Chemical and Physical Properties of Mucilage from Canola (Brassica campestris) cv. Candle

by

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ABSTRACT

The presence of mucilaginous material in the seed coat of canola cv. Candle has been associated with storage and mold infestation problems. This study examined the yield of mucilage from canola cv. Candle and some of its chemical and physical properties.

Canola seeds were extracted for 16 hours with water using a seed:water ratio of 1:18 or heat-treated prior to water extraction using a seed:water ratio of 1:7. The mucilage was precipitated with 4 volumes of 95% ethanol and recovered by centrifugation at 3500 x g and 23 C for 10 minutes. A twofold increase in the yield of mucilage (1.33 %) was obtained for the heat-treated seeds compared to (0.69-0.72 %) for the unheated canola seeds.

The heat-treated canola seeds were subjected to different extraction times from 4 to 16 hours using a seed:water ratio of 1:7. Approximately 63.8% of the total extractable mucilage was obtained after the first 4 hours. No significant differences ($P<0.05$) were observed in the yield of mucilage between 4 hour extraction intervals. A significant difference ($P<0.05$) however,was evident in the yield of mucilage between 8 and 12 hour extraction intervals. The total yield of mucilage obtained at the end of the 16 hour extraction period was 1.16 % .

Proximate analysis of canola mucilage showed it contained moisture (8.2 %),protein (18.2 %), carbohydrate

(30.6 %),ash (29.4%) with the remainder (13.6 %) unidentified. No significant differences ($P<0.05$) were observed in either the protein or carbohydrate content of mucilage throughout the 16 hour extraction period. This suggested the mucilage was homogeneous with respect to protein and carbohydrate content.

Analysis of the carbohydrate composition by gas chromatography showed that, with the exception of rhamnose, no significant ($P<0.05$) differences between the individual monosaccharides over the different extraction intervals. The monosaccharide composition of canola mucilage was galactose (31.7 %),arabinose (28.9 %), glucose (14.9 %), mannose (9.7 %),rhamnose (4.5 %), xylose (3.7 %) with the remaining 7.1 % unidentified . Purification of mucilage was accompanied by a two and threefold increase in rhamnose and unidentified fractions respectively. A marked decrease in the other monosaccharides was observed for the purified material.

The flow curve of a 0.5 % solution of canola mucilage exhibited a shear thickening property. Canola mucilage was non-Newtonian in behaviour up to a shear rate corresponding to 350 rpm while at higher rate of shear it was Newtonian. The viscosity of a 0.5 % solution of canola mucilage within the Newtonian range was 4 cp. Canola mucilage reduced surface tension and interfacial tension of a corn oil:water system . The interfacial tension value using canola mucilage was 9.5 dynes/cm which was similar

to mustard mucilage (8.5 dynes/cm). The ability of canola mucilage to reduce interfacial tension was responsible for the formation of a stable salad dressing incorporating canola mucilage as emulsifier.

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CHAPTER 1

INTRODUCTION

Canola , an oil-bearing seed from the genus Brassica, is the product of systematic breeding programs of rapeseed by plant breeders. Canada has been the major exporter of canola in the world. The average total production of the crop in Canada between 1982-1986 was 3.16 million tones per year (Vaisey-Genser and Eskin 1987). The oil from canola is the major source of edible oil in Canada while the meal is used extensively in the animal feed industry. The domestic use of canola oil includes shorteninig,tablespreads, salad dressing and cooking oil.

When stored in bulk, canola seeds spoil rapidly under moisture and temperature conditions favorable to mold growth. The appearance of a mucilage in the epidermal layer of the seed coat of canola cultivar, Candle, provides a potential substrate for growth of postharvest fungi. Canada remains a major importer of gum and mucilages for use by the food industry. The major ones imported include such water-soluble gums as guar and acacia gums. The only indigenous mucilage has been reported in yellow mustard seed (Sinapus alba). The presence of mucilage in canola seeds could provide an alternative gum for use by the food industry in Canada. It

appears prudent therefore to examine the physical and chemical properties of canola mucilage and to assess its potential as a food gum. The objectives of the present study were as follows:

1. To examine the effect of heat-treatment and soaking time on the yield of mucilage from canola cv. Candle.
2. To investigate the homogeneity of the extracted mucilage at different soaking times.
3. To investigate the physical properties of mucilage from canola cv. Candle.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Canola

The oilseed summer rape (Brassica napus) and summer turnip rape (Brassica campestris) are the predominant Brassica oilseed crops grown in Canada. These crops are able to survive and grow at relatively low temperatures making them ideal for Canadian growing conditions. Of these,summer rape accounts for approximately 55% of the total production in Canada (Downey,1983). The conversion to low erucic acid rapeseed began in 1974 in Canada with the licensing of the first canola variety, Tower. Low erucic varieties were first described by such terms as LEAR (low erucic acid rapeseed) and Canbra (Canadian Brassica) to identify rapeseed with less than 5% erucic acid. This lead to the introduction in Canada of the term canola. Canola now refers to seeds of species Brassica napus ar Brassica campestris in which the oil component of the seed has been less than two percent erucic acid and the solid component of the seed contains less than 30 micromoles of any one or any mixture of glucosinolates per gram of air dry,oil free solid. The major canola varieties currently grown in Canada are Westar (B. napus) and Tobin (B.campestris) each accounting

for 53.2 and 37.2% of the total crops respectively (Vaisey-Genser and Eskin, 1987).

2.2 Taxonomy

Canola and rapeseed varieties belong to the genus Brassica and the family Cruciferae. The varieties grown in Canada belong to the summer rape (Brassica napus) and the summer turnip rape (Brassica campestris) species. Other species of Cruciferae family include Brassica juncea mustard. Yellow or white mustard belongs to the genus Sinapis although it was previously referred to as Brassica hirta (Vose, 1974). The taxonomy of canola is outlined in Figure 1.

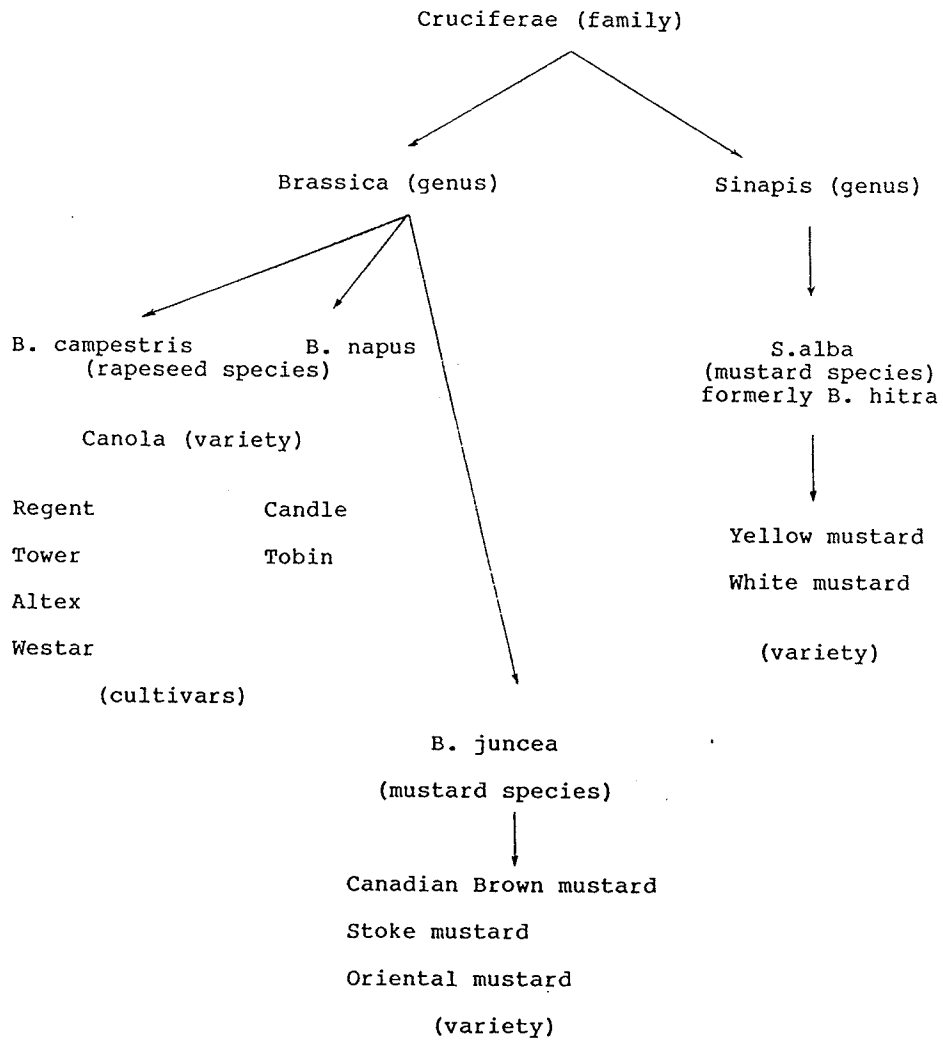


Figure 1. Taxonomy of the Cruciferae.

2.3 Mucilage in Canola and Related Species

The presence of abundant mucilaginous material was recently identified in the epidermal layer of the seed coat of the canola cv. Candle (Van Caeseele et al.,1981). While this is the first report of mucilage in canola its presence in white or yellow mustard seeds,Brassica alba L., Brassica hitra L. or Sinapis alba L.,was observed over 60 years ago by Bailey and Norris (1932). Mucilage was referred to by these researchers as the amorphous material laid down in the epidermal cells of white or yellow mustard seeds. Van Caeseele et al.(1981) noted a similarity in the cytological appearance of the mucilage produced in the seed coat of Brassica campestris L. cv. Candle with that of yellow mustard. The common feature noted was the stratified appearance of mucilage deposition. Grant et al.(1969) reported seed mucilages contained a crystalline formation of cellulose fibers which have been solubilized in some way by acidic polysaccharides . Van Caeseele et al.(1981) noted the development of a weak pink color in the canola seed coat in the presence of toluidine blue, which suggested the presence of mucopolysaccharides (Heath 1961). Mucopolysaccharides are polysaccharides that contain , in addition to simple sugars, such derivatives as amino sugars and uronic acids. Based on this observation these researchers referred to the amorphous material in canola as mucilage even though the precise content remained unknown.

2.4 Deposition of Mucilage in the Seed Coat of Canola and Other Species

The development of mucilage in the epidermal cells of canola seeds (Brassica campestris L.cv. Candle) was examined by Van Caeseele et al. (1981) with light and electron microscopy from 5 days after pollination to maturity. Starch deposition in the amyloplast occurred during the first 17 days at which time mucilage was observed between the plasmolemma and the outer tangential wall of the epidermal cells. Starch grains started to disappear with the expansion of mucilage and were totally absent at 25 days. Adjacent to the site of mucilage deposition,membrane bound structures and Golgi bodies were observed. Once the seed had fully matured the epidermal cells were totally devoid of cytoplasm and completely filled with mucilage. The seed coat of mature canola cv. Candle was shown by Van Caeseele et al.(1981), using phase contrast microscopy, to consist of an epidermal layer, a palisade layer and an aleurone layer as found in other Brassica campestris L. and Brassica napus seeds (Mills and Chong,1977). Candle canola was unique in having an epidermal layer completely filled with mucilage. The epidermal layer appeared to vary in the amount of mucilage deposited as shown by Schans et al.(1982) using calcoflour white M 20 staining technique.

The development of mucilage in other plants has been widely reported. The process of mucilage deposition

in Candle was reported by Van Caeseele et al.(1981) to be similar to that for Plantago ovata Forsk seeds (Hyde 1970). In the case of Candle , however, the cytoplasmic organelles disintegrated more dramatically with the result that the remnants of protoplasts were not evident in other epidermal cells at maturity. The appearance of mucilage was also found in yellow mustard seed (B. alba, B. hitra or S. alba) but not in the seed coats of either brown or oriental mustard (Brassica juncea) (Weber et al.,1974). These researchers did not observe mucilage in rapeseed (B.napus and B. campestris).

2.5 Mucilage Content of Canola and Related Seeds

Very little data is available on the level of mucilage in canola seeds as well as other closely related seeds. Woods and Downey (1980) studied the level of mucilage in four yellow mustard seed cultivars grown at four different locations in Western Canada. Their results (Table 1) showed significant differences ($p < 0.05$) in the levels of mucilage between cultivars ,years and locations. The range of mucilage levels detected was 0.34 % to 2.05 % with an overall mean of 1.28 % and a standard deviation of 0.38% . This compared to the value of 2.0% for white mustard seed mucilage reported by Bailey and Norris (1932).

