EFFECT OF CULTIVAR AND SEQUENTIAL ETHANOL PRECIPITATION ON THE PHYSICOCHEMICAL PROPERTIES OF FLAXSEED MUCILAGE

By

Tricia Laini Chornick

A Thesis
Submitted to the Faculty of Graduate Studies
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FACULTY OF GRADUATE STUDIES

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ABSTRACT

Flaxseed mucilage is an excellent source of dietary fiber and due to its highly viscous nature also shows potential for being used as a food gum. Flaxseed mucilage is a complex mixture of two polysaccharides which differ in monosaccharide composition, molecular size, structural conformation, and rheological properties. Further investigation on the physicochemical properties of these two polysaccharides is required and only limited information is available on the composition and rheological properties of mucilage extracted from flaxseed cultivars grown in Canada.

Sequential ethanol precipitation is a fractionation technique that has been used successfully for separating mixtures of proteins and polysaccharides. It was therefore believed that it may be a useful technique for extracting and separating the mixture of polysaccharides present in flaxseed. The first objective of this study was to determine whether sequential ethanol precipitation is an effective method for separating the neutral and acidic polysaccharides from flaxseed mucilage. The second objective of this study was to examine the effect cultivar has on the composition, rheological, and emulsifying properties of mucilage extracted from seven cultivars commonly grown in Canada.

Complete mucilage precipitation was carried out by adjusting the aqueous extract to 75% ethanol (UNF) and additional fractions were collected at 40% ethanol (40F) and 60% ethanol (60F) using sequential ethanol precipitation. The intrinsic viscosity values varied among the three mucilage extracts and the average molecular weight of the polymers present in the 40F extract was higher than those present in the 60F extract. The 40F and UNF

extracts exhibited more pronounced shear thinning and weak-gel behavior compared to the 60F extract. The 40F extract also exhibited superior emulsion stability properties compared to the UNF and 60F extracts. Differences in the monosaccharide composition of the extracts was also found but complete separation of the two polysaccharide fractions was not achieved. Nevertheless, sequential ethanol precipitation of flaxseed mucilage may be an effective method for obtaining polysaccharide fractions with different physicochemical characteristics.

Cultivar appeared to influence the carbohydrate and protein content as well as monosaccharide composition of the three mucilage extracts. Although cultivars containing higher levels of xylose (major sugar of the neutral polysaccharide) tended to exhibit increased viscosity and weak-gel properties, this parameter alone could not fully account for the variation in the rheological behavior observed among the cultivars. Intrinsic viscosity values and rates of shear thinning also varied among the cultivars which suggests that differences in molecular weight and structural conformation of the extracted polysaccharides likely existed. In general, those cultivars which exhibited higher intrinsic viscosity values showed the greatest potential for stabilizing an oil-in-water emulsion. The diversity observed among the seven cultivars provides evidence that genetic variability exists in the physicochemical properties of flaxseed mucilage. This variability may allow plant breeders to develop flaxseed cultivars that contain mucilage with rheological and functional characteristics for specific end uses.

1. INTRODUCTION

Flaxseed mucilage is an excellent source of dietary fiber and due to its highly viscous nature, also shows potential for being used as a food gum (Cui and Mazza, 1996; Mazza and Biliaderis, 1989). It has been suggested that flaxseed mucilage could be used as a thickening agent or emulsion stabilizer (Fedeniuk, 1993; Wannerberger et al., 1991; Mazza and Biliaderis, 1989; BeMiller, 1973), but information on the functionality of flaxseed mucilage remains quite limited. The functionality of a food gum is affected by a number of factors including: molecular size, structure, orientation and molecular association of the polymers, water binding and swelling, concentration, particle size, and degree of dispersion (Ward and Andon, 1993). As a result, detailed studies which examine the structure-property relationships of a potential food gum like flaxseed mucilage are required in order to fully understand and elucidate its functional role as a food ingredient.

Flaxseed mucilage is composed primarily of two polysaccharides: a neutral arabinoxylan-like polymer and an acidic pectic-like polymer (Cui et al., 1994a; Muralikrishna et al., 1987). Previous studies have reported that the composition and rheological properties of flaxseed mucilage can vary among flaxseed cultivars (Cui and Mazza, 1996; Cui et al., 1996; Oomah et al., 1995; Wannerberger et al., 1991). It has been reported that the monosaccharide composition of the mucilage has a direct effect on its rheological properties in an aqueous solution. In general, cultivars having higher levels of neutral polysaccharides exhibit higher apparent viscosity and stronger gel-like properties (Cui et al., 1996; Wannerberger et al., 1991). Other studies have examined the functionality of flaxseed

mucilage to some degree or have characterized its structure, composition and rheological properties to a greater extent by separating the neutral and acidic polysaccharides through various fractionation techniques. Ion exchange chromatography (Cui et al., 1994a) and selective precipitation with cetyltrimethylammonium bromide (CTAB) (Fedeniuk and Biliaderis, 1994; Hunt and Jones, 1962) have been used to separate the two polysaccharides based on differences in charge, but complete separation was often difficult to achieve. Analysis of the two polysaccharide fractions revealed that they differed in monosaccharide composition, molecular size, structural conformation and rheological properties (Cui et al., 1994a; Fedeniuk and Biliaderis, 1994; Muralikrishna et al., 1987), but limited information is available on the functional characteristics of these two fractions and in both cases only single cultivars were examined. Testing the functionality of the mucilage along with the composition and rheological properties has not yet been carried out on a number of cultivars. This type of study is required in order to determine what effect these factors may have on the functional characteristics of flaxseed mucilage. In addition, there is limited information available on the composition and rheological properties of flaxseed mucilage extracted from cultivars that are currently grown in Canada.

The use of CTAB and ion exchange chromatography for separation of the two polysaccharide fractions found in flaxseed mucilage tends to be labor intensive, tedious, and time consuming. Sequential ethanol precipitation is a fractionation technique that has been used for separating mixtures of proteins and polysaccharides. This technique does not require the use of a lot of chemicals other than ethanol, is not labor intensive, does not require the preparation and maintenance of packed columns, can be carried out in both large and small

scale, and requires very little clean-up of the fractions. Given that the two polysaccharides present in flaxseed mucilage differ in molecular size and structural conformation it is expected that sequential ethanol precipitation would be an effective fractionation technique. To determine how well this technique is able to separate the mixture of polysaccharides present in flaxseed mucilage the chemical composition, molecular size, rheological and functional properties of the resulting mucilage extracts could be examined. Depending on the effectiveness of this technique it may be beneficial to use sequential ethanol precipitation when examining the effect cultivar has on the physicochemical properties of flaxseed mucilage.

In view of the above considerations, the objectives of this study were:

1. To determine whether sequential ethanol precipitation is an effective method for separating the neutral and acidic polysaccharides from flaxseed mucilage.
2. To determine the effect cultivar has on the composition, rheological and emulsifying properties of mucilage extracted from cultivars commonly grown in Canada and to determine whether relationships exist among these parameters.

2. LITERATURE REVIEW

2.1. Composition and Characteristics of Flaxseed

Canada has been the world leader in the production and export of flaxseed (*Linum usitatissimum L.*) since 1994, with exports going mainly to Europe, the United States, Japan and South Korea (Flax Council of Canada, 2000). Traditionally, flaxseed was cultivated mainly for its oil to be incorporated into paints, varnishes and linoleum, and its fiber to be used in the production of linen and paper. Although flax is still being grown for these purposes, recently, renewed interest has been shown for the use of flaxseed as a food ingredient. As a result of this renewed interest, the nutritional and functional properties of flaxseed and its components have been examined more closely.

According to Statistics Canada the total seeded area for flaxseed in Canada for 2001 was 662 900 hectares which yielded approximately 702 300 tonnes of flaxseed (Statistics Canada, 2002). It has been found that the composition of flaxseed can vary due to differences in genotype, growing conditions, environment, seed processing and analytical methods (Vaisey-Genser and Morris, 1997a). In a survey of flaxseed cultivars grown in Western Canada in the year 2000, the average oil and protein content expressed as a dry weight basis (dwb) was 44% and 23%, respectively (Daun and DeClercq, 2001). Flaxseed is also a good source of both soluble and insoluble dietary fiber and in total it accounts for approximately 28% of the dry seed weight. The ratio of soluble to insoluble fiber ranges from 20:80 to 40:60 depending on the methods of extraction and chemical analysis used (Vaisey-Genser and Morris, 1997a). A mature flaxseed consists of a hull and embryo tissue, where the hull

constitutes about 40% of the total seed (Oomah et al., 1996). The seed coat (hull) consists of four layers and the outer most layer (epidermal layer) of the seed contains the soluble fiber portion of flaxseed also known as mucilage (Mazza and Biliaderis, 1989). The embryo tissue consists of an endosperm layer and the cotyledons. While the outer hull contains no oil and protein, some oil and protein can be found in the endosperm layer, but the highest concentration of these two components is found in the cotyledons (Oomah et al., 1996). Given that the mucilage is present in the outer most layer of the hull, attempts have been made to mechanically separate the hull from the embryo tissue in order to get a fraction that is concentrated with the mucilage and crude fiber (Tostenson et al., 2000; Oomah and Mazza, 1997; Oomah et al., 1996). One of the benefits of being able to obtain a fraction such as this would be for its use in human nutrition studies, to examine the beneficial physiological benefits of flax components that tend to be concentrated in the hull portion of the flaxseed (mucilage, lignans, crude fiber). Results from the fractionation studies suggest that a fairly good separation can be obtained, but given that the endosperm adheres tightly to the hull, separation is difficult, and it is not uncommon to have an oil content of greater than 20% in the hull fraction (Tostenson et al., 2000). Although some progress has been made in the area of mechanical fractionation, further work is required in order to improve the effectiveness and efficiency of the process. Flaxseed mucilage is a water soluble gum, therefore the most common method used for its isolation is an aqueous extraction procedure followed by ethanol precipitation. The mucilage comprises approximately 8% of the seed weight, but its yield depends greatly on the method used for extraction (Oomah et al., 1995) and varies among cultivars (Oomah et al., 1995; Cui et al., 1996).

2.2. Food Hydrocolloids

2.2.1. Description and Applications for the Food Industry

Food hydrocolloids, also known as food gums have been described as being complex nondigestible polysaccharides (complex carbohydrates) which dissolve or disperse in water to give a thickening or viscosity-building effect (Anderson and Andon, 1988; Dziezak, 1991). Food gums can be extracted from numerous sources including plants, trees, seeds and marine life or synthesized biologically or chemically (Sharma, 1981; Anderson and Andon, 1988). Flaxseed mucilage is an example of a gum that can be extracted from seeds, but at this time it is not a process that is being carried out commercially. It has been suggested that there is potential for flaxseed mucilage to be used as a food gum as a result of its thickening and emulsifying properties (BeMiller, 1973; Wannerberger et al., 1991; Fedeniuk, 1993). It appears as though some of the functional properties of flaxseed mucilage are similar to those of gum arabic and it is possible that flaxseed gum may be able to replace gum arabic as an emulsion stabilizer (BeMiller, 1973). One advantage flaxseed gum has over some of the commercial gums is its relatively low cost and stable world-wide supply (Cui and Mazza, 1996).

Gums are used widely in the food industry at relatively low concentrations (0.1% to 1.2%) in order to achieve a desired effect. Depending on the application, a gum can be added to a food product to act as an adhesive, emulsifier, foam stabilizer, binding agent, clarifying agent, coating agent, gelling agent, swelling agent, suspending agent, thickening agent and whipping agent (Sharma, 1981; Glicksman, 1982a). They may also be used to control crystallization, inhibit syneresis (the release of water from fabricated foods), or for

encapsulation and formation of films (Dziezak, 1991). Commercially there are numerous food gums available, but as a result of their differing structural and functional properties certain gums are used for specific applications and careful selection is required in order to obtain the desired effect. The functionality of a food gum is affected by many factors, some of which include molecular size, orientation and molecular association of the polymers, water binding and swelling, concentration, particle size and degree of dispersion (Ward and Andon, 1993). As a result, detailed studies which examine the structure-property relationships of a potential food gum like flaxseed mucilage are required in order to fully understand and elucidate its functional role as a food ingredient.

2.2.2. Nutritional Benefits

Food hydrocolloids are a good source of soluble dietary fiber (Ward and Andon, 1993). The health benefits of dietary fiber are well documented and over the last number of years consumers have become more aware of the important role dietary fiber plays in the human diet (Olson et al., 1987). It has been shown that certain forms of soluble dietary fiber promote beneficial physiological effects which include lowering the cholesterol/triglyceride lipid level in the blood and/or blood glucose attenuation (Glicksman, 1982b; Anonymous, 1984; Ink and Hurt, 1987; Rickard and Thompson, 1997; DFSRC, 2000).

Bile salts are synthesized in the liver from cholesterol and are used to solubilize and disperse dietary lipids. It is believed that some hydrocolloids have the ability to bind bile salts. This action prevents the bile salts from being recycled back to the liver and as a result the body is forced to synthesize more, leading to a cholesterol lowering effect (Glicksman,

1982b).

Studies have shown that the presence of viscous compounds such as gums, pectins, and some hemicelluloses in the small intestine slows down the digestion and absorption process of carbohydrates, in particular starch (Olson et al., 1987; Coultate, 1989). This causes a reduction in the rate of glucose flowing into the bloodstream after a carbohydrate-rich meal, as well as reducing the insulin and other endocrine responses (Rickard and Thompson, 1997). As a result of these findings, it has been suggested that water-soluble fibers should be incorporated into the diets of people with Type 2 diabetes in an attempt to lower post-prandial blood glucose levels (Ink and Hurt, 1987).

Cunnane et al. (1993) investigated the effect of milled flaxseed (oil removed) and flaxseed mucilage on reducing the post-prandial blood glucose response of people without diabetes. Within 60 minutes of consuming the flaxseed bread test meal containing milled flaxseed (contained 1.5 to 2 g of mucilage), the area under the blood glucose curve was 28% lower than what was observed after the control white bread test meal ($p < 0.01$). A similar response (27%) was observed when a solution containing 25 g flaxseed mucilage and 50 g of glucose was consumed. From this study it would appear that flaxseed mucilage does have a positive effect in lowering the blood glucose response of people without diabetes, but whether soluble fiber is the only component of flaxseed responsible for the reduction remains unresolved at the present time.

2.2.3 Rheological and Molecular Characteristics

2.2.3.1. Intrinsic Viscosity and Molecular Weight

Intrinsic viscosity is an important characteristic of a food hydrocolloid which can be used to compare the viscosities of dilute polysaccharide solutions. The intrinsic viscosity given by a particular polysaccharide is a reflection of its size or extension in space which is also referred to as its hydrodynamic volume (Billmeyer, 1984a). Molecules which take up more space in a solution are said to have a higher hydrodynamic volume and a greater viscosity building effect. The following equations can be used to measure the viscosity of a dilute polysaccharide solution (Launay et al., 1986).

$$\eta_{sp} = (\eta - \eta_o) / \eta_o$$

$$\eta_{red} = \eta_{sp} / c$$

Where:

η_{sp} is the specific viscosity

η is the solution viscosity

η_o is the solvent viscosity

η_{red} is the reduced viscosity (dl/g)

c is the concentration (g/dl) of the polysaccharide in the solvent

These viscosity measurements of dilute polymer solutions are usually carried out in capillary viscometers. Measurements of solution viscosity are made by comparing the times required for a specified volume of polymer solution and solvent to flow through the capillary. The following equation is used to obtain the specific viscosity of a polysaccharide solution (Billmeyer, 1984a):

$$\eta_{sp} = (t - t_o) / t_o$$

Where:

t is the time in seconds required for the polymer solution to flow through the capillary

t_o is the time in seconds required for the solvent to flow through the capillary

After calculating the reduced viscosity of a polymer solution at several concentrations, these values are plotted against concentration. The sample concentration can not be too large because additional effects may then arise from intermolecular forces and entanglements between polymers chains. Using the Huggins equation (Huggins, 1942) the data can be extrapolated to infinite dilution in order to obtain the intrinsic viscosity (dl/g) of a given polymer. Intrinsic viscosity is independent of concentration due to the fact that the data is extrapolated to c = 0 using the Huggins equation (Billmeyer, 1984a):

$$\eta_{sp} / c = [\eta] + k'[\eta]^2 c$$

Where:

$[\eta]$ is intrinsic viscosity (dl/g)

k' is a constant for a series of polymers of different molecular weights

in a given solvent

c is the concentration (g/dl) polysaccharide in the solvent

The intrinsic viscosity of a polymer depends primarily on molecular weight, chain rigidity and solvent quality (Launay et al., 1986). For polydisperse linear polymers an empirical relationship between viscosity and average molecular weight is given by the Mark-Houwink equation (Morris, 1984):

$$[\eta] = KM^a$$

Where:

K and a are constants for a particular polymer-solvent system which depend on the shape of the polymer, the solvent used and the temperature of the measurement

M is the molecular weight

The constants K and a must be determined using an absolute method for molecular weight analysis like light scattering. Once parameters K and a are known for a particular polymer-solvent system molecular weight can easily be determined from intrinsic viscosity measurements (Morris, 1984). As a result viscosity measurements are an extremely valuable tool for the molecular characterization of polymers (Billmeyer, 1984a).

2.2.3.2. Steady-Shear and Small Strain Oscillatory Flow Properties

Food hydrocolloids are used in the food industry due to their ability to modify the functional and textural properties of a food system. The degree to which a particular polysaccharide can accomplish such a task is highly dependent on its rheological properties. Rheology is defined as the science of deformation and flow of matter (Billmeyer, 1984b). Understanding the rheological properties of a polysaccharide and how these properties will effect its behavior in a given food system is essential when selecting a hydrocolloid that will satisfy the required functional properties of a given food product. Steady shear flow and small strain oscillatory measurements are two rheological methods that are commonly used to characterize the flow behavior of polysaccharide solutions.

All fluids can be classified as being either Newtonian or non-Newtonian. A

Newtonian fluid, also known as an ideal viscous fluid, is described as being incompressible and isotropic (similar throughout) with no structure or elastic properties. Newtonian behavior is shown by all gases, water, most oils, simple solutions like sugar syrups, salt brines, solutions of low molecular weight and dilute colloidal dispersions (Glicksman, 1982b; Tung 1988). All remaining fluids that do not exhibit ideal viscous behavior are classified as non-Newtonian fluids. Non-Newtonian fluids can be further classified as: time-independent, time-dependent, or viscoelastic fluids, but due to the complex nature of these fluids they often fall into more than one of these categories (Tung, 1988).

The basic rheological property which characterizes the flow behavior of a liquid system is that of viscosity, also referred to as internal friction or resistance to flow (Glicksman, 1982b). Viscosity is defined as the proportionality constant between shear stress and shear rate (Tung 1988):

$$\eta = \sigma/\gamma$$

Where:

η is viscosity (Pa·s)

σ is shear stress, the force per unit area of the fluid plane (Pa)

γ is shear rate, the velocity difference per unit thickness of the fluid (s^{-1})

Steady shear flow curves are obtained by measuring the viscosity of a polysaccharide solution as a function of shear rate. As Figure 2.1 shows, the steady shear rheological flow curves for a Newtonian and non-Newtonian fluid are distinctly different from each other. For Newtonian fluids shear stress is directly proportional to shear rate therefore viscosity is constant under changing conditions of shear (Glicksman, 1982b). On the other hand, for non-

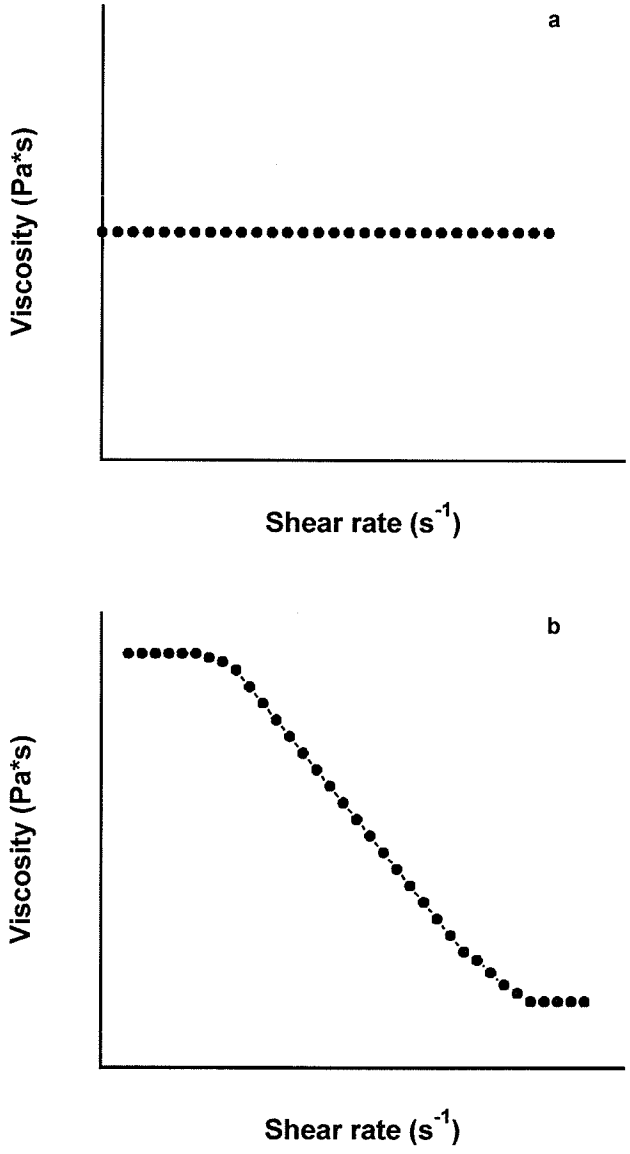

Figure 2.1. Typical steady shear rheological flow curves for (a) a Newtonian fluid and (b) a non-Newtonian fluid.

