PREPARATION AND PROPERTIES OF A DIETARY FIBRE (PLANTIX) FROM APPLES

by

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ABSTRACT

Preparation and Properties of a Dietary Fibre (Plantix) from Apples

M.Sc. Jonathan Farber Agricultural Chemistry and Physics

A procedure was developed for the preparation of a dietary fibre from apple (Cortland cultivar) core material. Mean particle size of the fibre preparation was 307 µm. Acid and neutral detergent residues of apple core fibre were prepared. Water holding capacity, settling volume and cation exchange capacity of apple core fibre and its detergent residues were measured; structures of the three preparations were observed by scanning electron microscopy. Proximate analysis of apple core fibre indicated a pectin content of 7.9% and a protein content of 6.8%. Apple core fibre was hydrolyzed with 5% and 72% sulfuric acid; neutral sugars in the resultant hydrolysates were identified by cellulose thin layer chromatography and determined by gas chromatography; uronic acids were determined colourimetrically. The apple core fibre comprises 70.8% neutral sugars and 11% uronic acids. Dye binding capacity of the apple core fibre and its detergent residues was measured; the results indicated that dye binding was not related to cation exchange capacity.

RESUME

Préparation et Propriétés de la Fibre Alimentaire de la Pomme

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Un protocole a été developpé pour la préparation de la fibre alimentaire dans le coeur de la pomme. La grosseur moyenne des particules de la préparation de fibre est 307 μm. Les structures des résidus de la préparation de fibre après extraction aux détersifs acides et neutres et la préparation de fibre sont analysées par microscopie électronique à balayage. La capacité de rétention d'eau, le volume de sédimentation et la capacité d'échange de cations sont mesurés chez les trois préparations. La pectine constitue 7.9% et les protéines 6.8% de la préparation de fibre. La préparation de fibre est hydrolysée avec de l'acide sulfurique à 5% et à 72%. Les glucides neutres résultants de l'hydrolyse sont identifiés par chromatographie en couche mince (cellulose) et sont quantifiés par chromatographie en phase gazeuse. La teneur en acides uroniques est determinée par colorimétrie. La préparation de fibre contient 70.8% de glucides neutres et 11% d'acides uroniques. L'affinité pour les colorant de la préparation de fibre et des résidus des détersifs n'est pas reliée à la capacité d'échange de cations.

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GENERAL INTRODUCTION

The food processing industry is a large and important aspect of our lives. It provides a year-round supply of an assortment of canned, frozen, dried, dehydrated and refrigerated foods. There is linked to this benefit, however, the production of vast quantities of waste materials.

The apple industry, apart from producing apples for direct consumption, processes millions of pounds of apples each year; in the United States alone, the weight of raw, processed apple is 1.05 x 10^6 tons (U.S. E.P.A. 1971). Pomace, which is the press cake left from pressing apples for juice includes peel and core wastes. The yield of wet pomace is 500 to 700 pounds per ton of apples pressed in manufacturing juice, concentrate, cider brandy and vinegar (Smock and Neubert, 1950). It is not surprising that industrial uses have been found for vast quantities of this waste material; these include the use of apple pomace as a cattle feed and as a source of pectin (Smock and Neubert, 1950).

Apple pomace was the first major source of pectin in the United States and Europe; commercial production began during the period 1915-1917 (Kertesz, 1951). Pectin production in the United States is now predominantly from citrus peel; it is 20-50% pectin on a dry weight basis (Kertesz, 1951).

Apple pomace and apple pectin pulp, which is the residue from pectin extraction, have been used as animal feeds directly, with or without added flavours and protein supplements or after having been ensiled (Morrison, 1956).

In Quebec, the wet apple pomace, which frequently contains added cellulose fibre and rice hulls to improve juice extraction and filtration, is discarded (M. Girard, personal communication).

The aim of the present investigation is to isolate and characterize the chemical and physical properties of apple core fibre.

I. REVIEW OF LITERATURE

A. Dietary Fibre: History and Definition

Hippocrates in 400 B.C., recognized the value of fibre in the diet as he suggested the use of bran as a laxative (Colmey, 1978). Trowell (1977b) in an exhaustive bibliography gave numerous examples of the use of fibre in the diet. Galton (1976) stated that T.L. Cleave first proposed that a link might exist between the amount of refined food in the diet and the incidence of lower bowel problems. Dennis Burkitt's studies on African populations led to the theory that fibre was essential in the prevention and treatment of many colonic disorders, as well as diseases related to metabolic levels of cholesterol, metal ions and glucose (Burkitt and Trowell, 1975).

The word 'fibre' was first used by Grew in 'The Anatomie of Plantes' (1682) in relation to the structure of the cell wall of plants (Preston, 1974; Trowell, 1977a). 'Crude Fiber' was developed as a method for the determination of indigestible residue in forage material. It is the residue of plant food which, after extracting lipid, is resistant to the sequential extraction with hot dilute acid followed by hot dilute alkali. According to Van Soest (1978) it was John Gorham (1820) and not Einhof (1806) who first introduced this method; the method, after several modifications (Browne, 1940) was accepted by the A.O.A.C. Hipsley (1953) introduced the term 'dietary fibre' to describe what McCance had called the unavailable or indigestible carbohydrates of plant food (Trowell, 1979). Trowell (1972) defined dietary fibre as "the remnants of vegetable cell walls which are not hydrolysed by the alimentary enzymes of man." This definition was later revised to read "the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man" (Trowell et al., 1976). Trowell's terminology has been the subject of debate (Spiller and Amen, 1975; Spiller and Fassett-Cornelius, 1976; Furda, 1977; Fassett-Cornelius and Spiller, 1978) and workers have proposed the terms "Plantix" (Spiller and Fassett-Cornelius, 1976) and "Partially Digestible Biopolymers" (Furda, 1977). Dietary fibre, however, has become a widely used term and it is likely that it will remain a part of our vocabulary for some time to come.

More recently, Southgate et al. (1978) defined dietary fibre as "the sum of the lignin and polysaccharides that are not digested by the endogenous secretions of the human digestive tract." This definition has a physiological basis and Schweizer and Würsch (1979) considered that the term should include the biosynthetic or synthetic fibre components of food such as hydroxy-methyl cellulose and carboxymethyl cellulose.

B. Dietary Fibre: Composition

There are complex relationships that exist between the various components of dietary fibre; it is not surprising, therefore, that the isolation and characterization of these components lead to many difficulties.

There are two methods of approach to the study of plant cell anatomy; namely a morphological approach and a physico-chemical approach. Within the last hundred years, a combination of these methods has led to the elucidation of the fine anatomical structure of the plant cell wall and to an extensive knowledge of its components.

Cell wall synthesis begins during telophase when the cell plate forms between daughter nuclei and extends to meet the existing cell wall;

this creates a middle lamella, a structure which is rich in pectic polygalacturonans (Southgate, 1975; Theander, 1977). The primary wall is formed in the middle lamella by cellulose fibrils laid in a network. These fibrils are bundles of micro-fibrils, each in turn, composed of elementary fibrils consisting of about forty cellulose molecules arranged in a micella (Fahn, 1974). Cellulose is a β-glucan that is composed of glucose units linked by β-(1-4) glycosidic bonds. The three-dimensional arrangements of fibrils favour inter- and intra-molecular hydrogen bonding thus fibrils of high mechanical strength and chemical resistance are formed (Theander, 1977). These fibrils are covered with an amorphous matrix of hemicellulose and pectin.

The hemicelluloses are perhaps the least understood of all the plant wall components (Van Soest and McQueen, 1973). The old classification defines hemicelluloses as those substances that are preferentially solubilized by dilute alkali after removal of water solubles and pectic polysaccharides. Hemicellulose is similar to cellulose in that it has a backbone of β-(1-4) linked pyranoside sugars (Theander and Aman, 1979a); it differs from cellulose in that it has a low degree of polymerization (50-200 residues) and has varying degrees of branching and sugar substitution (Kay and Strasberg, 1978). Individual classifications of the various hemicelluloses are now based on the predominant monomeric sugar residue(s), e.g. xylans, glucuronoxylans, galactoglucomannans, etc. (Theander and Aman, 1979a) rather than the acidity or neutrality of the hemicellulose. The acidic residues, glucuronic acid and galacturonic acid are available for reaction with metal ions, methylation and amidation. The hemicelluloses exist in close association with cellulose fibrils through covalent bonds and may act as plasticizers (Eastwood, 1973). The hemicelluloses are somewhat more digestible than cellulose (Theander and Aman, 1979a).

Pectins are a group of substances, the main constituent of which is D-galacturonic acid. The acid residues are bound by α-(1-4) glycosidic linkages interspersed with α-(1-2) linked rhamnose residues to form a backbone to which are attached either arabinans, galactans or arabinogalactans. Pectin exists in the cell wall as insoluble protopectin. These pectic substances are made insoluble by the presence of divalent calcium ions but may be extracted with ammonium oxalate or EDTA (Theander and Aman, 1979a). Joseph (1955) proposed a chemical extraction method which characterizes pectin according to its degree of esterification. Pectin acts as a support material that forms a cross-linked gel that is able to withstand osmotic repulsions. Its properties of adhesion and hydrophilicity, its binding of metal ions and lipids and its capacity to form hydrogen bonds are influenced by its degree of methylation and acetylation (Furda, 1979; Aspinall, 1970; Eastwood, 1973).

Growth of the cell wall involves the two following mechanisms:

1) Intussusception: this consists of a laying down of new material between particles of existing substance. The resultant "mosaic growth" (Fahn, 1974) occurs when thin areas of fibrils are penetrated by cytoplasm and distended by cytoplasmic synthesis; new fibrils are added to fill the gap.

2) Apposition: this type of growth is due to centripedal addition of new layers, one upon the other. The resultant "multinet growth" (Fahn, 1974) gives rise to a thickening and an increase in surface area of the primary

walls. The thickening is a result of fibril reorientation in the earliest formed lamella, from transverse to longitudinal; successive lamellae, with denser fibrils are then added transversely.

The secondary cell wall is formed by layering on the inner surface of the primary wall; it begins in cells or parts of them that have ceased growth. It is a trilaminar structure with successively apposed layers of fibrils that are orientated at an angle to the axis of the cell. This structure is surrounded by a hemicellulose matrix.

Lignification is initiated at the cell corners within or just inside the primary wall; it extends rapidly to the middle lamella then along the middle lamella to the secondary wall (Wardrop, 1971). Sarkanen and Ludwig (1971) have defined lignin as the "polymeric natural products arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors, namely trans-coniferyl, trans-sinapyl and trans-coumaryl alcohols." Lignin is a three dimensional matrix which varies in composition and chemical reactivity depending on the plant source and method of isolation. Lignin has a multiple function and is essential to plant life. It acts as a cementing and anchoring agent and imparts rigidity to cell walls and resistance to impact, bending and compression. It is involved in internal transport of water, nutrients and metabolites by decreasing water permeability in vascular tissue such as xylems. Lignified cells impede the penetration of destructive organisms and hence resist microbial attack (Sarkanen and Ludwig, 1971); the lignin itself may also be toxic to microorganisms (Gordon, 1978). Lignin exists in the cell in close association with hemicellulose and is covalently bound to it (Sarkanen and Ludwig, 1971). The exact nature of the covalent bonding is unknown. Morrison (1974) has postulated that three types of bonds exist; one type is cleaved by borohydride reduction; one is resistant to alkali and another is alkali labile. Complete lignification is associated with cell death.

C. Analytical Methods

(i) Crude Fibre

Gorham (1820) described a method for the analysis of crude fibre of Indian corn as early as 1820 (Van Soest and Robertson, 1977). Successive refinements and standardizations of the crude fibre method led to the adoption of this method by the A.O.A.C. in 1887 (Browne, 1940). The crude fibre thus determined was supposed to represent the indigestible fractions of food (Spiller and Amen, 1975). According to Van Soest and McQueen (1973) this method led to the elimination of 20 to 50 percent of the cellulose, 50-90 percent of the lignin and about 85 percent of the hemicellulose.

(ii) Detergent Extraction

Van Soest and coworkers developed methods involving the use of an acid detergent and a neutral detergent to determine crude fibre (Van Soest, 1963a, 1963b; Van Soest and Wine, 1967; Van Soest, 1973); They considered that the results obtained by these methods gave a more reliable measure of the cellulose and lignin content of feed than did the previous method. A list of the apparatus and reagents as well as the procedures is given in U.S.D.A. Handbook #379 (Goering and Van Soest, 1970). Other methods that have been suggested include a buffered-acid detergent method (Baker, 1977), an ammonium oxalate method (Collings and Yokoyama, 1978) and neutral detergent methods involving the use of an α-amylase to aid in the removal of starch (Schweizer and Wursch, 1979; Belo and deLumen, 1981; Schaller, 1977; Collinge et al., 1980). Although these methods provide an estimate of the indigestible residue, they do not give an accurate estimate of dietary fibre; non-cellulosic polysaccharides are solubilized by the detergent system.

(iii) Enzyme Digestion (Biochemical)

Williams and Olmsted (1935) were dissatisfied with the relevance of their crude fibre measurements (Olmsted et al., 1935); they developed a method which involved treating their samples with a pancreatin-bile salt mixture and subsequently with cold 21.4 N sulphuric acid, to more closely approximate the action of the human digestive system. A further modification by Hellendoorn et al. (1975) involved the use of pepsin in addition to pancreatin. Southgate et al. (1978) considered that the modified method does not give a reliable measure of all dietary fibre components or all water solubilized components.

(iv) Unavailable Carbohydrates

The Southgate method for unavailable carbohydrates (Southgate, 1969) is based on the work of McCance and Lawrence (1929). It is an extensive method which isolates and quantifies cellulose, lignin and non-cellulosic polysaccharides. The method is lengthy and lacks specificity because of the mutual interferences of hexose, pentose and uronic acids (Hudson and Bailey, 1980). This procedure has been modified by Englyst* to include the determination of hexoses and pentoses by gas liquid chromatography. Southgate et al. (1978) have proposed an analytical scheme for complete characterization of dietary fibre.

* In Southgate et al. (1978).

There are several other methods for fibre determination that involve solvent extraction, enzymatic removal of starch, sequential acid hydrolysis, colourimetric or gas chromatographic estimation of sugars and colourimetric or decarboxylative estimation of uronic acids (Anderson and Clydesdale, 1980; Jeltema and Zabik, 1980; Theander and Aman, 1979b).

Several authors (McConnell and Eastwood, 1974; Schweizer and Wursch, 1979; Saunders and Hautala, 1979) have made comparative studies of the above analytical techniques in connection with the use of these methods for the estimation of dietary fibre in diets. James and Theander (1981) have edited a review of recent advances in dietary fibre determination.

(v) Cell Wall Material

Selvendran et al. (1979a) have developed a technique which selectively removes cytoplasmic material; it is based on the use of organic solvents with a high degree of affinity for fibre contaminants. Plant material is ball-milled with a solution of 1% aqueous sodium deoxycholate. The residue from this extraction is then extracted twice with a solution of phenol/acetic acid/water, (2:1:1, w/v/v). A final extraction is performed with aqueous DMSO* (90% v/v). The residue, which is pure cell wall material (CWM), is free of starch and intracellular proteins. The small amounts of solubilized pectic substances (β-(1-3) and β-(1-4) glycans) can be recovered from the extracts and included with the cell wall material (Selvendran and Dupont, 1980a; Selvendran et al., 1979a).

D. Apple Research

Apples are fruits formed by the enlargement of the receptacle

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* DMSO = Dimethylsulfoxide

which becomes fleshy and surrounds the carpels (Jacobs, 1944). They are composed of water (84%) and carbohydrate (11-14%); they also contain small amounts of lignin, tannin, pectin, cellulose, hemicellulose and organic acids (Chatfield and MacLaughlin, 1931).

The edible portion and pulp from apples have been analyzed by several workers (Southgate et al., 1978; Schweizer and Würsch, 1979; Theander and Aman, 1979b). To the author's knowledge there is only one report on the separate analysis of peel and flesh (Southgate et al., 1976).

Table 1 shows the composition of apple pomace and apple pectin pulp in regard to digestible nutrients. Table 2 summarizes the results of several workers on the non-digestible (mono-gastric) portion of edible apple tissue. To the author's knowledge, there is no published information on the constituents of the dietary fibre portion of apple pomace or apple pectin pulp.

E. Binding Studies:

The physical properties and dietary functions of plant fibre as well as the various components of plant fibre have been the subject of much research. Several workers (Eastwood and Hamilton, 1968; Chang and Johnson, 1980; Kay et al., 1979; Thiffault et al., 1970; Judd et al., 1976) have reported that the lignin component of dietary fibre is involved in the adsorption of bile salts and acids and may influence serum cholesterol levels; pectin may react with sterols, lipids and bile salts (Furda, 1979; Selvendran, 1979; Kay et al., 1978; Pfeffer, 1981). It has been suggested that hemicellulose may interfere with protein absorption (Kies and Fox, 1977, 1978) and may increase bile acid excretion (Huang et al., 1978). Many

TABLE 1

Average Composition and Digestible Nutrients of Apple Tissue[1]

	Total Dry Matter %	Digestible Protein %	Total Digestible Nutrients %	Nutritive Ratio 1:	Average Total Composition				
					Protein %	Fat %	Fiber %[2]	N-Free[3] Extract %	Mineral Matter %
Dried Apple Pectin Pulp	91.2	2.6	62.4	23.0	7.0	7.3	24.2	49.4	3.3
Dried Apple Pomace	89.6	1.6	64.5	39.3	4.3	4.6	15.2	63.5	2.0

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[1] From Morrison, 1956

[2] As crude fiber

[3] Contains starch, sugars, soluble pentosans, complex carbohydrates, organic acids and lignin

TABLE 2

Composition of Apple Tissue

Material	Total Dietary Fiber	Non Cellulose Polysaccharides[III]	Cellulose	Lignin[II]	ADF	NDF
	Composition (gm/100 gm dry matter)					
Apple [1] (Flesh only)	9.2	6.1	3.0	Trace		14.7
Apple Pulp[2,VI] (Dried)	87.6	53.2	31.9	2.5		
Apple[3] (Homogenized)	17.0				8.5	10.1
Apple[4] (Homogenized)	17.5			1.3		
Apple[5,IV] (Flesh)	1.42	0.94	0.48	0.01		
Apple[5,IV] (Peel)	3.71	2.21	0.01	0.49		
Apple[6] (Dehydrated)	13[VII]	7.5[V]	3	2.5		
Apple[7] (Pulp)			5.3	0.7[I]	6.35	9.50
Apple[8] (Pulp)	10	5.8	3.2	1.0		

1) Southgate *et al.*, 1978
2) Cummings *et al.*, 1978
3) Schweizer and Wursch, 1979
4) Theander and Aman, 1979b
5) Southgate *et al.*, 1976
6) Manville *et al.*, 1939
7) Robertson, J.B., and Van Soest, P.J., 1981
8) Southgate and White, 1981

I) Lignin determined by $KMNO_4$ oxidation
II) Determined as Klason Lignin
III) Sum of hexoses, pentoses and uronic acids
IV) Values given are gms/100 gm edible portion
V) Sum of hemicellulose and pectin as determined
VI) gm/100 gm of material used
VII) Total of columns 2, 3 and 4

of these studies have involved the use of fibre preparations of undefined chemical composition (Story et al., 1979; Eastwood et al., 1976; Kritchevsky and Story, 1974; Vahouny et al., 1980; Kies and Fox, 1977). Holloway et al. (1980) in a study of hemicellulose digestion determined the monosaccharide profile of their material, at each stage of isolation. Selvendran (1979) studied the effects of pH, particle size and composition of fibre on the binding of bile salts; the author reported on the detailed analysis of the components of parenchymatous, lignified and cutinised tissue isolated from runner beans. Several workers (James et al., 1978; Reinhold et al., 1976; Davies, 1979) studied the binding of certain metals by dietary fibre.

McLean (1977) has reported that diet can modify chemical toxicity. Several workers (Ershoff and Marshall, 1975; Kimura et al., 1980; Takeda and Kiriyama, 1979; Tsujita et al., 1979) have shown that the addition of certain fibre preparations reduced the toxicity of cyclamate, Tween 60, Tween 20, polyethylene glycol 4000, amaranth and certain other food dyes. Kritchevsky (1977) pointed out that the mechanism of detoxification by dietary fibre has not yet been elucidated. Ershoff and Marshall (1975) noted that dietary fibre might be an effective detoxicant for several different toxic materials but the mode of action could be quite different.

