 3 N HCl at 100 C for 1 hr. After acid hydrolysis, the acid was removed by passing the hydrolysate through a short column of Duolite A4 ($OH^-$) resin.

Invertase was purchased from Difco Laboratories as Invertase Analytical.

### Isolation of Sugars From Kernel

Cleanly separated kernel (43 g) was ground in a mortar then heated under reflux for 1 hr with 250 ml of 70% aqueous ethanol. The mixture was cooled and filtered, and the residue washed with 100 ml of the same solvent. The combined filtrates were concentrated to about 100 ml. The solution was twice extracted with 100 ml of chloroform, then concentrated to about 20 ml and lyophilized. One quarter of the product was chromatographed on a column (120 × 4.5 cm) of 200–400 mesh Dowex 50W × 4 ($K^+$ form) (5). The column was eluted with water at a rate of 0.7 ml/min and fractions of 5 ml were collected. Aliquots of 0.2 ml from alternate tubes were assayed for carbohydrate content. Four peaks were obtained.

The first peak on evaporation yielded a yellow solid material. Mild acid hydrolysis followed by paper and thin layer chromatographic (TLC) investigation of the neutralized hydrolysate indicated both galacturonic and glucuronic acids (galacturonic > glucuronic), glucose, fructose, arabinose and fucose. In a partial quantitative assay, glucose was shown to comprise about 7% and the uronic acids about 56% of Peak I. Very small amounts of the other sugars were present; the remainder of this peak was noncarbohydrate material.

Paper chromatographic investigation of the second peak showed one component behaving like raffinose. With galactose oxidase, the component assayed as 100% raffinose. Mild acid hydrolysis yielded only two products which behaved chromatographically like fructose and melibiose. The material crystallized from aqueous ethanol. It had mp 78 C (Ref. 6 for raffinose pentahydrate, mp 80 C). An x-ray pattern was identical to that of authentic raffinose pentahydrate.

With the third peak, paper chromatography and TLC showed a single component behaving like sucrose. Invertase hydrolysis or mild acid hydrolysis yielded only glucose and fructose. The material was crystallized from aqueous ethanol and had mp 185–186 C (Ref. 7 for sucrose, mp 160–188 C). An x-ray pattern was identical to that of authentic sucrose.

With the fourth peak, prolonged irrigation (72 hr) with solvent A on a paper chromatogram indicated one major component and one minor component. The compounds were quantitatively separated on Whatman 3MM paper in solvent A, irrigating for 72 hr. The components were eluted with water. The $R_f$ value of the major component coincided with that of galactinol. It also had the same $K_D$ value as that shown by galactinol on the Dowex 50W resin (5,8). Mild acid hydrolysis of the component corresponding to galactinol yielded two components with $R_f$ values the same as those shown by galactose and myoinositol. In a quantitative assay the products of strong acid hydrolysis were found to be D-galactose and myoinositol in the ratio 1:1.03. This major component crystallized from aqueous ethanol after seeding with galactinol. It had mp 205 C (Ref. 9 for galactinol, mp 220 C). An x-ray pattern was identical to that of authentic galactinol. The minor component of this peak remains unidentified.

A quantitative analysis of the sugar components in safflower kernel is shown in Table I.

TABLE I
Identification and Distribution of Sugars in Safflower Hull and Kernel

| Safflower component | Sugars present | % Distribution of sugars | % Sugars | % Sugars on defatted basis (calc.) |
|---|---|---|---|---|
| Kernel | | | | |
| | Uronic sugar glycosides | 14.3 | 0.43 | |
| | Raffinose | 35.8 | 1.08 | |
| | Sucrose | 46.9 | 1.42 | |
| | Galactinol | 3.0 | 0.09 | |
| | Total | | 3.02 | 7.74 |
| Hull | | | | |
| | Uronic sugar glycosides | 38.9 | 0.37 | |
| | Raffinose | 6.8 | 0.06 | |
| | Sucrose | 22.6 | 0.21 | |
| | Galactinol | 2.7 | 0.025 | |
| | D-Glucose | 14.6 | 0.14 | |
| | D-Fructose | 14.2 | 0.13 | |
| | Total | | 0.94 | 0.97 |

**Isolation of Sugars From Hull**

This was carried out in a similar manner to the method described for the kernel except that 32.7 g of hull was extracted. The total lyophilized extract was chromatographed on the Dowex column in the same manner. Six peaks were obtained.

With the first peak, mild acid hydrolysis and chromatographic investigations indicated both galacturonic and glucuronic acids (glucuronic > galacturonic), glucose and fructose. The peak comprised approximately 48% uronic acids, 3.5% glucose, a trace of fructose and about 48% noncarbohydrate material.