Newtonian fluids the ratio of shear stress to shear rate does not remain constant and the resulting viscosity is referred to as apparent viscosity (Figure 2.2). For time-dependent flow the resulting shear stress depends on the magnitude and duration of shear, whereas for time-independent flow, shear stress is only dependent on shear rate. Most food gums including flaxseed mucilage fall within the time-independent classification.

The most common type of non-Newtonian behavior is pseudoplastic flow, also known as shear thinning, where the fluid exhibits a decrease in apparent viscosity as the shear rate increases (Glicksman, 1982b; Tung, 1988). The reason why shear thinning is observed is related to the nature of the polysaccharides. At low shear rates the polysaccharide polymers in a concentrated solution are in a dynamic entangled network structure which gives rise to high apparent viscosities. As the shear rate increases the rate of externally imposed movement is greater than the rate of forming new entanglements so the polymers become aligned into a shear orientated structure. This leads to a decrease in resistance to flow which is observed as a decrease in apparent viscosity (Tung, 1988). Shear thinning is an reversible process, the polymers return to a disorientated network once the imposed shear force is removed. At extremely high and low shear rates many non-Newtonian systems approach Newtonian behavior, it is only at the intermediate shear rates that shear thinning is observed (Glicksman, 1982b). Differences in shear thinning behavior among polysaccharide solutions can be quantified using the power law model (Launay et al, 1986; Fedeniuk and Biliaderis, 1994; Medina-Torres et al., 2000):

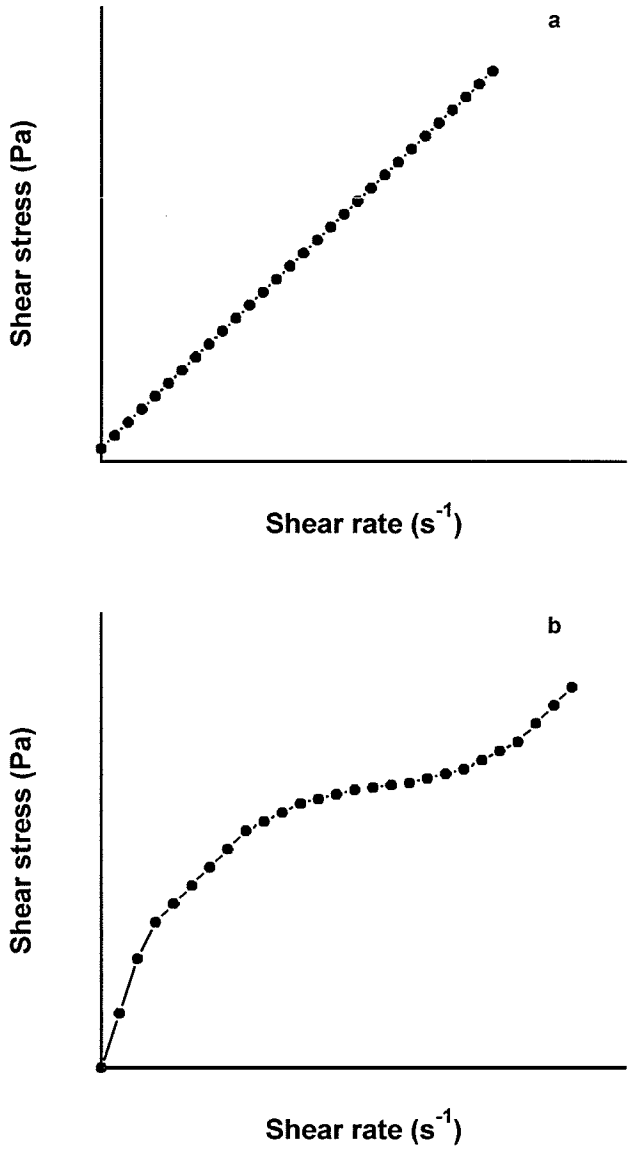

Figure 2.2. Relationship of shear stress vs. shear rate for (a) a Newtonian fluid and (b) a non-Newtonian fluid.

$$\eta = m\gamma^{n-1}$$

Where:

η is apparent viscosity (Pa·s)

γ is shear rate (s^{-1})

n is the flow behavior index

m is the consistency coefficient

For a given set of viscometric data the parameters n and m may be evaluated by plotting logη vs logγ.

$$\log \eta = \log m + (n-1) \log \gamma$$

For polysaccharide solutions exhibiting shear thinning behavior the flow behavior index (n) is always less than one. The effect concentration has on n has been studied for a number of food gums and in general it was found that as the concentration increases the value for n decreases meaning that the solution exhibits a higher degree of shear thinning (Launay et al., 1986). Mazza and Biliaderis (1989) and Cui et al. (1994a) observed similar findings for flaxseed mucilage. As the concentration decreased, the steady shear flow curves exhibited less shear thinning and started to behave more like a Newtonian fluid. A lower degree of shear thinning is observed for dilute polymer concentrations since the polymers are widely separated and do not interact to any significant effect. The frequent collisions and mutual interferences that are normally apparent among polymers in a concentrated solution no longer exist and the shear rate does not have as great of an effect on the apparent viscosity (Glicksman, 1982b). The concentration at which a polysaccharide solution will start to show Newtonian behavior over non-Newtonian behavior varies among the polysaccharides due to

differences in molecular size and structural conformation.

Small strain oscillatory testing (mechanical spectroscopy) is used to characterize the rheological properties of viscoelastic fluids. The viscoelastic aspect of rheological behavior becomes important in situations of unsteady motion; such that the stresses and strains are changing with time (Tung, 1988). A viscoelastic fluid is one that possesses both viscous and elastic properties. As shown in the equation given below the stress exhibited by a viscoelastic fluid is made up of two components, a viscous portion and an elastic portion.

$$\sigma = \gamma_o [G' \sin(\omega t) + G'' \cos(\omega t)]$$

Where:

σ is the stress exhibited by a viscoelastic fluid (Pa)

γ_o is the maximum strain amplitude

ω is the oscillatory frequency (rad/s)

t is time (s)

G' is the storage (elastic) modulus, a measure of the energy recovered per cycle of sinusoidal shear deformation (Pa)

G" is the loss (viscous) modulus, a measure of the energy dissipated as heat per cycle of sinusoidal shear deformation (Pa)

Using small strain oscillatory testing, G' and G" can be measured as a function of frequency. The resulting mechanical spectra can then be used to determine whether the material being tested is behaving as a strong gel, a weak gel, a concentrated polymer solution or a dilute polymer solution. For a strong gel, G' is ten times greater than G" over the entire frequency range examined and both moduli are essentially independent of frequency, as one would

expect for a perfectly elastic network (Tung, 1988). If G' is greater than G" over the entire frequency range but is less than ten times greater than G" the material is characterized as being a weak gel. In a weak gel system, G' and G" show a slight dependency on frequency. For a concentrated polymer solution, G" is greater than G' at lower frequencies and as the frequency increases the reverse is observed and the material exhibits more solid-like characteristics. For dilute polymer solutions, G" remains superior to G' over the entire frequency range indicating that the material is showing mainly viscous properties instead of a tendency to form a gel (Medina-Torres et al., 2000). However, as the frequency increases G' does start to approach G". The overall response of a viscoelastic fluid can be characterized by the complex modulus G*. Using G*, another parameter known as dynamic viscosity can be calculated and plotted as a function of frequency along with G' and G" (Morris, 1984):

$$\eta^* = G^* / \omega$$

Where:

η^* is the dynamic viscosity (Pa·s)

G* is the complex modulus and equal to $(G'^2 + G''^2)^{1/2}$ (Pa)

ω is the frequency (rad/s)

In addition, the ratio of G" to G' is often calculated and used as a measure of the energy lost compared to energy stored. This ratio is known as the loss tangent (Tung, 1988). As a polymer begins to exhibit more elastic than viscous behavior, the loss tangent starts to decrease.

2.2.4. Functional Properties

2.2.4.1. Emulsifying Ability

An emulsion is a two-phase system consisting of two immiscible liquids, one being dispersed as finite globules within the other (Glicksman, 1982b). These systems do not have a high degree of stability therefore, emulsifiers and/or stabilizers are generally added to prevent the breakdown of the emulsion (DeMan, 1976). In any emulsion the suspended droplets are referred to as the dispersed phase while the medium in which they are suspended is known as the continuous phase (St. Angelo, 1989). There are two types of emulsions that are commonly found in the food industry: the oil-in-water (O/W) emulsion where the oil phase is dispersed in small droplets throughout the water phase, and the water-in-oil (W/O) emulsion where the water is dispersed in small droplets throughout the oil phase (Glicksman, 1982b).

An emulsifier is described as a molecule that contains two distinct sections, one having polar or hydrophillic character, the other having nonpolar or hydrophobic properties (DeMan, 1976). Given its structure, an emulsifier has the ability to adsorb at the oil-water interface and become the bridge between the two phases. This type of orientation causes a lowering of the interfacial tension between the two phases and reduces the tendency for dispersed droplets to coalesce (St. Angelo, 1989). In addition, stabilizers are often added to enhance the action of an emulsifier (DeMan, 1976).

An oil-in-water emulsion starts to break down when the oil droplets start to come together to form larger droplets; this process is referred to as either creaming (settling) or coalescing depending on whether the process is reversible. In creaming the oil droplets

aggregate and rise to the surface but in doing so they maintain their size and shape and the emulsion can be restored with gentle stirring. In contrast, the breakdown of the emulsion is irreversible when coalescence occurs. In the first stage of emulsion breakdown the dispersed droplets come together to form clusters; this process is similar to creaming but is known as flocculation. In the coalescence stage, the clusters join together to form larger droplets which eventually cause the two phases to separate (St. Angelo, 1989).

In general, most food gums do not have the ability to act as an emulsifier because they are primarily hydrophillic in nature and do not contain sufficient hydrophobic moieties to be able to adsorb at the oil-water interface (Garti and Reichman, 1994). Instead, these hydrocolloids are added to an oil-in-water emulsion system to stabilize emulsions by increasing the viscosity of the continuous phase which inhibits the rate of coalescence between the oil droplets (Gaonkar, 1991). However, there are some food gums that do contain both hydrophobic and hydrophillic groups which have been identified as possessing emulsifying properties. Research has shown that gum arabic can function as an oil-in-water emulsifier due to the presence of protein within its structure (Anderson and Andon, 1988) Protein-containing hydrocolloids are considered to be good steric stabilizers because they contain sufficient amounts of hydrophobic groups from the protein portion which act as anchoring points and they contain sufficient hydrophillic groups from the carbohydrate section which behave like stabilizing chains (Garti and Reichman, 1994). In addition, common gums like alginates and cellulose derivatives which are also amphoteric in nature have been reported to lower the interfacial tension of oil-in-water emulsions by being able to adsorb at the oil/water interface (Gaonkar, 1991).

2.2.4.2. Foam Stability

A foam, like an emulsion, is a two phase system which results when air is incorporated and finely dispersed into a continuous liquid phase (St. Angelo, 1989). Formation of stable foams is essential for the preparation of many food systems because they have a distinct effect on the appearance, texture, consistency, size, density and overall organoleptic acceptability of the final product (Glicksman, 1982b). Foams are generally formed due to the presence of surface-active foaming agents like proteins (Kitabatake and Doi, 1982). Proteins have an amphoteric nature and are able to adsorb at the air-water interface such that the polar moieties orient themselves toward the water phase and the nonpolar groups unfold towards the air phase. In general, each protein molecule exhibits different foaming properties due to the fact that their molecular characteristics vary significantly. Protein molecules that have a greater ability to rapidly adsorb, unfold, and reorient themselves at the air-water interface exhibit superior foaming properties. In a protein-based foam, the protein molecules form a continuous cohesive film which entraps air in the form of a bubble. The bubbles are separated by thin capillaries of the liquid phase where each capillary is referred to as a lamella. The main destabilizing force of a foam is the drainage of fluid from the lamella which then allows the air bubbles to aggregate. Therefore, strong, but flexible films around the air bubbles are required to produce a stable foam (Phillips et al., 1994).

Food hydrocolloids have been found to be very effective functional agents for the stabilization of a foam (Glicksman, 1982b). It is believed that polysaccharides stabilize foams by retarding the diffusion of gas and/or by acting as steric stabilizers of the films surrounding the gas bubbles (Izydorczyk et al., 1991). Like polysaccharides, even simple sugars are

considered to enhance foam stability by increasing the viscosity of the lamellar fluid. This causes a reduction in the drainage rate of the fluid in the lamella and therefore prevents or slows down the complete destruction of a foam structure (Phillips et al., 1994).

Using defatted oilseed flours as the source of surface-active protein Susheelamma (1986) tested the foam stabilizing ability of flaxseed mucilage at different concentrations. When lower concentrations were used, it was found that the foam column showed considerable expansion but it was unstable to heat. As the concentration of mucilage increased, the viscosity of the aqueous phase also increased which minimized the free expansion of the foam columns causing the foam to be more compact and heat stable.

Combinations of food gums like carrageenan, sodium alginate, guar and carboxymethylcellulose have been added to whipped topping products to allow for multipurpose functionality, freeze thaw stability and resistance to syneresis (Glicksman, 1982b). There are numerous factors and interactions that control and influence foam formation and stability therefore; the food gum or combination of food gums which will provide the greatest stability will vary depending on the type of food product being produced and the processing conditions (pumping, spreading, stirring, extrusion, heating, freezing) the foam may be subjected to.

2.3. Flaxseed Mucilage

2.3.1. Extraction and Fractionation

Flaxseed mucilage is a complex mixture of both hemicellulose and pectic-like polysaccharides. Given that both polysaccharides are water soluble, the most common

method used for the isolation of flaxseed mucilage is an aqueous extraction procedure followed by ethanol precipitation. Alcohol precipitation is not only used for the collection of the dissolved polysaccharides, but it also removes potentially toxic cyanogenic glycosides that are likely to be extracted along with the mucilage (Rickard and Thompson, 1997). A number of researchers have reported that it is more favorable to extract the mucilage from whole seed as opposed to ground meal in order to reduce the amount of protein contamination. It has been found that grinding of the seed increases the surface area and makes the proteins more available to the extraction media (Bhatty, 1993; Fedeniuk and Biliaderis, 1994). At this time, the exact contribution of soluble proteins to the viscosity of the mucilage extract is not known but it is believed the functional properties of the mucilage are undoubtedly affected by the presence of proteins (Bhatty, 1993). In addition, other factors such as temperature, pH and water to seed ratio have been shown to have an effect on the yield, purity, composition and rheological properties of the extracted mucilage (Mazza and Biliaderis, 1989; Cui et al., 1994b; Fedeniuk and Biliaderis, 1994). In response to these findings, Cui et al. (1994b) found it necessary to optimize the extraction process with respect to several parameters including yield, purity, viscosity and energy cost. Using response surface methodology, the optimum extraction conditions were determined to be: temperature 85-90°C, pH 6.5 -7.0, and a water to seed ratio of 13:1 (Cui et al., 1994b). This optimized method or slight modifications to it have been used by proceeding researchers studying the characteristics of flaxseed mucilage.

Flaxseed mucilage is primarily composed of two polysaccharides, a neutral and an acidic fraction (Muralikrishna et al., 1987; Cui et al., 1994a; Fedeniuk and Biliaderis, 1994). The neutral fraction is comprised of a (1→4)-linked β-D-xylose backbone which contains side

chains of arabinose and galactose at positions 2 and/or 3. Evidence suggests that the main chain of the acidic fraction consists of a (1→2)-linked α-L-rhamnopyranosyl and a (1→4)-linked D-galactopyranosyluronic acid residues, with side chains of fucose and galactose (Muralikrishna et al., 1987; Cui et al., 1994a). A number of researchers have set out to separate the two polysaccharides using either ion exchange chromatography or selective precipitation with cetyltrimethylammonium bromide (CTAB) in order to study the composition and physicochemical properties of the two fractions (Muralikrishna et al., 1987, Cui et al., 1994a; Fedeniuk and Biliaderis, 1994). Cui et al (1994a) extracted mucilage from the flaxseed cultivar NorMan and then proceeded to separate the two polysaccharide fractions by ion exchange chromatography. Table 2.1 shows the monosaccharide composition of the neutral and acidic fractions that were obtained. Fedeniuk and Biliaderis (1994) used selective precipitation with CTAB to fractionate the two polysaccharides. Although the monosaccharide composition of the two fractions differed such that one was enriched with sugars that make up the neutral polysaccharides while the other contained mainly monosaccharides found in the acidic polysaccharides, it was reported that complete separation of the two fractions was not possible with this method.

2.3.2. Rheological and Molecular Characteristics

The rheological behavior of both the neutral and acidic polysaccharide fractions from flaxseed mucilage obtained by ion exchange chromatography (Cui et al., 1994a) and selective precipitation with CTAB (Fedeniuk and Biliaderis, 1994) have been studied. Steady shear flow curves were obtained by measuring the apparent viscosity of the polysaccharide solution

Table 2.1. Relative monosaccharide composition (%) of neutral and acidic fractions of mucilage extracted from cv. NorMan and separated by ion exchange chromatography

Monosaccharide	Neutral Fraction	Acidic Fraction
Rhamnose	0	54.5 ± 0.2
Fucose	0	10.1 ± 0.7
Arabinose	16.2 ± 0.2	2.0 ± 0.0
Xylose	62.8 ± 5.2	5.5 ± 1.2
Galactose	7.4 ± 0.0	23.4 ± 1.5
Glucose	13.6 ± 1.1	4.5 ± 0.0

Adapted from Cui et al. (1994a)

as a function of shear rate. Cui et al. (1994a) found that at concentrations above 0.5%, shear thinning behavior was observed for the neutral mucilage fraction. At concentrations below 0.5%, the neutral fraction exhibited more of a Newtonian-like behavior where the apparent viscosity was independent of shear rate. In contrast, the flow behavior of the acidic fraction was different from the neutral fraction because it exhibited lower apparent viscosity values and no shear thinning for any of the concentrations (0.3-2.0%) tested (Cui et al., 1994a). When CTAB was used as the fractionation technique, both the neutral and acidic fraction exhibited some shear thinning, but differences in the degree of shear thinning between the fractions were observed (Fedeniuk and Biliaderis, 1994). Using the power law model, Fedeniuk and Biliaderis (1994) reported that the neutral fraction had a higher m and lower n value compared to that of the acidic fraction implying that in solution the neutral fraction was more viscous and exhibited a higher degree of shear thinning. Compared to the rheological properties reported by Cui et al. (1994a), differences between steady shear flow curves for the two fractions were not as evident when CTAB was used as the fractionation technique. This was likely due to the fact that selective precipitation with CTAB was not as effective as ion exchange chromatography when it came to separating the two polysaccharides. Small strain oscillatory measurements were also carried out on the two fractions and it was found by both Cui et al. (1994a) and Fedeniuk and Biliaderis (1994) that the neutral fraction exhibited rheological characteristics more typical of a concentrated polymer solution whereas the rheological pattern of the acidic fraction was more typical of a dilute polymer solution.

The molecular size distribution of flaxseed mucilage and its fractions (neutral and

acidic) extracted from flaxseed cultivar NorMan have been examined by gel filtration chromatography (Cui et al., 1994a). The chromatograms showed polymers eluting over a range of molecular sizes indicating that flaxseed mucilage as a whole is highly polydisperse. Further examination of the neutral and acidic fractions revealed that the neutral fraction contained mainly high molecular weight species but some lower molecular weight polymers were also detected. The molecular size distribution of the acidic fraction revealed that it consisted of polymers with a smaller hydrodynamic volume than those found in the neutral fraction (Cui et al., 1994a).

The intrinsic molecular parameters of size and structure are the primary factors contributing to the rheological properties of a particular polysaccharide. In comparison to the acidic polysaccharides it has been reported that the neutral polysaccharides have a larger hydrodynamic volume (Fedeniuk, 1993; Cui et al., 1994a; Cui and Mazza, 1996) and a greater tendency for structure formation in an aqueous environment (Fedeniuk and Biliaderis, 1994). Thus, it has been concluded that the neutral polysaccharides are the major component responsible for the viscous nature and shear thinning behavior of flaxseed mucilage (Cui et al., 1994a).

2.3.3. Varietal Effects on Composition and Rheological Properties

Compositional analysis has revealed that the monosaccharide composition of mucilage as a whole (without separating the neutral and acidic polysaccharides) varies significantly among cultivars (Wannerberger et al., 1991; Oomah et al., 1995; Cui and Mazza, 1996; Cui et al., 1996). Xylose and rhamnose are the major components of the neutral and

acidic polysaccharides respectively, and are therefore the two monosaccharides that receive the most focus. The ratio of rhamnose to xylose has most commonly been used to estimate the ratio of acidic to neutral polysaccharides in flaxseed mucilage (Fedeniuk and Biliaderis, 1994; Oomah et al., 1995; Cui et al., 1996). Oomah et al. (1995) studied the monosaccharide composition of mucilage extracted from 109 different flaxseed accessions and found that the ratio of rhamnose to xylose ranged from 0.3 to 2.2 indicating that the content of acidic and neutral polysaccharides varied significantly among the cultivars examined. Wannerberger et al. (1991) measured the monosaccharide composition of 23 cultivars of flaxseed and reported that the amount of galacturonic acid (21-36%) and xylose (19-38%) varied considerably among the cultivars. In addition to rhamnose, galacturonic acid is also only found in the acidic polysaccharide and therefore has been used by some researchers (Wannerberger et al., 1991; Cui and Mazza, 1996) to estimate the amount of acidic polysaccharides.