II. MATERIALS AND METHODS

A. Measurements of Physical Characteristics of Apple Core Fibre

1. Determination of Mean Particle Size

Mean particle size was determined (ASAE Standard S319 ; Agricultural Engineers Yearbook, 1976) using U.S. Standard Sieves (Endecotts Filters Limited) numbers 10 (2 mm); 18 (1 mm); 35 (500 µm); 60 (250 µm); 100 (149 µm) and 200 (74 µm). A sample (30 gm approx.) of fibre material was placed in the uppermost sieve and the sieves tightly covered. The sieves were secured to a shaking device which had a 3.5 cm lateral shake and a 0.7 cm drop-tap and a running speed of approximately 265 rpm. The sieves were shaken for 15 minutes; the nested sieves were disassembled and the walls of each sieve brushed down. The sieving operation was continued for 10 minutes; the walls were again brushed down and the sieving operation continued for an additional 5 minutes. The fibre was collected from each sieve and the tray and then weighed. The mean particle size (mean diameter) and geometric standard deviation were calculated following the equations provided in the Agricultural Engineers Yearbook (1976).

2. Water Holding Capacity (Method #1)

Water holding capacity was determined using the method of Robertson et al. (1980) with a few modifications. A sample (0.3 gm approx.) was placed in a centrifuge tube (40 ml) along with 25 ml of glass distilled water; the mixture was stirred (Vortex Genie, Scientific

Industries Ltd.) and the tubes were then closed with parafilm. The tubes were placed in a water bath (25°C or 37°C) and allowed to stand for 24 hours. The supernatant was removed using a siphon; traces of solvent that remained were removed by using filter paper wicks. The tubes were weighed, the fibre frozen in liquid air and freeze dried. The difference in the weights of material before and after drying, divided by the weight of material after drying, was taken as the water holding capacity of the material.

3. Water Holding Capacity (Method #2)

Water holding capacity was determined using a filter paper method (Robertson et al., 1980). A sample (0.5 gm approx.) was soaked (25°C or 37°C) overnight (16 hrs.) in 100 ml of glass distilled water; the mixture was then filtered on a preweighed filter paper. The fibre was allowed to drain completely and the filter paper was then allowed to stand on a paper towel for 20 minutes. Filter papers (6) of known weight were wetted with water and weighed to determine the wet weight, dry weight ratio of the filter papers. The papers were weighed in beakers closed with parafilm and allowance was made for the increased weight of the filter paper. The wet weight of fibre minus the dry weight, divided by the dry weight was taken as a measure of the water holding capacity of the fibre.

4. Settling Volume in Water

Settling volume was measured using the method of Takeda and Kiriyama (1979). A sample (0.5 gm. approx.) of fibre was placed in a round bottom flask (100 ml) and suspended in deionized water (40 ml , 25°C).

The flask was evacuated (2-3 cm Hg) and allowed to stand overnight. The sample was transferred to a graduated cylinder (100 ml) and the solid material allowed to settle for 5 hrs. The supernatant was removed using a siphon and the sample transferred to a graduated cylinder (25 ml), along with sufficient water to give a total volume (fibre and water) of 25 ml. The mixture was stirred by agitation and the covered (parafilm) cylinder was allowed to stand overnight. The volume of the fibre layer was measured; the settling volume was expressed as mls of fibre layer divided by grams of fibre.

5. <u>Cation Exchange Capacity</u> (C.E.C.)

A sample (4 gm. approx.) of fibre was mixed with an excess of hydrochloric acid solution 0.1 M (Robertson <u>et al</u>., 1980) or 2.0 M(Mc Connell <u>et al</u>., 1974) and allowed to stand (25°C) for 48 hours. The mixture was filtered (Buchner funnel) and washed with glass distilled water until the filtrate was free of chloride (silver chloride test). A sample (0.25 gm approx. dry weight) of the acid charged material was suspended in sodium chloride solution (150 ml , 5%) and allowed to stand overnight. The suspension was stirred mechanically (magnetic stirrer) while it was titrated with sodium hydroxide (0.0511 N) using a Harvard Apparatus Infusion Pump (Model 600) equipped with a 50 ml syringe and a Beckman Zeromatic II pH meter (Beckman Instruments). Samples of acid detergent fibre and neutral detergent fibre prepared from the apple core fibre were soaked in 2 M HCl and treated as above. A sample of pure pectin (Sunkist Pure Citrus Pectin N.F.) was titrated without prior treatment.

6. Scanning Electron Microscopy of Fibre Samples

Double stick aluminum tape was placed at the centre of Scanning Electron Microscope specimen stubs. The surface of the stubs around the aluminum tape, was coated with silver paint; approximately 2 mg of air dried fibre sample, sieved to pass a 250 μm screen, was placed on the aluminum tape. The sample was coated with gold and observed under a Scanning Electron Microscope (Cambridge Stereoscan 600). Mr. Louis Thauvette of the Plant Science Department, Macdonald College assisted in the observation and photography of the samples.

B. Chemical Analysis of Apple Core Fibre

1. Acid Detergent Fibre (ADF) and Lignin

ADF was determined according to the A.O.A.C. method (A.O.A.C. Handbook, 1975). Lignin and ash are reported as lignin.

2. Neutral Detergent Fibre (NDF)

NDF was determined according to the method described by Robertson (1978).

3. Nitrogen

Nitrogen was determined by a micro-Kjeldahl method (A.O.A.C. Handbook, 1975).

4. Moisture Content

Moisture was determined using a vacuum oven (A.O.A.C. Handbook, 1975).

5. Pectin Extraction

Pectin was extracted from the apple core fibre using the method of McCready and McComb (1952) with some modifications. A sample (1.0 gm approx.) of apple core fibre was placed in a beaker (250 ml) along with 95% ethanol (1-2 ml) and EDTA solution (130 ml, 0.5% tetrasodium EDTA adjusted to pH 11.6). The suspension was stirred mechanically (magnetic stirrer) for 30 minutes at room temperature. The reaction of the suspension was adjusted to pH 5.4 (approx.) with glacial acetic acid. Pectinase (0.1 gm, Nutrition Biochemicals Corp.) was added and the solution was stirred for one hour. The suspension was transferred to a volumetric flask (250 ml) and the volume adjusted to 250 ml using distilled water. The suspension was mixed thoroughly and then filtered (Whatman #1); the first few mls. of filtrate were discarded and the remaining filtrate (Solution A) was collected for analysis. A sample of pectinase and a sample of pure pectin (Sunkist Pure Citrus Pectin N.F.) were subjected to the extraction procedure outlined above.

6. Pectin Estimation; Modified Carbazole Method for Uronic Acids

Uronic acid in solution A (see above) was determined using the method of Bitter and Muir (1962). A sample (1 ml) of solution A was added to a volumetric flask (50 ml) and the volume adjusted to 50 ml using deionized water. Aliquots (1 ml) of the diluted solution A were placed in test tubes (16 x 150 mm glass stoppered) and the liquid was frozen using liquid air. Tetraborate solution (5 ml , 0.025 M sodium tetraborate in concentrated sulfuric acid A.R.) was added, and the tubes were shaken gently at first and then the mixture was stirred vigourously using a vortex mixer. The tubes were heated (10 min) in the boiling water bath and then cooled to room temperature. Carbazole reagent (0.2 ml, 0.125% carbazole in absolute ethanol) was added, the solutions were mixed thoroughly and then heated (15 min) in the boiling water bath. The tubes were cooled to room temperature and the optical density (530 nm; 1 cm cell) of the solutions was measured using a Perkin Elmer Lambda 3 UV/VIS Spectrophotometer. Sulfuric acid (Conc., A.R.) was used as a blank.

Standard solutions (4–40 μg/ml) of galacturonic acid were prepared by dissolving galacturonic acid (Nutritional Biochemicals Corp.) in deionized water that had been saturated with benzoic acid. Standard curves (Figs. 1 and 2) were prepared using 0.5 ml and 1.0 ml samples. The purity of the galacturonic acid was determined by titration. Blank determinations were made using the saturated solution of benzoic acid and deionized water.

7. Pectin Estimation: Hydroxy Biphenyl Method for Uronic Acids.

Uronic acid in solution A (see above) was determined using a modification of the method described by Blumenkrantz and Asboe-Hansen (1973). An aliquot (0.1 ml) of solution A was placed in a test tube (16 x 150 mm, glass stoppered) along with 0.4 ml of glass distilled water. The resultant solution was frozen in liquid air. Tetraborate solution (3 ml, 0.0125 M tetraborate in concentrated sulfuric acid A.R.) was added and the tubes shaken gently at first and then the mixture was stirred vigorously using a vortex mixer. The tubes were heated (5 min.) in a boiling water bath and then cooled to room temperature. o-hydroxy biphenyl solution (100 μl; 0.3 gm /200 ml, 0.5% sodium hydroxide, lab grade) was added

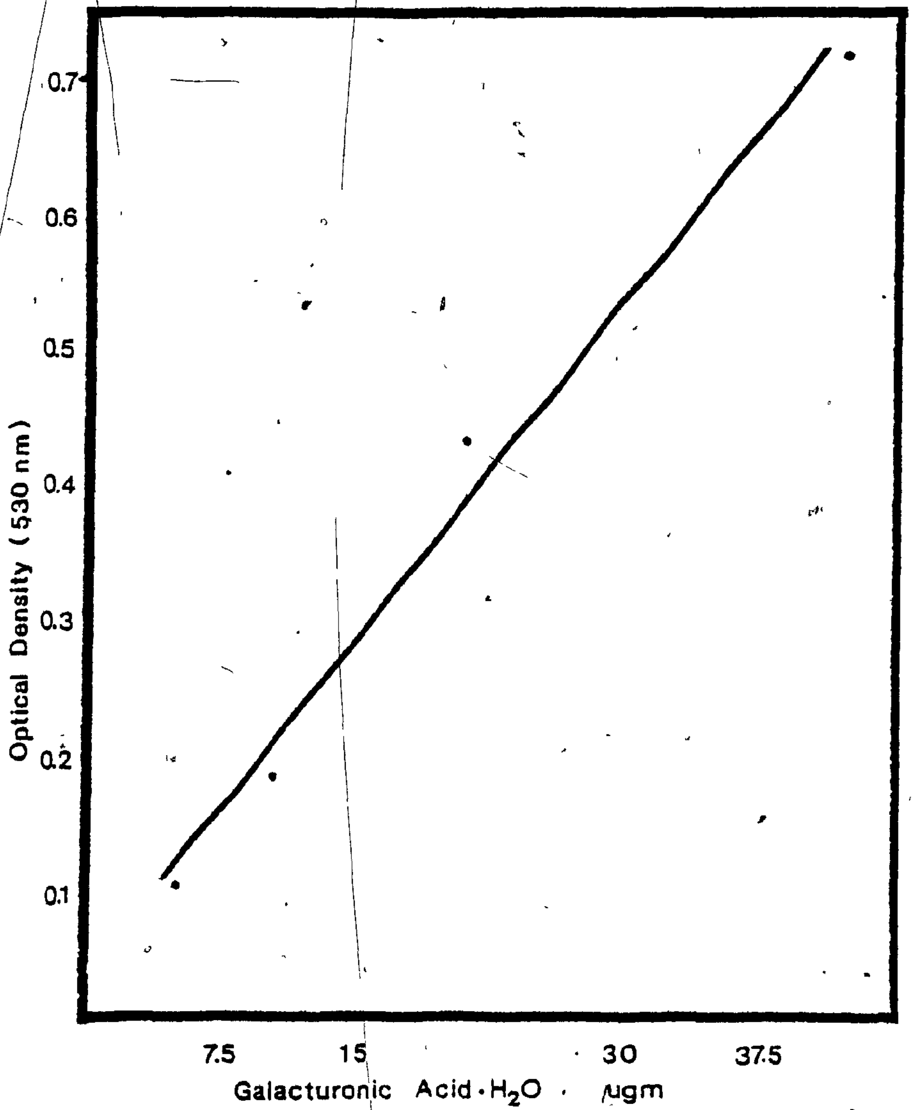

Figure 1. Calibration curve for Carbazole Test (0.5 ml Sample Size).

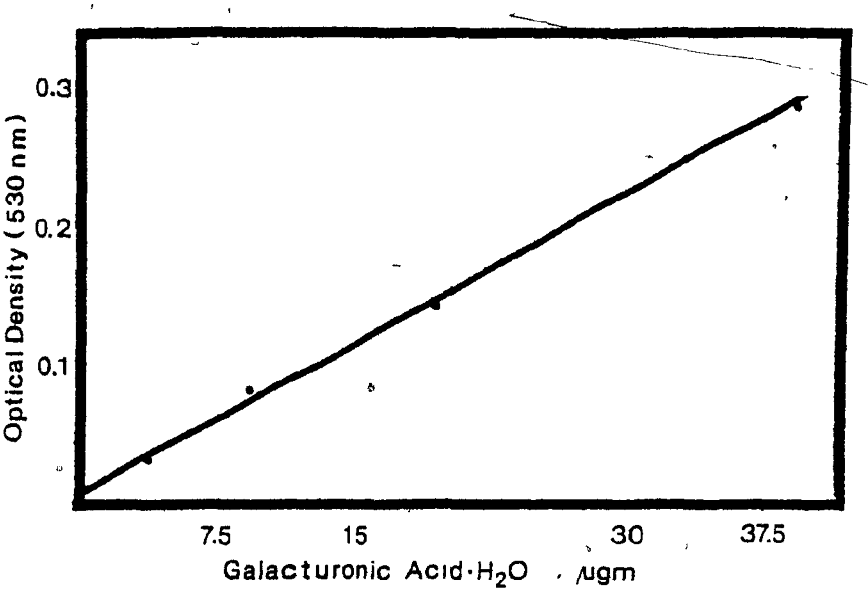

Figure 2. Calibration curve for Carbazole Test. (1 ml Sample size).

and the mixture was stirred thoroughly and then allowed to stand for 15 minutes. The optical density (477 nm; 1 cm cell) of the solutions was measured using a Perkin Elmer Lambda 3 UV/VIS Spectrophotometer. Sulfuric acid (concentrated, A.R.) was used as a blank.

Standard solutions (5-50 µg/0.5 ml) of galacturonic acid were freshly prepared. Blank determinations were made on aliquots in which sodium hydroxide solution (100 µl; 0.5%) was used instead of the reagent. A standard curve is shown in Figure 3.

8. Amino Acid Analysis

Samples (15 mg approx.) of apple core fibre were added to test tubes (13 x 100, Pyrex) along with hydrochloric acid (1 ml, constant boiling, glass distilled). The contents of the tubes were frozen in liquid air and were then thawed using a stream of nitrogen. This procedure was repeated three times, and then the tubes were sealed under nitrogen. The tubes were heated (110°C), for 22 hours in a Stabil-Therm Gravity Oven Model RS 12 1AX (Blue M Electric Company, Blue Island, Ill.) The tubes were cooled to room temperature and opened; the contents were filtered (fritted glass, porosity F) under moderate suction. The tubes were rinsed with deionized water (1 ml) and the filtrates collected. The filtrates were evaporated to dryness using sodium hydroxide as described in the Beckman Instruction Manual (Beckman Model 120c Amino Acid Analyzer Instruction Manual, Section 7, Spinco Division Beckman Instruments, Palo Alto, Cal.) Citrate buffer (1.7 ml , pH 2.2) and norleucine standard (0.3 ml , 0.0787 gm/litre in citrate buffer pH 2.2) were added to the residue. A sample (0.3 ml) was used for amino acid analysis (Beckman

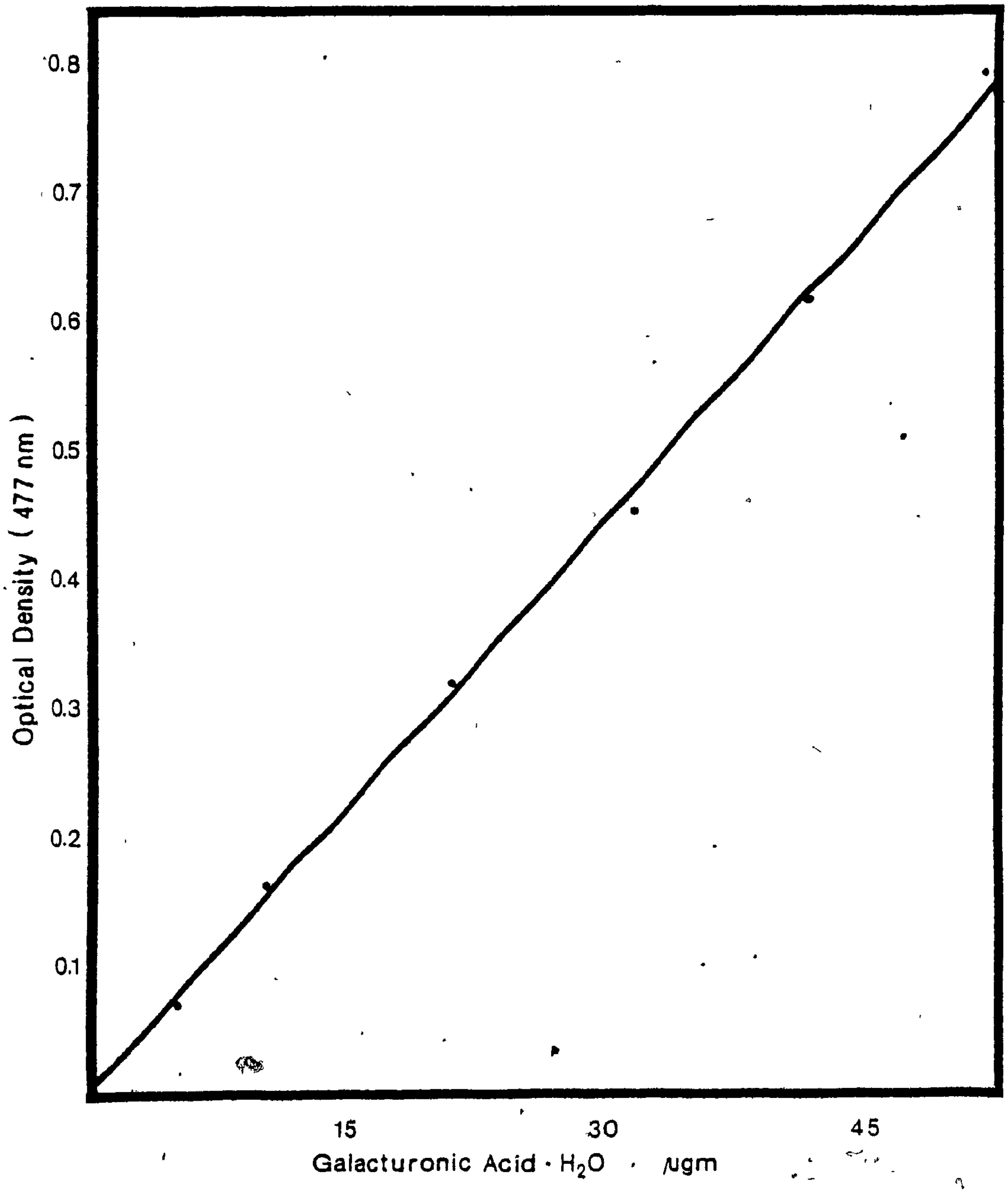

Figure 3. Calibration curve for Hydroxy Biphenyl Test.

Model 120c Amino Acid Analyzer modified to a Model 119, equipped with a Beckman Model 125 Integrator; Beckman Instruments, Palo Alto, Cal.) The citrate buffers, the operating parameters and integrator responses for amino acid standards are reported in Tables 3, 4, and 5. The ninhydrin colour reagent was prepared as follows: Sodium acetate solution (974 ml, 4 N; Reagent Grade, BDH) and Pier Solv (2366 ml; Glass-bottled, Pierce) were mixed under nitrogen. Ninhydrin (75.76 gm; Reagent Grade, Pierce) and Stannous Chloride Dihydrate (1.515 gm, A.C.S. Fisher) were dissolved in the resultant solution. The ninhydrin colour reagent was stored under nitrogen during preparation and use to prevent deterioration.

9. Acid Hydrolysis

(a) Hydrolysis with 5% Sulfuric Acid

The apple core fibre preparation was hydrolyzed using the method of Anderson and Clydesdale (1980) with several modifications. Samples (6; 150 mg each) of apple core fibre were placed in tared centrifuge tubes (40 ml, Pyrex) along with β-d-Allose (10 mg approx.; Sigma) as an internal standard and sulfuric acid (25 ml, 5% w/v) was added. The tubes were sealed with rubber septa secured with copper wires. The contents of the tubes were mixed (Vortex mixer) and the tubes were heated (2.5 hours) in a boiling water bath. The tubes were then cooled to room temperature and the septa removed. Deionized water (10 ml approx.) was used to wash down the walls of the tubes then the tubes were centrifuged (2500 rpm, 20 min. at 40°C; IEC centrifuge model PR-1, equipped with head #812). The supernatants were removed using a siphon and were transferred to volumetric flasks (100 ml). The residues were washed with

TABLE 3

Citrate Buffer for Amino Acid Analysis[1,2,3,4]

Buffer	Na^+	pH	Running time (Mins.)
A	0.2N	3.1	65
B	0.2N	4.0	35
C	1.35N	5.3	82

1 Buffers are made with Sodium Citrate $.2H_2O$ ('Baker Analyzed' Reagent, Baker Chemical Company) and freshly deionized water prepared from distilled water.