Table 1

Percentage Mucilage in the Seeds of Four Yellow Mustard[1] Cultivars Grown at Four Locations Over Two Years.

Location	Average %	Cultivars	Average %	Year	Average %
Melford	2.15	BHL3926	1.94	1977	1.79
Sidney	1.78	Sabra	1.55	1978	1.41
Winnipeg	1.31	Gisilba	1.49		
Saskatoon	1.17	Yellow-2	1.41		

LSD at 5% levels;locations and cultivars 0.37% ; Year 0.26% .

1. Woods and Downey (1980).

Vose (1974) obtained polysaccharide fractions from defatted rape and mustard hulls using a number of different sequential solvent extractions. His results (Table 2) for the mucilaginous polysaccharide material extracted by cold water showed that it constituted 22.6 % of the total hull weight for yellow mustard compared to 1 % or less for the rapeseed hulls.

2.6 Biological Functions of Mucilage

There are no clear biological reasons for the presence of mucilage in canola seeds. Nevertheless Van Caeseele et al.(1981) suggested it may have certain adaptive advantages,including reduction of specific weight in water and decreased desiccation during germination. Mucilage may also restrict the passage of oxygen thereby preventing germination in water-logged conditions (Fahn,1982).

Canola seeds stored in bulk are particularly succeptible to molds. Van Caeseele et al.(1987) suggested that the presence of mucilage would provide a suitable location for the multiplication of fungi causing spoilage during storage. Schans et al. (1982) using fluorescence microscopy examined the course of invasion of Brassica campestris L. Candle seeds by fungi. This technique permitted differentiation of fungal and rapeseed host tissues. These researchers also observed differences in

Table 2

Percent Mucilaginous Polysaccharide Extracted With Cold Water from Deffated Mustard and Rapeseed Hulls.[1,2]

S. alba (Yellow mustard)	22.6
B. Juncea (Brown mustard)	3.6
B. Juncea (Stoke mustard)	3.2
B. juncae (Oriental mustard)	2.2
B. campestris (Polish rape)	1.2
B. napus (Argentina rape)	0.9

1. Adapted from Vose (1974).
2. Dry weight basis.

seed structure which affected the time required for fungal penetration. Seeds containing unusually dense mucilage appears to delay the penetration of cell layers beneath the epidermis by fungi.

2.7 Extraction of Polysaccharides from Brassica Seeds

Vose,(1974) extracted polysaccharides from rape and mustard hulls using different sequential solvent extractions. This was based on the isolation procedures used by Aspinal et al.(1945) for obtaining polysaccharides from soybeen hulls. Hulls were defatted by Soxhlet extraction with acetone for 8 hours; hexane for 24 hours or ethanol:water (4:1 v/v) for 48 hours. The defatted hulls were then micromilled through a U.S. No. 40 mesh sieve and the hull flour extracted with the following series of solvents: water at 20 C; water at 60 C and 0.5% ammonium oxalate at 80 C. The remainder of the hulls was subjected to four additional stepwise treatments producing seven major fractions.

In a study conducted by Theander et al.(1977), hulls from rape and mustard seeds were extracted with 80 % ethanol to remove low molecular weight substances. The extracts were then evaporated and separated into chloroform soluble material (A) and watersoluble material. The water soluble extracts were further fractionated into neutral (B),basic as chlorides(C), and acidic (D) fractions.

Woods and Downey (1980) extracted whole yellow mustard seeds overnight with cold water in a ratio of 1:18 of seeds to water. Chloroform (2.5 ml/l) was added in order to prevent microbial growth. The extract referred to as mucilage was precipitated by the addition of acidified acetone to the extract which was then filtered and dried under vacuum at room temperature. Van Caeseele et al.(1987) soaked canola seeds in a seed:water ratio of 1:3.5 for 2.5 hours to extract mucilage. In a recent study by El-Mahdy and El-Sebaiy (1984) ground Fenugreek seeds were heat-treated at 70 C for 15 minutes to inactivate enzymes prior to soaking in water (1:6 seed:water ratio). It may also be appropriate to heat-treat the canola seeds prior to extraction to inactivate myrosinase.

2.8 Chemical Composition of Canola Mucilage and Related Species

Mucilage is deposited in the seed coat (hulls) of canola seed but little information is available on the chemical composition of canola seed coat except for that reported for rapeseed and mustard hulls by Vose (1974). The chemical composition of rapeseed and mustard hulls are given in Table 3. The carbohydrate content of rapeseed hulls was considerably lower than that from mustard hulls.

Van Caeseele et al. (1987) reported the presence of neutral sugars in the mucilage from canola seeds. The

Table 3

Chemical Composition of Rapeseed and Mustard Hulls.[1,2]

Component (%)	Rapeseed	Mustard
Total hulls	16-19	12-20
Carbohydrate	39-43	52-82
Crude fiber	26-30	15-20
Protein	14-16	14-21
Fat	8-15	8-15
Total ash	3.5-5	3.5-5

1. Adapted from Vose (1974)
2. Dry weight basis.

mucilage was extracted from canola seeds with cold water for 2.5 hours. It was composed of rhamnose (5.8%), mannose (9.55%), glucose (10.7%), arabinose (20%),galactose (34%). The major monosaccharides of canola mucilage were galactose and arabinose.

The main constituents of neutral water-soluble polysaccharides extracted from turnip rapeseed and mustard hulls were examined by Theander et al. (1977). The results presented in Table 4 indicate considerable differences in monosaccharide composition between White mustard (S. alba) and Turnip rapeseed (B. campestris). Glucose and arabinose constituted the major sugars of the polysaccharides of Turnip rapeseed and White mustard hulls, followed by galactose and xylose.

Vose (1974), used a number of different sequential solvent system to extract polysaccharides from the defatted hulls of rapeseed (B. campestris and B. napus) and mustard (B. juncea and S. alba). Of these seeds only Yellow mustard (S. alba) differed from the Brassica species by the presence of 60-70 % cellulose which yielded B-D-glucose on hydrolysis. The remaining polysaccharide was composed of galacturonic acid (30 %),arabinose (20 %), glucose (20 %),xylose (6 %),fructose (6 %),rhamnose (6 %), together with traces of galactose and mannose. The carbohydrate composition of the hulls from rapeseed (B. campestris and B. napus) and B. juncea mustard were very similar. The cold water-soluble polysaccharide

Table 4

Carbohydrate Content in Turnip Rapeseed and White Mustard Hulls.[1]

	Turnip Rapeseed Cultivars (B. campestris)			White Mustard (S. alba)
	Bele	Lute	Sv. 72/60029	Sv. 67/670
Total Carbohydrate[2]	26.0	33.1	38.1	38.4
Monosaccharides[3]				
Glucose	39.5	42.8	34.7	39.3
Arabinose	32.4	33.1	39.9	25.4
Galactose	12.6	9.8	6.9	17.9
Xylose	8.0	8.2	9.8	7.0
Mannose	3.6	3.0	2.9	5.4
Rhamnose	2.5	2.1	3.9	4.0
Fucose	1.5	1.0	1.9	1.0

1. Adapted from Theander et al.(1977).
2. Given as percent of dry, 80% ethanol extracted and benzene-ethanol (2:1) extracted hulls.
3. Given as relative percent of neutral carbohydrates in extracted hulls after hydrolysis (dry basis).

fractions from the hulls of these seeds contained arabinose (40-50%),xylose (18-25%) and glucose (10-14 %), and uronic acid (6-10 %) in addition to trace amounts of fructose,galactose and rhamnose.

The data published so far indicate considerable variation in monosaccharide content between rapeseed and mustard species as well as among varieties. Xylose was not reported in canola mucilage whereas it was detected in rapeseed and mustard seed. Possible differences in carbohydrate composition may reflect variation in the method of extraction of the mucilage from these seeds. During the process in which the hulls are removed from the seed prior to extraction of mucilage possible contamination with the polysaccharides of the testa and embryos of the seeds may occur. An alternative method used by Van Caeseele and Mills (1982) involved soaking whole canola seeds in cold water and collecting the water-soluble exudates from the seeds directly .

2.9 Hydrolysis of Polysaccharides

The experimental conditions reported for the hydrolysis of polysaccharides of the Brassica family appear somewhat arbitrary. For example Vose (1974) refluxed with 1M sulfuric acid for four hours to completely hydrolyse the cold water-soluble polysaccharide

fraction obtained from rapeseed and B.juncea mustard hulls . Only 35% of this polysaccharide was hydrolised in yellow mustard as it constituted 60 to 70% native cellulose. Theander et al.(1977) hydrolysed the neutral polysaccharide fractions extracted from several varieties of Turnip rapeseed and mustard hulls with 12M sulfuric acid at room temperature for two hours followed by refluxing for six hours after dilution to 0.35M. Weber et al.(1974) subjected the mucilage extracted from yellow mustard seed hulls to acid hydrolysis by refluxing with a 5% (0.5M) solution of sulfuric acid for four hours. Siddiqui and Woods,(1971) reported complete hydrolysis of an amyloid isolated from water-soluble rapeseed polysaccharide with 1 M sulphuric acid at 100 C for three hours.

2.10 Functional Properties of Mucilages

Gum, mucilages or hydrocolloids are defined by industry as those materials which in water give viscous solutions or dispersions. While there are many exceptions, in general, this criterion of water solubility to yield increased viscosity covers the majority of gums used in the food industry (Glicksman,1982). Most of these materials have colloidal properties and are usually high molecular weight polymers. Chemically most of them are polysaccharides while a few are proteins such as gelatin

and casein. The importance of hydrocolloids in food applications is due to their unique functional properties such as water binding capacity,reduction of evaporation rate, alteration of freezing rate,modification of ice crystal formation, regulation of rheological properties, participation in chemical transformations,suspension of insoluble particles and stabilization of foams and emulsions (Glicksman,1982).

There has been no research conducted on the functional properties of canola mucilage although some work has been reported on mucilage from yellow mustard (Weber et al.,1974). Vose,(1974) recognized the commercial value of mustard hulls in so far as their functional properties are concerned. The latter included water absorption,emulsification and thickening properties in addition to their flavour components. Differences in thickening properties among various mustard and rapeseed hulls are shown in Table 5. It was apparent that slurries made from wet-milled yellow mustard hulls yielded the most viscous preparations.

The presence of a 22.6% cold water soluble mucilage in yellow mustard was attributed for the high thickening properties of this species of mustard. Two species of rapeseed were among the least viscous materials. Viscosities of other fractions of polysaccharides from yellow mustard were low and comparable to other species of mustard and rapeseed.

Table 5

Viscosities of Stone-milled 5% (w/v) Aqueous Dispersions of Mustard and Rapeseed Hulls.[1]

Species	Viscosity cp[2]
S. alba (Yellow Mustard)	5200
B.juncea (Brown Mustard)	1845
B.juncea (Stoke Mustard)	218
B.juncea (Oriental Mustard)	82
B.campestris (Polish Rape)	55
B. napus (Argentinian Rape)	45

1. Adapted from Vose (1974).

2. Viscosities expressed in centipoises (cp) units were taken using a Brookfield Viscometer Model RVT at 20 r.p.m. at room temperature.

Weber et al.(1974) examined the functional properties of mucilage extracted from defatted dried yellow mustard seed hulls. The mucilage swelled in cold water and increased in viscosity after heating or upon standing on room temperature, to yield a low to medium viscosity solution. The viscosity of a solution of 1% mustard mucilage at 20 r.p.m. and room temperature was 520 cp. using a Brookfield Synchro-electric Viscometer Model RVT. Yellow mustard mucilage was found to be a thixotropic hydrocolloid. Viscosity of a 5% (w/v) slurry made from stoned-milled yellow mustard hulls was 5200 cp. at 20 r.p.m. measured with a Brookfield viscometer at room tempersture. Yellow mustard mucilage has been known for its high viscosity (Weber,et al.1974 and Vose,1974).

Gum tragacanth has been known as a stabilizer for oil in water emulsion (Whistler,1974). Stauffer and Andon,(1975) showed gum tragacanth reduced the surface tension and interfacial tension between water and oil by acting as an emulsifier. Among all the industrial food gums yellow mustard mucilage has the ability to reduce the surface tension and interfacial tension substantially in forming stable emulsions (Weber et al. 1974).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Seeds

A representative sample of canola seed (B. campestris) c.v. Candle was obtained from Dr.B. Stefansson of the Department of Plant Science University of Manitoba, Canada.