Further examination of the physical properties of flaxseed mucilage from different cultivars suggests that a relationship may exist between monosaccharide composition and the rheological properties of the mucilage. In the study by Wannerberger et al. (1991) the viscosity of the mucilage extracted from 23 cultivars was measured in addition to the monosaccharide compositions. The viscosities reported were measured at a shear rate of 10 s^{-1} and the values ranged from 0.02-0.28 Pa•s. The cultivars containing higher amounts of xylose also had higher viscosity readings. The two cultivars whose rheological properties differed the most were Szegedi 62 and Liflora. Szegedi 62 was described as forming a nearly elastic gel at a concentration of 2% and Liflora exhibited rheological characteristics that were more typical of a viscous fluid. These large differences in rheological properties

corresponded well with the differences observed in the monosaccharide composition. Monosaccharide analysis revealed that Szegedi 62 contained considerably more neutral polysaccharides than Liflora as indicated by its higher xylose content. In addition, it was speculated that the average molecular weight of polysaccharides in Szegedi 62 mucilage was considerably higher than Liflora. This is in agreement with the intrinsic viscosity results obtained by Cui and Mazza (1996). In their study, the cultivar containing the highest amount of neutral polysaccharides (highest amount of xylose) exhibited the highest intrinsic viscosity. The intrinsic viscosity of the fractions obtained by ion exchange chromatography from flaxseed cultivar NorMan was measured and the neutral fraction (5.3 dl/g) was found to exhibit a higher intrinsic viscosity than the acidic fraction (2.48 dl/g). Given the relationship between intrinsic viscosity and average molecular size as described by the Mark-Houwink equation, it was suggested by Cui and Mazza (1996) that the intrinsic viscosity value of flaxseed mucilage reflects the average molecular size of the constituent polysaccharides. Therefore, it was concluded that the neutral polysaccharides had a higher molecular weight than their acidic counterparts and as a result those cultivars containing higher amounts of neutral polysaccharides exhibited higher intrinsic viscosities (Cui and Mazza, 1996).

A similar study to that of Wannerberger et al. (1991) was conducted by Cui et al. (1996) who examined the monosaccharide composition and rheological properties of mucilage from 12 flaxseed (6 brown seeded and 6 yellow seeded) cultivars. The rheological properties were found to vary among the cultivars such that those containing higher amounts of neutral polysaccharides exhibited weak-gel properties and a higher degree of shear thinning. In contrast, rheological behavior more typical of a viscoelastic fluid was observed

for those cultivars containing higher amounts of acidic polysaccharides. They also reported that in general, the mucilage extracted from the yellow seed coat cultivars exhibited stronger rheological properties than that from the brown seed coat cultivars.

In addition to analyzing the yield and composition of the mucilage extracted from the 109 accessions of flaxseed, Oomah et al. (1995) also measured the oil and protein content of the seeds. Comparisons were made between various carbohydrate parameters (monosaccharide composition, arabinose/xylose ratio, rhamnose/xylose ratio and carbohydrate yield) and the oil and protein contents to determine if any of the carbohydrate parameters were related in any way to the other major components of flaxseed. The Pearson correlation coefficients between the carbohydrate parameters and the protein and oil contents ranged from -0.004 to 0.145 and -0.041 to -0.289, respectively (significance not given). These weak associations between the carbohydrate parameters and the oil and protein contents suggest that if flax breeders were able to alter the carbohydrate composition and content through their breeding program to meet certain rheological and functional characteristics, it should have little effect on the oil and protein content of the seed.

3. EFFECT OF SEQUENTIAL ETHANOL PRECIPITATION ON THE PHYSICOCHEMICAL PROPERTIES OF FLAXSEED MUCILAGE

3.1. Abstract

Flaxseed mucilage is a complex mixture of two polysaccharides: a neutral arabinoxylan-like polymer and an acidic pectic-like polymer. It has been shown that the neutral polysaccharide is primarily responsible for the viscosity building characteristics of flaxseed mucilage. Characterization of a polymers composition, rheological and molecular characteristics is difficult when a mixture of two or more polymers are present. For this reason, techniques such as CTAB and ion exchange chromatography have been used somewhat successfully to separate the two polysaccharides present in flaxseed mucilage. The objective of this study was to determine whether sequential ethanol precipitation is an effective method for separating the neutral and acidic polysaccharides from flaxseed mucilage. Mucilage was extracted from two flaxseed cultivars (AC Emerson and Vimy). An aqueous extraction procedure was used followed by sequential ethanol precipitation which resulted in the collection of two fractions: one at 40% ethanol (40F) and a second at 60% ethanol (60F). In addition, complete mucilage precipitation was also carried out by adjusting the aqueous extract to 75% ethanol (UNF). The chemical composition, molecular size, rheological and functional properties of the resulting mucilage extracts were examined to determine the effectiveness of sequential ethanol precipitation for separating the mixture of polysaccharides. The yield and chemical composition of the mucilage extracts was influenced by the percentage of ethanol used for precipitation. The three extracts differed significantly in monosaccharide

composition. The intrinsic viscosity values also varied among the mucilage extracts suggesting that the polysaccharides present in 40F and 60F differed in molecular weight and/or structural conformation. Molecular weight analysis by light scattering revealed that the average molecular weight of polymers eluting in the high molecular weight region of 40F was significantly higher than that of the corresponding polymers in 60F. Differences in the molecular characteristics were reflected in the rheological behavior of the three extracts, with UNF and 40F exhibiting more pronounced shear thinning and weak-gel behavior compared to 60F. The emulsifying ability of the three extracts also differed such that the initial quality of the emulsion was greater for those systems containing the 40F and UNF extracts, but the 40F extract exhibited superior emulsion stability properties compared to the UNF and 60F extracts. It is evident from the monosaccharide analysis results that sequential ethanol precipitation was able to separate the neutral and acidic polysaccharides to some degree but complete separation of the two polysaccharide fractions is likely not possible using this fractionation technique.

3.2. Introduction

Flaxseed mucilage is an excellent source of dietary fiber, which, due to its highly viscous nature, also shows potential for being used as a food gum. (Cui and Mazza, 1996; Mazza and Biliaderis, 1989). Flaxseed mucilage is composed primarily of two polysaccharides: a neutral arabinoxylan-like polymer and an acidic pectic-like polymer (Cui et al., 1994a; Muralikrishna et al., 1987). Previous studies have shown that the ratio of acidic to neutral polysaccharides in flaxseed mucilage is influenced by cultivar (Cui et al., 1996;

Oomah et al., 1995; Wannerberger et al., 1991). It has been reported that monosaccharide composition of the mucilage has a direct effect on the rheological properties of the mucilage in aqueous solutions. In general, cultivars having higher levels of neutral polysaccharides exhibit higher apparent viscosity and stronger gel-like properties (Cui et al., 1996; Wannerberger et al., 1991). The differences in rheological properties of mucilage from various cultivars have lead researchers to examine the two polysaccharide fractions independently and in greater detail.

Ion exchange chromatography (Cui et al., 1994a) and selective precipitation with CTAB (Fedeniuk and Biliaderis, 1994; Hunt and Jones, 1962) have been used to separate the two polysaccharides based on differences in charge, but complete separation was often difficult to achieve. Analysis of the two polysaccharide fractions revealed that they differed in monosaccharide composition, molecular size, structural conformation, and rheological properties (Cui et al., 1994a; Fedeniuk and Biliaderis, 1994; Muralikrishna et al., 1987), but limited information is available on the functional characteristics of these two fractions.

Given that the two polysaccharides differ in molecular size and structural conformation, it should be possible to achieve separation based on these parameters rather than on charge. The objective of this study was to determine whether sequential ethanol precipitation is an effective method for separating the neutral and acidic polysaccharides from flaxseed mucilage. The chemical composition, molecular size, rheological and functional properties of the resulting mucilage extracts were examined to determine how well sequential ethanol precipitation separated the mixture of polysaccharides present in flaxseed mucilage and to determine whether it would be beneficial to explore this technique further when

examining the physicochemical properties of mucilage extracted from several flaxseed cultivars. Molecular characteristics of the resulting mucilage extracts were also compared to those reported previously by Cui et al. (1994a) and Fedeniuk and Biliaderis (1994) for mucilage fractions obtained by fractionation techniques based on differences in charge

3.3. Materials and Methods

3.3.1. Materials

Flaxseed cultivars AC Emerson and Vimy were obtained from the 1999 harvest at the Morden Research Station, Agriculture and Agri-Food Canada, located in Morden, Manitoba. These two cultivars were chosen based on results from preliminary experiments which revealed that their monosaccharide composition and rheological properties were different from each other. All chemicals used were of analytical reagent grade.

3.3.2. Extraction and Fractionation of Mucilage

The extraction and fractionation procedure that was used is summarized in Figure 3.1. The mucilage was isolated by mixing whole flaxseed in distilled water (20:1 water:seed ratio) for 3 hours (2 hours with a magnetic stirring bar and 1 hour with a standard lab batch automated mixer, Silverson Machines Ltd., East Long Meadow, MA), at a controlled temperature (80 ± 2°C), and pH (6.5-7.0). The pH was monitored and adjusted with 0.1N NaOH as required. The seeds were separated from the aqueous extract using a 40-mesh sieve and rinsed with distilled water (80°C). Water insoluble material was removed by filtering the hot aqueous extract through a silk mesh (136 μm) on a Buchner funnel. A portion of the

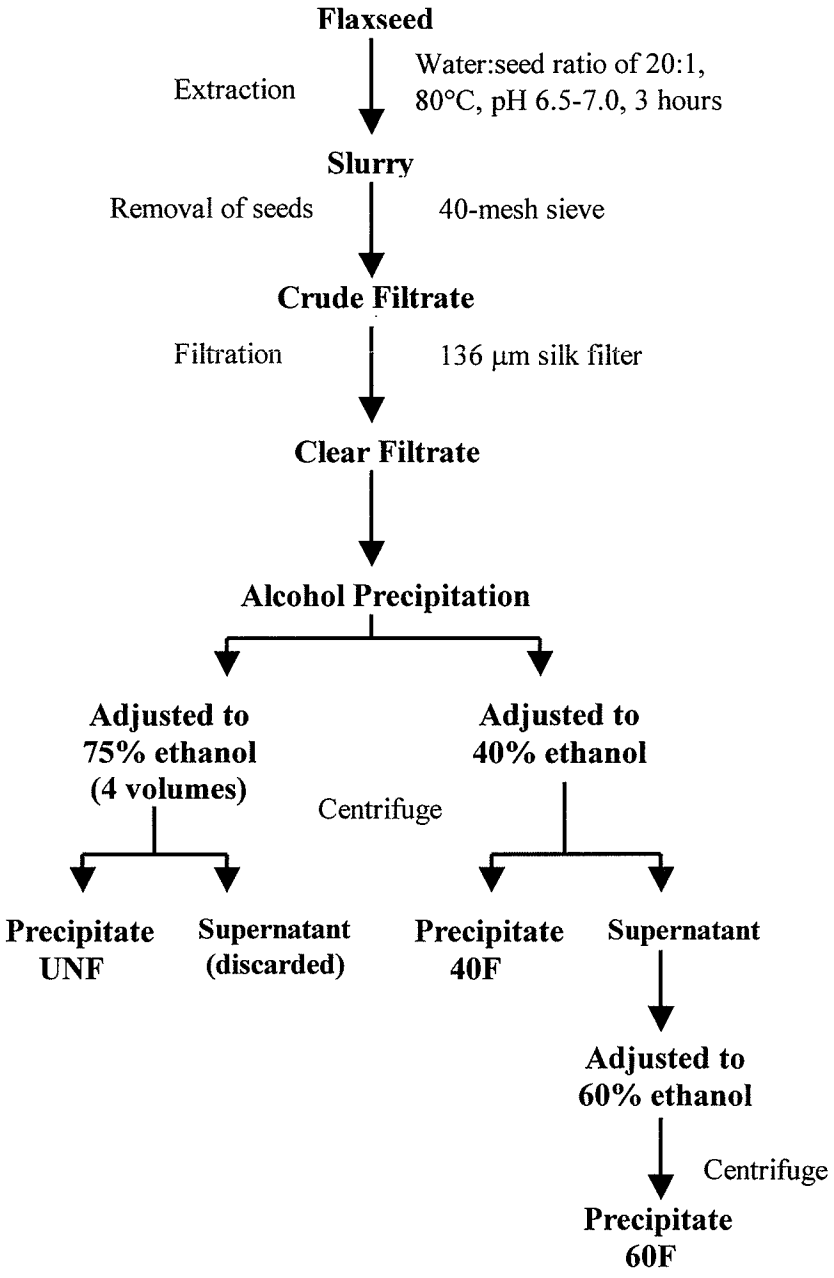

Figure 3.1. Extraction and fractionation of flaxseed mucilage.

aqueous extract was adjusted to 75% ethanol (the equivalent to approximately 4 volumes of 95% ethanol) and left for 1.5 hours prior to centrifugation. This precipitate was designated as the unfractionated mucilage (UNF). The remaining portion of the aqueous extract was first adjusted to 40% ethanol and left at room temperature for 2 hours; the precipitated material collected by centrifugation was designated as 40F. The supernatant fraction was adjusted to 60% ethanol and left overnight at room temperature. The material that precipitated at 60% ethanol was designated as 60F. All precipitates were collected by centrifugation (RC2-B Sorvall Centrifuge, 8300 x g, 20 min.), redissolved in deionized water and heated (80°C, 1 hour) to remove any residual ethanol. The hot aqueous solutions were filtered once again through the silk mesh (136 μm) on a Buchner funnel prior to freeze-drying.

3.3.3. Chemical Analyses

Protein content of the mucilage extracts was determined according to the method of Lowry et al. (1951), using bovine serum albumin as a standard (Sigma-Aldrich, Oakville, ON). Galacturonic acid content was determined according to the method of Blumenkrantz and Asboe-Hansen (1973), and total ash content was analyzed according to AOAC approved method 7.009 (AOAC, 1980). Amino acid composition was analyzed according to the method of Andrews and Baldar (1985). Relative amounts of component monosaccharides in the mucilage extracts were determined by gas-liquid chromatography following the procedure of Fedeniuk and Biliaderis (1994). The polysaccharides were hydrolyzed in 1 M sulfuric acid for 2 and 6 hours at 100°C. The free sugars were converted into their alditol acetate derivatives and were separated on a glass capillary column (SP-2330, 30 m x 0.25 mm

i.d.). All samples (2 µl) were injected into a Star 3400 CX Varian chromatograph equipped with a flame ionization detector (injector temperature 250°C, column temperature 185-235°C at 12.5°C/minute, detector temperature 250°C). Erythritol (Sigma-Aldrich, Oakville, ON) was used as an internal standard. Retention times, peak areas and sugar contents were calculated using Varian Star 4.02 chromatography software. The values of fucose, arabinose, xylose, mannose, galactose and glucose were calculated after 2 hours of hydrolysis, while rhamnose was calculated after 6 hours. The relative amounts of component monosaccharides present in the mucilage extracts are reported as relative mole percent. All analyses were done in duplicate.

3.3.4. Rheological Measurements

Viscosity measurements of aqueous solutions of Vimy UNF (0.01-0.03%, w/v), Vimy 40F (0.03-0.06%, w/v) and Vimy 60F (0.01-0.02%, w/v) in deionized water were performed using a number of Ubbelohde viscometers (International Research Glassware, Kenilworth, NJ) at 24 ± 0.05°C. The effect of different solvents (1M NaCl and 0.2 M sodium acetate buffer, pH=3) on the viscosity of the mucilage solutions (0.06-0.10%, w/v) was also determined for the three Vimy extracts. Viscosity measurements of aqueous solutions (0.03-0.06%, w/v) of AC Emerson 40F in deionized water were also carried out. In addition, aqueous solutions (0.06-0.1%, w/v) of AC Emerson UNF and 60F and solutions (0.03-0.05%, w/v) of xanthan gum were prepared in 1 M NaCl and measured in order to make comparisons between the various gums. The reduced viscosities were plotted against polymer concentration and extrapolated to zero concentration, using the Huggins equation (Huggins,

1942), to obtain the intrinsic viscosities. Steady shear flow curves and small strain oscillatory measurements of the mucilage extracts were carried out using a Bohlin VOR rheometer (Bohlin Rheology, Edison, NJ). All measurements were taken at 23 ± 0.1 °C using a concentric cylindrical geometry (the radii of the inner rotor and outer container were 7.8 and 19.5 mm, respectively; the rotor length was 64 mm) and the 16.91 g·cm torsion element. Steady shear rheological measurements were carried out on aqueous solutions of UNF (0.5%, 1.0%, 1.5%, w/w), 40F (0.25%, 0.5%, 1.0%, and 1.5%, w/w) and 60F (0.5%, 1.0%, 1.5%, 2.0%, w/w) for both cultivars. The samples were subjected to shear rates of 0.0291 to 116s^{-1} and the results are reported as averages of two measurements. The mechanical spectra of 40F (1.5%, w/w) and 60F (2.0%, w/w) extracts were obtained between 0.01-10 Hz and less than 2% strain. Results are reported as averages of three measurements.

3.3.5. Mucilage Fraction Interactions

Interactions between the two mucilage fractions were monitored according to the procedure outlined by Morris (1984). The procedure involved preparing solutions of AC Emerson 40F and 60F in deionized water so that they gave approximately the same specific viscosity; followed by mixing the two solutions in various ratios. As the two solutions were mixed, the specific viscosity was measured and any departure from the original viscosity was monitored. All viscosity measurements were made using the Ubbelohbe viscometer (23 ± 0.1 °C).

3.3.6. Molecular Weight (M_w) Analysis

Aqueous solutions of mucilage (0.2 mg/ml) were dissolved with heating (85°C) in deionized water. The hot solution was filtered through a 3 μm cellulose acetate membrane and injected into a high- performance size-exclusion chromatography (HPSEC) multi-angle laser light scattering (MALLS) refractive index (RI) and UV detector system.

The calibration constants for the refractive index and multi-angle laser light scattering detectors were determined according to the method of You et al. (1999). Bovine serum albumin was used for the normalization of the photodiodes located around the scattering cell. The high performance size exclusion chromatography (HPSEC) system consisted of a pump (Model 510; Waters, Milford, MA), an injection valve (Model 7010; Rheodyne, Rohnert, CA) with a 200 μl sample loop, a TSK PWH guard column (Tosoh Corp., Tokyo, Japan), a MALLS detector (Wyatt Technology, Santa Barbara, CA), a RI detector (Model 410; Waters, Milford, MA) and a UV detector (Model 484; Waters, Milford, MA). A TSK G5000 PW column, 7.8 x 600 mm (Tosoh Corp., Tokyo, Japan), kept at room temperature, was used to determine the molecular weight profiles of 40F and 60F. The flow rate of the mobile phase (0.15M $NaNO_3$ containing 0.02% NaN_3), which was filtered through a 0.2 μm cellulose acetate membrane followed by a 0.1 μm cellulose acetate membrane, was 0.4 ml/min. Weight-average molecular weight (M_w), root-mean square of the radius of gyration (R_g), and polydispersity (M_w/M_n) values were calculated using Astra 4.72 software (Wyatt Technology, Santa Barbara, CA) and a dn/dc value of 0.147 was used. Results reported are averages of duplicate measurements.

3.3.7. Evaluation of Emulsifying Ability

Emulsifying properties of aqueous mucilage solutions were determined according to the turbidimetric method of Pearce and Kinsella (1978) with some modifications. Mucilage solutions (0.1 and 0.25%, w/w) were prepared in duplicate using 0.1 M citric acid disodium hydrogen phosphate buffer (pH 7.0) as the solvent. In addition, aqueous solutions of gum arabic (0.1% and 2.0%, w/w) were prepared and evaluated for comparison purposes. In a 10-ml test tube, 1 ml of corn oil (Mazola, purchased from a local supermarket) was emulsified with 4 g of polysaccharide solution by means of a hand held tissue tearor (model 985-370, Biospec Products Inc., Bartlesville, OK) for two minutes at 11 000 RPM. The emulsifying ability of each solution was measured in duplicate. At various times after homogenization 50 µl of the emulsion was taken from the bottom of the test tube and diluted to 2.5 ml with 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance (A) of the diluted emulsions was measured at 500 nm on a Spectronic 601 spectrophotometer (Milton Roy, Rochester, NY). The interfacial area covered by the available polysaccharide is directly related to the absorbance and was calculated according to the relationship given by the Mie theory for light scattering (Kerker, 1969):

$$\text{Interfacial area} = 2T$$

Where:

$T = 2.303A/\ell$

A is the absorbance at 500 nm immediately after emulsification

ℓ is the light pass (1 cm)

The rate of emulsion breakdown was evaluated using a first-order model equation. For a first-order reaction, a plot of the natural log of the absorbance (ln[A]) as a function of time (t) yields a straight line with a slope of $-k_1$ (k_1 = rate of decay of the emulsion). The initial rapid decrease in the absorbance is an indication of the rate of decay of an emulsion, therefore, the time interval of 0 to 8 minutes was used to evaluate the rate of emulsion decay in this study.

3.3.8. Foam Stabilization

The effect of aqueous mucilage solutions on the formation and stabilization of foam formed by surface-active protein (bovine serum albumin) was studied according to the procedure of Izydorczyk et al. (1991) with some modifications. Mucilage solutions were prepared at concentrations ranging from 0.25 to 1.0% (w/w) and 0.5 g of the aqueous solution was added to 1 ml of bovine serum albumin (2.0%, w/v) and 0.25 ml of $NaHCO_3$ (5.0%, w/v) in a 15-ml graduated test tube. The test tube was shaken for two minutes using a Burrell Wrist-Action shaker (Burrell Corp., Pittsburgh, PA) and the volume of foam was recorded immediately. Then 0.25 ml of citric acid (5.0%, w/v) was added, the tube was shaken for an additional 2 minutes, and the volume of foam was then recorded immediately and after 10 minutes. The test tube was subsequently placed in a water bath at 95°C and the change in foam volume was noted after one, two, and three minutes of heating. Aqueous solutions of xanthan gum (0.25 and 1.0% , w/w) and gum arabic (1.0% w/w) were also prepared and tested for comparison purposes. Reported values are the means of at least two measurements.