2 The pH of the buffers was adjusted using constant boiling, glass distilled, HCl.

3 All buffers contain caprylic acid (0.01% v/v) and phenol (0.1% w/v).
Buffers A & B contain isopropanol (2% v/v)
Buffer C contains isopropanol (4% v/v)

4 Prepared buffers were filtered through a millipore filter and degassed.

TABLE 4

Amino Acid Analyzer Running Parameters

Column Dimension	6 mm Width 32 cm Length
Resin Type	Aminex A-9 (Biorad) Cation Exchange
Resin Bead	11 ± 0.5 μ
Flow Rate	37 mls/hr Buffer 12 mls/hr Ninhydrin
Running Time	220 minutes
Integrator Range	200 counts/O.D./sec.
Integrator Plateau	20 seconds
Integrator Sensitivity	0.005 O.D./min.
Integrator Baseline Delay	50 seconds
Integrator Baseline Correction	0.02 O.D./min.

TABLE 5

Integrator Response for Amino Acids[1]

Amino Acid	Response
Aspartic acid	3195
Threonine	2994
Serine	4131
Glutamic acid	3988
Glycine	4691
Alanine	3726
Valine	1727
Cysteine	3564
Methionine	4725
Isoleucine	3663
Leucine	4472
Norleucine[2]	4345
Tyrosine	4553
Phenylalanine	4799
Lysine	8301
Histidine	5254
Arginine	3974
Proline	0.825[3]

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[1] All responses are for 0.5 moles of amino acid except cysteine which is for 0.25 moles.

[2] Internal standard

[3] The response for proline was calculated using the "Height Width" method outlined in the Beckman Model 120c Amino Acid Analyzer Instruction Manual, Section 8.

distilled water (3 x 15 ml) and the washings were added to the supernatants. The volumes were adjusted to 100 ml with distilled water; the solutions (hydrolysates H_1 to H_6) were stored at 5°C. The residues (R_1-R_6) were washed with acetone (3 x 15 ml; Lab Grade Caledon) and diethyl ether (2 x 15 ml; Anhydrous A.C.S., Anachemia). The tubes were heated (40°C) in a water bath and the ether was removed using a stream of air that had been dried over calcium chloride. The tubes were then heated (110°C, 10 min) and cooled (20 min) in a desiccator (silica gel) and weighed. The recovery was measured using purified hemicellulose (Gum Arabic; Brinkman & Co.)

(b) Hydrolysis with 72% sulfuric acid

β-d-Allose (10 mg approx.) was added to the residues (R_1-R_6) along with sulfuric acid (17.5 ml, 72% w/v, 4°C; A.R. Anachemia). The contents of the tubes were mixed occasionally (Vortex mixer) while the tubes were allowed to stand for 48 hours (4°C). The mixtures were filtered (tared, 50 ml, sintered glass crucible, porosity C) and the residues were washed (2 x 25 ml) with distilled water. The acid and water filtrates were transferred to volumetric flasks (250 ml) and the volumes were adjusted to 250 ml. The resultant solutions (hydrolysates h_1 to h_6) were mixed thoroughly and stored at 5°C. The residues in the crucibles were then washed with acetone (3 x 15 ml) and with diethyl ether (2 x 15 ml). The crucibles were then heated (110°C, 15 min) and cooled (30 min) in a desiccator (silica gel) and then weighed. A recovery experiment was performed using a sample of purified cellulose (Cellulose MN 300, Macherey Nagel).

10. Analysis of Acid Hydrolysates

(a) Qualitative Estimation of Monosaccharides and Uronic Acids

Samples (10 ml) of the hydrolysates (H_1 - H_6; h_1 - h_6) were neutralized with solid barium carbonate and were then filtered (Whatman #1); the filtrates were dried on a rotary evaporator (40°C, 2-3 cm Hg). The residues were dissolved in ethanol (10 ml, 70%), the solutions were filtered and the filtrates dried using a rotary evaporator. The residues were dissolved in ethanol (1 ml, 70%) and the resultant solution was analyzed by thin layer chromatography (T.L.C.) (Polygram Cel 300, 0.1 mm Cellulose MN 300, Macherey Nagel) using a modification of the method described by Hoton-Dorge (1976). Samples (5-10 μl) of each solution and each standard sugar (2-4 μl) [1 mg/ml approx. of β-d-Allose (Sigma); L-(-)-Rhamnose·H_2O (Eastman); L-(-)-Fucose, d-(-)-Arabinose, d-(+)-Xylose, d-(+)-Mannose, d-(+)-Galactose, anhydrous (Nutritional Biochemicals Corp.); d-(+)-Glucose, anhydrous (Anachemia) in 70% Ethanol] as well as mixtures, were spotted on T.L.C. plates. The chromatograms were developed twice (40°C ± 1.5°C, 2 hours) in a lined tank (Dasaga) which was equilibrated for 15 minutes with the solvent [50 ml ; acetonitrile, pyridine, (A.C.S., Anachemia), 1-butanol, glacial acetic acid (A.C.S., Caledon), water; 4:5:9:3:4]. The chromatographic plates were dried after each development using an air dryer (Oster Airjet Model 202). Residual solvent was removed from the chromatogram by heating (100°C, 1 min) them in an oven. Monosaccharides and uronic acids were visualized using p-anisidine-phthalic acid spray reagent (1.23 gm p-anisidine, 1.66 gm phthalic acid in 100 mls absolute methanol; Handbook of Chromatography, 1972).

(b) Determination of Uronic Acids

The uronic acid content of the hydrolysates was determined using the methods described above (see Sections II.B. 6&7, pages 19-20 and Figs. 1&2, pages 21-22). Samples (0.5 ml) of undiluted hydrolysates were used in the modified carbazole method (Section II.B.6, page 19) in order to conserve reagents (see Bitter and Muir, 1962). Samples (0.5 ml) of hydrolysates h_1-h_6 and samples (0.25 ml) of hydrolysates H_1-H_6, diluted to 0.5 ml with distilled water were used in the hydroxy-biphenyl method (Section II.B.7, page 20).

(c) Determination of Monosaccharides by Gas Liquid Chromatography (G.L.C.)

Samples (2 ml.) of the pectin extracts (solutions A, Section II.B.5, page 19) including the pure pectin sample, were added to test tubes (13 x 100 mm, Pyrex) along with β-d-Allose solution (1 ml, 0.0434 gm/50 ml of 15% (w/v) Sulfuric Acid) that was freshly prepared. The tubes were sealed in a flame and then heated (2.5 hours) in a boiling water bath. The tubes were cooled to room temperature and opened. Samples (3 ml) of hydrolysates h_1-h_6 were added to test tubes (13 x 100 mm, Pyrex); the tubes were sealed and then heated (2.5 hours) in a boiling water bath. The tubes were cooled to room temperature and opened. Samples (2 ml) of hydrolysates H_1-H_6 were added to test tubes and were used without further hydrolysis. Samples (2 ml) of a standard sugar solution [50 mg approx. of each sugar in Section II.B.10, p.30 dried *in vacuo* (2-3 cm Hg) at 25°C over calcium chloride, dissolved in 50 mls distilled water] were added to test tubes (13 x 100 mm, Pyrex) along with sulfuric acid (1 ml, 15%(w/v)). The tubes were sealed then heated (2.5 hours) in a boiling water bath. The tubes were cooled to room temperature and opened.

All the samples were neutralized with solid barium carbonate and then filtered (sintered glass funnel, porosity F, lined with Whatman #1) using moderate suction. Residues were washed with distilled water (5 ml) and the filtrates were pooled and added to test tubes. Sodium carbonate decahydrate was added to adjust the concentrations at 0.01 M. The solutions were allowed to stand 20 minutes. Sodium borohydride (25 mg, 98%, Fisher) was added and the resultant solutions were allowed to stand (25°C) for 3 hours. Glacial acetic acid (A.C.S.) was added dropwise to neutralize the excess borohydride. The solutions were then decationized using ion exchange columns (3 ml Amberlite IR-120 (H^+), 1.9 meq H^+/ml). The effluent and three bed volumes were collected in round bottom flasks (50 ml) and dried on a rotary evaporator (40°C, 2-3 cm Hg). The residues were washed with acidified methanol (10 ml, glacial acetic acid: absolute methanol, 1:9) and then evaporated to dryness. The residues were washed with absolute methanol (3 x 10 ml) and transferred with absolute methanol (5-8 ml), using a Pasteur pipette, to test tubes (13 x 100 mm, Teflon-lined screw cap). The solvent was evaporated (40°C) using a stream of air that had been dried over calcium chloride. Acetic anhydride (0.5 ml, A.C.S. Anachemia) and anhydrous sodium acetate (15 mg, A.C.S. Anachemia) were added to the tubes. The tubes were sealed and the contents mixed vigorously (Vortex mixer). The tubes were then heated (105°C) for three hours. The reaction mixtures were cooled and aliquots (2.5 1) were analyzed by injecting them directly on to the G.L.C. column. The gas chromatograph (Packard, 7400 Series) was equipped with a flame ionization detector and a six foot glass column packed with Supelco SP-2340 on 100/120 Supelcoport (Supelco). The conditions of operation are reported in Table 6. Molar response factors for each sugar were calculated according to the method of Sloneker (1972) using the standard sugar solution and are reported in Table 7. Peak area was calculated by multiplying peak height by the width at half height. A sample gas chromatogram is shown in Fig. 4.

11. Binding of Food Dyes

Stock solutions (250 ml) of each dye [Brilliant Blue FCF (C.I. #42090; FD&C Blue #1); Erythrosine (C.I. #45430; FD&C Red #3); Ponceau SX (C.I. #14700; FD&C Red #4)] were prepared using each of the following buffers. Buffer 1 - Potassium biphthalate-sodium hydroxide at pH 4.8. Buffer 2 - Potassium dihydrogen phosphate-sodium hydroxide at pH 6.5. Buffer 3 - Tris hydroxymethyl aminomethane-hydrochloric acid at pH 8.2. The concentrations of stock solutions were as follows: 50 and 100 ppm for Brilliant Blue FCF; 50 and 150 ppm for Ponceau SX; 50 and 300 ppm for Erythrosine. The dye-buffer combinations are listed in Table 8. Dye binding was measured using a modification of the method described by Takeda and Kiriyama (1979). Samples (0.2 gm approx.) of apple core fibre, the residues from acid and neutral detergent fibres or cellulose MN 300 (Macherey Nagel) were added to centrifuge tubes (40 ml , Pyrex) along with 15 ml of a dye-buffer solution. Dye-buffer solutions were placed in tubes containing no test material and all the tubes were covered with parafilm. The contents of the tubes were mixed (1 min, Vortex mixer) and then the tubes were heated (37°C, 18 hours) in a water bath. The contents of the tubes were mixed (1 min, Vortex mixer) at hourly intervals for the

TABLE 6

Operating Conditions for Packard 7400 Gas Chromatograph

Carrier Gas Flow Rate	Helium, 20 ml./min[1]
Temperature Programme	195°C hold 5 min. 2.5°C/min to 235°C Hold 5 min.
Injection Port Temperature	230°C
Detector Port Temperature	250°C
Attenuation	3×10^{-10} amps
Supply Voltage	150

---

[1] Flow Rate was measured at the column outlet using a soapfilm type flowmeter.

TABLE 7

Molar Response Factor[1] for Sugar Standards

Sugar	Molar Response Factor ± S.D.[2]
Rhamnose	1.01 ± 0.03
Fucose	1.01 ± 0.02
Arabinose	0.93 ± 0.02
Xylose	0.39 ± 0.02
Mannose	1.00 ± 0.01
Galactose	0.94 ± 0.03
Glucose	0.96 ± 0.03

1. Response factors were calculated from the equation

$$K\ (\text{response}) = \frac{\text{area of sample peak}}{\text{moles of sample}} \times \frac{\text{moles of Standard (Allose)}}{\text{area of Standard}}$$

2. Response factors were determined using 6 peak area measurements for each sugar.

Figure 4

Sample Gas Chromatogram

1. Rhamnitol Acetate
2. Fucitol Acetate
3. Arabitol Acetate
4. Xylitol Acetate
5. Allitol Acetate
6. Mannitol Acetate
7. Galactitol Acetate
8. Glucitol Acetate

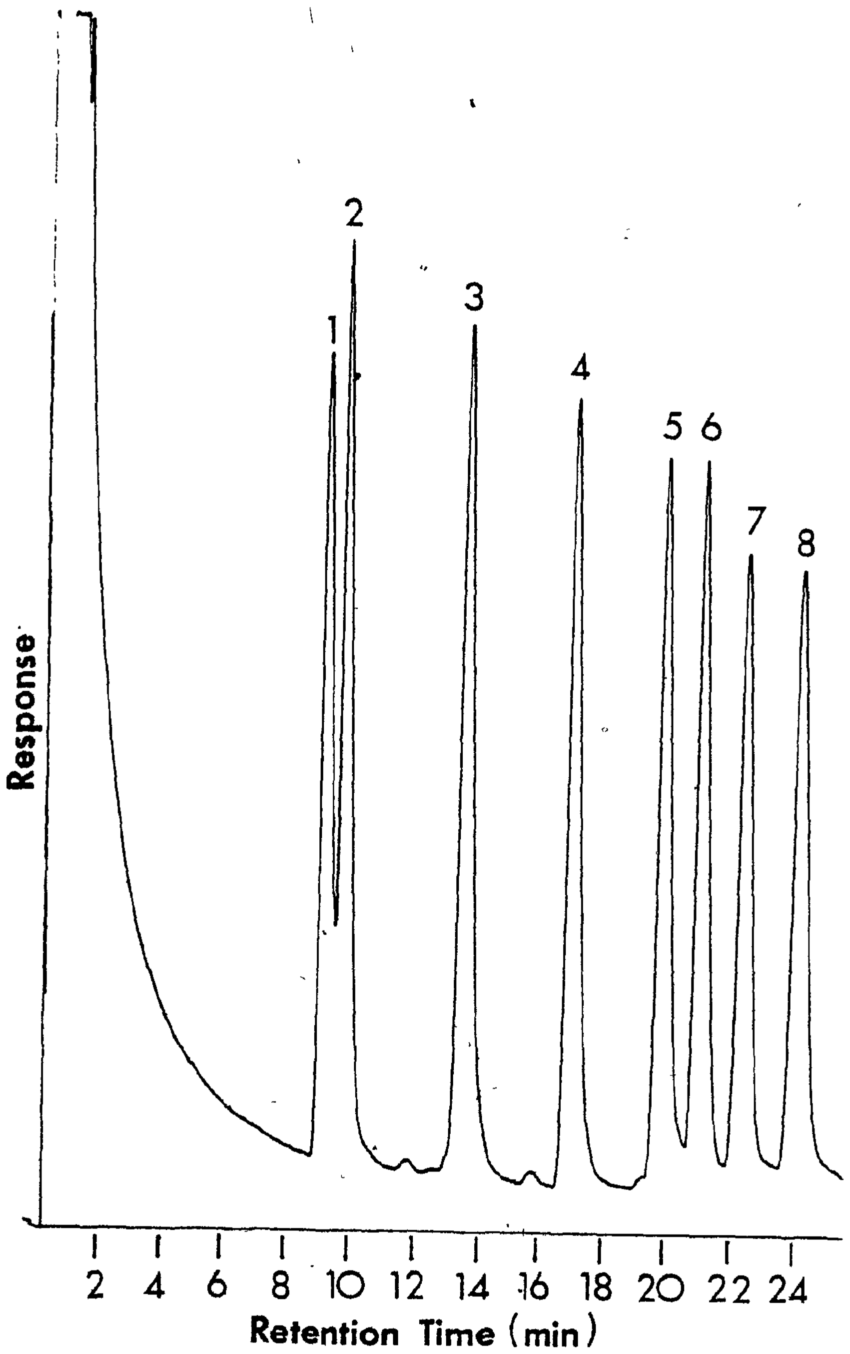


Figure 4. Sample Gas Chromatogram.

TABLE 8

Stock Solutions used in Dye Binding

Dye-Buffer Combinations

Dye	Buffer	Dye Concentration (ppm)
Brilliant Blue FCF	1	100
	2	100
	3	100
	1	50
	2	50
	3	50
Ponceau SX	1	150
	2	150
	3	150
	1	50
	2	50
	3	50
Erythrosine	2	300
	3	300
	1	50
	2	50
	3	50

---

1. Buffers were prepared according to the data in the Handbook of Chemistry and Physics, 57th edition, R.D. Weast editor; C.R.C. Publishing pg. D-134.

Buffer 1 Potassium Biphthalate - Sodium Hydroxide, pH 4.8
Buffer 2 Potassium Dihydrogen Phosphate - Sodium Hydroxide, pH 6.5
Buffer 3 THAM - Hydrochloric Acid, pH 8.2

first four hours. The tubes were removed from the bath, were mixed (1 min, Vortex mixer) and then allowed to stand (25°C) for one hour. The parafilm was removed and aliquots of the supernatant were diluted (1 ml of supernatant in 24 ml of buffer or 3 ml of supernatant in 22 ml of buffer) before their optical density was measured [630 nm (Brilliant Blue FCF); 536 nm (Erythrosine); 501 nm (Ponceau SX); 1 cm cell] using a Perkin Elmer Spectrophotometer (Model Lambda 3 uv/vis). Standards [1-5 ppm (Brilliant Blue FCF); 3-15 ppm (Erythrosine and Ponceau SX)] were prepared by dilution of the stock solutions with the appropriate buffer. Standards were heated (37°C, 18 hours) in a water bath and were mixed thoroughly before their optical density was measured. Standard curves were prepared for each stock solution.

Representative standard curves for each dye are shown in Figs. 5, 6, and 7.

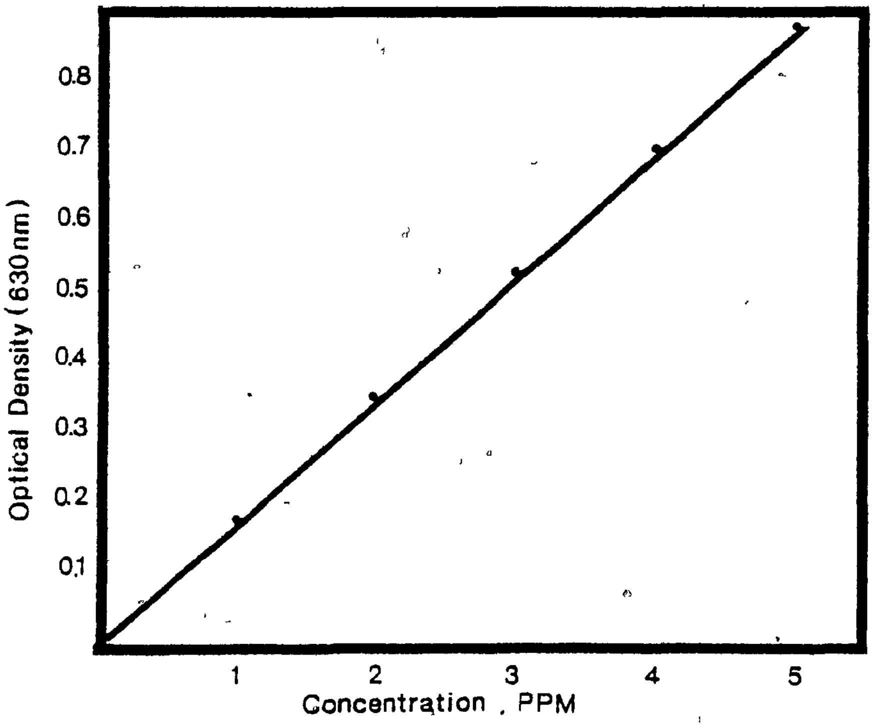


Figure 5. Representative Calibration Curve for Brilliant Blue FCF.