3.1.2 Reagents

All the reagents used in this study were of the highest purity and analytical grade. The standard sugars for GC analysis were of 99% purity. Rhamnose and 3-O-methyl glucopyranose (internal standard) were obtained from Sigma Chemical Company U.S.A. Glucose,galactose, mannose,arabinose and xylose were purchased from Mandel Scientific Company Canada. The sillylation reagents were also obtained from Sigma Chemical Company,U.S.A.

3.1.3 Industrial Gums

Gum Tragacanth G-1128, Xanthan gum G-1253 and Guar gum G- 4129 were purchased from Sigma Chemical Company,U.S.A. Mustard mucilage was provided by Dr. J. Jones, Food Research Institute,Ottawa.

3.2 METHODS

3.2.1 Sample Preparation

A representative sample of Candle seeds was mixed throughly and divided into two 2.5 kg lots (A and B) using a plastic container (50 grams). Lot B was kept in cold storage while the contents of lot A were mixed thoroughly and divided in 4 sub-lots of 625 grams (A1,A2,A3,A4). From each of these sub-lots seeds were transferred with a small scoop (10 grams) to each of the six small plastic bags. This resulted in 24 sub-samples of 100 g. The procedure is shown schematically in Figure 8.

Figure 2. Preparations of Sub-samples

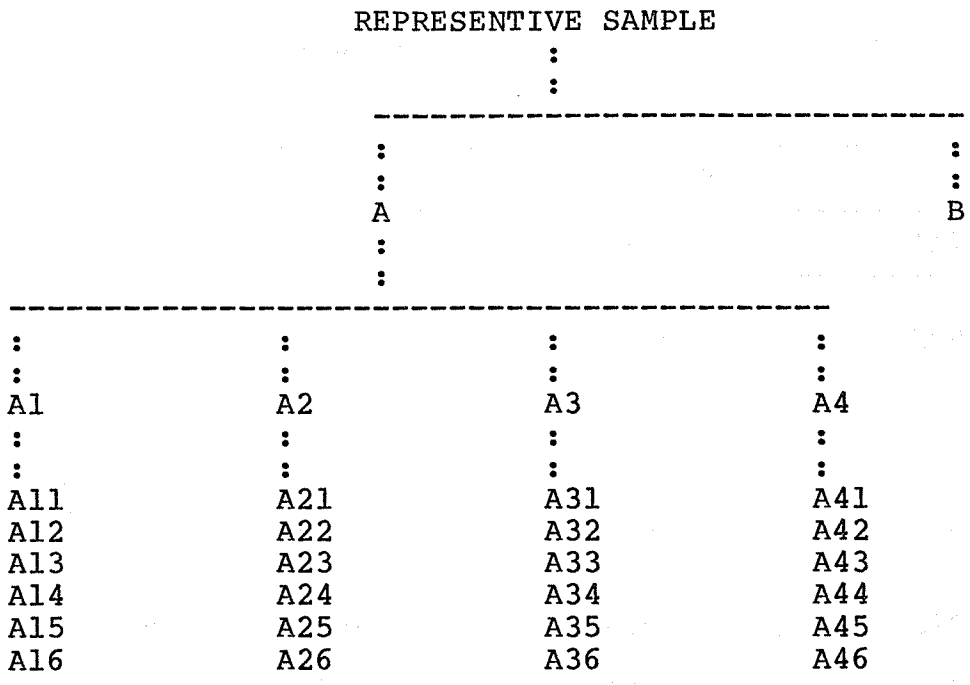


Table 6

Experimental Design for Experiment 1

	Treatment		
	I	II	III
Weight of Seeds	5 g	100 g	100 g
Seed:Water Ratio	1:18	1:18	1:7
Samples	A46	A15	A16
	A46	A25	A26
	A46	A35	A36

Table 7

Experimental Design for Experiment 2

Treatment			
I	II	III	IV
A11	A12	A13	A14
A21	A22	A23	A24
A31	A32	A33	A34
A41	A42	A43	A44

3.2.2 Effects of Temperature and Volume of Water on Yields of Mucilage

Triplicate subsamples of seeds were each subjected to the following treatments (Table 6). The first treatment (I) followed that described by Woods and Downey (1980) in which 5 grams of seeds were soaked in 90 ml of cold distilled water (1:18, seed:water ratio) and stirred with a magnetic stirrer for 16 hour at room temperature. The second treatment (II) was a scaled up model of treatment (I) in which 100 g of seed was used. The third treatment (III) was adapted from Woods and Downey (1980),Van Caeseele et al.(1987), El-Mahdy and El-Sebaiy (1984). 100 g of seeds were placed in a 1 liter flask to which was added 250 ml of boiling distilled water. The temperature dropped to 75 C within a minute and the mixture was held in a water bath at this temperature for 25 minutes. The flask was removed from the water bath and 450 ml of cold distilled water was added and the suspension stirred with a magnetic stirrer for 16 hours at room temperature (seed:water ratio 1:7). In all treatments 2.5 ml of chloroform was added per liter to prevent microbial growth (Woods and Downey,1980).The slurry was passed through three layers of thick cheese cloth. The mucilage was then precipitated with 4 volumes of 95% ethanol and the precipitate recovered by centrifugation at 3500 x g at 23 C for 10 minutes. The precipitate was washed three times with ethanol and

acetone, lyophilized and ground by hand in a mortar to a fine powder.

3.2.3 Effects of Soaking Time on Yield and Composition of Mucilage.

Four subsamples of seeds (100 g) were each subjected to different soaking times using a seed:water ratio of 1:7 . The soaking times used were 4,8,12,and 16 hours. The seeds were heat-treated as described earlier and the suspension stirred with a magnetic stirrer at room temperature for the period specified for each treatment. At the end of each treatment the slurry was passed through three layers of thick cheese cloth. The mucilage was precipitated with 4 volumes of 95% ethanol and the precipitate recovered by centrifugation at 3500 x g at 23 C for 10 minutes. The precipitate was washed three times with ethanol and acetone, lyophilized, weighed and ground by hand in a mortar to a fine powder for further chemical analysis.

3.2.4 Chemical Analysis

3.2.4.1 Crude Protein

Protein (%N) content of crude mucilage obtained from each sub-sample was determined in duplicate using the micro-Kjeldahl,method (A.A.C.C.1985).See Appendix (I).

3.2.4.2 Total Carbohydrate

The total carbohydrate content of the crude mucilage obtained from each sub-sample was determined in duplicate using the phenol-sulphuric acid method of Barnett and Towab (1957). See Appendix (II).

3.2.4.3 Moisture, Ash and Mineral

Moisture content of crude canola mucilage was determined according to the A.A.C.C. method 44-15A (1985). Ash and mineral content of crude canola mucilage was determined following the method of Chapman and Pratt (1961).

3.2.4.4 Gas Chromatography of Monosaccharides

Analysis was performed by Gas Liquid Chromatograpy (Perkin Elmer Sigma 3B), equipped with a flame ionization detector, and fused silica glass capillary column (30 m x 0.314 mm I.D.) coated with SE 30 (Chromatography Specialties Canada). The column oven temperature was programmed for the first 25 minutes at 200 C followed by 210 C for the next 20 minutes . Injector and detector temperatures were operated at 225 and 245 C respectively.

The flow-rate of the carrier gas (nitrogen) was regulated at 1 ml/min by the use of a 100/1 splitter. The eluate was continuously mixed with the scavenger gas (nitrogen),38 ml/min. and the mixture was introduced into the detector. Peaks were integrated with Hewlett Packer electronic integrator.

3.2.4.4.1 Hydrolysis of Carbohydrate

Each sample of mucilage (1-2 mg) was dissolved in 4 M hydrochloric acid (100 ul) in an ampule. The ampule was then flushed with nitrogen,sealed and heated for one hour in a boiling water bath. 100 ul of 1% 3-O-methyl glucopyranose was added to the mixture prior to heating as the internal standard. After hydrolysis the materials were transferred to a reaction tube and evaporated to dryness under reduced pressure in a desiccator containing sodium hydroxide.

3.2.4.4.2 Methylation of Monosaccharides

The method of Honda et al.(1979) was utilized for qualitative and quantitative determination of monosaccharides in the hydrolyzate . Twenty ul of ethanethiol-trifluoroacetic acid (2:1,v/v) was added to the residue in a reaction tube. The reaction tube was then closed tightly with a polyethylene stopper, and kept for 10 min at 25 C. Pyridine (50 ul), hexamethyldisilazane (100 ul), and trimethylchlorosilane (50 ul) were then added and the mixture incubated for 30 min at 50 C with occasional shaking. The mixture was centrifuged, and the 1 ul of the supernatant analysed by gas chromatography. A mixture of standard sugars was treated in an identical manner. The amounts of sugars in the sample were calculated by comparing the peak areas of sugar dervatives of samples, to that of the internal standard.

3.2.4.5 Purification of Mucilage.

The method used is that described by Woolfe et al.(1977), which is an adaptation of a general method of gum isolation from foods (Glicksman,1969). The crude mucilage (1%) was homogenized (Polytron homogeniser) with cold dilute trichloroacetic acid solution, (5%). The solution was centrifuged (3500 x g for 20 min),neutralized with sodium hydroxide by dropwise addition, and then dialyzed for 30 hr against distilled water at 4 C. The dialysis water was changed every 6 hours and the mucilage

precipitated with ethanol (three volumes), washed successively with ethanol and acetone then lyophilized, and finally ground by hand in a mortar to a fine powder.

3.2.5 Measurements of Physical Properties of Canola Mucilage

3.2.5.1 Viscometric Properties.

The viscosity of extracted mucilage as well as guar gum,tragacanth, and xanthan were measured with a Haake Rotovisco RV3 viscometer system, and MK50 sensorsystem. Solutions of various gums were prepared at 0.5% (w/w) and the measurements were conducted at 25 C in a constant water bath.Samples to be tested were placed in the cylinder sensor system. The shear rate was varied from 0 rpm to 720 rpm uniformly with time by a shear rate programmer (250 rpm/min) for obtaining the up curve. In order to record the down curve after recording the up curve, the shear rate was decreased from 720 rpm to 0 rpm uniformly with time by the shear rate programmer (250 rpm/min). The two measured values proportional to shear stress (t) and shear rate (D) were recorded on the two axes of an XY-recorder to give an automated plot of the sample material's flow curve.

3.2.5.2 Surface and Interfacial Tension

Surface tension of solutions (0.5% w/w) of crude canola mucilage, mustard mucilage ,tragacanth, xanthan,and guar gum were measured with a Cenco tensiometer NO. 70520. For measurement of interfacial

tension, corn oil was floated to the surface of gums and left for 60 seconds to establish two phases prior to any measurements. All measurements were carried out at room temperature.

3.2.6 Application of Canola Mucilage to Product Development

The following model salad dressing system was developed based on the formulation by Stauffer and Andon,(1975).

Corn oil	40%
Water	53.25%
Salt	4.25%
Acetic acid	2.0%
Gum	0.5%
Total	100%

This formulation was examined using Candle mucilage at 0.5% concentration (w/w) . The two measured values proportional to shear stress (t) and shear rate (D) were recorded on the two axes of an XY-recorder to give an automated plot of a the salad dressing flow curve.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Effects of Temperature and Volume of Water on Yield of Mucilage from Canola Seed

The yields of mucilage from Candle canola seed obtained by different treatments are summarized in Table 8. Analysis of variance showed there were significant differences ($p<0.05$) between the heat-treated and unheated seeds. This was evident for the heated canola seeds (treatment III) in which the yield of crude mucilage increased twofold to 1.33 % (sd= $\overline{+}$ 0.15) compared to 0.69-0.72 % (sd= $\overline{+}$ 0.02 - 0.04) for the unheated seeds. The ratio of seed to water for treatment I and II was 1:18 compared to 1:7 for treatment III. The heat treatment of seeds substantially increased the yield of mucilage even though the volume of water used was reduced by a factor of 2.57. Van Caeseale et al. (1987) extracted their mucilage from Candle seeds in cold water with a seed:water ratio of 1:3.5 for 2.5 hours but did not report the yield of mucilage obtained. A particular advantage of heat treatment of seeds is the inactivation of myrosinase in canola seed to prevent the hydrolysis of intact glucosinolate.

Table 8

Percent Yield of Lyophilized Mucilage from Candle Canola Seeds.

