# Neutral Sugar Analysis of Polysaccharides From the Seed Epidermis of *Brassica campestris*

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**A mucilaginous material was extracted from the epidermal cells of seed of *Brassica campestris* L. cv. Candle canola. Hydrolysis and gas chromatographic analysis showed that it contained the following neutral sugars: arabinose, rhamnose, glucose, mannose and galactose. Total hydrolysis of the extract was not realized. The residue contained carbohydrate, protein and lipid.**

The presence of mucilaginous material in the epidermal layers of several cultivars of canola and rapeseed has been shown (1). The importance of eliminating this gummy material in the oil extraction process and its possible effect on long-term storage of canola seeds prompted an analysis of the polysaccharide. Also, the economic importance of canola and rapeseed as an oil source required an analysis of by-products of the oil extraction process for possible commercial use. Recent studies (2,3) have shown that the mucilages of yellow mustard, oriental mustard and rapeseed were different in hydrolytic properties and neutral sugar profiles. This study was initiated to determine the neutral sugar profile of mucilage from Candle canola.

## EXPERIMENTAL PROCEDURES

*Extraction.* Mucilage was extracted from the epidermal cells by placing 400 g of untreated seeds in a 2-l Erlenmeyer flask with 1.4 l deionized water. The flask was placed on a mechanical shaker and rotated at 150 rpm for 2 hr. The resulting slurry was filtered through six layers of fine-mesh dacron. Long-chain polysaccharides were precipitated by adding four volumes of 95% ethanol to the filtrate. The precipitate was collected by centrifugation, washed three times in 95% ethanol, lyophilized, and stored in a dessicator at −10 C.

*Hydrolysis.* A 7-8 mg sample was dissolved in water and placed in a hydrolyzing tube with an equal volume of 4N trifluoroacetic acid (TFA). The tube was sealed and placed in an autoclave at 121 C for two hr. After cooling, the tube was placed in a fume hood, evaporated to near dryness under a jet of filtered air and lyophilized. Precipitate which remained after the first hydrolysis was subjected to a further three hr of hydrolysis using fresh 2N TFA at 121 C.

The lyophilized precipitate was resuspended in water and passed through a 1.75 × 230 cm column containing Bio-Gel P-2, 200-400 mesh (Bio-Rad Laboratories, Richmond, California) using deionized, oxygen-free water as an eluting agent (5) at a flow rate of 24 ml/hr. This procedure was included to separate monosaccharides from polymers of saccharides which may have resisted hydrolysis.

*Gas chromatography.* The monosaccharide fractions were determined by gas liquid chromatography (GLC) according to the method of Honda et al. (6) with the following modifications. A 0.1 M aqueous solution of 3-0-methyl-glucose (internal standard) was added to the reaction vials containing the samples and evaporated to dryness under reduced pressure. To the residue 100 μl of ethanethiol-trifluoroacetic acid (2:1, v/v) was added, and the tubes were closed and kept at room temperature for 30 min. The further steps of derivatization were the same as described by Honda et al. (6). The derivatized samples were quantitatively analyzed by gas chromatography (FID detector) using a fused silica capillary column (30 m × 0.314 mm, coated with SE-30; Chromatographic Specialties, Canada) operated at 225 C. Injector and detector were operated at 235 and 245 C, respectively. The carrier gas (helium) was regulated at 1 ml/min with the splitter set at 100:1. The peaks were analyzed with an electronic integrator (Model 3380S, Hewlett Packard).

*Assays.* Total carbohydrate was determined by a modified anthrone method (7). Protein was determined by a modified Kjeldahl method (8). Fatty acids were determined by GLC using the method of Kovacs et al. (9).

## RESULTS AND DISCUSSION

When intact Candle canola seeds were placed in water at 20 C those epidermal cells which contained mucilage ruptured, and some of the mucilage became suspended and/or dissolved in the water (1). Extraction with water did not result in total removal of epidermal mucilage but yielded a viscous slurry containing adequate material for analysis. Since long chain polymers were of prime interest in this study, they were precipitated by adding four volumes of 95% ethanol to the initial filtrate. The ethanol precipitate could best be described as fibrous, but it dispersed to form a clear solution in water. When lyophilized, a greyish-white fibrous residue resulted.