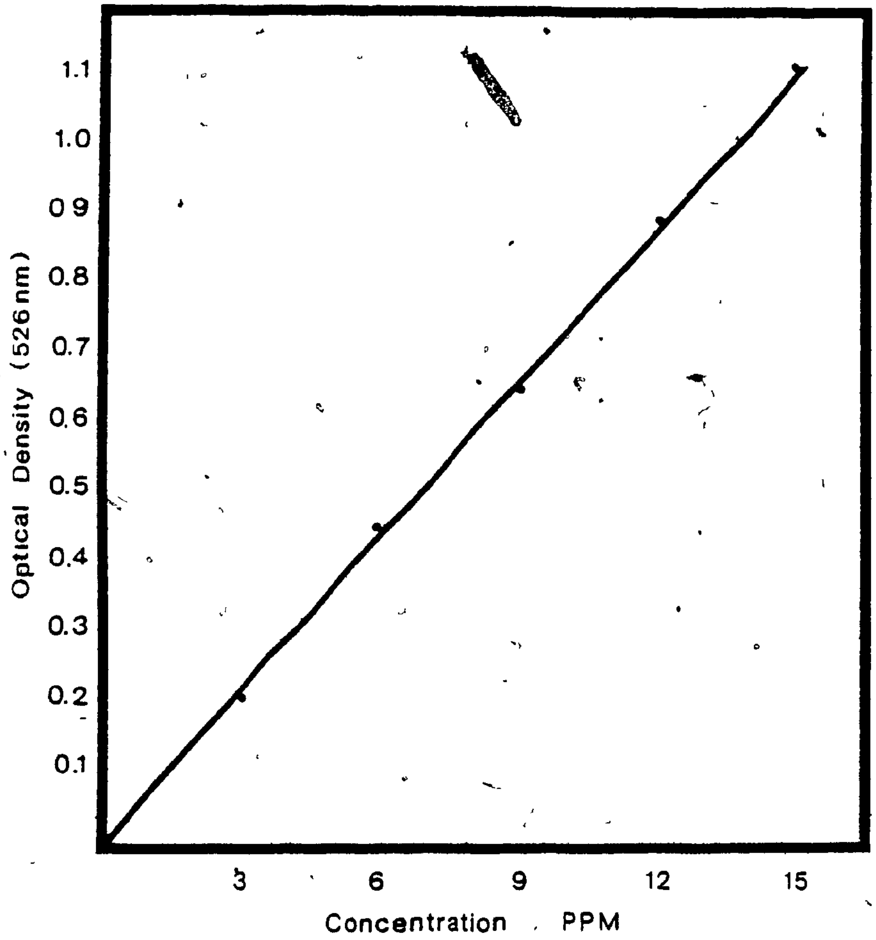


Figure 6. Representative Calibration Curve for Erythrosine.

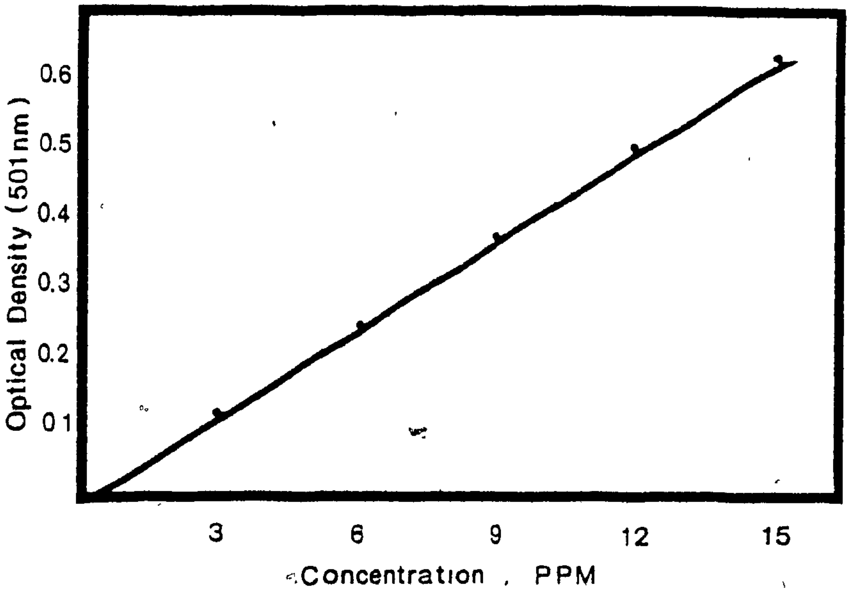


Figure 7. Representative Calibration Curve for Ponceau SX.

III. RESULTS AND DISCUSSION

A. Preparation of Apple Core Fibre

1. Preliminary Experiments

To determine the optimum dimensions of the core to be taken, the apples (Cortland), were sectioned both vertically and horizontally using a razor. Vertical (polar) sections from the center of the apple were used to obtain an average measurement of the carpel blade length (Tukey and Young, 1942). Horizontal (cross) sections through the center of the apple were used to obtain an average measurement of the core diameter (Tukey and Young). The resultant measurements defined a cube approximately 1.25 to 1.50 inches on a side. The core sample would contain tissues from the pith, the fleshy pericarp and the cartilagenous pericarp (Tukey and Young, 1942).

The use of antioxidant to prevent oxidative browning of the apple tissue was investigated. Sutton and Lauck (1967) reported that a synergistic mixture of phosphoric acid and sodium bisulfite or sodium sulfite inhibited enzymatic darkening and fermentation of fresh fruit. It was found that apple slices, exposed to air for 7 days at room temperature would not brown if covered with a solution of 2 gm sodium bisulfite and 4 gm o-phosphoric acid in 2 litres of distilled water. Slurries of apple core tissue with hot (85°-90°C) antioxidant solution did not brown when maintained at 85°-90°C for 4 hours. The hot antioxidant solution also proved to be an effective means of extracting pectic substances as indicated by the formation of a gel upon cooling of a filtrate of the slurry.

In order to remove tannins and free sugars, as well as less soluble pectin, 80% methanol and EDTA solution (0.08M Tetra Sodium EDTA, adjusted to pH 6.8) were used. It was found that if EDTA extraction was followed by methanol extraction, a coarse matted material was produced which browned rapidly during air drying. Methanol extraction followed by EDTA extraction resulted in a product which was light beige in colour.

Acetone extraction followed by air drying was used initially in this work. It was found that the acetone dried product was very electrostatic. Acetone extraction followed by an ether wash and air drying eliminated this problem.

2. Procedure

Cortland apples (Malus pumila Mill. cv. Cortland), were obtained from the Macdonald College orchards. The outer portions of the apples were removed by hand to leave 1¼ to 1½ inch cores. The seeds were removed and the cores were chopped finely and then suspended in a solution 500 gm core material/500 ml solution) of antioxidant (2 gm Sodium Bisulfite and 4 gm O.Phosphoric acid/2 litres distilled water, adjusted to pH 2; Pintauro, 1974).

The mixture was allowed to stand (5°C) overnight: it was then heated (85°-90°C) for thirty minutes. The cores were macerated (G.E. Portamix) and the hot (85°-90°C) slurry filtered using a lined Buchner funnel (Whatman #1) and moderate suction. The slurry was resuspended in an equal volume of methanolic antioxidant solution (80:20 (V/V) absolute methanol, antioxidant solution) and allowed to stand (5°C) overnight. The mixture was heated (85°-90°C), macerated and filtered as described above.

The extraction procedure was repeated twice using an EDTA solution (0.08M tetrasodium EDTA, pH 6.8), then twice with glass distilled water. The slurry was suspended in 1.5 volumes of acetone and allowed to stand (5°C) for 48 hours. The material was recovered by filtration and then resuspended in acetone and the acetone extraction procedure repeated until there was no more extraction of coloured material. The material was allowed to stand (5°C) overnight in an equal volume of diethyl ether; the suspension was filtered and the residue washed with ether to remove traces of acetone. The ether was removed from the washed material by allowing air, that had been dried with calcium chloride, to pass through the Buchner funnel. The dried apple core fibre was ground in a Mikro sample Mill (Pulverising Machinery Company) fitted with a 2 millimeter mesh screen. The ground samples were placed in Mason jars, sealed and stored at room temperature.

The fibre prepared from apple core material was a light beige powder which was interspersed with light yellow particles. The yield of apple core fibre based on the weight of air dried material was 2 percent of the weight of fresh core material. Cortland apples were chosen for this work because of their low tannin content (Smock and Neubert, 1950). The tannins, which represent from 0.1 to 0.3 percent of the fresh weight of apples (Overholser and Cruess, 1923), can interfere in the determination of lignin by forming protein-tannin complexes which are insoluble in 72 percent sulfuric acid (Hartley, 1978). The oxidation of tannins by the peroxidase and polyphenoloxidase present in apple tissue (Smock and Neubert, 1950) renders tannins insoluble in alcohol and prevents their removal. The use of antioxidant solution prevented the enzymatic browning of the apple tissue, and the tannins were removed by a subsequent treatment with alcohol. The combination of high acidity (pH 2) and moderately high temperature (80^{o}-90^{o}C) also promoted the solubilization of protopectin (McCready and McComb, 1952; Paton, 1974).

The methanolic antioxidant solution was used to extract the tannins, the free sugars (monosaccharides, disaccharides and higher oligosaccharides), some lipid and amino acid pigments (Southgate, 1981). Pectic substances precipitated in the 80% alcohol (Barrett and Northcote, 1965) and formed a layer, over the filter paper, which interfered with the filtration procedure.

The EDTA solution was used to dissolve the low-methoxyl pectins and pectinates (McCready and McComb, 1952). Albersheim et al. (1960) believed that this extraction was enhanced by the degradation of the pectin polymer during the extraction procedure.

The water wash was used to remove the soluble pectins, any residual sugars and to remove residual EDTA.

Anderson et al. (1961) indicated that hot alcohol, hot water, and hot oxalate solutions caused varying degrees of decarboxylation of pectin. However, the duration of each treatment used by the authors was at least 2 hours and temperatures varied from 85^{o}-100^{o}C. The present work was carried out with a heating time not in excess of 30 minutes and with temperatures never exceeding 90^{o}C.

The acetone extraction was used to remove any lipids and colour materials. The acetone extract was yellow; the yellow pigment which is considered to occur in all apple varieties is 3-galactosidyl quercetin

(Smock and Neubert, 1950). It is essential to remove this flavone in order to reduce the interference in the measurement of acid detergent fibre and lignin. The pigment is converted to an insoluble reddish brown substance, similar to phlobaphene, on treatment with mineral acid.

Diethyl ether was used to remove the acetone. The residual ether was eliminated from the fibre preparation by passing air through the product at room temperature. A higher temperature was not used so as to avoid the possible formation of Maillard reaction products which interfere in the determination of the lignin component of dietary fibre (Hartley, 1978).

B. Particle Size Analysis

Four samples (30 gm each) of apple core fibre were subjected to the sieving procedure as described in Section II. 1, page 15. The results are shown in Table 9 and in graphic form in Figs. 8 and 9. It will be noted that a high proportion of the particles range in diameter from 250 to 1000 μm. The data when plotted as a log-probability distribution, may be approximated by a straight line (Lapple, 1968) with a standard geometric deviation of 2.19 and a mean particle size (diameter) of 307 μm.

The sieving procedure is influenced by the particle shape; rod-like particles will be measured by their small dimension, flat particles by their large dimension (Waldo et al., 1971; Lapple, 1968). A small portion of the apple core fibre preparation is rod-like; the cartilagenous pericarp, that tissue which surrounds the seeds, is sheared during the final grinding step.

TABLE 9

Particle Size Distribution of Apple Core Fibre

Sieve No.	Aperture (μm)	Log Aperture	Cumulative[1] Percent Undersize	Weight[2] Fraction
10	2,000	3.3	100	0
18	1,000	3.0	98.2	0.0186
35	500	2.7	65.8	0.3232
60	250	2.4	33.9	0.3199
100	149	2.2	18.9	0.1497
200	74	1.9	8.7	0.1014
230[3]	63	1.8		0.0873

1. The percentage of the total weight of material which passed through indicated sieve.

2. These figures represent the fraction of the total weight of material which is related by the indicated sieve.

3. The tray is considered a sieve of the next smaller size.

4. The values are the average of 4 trials.

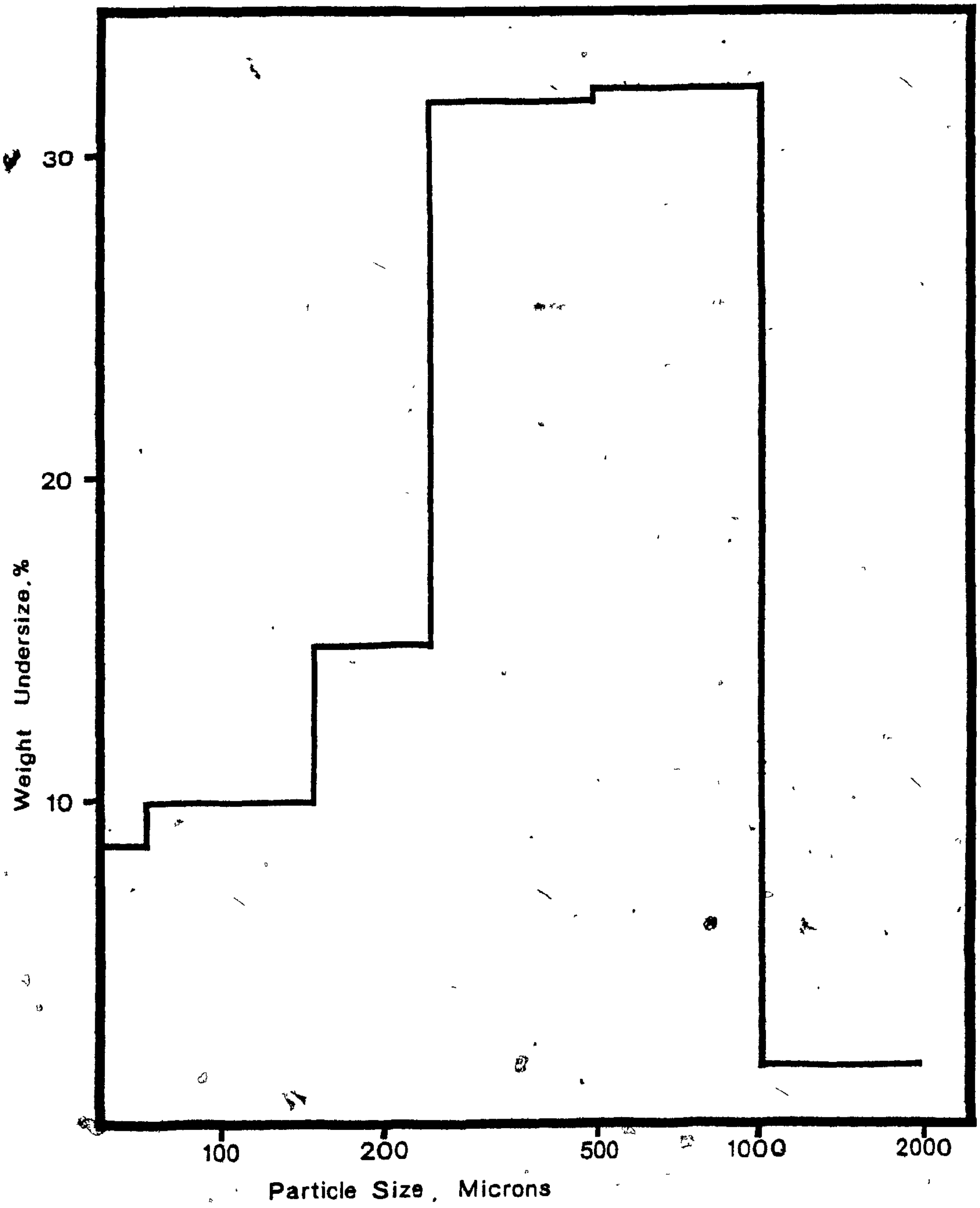


Figure 8. Particle Size Distribution of Apple Core Fibre.

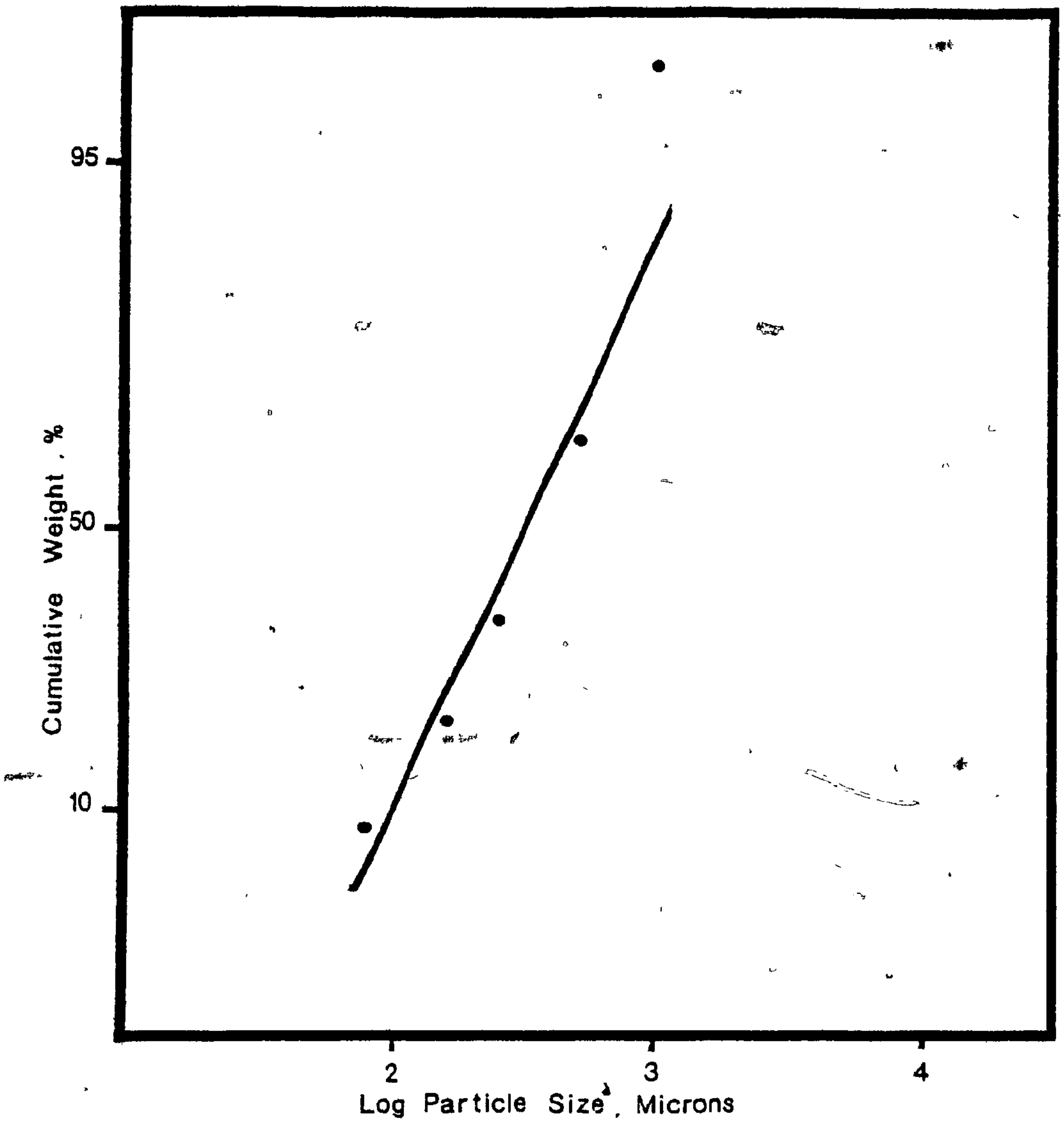


Figure 9. Log-Normal Distribution for Sieving Data of Apple Core Fibre.

Particle size is important at both the chemical and physical levels of digestion. Schwerdtfeger (1979) determined that well-chewed food passed a 3 mm sieve; analyses of dietary fibre are generally performed on material ground to pass a 1 mm sieve (Theander and Aman, 1979b; McConnell et al., 1974; Goering and Van Soest, 1970). Particle size may influence digestion (Lewis, 1978; Thomas and Elchazley, 1976). George et al. (1980) concluded that the particle size may be reduced to a point of optimum fibre digestibility and hydration capacity. The former is influenced by the surface area; the latter is a complex function of surface area, which increases with grinding, and interior cell space, which decreases with grinding (Van Soest, 1978). Reduction of the particle size below this optimum, results in compaction which prevents enzymatic activity, increases feed density and allows the finer particles to flow with liquid, thereby reducing the bulk effect and decreasing transit time (George et al., 1980; Van Soest and Robertson, 1976).

Heller et al. (1977) demonstrated that the reduction of particle size resulted in lower hemicellulose values. Lewis (1978) suggested that excessive grinding may cause hydrolysis of some polysaccharides. Selvendran (1979) concluded that, for a preparation of cell wall material with a particle size of 25 µm-50 µm, polysaccharide degradation had not occurred.