Samples	Treatments		
	I	II	III
1	0.77	0.67	1.24
2	0.71	0.71	1.51
3	0.69	0.68	1.25
[1] Mean $\pm$ sd	0.72 ± 0.04^{a}	0.69 ± 0.02^{a}	1.33 ± 0.15^{b}

1. Means with the same superscript are not significantly different ($P<0.05$).

4.2 Effects of Soaking Time on Yield of Mucilage From Canola Seeds

In the previous experiment it was found that the heat-treated seed produced the highest yield of mucilage. Further experiments were conducted to establish whether different soaking times would also affect the amount and composition of mucilage. The yields of mucilage obtained from the heat-treated canola seeds extracted with cold water for 4,8,12 and 16 hours are presented in Table 9. This is graphically presented in Figure 3. The results indicate that soaking time influenced the yield of mucilage. An increase in soaking time resulted in an increase in yield of mucilage.

Approximately 63.8 % of the total extractable mucilage was obtained after the initial 4 hours extraction period. The amounts extracted after 8,12, and 16 hours resulted in a further increase of 12.1% ,10.3% and 13.8% respectively. No significant differences ($P<0.05$) in the amount of mucilage were obtained between any of the 4 hour extraction intervals. A significant difference ($P<0.05$), however, was evident between 8 and 12 hours of extraction intervals. Previous studies on canola,rapeseed or mustard seed did not examine the effect of extraction time on yield of mucilage. The total amount of mucilage obtained at the end of 16 hours extraction period was 1.16% of the whole canola seed. The amount of mucilage in canola seed however, was within the lower range (0.34-2.05 %) reported

Figure 3. Effect of Extraction Time on Yield of Mucilage

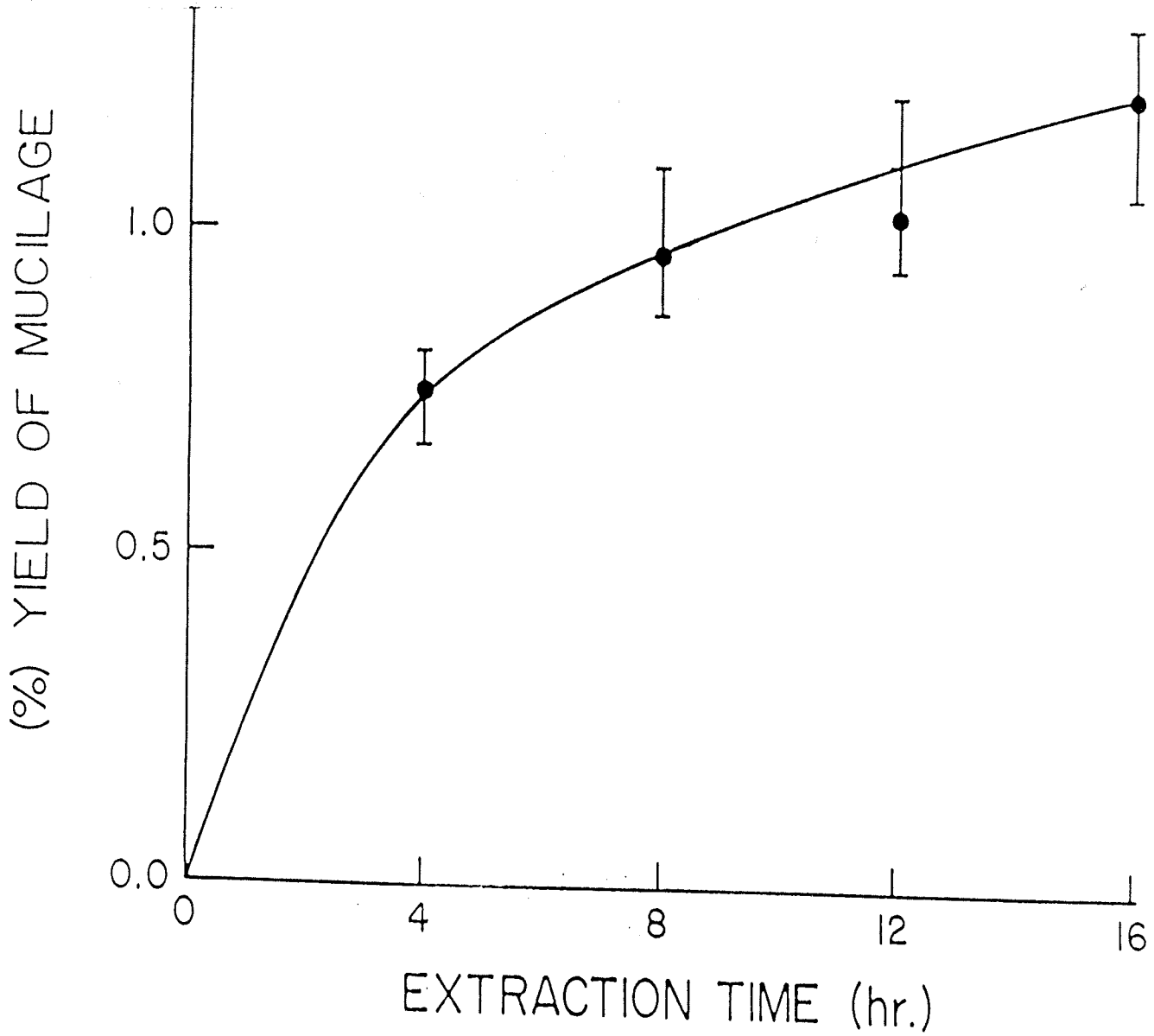

(%) YIELD OF MUCILAGE
1.0
0.5
0.0
0
4
8
12
16
EXTRACTION TIME (hr.)

Table 9

Percent Yield of Mucilage from Candle Canola Seeds at Different Soaking Times

Samples	Extraction Time (h)			
	4	8	12	16
1	0.66	1.01	0.94	1.28
2	0.74	0.70	1.10	1.16
3	0.78	0.87	0.99	1.06
4	0.79	0.95	0.98	1.16
[1] Mean $\pm$sd	$0.74^{a} \pm 0.7$	$0.88^{ab} \pm 0.13$	$1.00^{bc} \pm 0.07$	$1.16^{c} \pm 0.09$

1. Means with the same superscripts are not significantly different ($P<0.05$).

for mustard mucilage by Woods and Downey (1980).

Vose (1974) previously reported mucilage levels extracted with cold water from the hulls of Polish rape (B. campestris) and Argentinian rape (B. napus) to be 1.2 and 0.9 % of the total defatted hull weight respectively. This compared with 22.6 % reported for yellow mustard by the same researcher. Since hulls account for 16-19% of the total seeds,thus the mucilage values reported would be equivalent to 0.16-0.2 % of the total seed for the rapeseed and 2.7- 4.3 % for yellow mustard. This study showed much higher levels of water-soluble extract from the whole canola seed compared to that reported from the rapeseed hulls by Vose (1974). This could be explained in part by loss of mucilage during the dehulling process and/or varietal differences.

4.3 Chemical Analysis of the Crude Mucilage from Canola Seeds

4.3.1 Total Protein

Total protein content of crude mucilage from canola seeds was determined by the micro-Kjeldahl method of (A.A.C.C. 1985). Duplicate samples were analysed for total nitrogen content and the % total nitrogen converted to the % total protein using 6.25 as the conversion factor (Siddiqui and Woods, 1977). These results are presented in Table 10.

The protein content of extracted mucilage increased slightly from 17.7 $\bar{+}$ 0.7 to 18.9 $\bar{+}$ 0.5 over the 16 hours extraction period. However no significant difference ($P<0.05$) in protein content of the mucilage was observed between the four and sixteen hours of extraction time. This increase in protein content of the mucilage extracted was quite modest and could be accounted for by the solubility of the more soluble proteins during the prolonged extraction period. The possibility of some non-protein nitrogen being present, however, cannot be ruled out. The mean protein content of crude canola mucilage was 18.15 with a standard deviation of $\bar{+}$ 0.73. Vose (1974) showed rapeseed hulls had 14-16 % protein while mustard hulls contained 14-21% . Siddiqui and Woods (1971) isolated a crude neutral polysaccharide from rapeseed meal which contained 7.03% nitrogen or 45.75% protein using a

Table 10

Percent Total Protein Content of Crude Mucilage from Candle Canola Seeds.

Samples	Extraction Time (h)			
	4	8	12	16
1	16.88	18.19	17.83	19.48
2	17.96	18.11	17.73	18.70
3	18.49	16.89	18.58	18.93
4	17.35	17.89	18.93	18.42
[1] Mean $\bar{+}$sd	17.67^{a} $\bar{+}0.7$	17.77^{a} $\bar{+}0.6$	18.26^{a} $\bar{+}0.58$	18.88^{a} $\bar{+}0.45$

1. Means with the same superscript are not significantly different ($P<0.05$).

6.25 conversion factor. This polysaccharide was further purified by extraction with 90% aqueous phenol resulting in a polysaccharide fraction and a protein fraction. The separation of this protein fraction indicated that it was not bound to the polysaccharide. Churms and Stephen (1984) reported the presence of protein (18% w/w)in the gum exudate from Acacia robusta which was bound to an arabinogalactan. In this study, however, it was not clear whether protein in the exudates from canola was bound to polysaccharides or present in the free form. Further clarification of the nature of the bonding between protein and polysaccharide is needed.

4.3.2 Total Carbohydrate

The total carbohydrate content of crude mucilage extracted from the canola seeds was determined with the phenol-sulphuric method of Barnett and Towab (1957). The results summarized in Table 11 ,represent the average of duplicate analyses for each sample and are reported as % of the total dried crude mucilage. Prolonged extraction time resulted in a slight increase (30.21 to 31.50) in the total carbohydrate content of canola mucilage following 16 hours of extraction. Analysis of variance showed no significant differences ($P<0.05$) between the sugar content of mucilage extracted at different extraction

Table 11

Percent Total Carbohydrate of Crude Mucilage from Candle Canola Seeds.

Samples	Extraction Time (h)			
	4	8	12	16
1	29.33	32.17	30.52	32.04
2	30.40	30.44	29.30	30.56
3	31.47	30.28	30.42	31.87
4	29.65	29.36	31.07	31.53
[1] Mean $\bar{+}$sd	$30.21 \bar{+}0.95^{a}$	$30.56 \bar{+}1.17^{a}$	$30.32 \bar{+}0.74^{a}$	$31.50 \bar{+}0.66^{a}$

1. Means with the same superscript are not significantly different ($P<0.05$).

Table 12

Ratio of Total Carbohydrate to Protein in Crude Mucilage from Candle Canola Seeds.

Samples	Extraction Time (h)			
	4	8	12	16
1	1.74	1.77	1.71	1.64
2	1.69	1.68	1.65	1.64
3	1.70	1.79	1.64	1.68
4	1.71	1.64	1.64	1.71
[1] Mean ±sd	1.71 ±0.02[a]	1.72 ±0.07[a]	1.66±0.03[a]	1.67 ±0.03[a]

1. Means with the same superscripts are not significantly different ($P<0.05$).

time. The mean carbohydrate content of canola mucilage was 30.65 % with a standard deviation of $\pm$0.96.

Calculation of the ratios of total sugar to total protein content of mucilage obtained for different extraction periods is summarized in Table 12. No significant differences ($P<0.05$) were evident between the ratios which suggested canola mucilage extracts was homogeneous with respect to protein and carbohydrate content.

4.3.3 Moisture,Ash and Mineral

Preliminary analyses of ash ,mineral and moisture content of canola mucilage are shown in Tables 13 and 14. The average moisture and ash contents of dried canola mucilage for the four extraction periods was 8.16 $\pm$0.28 and 29.4 $\pm$ 3.4 % respectively. The average ash content is equivalent to approximately 10 % of the total ash in rapeseed (Vose, 1974). The ash content of white mustard mucilage was reported by Bailey and Norris (1932) to vary from 5.7 to 16.2 % . The range in ash content was attributed by these researchers to the different extraction procedures used. The ash content of a crude water-extracted polysaccharide from rapeseed meal was reported by Siddiqui and Woods (1977) to be 27 % .