Trifluoroacetic acid has been used as a hydrolyzing agent (4) since it is easily removed by evaporation after hydrolysis. In this study the percent distribution of neutral sugars in hydrolysates of sulfuric acid and trifluoroacetic acid did not differ; consequently, the latter acid was used routinely. Treatment in either 2N trifluoroacetic acid or 2N sulfuric acid did not totally hydrolyze the above precipitate.

Table 1 shows the recovery of the extraction, hydrolysis and column steps in preparing a solution of monosaccharides for analysis using GLC.

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**TABLE 1**

**Percent Carbohydrate Recovery from Individual Procedures During Preparation of Monosaccharide Fraction**

| Procedure | Recovery (%) |
|---|---|
| Water extract at 20 C | 100[a] |
| Ethanol precipitation | 60 |
| Acid hydrolysis | 12 |
| Separation on Bio-gel Column | 12 |

[a]Arbitrarily set at 100%.

**TABLE 2**

**Percent Distribution of Neutral Sugars in Crude Extract and Ethanol Precipitate of Canola Mucilage**

| Mono-saccharide | Crude extract (%) | Ethanol precipitate (%) | Ratio of sugars (ethanol precipitate/crude extract) |
|---|---|---|---|
| Arabinose | 14.9 | 23.0 | 1.54 |
| Rhamnose | 4.9 | 6.7 | 1.37 |
| Glucose | 32.9 | 12.3 | 0.37 |
| Mannose | 7.1 | 11.0 | 1.55 |
| Galactose | 31.3 | 40.0 | 1.28 |
| Total | 91.1 | 93.0 | |

Table 2 summarizes the carbohydrate percentages in the crude extract and the fraction obtained by ethanol precipitation.

With the exception of glucose, the percent distribution of the individual sugars is similar in both sample types, as indicated by the ratio of sugar in the ethanol precipitate to that in the crude extract.

The crude extract hydrolysate contained significantly more glucose than the ethanol precipitate hydrolysate. This result can be explained if the origin of the glucose is traced. During development of the seed hull, large starch grains are deposited in amyloplasts inside the epidermal cells. As the seed matures the starch grains disappear concurrently with the formation of mucilage in the outer epidermal cell (1). It is reasonable to assume that some glucose released during starch hydrolysis remained unbonded, not forming polymers, and/or failed to be translocated from collapsing epidermal cells.

Cold-water extracted hull mucilages of yellow mustard and rapeseed have been analyzed for neutral sugar content by Weber et al. (3) and Vose (2), respectively. Both studies found arabinose, xylose and glucose in similar percentages. The former study reported two unidentified saccharides, while the latter identified traces of fructose, galactose and rhamnose. These studies have reported markedly different sugar profiles from Candle canola. This is reasonable in the case of mustard, which is related to Candle only at the generic level. The parentage of Candle, however, includes four cultivars of rapeseed and *Brassica juncea* (oriental mustard); it was expected, therefore, that the neutral sugar profiles of rapeseed and Candle would be similar.

Vose (2) reported that mucilage extracted from rapeseed and *B. Juncea* hulls hydrolyzed completely in 2N sulfuric acid after refluxing 4 hr while yellow mustard mucilage was hydrolyzed only 35% under the same conditions. The unhydrolyzable fraction was identified as cellulose. Weber et al. (2) also reported an unhydrolyzable fraction in water-extracted yellow mustard mucilage which was identified as cellulose. In this study, an unhydrolyzable fraction remained containing carbohydrate (89%), protein (13.3%) and fatty acids (0.23%).

When new cultivars of canola and rapeseed were produced to improve the oil constituents and disease resistance of the traditional *Brassica napus* and *Brassica campestris* cultivars, some different hull characteristics were also introduced. Whether a characteristic such as the presence of mucilage in epidermal cells is useful or disadvantageous remains to be seen. The analysis of such components represents an initial step in evaluating their usefulness.

## ACKNOWLEDGMENT

This research was funded in part by the University of Manitoba Research Grants Committee.

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[Received August 8, 1986]



JAOCS, Vol. 64, no. 5 (May 1987)