C. Water Holding Capacity

The water holding capacity of apple core fibre was measured by the methods (Methods 1 and 2) described in Section II.A.2&3, pages 15 and 16. The results are shown in Table 10. Acid detergent fibre and neutral

TABLE 10

Water Holding Capacity of Fibre Samples

	Water Holding Capacity (gm H_2O/gm fibre (dry wt.)			
Temp. °C	Apple Core Fibre[1]	NDR[2]	ADR[2]	Cellulose MN 300[3]
Method #1				
25°	16.4[4]	12.8[5]	8.6	6.2
37°	20.9[4]	14.7[5]	8.6	6.1
Method #2				
25°	15.7	10.3[6]	5.6[7]	5.3
37°	16.3	13.6[6]	7.2[7]	5.0

1. Values are the averages of six replicates.
2. Residues from neutral detergent fibre (NDR) and acid detergent fibre (ADR) of apple core fibre.
3. Values are the average of three replicates.
4. These values are significantly different, $p = 0.05$
5. These values are significantly different, $p = 0.05$
6. These values are significantly different, $p = 0.05$
7. These values are significantly different, $p = 0.05$

detergent fibre were prepared from apple core fibre as described in Section II.B.1&2, page 18. The residues were dried at 100°C and ground to pass a 2 mm sieve. The water holding capacity of each residue and the water holding capacity of a purified cellulose (Cellulose MN 300, Macherey Nagel) were also measured by the two methods. The results of the analyses are given in Table 10. Differences between the four types of fibre were evaluated using the Duncan's Multiple Range Test (Steel and Torrie, 1960); differences between treatments were evaluated using Student's t-Test (Steel and Torrie, 1960). It will be noted that the values obtained using Method #1 (freeze-drying) are higher than those obtained by Method #2 (filtration). This finding is in agreement with that of Robertson et al. (1980). The centrifugation step used by these authors was found to be of little use in the present study as the apple core fibre and the NDF preparation did not pellet when centrifuged. McConnell et al. (1974) suggested that the use of the same conditions for the measurement of water holding capacity for a range of substances, may not give a true measure of water holding capacity.

There are several methods for the measurement of water holding capacity, other than the two used in the present study (Parrott and Thrall, 1978; Lapsley, 1980; Robertson et al., 1980). Several terms are used to denote this property of fibre (Rasper, 1979a,b; Van Soest, 1978; McConnell et al., 1974). Water holding capacity is usually defined by the methodology (Robertson et al., 1980) but "hydration capacity" can be measured by various techniques (Rasper, 1979b; Van Soest and Robertson, 1976) which measure different properties.

Water holding capacity is a complex function of fibre source and structure (Robertson and Eastwood, 1981); it is governed by the crystallinity and particle size (Lewis, 1978), the surface characteristics and the bulk volume (Van Soest and Robertson, 1976). Cummings et al. (1978) correlated increase in faecal weight with the pentose fraction of non-cellulosic polysaccharides (hemicelluloses). The effect of the particle size on the measured amounts of hemicellulose (Heller et al., 1977) has been discussed previously. McConnell et al. (1974) noted that water holding capacity was increased by increasing temperature. Parrott and Thrall (1978) noted that the shape of fibre particles was affected by temperature.

The results in Table 10 demonstrate a decrease in water holding capacity in the order; apple core fibre, NDF, ADF. These results are in agreement with the work of Lapsley (1980). The decrease may be due in part to differences in composition; NDF and ADF are more or less equivalent to the apple core fibre less pectin and apple core fibre less pectin and hemicellulose, respectively. The preparation of the NDF and ADF residues included drying at 100°C and grinding to pass a 2 mm sieve. Robertson and Eastwood (1981) indicated that severe drying lowers the water holding capacity. Van Soest and Robertson (1976) demonstrated that fibre from wheat bran increased its hydration capacity as particle size decreased but Lapsley (1980) has shown that in most instances with apple mesocarp and its fibre residues, particle size had no effect on the water holding capacity. McConnell et al. (1974) indicated that grinding reduced water holding capacity but that the effects were small.

The water holding capacity measures bound and trapped water. Robertson and Eastwood (1981) stated that trapped water does not remain associated with fibre. This phenomenon may be reflected in the difference in values obtained with the two techniques used in the present study to measure water holding capacity.

It should be pointed out that the values reported for the water holding capacity of cellulose MN 300 are valid for the specific cellulose used in the present study; other workers (Schaller _et al._, 1978; Parrott and Thrall, 1978) have pointed out that samples of commercial purified celluloses differ in composition and may differ in particle size as well as ultimate source.

D. Settling Volume

The settling volume of the apple core fibre, the residues from the acid and neutral detergent fibre determinations and a purified cellulose (Cellulose MN 300) was measured by the method described previously (Section II.A.4, page 16). The results are shown in Table 11. The bulk volume and the water saturation capacity (Middleton and Byers, 1934) are reported in Table 12. Settling volume (SV) and water saturation capacity (WSC) are related by the equation

$$\frac{\text{SV (ml)} - \text{Bulk Volume (ml/gm)} \times \text{Dry Weight (gm)}}{\text{Dry Weight (gm)}} = \text{WSC (gm/gm)}$$

The volume of water is numerically equal to its weight (Middleton and Byers, 1934). The bulk volume of the fibre samples was determined from the bulk density; the bulk density was measured according to the method described by Montgomery and Baumgardt (1965), using a 10 ml graduated cylinder.

TABLE 11

Settling Volume of Fibre Samples

Settling Volume (ml/gm Fibre)[4] (25°C)			
Apple Core Fibre[1]	NDF	ADF	Cellulose MN 300
23.2 (1.6)[2,3]	22.4 (0.4)[2]	12.8 (0.2)	11.4 (0.2)

1. Values for apple fibre are the average of six replicates, all others are the average of three replicates.
2. These values are not significantly different (alpha = 0.05).
3. The numbers in the brackets represent standard error.
4. Values are on a dry weight basis

TABLE 12

Bulk Volume and Water Saturation Capacity of Fibre Samples

Method	Apple Core Fibre	NDF	ADF	Cellulose MN 300
Bulk Volume[1]	7.1	5.5	5.7	5.2
W.S.C.[2]	16.1 (1.7)[3,4]	16.9 (0.5)[3]	7.1 (0.3)	6.1 (.2)

1. Values of Bulk Volume are expressed as ml/gm fibre on a dry weight basis. Values are the averages of three replicates.
2. Values of Water Saturation Capacity are expressed as gm of water/gm fibre, on a dry weight basis.
3. These values are not significantly different (alpha = 0.05).
4. The numbers in brackets represent standard error.

It will be noted that the values for the NDF and apple core fibre are not markedly different. A comparison of pooled values of the water holding capacity from Method #1 and #2 with the values for WSC (Table 13) demonstrates a large difference in the values for the NDF residue. It is possible that the solubilization in the neutral detergent of specific cell wall components (pectin and some hemicellulose) alters the ability of the fibre to retain water under the mild compaction which would occur in the measurement of the water holding capacity.

E. Cation Exchange Capacity.

An initial experiment was conducted to determine if a difference existed between the two following methods of acid-charging recommended in the literature,- a 48 hr. soaking of sample in 0.1 M HCl as suggested by Robertson <u>et</u> <u>al</u>. (1980), a 48 hr. soaking in 2.0 M HCl as suggested by McConnell <u>et</u> <u>al</u>. (1974). Samples (4 gm approx.) of apple core fibre were mixed with an excess of either 0.1 M or 2.0 M HCl and allowed to stand (25°C) for 48 hours: The samples were filtered (Buchner funnel) and washed with glass-distilled water until the filtrates were free of chloride (silver chloride test). Samples (6; 0.25 gm, dry weight, approx.) of each treated fibre were titrated as described in Section II.A.5, page 17. Readings were taken every two minutes and the average of the six samples used to plot a titration curve. The titration curves for the acid-charged apple core fibres are presented in Fig.10. It is evident from the graph that the 2.0 M HCl was more effective than the 0.1 M HCl in converting the fibre to a fully protonated state. All subsequent samples were acid-charged using 2.0 M HCl.

TABLE 13

Comparison of Pooled Values of Water Holding Capacity with Water Saturation Capacity for Fibre Samples.

Method	Apple Core Fibre	NDF	ADF	Cellulose MN 300
W.H.C.[1]	16.1 (1.9)[2]	11.5 (1.5)	7.1 (1.7)	5.8 (0.6)
W.S.C.[1]	16.1 (1.7)	16.9 (0.5)	7.1 (0.3)	6.1 (0.2)

1. Values of W.H.C. and W.S.C. are expressed as gm H_2O/gm fibre on a dry weight basis.
2. The numbers in the brackets represent standard error.

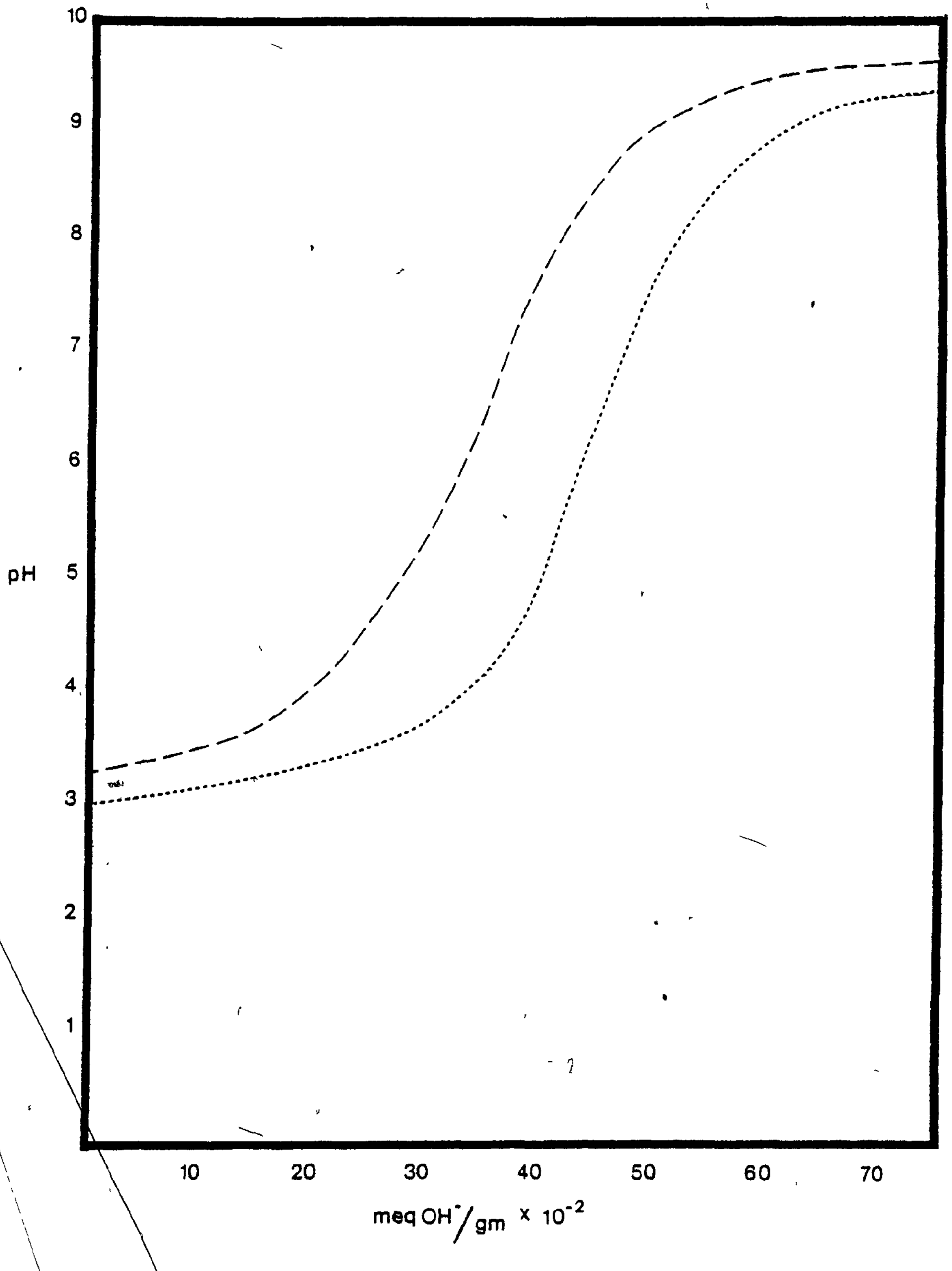


Figure 10. Titration Curves of Apple Core Fibre, acid charged with 0.1M HCl (---) and 2.0M HCl (...)

The cation exchange capacity of the residues from the NDF and ADF was measured, after acid-charging in 2.0 M HCl, by the method described in Section II.A.5, page 17. The results of the analysis are given in Table 14. The cation exchange capacity of citrus pectin (Sunkist Pure Citrus Pectin N.F.) was measured without acid-charging. This value is reported in Table 14 along with values reported in the literature for apple tissue, cellulose (Whatman #41) and Solka Floc (Brown's Co., Ill.) It should be pointed out that the values for both the cellulose and the Solka Floc were determined by a calcium binding method (Van Soest and Robertson, 1976; James et al., 1978) and hence one might expect different values from those obtained with the technique used in the present study. The ADF residue contains 94% cellulose and a small quantity of lignin. Small amounts of pectin (Bélo and deLumen, 1981) and hemicellulose (Morrison, 1980) may also be present in the ADF residue. Van Soest and Robertson (1976) indicated that preparations of cellulose from wood may contain up to 15% pentosans. Parrott and Thrall (1978) demonstrated that two Solka Floc preparations (BW100 and SW40) contain protein and components soluble in acid detergent. The exchange capacity of these preparations may be due to their non-cellulosic components.

The titration curves of the apple core fibre and the residues of NDF and ADF are compared in Fig.11. Robertson et al. (1980) suggested that the pectins are mainly responsible for the cation exchange capacity since pectins have a high proportion of uronic acids. Van Soest (1978) and Van Soest and Robertson (1976) suggested that the components of fibre responsible for exchange were pectin, hemicellulose, lignin, and nitrogenous compounds such as Maillard polymers. The titration curves for the

TABLE 14

Cation Exchange Capacity of Fibre Samples

Sample	meq/gm
Apple Core Fibre	0.47[4]
Apple Core Fibre NDF	0.14[5]
Apple Core Fibre ADF	0.05[5]
Citrus Pectin[1]	1.01[6]
Apple Tissue[2]	1.9
Cellulose (Filter paper)[3]	0.00
Solka Floc[3]	0.06

1. Cation Exchange Capacity was determined using uncharged Sunkist Pure Citrus Pectin N.F.
2. Water washed, acetone dried;(McConnell <u>et al</u>., 1974.
3. Cellulose is Whatman #41 filter paper; Van Soest and Robertson, 1976
4. Average of six replicates.
5. Average of three replicates.
6. Average of duplicate measurement

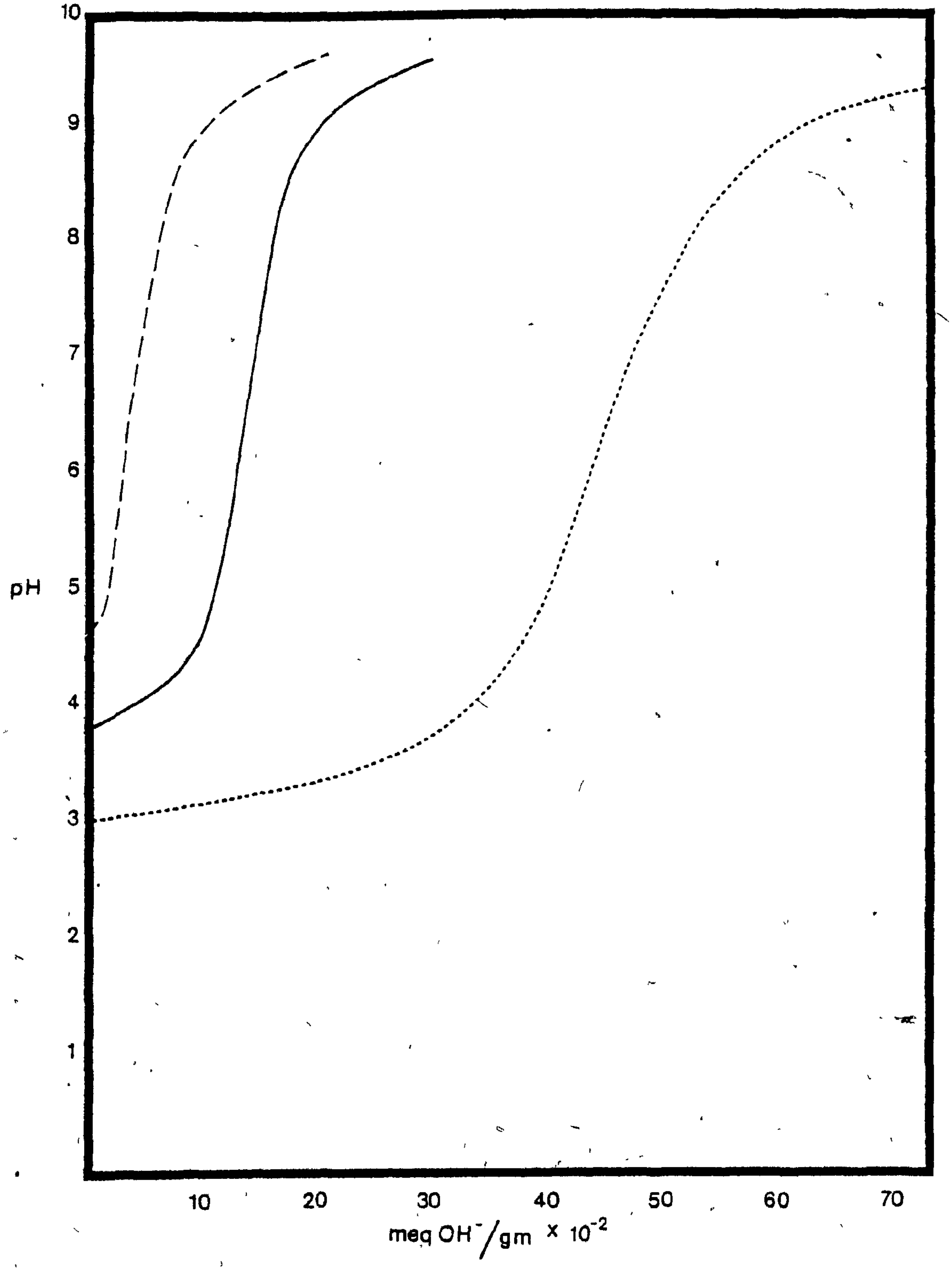


Figure 11. Titration Curves of ADF (----), NDF (——) and Apple Core Fibre (....)

apple core fibre and its detergent residues are similar to the curve obtained for citrus pectin (Fig.12). The pK's for the apple core fibre, the NDF and ADF residues and the citrus pectin, calculated from the titration curves and the pK of galacturonic acid (determined separately) are 3.5; 4.2; 5.1; 3.7 and 3.4 respectively. Kertesz (1951) reported that the titration curve of pectin resembled that of a monobasic acid having only one point of inflection. Bonner (1936) indicated that the pK of pectin increases constantly from 2.7 to over 4.0 during a titration. Hinton (1940) reported pK values for pectin ranging from 2.56 to 4.16. Robertson et al. (1980) stated that uronic acids have pK values which range from 4.0 to 6.0. It seems reasonable to assume the uronic acids in the apple core fibre are responsible for the cation exchange capacity since the apple core fibre has three times the exchange capacity of the NDF (apple core fibre minus pectin) and nearly ten times the exchange capacity of the ADF (apple core fibre minus pectin and hemicellulose).