3.3.9. Statistical Analysis

One-way analysis of variance (ANOVA) and Tukey's studentized range test were used to determine differences in mean values based on data collected from two replications of each measurement. ANOVA was preformed using JMP IN Statistical software (SAS Institute Inc., 1996). Significance was established at $p \leq 0.05$.

3.4. Results and Discussion

3.4.1. Extraction and Fractionation of Mucilage

The yields of the extracted mucilage based on seed weight are presented in Table 3.1. When the concentration of ethanol in the aqueous extract was adjusted to 75% (approximately 4 volumes), the yields of the precipitated mucilage were 6.8 and 7.0% for AC Emerson and Vimy, respectively. These values fall within the range of yields (5.5 to 7.9%) reported by Cui et al. (1996), who extracted mucilage from six flaxseed cultivars using four volumes of ethanol. Sequential ethanol precipitation resulted in two fractions being collected, one at 40% (40F) and the second at 60% (60F) ethanol. When the yields for the two fractions were added together, the total yields were almost identical to the yields for the unfractionated mucilage. These results indicate that very little mucilage was precipitated above 60% ethanol, and therefore four volumes of ethanol may not be necessary for full mucilage precipitation. For both cultivars, the yield of 40F was lower than that of 60F.

Although no official solubility tests were carried out, some general observations on the ability to dissolve each of the extracts were noted. In order to dissolve the 40F extract, hydration overnight was required followed by vortexing and heating in a water bath (80°C)

Table 3.1. Yield[a] and composition[b] of mucilage extracts

Cultivar and fraction	Yield (%)	Carbohydrate (%)	Protein (%)	Ash (%)
AC Emerson				
UNF	6.8	50.0 ± 1.5 [ab]	28.7 ± 1.0 [a]	6.2 ± 0.2 [a]
40F	2.7 (41.5)	54.9 ± 2.0 [a]	20.2 ± 0.9 [b]	4.6 ± 0.2 [b]
60F	3.8 (58.5)	44.2 ± 1.5 [b]	24.3 ± 0.2 [c]	7.8 ± 0.1 [c]
Vimy				
UNF	7.0	45.5 ± 0.3 [ab]	27.5 ± 0.5 [a]	7.5 ± 0.1 [a]
40F	3.0 (42.5)	49.3 ± 1.7 [a]	24.6 ± 0.2 [b]	6.4 ± 0.1 [b]
60F	4.0 (57.5)	43.2 ± 1.0 [b]	19.5 ± 0.5 [c]	7.8 ± 0.2 [a]

[a] Yields expressed as a % of seed weight. In brackets, yields are expressed as % of total mucilage collected at 40% and 60% ethanol.

[b] means ± SD (n = 2), presented on an as is % wt basis; means from the same cultivar within a column followed by a different superscript are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

for varying lengths of time depending on the cultivar. In contrast, the 60F extract dissolved almost instantly with a small amount of heat while the UNF extract required a slightly longer period of time in the water bath. The appearance of the aqueous solutions also differed among the extracts. The aqueous solution of the 60F extract was very clear while the 40F extract was opaque and often contained tiny insoluble particles. The appearance of the UNF extract was slightly more opaque than 60F but did not contain any insoluble particles. The varying degrees of solubility observed among the mucilage extracts most likely reflects differences in structure and molecular weight of the constituent polysaccharides. According to Glicksman (1982a), branched molecules tend to be more soluble than linear molecules because the extended side chains prevent close polymeric association resulting in improved solubility. In contrast, linear polymers tend to orientate themselves in a planar association making it more difficult to solubilize them and keep them in solution. Mazza and Biliaderis (1989) examined the solubility of flaxseed mucilage extracted from the flaxseed cultivar Linott and precipitated with 80% ethanol. It was reported that the mucilage could be readily solubilized at concentrations up to 0.5% (w/v).

3.4.2. Chemical Composition

The differing chemical compositions found for the mucilage extracts (Table 3.1) suggests that the level of ethanol used for precipitation had an effect on the composition of the mucilage. For both AC Emerson and Vimy, 40F had a significantly higher ($p \leq 0.05$) carbohydrate content than 60F while the carbohydrate content of 40F and 60F was not significantly different ($p \leq 0.05$) from that UNF.

For both cultivars the highest protein content was observed in the UNF extract. As the ethanol concentration increased from 40% to 60%, the protein content for AC Emerson increased from 20.2% to 24.3%, respectively. The opposite was observed for Vimy such that 40F had significantly higher protein content than 60F. These results for protein content are significantly higher than those reported by Cui et al. (1994a) and may reflect differences in cultivars and extraction conditions. It has been reported that pH, temperature, and water to seed ratios have a significant effect on the final composition of the extracted mucilage (Cui et al., 1994b). The extraction conditions used in the present study were similar to those used by Fedeniuk and Biliaderis (1994), who reported a protein content of 22.8%, comparable to the results obtained in this study.

It is believed that the majority of the protein was not chemically bound to the polysaccharides. The high protein contamination was one of the draw backs of using the higher water to seed ratio. Preliminary experiments showed that a water to seed ratio of 20:1 was required because anything lower resulted in an aqueous extract that was extremely viscous and difficult to work with. Fedeniuk and Biliaderis (1994) successfully removed approximately 80% of the proteins from a mucilage extract using vega clay, but in doing so the yield of the treated mucilage was substantially lowered and the ash content increased. At the risk complicating the extraction procedure and losing some of the polysaccharides which are significant to the rheological and functional characteristics of the mucilage, it was decided that the mucilage would be examined on an as is basis, without the removal of protein.

The ash contents ranged from 4.6 to 7.8%, and these values are comparable to those reported by other researchers (Cui and Mazza, 1996; Fedeniuk and Biliaderis, 1994; Mazza

and Biliaderis, 1989). For both AC Emerson and Vimy, the ash content of 40F was lower than that of 60F, with UNF falling between the two fractions.

3.4.3. Monosaccharide Composition

The monosaccharide composition of the mucilage extracts is presented in Table 3.2. For both cultivars all three extracts contained a large number of sugars, a strong indicator of the complex nature of flaxseed mucilage. AC Emerson 40F contained mainly xylose followed by arabinose, galactose and much smaller amounts of rhamnose and fucose. In comparison, AC Emerson 60F contained significantly ($p \leq 0.05$) higher amounts of rhamnose, fucose, galactose, and galacturonic acid, and significantly lower ($p \leq 0.05$) amounts of xylose, arabinose, and glucose. Similar findings were also observed between the 40F and 60F extracts of Vimy.

When the monosaccharide composition for each of the extracts was compared between cultivars differences were observed (statistical comparisons among the cultivars are given in section 4.4.3). Vimy 40F contained lower amounts of xylose and arabinose, and higher amounts of rhamnose, glucose, and galacturonic acid compared to AC Emerson 40F. In contrast, the monosaccharide composition of the 60F extract did not differ greatly between cultivars, although, Vimy 60F contained slightly higher amounts of fucose and glucose and a slightly lower amount of xylose compared to AC Emerson 60F. Interestingly, a significant amount of glucose was found in 40F for both cultivars. Previous studies have suggested that glucose is not an integral component of flaxseed polysaccharides and that its presence resulted from some soluble, cellulose-like material which had been extracted from the cell wall

Table 3.2. Relative monosaccharide composition[d] of mucilage extracts from AC Emerson and Vimy

	AC Emerson			Vimy		
Monosaccharide (mole %)[a]	**UNF**	**40F**	**60F**	**UNF**	**40F**	**60F**
Rhamnose	22.0 ± 0.2 [a]	8.3 ± 0.0 [b]	37.4 ± 0.3 [c]	28.1 ± 0.7 [a]	13.1 ± 0.4 [b]	37.4 ± 0.0 [c]
Fucose	4.1 ± 0.1 [a]	0.4 ± 0.0 [b]	7.3 ± 0.0 [c]	5.2 ± 0.0 [a]	0.6 ± 0.0 [b]	9.3 ± 0.2 [c]
Arabinose	12.6 ± 0.0 [a]	19.8 ± 0.2 [b]	5.6 ± 0.2 [c]	10.8 ± 0.2 [a]	17.1 ± 0.2 [b]	5.5 ± 0.0 [c]
Xylose	40.3 ± 0.0 [a]	54.7 ± 0.2 [b]	19.2 ± 0.3 [c]	32.2 ± 0.4 [a]	48.2 ± 0.9 [b]	17.0 ± 0.2 [c]
Mannose	ND[b]	ND	ND	ND	0.5 ± 0.0	ND
Galactose	19.0 ± 0.0 [a]	11.2 ± 0.1 [b]	27.9 ± 0.1 [c]	19.8 ± 0.1 [a]	10.9 ± 0.1 [b]	27.5 ± 0.2 [c]
Glucose	2.0 ± 0.1 [a]	5.6 ± 0.2 [b]	2.6 ± 0.1 [a]	3.8 ± 0.0 [a]	9.6 ± 0.5 [b]	3.4 ± 0.1 [a]
Galacturonic Acid[c]	25.0 ± 0.2 [a]	16.9 ± 0.4 [b]	32.3 ± 0.3 [c]	26.5 ± 0.1 [a]	21.8 ± 0.5 [b]	32.0 ± 0.2 [c]

[a] Relative monosaccharide composition expressed as mole %, means ± SD (n = 2)
[b] ND, none detected
[c] Galacturonic acid content on a polysaccharide weight basis, means ± SD (n = 2)
[d] Means from the same cultivar within a row followed by a different superscript are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

(Erskine and Jones, 1957; Cui et al., 1994a). It is possible that a similar type of material may have been extracted in this study but the exact source of the detected glucose remains unresolved at the present time. A trace amount of mannose (0.50%) was found in the 40F extract of Vimy. Small amounts of mannose (1–2%) have also been reported by Wannerberger et al. (1991). The variation in monosaccharide composition between cultivars confirmed findings of previous studies (Wannerberger et al., 1991; Oomah et al., 1995; Cui and Mazza, 1996; Cui et al., 1996), which showed that genotype has an influence on monosaccharide composition.

The monosaccharide analysis revealed that for both cultivars the 40F extract was enriched with neutral polysaccharides, whereas the 60F extract contained significantly more acidic polysaccharides. However, when comparisons were made to the results reported by Cui et al. (1994a), it appears as though ion exchange chromatography may have been more effective than sequential ethanol precipitation in separating the neutral and acidic polysaccharides. Cui et al. (1994a) reported no rhamnose or fucose in the neutral fraction and relatively little arabinose and xylose in the acidic fraction. On the other hand, Fedeniuk and Biliaderis (1994) reported that complete separation of the two polysaccharides was not possible when CTAB precipitation was used.

3.4.4. Amino Acid Composition

The amino acid composition of the mucilage extracts is presented in Table 3.3. Glutamic acid was the major amino acid present in all the extracts examined. This finding is in agreement with the results reported by Cui and Mazza (1996) for mucilage extracted from

Table 3.3. Amino acid composition of mucilage extracts from AC Emerson and Vimy

Amino Acid (g/100g protein)[a]	AC Emerson			Vimy		
	UNF	40F	60F	UNF	40F	60F
Aspartic Acid	6.1 ± 0.1	6.1 ± 0.0	5.8 ± 0.2	6.2 ± 0.0	6.7 ± 0.1	5.6 ± 0.1
Threonine	2.7 ± 0.0	2.3 ± 0.2	2.4 ± 0.1	2.8 ± 0.0	2.8 ± 0.2	2.3 ± 0.2
Serine	4.6 ± 0.1	4.5 ± 0.0	4.2 ± 0.1	4.6 ± 0.0	4.8 ± 0.1	4.5 ± 0.0
Glutamic Acid	34.6 ± 0.3	36.6 ± 0.6	38.5 ± 0.5	34.8 ± 0.2	33.6 ± 0.3	36.6 ± 0.7
Proline	2.4 ± 0.1	2.5 ± 0.3	2.0 ± 0.4	2.4 ± 0.1	2.8 ± 0.2	2.2 ± 0.1
Glycine	10.1 ± 0.1	12.9 ± 0.3	8.9 ± 0.1	9.6 ± 0.1	10.9 ± 0.2	9.2 ± 0.4
Alanine	2.9 ± 0.1	2.8 ± 0.0	2.8 ± 0.0	3.1 ± 0.0	3.2 ± 0.0	2.4 ± 0.0
Cystine	4.7 ± 0.1	4.0 ± 0.1	4.6 ± 0.0	5.0 ± 0.1	4.3 ± 0.1	5.4 ± 0.1
Valine	2.7 ± 0.2	2.5 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	2.9 ± 0.1	2.7 ± 0.2
Methionine	1.2 ± 0.0	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.0	1.2 ± 0.0	1.3 ± 0.1
Isoleucine	2.6 ± 0.2	2.2 ± 0.0	2.4 ± 0.2	2.6 ± 0.2	2.8 ± 0.2	2.6 ± 0.6
Leucine	5.3 ± 0.2	4.7 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.4 ± 0.2	5.2 ± 0.2
Tyrosine	0.9 ± 0.3	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	0.6 ± 0.2
Phenylalanine	2.2 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.0	2.5 ± 0.0	2.2 ± 0.1
Histidine	1.2 ± 0.1	1.2 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.0 ± 0.0
Lysine	5.1 ± 0.1	4.9 ± 0.0	5.1 ± 0.1	4.8 ± 0.0	4.9 ± 0.0	5.3 ± 0.0
Arginine	10.7 ± 0.3	8.6 ± 0.3	10.3 ± 0.3	10.8 ± 0.1	9.0 ± 0.3	10.9 ± 0.2

[a] Amino acid composition expressed as g/100g protein, means ± SD (n = 2)

four flaxseed varieties. In a previous study by Oomah and Mazza (1993), the amino acid profile of whole seed from the same four varieties was examined. They found high levels of glutamic acid in the whole seed followed by relatively large amounts of arginine, aspartic acid, leucine and glycine. Overall, Cui and Mazza (1996) observed that the distribution of amino acids in the mucilage extracts was similar to the distribution observed by Oomah and Mazza (1993) in the whole seed. The amino acid composition reflects the nature of the proteinaceous components of the flaxseed mucilage which may affect the functional properties of the mucilage extracts. The results presented in Table 3.3 show that the amino acid composition did not vary greatly among the mucilage extracts. Minor differences among the extracts were more easily observed once the amino acids were separated into two groups; non-polar or lipophilic in nature and polar or hydrophilic in nature (Table 3.4). For both cultivars the 40F extract contained a higher amount of non-polar amino acids and a lower amount of polar amino acids compared to the other two fractions. The opposite was found for the 60F extracts. The amount of polar and non-polar amino acids found in the UNF extracts was between that of the 40F and 60F extracts. Overall, large differences in the amino acid composition of the mucilage extracts were not observed suggesting that sequential ethanol precipitation had more of an effect on the quantity of protein present rather than on the composition.

3.4.5. Rheological Measurements

The calculated intrinsic viscosity values for Vimy UNF, 40F and 60F in various solvents are presented in Table 3.5. The highest intrinsic viscosity values were obtained

Table 3.4. The total amount of polar and non-polar amino acids found in the mucilage extracts[a] from AC Emerson and Vimy

Mucilage extract	Sum of polar amino acids (g/100g protein)	Sum of non-polar amino acids (g/100g protein)
AC Emerson		
UNF	66.1	32.6
40F	65.5	33.2
60F	68.4	30.6
Vimy		
UNF	66.3	32.6
40F	64.6	34.1
60F	67.5	31.5

[a] It is unclear as to which category histidine falls into therefore it was not included in the calculation

Table 3.5. The effect of different solvents on the intrinsic viscosity of mucilage extracts from Vimy (dl/g)

Solvent	UNF	40F	60F
Deionized water	23.58	5.26	43.86
1 M NaCl	4.75	1.93	5.09
0.2 M Sodium Acetate buffer, pH = 3	5.19	2.48	6.13

Table 3.6. Intrinsic viscosities of mucilage extracts from AC Emerson and Vimy (dl/g)

Cultivar	UNF[a]	40F[b]	60F[a]
AC Emerson	5.51	7.76	4.43
Vimy	4.75	5.26	5.09

[a] 1 M NaCl was used as the solvent for the intrinsic viscosity measurements
[b] Deionized water was used as the solvent for the intrinsic viscosity measurements

when deionized water was used as the solvent, with 60F having the highest value followed by UNF and 40F. Compared to deionized water, the intrinsic viscosity values in 1M NaCl were lower for all three extracts, but the effect of salt was most prominent for 60F revealing the polyelectrolyte nature of this fraction. The higher intrinsic viscosity values exhibited by 60F and UNF in deionized water were likely due to coil expansion caused by electrostatic repulsion between the acidic polymers that were predominant in 60F. The effect of salt on the intrinsic viscosity of 40F was not as great due to the high concentration of neutral polymers and a smaller amount of acidic polymers compared to the other two extracts. The intrinsic viscosity values obtained when 0.2M sodium acetate buffer (pH =3) was used as the solvent were only slightly higher than those obtained when 1M NaCl was used. This suggests that the two solvents had a similar charge suppression effect. Although repulsive forces were suppressed by the presence of salt, 60F and UNF had higher intrinsic viscosity values compared to 40F. This finding is not in agreement with the results reported by Cui and Mazza (1996) and Fedeniuk and Biliaderis (1994) since both found higher intrinsic viscosity values for the neutral fraction compared to the acidic fraction in the presence of salt. The conflicting results could be due to differences in the extraction and fractionation techniques used and the fact that the mucilage extracts in this study contained a significant amount of protein. For polydisperse linear molecules, a direct relationship between molecular weight and intrinsic viscosity exists using the Mark-Houwink equation (Launay et al., 1986), but researchers have also found that large differences in intrinsic viscosity values among polymers are not only the result of differences in molecular weight but also due to differences in structure and conformation (Schooneveld et al., 1999a). Researchers have reported that the

two polysaccharides (neutral and acidic) which make up flaxseed mucilage vary in composition, structure and molecular weight (Muralikrishna et al., 1987; Fedeniuk and Biliaderis, 1994; Cui et al., 1994). Muralikrishna et al. (1987) reported that the neutral polymers are mainly linear while the acidic polysaccharides are highly branched. It is believed that the higher intrinsic viscosity value exhibited by 60F compared to that of 40F in the presence of salt was caused by the differences in structure and conformation rather than molecular weight. It is assumed that the majority of the polysaccharides in 60F were highly branched causing the backbone to have a rigid extended conformation. As a result, the hydrodynamic volume of 60F was greater than that of 40F which was reflected in the intrinsic viscosity values. In all the solvents tested, the intrinsic viscosity values for UNF fell between those of 40F and 60F which was expected given that UNF is a mixture of the two polysaccharides.

When the intrinsic viscosity values for the flaxseed mucilage extracts in 1M NaCl (Table 3.5) were compared to the literature values reported for other food gums in 1 M NaCl, the three flaxseed mucilage extracts had lower intrinsic viscosities than guar gum (11.35 dl/g) and xanthan gum (13.55 dl/g), but had higher intrinsic viscosities than gum arabic (0.14 dl/g) (Cui and Mazza, 1996). Differences in the intrinsic viscosity values for the three extracts in either 1M NaCl or deionized water were found between the two cultivars, AC Emerson and Vimy (Table 3.6). AC Emerson exhibited higher intrinsic viscosity values for UNF and 40F, whereas Vimy exhibited a higher intrinsic viscosity value for 60F. The greatest difference between the two cultivars was observed in the 40F extract.