Table 13

Percent Total Ash,Mineral and Moisture Content of Crude Candle Canola Mucilage

Minerals	Extraction Time (h)			
	4	8	12	16
Total ash	25.30	33.60	28.90	29.80
Moisture	8.53	8.02	8.21	7.89
Potassium	6.10	8.40	6.60	7.90
Nitrogen	2.83	2.84	2.92	3.01
Calcium	2.90	2.60	2.50	2.30
Phosphorus	2.05	2.29	2.52	2.30
Sulphur	1.60	2.80	2.20	2.60
Magnesium	1.60	1.90	1.90	1.90

Table 14

Conentrations of Trace Metals in Crude Candle Canola Mucilage [1]

Metals	Extraction Time (h)			
	4	8	12	16
Iron	8100	5400	4700	2900
Aluminium	3500	1760	1560	1400
Manganese	162	25	44	33
Zinc	151	38	62	33
Copper	74	29	31	33
Lead	24	13	17	10
Cadmiu	3.1	N.D.	0.7	0.4

1. ppm.

N.D. - not detectable

The major elements in Candle canola mucilage were potassium,calcium,phosphorus,magnesium,sulfur in order of decreasing concentration (Table 13). The presence of other elements including sodium would account for the remainder of the ash content but this remains to be confirmed. The concentration of trace metals cited in Table 14 indicated iron and aluminium to be present at substantially higher levels compared to the other trace elements. Little work has been reported on the mineral content of canola/rapeseed mucilage with the exception of an early study on white mustard mucilage by Bailey and Norris (1932). These researchers reported the presence of abundant amount of calcium,magnesium and iron in addition to aluminium, strontium,silicon and phosphorus. No sodium or potassium was detected in their studies which may be due to the relatively poor methods of analyses available at that time.

4.3.4 Monosaccharide Composition of Crude Canola Mucilage

Samples of crude mucilage extracted from Canola seeds were hydrolysed for one hour at 100 C ,dried and methylated according to the method of Honda et al.(1979). The particular advantage of this method discussed earlier is that aldoses lose their assymetric center at C-1 on derivatization to dithioacetals. By preventing isomeric

aldose formation, pentoses and hexoses can be separated. A programmed temperature of 200 C for 25 min followed by 210 C for 20 min was established which effectively separated the monosaccharides. A typical chromatogram of a known mixture of neutral monosaccharides is shown in Figure 4 compared to the canola mucilage sample in Figure 5. Six major peaks corresponding to xylose , arabinose,rhamnose, glucose,mannose and galactose were detected in mucilage extracted from canola seeds. While the retention times for the xylose and arabinose peaks were very close together,their resolution was adequate to permit quantitation. Decreasing the oven temperature slightly improved the resolution of xylose and arabinose peaks although the retention time was too long to be practical.

The effect of extraction time on the composition of canola mucilage monosaccharides is presented in Table 15. The six major monosaccharides detected included galactose, arabinose,glucose,mannose,rhamnose,and xylose in order of decreasing concentration. Of these only rhamnose was slightly affected by the increase in extraction time. A small but significant ($P<0.05$) increase in rhamnose concentration from 3.85 to 4.38% occurred after 8 hours extraction with a further modest but significant ($P<0.05$) increase to 5.08% after 12 hours which then levelled off. Rhamnose is one of the monosaccharides of pectic substances which comprise a

Figure 4. Gas Chromatogram of Standard Mixture of Monosaccharide.

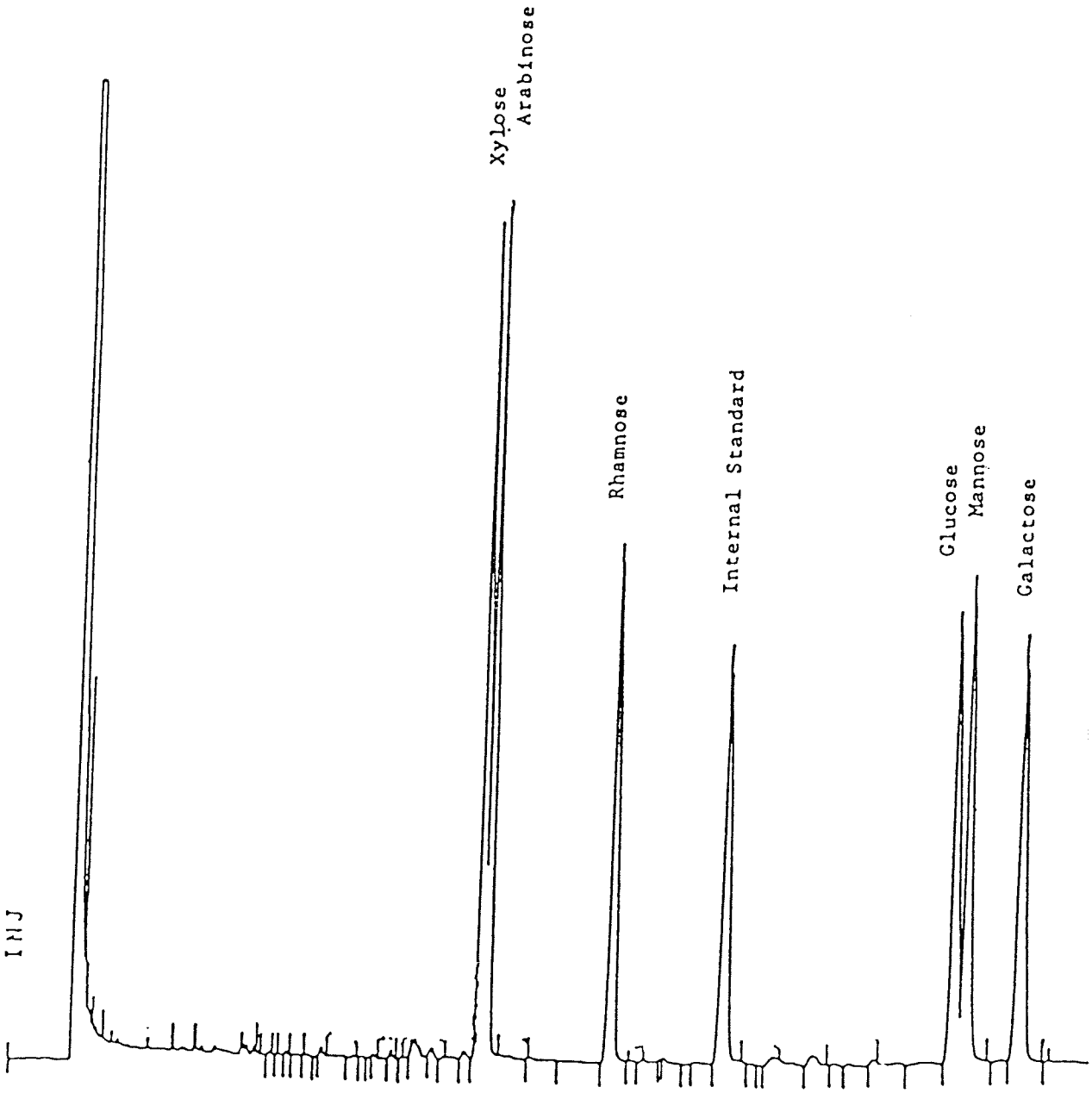

INJ
Xylose
Arabinose
Rhamnose
Internal Standard
Glucose
Mannose
Galactose

Figure 5. Gas Chromatogram of Monosaccharides in a Sample of Crude Canola Mucilage.

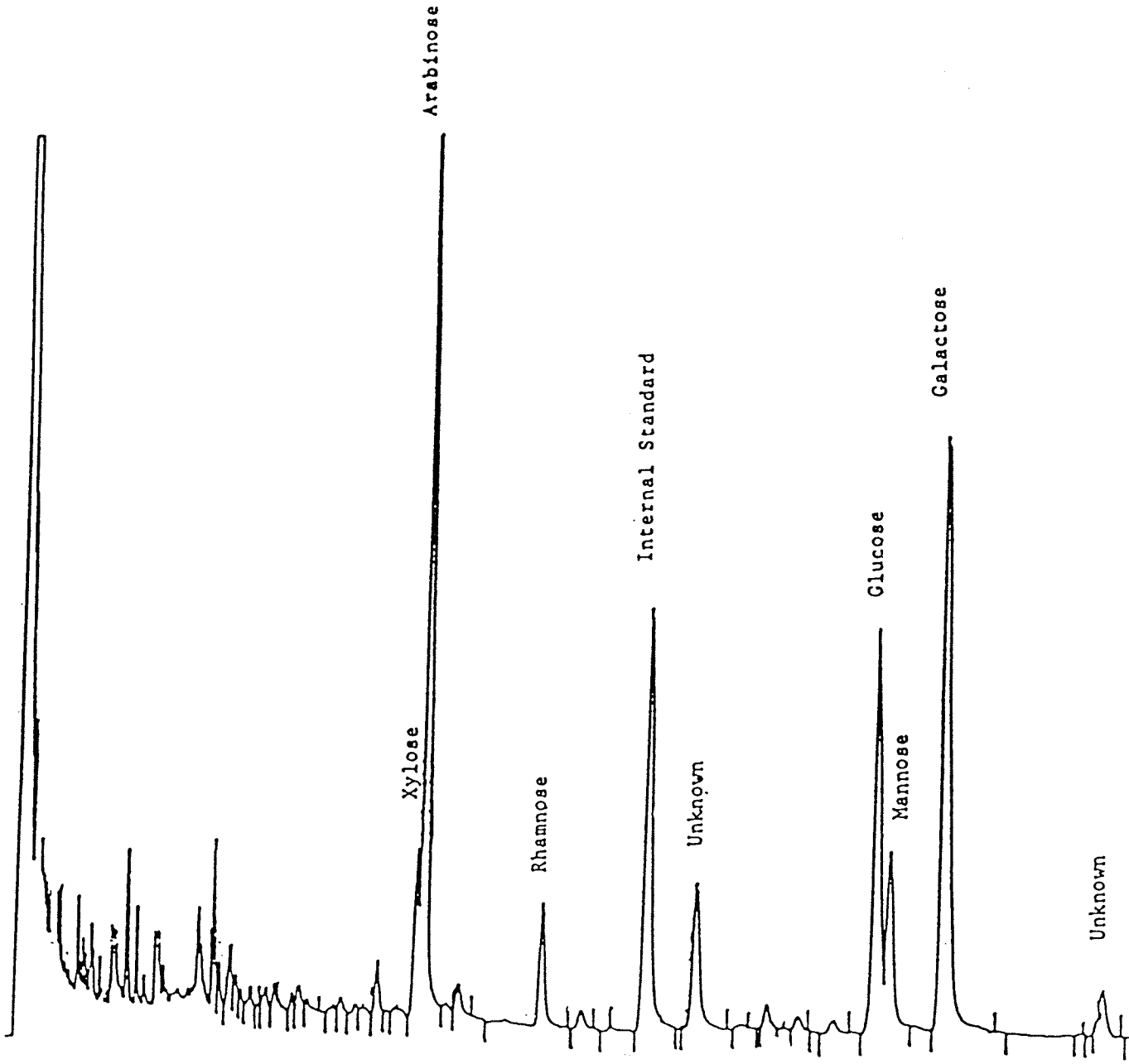

Arabinose
Xylose
Rhamnose
Internal Standard
Unknown
Glucose
Mannose
Galactose
Unknown

Table 15

Percent Monosaccharide Composition of the Total Carbohydrate in Crude Mucilage

Sugars	Extraction Time (h)			
	4	8	12	16
Xylose	3.49 ± 0.42 [a]	3.59 ± 0.19 [a]	4.02 ± 0.62 [a]	3.68 ± 0.29 [a]
Arabinose	30.22 ± 2.34 [a]	28.53 ± 2.70 [a]	28.22 ± 1.79 [a]	28.97 ± 2.89 [a]
Rhamnose	3.85 ± 0.32 [a]	4.38 ± 0.60 [ab]	5.08 ± 0.53 [c]	4.7 ± 0.42 [cb]
Glucose	15.27 ± 0.96 [a]	14.12 ± 2.35 [a]	15.43 ± 0.66 [a]	15.03 ± 0.83 [a]
Mannose	9.00 ± 0.51 [a]	10.06 ± 0.96 [a]	9.74 ± 1.3 [a]	8.99 ± 1.28 [a]
Galactose	32.48 ± 1.56 [a]	32.07 ± 1.35 [a]	31.73 ± 2.29 [a]	30.62 ± 2.53 [a]
Unknown	6.43 ± 2.35 [a]	7.18 ± 2.31 [a]	6.85 ± 3.2 [a]	7.92 ± 4.41 [a]

Means with the same superscripts are not significantly different ($P<0.05$).