F. Scanning Electron Microscopy of Fibre Samples

The electron microscope has been used to study the submicroscopic structure of muscle and the effects of mechanical stress on the structural characteristics of dough (Varriano-Marston, 1977). Robertson and Eastwood (1981), Lapsley (1980) and Parrott and Thrall (1978) have used Scanning Electron Microscopy to examine differences in fibre preparations. Varriano-Marston (1977) stated that "morphological structure is often a clue to the functionality of a food material." Robertson and Eastwood (1981) stated that fibre structure influenced water holding capacity. The apple core fibre, the acid and neutral detergent residues and

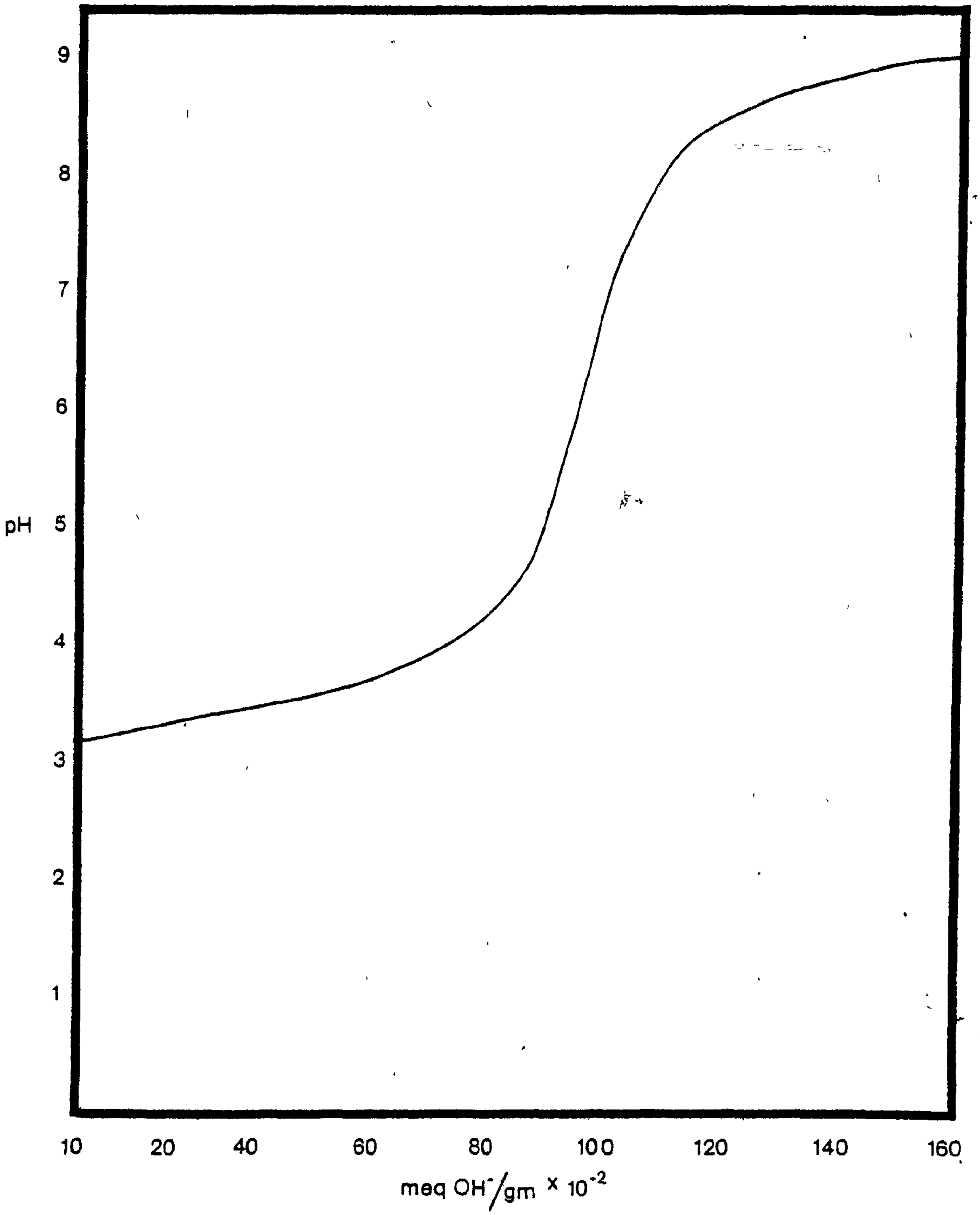


Figure 12. Titration Curve of Pure Pectin.

the residue from the determination of the pectin content (Section II.B.5, page 19) were observed under the Scanning Electron Microscope.

Fig. 13 shows the electron micrograph of apple core fibre. The cell walls are somewhat compressed, thickened and smooth. In contrast to this, the cell walls of the apple core fibre after pectin extraction (Fig. 14) are finely detailed and appear to be thinner. The electron micrograph of the neutral detergent residue of apple core fibre (Fig. 15a) indicates that the material is similar to the fibre after pectin extraction; the cells, however, are more highly compressed. The same material at higher magnification (Fig. 15b) appears as a coarse mat of cells which is cracked in several places. The acid detergent residue (Fig. 16a) is very compressed and lacks any fine detail. A higher magnification of the same material (Fig. 16b) shows coarse, fragmented and compressed cell walls.

It is possible that some of the differences in the structures of the fibre preparations is a result of the drying techniques. Apple core fibre was washed with acetone and ether and air-dried at room temperature. The residue from the pectin extraction was washed with acetone and dried in an oven at 40°C. The residues from the acid and neutral detergent fibre determinations were washed with acetone and dried at 100°C. Varriano-Marston (1977) demonstrated that different dehydration procedures affected the fine structure and the spatial and topographical relationships of dough samples. Robertson and Eastwood (1981) demonstrated that freeze-drying and air-drying of potato fibre caused differences in its cellular appearance from that of frozen potato fibre.

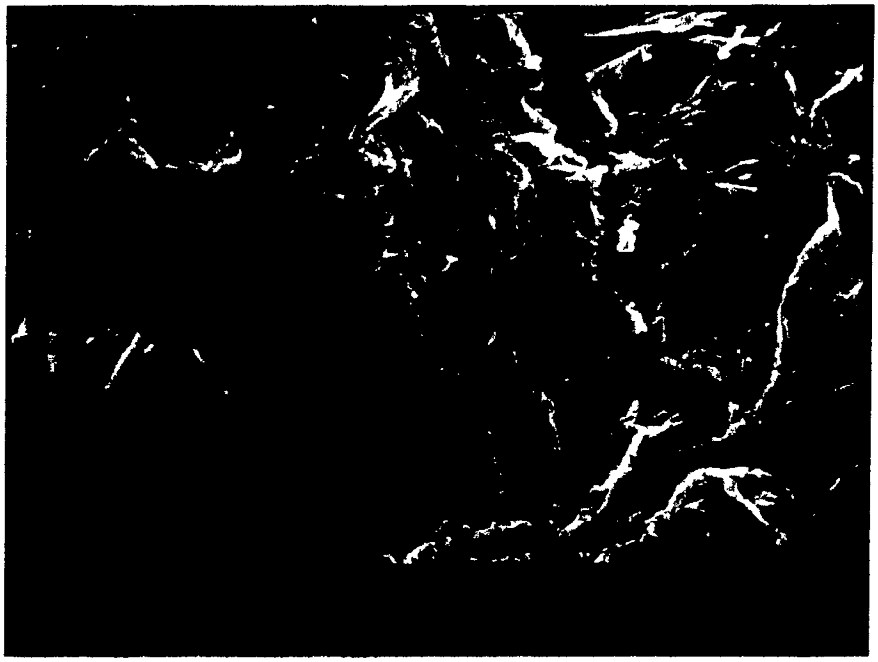


Figure 13. Electron Photomicrograph of Apple Core Fibre
Mag. x 1000

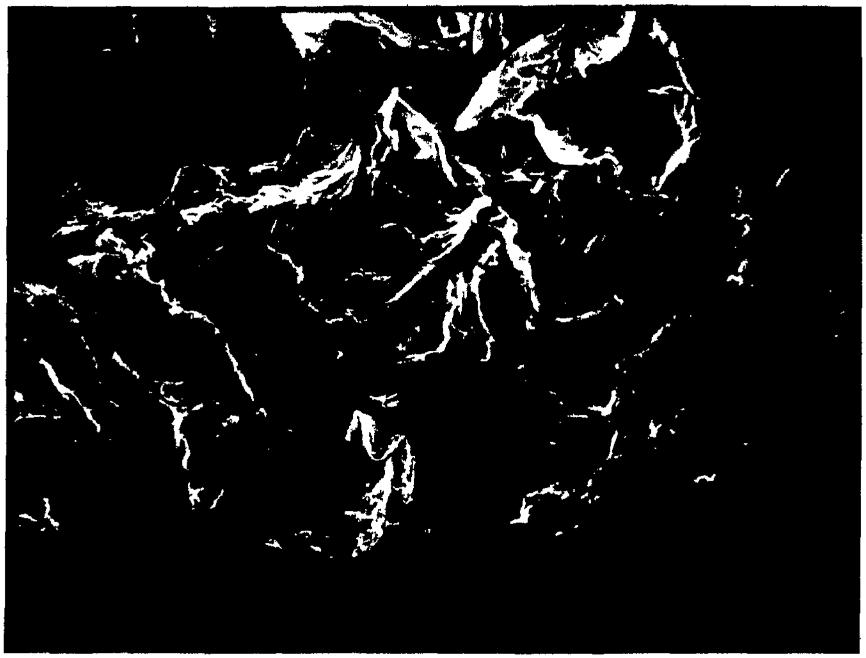


Figure 14. Electron Photomicrograph of Apple Core Fibre after extraction of Pectin by EDTA and Pectinase. Mag. x 500.

Figure 15a. Electron Photomicrograph of the neutral detergent residue of Apple Core Fibre. Mag. x 1000.

Figure 15b. Electron Photomicrograph of the neutral detergent residue of Apple Core Fibre. Mag. x 2000.

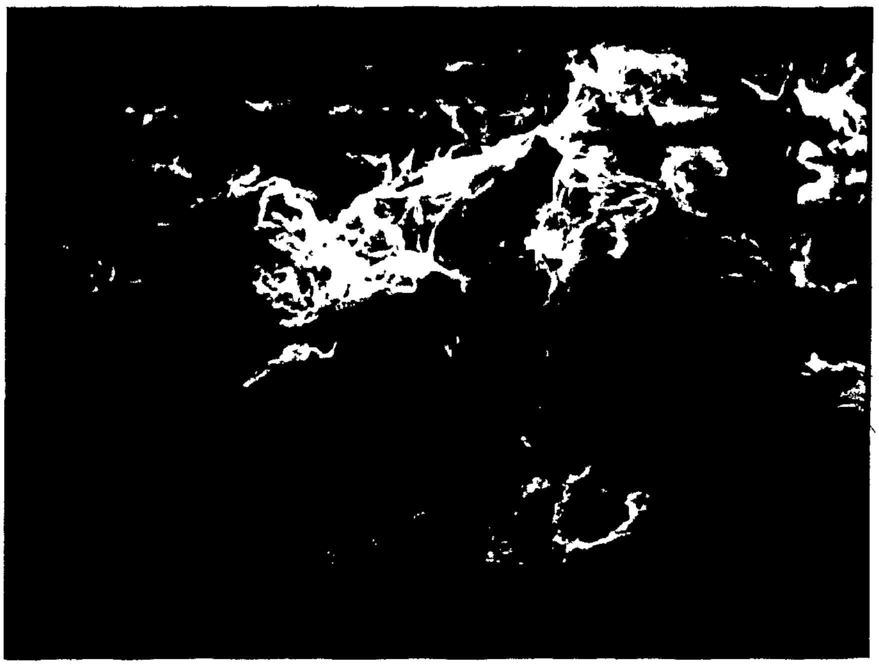


Figure 16a. Electron Photomicrograph of the acid detergent residue of Apple Core Fibre. Mag. x 500.

Figure 16b. Electron Photomicrograph of the acid detergent residue of Apple Core Fibre. Mag. x 1000.

S

G. Amino Acid Analysis of Apple Core Fibre

Four samples (15 mg) of apple core fibre were hydrolyzed with hydrochloric acid and prepared for amino acid analysis as described in Section II.B.8, p.23. The results of the amino acid analysis expressed as grams of amino acid in 100 grams of apple core fibre and as grams of nitrogen in each amino acid in 100 grams of apple core fibre are reported in Table 15. The nitrogen content, calculated from the nitrogen content contributed from each amino acid, is 0.67% on a dry weight basis. The nitrogen content as determined by a micro-Kjeldahl method is 1.1% on a dry weight basis. Based on the nitrogen content and using a factor of 6.25 the protein content of the apple core fibre is 6.8%. The protein content reported in the present study, agrees closely with the value reported by Morrison (1956) for dried apple pectin pulp. The discrepancy between the nitrogen content of apple core fibre determined by the micro-Kjeldahl method and that calculated from the amino acid content may be the result of (a) the presence of non-protein nitrogen such as nucleic acid and (b) the destruction of certain amino acids during acid hydrolysis of proteins in the presence of carbohydrates (Blackburn, 1978; Lugg, 1933, 1938; Block and Bolling, 1951).

Davis et al. (1949) recommended that a conversion factor of 11.76 instead of the usual factor of 6.25 should be used to convert the nitrogen to protein in connection with apple protein. Based on this factor, the protein content of apple core fibre is 13%.

TABLE 15

Amino Acid and Nitrogen Content of Apple Core Fibre

Amino Acid	gm Amino Acid/[1] 100 gm fibre	gm Nitrogen/ gm Amino Acid/ x 10^2 100 gm fibre
Aspartic Acid	.56	5.89
Threonine	.23	2.70
Serine	.21	2.80
Glutamic Acid	.59	5.62
Glycine	.24	4.48
Alanine	.27	4.24
Valine	.61	7.30
Isoleucine	.27	2.88
Leucine	.45	4.81
Tyrosine	.11	.85
Phenylalanine	.23	1.95
Lysine	.44	8.43
Arginine	.22	7.08
Histidine	.18	4.87
Proline	.23	2.80

---

[1] Average of 4 replicates.

H. Identification of Neutral Sugars in Apple Core Fibre

Samples (6; 150 mg each) of apple core fibre were hydrolyzed with sulfuric acid (5%) for 2.5 hours as described in Section II.B.9, p.25. The hydrolysates were allowed to settle and the supernatants were removed by siphoning. These were labeled H_1 to H_6 and were set aside for sugar analysis. The residues were again hydrolyzed with sulfuric acid (72% w/v, 48 hr, 4°C) and the mixture filtered to give an additional six samples of hydrolysates. These were labeled h_1 to h_6 and were set aside for sugar analysis.

Previous workers (Theander and Aman, 1979b) have shown that apple tissue contains rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose. The literature was reviewed to find a suitable method for the identification of the sugars mentioned above. Jermyn and Isherwood (1949) separated seven sugars as well as galacturonic acid by paper chromatography. The method gave an unsatisfactory separation of arabinose and mannose. Schweiger (1962) using the solvent system of Jermyn and Isherwood (1949) and cellulose as the supporting material (thin layers) failed to improve the separation of arabinose and mannose. Raadsveld and Klomp (1971) achieved a good separation of mannose, arabinose and glucose by thin layer (cellulose) chromatography using a mixture of ethyl acetate, pyridine and water. Hoton-Dorge (1976) separated the seven sugars (rhamnose, fucose, xylose, arabinose, mannose, galactose, glucose) that were found to be present in apple tissue (Theander and Aman, 1979b) by thin layer chromatography (Cellulose MN 300) using a mixture of ethyl acetate, pyridine, water, n-butanol, acetic acid (5:4:4:10:2). Several unsuccessful attempts were made to separate the seven sugars mentioned above using cellulose plates (Brinkman Polygram Cel 300), the solvent system used by Hoton-Dorge (1976) and a development time of 7.0 hours (2 developments of 3.5 hr. each). Experiments showed that a mixture of acetonitrile, pyridine, water, n-butanol and acetic acid (4:5:4:9:3) and two developments (2 hr. each, 40°C) using cellulose plates (Brinkman Polygram Cel 300) gave a good separation of the seven sugars.

Fig. 17 shows a photograph of a chromatogram which was prepared using solutions of the eight individual sugars, mixtures of these sugars as well as the apple core fibre hydrolysates (H_1 and h_1). It will be noted that H_1 (5% sulfuric acid hydrolysate) contained small amounts of rhamnose and fucose, and somewhat larger amounts of mannose, galactose and glucose; it contained relatively larger amounts of xylose and arabinose. Hydrolysate h_1 (72% sulfuric acid hydrolysate) contained xylose and glucose. Both hydrolysates contained uronic acids. The chromatogram indicated that hydrolysate h_1 contained both glucuronic and galacturonic acid. Table 16 lists the Rf and Rg values of the various sugars as well as the colour of the individual sugars on the chromatogram. The Rg values for the uronic acids in hydrolysate h_1 are 41 and 47; the Rg value of the uronic acid in hydrolysate H_1 is 41. These values are in agreement with the values cited in Table 16.

1. Gas Chromatographic Determination of Neutral Sugars in Apple Core Fibre

Samples from the 5% sulfuric hydrolysates (H_1-H_6; Section II.B.9, p.25) and samples from the 72% sulfuric hydrolysates (h_1-h_6; Section II.B.9, p.29) were prepared for gas chromatographic analysis as described in Section II.B.10,p.30. Samples of gum arabic which were hydrolyzed with 5% sulfuric

Figure 17

Chromatogram of Sugar Standards and Acid Hydrolysates

1. Galactose
2. Glucose
3. Allose
4. Mannose
5. Arabinose
6. Xylose
7. Fucose
8. Rhamnose
9. Sugars 1, 2, 4, 5, 6, 8
10. Sugars 1, 2, 4, 5, 6, 7, 8
11. Hydrolysate h_1
12. Hydrolysate H_1
13. Galactose

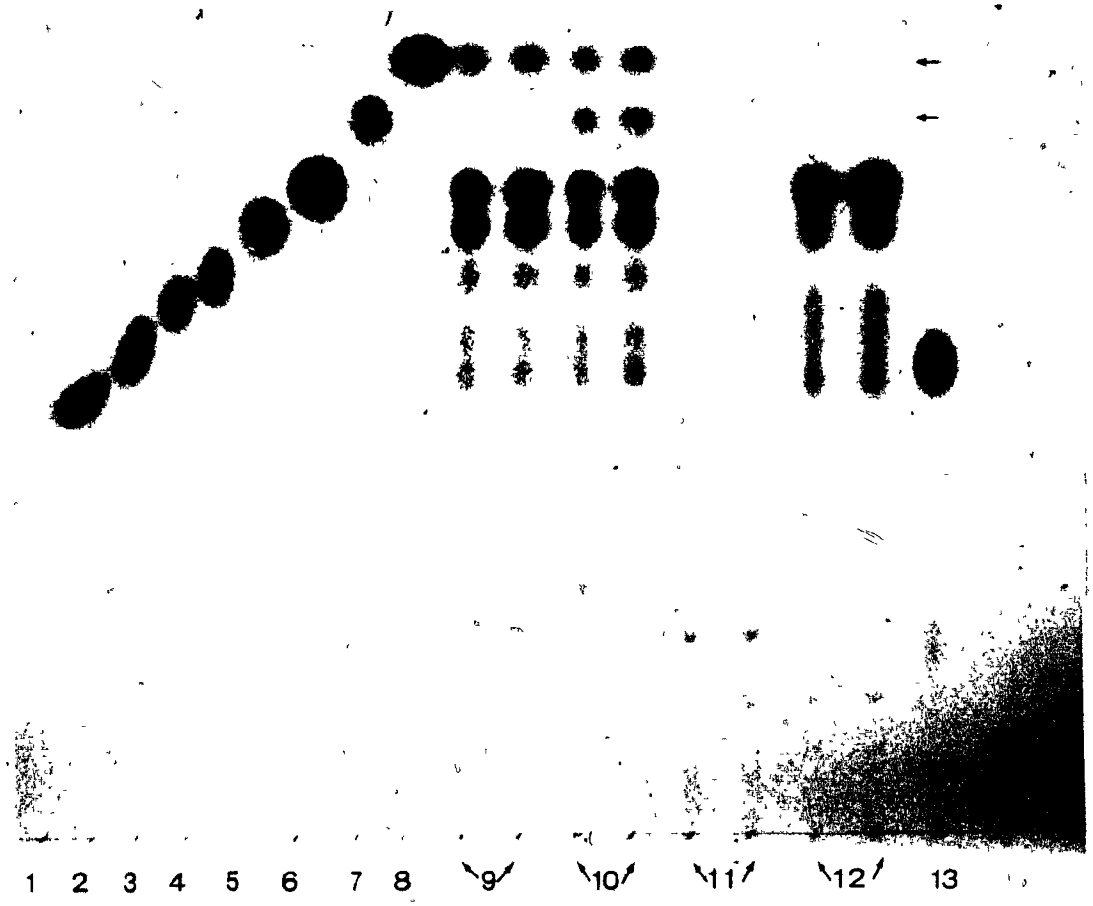


Figure 17. Chromatogram of Sugar Standards and Acid Hydrolysates.

TABLE 16

Rf and Rg Values for Sugars

Sugar	Rf x 100	Rg x 100	Rg x 100[1]	Colour Response
Galactose	43	90	86	Green
Glucose	48	100	100	Green
Allose	52	108		Green
Mannose	55	115	109	Green
Arabinose	61	127	119	Pink
Xylose	64	133	131	Pink
Fucose	71	148	143	Green
Rhamnose	77	160	171	Green
Galacturonic Acid[2]	23	42		Brown
Glucuronic Acid[2]	25	46		Brown

1. Values calculated from the Rf values reported in Hoton-Dorge (1976).
2. Determined in several previous runs as components of a sugar mixture.

acid and samples of a purified cellulose (Cellulose MN 300, Macherey Nagel) which had been hydrolyzed with 72% sulfuric acid, were prepared for analysis as well. Samples of the pectin extracts (P1 to P4) of apple core fibre and the pectin extract of citrus pectin, prepared as described in Section II.B.5, p.19, were hydrolyzed and prepared for analysis by gas chromatography as described in Section II.B.10, p.31.