The second, third and fourth peaks were raffinose, sucrose and galactinol (plus an identified minor component) respectively, identified in a similar manner already described for the equivalent peaks for the kernel.

The fifth peak component had a $K_D$ value corresponding to that of D-glucose. Paper chromatography in solvent A and TLC showed a single component behaving like glucose. With glucose oxidase, the peak assayed as 100% D-glucose. A UV spectrum of the material in HCl (10) was identical to the UV spectrum of authentic D-glucose in HCl.

The sixth peak contained material with a $K_D$ value corresponding to that of D-fructose (5). Paper chromatography in solvents A and B and TLC showed a single component with the same $R_f$ value as that of D-fructose. A UV spectrum of the material in HCl (10) was identical to the UV spectrum of authentic D-fructose in HCl.

A quantitative analysis of the sugar components in safflower hull is shown in Table I.

## Discussion

In the earlier report from this laboratory on the composition of safflower seeds (2), workers suspected the presence of sucrose and raffinose and a small amount of reducing sugar. This report confirms that in the kernel, raffinose and sucrose account for about 83% of the sugars, but no reducing sugars are present. The total sugar content calculated for the defatted kernel, assuming 60.9% oil (2), is 7.74%. This compares with 6.3% quoted by the previous workers. The components in the first peak in both the hull and kernel separations were not fully identified but the carbohydrate components were tentatively identified. From experience with this chromatographic system in separating plant sugar extracts (8,11), the material in the void volume (equivalent to the first peak) is a very complex mixture. The relatively large amount of glucose found here may be indicative of glycosides. Several glycosides containing glucose have recently been identified in safflower kernel in our laboratory (Palter, private communication). Fucose has been found in other plant sources including corn, flax and soybean, where it is part of a complex phytoglycolipid (12). Possibly the presence of fucose in safflower kernel is indicative of a similar complex material. In the phytoglycolipid found in these other plant sources, myoinositol, arabinose and glucuronic acid also occur. In the safflower here, arabinose and glucuronic have been found and also myoinositol but in the glycoside galactinol. Previously galactinol has been found in sugar beets (9), potatoes (13), and in the leaves of several higher plants (14). The compound occurring along with galactinol in the column chromatographic separations has not been identified yet but it appears to be a glycoside; further work on this material will be reported in the future.

In the hull, a smaller amount of the same sugars was found, except for a larger amount of material containing uronic acids and a small amount of D-glucose and D-fructose. The complete absence of glucose and fructose in the kernel, and their equal amounts in hull, suggest that the presence of these sugars in the hull could be an artifact produced by invertase hydrolysis of sucrose. The invertase could be a seed coat constituent or contaminant that is absent in kernel tissue.

Knowledge of sugar constituents of safflower hull and kernel should contribute to nutritional studies and may assist in understanding the various physiological effects of safflower fractions.

ACKNOWLEDGMENT

Miss Margaret Connor extracted the sugars from safflower, H. J. Burkhardt donated the safflower, R. M. McCready donated a sample of galactinol, Miss Kay Lee performed the x-ray analyses, and H. G. Walker, Jr., assisted in preparation of the manuscript.

REFERENCES

1. Kohler, G. O., "World Protein Resources," Advances in Chemistry Series No. 57, American Chemical Society, Chicago, 1966, p. 243–253.
2. Guggolz, J., D. D. Rubis, V. V. Herring, R. Palter and G. O. Kohler, JAOCS *45*, 689–693 (1968).
3. Burkhardt, H. J., Ibid. *45*, 96–99 (1968).
4. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Anal. Chem. *28*, 350–356 (1956).
5. Saunders, R. M., Carbohydrate Res. *7*, 76–79 (1968).
6. Pigman, W. W., and R. M. Goepp, "Chemistry of the Carbohydrates," Academic Press Inc., New York, 1948, p. 456.
7. Levi, I., and C. B. Purves, "Advances in Carbohydrate Chemistry," Vol. 4, Academic Press Inc., New York, 1949, p. 1.
8. Walker, H. G., Jr., and R. M. Saunders, Cereal Sci. Today, in press.
9. Brown, R. J., and R. F. Serro, J. Amer. Chem. Soc. *75*, 1040–1042 (1953).
10. Doyle, R. J., and H. M. Burgan, Anal. Biochem. *17*, 171–174 (1966).
11. Saunders, R. M., and H. G. Walker, Jr., Cereal Chem. *47*, 85–92 (1969).
12. Carter, H. E., B. E. Betts and D. Strobach, Biochemistry *3*, 1103–1107 (1964).
13. Pressey, R., and R. Shaw, Europ. Potato J. *12*, 64–66 (1969).
14. Senser, M., and O. Kandler, Phytochemistry *6*, 1533–1540 (1967).

[Received February 4, 1970]