Each mean is average of 8 values

substantial portion of the cell walls of the plant tissue (Aspinall,1970). One possible explanation is that the increase in soaking time and shaking of the seeds may have facilitated solubilization and/or separation of a small portion of soluble pectic substances from the seed coat mucilage. With respect to the other monosaccharides no significant differences ($P<0.05$) were observed. These results suggest that the extracted material from canola seeds was fairly homogeneous with respect to its carbohydrate composition.

In all the samples examined two unidentified peaks were observed. One of these peaks eluted immediately after the internal standard while the other followed the galactose peaks. These two peaks however, constituted only 7% of the total sugars and need to be identified.

Van Caeseele et al. (1987) analyzed the neutral monosaccharides in the mucilage extracted from Candle canola seed with cold water for 2.5 hours. They identified the presence of galactose (34 %), arabinose (20.03 %), glucose (10.69 %) ,mannose (9.55 %), rhamnose (5.82 %), in addition to 4% unidentified sugars. Their results were consistant with the analysis of the mucilage conducted this study (Table 15) in which arabinose and galactose were the major monosaccharides present. Differences were apparent between the relative levels of some of the monosaccharides. For example the levels of arabinose and glucose were higher in this study while

galactose and rhamnose were slightly lower. The presence of xylose was also reported in this study. The absence of xylose in canola mucilage reporterd by Van Caseele et al. (1987) may be attributed to a number of factors including differences in extraction time, treatments and the chromatographic conditions. The method of sugar analysis used by Van Caeseele et al. (1987) was based on the procedure of Honda et al.(1979). As discussed earlier the retention time of xylose and arabinose is very close and at temperatures above 200 C these two peaks overlap in the chromatogram. Since the concentration of xylose in canola mucilage was low, the establishment of optimum oven temperature conditions was very important for its separation. This could explain the inability of previous researchers to detect xylose in canola mucilage.

Xylose ,arabinose ,rhamnose ,glucose ,mannose , galactose, and fucose were reported by Theander et al.(1977) in the water-soluble extracts from turnip rapeseed hulls. Fucose was found to be about 1-2% of the total polysaccharide extracted. Fucose was not reported in this study although the presence of a peak after rhamnose may possibly be fucose ,but this remains to be confirmed. While galactose and arabinose were the major polysaccharides of the canola mucilage, glucose and galactose were the major monosaccharides of turnip rapeseed. The concentration of glucose in water-extracts of turnip rapeseed hulls was 2.6 times higher than water extracts of canola mucilage. In contrast, galactose in

canola mucilage was present at three times the level found in turnip rapeseed. The other major monosaccharide, arabinose, was only slightly higher in turnip rapeseed hull extract compared to canola mucilage. The presence of xylose although quite low was still substantially higher in turnip rapeseed hull extract compared to canola mucilage.

Vose (1974), extracted a water-soluble polysaccharide fraction from the defatted hulls of several rapeseed varieties (B. campestris and B. napus) which consisted of arabinose (40-50 %), xylose (18-25 %), glucose (10-14%) uronic acids (6-12 %) and traces of fructose,galactose, and rhamnose. The monosaccharide composition of canola mucilage was quite different from the polysaccharide fraction analyzed by Vose (1974). The differences could be attributed to varietal differences and/or the method of extraction used.

The monosaccharide composition of the cold water-soluble fractions from the defatted hulls of Yellow mustard (S. alba),Canadian Brown,Stoke, and Oriental mustard (B. juncea) were also examined by Vose (1974). While the B. Juncea mustards were similar in monosaccharide composition, yellow mustard,however, was quite different as it contained 60-70% cellulose. Canola mucilage differed from mustard in having lower levels of xylose and arabinose and higher levels of galactose.

Several neutral and acidic polysaccharides have been isolated from rapeseed meal (B. campestris). Larm et al.(1976) isolated an arabinogalactan from rapeseed (B.campestris) with a ratio of arabinose to galactose of 9:1 which accounted for 95% of the polysaccharide. Siddiqui and Woods (1971,1977) reported the presence of several neutral and acidic polysaccharides in rapeseed meals. One rapeseed arabinan isolated was composed largely of homogenous material containing approximately 4% of a galactan or arabinogalactan contaminant. An acidic arabinogalactan was also isolated from rapeseed meal and contained L-arabinose,D-galactose and D-glucuronic acid in molar ratio of 1:1.05:0.13 . The extraction procedure used in this study was quite different from that reported by Theander et al.(1977) and Vose (1974) which might explain the dissimilarities between levels of monosaccharide determined. These researchers extracted their polysaccharides from the hulls which may contain some of the polysaccharide fractions of the seed. Possible differences in carbohydrate compositions can occurr depending on the amount of polysaccharide seed contaminant present in the hulls. This study only examined the exudate from the whole seed and probably reflects those polysaccharides deposited in the seed coat. Examination of the monosaccharide ratios suggest that any possible contamination of the extracted mucilage with the polysaccharides from the testa of the canola seed was minimized using this method.

4.3.5 Monosaccharide Composition of Purified Canola Mucilage

A sample of crude mucilage extracted at 16 hours was further purified according to the method of Woolfe et al.(1977) which is an adaptation of general method of gum isolation from foods (Glicksman,1969). This method removed the insoluble portion of the crude mucilage while the soluble fraction remaining in the trifluoroacetic acid solution was precipitated by the addition of ethanol and recovered by centrigugation. The purified mucilage was cream in color compared to the dark colored crude material. It was hydrolysed and methylated according to the method of Honda et al. (1979). The results of the sugar analysis are summarized in Table 16 with a typical chromatograph for the monosaccharides shown in Figure 6.

With the exception of rhamnose all other monosaccharides were present in substantially reduced amounts in the purified mucilage. The monosaccharide rhamnose doubled in concentration in the purified mucilage . Two unidentified peaks previously detected in crude mucilage increased threefold in the purified material. The major monosaccharides in purified mucilage were galactose and arabinose which together accounted for 45.9% of the total carbohydrate present. Removal of the trifluoroacetic acid insoluble fraction during purification resulted in lower levels of arabinose, galactose,and a higher level of rhamnose in the purified mucilage. Galactose and

Figure 6. Gas Chromatogram of Monosaccharides in a Purified Sample of Canola Mucilage.

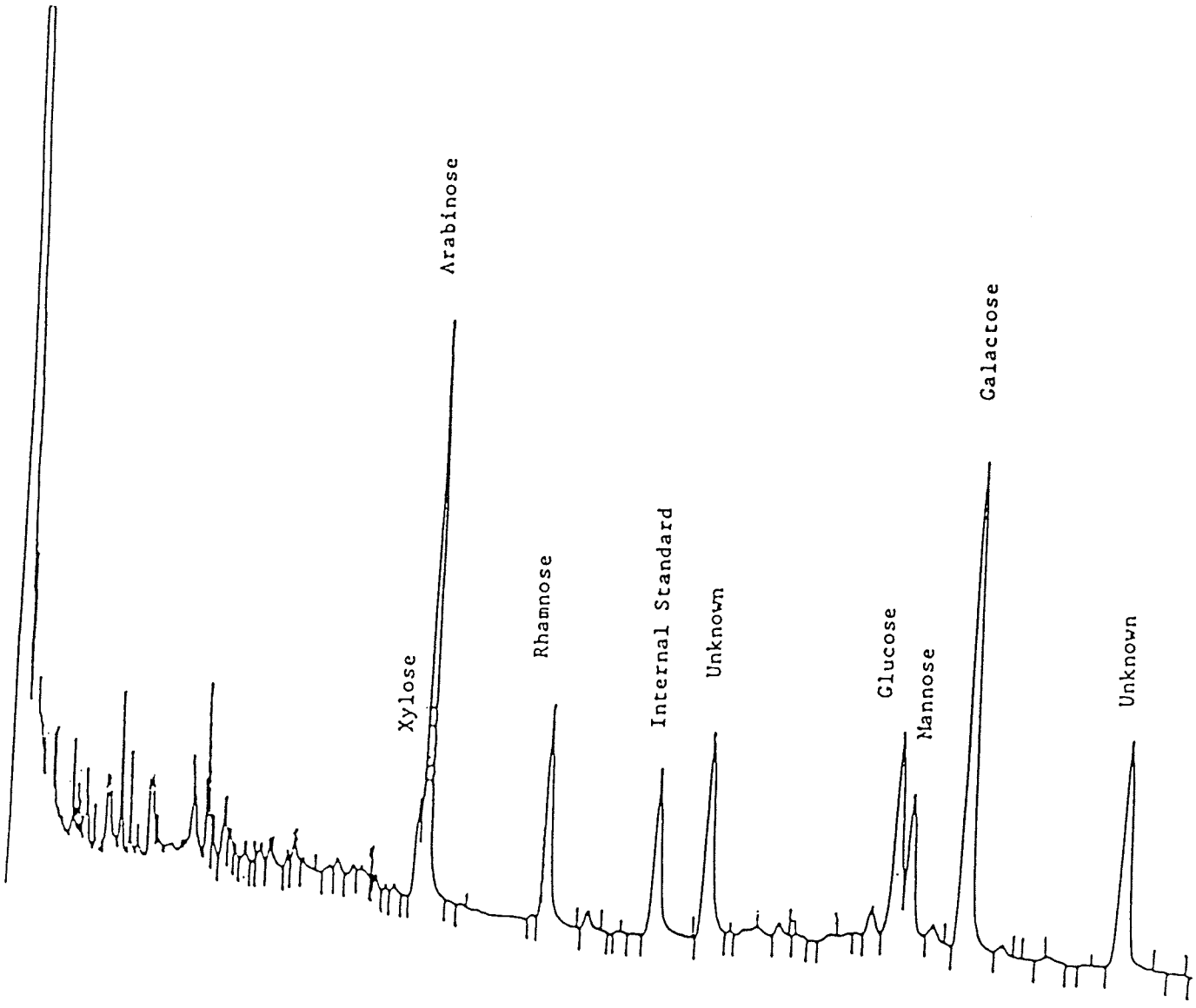

Xylose
Arabinose
Rhamnose
Internal Standard
Unknown
Glucose
Mannose
Galactose
Unknown

Table 16

Monosaccharide Composition of Crude and Purified Mucilage.

Sugars (% Total Carbohydrate)	Crude	Purified
Xylose	3.70	3.08
Arabinose	28.97	18.54
Rhamnose	4.52	10.31
Glucose	14.96	10.08
Mannose	9.45	7.26
Galactose	31.73	27.31
Unknown	7.10	23.40

arabinose were present in a ratio of 1.47:1 compared to 1.1:1 for the crude mucilage. The ratio of these sugars in purified mucilage differed substantially from arabinogalactan isolated from rapeseed meal by Larm et al.(1976) in which the ratio of galactose to arabinose was 1:9 . In the acidic arabinogalactan isolated from rapeseed meal by Siddiqui and Woods (1977) the ratio of L-arabinose:D-galactose:D-glucuronic acid was 1:1.05:0.13. The variation in monosaccharide ratios reported probably reflect the different polysaccharide fractions present in the seed coat and in the seed of canola/rapeseed as well as differences in the extraction procedures used.