The steady shear flow curves for the UNF, 40F, and 60F (1.5%, w/w) extracted from

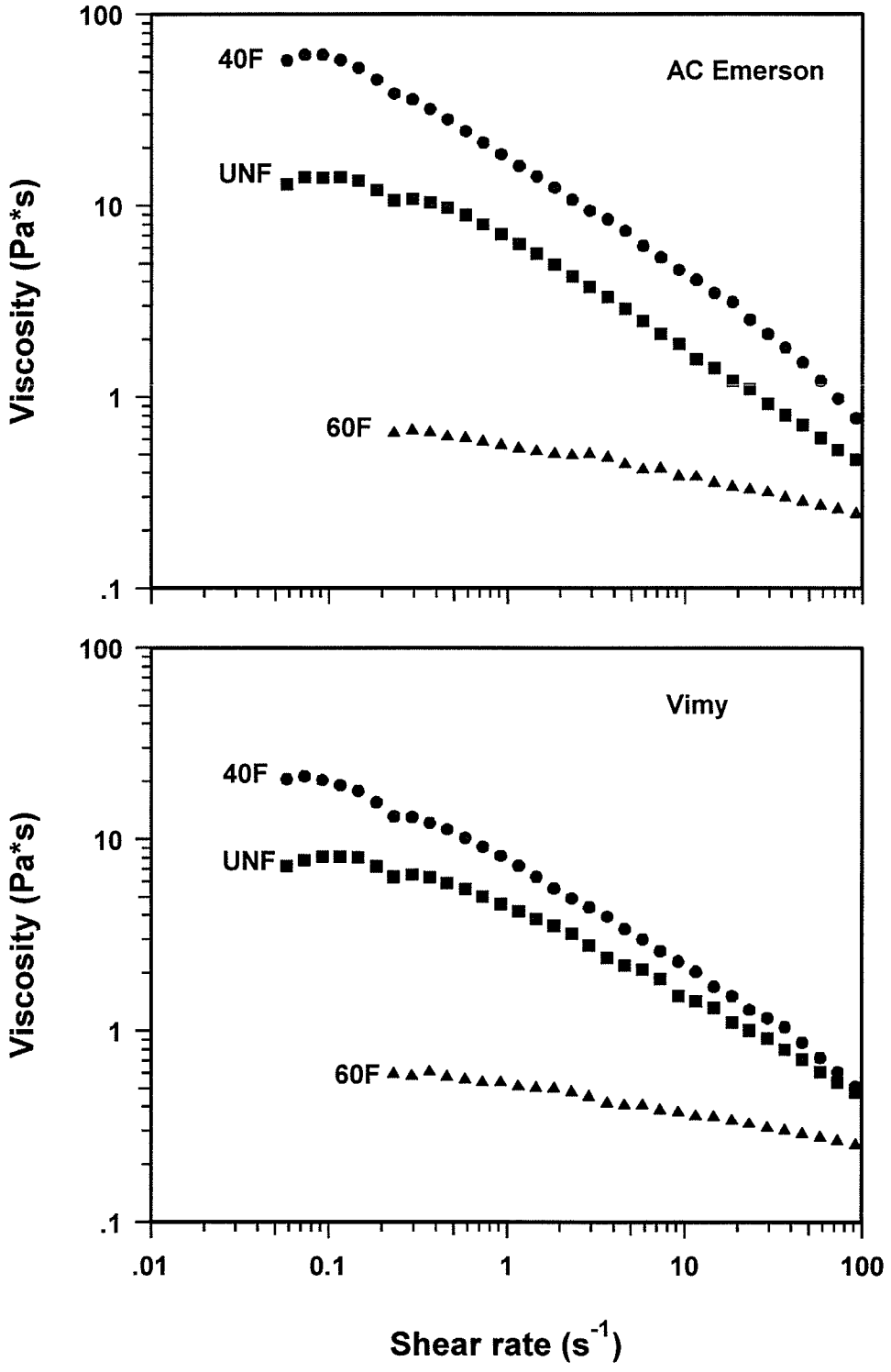

Figure 3.2. Steady shear rheological flow curves of UNF, 40F, and 60F (1.5% w/w) extracts from AC Emerson and Vimy.

AC Emerson and Vimy are shown in Figure 3.2. For both cultivars, UNF and 40F showed a pronounced decrease in viscosity (shear thinning) with an increase in shear rate, typical behavior of a pseudoplastic material. In contrast, the 60F extracts more closely resembled a Newtonian fluid since the steady shear flow curves showed very little shear thinning. For AC Emerson, the apparent viscosity values for the 40F extract at the low shear rates were approximately four times greater than those found for UNF and 60 times greater than those found for 60F. A similar order of decreasing viscosity was observed for the extracts from Vimy, although the apparent viscosities of 40F at low shear rates were not as high as those of AC Emerson 40F. In contrast, Cui et al., (1994a) reported the overall ranking of apparent viscosity to be in the order of unfractionated mucilage > neutral fraction > acidic fraction. The apparent viscosity values exhibited by the 40F extracts for both cultivars in the present study were higher than those reported by Cui et al., (1994a) and Fedeniuk and Biliaderis (1994) for their neutral fraction. The steady shear rheological flow curves for various concentrations of UNF, 40F and 60F are shown in Figures 3.3 and 3.4 for AC Emerson and Vimy, respectively. From the data presented, it is apparent that there is an increase in viscosity with concentration. This observation is in agreement with results reported by a number of researchers who have studied the rheological properties of flaxseed mucilage (Cui et al., 1994a; Cui et al., 1994b; Fedeniuk and Biliaderis, 1994; Wannerberger et al., 1991; Mazza and Biliaderis, 1989). The greatest difference in apparent viscosities between AC Emerson and Vimy was observed in 40F and this became more evident as the steady shear rheological flow curves were studied at different concentrations. The steady shear flow curve for AC Emerson 40F at 0.25% (w/w) was similar to that of Vimy 40F at 0.50% (w/w). As

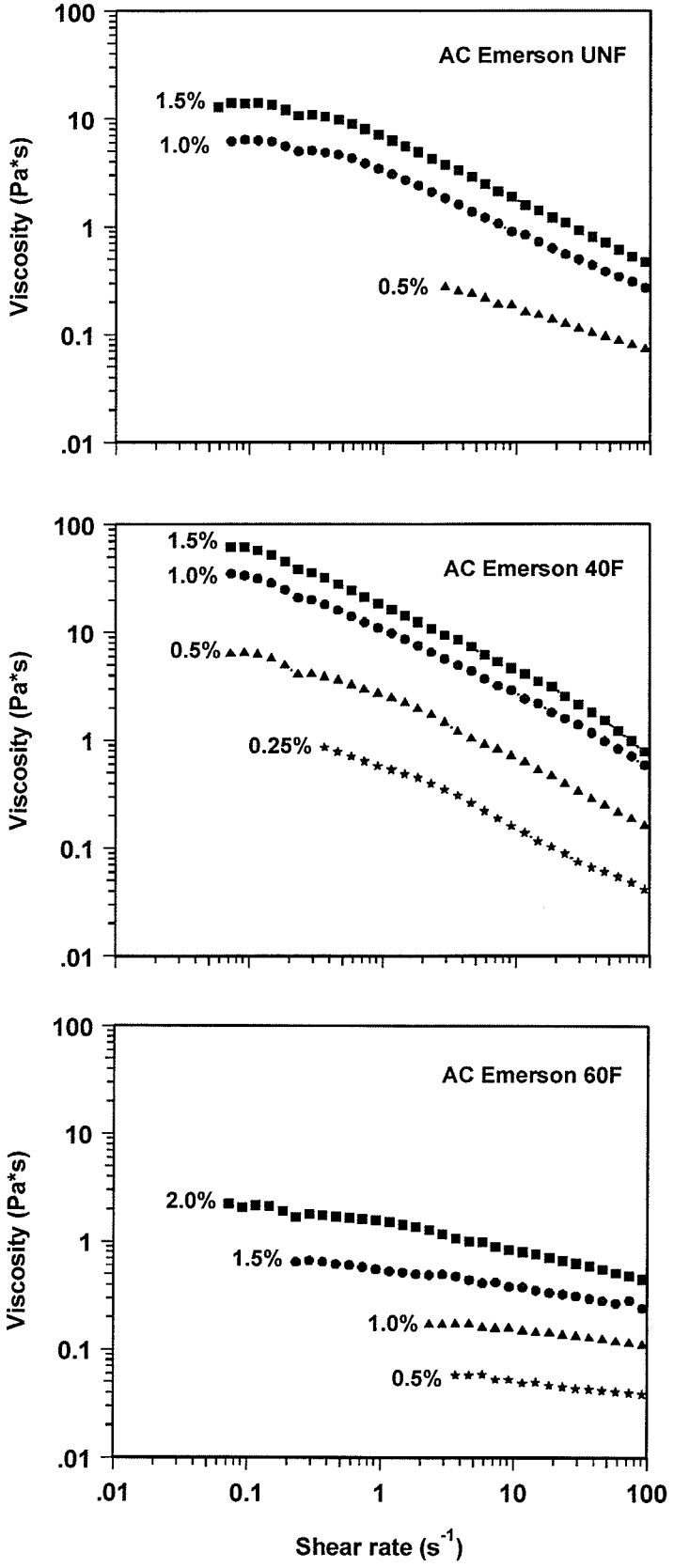

Figure 3.3. The effect of concentration on the steady shear rheological flow curves of AC Emerson UNF, 40F, and 60F.

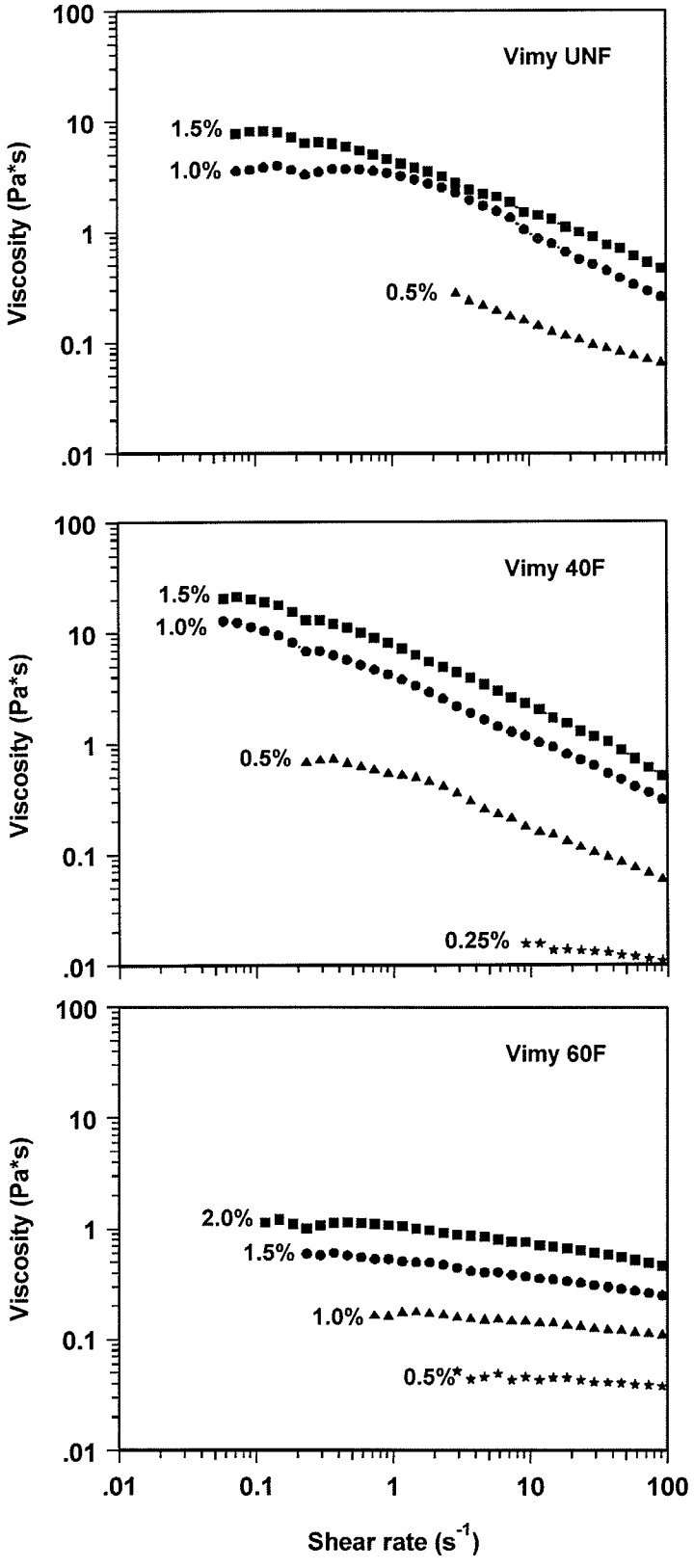

Figure 3.4. The effect of concentration on the steady shear rheological flow curves of Vimy UNF, 40F, and 60F.

the concentration of Vimy 40F was decreased to 0.25% (w/w), the flow curve showed characteristics of a Newtonian fluid, whereas AC Emerson 40F at 0.25% (w/w) continued to exhibit shear thinning behavior.

The small strain oscillatory measurements revealed a gel-like behavior for 40F from both AC Emerson and Vimy, with the storage modulus (G') greater than the loss modulus (G") over the entire frequency range examined (Figure 3.5). The mechanical spectra for 60F, from both cultivars, were typical of a dilute polymer solution with G" greater than G' over the entire frequency range (Figure 3.5). These results are different from those presented by others (Cui et al., 1994a and Fedeniuk and Biliaderis, 1994) in that weak gel properties were not observed for the neutral fraction when either ion exchange chromatography or CTAB precipitation was used. It appears that sequential ethanol precipitation is more effective in obtaining mucilage extracts with very high apparent viscosity and gel-like properties compared to other fractionation techniques. It is believed that sequential ethanol precipitation fractionates polymers mainly on the basis of molecular weight (Scopes, 1982). It has been reported that high molecular weight proteins require a lower percentage of ethanol to induce their precipitation than their low molecular weight counterparts (Scopes, 1982). On the other hand, inconsistent findings have been reported for the fractionation of polysaccharides. Gruppen et al. (1992) found that arabinoxylan fractions which precipitated at higher alcohol concentrations had higher molecular weights than those obtained at lower ethanol concentrations. However, recent studies by Schooneveld-Bergmans (1999a, 1999b) indicated that the molecular weight, radius of gyration (R_g), and intrinsic viscosity of glucuronoarabinoxylans decreased for fractions precipitated at higher ethanol concentrations.

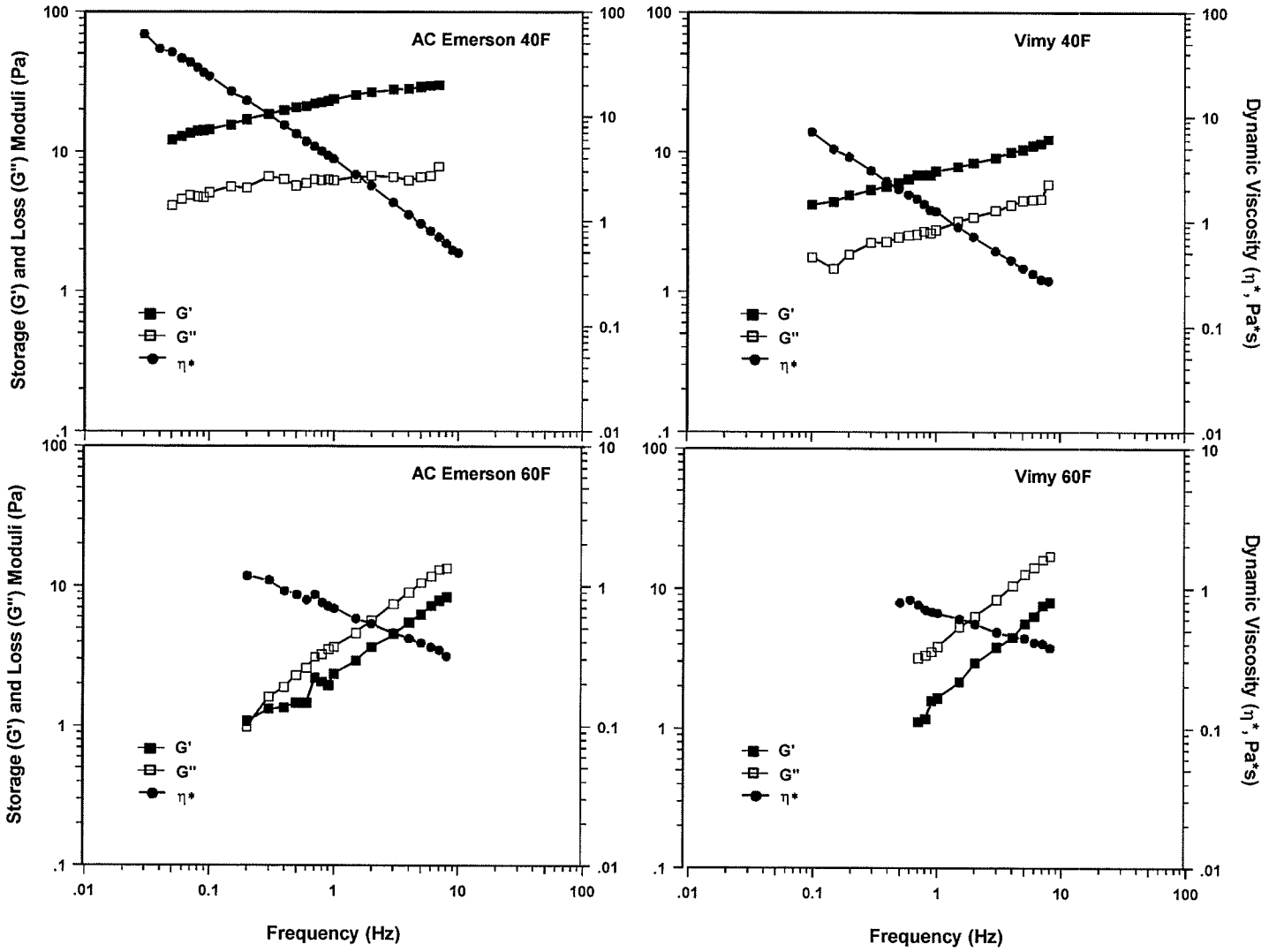

Figure 3.5. Mechanical spectra of 40F (1.5% w/w) and 60F (2.0% w/w) extracts from AC Emerson and Vimy.

By examining the intrinsic viscosity results in combination with the rheological flow curves, it is evident that the polysaccharides present in the 40F and 60F flaxseed extracts differ in structure and conformation along with possible differences in molecular weight. The high apparent viscosity and shear thinning behavior exhibited by the 40F extracts indicates that the polysaccharides present in these extracts have a greater tendency to form a dynamic entangled network in an aqueous environment. This observation is in agreement with the results reported by Fedeniuk and Biliaderis (1994) for the neutral polysaccharide fraction. In contrast, the 60F extracts had more of a branched structure which is not conducive to the formation of an entangled network thereby resulting in properties of low viscosity and very little shear thinning. The polysaccharides of the 60F extracts may be showing characteristics similar to gum arabic, since the highly branched compact structure of gum arabic is believed to be responsible for its low viscosity (Ward and Andon, 1993).

3.4.6. Mucilage Fraction Interactions

Drastic increases in the viscosity of mixed systems are indicative of the synergistic interactions between polymers, whereas significant decreases of the viscosity indicate their incompatibility (Kasapis et al., 1994). Although no large changes in the specific viscosity (η_{sp}) were observed when the 40F and 60F extracts of AC Emerson were mixed together, a small decrease in η_{sp} was observed especially when the polymers were mixed in almost equal ratios (Figure 3.6). These results, therefore, eliminate the possibility of any synergistic interactions between the fractions, indicating instead slight incompatibility. Considering the different molecular composition of the two fractions, these results are not too surprising.

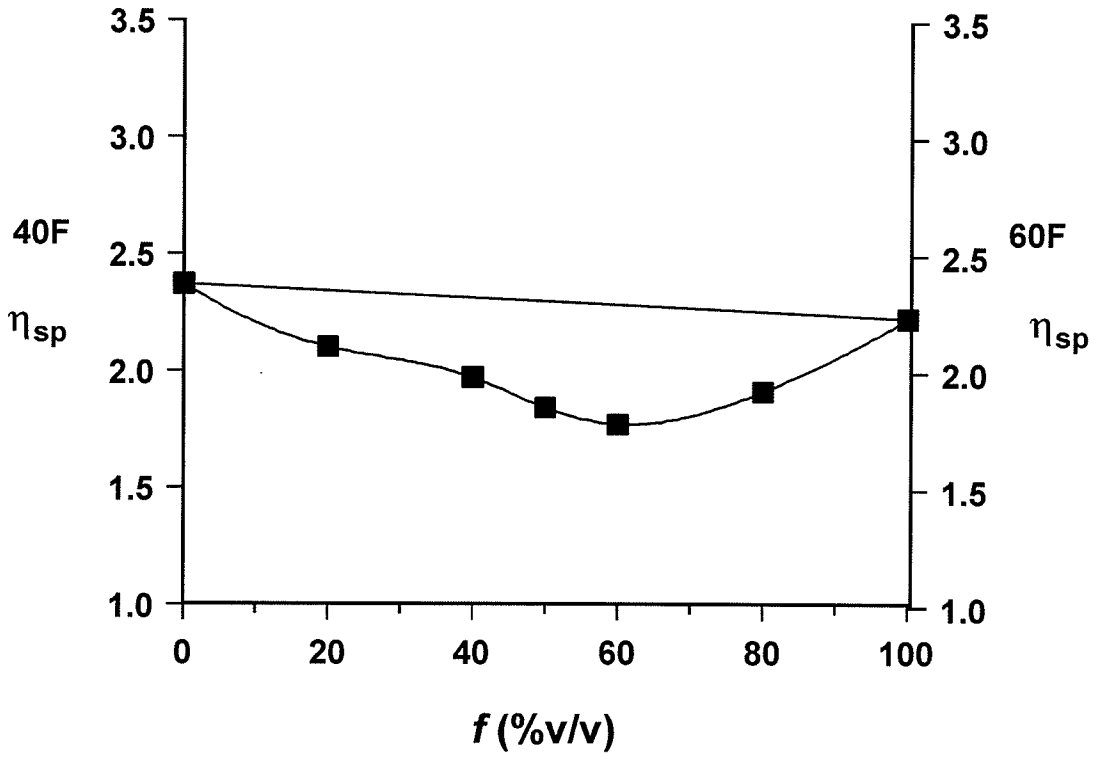

Figure 3.6. Variation in specific viscosity (η_{sp}) of mixtures of AC Emerson 40F and 60F in various proportions; *f* denotes the proportion of 60F solution in each mixed system, with $f = 0$ and $f = 100\%$ (v/v) corresponding to the starting solutions of 40F and 60F respectively.

Incompatibility and phase separation phenomenon are common among unlike polymers (Tolstoguzov, 1986).

3.4.7. Molecular Weight Determination

The size exclusion chromatography profiles for Vimy 40F and 60F are presented in Figure 3.7. Three distinguished polymer populations were observed in 40F. The highest molecular weight population (I), eluting between 11 and 13.5 ml, was followed by two additional intermediate (II) and low molecular weight (III) populations, eluting from 14-17 ml and 21-23 ml, respectively. In contrast, only two polymer populations were observed in 60F, corresponding to the high and low molecular weight populations.