Trifluoroacetic acid (TFA) and sulfuric acid hydrolysis have been used extensively to release the sugars from complex mixtures of polysaccharides. Sugars are easily isolated from TFA hydrolysates by evaporation of the acid (Albersheim _et al._, 1967). TFA will release the neutral sugars from aldobiouronic acids which are resistant to sulfuric acid hydrolysis (Talmadge _et al._, 1973). The authors indicated, however, that TFA will not hydrolyze cellulose or the β-(1-4)-glycosyl linkages in a xyloglucan backbone. Further, TFA hydrolysis will degrade uronosyl residues in cell wall polymers. Bittner _et al._ (1980) indicated that as a consequence of these limitations the analysis of cell walls conducted by Collings and Yokoyama (1979), may have under-estimated the non-cellulosic components and over-estimated the cellulose component.

Sulfuric acid hydrolysis is the basis for the analysis of the neutral sugars in the present work. The neutral sugar composition of the sulfuric acid hydrolysates of apple core fibre, expressed as grams of anhydro sugar in 100 grams fibre (dry weight) are reported in Table 17. The neutral sugar composition of apple core fibre is reported in Table 17 as well. Southgate (1969) demonstrated that the release of hexose and pentose from food samples reach a maximum after 2.5 hours in 5% (v/v) sulfuric acid at 100°C. The author considered that this hydrolysis pro-

TABLE 17

Neutral Sugar Composition of Apple Core Fibre

Sugar	Composition of Apple Core Fibre (gm/100 gm, dry weight)[1] 5% Sulfuric Acid Hydrolysate	72% Sulfuric Acid Hydrolysate	Total of 5% and 72%
Rhamnose	0.6 (0.1)[2]	-	0.6 (0.1)
Fucose	0.9 (0.1)	-	0.9 (0.1)
Arabinose	5.3 (0.5)	-	5.3 (0.5)
Xylose	10.7 (1.2)	1.3 (0.2)	12.0 (1.4)
Mannose	0.8 (0.1)	1.5 (0.2)	2.3 (0.3)
Galactose	6.4 (0.8)	-	6.4 (0.8)
Glucose	5.2 (0.5)	38.0 (2.9)	43.3 (3.2)
Total	29.9	40.8	70.8

1. These results represent the average of 6 samples; each sample was analyzed in triplicate and aliquots of each replicate were analyzed in duplicate on the gas chromatograph.
2. The numbers in the brackets represent the standard error.

cedure released the hemicellulose fractions of food samples. Cellulose was solubilized by further hydrolysis with 72% (w/w) sulfuric acid. Saeman et al. (1963) indicated that complete hydrolysis of cellulose required a primary hydrolysis to form a mixture of oligosaccharides and a secondary hydrolysis to complete the conversion to sugar monomers. The technique of Anderson and Clydesdale (1980) which was used with slight modification in the present study, is essentially a combination of these methods. Selvendran et al. (1979b) recommended Saeman hydrolysis as a method for the measurement of the total neutral sugars in cell wall material. Theander and Aman (1979b) used a similar procedure in their studies on dietary fibre. The authors argued that since some cellulose is hydrolyzed by dilute sulfuric acid and some fractions of hemicellulose are difficult to hydrolyze, the total polysaccharides should be measured rather than be measured as hemicellulose and cellulose (Theander and Aman, 1981).

Acid hydrolysis of polysaccharides can lead to the degradation of the liberated sugars (Adams, 1965). Selvendran et al. (1979b) reported small losses of sugars after heating (100°C, 5 hr.) mixtures of sugars in sulfuric acid (2N). Hough et al. (1972) indicated that the neutralization step following acid hydrolysis, can result in losses of sugars. In the present study, a standard sugar mixture was heated (100°C, 2.5 hr.) in sulfuric acid (5% w/v); the mixture was then neutralized and the sugars derivatized as described in Section II.B.10, p.31. The molar response factors were determined using β-d-Allose (Sigma) as the internal standard and are reported elsewhere (see Table 7). Although myo-inositol has been used by several authors (Oades, 1967; Borchardt and Piper, 1970; Jones and Albersheim, 1972), Theander and Aman (1979b) reported difficulty in securing results. Anderson and Clydesdale (1980) reported variability in recovery (94.7 ± 7.4%) using myo-inositol as the internal standard. Torello et al. (1980) suggested the use of ribose. Preliminary experiments carried out in the present study using ribose as an internal standard, indicated a low recovery after hydrolysis in sulfuric acid (5%, 100°C) for 2.5 hours.

The advantage of an internal standard as outlined by Laker (1980) are (1) more certain identification of peaks; (2) correction for solvent losses due to evaporation; (3) correction for fluctuation of the injection volume (microlitres). The criteria for the selection of an internal standard are (1) complete separation, as a single peak, from other components; (2) it should be stable and of high purity; (3) it should have identical or at least similar detector response and (4) it should never occur in the sample to be analyzed.

An important consideration in the determination of the response factors for each sugar is the assurance that the sugars and internal standard are dried before they are weighed. Jones and Albersheim (1972) dried their internal standard at 50°C for 48 hr. in a vacuum and their reference sugars were stored for 72 hr. at 50°C in a vacuum oven prior to weighing. Southgate (1969) dried monosaccharide standards over P_2O_5 under reduced pressure at 37°C. In the present study, the sugars were dried over calcium chloride under reduced pressure (2-3 cm Hg) at 25°C. Karma (1980) indicated that calcium chloride is less effective than P_2O_5 as a desiccant.

The sugars released by acid hydrolysis were converted to their corresponding alditol acetates by reduction with sodium borohydride and acetylation with acetic anhydride and anhydrous sodium acetate (Sloneker, 1972). This method eliminates the formation of sugar anomers. Preliminary work using pyridine and acetic anhydride to acetylate the alditols (Sawardeker et al., 1965) resulted in dark reddish-brown solutions which produced tailing of the solvent front and caused rapid deterioration of the first few centimeters of the column packing. The method employed in the present study produced solutions which were light yellow to light brown in colour. The solutions caused little deterioration of the column packing and reduced the tailing of the solvent front. Recently, Bittner et al. (1980) reported a new acetylation technique using N-methylimidazole and acetic anhydride which reduces acetylation time to 5 minutes.

J. The Uronic Acid Content of Apple Core Fibre

Samples (4; 1 gm each) of apple core fibre were suspended in EDTA (130 ml, 0.5%, pH 11.6) and stirred mechanically for 30 minutes. The reaction of the suspensions was adjusted to pH 5.4 (approx.), pectinase (0.1 gm, Nutritional Biochemicals Co.) was added and the suspensions were stirred for one hour. The suspensions were transferred to volumetric flasks (250 ml), the volume adjusted and then mixed thoroughly. The suspensions were filtered (Whatman #1); the first few millilitres of the filtrates were discarded and the remaining filtrates (pectin extracts P1-P4) were collected for analysis. A sample of citrus pectin (Sunkist Pure Citrus Pectin, N.F.) was also subjected to the same extraction procedure. Samples of apple core fibre (6; 150 mg each) were hydrolyzed (100°C, 2.5 hr) with sulfuric acid (5% w/v) and the supernatant collected by siphoning, to give six samples of hydrolysate (H_1-H_6). (See Section II.B.9, p.25). The residues were hydrolyzed with sulfuric acid (72% w/v, 48 hr. 4°C) and filtered (sintered glass crucible, porosity C). The filtrates were collected to give six samples of hydrolysate (h_1-h_6) (See Section II.B.9 p.29). A sample of gum arabic was hydrolyzed with sulfuric acid (5% w/v) and the supernatant collected as described above. A sample of purified cellulose (Cellulose MN 300, Macherey Nagel) was hydrolyzed with sulfuric acid (72% w/v) and the filtrate collected as described above. The uronic acid content of all samples was determined by the two methods described in Section II.B.6&7,p.19,20. The modified carbazole method (Bitter and Muir, 1962) and the hydroxy-biphenyl method (Blumenkrantz and Asboe-Hansen, 1973) . Several corrections have been applied to the values determined by the carbazole method. A sample blank correction was used as recommended by Southgate (1981). Corrections were made for the neutral sugar content of the samples as determined by gas chromatography. Hudson and Bailey (1980) indicated that only the hexoses interfered in the carbazole reaction; therefore a set of optical density (O.D.) values corresponding to the neutral sugar content of each sample, was calculated using the combined weight of the hexoses (mannose, glucose, galactose, rhamnose and fucose). These O.D. values were used to correct the O.D. readings obtained for each sample. Selvendran et al. (1979b), Barrett and Northcote (1965) and McComb and McCready (1952) reported interferences from pentose (arabinose) as well as hexoses. Selvendran et al. (1979b) reported that the interference due to glucose was different from that due to galactose. In the present work, a set of O.D. values corresponding to the neutral sugar content of each sample, was calculated using the combined weight of pentoses (arabinose and xylose), the weight of galactose and the combined weight of the remaining hexoses. This set of O.D. values was used to correct the O.D. readings of the samples. Finally a set of O.D. values corresponding to the neutral sugar content of each sample was calculated using the weight of galactose and the combined weight of the remaining hexoses. The three sets of O.D. values corresponding to the neutral sugar content of each sample are reported in Table 18. The O.D. readings of the samples were corrected in the following manner:- (1) the O.D. readings were corrected using one of the three sets of O.D. values corresponding to the neutral sugar content. The corrected O.D. values were used to calculate the uronic acid content of the samples. The values for gum arabic, Cellulose MN 300, and citrus pectin, as well as the mean values for the pectin extracts, hydrolysates H_1-H_6 and hydrolysates h_1-h_6 are reported in Table 19. The mean values for hydrolysates h_1-h_6 have been calculated in two ways; the first is based on the dry weight of the residue subjected to hydrolysis with 72% (w/v) sulfuric acid; the second is based on the dry weight of the apple core fibre. The total uronic acids in the apple core fibre are also reported. (2) The O.D. readings for the samples were corrected with a sample blank and further corrected using one of the three sets of O.D. values corresponding to the neutral sugar content. The corrected O.D. values were used to calculate the uronic acid content of the samples. The uronic acid content of the samples is reported in Table 20.

Theander and Aman (1981) indicated that accurate corrections are difficult for the colour compounds produced by the degradation of uronic acids and sugars and their condensation products. The neutral

TABLE 18

Optical Density Values Calculated from the Neutral Sugar Content of Fibre Samples

Sample	Optical Density (Set 1)	Optical Density (Set 2)	Optical Density (Set 3)
Citrus Pectin	0.006	0.006	0.015
Gum Arabic	1.108	0.935	1.297
Cellulose MN 300	0.411	0.411	0.476
Pectin Extracts			
1	0.006	0.005	0.014
2	0.007	0.006	0.015
3	0.007	0.006	0.015
4	0.007	0.006	0.015
Hydrolysates			
H_1	0.551	0.425	0.530
H_2	0.647	0.521	0.639
H_3	0.505	0.396	0.500
H_4	0.553	0.403	0.505
H_5	0.522	0.399	0.498
H_6	0.536	0.403	0.505
h_1	0.467	0.461	0.520
h_2	0.456	0.451	0.509
h_3	0.377	0.372	0.425
h_4	0.427	0.420	0.476
h_5	0.372	0.367	0.420
h_6	0.476	0.469	0.529

Set 1 calculated for hexoses and pentoses according to Selvendran et al. (1979b)
Set 2 calculated for hexoses only according to Selvendran et al. (1979b)
Set 3 calculated for hexoses only according to Hudson and Bailey (1980)

TABLE 19

The Uronic Acid Content of Fibre Samples

Sample	Uronic Acid Content (gm Anhydrouronic Acid/100 gm fibre (dry weight)			
	Carbazole Method (without sample blank correction)			m-OH-Biphenyl Method
	Corrected with O.D. value Set 1	Corrected with O.D. value Set 2	Corrected with O.D. value Set 3	
Pectin Extracts	8.7 (1.1)	8.9 (1.1)	7.5 (1.2)	7.9 (0.3)
Citrus Pectin	96.3	96.3	90.4	76.6
Hydrolysates (H_1-H_6)	7.6 (1.0)	8.4 (1.0)	7.7 (1.2)	9.4 (0.8)
Gum Arabic	9.9	11.2	8.5	5.6
Hydrolysates (h_1-h_6)^1	3.5 (1.0)	3.7 (1.0)	2.0 (1.0)	3.2 (0.9)
Hydrolysates (h_1-h_6)^2	1.7 (0.5)	1.8 (0.5)	1.0 (0.5)	1.6 (0.3)
Cellulose MN 300	9.2	9.2	7.3	3.2
Total Uronic Acids	9.3 (1.2)	10.2 (1.3)	8.7 (1.4)	11.0 (0.9)

1. Based on the dry weight of residue from hydrolysis with sulfuric acid (5% w/v).
2. Based on the dry weight of apple core fibre.
3. The numbers in the brackets are standard error.

TABLE 20

The Uronic Acid Content of Fibre Samples

Sample	Uronic Acid Content (gm Anhydrouronic Acid/100 gm fibre (dry weight) Carbazole Method (with sample blank correction) Corrected with O.D. value Set 1	 Corrected with O.D. value Set 2	 Corrected with O.D. value Set 3	 m-OH-Biphenyl Method
Pectin Extracts	8.0 (1.1)[3]	8.2 (1.1)	6.7 (1.2)	7.9 (0.3)
Citrus Pectin	91.1	91.1	86.7	76.6
Hydrolysates (H_1-H_6)	6.0 (1.0)	6.8 (1.0)	6.2 (1.0)	9.4 (0.8)
Gum Arabic	3.3	4.7	2.3	5.6
Hydrolysates (h_1-h_6)[1]	0.0	0.0	0.0	3.2 (0.9)
Hydrolysates (h_1-h_6)[2]	0.0	0.0	0.0	1.6 (0.3)
Cellulose MN 300	0.0	0.0	0.0	3.2
Total Uronic Acids in Hydrolysates	6.0 (1.0)	6.8 (1.0)	6.2 (1.0)	11.0 (0.9)

1. Based on the dry weight of residue from hydrolysis with sulfuric acid (5% (w/v).
2. Based on the dry weight of apple core fibre.
3. The numbers in the brackets are standard error.

sugars may be underestimated by a failure to analyze for unusual sugars such as apiose or 2-O-methylfucose (Darvill et al., 1978) or by sugars being trapped in the form of acid resistant aldobiouronic acids (Theander and Aman, 1981).

The uronic acid contents of the samples were measured by the method of Blumenkrantz and Asboe-Hansen (1973) using o-hydroxy biphenyl reagent instead of m-hydroxy biphenyl reagent. A preliminary study indicated that the peak maximum for the colour reaction was in the region 475-480 nm. The wavelength chosen for the analyses was 477 nm. An evaluation of the interference from the neutral sugars was not undertaken. The values for the uronic acid content of the various samples are reported in Tables 19 and 20 for comparison with the results obtained with the carbazole method.

The uronic acid values (corrected, different methods of determination) reported for the pectin extracts are not significantly different (Student's t-Test, alpha = 0.05). This is probably due to the low content of the neutral sugars in the samples as determined by gas chromatography. The mean weight of the residue from the pectin extraction, was 70% of the original dry weight of apple core fibre. The results indicate that apple core fibre contains 8% pectin and 7% neutral sugars as determined by gas chromatography; fifteen percent of the apple core fibre is unaccounted for. Examination of the results of the analysis of citrus pectin indicates that the pectin contains 21% neutral sugar; the pectin content as determined by the m-hydroxy biphenyl method is 76%. Theander and Aman (1981) reported a value of 76.1% for a citrus pectin (EEC/IARC reference material, James and Theander, 1981). The recovery of 97% (21% neutral sugar, 76%

pectin) of the sample precludes an under estimation of the neutral sugars or the uronic acid content. Theander and Aman (1981) could not account for approximately 6% of the citrus pectin sample after complete analysis. It is possible that in the present work, protein and protein-tannin complexes were solubilized along with the pectins; Hulme (1956) used alkaline buffers to isolate undenatured protein and Davis et al. (1949) reported that protein was isolated as a protein-phlobaphene complex. It is also possible that certain substances in the residue were extracted by the acetone used to dry the residue prior to oven drying at 40°C.

The uronic acid content of the apple core fibre (Table 20) as determined from the sulfuric acid hydrolysates is lower than the results obtained by the method involving the uronic acid determination of the extracted pectin. The hemicellulose component of apples contains some uronic acid (Theander and Aman, 1981). One would expect that the uronic acid content of the apple core fibre determined from the acid hydrolysate which contains hydrolyzed pectin and hemicellulose, would be higher, not lower. This discrepancy may be the result of several factors. For example, the repeated siphoning of the supernatant and water washes from the 5%(w/v) sulfuric acid hydrolysis, withdrew small amounts of particulate matter; this particulate matter could have contained small amounts of uronic acid. Selvendran et al. (1979b) reported that particles of undissolved material, especially lignified tissue, caused interference in the carbazole reaction; the authors recommended that the acid hydrolysates be filtered before they were used for analysis of uronic acids. Filtration of the hydrolysates was not included in the present study; visible particulate matter was allowed to settle out of solution. Treatment of apple core fibre with 5%

(w/v) sulfuric acid produced a rose-pink coloured supernatant. This colour may interact with the carbazole or hydroxy biphenyl reagents or the 'uronic acid-reagent complexes'. Several authors (Warren and Woodman, 1973; Anderson et al., 1961) have suggested that the uronic acids are decarboxylated during hydrolysis. Selvendran et al. (1979b) demonstrated that treatment (20°C, 3 hr) of uronic acids with sulfuric acid (72% w/w) had "no significant effect on the yield of uronic acid." The use of a sample blank correction and a correction for the neutral sugars could have overcorrected the O.D. values and hence led to very low values for the uronic acid content of the apple core fibre. Use of the sample blank correction without a correction for the neutral sugars, however, gave unreasonably high values for the uronic acid content and these values have not been reported. The values reported for the uronic acid content of the acid hydrolysates of apple core fibre, using the m-hydroxy biphenyl method, are likely inflated since no correction was made for the neutral sugar content of the samples. The values for the uronic acid content were calculated using calibration curves based on galacturonic acid. Hemicelluloses contain glucuronic acid (McCready and McComb, 1952). Selvendran et al., (1979b) and Blumenkrantz and Asboe-Hansen (1973) reported a higher response for glucuronic acid than galacturonic acid using the carbazole and m-hydroxy biphenyl methods. Therefore the value for the total uronic acids calculated by both methods, may be too high.

It is difficult to suggest which colourimetric technique and correction technique would give the most reliable measurements of the uronic acid content of apple core fibre. Both the methods suffer from interferences which are influenced by the nature of the uronic acids and the composition of the material from which they are isolated (Helbert and Brown, 1956). Galambos (1967) reduced the interferences of hexose by the introduction of sulfamate in the carbazole reaction. The method of extraction of the uronic acids further complicates their measurement. McCready and McComb ([illegible]952) reported that the extraction of uronic acids by EDTA and the release of the uronics by pectolytic enzyme was incomplete. Selvendran _et al_. (1979a) indicated that only 80-90% of the total pectic substances can be extracted by the various solutions [illegible]% hexametaphosphate, 0.5% ammonium oxalate, 0.05M EDTA) that have been employed.

Several methods have been developed to measure the total uronic acids in samples without extraction or digestion (Warren and Woodman, 1973; McCready _et al_., 1946; Barker _et al_., 1958; Castagne and Siddiqui, 1975; Theander and Aman, 1979b). The decarboxylation technique of Theander and Aman (1979b) can be corrected graphically for errors due to non-uronic acid material. An important feature of these techniques is that the uronic acids all give similar responses, whether they exist as monomers, polymers, or are bound to hemicellulose by methyl ester linkages. This is not the situation when uronic acids are measured colourimetrically.

K. Composition of Apple Core Fibre

A summary of the results of the analysis of apple core fibre is presented in Table 21. It will be noted that 91.8% of the fibre is accounted for by the constituents which were determined. In connection with the other materials that were analyzed, 87.7% of the gum arabic, 89.7% of the purified cellulose and 97.8% of the citrus pectin were accounted for by the constituents which were determined. Glucose and galactose constituted

TABLE 21

Composition of Apple Core Fibre[1]

	gm/100 gm fibre (dry weight)
Total Anhydro Sugars	70.8 (3.7)[2]
Crude Protein[3]	6.8 (0.9)
Crude Lignin + Ash	3.2 (0.3)
Total Uronic Acids	11.0 (0.9)
Total	91.8

1. Values are based on the results of sulfuric acid hydrolysis
2. The numbers in brackets are standard error
3. Protein was determined separately on apple core fibre samples by a micro-kjeldahl method. Value is the average of 6 replicates.