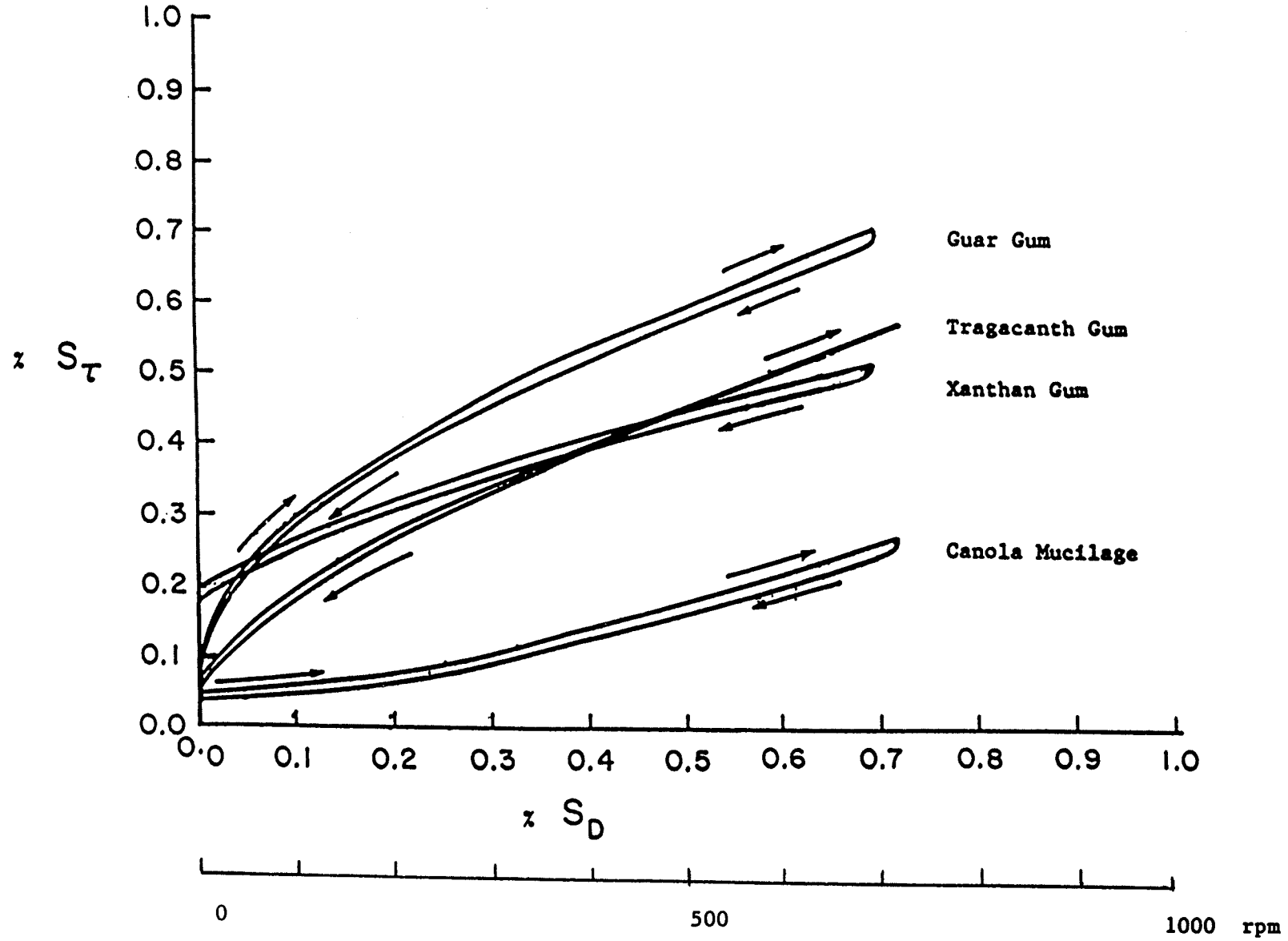
4.4 Physical Properties of Canola Mucilage

4.4.1 Viscometric Measurements

In order to compare the rheological properties of canola mucilage with xanthan,guar gum,and tragacanth, solutions of these gums were prepared at 0.5 % (w/w). The two measured values proportional to shear stress (t) and sheer rate were recorded on the two axes of an XY-recorder to give an automated plot of the sample material's flow curve using the Haake viscometer.

While the 0.5 % solutions of xanthan,tragacanth and guar gums showed a shear thininig effect the flow curve of the 0.5 % solution of canola mucilage was quite different by exhibiting a shear thickening property. Canola mucilage was non-Newtonian in behaviour up to a shear rate corresponding to 350 rpm while at higher rate of shear it was Newtonian. A typical hysteresis loop in the rheogram of 0.5 % solution of canola mucilage was also observed between up curves and down curves (Figure 7). The viscosity of a 0.5 % solution of canola mucilage within the Newtonian range was 4 cp which was quite low compared to yellow mustard. The viscosity of canola mucilage,however,was within the range reported for other varieties of mustard and rapeseed (Vose,1974).

Figure 7. Rheological Properties of Canola Mucilage and Other Industrial Food Gums.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
x S_T
Guar Gum
Tragacanth Gum
Xanthan Gum
Canola Mucilage
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
x S_D
0
500
1000 rpm

4.4.2 Surface and Interfacial Tension

The results obtained from surface tension and interfacial tension measurements are summarized in Table 17. Xanthan gum exhibited the highest surface tension while Candle and mustard mucilage were slightly higher than guar and tragacanth gums. Gum tragacanth was reported to reduce the surface tension and interfacial tension acting as surface active agent (Stauffer and Andon,1975). Candle and mustard mucilage both had similar surface tension properties. The value of 55.2 dynes/cm for the surface tension of mustard mucilage was in agreement with that reported by Webber et al. (1974).

The interfacial tension values were much lower for both Candle and mustard mucilages compared to the other commercial gums. The value for mustard mucilage of 8.5 dynes/cm using corn oil was very similar to 9 dynes/cm reported for soybean oil by Webber et al,(1974). They attributed the ability of yellow mustard to form stable emulsions to the reduction of interfacial tension rather than to its thickening properties. Grant et al.(1969) reported a water-soluble polysaccharide extracted from yellow mustard which contained crystalline bundles of cellulose chains solubilized by association with, or perhaps encapsulation by, other polysaccharides. Vose,(1974) reported the presence of cellulose in the water-soluble extract from yellow mustard hulls whereas they did not find cellulose in B. juncea mustard or either

Table 17

Surface Tension and Interfacial Tension of Gums (0.5% w/w)

Gums	Surface Tension dynes/cm	Interfacial Tension dynes/cm
Tragacanth	51.4 ±.85	12.5 ±0.31
Guar gum	52.5 ±0.57	11.6 ±0.2
Xanthan	74.6 ±0.47	18.2 ±0.25
Candle mucilage	56.6 ±0.28	9.5 ±0.25
Mustard mucilage	55.2 ±0.29	8.5 ±0.14

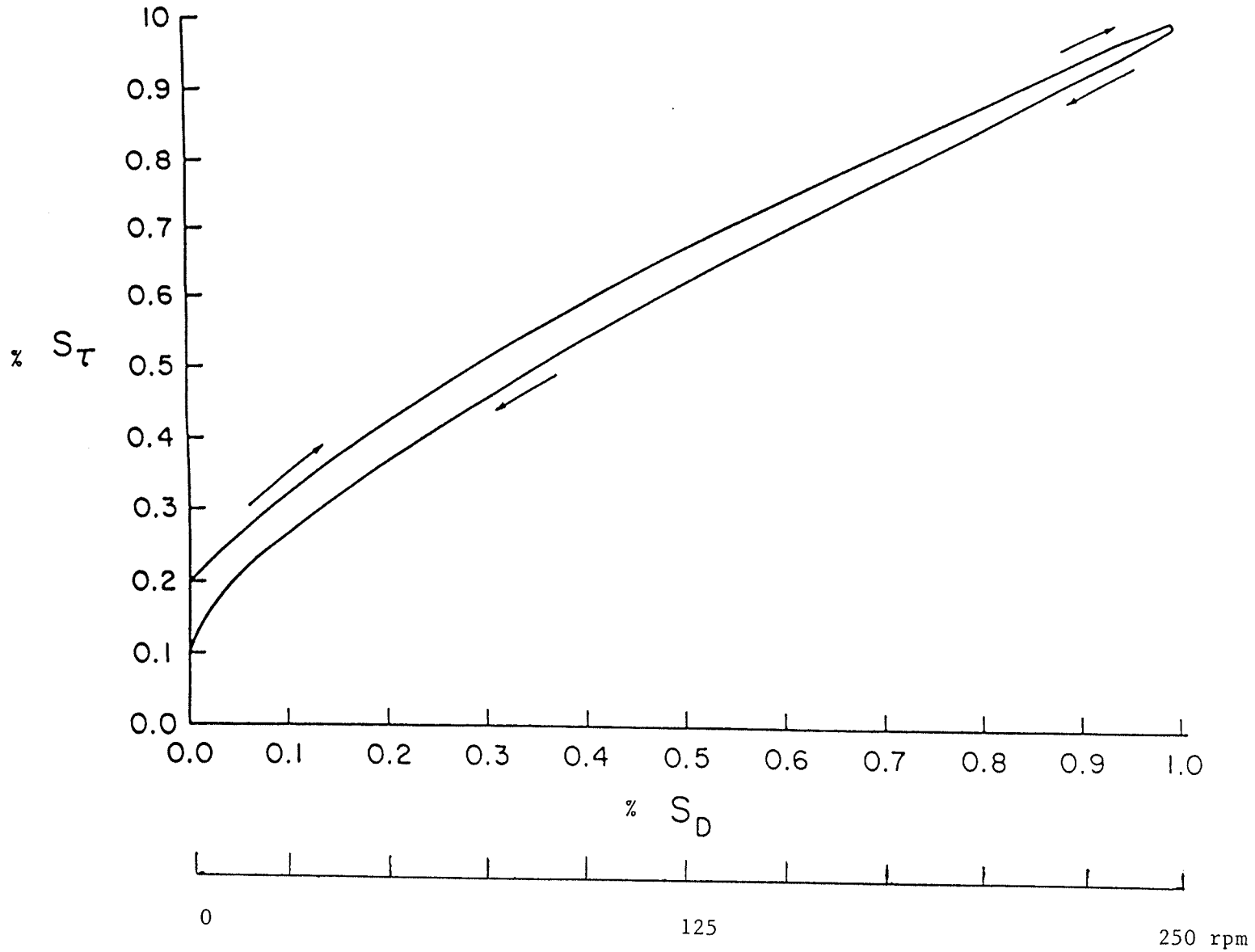
Mean ±sd

of the rapeseed species examined. This study showed the monosaccharide composition of the mucilage from canola seed to be quite different from other related rapeseed/mustard species (Vose,1974 ; Weber et al.1974). The presence of cellulose in the canola mucilage was not investigated although the detection of only 15 % glucose in the canola mucilage suggests it is a minor component. Nevertheless the presence of cellulose still remains to be confirmed. Candle mucilage reduced the surface tension and interfacial tension of a corn oil system indicating its ability to acts as an emulsifier. Further studies are needed,however, to relate the physical properties of canola mucilage to its chemical structure.

4.5 Product Development

Canola mucilage reduced both surface tention and interfacial tension of corn oil system. Based on these results it was incorporated as the emulsifier in a salad dressing using the formulation of Stauffer and Andon (1975). Canola mucilage produced a stable emulsion with the rheogram of the emulsion shown in Figure 8. The thixotropic behaviour of the emulsion is clearly evident from the presence of the loop in the rheogram of the emulsion. In this study the particle size of the oil droplet was not investigated. More work is needed in the area of product development which was beyond the scope of this study.

Figure 8. Rheogram of Salad Dressing With Canola Mucilage.


10
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
% S_T
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
% S_D
0
125
250 rpm

CHAPTER 5

SUMMARY AND RECOMMENDATIONS

This study showed that the heat-treatment of seeds prior to extraction increased the yield of mucilage from canola seed. Approximately 63% of mucilage was extracted from canola seeds with water (seed:water ratio of 1:7) after 4 hours with the remaining 36% extracted after 16 hours.

Chemical analyses showed no significant differences ($P<0.05$) in protein or carbohydrate content of canola mucilage extracted during 4 to 16 hours. No significant differences ($p<0.05$) in the ratios of carbohydrate to protein were observed during the 4 to 16 hours extraction period. These results suggested that canola mucilage extracted at different times (4-16 hours) were homogeneous with respect to both carbohydrate and protein content.

The proximate analysis of canola mucilage appeared to be moisture (8.2 %), protein (18.2 %), carbohydrate (30.6 %),ash (29.4 %),with the remainder (13.6 %) unidentified.

The monosaccharide composition of crude canola mucilage was xylose (3.7 %),arabinose (28.9 %), rhamnose (4.5 %),glucose (14.9 %),mannose (9.4 %),galactose(31.7 %) with a remaining (7.1 %) unidentified substances. With the exception of rhamnose, extraction time did not

significantly affect ($P<0.05$) the monosaccharide composition of the canola mucilage. Purification of mucilage increased rhamnose and the unidentified sugars while decreasing the remaining monosaccharides. A more detailed characterization of the unidentified sugars needs to be undertaken.

The viscosity of canola mucilage was very low compared to other industrial food gums. The mucilage from canola was able to reduce the surface tension and interfacial tension between a corn oil and water phase. Thus canola mucilage appears to have considerable potential for use in food emulsion systems.