The three polymer populations observed in 40F are indicative of the complex nature of this fraction. The response from the UV detector provided evidence that the low molecular weight population contained primarily proteins. According to the findings of Madhusudhan and Singh (1985), the water-soluble proteins of flaxseed have relatively small molecular weights and therefore could account for the later eluting peak observed after the majority of the carbohydrates had eluted. This conclusion is in agreement with the gel permeation chromatography results reported by Fedeniuk and Biliaderis (1994), which showed carbohydrates eluting mainly in the void volume while proteins were detected in both the void and total volumes. It is interesting to note that some protein material was also found to be associated with the highest molecular weight population. These results might indicate covalent linkages between a portion of the protein material and the high molecular weight polysaccharides. It is believed that the neutral polymers containing arabinose and xylose

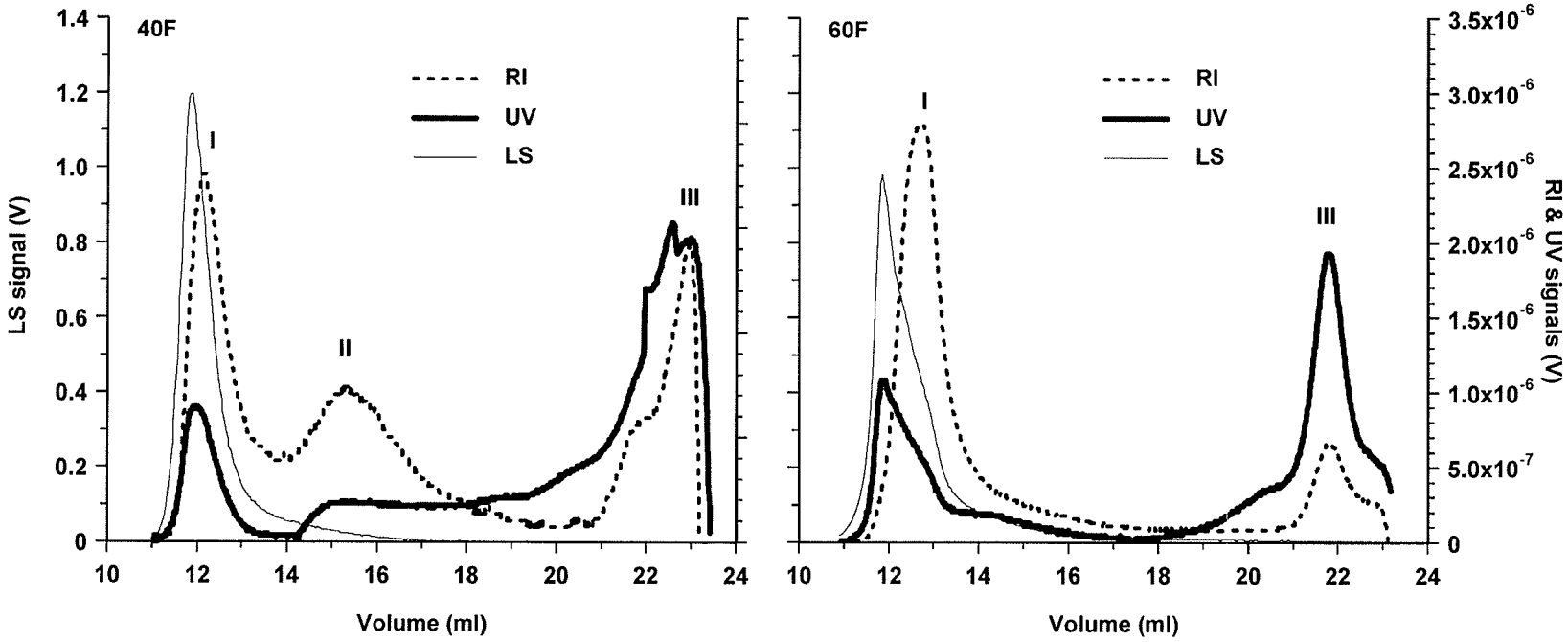

Figure 3.7. Superimposed chromatograms of multiangle light scattering (LS), refractive index (RI), and UV detector outputs for Vimy 40F and 60F.

constituted the majority of the high molecular weight population. It is possible that the acidic polymers in 40F, as indicated by the presence of rhamnose and galacturonic acid in this fraction, eluted either with the high or intermediate molecular weight populations. The exact composition of the high and intermediate molecular weight populations in 40F remains unresolved at the present time. The high concentration of glucose found in 40F suggests that a cellulose-like polymer might have been extracted with the arabinoxylan and pectic-like polymers from flaxseed.

The calculated values for average molecular weight (M_w), radius of gyration (R_g), and polydispersity (M_w/M_n) of the various polymer populations in the 40F and 60F extracts are presented in Table 3.7. The high molecular weight population in AC Emerson 40F exhibited a higher M_w than the corresponding population in Vimy 40F. The M_w of the intermediate material in AC Emerson 40F was also higher than in Vimy 40F. For the high molecular weight and intermediate molecular weight populations the R_g values (a measure of the amount of space occupied by the polysaccharide) for AC Emerson 40F were greater than those of Vimy 40F. These differences in M_w and R_g correspond well with the rheological properties and intrinsic viscosity values observed for AC Emerson 40F and Vimy 40F.

The response from the UV detector indicates that the distribution of protein material in 60F (Figure 3.7) was similar to that in 40F. Some proteins were associated with the high molecular weight population, but the majority eluted in the low molecular weight region. The average M_w of polymers eluting in the high molecular weight region of 60F was significantly lower than the corresponding polymers in 40F for both cultivars (Table 3.7). The R_g was also lower but not by the same order of magnitude as seen for the M_w values (Table 3.7). This

Table 3.7. Molecular weight (M_w), radius of gyration (R_g) and polydispersity (M_w/M_n) values for the mucilage extracts

Cultivar and extract	High Molecular Weight Population (I)			Intermediate Molecular Weight Population (II)		
	$M_w \times 10^6$	R_g (nm)	(M_w/M_n)	$M_w \times 10^6$	R_g (nm)	(M_w/M_n)
AC Emerson						
40F	23.3 ± 2.9	129.9 ± 11.5	1.09 ± 0.04	5.0 ± 0.4	108.2 ± 6.7	1.07 ± 0.02
60F	4.4 ± 0.3	95.4 ± 0.7	4.62 ± 0.59			
Vimy						
40F	13.1 ± 0.1	105.6 ± 2.1	1.34 ± 0.05	1.6 ± 0.1	79.1 ± 1.8	1.15 ± 0.07
60F	3.1 ± 0.0	80.2 ± 0.1	1.89 ± 0.43			

Means ± SD (n=2)

may imply different conformation of the neutral and acidic polymers. Again, these results corroborate the rheological data which showed 60F having very low apparent viscosities compared to 40F. In addition, the polydispersity values for polymers in the high molecular weight population of 60F were greater than those reported for the corresponding polymers in 40F (Table 3.7).

3.4.8. Evaluation of Emulsifying Ability

The emulsifying ability of the three mucilage extracts from AC Emerson and Vimy (0.1 and 0.25%, w/w) was evaluated by monitoring the absorbance of the diluted emulsions as a function of time (Figures 3.8 and 3.9 respectively). Although the performance of each polysaccharide solution varied, they all showed some degree of emulsifying ability as shown by the higher absorbance reading at each time interval compared to that of the control (which had no polysaccharide added). Emulsion quality is often estimated by the interfacial area of the emulsion (Pearce and Kinsella, 1978). AC Emerson UNF (0.1%) and 40F (0.1%) had similar initial absorbance readings and as a result had interfacial area values that were almost identical (Table 3.8). As the concentration increased to 0.25% for both AC Emerson UNF and 40F the quality of the emulsions increased similarly as indicated by the higher absorbance and interfacial area values. The interfacial area values for Vimy UNF and 40F were lower than those of the respective AC Emerson extracts. Vimy 40F exhibited slightly higher interfacial area values than Vimy UNF at 0.1 and 0.25%. For both cultivars, 60F exhibited a lower interfacial area value than UNF and 40F. As the concentration of 60F increased from 0.1 to 0.25% the interfacial area values also increased for both cultivars. No differences

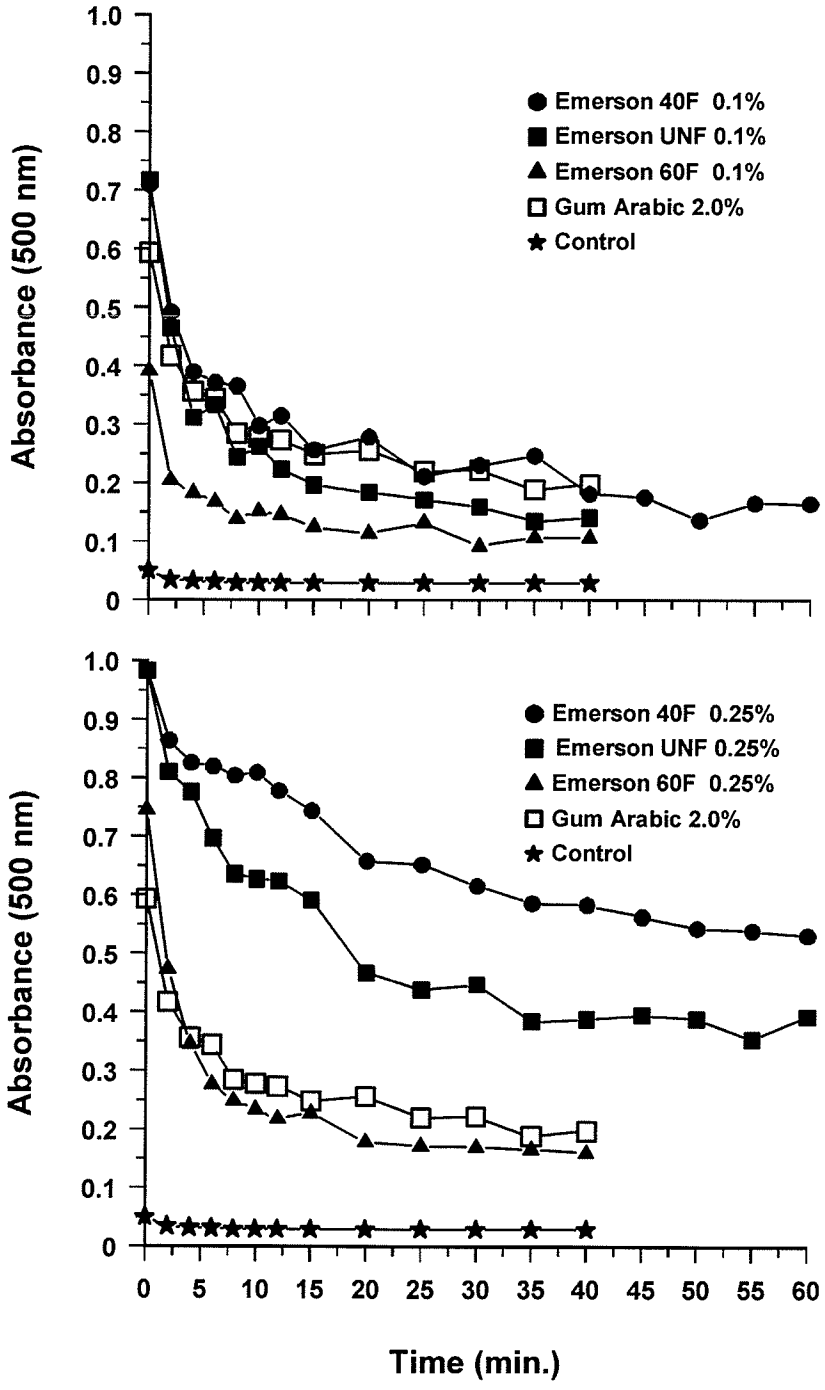

Figure 3.8. Emulsifying ability of gum arabic (2.0% w/w) and mucilage extracts (0.1 and 0.25%, w/w) from AC Emerson. The emulsions were diluted (50-fold) with 0.1% sodium dodecyl sulfate (SDS) solution, and the emulsifying ability was evaluated by measuring the absorbance at 500 nm.

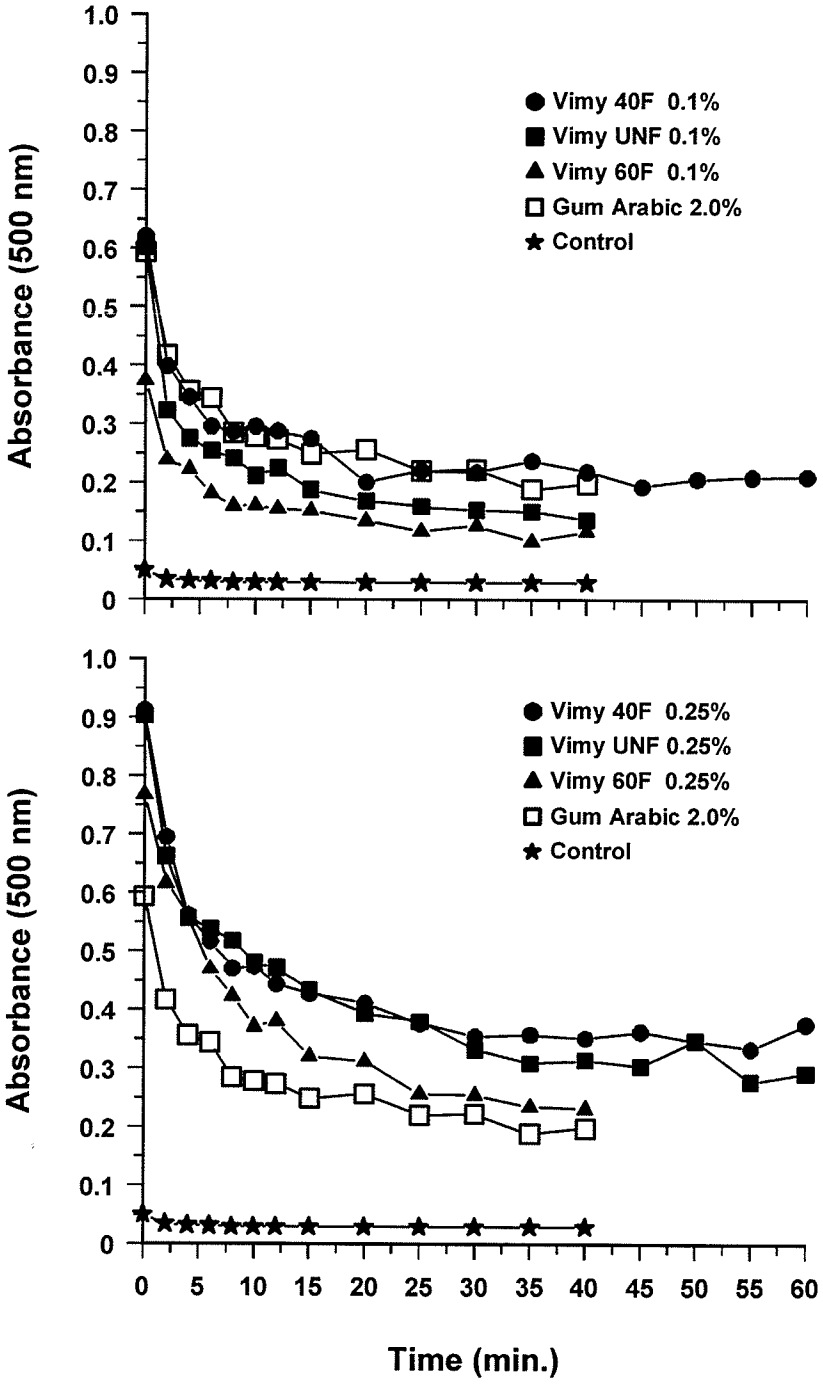

Figure 3.9. Emulsifying ability of gum arabic (2.0% w/w) and mucilage extracts (0.1 and 0.25%, w/w) from Vimy. The emulsions were diluted (50-fold) with 0.1% sodium dodecyl sulfate (SDS) solution, and the emulsifying ability was evaluated by measuring the absorbance at 500 nm.

Table 3.8. Interfacial area data for oil-in-water emulsions prepared using flaxseed mucilage at different concentrations

Polysaccharide	Interfacial area[a] x 10^3 m^{-1}	
	0.1% (w/w)	0.25 % (w/w)
AC Emerson		
UNF	16.5 ± 0.1	22.7 ± 0.3
40F	16.4 ± 1.0	22.7 ± 1.1
60F	9.0 ± 0.0	17.2 ± 0.2
Vimy		
UNF	13.9 ± 0.0	20.8 ± 0.0
40F	14.3 ± 0.8	21.0 ± 0.3
60F	8.6 ± 0.9	17.7 ± 0.4

[a]Means ± SD (n = 2)

Table 3.9. Rate constant (k) for emulsion decay for oil-in-water emulsions prepared using flaxseed mucilage at different concentrations

Polysaccharide	Rate constant[a] (k) min^{-1}	
	0.1% (w/w)	0.25 % (w/w)
AC Emerson		
UNF	0.124 ± 0.001 [b]	0.051 ± 0.002 [b]
40F	0.081 ± 0.006 [a]	0.023 ± 0.000 [a]
60F	0.114 ± 0.002 [b]	0.137 ± 0.003 [c]
Vimy		
UNF	0.103 ± 0.003 [a]	0.066 ± 0.002 [a]
40F	0.093 ± 0.016 [a]	0.081 ± 0.012 [a]
60F	0.100 ± 0.015 [a]	0.073 ± 0.005 [a]

[a]Means ± SD (n = 2). Means from the same cultivar within a column followed by a different superscript are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

between the cultivars were found. Since the mucilage extracts evaluated in this study varied in composition and solution viscosity, it is believed that these differences likely had an effect on the initial emulsification. According to Gaonkar (1991), an increase in the viscosity of the continuous phase facilitates emulsion formation by favoring oil drop breakage leading to higher turbidity and increased interfacial area values and a better quality emulsion overall. The differences observed in the interfacial area for the three extracts of AC Emerson and Vimy correspond well with the rheological properties observed for the same extracts. Fedeniuk (1993) also reported an increase in interfacial area with an increase in concentration after evaluating the emulsifying ability of unfractionated flaxseed mucilage extracted under similar conditions.

Measurement of the interfacial area of a polymer not only permits assessment of emulsion quality, but by monitoring this parameter as a function of time it is possible to evaluate a polymers ability to stabilize an oil-water interface (Pearce and Kinsella, 1978). All the mucilage emulsions showed a decrease in absorbance with time (Figures 3.8 and 3.9). The stability of the emulsions were evaluated as first-order models and the rate constants (k) for emulsion decay are reported in Table 3.9. The mucilage extracts which exhibited similar interfacial area values immediately after emulsification (Table 3.8) did not show the same ability to stabilize the emulsion. This is indicated by the different rates of emulsion decay (Table 3.9). In general, as the concentration increased from 0.1% to 0.25%, the rate of emulsion decay decreased, meaning the emulsion was more stable. AC Emerson 60F was the only extract for which the opposite was observed. When comparing the stabilizing effect of the two cultivars at 0.1% and 0.25%, the three extracts from AC Emerson differed greatly

from each other, whereas all three extracts from Vimy exhibited similar rates of emulsion decay. Overall, enhanced emulsification and emulsion stability were attained when AC Emerson 40F (0.25%) was incorporated into the aqueous phase.

Gum arabic is known for having excellent emulsifying properties and differs from other polysaccharides due to its unusually low viscosity. It is commonly used in bakery emulsions in combination with other gums like gum tragacanth (Ward and Andon, 1993). The emulsifying ability of gum arabic was evaluated along with the flaxseed mucilage extracts in order to make comparisons between the two hydrocolloids. The emulsifying ability of gum arabic (0.1 and 2.0%, w/w) was determined by monitoring the absorbance of the diluted emulsions as a function of time (Figure 3.10). As the concentration of gum arabic increased to 2.0% the interfacial area for the emulsion increased (Table 3.10) indicating that a higher quality emulsion was attained. Although the 2.0% solution of gum arabic gave a better quality emulsion, the rate of emulsion decay was faster than the emulsion containing only 0.1%.

When examining the emulsifying ability of gum arabic and the flaxseed mucilage extracts at a concentration of 0.1%, it was observed that the interfacial area value for gum arabic (Table 3.10) was lower than values for UNF and 40F from both cultivars but similar to the values reported for 60F (Table 3.8). Although the initial quality of the emulsion containing gum arabic (0.1%) was lower than the emulsion containing the UNF and 40F mucilage extracts (0.1%), gum arabic had a slower rate of decay indicating that it had a greater emulsion stabilizing effect. As the mucilage concentration increased to 0.25%, all of the extracts showed higher interfacial area values compared to gum arabic (0.1%) but only

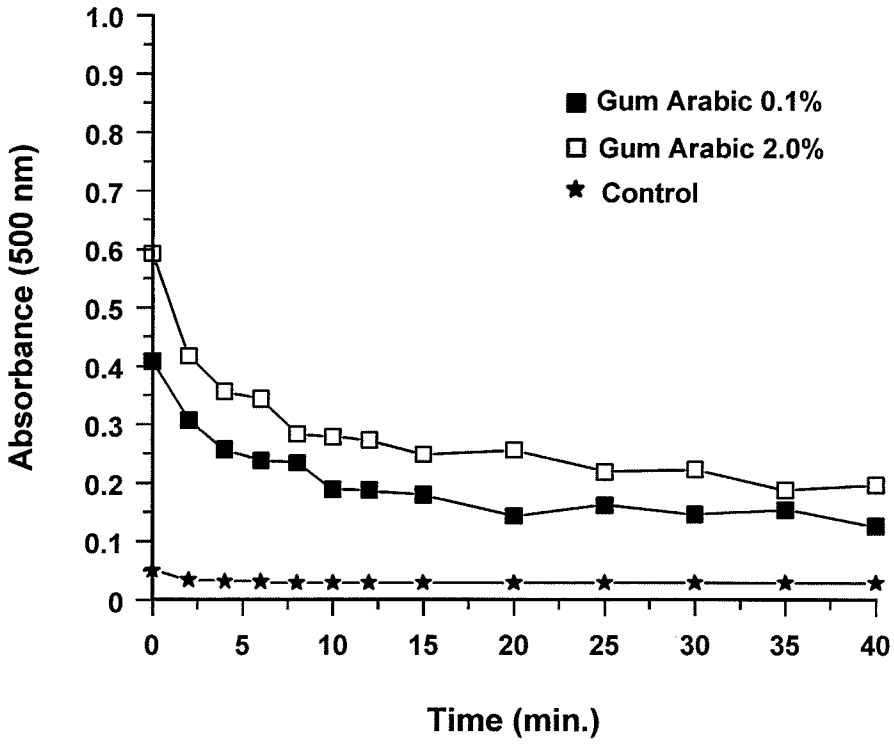

Figure 3.10. Emulsifying ability of gum arabic (0.1 and 2.0% w/w) preparations. The emulsions were diluted (50-fold) with 0.1% sodium dodecyl sulfate (SDS) solution, and the emulsifying ability was evaluated by measuring the absorbance at 500 nm.