27% of the citrus pectin; traces of rhamnose and arabinose were also present. The value for the uronic acid content of citrus pectin (o-hydroxy biphenyl method) may be somewhat high because no corrections were made for the neutral sugar content. The values obtained for the neutral sugar content (12% rhamnose, 27% arabinose, 42.5% galactose) of gum arabic agree closely with the values (14% rhamnose, 28% arabinose, 39% galactose) reported by Anderson et al. (1966). The authors however reported a value of 19% for the glucuronic acid content of gum arabic; a value of 5.6% for the uronic acid content of gum arabic was obtained in the present study. The uronic acid content of the fibre samples, determined using a galacturonic acid standard, would tend to overestimate the glucuronic acid content. It is possible therefore that all the material was not hydrolyzed by the mild acid treatment. Selvendran et al. (1981) reported that hydrolysis (100°C, 2.5 hr) with sulfuric acid (5%) may not liberate the uronic acid sugars from the non-cellulosic polysaccharides of fruits and vegetables. Selvendran and DuPont (1980b) reported that for potato powder, fresh potato and rye biscuit, the various constituents accounted for only 70-90% of the dry weight of starting material. They further indicated that only 60% of the rhamnose which is present in tissues is released by hydrolysis (100°C, 2.5 hr) in sulfuric acid (1N). The authors concluded that the material which was unaccounted for included residual water, sugars lost during hydrolysis or not measured due to incomplete hydrolysis. Theander and Aman (1981) indicated that sugars may condense with the degradation products of uronic acids and hence may not appear on the gas chromatogram.

A summary of the results of proximate analysis of apple core fibre is presented in Table 22. NDF residues contain lignin, cellulose, hemicellulose and cell wall protein (Van Soest and Robertson, 1976). ADF residues contain lignin, cellulose, heat damaged protein and protein-tannin complexes (Robertson and Van Soest, 1981; Van Soest and Robertson, 1976). The hemicellulose content of the apple core fibre is estimated from the difference in the NDF and the ADF residues. The residue from the sulfuric acid (72%) treatment of the ADF residue is reported as the crude lignin* plus ash since ashing of the residue was not performed. The cellulose content of the apple core fibre is estimated from the difference of ADF and crude lignin plus ash. Robertson and Van Soest (1981) reported that several factors influence the estimate of the hemicellulose content of fibre. Cell wall protein, which is dissolved in acid detergent, is recovered in the NDF residue, and this can lead to an increased estimate of the hemicellulose content. Pectin and tannins are dissolved in neutral detergent; pectin is partially precipitated and tannins precipitate as protein-tannin complexes in acid detergent. This can lead to a decreased estimate of the hemicellulose content.

Anderson and Clydesdale (1980) and Southgate (1969) considered that the hemicellulose component of dietary fibre was hydrolyzed by heating (100°C, 2.5 hr) in sulfuric acid (5%). Selvendran and DuPont (1980b) demonstrated that 5-10% of the cellulose is hydrolyzed by heating (100°C, 2.5 hr) in sulfuric acid (5%). The hemicellulose content of the apple core fibre is reported in Table 22. A comparison of the hemicellulose content with the sugar content of the 5% sulfuric acid hydrolysate (Table 21) shows a difference of nearly 7%. This difference is greater if the uronic acid content of the hydrolysate, corrected for the pectin content, is included.

* ADF-Lignin

TABLE 22

Composition of Apple Core Fibre

	Composition (%)			
Moisture[2]	9.8(0.1)[1]			
Dry Matter	90.2			
Dry Matter				
NDF[3]		71.0(1.4)[1]		
ADF[3]			48.0(0.8)[1]	
Cellulose				45.2
ADF-Lignin+Ash[3]				2.8(0.3)[1]
Hemicellulose (NDF-ADF)			23.0	
Pectin[2] (Hydroxy Biphenyl)		7.9(0.3)[1]		
Crude Protein[3] (N x 6.25)		6.8(0.9)[1]		

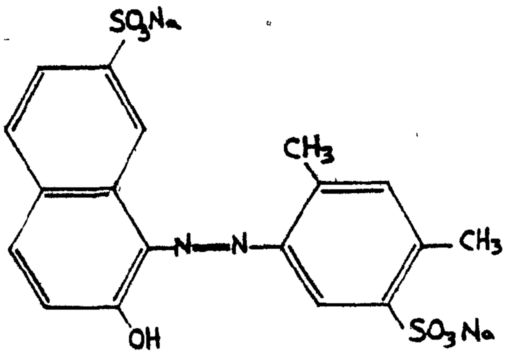
1. The numbers in brackets are standard error.
2. The value is the average of 4 replicates.
3. The value is the average of 6 replicates.

L. Binding Studies

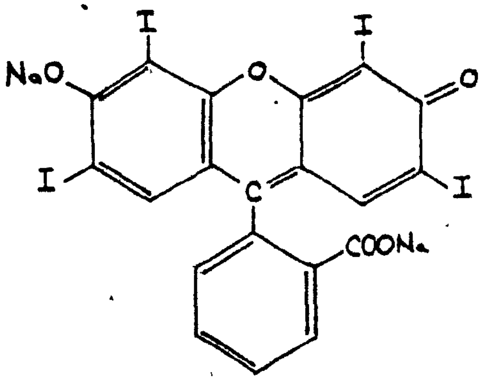
Samples (3; 0.2 gm) of apple core fibre, the residues from acid and neutral detergent fibre determinations and Cellulose MN 300 (Macherey Nagel) were placed in tubes (40-ml, Pyrex) along with 15 ml of one of the dye-buffer solutions as described in Table 8, p. 38. The same solutions were placed in tubes containing no test material. The tubes were covered with parafilm, the contents were stirred (vortex mixer) for one minute and then heated (37°C, 18 hr) in a water bath. The contents of the tubes were stirred (1 min., vortex mixer) at hourly intervals for the first four (4) hours. The tubes were removed from the bath, the contents again stirred for one minute and then the tubes allowed to stand for one (1) hour. The parafilm was removed and aliquots of supernatant were diluted with the appropriate buffer solution; the optical density of the diluted supernatant was measured against buffer solution. Standards were prepared by dilution of stock solutions with the appropriate buffer; the standards were heated (37°C, 18 hr) in a water bath, mixed thoroughly and the optical density measured. In this manner, standard curves for each dye-buffer solution could be prepared.

The three dyes (Brilliant Blue FCF, Ponceau SX, and Erythrosine) selected for this study, represent three major classes of water soluble, synthetic food dyes, namely, triphenylmethane dyes, sulfonated napththalene azo dyes and xanthene or fluorescein dyes. The structure of these dyes are given in Fig. 18.

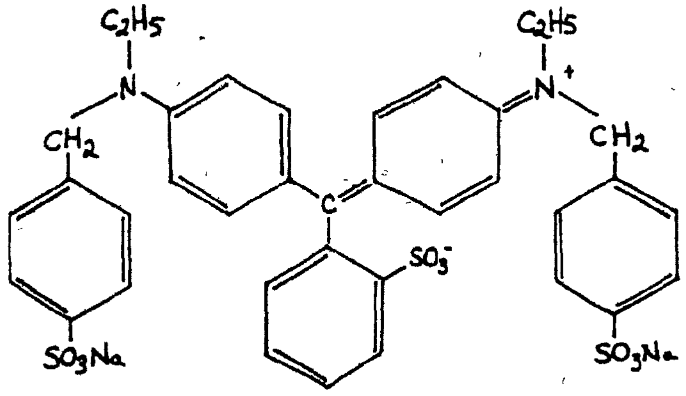
The triphenyl methane dyes are highly water soluble (Radomski, 1974). In general, water soluble food dyes are less toxic than fat



PONCEAU SX



ERYTHROSINE



BRILLIANT BLUE FCF

Figure 18. Structures of Ponceau SX, Erythrosine, and Brilliant Blue FCF.

soluble food dyes (Hansen et al. 1966). Radomski (1974) indicated that due to the acidic nature of this dye group, these dyes are poorly absorbed after oral administration and are excreted, unchanged in the faeces; Hansen et al. (1966) reported recoveries of 60-73% for these dyes. Brilliant Blue FCF is one of the least toxic dyes in this group (Radomski, 1974; Hansen et al., 1966).

The sulfonated naphthalene azo dyes are more toxic. Bowie et al. (1965) related the toxicity of the azo dyes to the metabolic products of reductive fission of the azo linkage caused by the azo reductase of intestinal bacteria. Rowland (1981) indicated that this ability to reduce azo dyes is widely distributed amongst intestinal bacteria including Streptococcus faecalis, Proteus, and Bacteroides fragilis ss. thetaiotaomicron. According to Radomski (1974) the toxicity of the metabolites is related to their structure. Sulfonated fission products which are poorly absorbed, are less toxic than the unsulfonated fission metabolites. Ponceau SX is slightly more toxic than Amaranth (FD and C Red#2). The toxicity and carcinogenicity of these dyes may be due to impurities (Radomski, 1974). Khanna et al. (1980) indicated that α- and β-naphthylamines, benzidine and 4-aminobiphenyl, considered to be carcinogens (Merck Index 1976) have been detected in certain azo dyes.

According to Radomski (1974) Erythrosine is one of the more non-toxic food colours, possibly due to the fact that most of the dye is excreted unchanged. It has been suggested, however, that Erythrosine plays a major role in the etiology of hyperkinesis or minimal brain dysfunction (Weiss et al., 1980; Lafferman and Silbergeld, 1979). Augustine and Levitan (1980) demonstrated that Erythrosine, in low doses, caused an irreversible increase in synaptic transmission at the neuromuscular synapse.

In Canada, the maximum permissible levels for Brilliant Blue FCF, Ponceau SX and Erythrosine are 100 ppm, 150 ppm, and 300 ppm, respectively. The binding studies were carried out at two levels of concentration namely, 50 ppm and the maximum permissible ppm for each dye. The studies were conducted at pH 4.8, pH 6.5, and pH 8.2; these reactions were chosen to represent the upper,mean and lower levels of acidity of the human small intestine (Weast, 1976). Coulson (1980) reported that Erythrosine precipitates as its free acid below pH 4.0. A preliminary study with the food dyes in our laboratories revealed that Erythrosine precipitated from a 300 ppm solution at pH 4.8. It is for this reason that this dye-buffer combination of Erythrosine (300 ppm, pH 4.8) was not used in our studies. The results of these experiments are reported in Tables 23-28.

The present work indicated that the binding of dye to all test samples was affected by the concentration of dye solution that was used. Within each dye group, the neutral detergent residue and Cellulose MN 300 gave the largest increases in adsorption with increasing dye concentration, with the exception of Ponceau SX at pH 8.2. These results conflict with those reported by Takeda and Kiriyama (1979) who used Amaranth. The authors demonstrated that only ADF and NDF bound more dye as the dye concentration increased.

The effect of pH on the dye binding was different for each dye. The binding of Erythrosine decreased with increasing pH at both levels of concentration. Ponceau SX was bound most strongly at pH 6.5 at both

TABLE 23

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[1,2]		
		Erythrosine	Ponceau SX	Brilliant Blue
Apple Core Fibre	0.47	3.40	0.77	0.94
Acid Detergent Fibre	0.05	2.73	0.91	0.35
Neutral Detergent Fibre	0.14	2.05	0.11	0.18
Cellulose MN 300	-	1.56	0.07	0.12

1. Concentration of Dye Solution - 50 ppm
2. Reaction of Dye Solution - pH 4.8

TABLE 24

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[4]		
		Erythrosine[1]	Ponceau SX[2]	Brilliant Blue[3]
Apple Core Fibre	0.47	-	2.18	1.67
Acid Detergent Fibre	0.05	-	2.28	0.54
Neutral Detergent Fibre	0.14	-	0.89	0.27
Cellulose MN 300	-	-	0.88	0.18

1. Concentration of Erythrosine - 300 ppm (Maximum permissible level in Canada)
2. Concentration of Ponceau SX - 150 ppm (Maximum permissible level in Canada)
3. Concentration of Brilliant Blue FCF - 100 ppm (Maximum permissible level in Canada)
4. Reaction of solution - pH 4.8

TABLE 25

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[1,2]		
		Erythrosine	Ponceau SX	Brilliant Blue
Apple Core Fibre	0.47	2.67	0.61	0.34
Acid Detergent Fibre	0.05	2.15	1.19	0.44
Neutral Detergent Fibre	0.14	1.31	0.31	0.07
Cellulose MN 300	-	1.47	0.48	-0.10

1. Concentration of Dye Solution - 50 ppm
2. Reaction of Dye Solution - pH 6.5

TABLE 26

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[4]		
		Erythrosine[1]	Ponceau SX[2]	Brilliant Blue[3]
Apple Core Fibre	0.47	13.49	1.58	0.63
Acid Detergent Fibre	0.05	9.34	2.67	0.70
Neutral Detergent Fibre	0.14	7.96	1.20	0.31
Cellulose MN 300	-	9.02	1.24	0.23

1. Concentration of Erythrosine - 300 ppm (Maximum permissible level in Canada)
2. Concentration of Ponceau SX - 150 ppm (Maximum permissible level in Canada)
3. Concentration of Brilliant Blue FCF - 100 ppm (Maximum permissible level in Canada)
4. Reaction of Solution - pH 6.5

TABLE 27

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[1,2]		
		Erythrosine	Ponceau SX	Brilliant Blue
Apple Core Fibre	0.47	1.33	-0.02	0.04
Acid Detergent Fibre	0.05	1.10	0.48	0.12
Neutral Detergent Fibre	0.14	0.55	-0.06	-0.02
Cellulose MN 300	-	0.43	-0.38	-0.02

1. Concentration of Dye Solution - 50 ppm
2. Reaction of Dye Solution - pH 8.2

TABLE 28

Cation Exchange Capacity and Dye Binding Capacity of Fibre Samples

Test Substance	Cation Exchange Capacity (meq/gm fibre)	Amounts of Dye Adsorbed (mg adsorbed/gm fibre)[4]		
		Erythrosine[1]	Ponceau SX[2]	Brilliant Blue[3]
Apple Core Fibre	0.47	5.94	0.17	0.12
Acid Detergent Fibre	0.05	4.35	0.89	0.31
Neutral Detergent Fibre	0.14	3.28	0.08	0.02
Cellulose MN 300	-	2.50	-0.37	0.10

1. Concentration of Erythrosine - 300 ppm (Maximum permissible level in Canada)
2. Concentration of Ponceau SX - 150 ppm (Maximum permissible level in Canada)
3. Concentration of Brilliant Blue FCF - 100 ppm (Maximum permissible level in Canada)
4. Reaction of Solution - pH 8.2

levels of concentration; apple core fibre bound this dye most strongly at pH 4.8. Brilliant Blue FCF, at 50 ppm, was bound most strongly at pH 4.8 with the exception of the ADF which bound best at pH 6.5. Brilliant Blue FCF, at 100 ppm, was bound most strongly at pH 6.5 with the exception of the apple core fibre which bound most strongly at pH 4.8.

The dye binding capacity of each fibre sample varied with pH and type of dye. The binding capacity of the various materials, using Erythrosine at pH 4.8 and pH 8.2 was in decreasing order, apple core fibre, ADF, NDF, Cellulose MN 300. The Cellulose MN 300 bound more dye than the NDF at pH 6.5. The order of binding with Ponceau SX at pH 4.8 and pH 8.2 was ADF > apple core fibre > NDF > Cellulose MN 300. Cellulose MN 300 bound a slightly greater amount of Ponceau SX than did NDF at pH 6.5. The order of binding at pH 4.8 with Brilliant Blue FCF was apple core fibre > ADF > NDF > Cellulose MN 300 while at pH 6.5 and pH 8.2 the order was ADF > apple core fibre > NDF > Cellulose MN 300.

The results indicated that the capacity of a substance to bind dye is not related to the cation exchange capacity since in many instances; ADF bound more dye than apple core fibre and always bound more dye than the NDF residue, even though its CEC is much smaller than that of apple core fibre or NDF. The capacity of ADF to bind large amounts of dye was noted by Takeda and Kiriyama (1979).

It is possible that dye binding is related to the anion exchange capacity of the fibre samples. The anion exchange capacity is related to the lignin, pectin, Maillard polymers and the protein-tannin complexes (Van Soest, 1978; Robertson and Van Soest, 1981), ADF contains lignin, cellulose, heat-damaged protein and protein-tannin complexes, precipitated pectins and Maillard polymers (Goering and Van Soest, 1970; Robertson and Van Soest, 1981). NDF contains lignin, cellulose, hemicellulose and cell wall protein. ADF would therefore contain more active components than NDF. One may assume that apple core fibre does not contain the Maillard polymers or the protein-tannin complexes; its lignin content is about half that of the ADF.

Dye binding by fibre is difficult to measure accurately. It will be noted that in certain instances the concentration of dye in the solution increased after the addition of the fibre sample (Note negative values reported in Tables 25, 27, 28). This could result from a situation in which more water was bound by the sample than was the dye. This would lead to an increase in the concentration of the dye in the solution. In those instances where the amount of water bound to the fibre is as great or greater than the amount of bound dye, there is no way of measuring the dye binding capacity of the fibre unless a correction can be made for the bound water. Karel (1975) described several methods for the measurement of bound water in foods including wideline proton-magnetic resonance and differential thermal analysis. To the author's knowledge, there are no published methods for the measurement of bound water in fibre preparations.

Selvendran (1979) suggested that decreased binding may be caused by anions (citrate, phosphate) which are preferentially adsorbed to the fibre samples. It is reasonable to assume therefore that the buffers which were used in the present work, could have influenced the dye binding of the test materials. Selvendran (1979) indicated that binding of sodium cholate was influenced by the degree of ionization of the acid groups of fibre samples. The author suggested that the smaller the number of ionized groups in the fibre, the better was the binding capacity. It will be noted from the pK values of apple core fibre, NDF and ADF (Section III.E., p.64) that at pH 4.8, more than half of the ionizable groups of apple core fibre and NDF would be ionized, while less than half of the ionizable groups in ADF would be ionized. This cannot fully account for the difference in bound dye that was noted because one would expect that the amount of ionizable groups would be very low in the ADF compared to the apple core fibre. Selvendran (1979) stated that there must be other factors, such as conformation of fibre components, which affect the binding of dye to fibre.

SUMMARY

(1) A procedure was developed for the preparation of apple core fibre. Core tissue was removed from apples (Cortland) and extracted sequentially with antioxidant solution, 80/20 (v/v) absolute methanol-antioxidant solution, EDTA solution, water, acetone and ether.

(2) The physical properties of the apple core fibre were examined. Particle size distribution revealed the average particle size to be 307 µm. The water holding capacity of the fibre preparation was determined by a filtration method and a method involving decantation and freeze-drying. The settling volume and water saturation capacity were also determined. The acid and neutral detergent residues of the apple core fibre were prepared and their water holding capacity, settling volume and water saturation capacity were determined. It was found that while the water holding capacities of the acid and neutral detergent residues were lower than that of the apple core fibre, the water saturation capacity of the neutral detergent residue did not differ markedly from that of the apple core fibre.

The cation exchange capacity of the apple core fibre and its detergent residues, were examined and shown to decrease in the order apple core fibre, NDF, ADF.

(3) The structure of apple core fibre, the acid and neutral detergent residues and the residue from the pectin determination of apple core fibre were observed under the scanning electron microscope. It was shown that the cell walls were altered by the various extraction techniques.

(4) The apple core fibre was subjected to acid hydrolysis using 5% and 72% sulfuric acid. The hydrolysates were analyzed for neutral sugar content and uronic acid content. The sugars present in the hydrolysates were identified by cellulose thin layer chromatography. The sugars were determined using gas liquid chromatography. The apple core fibre comprises 70.8% neutral sugars. The uronic acids present in the hydrolysates were measured using (a) the modified carbazole method that involved several correction factors, and (b) the m-hydroxy biphenyl method. The uronic acid content of the apple core fibre is 11%.

(5) The dye binding capacity of the apple core fibre and its acid and neutral detergent residues, was examined. The dye binding capacity was shown to be dependent on the concentration of dye solution. The neutral detergent residue demonstrated the largest increase in dye binding with increasing dye concentration. The effect of pH on the dye binding capacity was different for each dye. Experiments indicated that dye binding capacity was unrelated to the cation exchange capacity of the fibre.

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