The low content of mucilage in the seed coat together with low viscosity characteristics of this mucilage may limit it economically as a viable alternative to other industrial food gums. Nevertheless the presence and yield of mucilage in other canola varieties needs to be established to determine whether varietal and/or agronomic factors affect its development. Woods and Downey (1980), calculated the cost of production of one kilogram of mustard mucilage to be about $12.5 compared to $1.5 for guar gum. Taking into account the increase in labour cost and cost of production during the last 8 years the cost of production of canola mucilage would be higher than $12.5 per kilogram. Van Caseele et al. (1982) reported that mucilage may not be present in all the seeds from the Candle. Schans et al.(1982) studied the course of invasion of rapeseed by fungi which thrive well on the mucilage of

the seed coat. Taking into account these factors production of cultivars with high level of mucilage could result in undesirable storage problems (Mills and Sinha, 1980). These problems would be eliminated by the removal of mucilage from canola seeds.

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Appendix I

MICRO KJELDAHL DETERMINATION

Digestion:

1. Weigh sample (25 mg) onto 1/4 of a sheet of weighing paper. Fold paper over and push down the neck of a 30 ml digestion flask. Push into the bottom of the flask using a glass rod.
2. Add 1 gm of titanium dioxide catalyst.
3. Pipet 2 ml of conc. H2S04 into the flask so that it washes dowm the flask neck.
4. Place on digestion rack in the fume hood on full heat and digest for 1 hour after the samples are clear. The digestion rack should be set up in the fume hood and the glass manifold should be attached to a water aspirator. If the samples being analyzed are liquid it may be necessary to attach a rheostat to the digestion rack and run the samples at a lower heat to prevent froting until all the water has evaporated and then turn them up to full heat.
5. Cool slightly and then wash using a squirt bottle down the neck of the flask with 3-5 ml of distilled water to prevent precipitation.

Distillation:

6. Fill the steam generator 3/4 full with glass distilled water and plug in heating mantel. Leave the steam generator vent open.

7. Attach condensor to cold water line and turn on the tap.

8. Fill reservoir next to the steam generator with 8-9 mls of conc. NaOH solution.

9. Prepare receiving flask by placing 5 ml boric acid solution and 2 drops of indicator into a 50 ml erlenmeyer. Place under the condenser tip so that the end of the condenser is at the bottom of the flask.

10. Transfer sample from digestion flask to distilling flask rinsing 3 times with distilled water.

11. Attach the distilling flask containing the sample to the apparatus using spring clips and immediately clamp the the vent on the steam generator.

12. Open stopcock and allow conc. NaOH solution to slowly flow into the distilling flask. Close stopcock.

13. Distill about 15 ml of solution into the erlenmeyer and then lower the erlenmeyer so that the tip of the condenser is above the surface of the liquid and allow it to drip for 30-60 seconds. Rinse condenser tip with distilled water and remove erlenmeyer.

14. Unclamp vent on the steam generator and remove distilling flask.

15. If distilling another sample in a few minutes just leave the steam generator on. After 4 or 5 samples you will have to put more distilled water in the generator.

Conversely , after each sample you can add a small amount of water but not while the sample is distilling.

16. When finished distilling samples unplug the generator and shut off the water to the condenser. Also rinse out the sodium hydroxide reservior and remove the stopcock to prevent freezing.

Titration:

17. Titrate distillate with approximate 0.01 N HCl of which you know the exact normality using a 10 ml buret.

18. A blank containing 100 mg of sucrose must be run through the entire procedure starting at the digestion to determine the amount of nitrogen in the reagents.

19. Calculate the amount of the nitrogen in the sample using the following formula:

mg Nitrogen in sample =

$$(\text{Normality} \times \text{mls of acid} \times 14)_{\text{Sample}} - (\text{Normality} \times \text{mls acid} \times 14)_{\text{Blank}}$$

% Nitrogen in sample =mg Nitrogen x 100 / mg sample

% protein = % Nitrogen x 6.25 (usually)

20. When finished with the buret rinse with distilled water, loosen stopcock and leave down to dry.

Reagents:

50% NaOH - To 60 ml of distilled water in a 400 ml beaker sitting in a container of cold water add 50 gm of NaOH pelets. Stir with a stirring rod untill dissolved. Do not inhale fumes.

4% Boric acid - Weigh 4 gm of boric acid into a beaker. Add 96 ml water and stirring bar and place on a magnetic stirrer until disolved.

Indicator - Weigh 0.63 gm of methyl red and 0.33 gm methylene blue into a beaker and add 50 mls of 95% alcohole. Stir on magnetic stirrer for 1/2 hour.

Approximate 0.01 N HCl - Add 1.7 mls HCl to distilled water and dilute to 2000 ml. Exact normality must be determined by using a standardized base.

Reagents 1-3 from AACC method 46-13.

STANDARDIZING SODIUM HYDROXIDE

Reagents:

Carbone dioxide free water- Prepare by one of the following methods: 1) Boil H2O for 20 minutes. and cool with soda-lime protection. 2) bubble air ,free from CO2 by passing through H2O for 12 hours.

sodium hydroxide sol. (1+1) - to 1 part NaOH in a flask add 1 part H2O and swirl until soln. is complete. Close with rubber stopper and set aside until Na2CO3 has settled, leaving perfectly clear liquid (ca 10 days).

potasium acid phthalate- crush to a fine powder with a mortar and pestle and then dry for 2 hrs. at 120C. Cool in a desiccator and weigh samples that day.

Procedures:

add 2.6 ml of NaOH soln. (1+1) to 1 liter of CO2 free water. Mix and store with CO2 protection.

accurately weigh about 0.200 gm $KHC_8H_4O_4$ and record weight.

transfer to a 125 ml erlenmeyer swept free of Co2 and add 25 ml of CO2 free water. Stopper and swirl gently until sample dissolves.

add three drops of phthln. and titrate to a pink end-point. The solution should stay pink for at least 30 seconds.

titrate a blank containing the same volume of water and indicator.

subtract the blank from the first titration and calculate normality as follows:

$$\text{NORMALITY} = \frac{\text{gm acid phthalate} \times 1000}{\text{ml NaOH} \times 204.229}$$

do in triplicate.

Appendix II

Total Carbohydrates by the Phenol-Sulfuric Acid Method

Reagents and Apparatus:

5 % aqueous Phenol

Sulfuric acid, reagent grade

Spectrophotometer and cuvets

Vortex mixer

Rapid-delivery pipet for sulfuric acid

100 ml volumetric flasks

20 ml screw cap tubes

Standards: B-glucose

Stock std: 500 ug/ml (50 mg/100 ml)

Working Standards:

15 ug glucose/2 ml - 1.5 ml stock std. made up to 100 ml

30 ug glucose/2 ml - 3.0 ml stock std. made up to 100 ml

45 ug glucose/2 ml - 4.5 ml stock std. made up to 100 ml

60 ug glucose/2 ml - 6.0 ml stock std. made up to 100 ml

Standards must be made fresh daily.

Also run reagent blank of water and phenol and sulfuric acid.

Method:

Approximately 50 mg sample of mucilage was homogenised with a polytron homogenize and transferred into a 100 ml volumetric flask and made up to volume with

water. 10 ml of this diluted solution was transfered to another 100 ml volumetric flask and made up to volume with water.

Color reaction:

- pipet 2.0 ml diluted mucilage into screw cap tube (duplicate)
- add 1.0 ml 5% phenol
- add 5.0 ml sulfuric acid (add rapidly; for complete mixing direct the acid against the liquid surface rather than the side of the tube).
- cap tubes and let stand 10 min.
- mix for a few secounds on a vortex mixer
- let stand 30 min.; measure abs. at 485 nm against water

Calculations:

Subtract blank abs. from sample abs. value - read from standard curve to determine ug glucose/2 ml.

% Total carbohydrate in mucilage =

$$= \frac{\text{ug glucose/2 ml} \times 50}{\text{weight of mucilage (mg)}}$$

Notes:

1. When working with phenol and/or sulfuric acid, always wear safety glasses and gloves.
2. It is not necessary to run a complete standard curve with each set of samples; run one std. to verify that it is on the curve.

Table 18. One way analysis of variance for the effect of extraction time on yield of canola mucilage.

Source of variation	df	Ms	F
Treatments	3	0.128	14.98 **
Error	12	0.0086	
Total	15		

** P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	0.74 a	0.88 ab	1.00 bc	1.16 c

SEM = 0.046
Critical value of studentized range = 4.199
Minimum significant difference = 0.049 x 4.199 = 0.195
Alpha = 0.05 , df = 12

Table 19. One way analysis of variance for the effect of extraction time on concentration of total carbohydrate from canola mucilage.

Source of variation	df	Ms	F
Treatments	3	1.379	1.78
Error	12	0.773	
Totals	15		

P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	30.21 a	30.56 a	30.32 a	31.50 a

SEM = 0.44
Critical value of studentized range = 4.199
Minimum significant difference = 0.44 x 4.199 = 1.846
Alpha = 0.05 , df= 12

Table 20. One way analysis of variance for the effects of extraction time on concentration of protein in canola mucilage

Source of variation	df	MS	F
Treatments	3	1.234	3.54
Error	12	0.348	
Total	15		

$P<0.05$

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	17.67 a	17.77 a	18.26 a	18.88 a

SEM = 0.295
Critical value of studentized range = 4.199
Minimum significant difference = 0.295 x 4.199 = 1.24
Alpha = 0.05 , df = 12

Table 21. One way analysis of variance for effect of extraction time on the ratio of total carbohydrate to protein in canola mucilage.

Source of variance	df	MS	F
Treatments	3	0.0036	1.83
Error	12	0.00197	
Total	15		

P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	1.71 a	1.72 a	1.66 a	1.67 a

SEM = 0.022
Critical value of studentized range = 4.199
Minimum significant difference = 0.022 x 4.199 = 0.9325
Alpha = 0.05 , df = 12

Table 22. One way analysis of variance for the effects of extraction time on concentration of galactose in canola mucilage.

Sources of variation	df	MS	F
Treatments	3	5.11	1.28
Error	28	3.99	
Total	31		

P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	32.48 a	32.07 a	31.73 a	30.62 a

SEM = 0.706
Critical value of studentized range = 3.861
Minimum significant difference = 0.706 x 3.861 = 2.73
Alpha = 0.05 , df = 28

Table 23. One way analysis of variance for the effects of extraction time on concentration of mannose in canola mucilage.

Source of variation	df	MS	F
Treatments	3	2.31	2.03
Error	28	1.135	
Total	31		

$P<0.05$

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	9.0 a	10.06 a	9.74 a	8.99 a

SEM = 0.376
Critical value of studentized range = 3.861
Minimum significant difference = 0.376 x 3.861 = 1.45
Alpha = 0.05 , df = 28

Table 24. One way analysis of variance for the effects of extraction time on concentration of glucose in canola mucilage.

Source of variation	df	MS	F
Treatments	3	2.72	1.43
Errors	28	1.90	
Total	31		

$P<0.05$

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	15.27 a	14.12 a	15.43 a	15.03 a

SEM = 0.487
Critical value of studentized range = 3.861
Minimum significant difference = 0.487 x 3.861 = 1.88
Alpha = 0.05 , df = 28

Table 25. One way analysis of variance for the effect of extraction time on the concentration of rhamnose in canola mucilage.

Sources of variation	df	MS	F
Treatments	3	2.255	9.61 **
Error	28	0.234	
Total	31		

**P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	3.85 a	4.38 ab	5.08 c	4.77 cb

SEM = 0.171
Critical value of studentized range = 3.861
Minimum significant difference = 0.171 x 3.861 = 0.66
Alpha = 0.05 , df = 28

Table 26. One way analysis of variance for the effect of extraction time on concentration of xylose in canola mucilage.

Source of variation	df	MS	F
Treatments	3	0.428	2.40
Error	28	0.178	
Total	31		

P<0.05

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	3.49 a	3.59 a	3.68 a	4.02 a

SEM = 0.149
Critical value of studentized range 3.861
Minimum significant difference = 0.149 x 3.861 =0.576
Alpha = 0.05 , df = 28

Table 27. One way analysis of variance for the effect of extraction time on concentration of arabinose in canola mucilage.

Source of variation	df	MS	F
Treatments	3	6.16	1.01
Error	28	6.10	
Total	31		

$P<0.05$

Tukey Mean Separation

Treatments:	4 hours	8 hours	12 hours	16 hours
Means:	30.22 a	28.53 a	28.22 a	28.92 a

SEM = 0.873
Critical value of studentized range = 3.861
Minimum significant difference = 0.873 x 3.861 = 3.37
Alpha = 0.05 , df = 28