Table 3.10. Interfacial area data and rate constant (k) for emulsion decay for oil-in-water emulsions prepared using gum arabic at different concentrations

Polysaccharide	Interfacial area x 10^3 m^{-1}	Rate constant (k) min^{-1}
Gum Arabic		
0.1% (w/w)	9.4 ± 1.6	0.068
2.0% (w/w)	13.7 ± 0.5	0.083

Means ± SD (n = 2)

AC Emerson UNF, AC Emerson 40F and Vimy 40F exhibited slower rates of emulsion decay.

The emulsifying ability of gum arabic at a higher concentration was also evaluated because when used at equal concentrations gum arabic did not show similar emulsifying properties to the mucilage extracts. Due to the fact that gum arabic does not develop any significant viscosity at concentrations less than 30% (Anderson and Andon, 1988), it was possible to test the emulsifying ability of gum arabic at 2.0% and then compare its performance to that of the mucilage extracts at 0.25%. Even though gum arabic was evaluated at a higher concentration (2%), all of the flaxseed mucilage extracts at 0.25% exhibited greater interfacial area values (Tables 3.8 and 3.10). In addition, all the mucilage extracts (0.25%), except AC Emerson 60F, had slower rates of emulsion decay (Table 3.9 and 3.10). These results are in agreement with those of Fedeniuk (1993) who reported that flaxseed mucilage (0.2%) extracted from Linott at 80°C had a higher emulsifying activity index than gum arabic (0.2%).

According to Gaonkar (1991), gums generally stabilize emulsions by increasing the viscosity of the aqueous phase which inhibits the rate of coalescence between the oil droplets. Gum arabic exhibits very little solution viscosity, therefore it has been found that it is the small percentage (2-3%) of heat-sensitive proteins associated with gum arabic which are responsible for its emulsifying capabilities (Anderson and Andon, 1988). Protein molecules are amphoteric in nature and are therefore able to absorb at the oil-water interface, thereby decreasing the interfacial tension between the two phases (Jackman et al., 1989). Although the flax mucilage extracts do contain a significant amount of protein, it is believed that the stabilizing effect that has been observed is mostly due to the ability of the mucilage to increase

solution viscosity. This conclusion is in agreement with Fedeniuk (1993) who concluded that differences in emulsifying ability among flaxseed mucilage extracts could not be attributed to protein content alone since extracts with a higher protein content exhibited lower emulsifying activity index values. In addition, Fedeniuk (1993) reported that mucilage extracts which exhibited similar interfacial activity had significantly different emulsifying activity index values suggesting that another factor besides protein content is involved in determining the emulsifying ability of flaxseed mucilage. Regardless of the mechanism, the suggestion by BeMiller (1973) that flaxseed mucilage could replace gum arabic as a functional ingredient in oil-in-water emulsion systems is supported by the results from this study.

3.4.9. Foam Stabilization

The protective action of the mucilage extracts against the thermal disruption of a foam formed by a surface-active protein (bovine serum albumin) was measured and the results are shown in Table 3.11. Bovine serum albumin in the presence of $NaHCO_3$ and citric acid (control) formed a small layer of foam which increased in volume as it was exposed to heat. After 2 minutes of heating the foam started to break down and the volume decreased. The volume of the foam for the control and the mucilage containing systems was monitored for 5 minutes in a hot water bath. For some of the samples, it became difficult to determine whether a complete foam was still present after 3 minutes. This caused the test to become more subjective making it difficult to achieve reproducible results. As a result, the foam volumes are only reported up to 3 minutes of heating. The stabilizing effect of the three mucilage extracts for each cultivar at three different concentrations was examined. In general,

Table 3.11. Stabilization of foams by mucilage extracts from AC Emerson and Vimy

		Foam Volume (ml)					
			Acidification[a]		Heating[b]		
Polysaccharide[c]	% (w/w)	Initial*	Immediate	After 10 min.	After 1 min.	After 2 min.	After 3 min.
Control[d]		0.9 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.2	3.7 ± 0.3
AC Emerson UNF	1.0	0.7 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	4.2 ± 0.1	5.2 ± 0.0	4.4 ± 0.0
	0.5	0.9 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	3.8 ± 0.8	4.1 ± 0.5	---
	0.25	1.0 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	3.9 ± 0.0
AC Emerson 40F	1.0	0.7 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	6.3 ± 0.0	7.5 ± 0.0	7.3 ± 0.0
	0.5	0.9 ± 0.0	1.3 ± 0.1	1.1 ± 0.1	5.2 ± 0.2	5.5 ± 0.7	2.0 ± 0.0
	0.25	0.8 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	4.5 ± 0.0	4.6 ± 0.3	4.2 ± 0.5
AC Emerson 60F	1.0	0.9 ± 0.1	1.2 ± 0.2	1.0 ± 0.0	4.6 ± 0.2	4.9 ± 0.4	3.4 ± 0.8
	0.5	1.1 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	4.3 ± 0.3	4.3 ± 0.4	3.7 ± 0.3
	0.25	1.1 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	4.3 ± 0.0	4.2 ± 0.3	4.1 ± 0.4
Vimy UNF	1.0	1.0 ± 0.1	1.4 ± 0.3	1.2 ± 0.2	6.1 ± 0.2	5.5 ± 0.6	2.7 ± 0.1
	0.5	1.1 ± 0.2	1.4 ± 0.2	1.3 ± 0.1	5.4 ± 0.7	4.9 ± 0.4	4.5 ± 0.5
	0.25	1.0 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	5.0 ± 0.1	5.0 ± 0.2	4.7 ± 0.1
Vimy 40F	1.0	0.5 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	4.7 ± 0.5	6.1 ± 0.2	6.1 ± 0.1
	0.5	0.7 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	5.0 ± 0.0	5.8 ± 0.1	5.9 ± 0.1
	0.25	1.1 ± 0.2	1.5 ± 0.0	1.3 ± 0.0	5.9 ± 0.2	5.5 ± 0.1	5.0 ± 0.0
Vimy 60F	1.0	0.9 ± 0.1	1.5 ± 0.2	1.3 ± 0.2	5.2 ± 0.5	4.7 ± 0.1	1.6 ± 0.5
	0.5	1.2 ± 0.1	1.5 ± 0.0	1.4 ± 0.0	5.0 ± 0.1	5.0 ± 0.6	4.8 ± 0.4
	0.25	1.2 ± 0.1	1.5 ± 0.1	1.4 ± 0.0	5.1 ± 0.1	4.9 ± 0.0	4.7 ± 0.0
Xanthan Gum	1.0	0.6 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	11.6 ± 0.9	10.9 ± 1.3	8.7 ± 0.5
	0.25	0.9 ± 0.0	1.3 ± 0.4	1.2 ± 0.2	5.6 ± 0.7	6.5 ± 0.6	6.5 ± 0.6
Gum Arabic	1.0	1.1 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	4.7 ± 0.2	4.2 ± 0.4	3.7 ± 0.3

[a] Acidification by addition of 0.25 ml of citric acid solution (5%, w/v)
[b] Heating in a 95°C water bath
[c] Means ± SD (n = at least two replicates)
[d] Means ± SD (n = 8); Control foam formed by shaking (2 min.) 1 ml of 2.0% (w/v) bovine serum albumin and 0.25 ml of 5% (w/v) $NaHCO_3$
* Prior to the addition of citric acid

for both AC Emerson and Vimy, the 40F extracts tended to have lower initial volumes than UNF and 60F. This result is in agreement with Fedeniuk et al. (1993) who reported that the greatest reduction of initial foam formation occurred when the neutral fraction of flaxseed mucilage was incorporated into a foam forming system. The initial decrease in foam volume upon the addition of certain polysaccharides is assumed to be caused by the increased viscosity of the system (Izydorczyk et al., 1991).

It is believed that polysaccharides are able to stabilize foams by retarding the gas diffusion and/or by acting as steric stabilizers of the films surrounding the gas bubbles. Each of the extracts were able to stabilize the foam to some degree, but the 40F extracts which exhibited higher apparent viscosities and weak gel-like behavior were better able to stabilize the foams with AC Emerson 40F (1.0%) having the largest foam volumes during the heating process. This result is likely due to the fact that the 40F extracts were able to provide a certain level of viscosity and elasticity to the thin film surrounding the gas bubbles which is important for foam stabilization (Izydorczyk, 1991). When comparing the two cultivars the greatest difference in foam volumes during heating was observed with the 40F extract which corresponds well with the rheological data. These results are in agreement with those reported by Fedeniuk (1993) where it was found that mucilage fractions exhibiting the greatest solution viscosity were more effective in stabilizing the foam during heating. It is believed that more definitive conclusions regarding the performance of the various mucilage extracts in this study would have been possible if the reporting of the foam volumes could have been extended for a longer period of time. Xanthan gum and gum arabic, included for comparison purposes, were also effective at stabilizing the foam during heating, but xanthan

gum (1.0%) was the most effective. At a concentration of 1.0%, the 40F mucilage extracts gave similar results to that of xanthan gum (0.25%), with AC Emerson 40F performing slightly better.

3.5. Conclusions

Compositional analysis of the mucilage extracts revealed that sequential ethanol precipitation was able to separate the neutral and acidic polysaccharides present in flaxseed mucilage to some degree but it is believed that complete separation of the two polysaccharide fractions is likely not possible using this fractionation technique. Sequential ethanol precipitation does appear however to be an effective method for obtaining polysaccharides with different physicochemical characteristics. Examination of the UNF, 40F and 60F extracts for AC Emerson and Vimy revealed that these three extracts differed in composition, rheological properties and functional characteristics (emulsifying ability and foam stabilization). Further examination of the 40F and 60F revealed that the average molecular weight of polymers eluting in the high molecular weight region of 40F was significantly higher than the corresponding polymers in 60F. Results from molecular weight analysis and intrinsic viscosity measurements suggested that the polysaccharides in the two extracts must have also differed in structure and conformation. Differences in the molecular characteristics were reflected in the rheological behavior of the extracts, with UNF and 40F exhibiting more pronounced shear thinning and weak-gel behavior compared to 60F.

While investigating the effects of sequential ethanol precipitation, it was determined that no additional mucilage precipitated after the aqueous extract was adjusted to 60% ethanol (approximately 2 volumes), therefore it was concluded that 4 volumes of ethanol were

not necessary for complete mucilage precipitation.

4. EFFECT OF CULTIVAR ON THE PHYSICOCHEMICAL PROPERTIES OF FLAXSEED MUCILAGE

4.1. Abstract

The objective of this study was to determine the effect of cultivar on the composition, rheological, and emulsifying properties of mucilage extracted from cultivars commonly grown in Canada and to determine whether relationships exist among these parameters. Mucilage was extracted from seven flaxseed cultivars commonly grown in Canada (CDC Bethune, AC Carnduff, AC Emerson, AC Linora, AC McDuff, NorLin, Vimy). An aqueous extraction procedure was used followed by precipitation with ethanol at various concentrations. Complete mucilage precipitation was carried out by adjusting the aqueous extract to 75% ethanol (UNF). Sequential ethanol precipitation was also investigated and fractions were collected at 40% ethanol (40F) and 60% ethanol (60F) for each cultivar. The mucilage extracts were analyzed for carbohydrate and protein content as well as monosaccharide composition. The rheological properties were characterized using intrinsic viscosity, steady shear flow and small strain oscillatory measurements. The functionality of the mucilage extracts was assessed by examining their emulsifying ability. Significant differences in carbohydrate and protein content of the mucilage extracts were observed among the cultivars. The proportion of neutral and acidic polysaccharides present in the mucilage extracts also varied among the cultivars. Although cultivars containing a larger quantity of neutral polysaccharides (higher xylose content) tended to exhibit increased viscosity and weak-gel properties, the variation in the rheological behavior observed among the cultivars could not

be fully accounted for by the differences observed in xylose content. Differences in intrinsic viscosity and rates of shear thinning were also observed which suggested that the molecular weight and structural conformation of the extracted polysaccharides differed among the cultivars. It was concluded that the diverse rheological properties observed among the cultivars was caused by differences in the proportion of neutral and acidic polysaccharides as well as differences in molecular weight and structural conformation of the extracted polysaccharides. In general, those cultivars which exhibited increased viscosity and higher intrinsic viscosity values also showed the greatest potential for the stabilization of oil-in-water emulsions. The diverse rheological properties observed among the seven cultivars provides breeders with evidence that genetic variability exists in the physicochemical properties of flaxseed mucilage. This variability may allow plant breeders to develop cultivars that contain mucilage with rheological and functional characteristics for specific end uses.

4.2. Introduction

It has been suggested that there is potential for flaxseed mucilage to be used as a thickening agent or emulsion stabilizer due to its highly viscous nature (Fedeniuk, 1993; Wannerberger et al., 1991; Mazza and Biliaderis, 1989; BeMiller, 1973), but information on the functionality of flaxseed mucilage remains quite limited. The functionality of a food gum is affected by many factors, including molecular size, structure, orientation and molecular association of the polymers, water binding and swelling, concentration, particle size and degree of dispersion (Ward and Andon, 1993). For this reason, detailed studies which examine the structure-property relationships of a potential food gum like flaxseed mucilage

are required in order to fully understand and elucidate its functional role as a food ingredient.

Flaxseed mucilage is composed primarily of two polysaccharides: a neutral arabinoxylan-like polymer and an acidic pectic-like polymer (Cui et al., 1994a; Muralikrishna et al., 1987). Previous studies have reported that the composition and rheological properties of flaxseed mucilage can vary among flaxseed cultivars (Cui and Mazza, 1996; Cui et al., 1996; Oomah et al., 1995; Wannerberger et al., 1991). Other studies have examined the functionality of flaxseed mucilage to some degree (Fedeniuk, 1993; Mazza and Biliaderis, 1989) or have characterized its structure, composition and rheological properties to a greater extent by separating the neutral and acidic polysaccharides through various fractionation techniques (Cui et al., 1994a; Fedeniuk and Biliaderis, 1994; Fedeniuk 1993), but in all cases only single cultivars were examined. Testing the functionality of the mucilage along with the composition and rheological properties has not yet been carried out on a number of cultivars. This type of study is required in order to determine what effect these factors may have on the functional characteristics of flaxseed mucilage. In addition there is limited information available on the composition and rheological properties of flaxseed mucilage extracted from cultivars that are currently grown in Canada.

Once the relationships between composition, rheological properties and functionality are established, plant breeders may be able to use this information to establish methods which will aid them in screening for desired functional characteristics. Therefore, the objective of this study was to determine the effect of cultivar on the composition, rheological properties and emulsifying ability of mucilage extracted from several flax cultivars commonly grown in Canada and to determine whether relationships among these parameters exist . Sequential

ethanol precipitation was used in addition to complete mucilage precipitation. This approach has not yet been used on flaxseed mucilage and it is believed that fractionation of the mucilage may provide additional insight into any differences which may exist among the cultivars. In addition, data collected from the various cultivars will be used to try to establish which physicochemical properties have the greatest effect on the functionality of flaxseed mucilage, in particular as a thickening agent and as an emulsion stabilizer.

4.3. Materials and Methods

4.3.1. Materials

Flaxseed cultivars CDC Bethune, AC Carnduff, AC Emerson, AC Linora, AC McDuff, NorLin and Vimy were obtained from the 1999 harvest at the Morden Research Station, Agriculture and Agri-Food Canada, located in Morden, Manitoba. In addition, a seven cultivar composite containing equal proportions of all cultivars was made and evaluated along with the individual cultivars. All chemicals were of analytical reagent grade.

4.3.2. Chemical Analyses of Whole Flaxseed

Oil content was determined using pulsed nuclear magnetic resonance spectroscopy (NMR) according to the International Organization for Standardization method ISO 10565:1993(E) and the results are reported as a percent, calculated to a moisture-free basis. Protein content was measured by the Kjeldahl method according to the approved AOCS method Ba 4d-90(93). Prior to protein analysis the flax samples were defatted by shaking approximately 1.25 g of sample with 10 ml of petroleum ether in a swedish tube for 1 hour.

The sample was filtered and the meal was dried overnight at 50°C. Results are reported as percent protein (N*6.25) on an oil free and dry weight basis. Fatty acid profiles were determined by gas liquid chromatography according to the approved AOCS methods Ce-91 and Ce2-66(93). Results are reported as the relative percent of each fatty acid. The iodine number was calculated according to the approved AOCS method Cd 1c-85. Cyanogenic glucosides were identified and quantified by high performance liquid chromatography according to the procedure described by Oomah et al. (1992). All analyses were done in duplicate.

4.3.3. Extraction and Fractionation of Mucilage

The extraction and fractionation of the mucilage was carried out according to the procedure described in section 3.3.2.

4.3.4. Chemical Analyses of Flaxseed Mucilage Extracts

Protein content, galacturonic acid content and relative amounts of component monosaccharides in the mucilage extracts were determined according to the procedures outlined in section 3.3.3.

4.3.5. Rheological Measurements

The experimental work described in section 3.4.5 was used to determine which solvents would be best suited for measuring the intrinsic viscosity of the three extracts for the remaining cultivars. For the intrinsic viscosity measurements, aqueous solutions of UNF and

60F (0.06-0.10%, w/v) were prepared using 1 M NaCl while aqueous solutions of 40F extracts (0.03-0.06%, w/v) were prepared with deionized water. All measurements were preformed in Ubbelohde viscometers (International Research Glassware, Kenilworth, NJ) at 24 ± 0.05°C. The reduced viscosities were plotted against polymer concentration and extrapolated to zero concentration using the Huggins equation (Huggins, 1942) to obtain the intrinsic viscosities. Steady shear flow curves and small strain oscillatory measurements of the mucilage extracts were carried out using a Bohlin VOR rheometer (Bohlin Rheology, Edison, NJ). All measurements were taken at 23 ± 0.1 °C using a concentric cylindrical geometry (the radii of the inner rotor and outer container were 7.8 and 19.5 mm respectively; the rotor length was 64 mm) and the 16.91 g·cm torsion element. Steady shear rheological measurements were carried out on aqueous solutions of UNF (1.0%, w/w), 40F (1.0%, w/w) and 60F (2.0%, w/w). The samples were subjected to shear rates of 0.0291 to 116 s^{-1} and the results are reported as averages of two measurements. The mechanical spectra of the UNF (1.5%, w/w) and 40F (1.5%, w/w) extracts were obtained between 0.01-10 Hz and less than 2% strain. Results are reported as averages of three measurements. Mechanical spectra for the 60F extracts were not included because they did not possess weak gel properties and for the majority of the spectra contained a lot of noise which made it difficult to make comparisons among the cultivars.

4.3.6. Evaluation of Emulsifying Ability

The method described in section 3.3.7 was used for evaluating the emulsifying ability of the UNF, 40F, and 60F extracts at a concentration of 0.25% w/w.

4.3.7. Statistical Analysis

One-way analysis of variance (ANOVA) and Tukey's studentized range test were used to determine differences in mean values based on data collected from two replications of each measurement. ANOVA and Pearson correlation coefficients were preformed using JMP IN Statistical software (SAS Institute Inc., 1996). Significance was established at $p \leq 0.05$.

4.4. Results and Discussion

4.4.1. Chemical Analyses of Whole Flaxseed

The oil content for the seven flax cultivars ranged from 43.8 to 46.8% (dwb) (Table 4.1) and significant differences ($p \leq 0.05$) were found among all the cultivars. These values are in agreement with data obtained from the 1999 Canadian Grain Commission harvest survey where it was reported that the oil content for farm delivered samples ranged from 38.8 - 48.4% (dwb) (Daun and Przybylski, 2000). The protein contents for the cultivars used in the present study ranged from 44.9 to 47.6% (oil free and dry weight basis) (Table 4.1) and significant differences ($p \leq 0.05$) were observed among some of the cultivars. Differences in protein content among flax samples has been attributed to both genetics and environment (Oomah and Mazza, 1993). The cultivars used in this study were all grown at the same location so it is believed that differences in protein content were a result of genotype differences among the cultivars examined.

Linustatin and neolinustatin are the main cyanogenic glucosides present in flaxseed (Oomah et al., 1992). The linustatin and neolinustatin contents of the seven flax cultivars

Table 4.1. Chemical composition of seven flaxseed cultivars

			Cyanogenic Glucosides (mg/100g)		
Cultivar	**Oil (%)[a]**	**Protein (%)[b]**	**Linustatin**	**Neolinustatin**	**Total**
CDC Bethune	45.2 ± 0.0^{a}	47.6 ± 0.5^{a}	155.9 ± 2.0^{cd}	109.1 ± 1.6^{b}	265.0 ± 0.4^{e}
AC Carnduff	43.8 ± 0.0^{b}	45.7 ± 0.9^{bc}	141.2 ± 3.0^{d}	168.8 ± 3.7^{a}	310.1 ± 0.7^{bc}
AC Emerson	44.9 ± 0.0^{c}	44.9 ± 0.3^{c}	146.3 ± 1.2^{d}	168.5 ± 4.8^{a}	314.8 ± 6.0^{b}
AC Linora	46.4 ± 0.0^{d}	46.4 ± 0.1^{abc}	164.4 ± 6.4^{bc}	106.0 ± 2.5^{b}	270.4 ± 8.9^{de}
AC McDuff	46.8 ± 0.0^{e}	46.7 ± 0.2^{ab}	206.5 ± 7.0^{a}	68.1 ± 8.5^{c}	274.6 ± 1.5^{de}
NorLin	44.0 ± 0.0^{f}	45.3 ± 0.3^{bc}	172.7 ± 1.0^{b}	166.6 ± 1.6^{a}	399.3 ± 2.6^{a}
Vimy	45.8 ± 0.1^{g}	46.6 ± 0.2^{abc}	164.2 ± 3.0^{bc}	124.2 ± 6.3^{b}	288.4 ± 9.3^{cd}

[a] Means ± SD (n = 2), dry weight basis
[b] Means ± SD (n = 2), oil free and dry weight basis, N x 6.25
Means within the same column followed by a different superscript are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test

ranged from 141 to 207 mg/100g and 68 to 169 mg/100g respectively (Table 4.1). It has been reported that the amount of cyanogenic glucosides present in flaxseed is dependent on cultivar, location and year of production, with cultivar being the most important factor (Oomah et al., 1992). Thus, it is not surprising that significant differences ($p \leq 0.05$) were observed among the cultivars examined in the present study.

The fatty acid composition of the seven flax cultivars is shown in Table 4.2. Flaxseed oil is known for its high content of alpha-linolenic acid (ALA) which makes it unique from other edible oils. ALA is an essential fatty acid in the diet and recent research suggests that ALA along with other important components of flaxseed (fiber and lignan) can provide numerous health benefits some of which include preventing or reducing the risk of certain types of cancers, cardiovascular disease and diabetes (Vaisey-Genser and Morris, 1997b). The amount of ALA for the seven cultivars ranged from 53.7 to 59.0% with CDC Bethune having the lowest amount and AC Emerson having the highest. The iodine value, which is a measure of the degree of unsaturation, was also calculated for each cultivar from the fatty acid composition results and these values are provided in Table 4.2.

4.4.2. Extraction and Fractionation of Mucilage

The yield of unfractionated mucilage (UNF) for the seven cultivars ranged from 4.9 to 7.2% of the seed weight (Table 4.3) with the average yield being 6.3%. As expected, the yield for the seven cultivar composite was the same as the average yield for the seven individual cultivars. The yields obtained are comparable to those reported by Cui et al. (1996) where the same amount (4 volumes) of ethanol was used for the precipitation of mucilage

Table 4.2. Fatty acid composition[a] and iodine value for seven flaxseed cultivars[b]

Cultivar	C16:0	C18:0	C18:1	C18:2	C18:3	Iodine Value
CDC Bethune	4.8 ± 0.0^{e}	3.9 ± 0.1^{b}	23.6 ± 0.1^{b}	14.1 ± 0.1^{c}	53.7 ± 0.1^{d}	185.1 ± 0.4^{f}
AC Carnduff	4.7 ± 0.0^{e}	4.4 ± 0.1^{a}	20.1 ± 0.0^{d}	15.0 ± 0.1^{b}	55.9 ± 0.1^{b}	189.4 ± 0.3^{c}
AC Emerson	5.4 ± 0.0^{c}	2.8 ± 0.0^{e}	18.8 ± 0.4^{e}	14.1 ± 0.1^{c}	59.0 ± 0.4^{a}	194.9 ± 0.7^{a}
AC Linora	5.3 ± 0.0^{c}	2.8 ± 0.0^{e}	19.0 ± 0.0^{e}	16.4 ± 0.1^{a}	56.6 ± 0.0^{b}	192.7 ± 0.1^{b}
AC McDuff	5.8 ± 0.1^{a}	4.1 ± 0.1^{b}	19.3 ± 0.1^{e}	16.3 ± 0.2^{a}	54.8 ± 0.1^{c}	187.9 ± 0.1^{d}
NorLin	5.0 ± 0.0^{d}	3.2 ± 0.1^{d}	24.9 ± 0.0^{a}	11.9 ± 0.1^{d}	55.1 ± 0.2^{c}	186.1 ± 0.4^{ef}
Vimy	5.6 ± 0.0^{b}	3.6 ± 0.0^{c}	21.3 ± 0.2^{c}	14.7 ± 0.1^{b}	54.8 ± 0.1^{c}	187.0 ± 0.2^{de}

[a] C16:0 = palmitic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = alpha-linolenic acid

[b] Means ± SD (n = 2); means within the same column followed by a different superscript are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test

Table 4.3. Yield[a] and composition[b] of mucilage extract UNF

Cultivar	Yield (%)	Carbohydrate (%)	Protein (%)
CDC Bethune	6.3	44.8 ± 1.5^{b}	32.6 ± 1.2^{ab}
AC Carnduff	4.9	42.8 ± 1.2^{bc}	34.5 ± 0.9^{a}
AC Emerson	6.8	50.0 ± 1.5^{a}	28.7 ± 1.0^{bc}
AC Linora	6.4	43.9 ± 0.8^{b}	32.9 ± 1.3^{a}
AC McDuff	5.7	38.4 ± 0.6^{c}	34.3 ± 0.9^{a}
NorLin	7.2	45.3 ± 1.9^{ab}	27.2 ± 1.3^{c}
Vimy	7.0	45.5 ± 1.3^{ab}	27.5 ± 0.5^{c}
Average	6.3	44.4	31.1
C.V. (%)	12.7	7.8	10.2
7 Cultivar Composite	6.3	41.4 ± 0.3	29.1 ± 0.1

[a] Yields are expressed as a % of seed weight

[b] $n = 2 \pm$ SD, presented on an as is % wt basis, means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test

from 12 flaxseed cultivars. A single extraction was carried out for all the cultivars except for AC McDuff. When duplicate extractions were conducted on AC McDuff, the yields for UNF differed by only 0.02%. This result suggests that the method of extraction used in this study can provide reproducible results. Under similar extraction conditions Cui et al. (1996) also reported little variation in yield between duplicate extractions. Among the seven cultivars, NorLin was the highest yielding while AC Carnduff was the lowest. The results in section 3.4.1. suggest that very little mucilage precipitates above 60% ethanol. It is not known for sure whether all the mucilage present in the seed was extracted during the three hour extraction procedure, but since the yields were similar to those reported by other researchers it is believed that the yield of mucilage collected after adjusting to 75% ethanol could be used as an indicator for the amount of soluble fiber present in a particular cultivar. From the yields reported for the UNF extract, it is evident that the soluble fiber content varied among the seven cultivars. There is limited information available on the soluble fiber content of different cultivars because it is not a component of flaxseed that is commonly monitored, unlike oil, protein and fatty acid composition. However, Hettiarachchy et al. (1990) measured the total dietary fiber content of eleven flaxseed cultivars grown in North Dakota and found that the total dietary fiber content varied by 10% among the cultivars.

Chemical analysis of the UNF extracts revealed that the carbohydrate and protein contents ranged from 38.4 to 50.0% and 27.2 to 34.5% respectively (Table 4.3). Significant differences in carbohydrate and protein content were observed among the cultivars and may be a reflection of differences in seed composition. The carbohydrate and protein contents for the seven cultivar composite were slightly lower than the average carbohydrate and protein

contents for the seven cultivars. The carbohydrate content of AC McDuff was much lower than the seven cultivar average (38.4 vs. 44.4%) while the carbohydrate content of AC Emerson (50.0%) was significantly higher ($p \leq 0.05$) than the majority of the other cultivars. The results for protein content are higher than those reported by Cui et al. (1994a), Fedeniuk and Biliaderis (1994) and Wannerberger et al. (1991), while the results for carbohydrate content were lower than those reported by Cui et al. (1994a), Wannerberger et al. (1991), and Susheelamma (1987). These differences in composition may be a reflection of the different cultivars as well as differences in extraction conditions and methods of analyses.

Sequential ethanol precipitation resulted in two fractions being collected, one at 40% ethanol (40F) and a second at 60% ethanol (60F). The yields of 40F ranged from 1.2 to 3.0% of the seed weight for the seven cultivars (Table 4.4) with the average yield being 2.3%. The seven cultivar composite yielded a slightly lower amount than the average. When duplicate extractions were conducted on AC McDuff, the yields for 40F differed by 0.21%. Chemical analysis revealed that the carbohydrate and protein contents for 40F ranged from 48.8 to 54.9% and 20.2 to 28.8%, respectively (Table 4.4). Significant differences in carbohydrate and protein content were observed among the cultivars. The average carbohydrate content for the seven cultivars was 52.0% which is similar to the value obtained for the seven cultivar composite. The average carbohydrate content for the 40F fraction was considerably higher than that of the UNF fraction, but the variation among the cultivars was not as great. The average protein content for the seven cultivars was 24.5% which is considerably higher than the value obtained for the seven cultivar composite. Overall, the average protein content for the 40F extract was considerably lower than that of UNF. The composition results for the

Table 4.4. Yield[a] and composition[b] of mucilage extract 40F

Cultivar	Yield (%)	Carbohydrate (%)	Protein (%)
CDC Bethune	2.6 (47.7)	53.2 ± 0.9^{abc}	21.4 ± 0.6^{c}
AC Carnduff	1.5 (33.5)	53.9 ± 0.6^{a}	25.5 ± 0.1^{b}
AC Emerson	2.7 (41.5)	54.9 ± 2.0^{a}	20.2 ± 0.9^{c}
AC Linora	2.1 (40.4)	48.8 ± 0.4^{c}	26.1 ± 1.3^{ab}
AC McDuff	1.2 (22.1)	50.6 ± 0.6^{abc}	28.8 ± 1.2^{a}
NorLin	2.9 (44.2)	53.4 ± 1.0^{ab}	24.8 ± 0.5^{b}
Vimy	3.0 (42.5)	49.3 ± 1.7^{bc}	24.6 ± 0.2^{b}
Average	2.3	52.0	24.5
C.V. (%)	30.7	4.6	11.8
7 Cultivar Composite	2.0 (33.8)	52.6 ± 1.3	19.9 ± 0.5

[a] Yields are expressed as a % of seed weight. In brackets, yields are expressed as a % of total mucilage collected at 40% and 60% ethanol.

[b] n = 2 ± SD, presented on an as is % wt basis, means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test

40F extracts suggest that when a lower concentration of ethanol is used for precipitation, mucilage with a higher carbohydrate and a lower protein content can be obtained.

The yields for 60F were higher than 40F and ranged from 2.8 to 4.1% of the seed weight for the seven cultivars (Table 4.5) with the average yield being 3.5%. The seven cultivar composite yielded a slightly higher amount. When duplicate extractions were conducted on AC McDuff, the yields for 60F differed by 0.17%. Chemical analysis revealed that the carbohydrate and protein contents for 60F ranged from 39.1 to 44.6% and 19.5 to 31.5%, respectively (Table 4.5). Significant differences in carbohydrate and protein content were observed among the cultivars. The average carbohydrate content of 60F for the seven cultivars was 42.0% which is similar to the value obtained for the seven cultivar composite (41.5%). The average protein content of 60F for the seven cultivars was 27.7% which is slightly higher than the value obtained for the seven cultivar composite (23.1%). For each cultivar, the carbohydrate content of 60F was lower than that of 40F, and for all cultivars, except Vimy, the protein content of 60F was higher than that of 40F.

4.4.3. Monosaccharide Composition

The monosaccharide composition of UNF for the seven cultivars is presented in Table 4.6 and significant differences ($p \leq 0.05$) were observed among some of the cultivars. Xylose and rhamnose have commonly been used as indicators for the neutral and acidic polysaccharides respectively (Oomah et al., 1995; Fedeniuk and Biliaderis, 1994). For all cultivars, UNF contained a mixture of neutral and acidic polysaccharides as indicated by the considerable quantities of both rhamnose and xylose, but the amount of each polysaccharide

Table 4.5. Yield[a] and composition[b] of mucilage extract 60F

Cultivar	Yield (%)	Carbohydrate (%)	Protein (%)
CDC Bethune	2.8 (52.3)	39.6 ± 0.5^{cd}	29.9 ± 0.6^{c}
AC Carnduff	3.0 (66.5)	42.9 ± 1.1^{abc}	31.5 ± 0.2^{a}
AC Emerson	3.8 (58.5)	44.2 ± 1.5^{ab}	24.3 ± 0.2^{e}
AC Linora	3.2 (59.6)	39.1 ± 0.5^{d}	31.3 ± 0.2^{ab}
AC McDuff	4.1 (77.9)	40.6 ± 0.4^{bcd}	30.3 ± 0.2^{bc}
NorLin	3.7 (55.8)	44.6 ± 1.0^{a}	27.3 ± 0.1^{d}
Vimy	4.0 (57.5)	43.2 ± 1.0^{abc}	19.5 ± 0.5^{f}
Average	3.5	42.0	27.7
C.V. (%)	14.6	5.3	15.9
7 Cultivar Composite	3.9 (66.2)	41.5 ± 0.9	23.1 ± 0.1

[a] Yields are expressed as a % of seed weight. In brackets, yields are expressed as a % of total mucilage collected at 40% and 60% ethanol.

[b] $n = 2 \pm$ SD, presented on an as is % wt basis, means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test

varied among the cultivars. For the UNF extracts, the ratio of rhamnose to xylose ranged from 0.55 to 1.50 (Table 4.6) with AC Emerson having the lowest and AC McDuff having the highest. The remaining cultivars had rhamnose to xylose ratios between AC Emerson and AC McDuff and these ratios were quite similar to each other. The ratios fell within the range (0.3 to 2.2) reported by Oomah et al. (1995) for 109 accessions of flaxseed. The xylose content for AC Emerson (40.3%) was significantly higher ($p \leq 0.05$) than all cultivars, including AC McDuff (22.7%). Whereas the rhamnose content for AC McDuff (34.1%) was significantly higher ($p \leq 0.05$) than all cultivars including AC Emerson (22.0%). In addition to being the only cultivar having a rhamnose to xylose ratio greater than one, AC McDuff also had the highest content of galactose and fucose and the lowest content of arabinose among the seven cultivars, all indicators of its acidic nature. The average monosaccharide composition for each of the seven cultivars was comparable to the composition found for the seven cultivar composite. The monosaccharide levels found in the present study fall within the ranges reported by Cui et al. (1996) for mucilage extracted from several other flaxseed cultivars under similar extraction conditions.

The monosaccharide composition of 40F for the seven cultivars is presented in Table 4.7 and significant differences ($p \leq 0.05$) were observed among some of the cultivars. For all cultivars, the 40F extract contained larger quantities of neutral polysaccharides compared to acidic polysaccharides as indicated by the low rhamnose to xylose ratios. The ratios of rhamnose to xylose ranged from 0.15 to 0.27 with AC Emerson having the lowest and Vimy having the highest. Compared to their UNF counterparts, the rhamnose to xylose ratios of 40F were considerably lower. When the monosaccharide composition of the seven cultivar

Table 4.6. Monosaccharide composition[a] of mucilage extract UNF

Cultivar	Rhamnose	Fucose	Arabinose	Xylose	Galactose	Glucose	GalA[b]	R/X[c]
CDC Bethune	24.5 ± 0.8 [c]	4.7 ± 0.0 [d]	13.6 ± 0.1 [a]	37.8 ± 0.3 [b]	17.5 ± 0.5 [de]	1.9 ± 0.1 [de]	23.8 ± 0.5 [bc]	0.65
AC Carnduff	26.0 ± 0.5 [bc]	5.3 ± 0.3 [b]	12.4 ± 0.1 [b]	33.2 ± 0.4 [d]	18.4 ± 0.2 [cd]	4.6 ± 0.0 [a]	22.2 ± 0.1 [c]	0.78
AC Emerson	22.0 ± 0.2 [d]	4.1 ± 0.1 [e]	12.6 ± 0.0 [b]	40.3 ± 0.0 [a]	19.0 ± 0.0 [bc]	2.0 ± 0.0 [cd]	25.0 ± 0.2 [ab]	0.55
AC Linora	26.7 ± 0.8 [b]	4.8 ± 0.0 [cd]	12.5 ± 0.1 [b]	35.8 ± 0.6 [c]	18.3 ± 0.1 [cd]	1.9 ± 0.2 [de]	24.1 ± 1.3 [abc]	0.75
AC McDuff	34.1 ± 0.2 [a]	6.1 ± 0.1 [a]	9.2 ± 0.1 [d]	22.7 ± 0.2 [e]	25.6 ± 0.2 [a]	2.3 ± 0.0 [c]	24.7 ± 0.1 [ab]	1.50
NorLin	26.5 ± 0.1 [bc]	4.5 ± 0.0 [de]	12.7 ± 0.2 [b]	37.4 ± 0.1 [b]	17.3 ± 0.3 [e]	1.6 ± 0.1 [e]	25.2 ± 0.9 [ab]	0.70
Vimy	28.1 ± 0.7 [b]	5.2 ± 0.0 [bc]	10.8 ± 0.2 [c]	32.2 ± 0.4 [d]	19.8 ± 0.1 [b]	3.8 ± 0.0 [b]	26.5 ± 0.1 [a]	0.87
Average	26.8	5.0	12.0	34.2	19.4	2.6	24.5	0.80
C.V. (%)	13.9	13.1	12.3	16.9	14.7	44.3	5.5	37.8
7 Cultivar Composite	24.3 ± 0.7	4.8 ± 0.0	12.3 ± 0.1	36.0 ± 0.3	19.6 ± 0.2	2.6 ± 0.0	20.3 ± 0.0	0.68

[a] Relative monosaccharide composition expressed as mole %, n =2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[b] Galacturonic acid content determined colorimetrically and reported on a polysaccharide weight basis, n = 2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[c] R/X = rhamnose to xylose ratio

Table 4.7. Monosaccharide composition[a] of mucilage extract 40F

Cultivar	Rhamnose	Fucose	Arabinose	Xylose	Galactose	Glucose	GalA[b]	R/X[c]
CDC Bethune	10.5 ± 0.4 [b]	0.7 ± 0.0 [a]	19.8 ± 0.0 [a]	53.0 ± 0.2 [bcd]	11.6 ± 0.0 [a]	4.5 ± 0.1 [c]	15.2 ± 0.4 [e]	0.20
AC Carnduff	8.9 ± 0.1 [c]	0.6 ± 0.0 [ab]	18.3 ± 0.1 [bc]	51.3 ± 0.1 [d]	9.9 ± 0.0 [e]	10.7 ± 0.1 [a]	13.2 ± 0.3 [f]	0.17
AC Emerson	8.3 ± 0.1 [c]	0.4 ± 0.0 [d]	19.8 ± 0.2 [a]	54.7 ± 0.2 [ab]	11.2 ± 0.1 [bc]	5.6 ± 0.3 [bc]	16.9 ± 0.4 [d]	0.15
AC Linora	11.3 ± 0.3 [b]	0.5 ± 0.0 [c]	17.7 ± 0.1 [cd]	53.4 ± 0.6 [bc]	10.7 ± 0.2 [d]	6.0 ± 0.2 [b]	17.6 ± 0.3 [cd]	0.21
AC McDuff	11.4 ± 0.7 [b]	0.6 ± 0.0 [bc]	18.4 ± 0.3 [b]	51.5 ± 0.8 [cd]	11.5 ± 0.1 [ab]	6.3 ± 0.5 [b]	18.9 ± 0.3 [bc]	0.22
NorLin	10.6 ± 0.2 [b]	0.3 ± 0.0 [e]	17.5 ± 0.1 [d]	56.5 ± 0.1 [a]	9.6 ± 0.0 [e]	5.2 ± 0.1 [bc]	19.5 ± 0.6 [b]	0.19
Vimy	13.1 ± 0.4 [a]	0.6 ± 0.0 [ab]	17.1 ± 0.2 [d]	48.2 ± 0.9 [e]	10.9 ± 0.1 [cd]	9.6 ± 0.5 [a]	21.8 ± 0.5 [a]	0.27
Average	10.6	0.5	18.4	52.7	10.8	6.8	17.6	0.20
C.V. (%)	15.2	26.1	5.8	5.1	7.1	34.4	16.2	19.1
7 Cultivar Composite	10.7 ± 0.3	0.5 ± 0.0	19.3 ± 0.3	48.8 ± 0.3	12.3 ± 0.0	7.9 ± 0.3	16.4 ± 0.1	0.22

[a] Relative monosaccharide composition expressed as mole %, n = 2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[b] Galacturonic acid content determined colorimetrically and reported on a polysaccharide weight basis, n = 2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[c] R/X = rhamnose to xylose ratio

composite was compared to the average for the seven cultivars some differences were observed.

The monosaccharide composition of 60F for the seven cultivars is presented in Table 4.8 and significant differences ($p \leq 0.05$) were observed among some of the cultivars. For all cultivars, 60F contained larger quantities of acidic polysaccharides compared to neutral polysaccharides as indicated by the high rhamnose to xylose ratios. The ratios of rhamnose to xylose ranged from 1.86 to 3.97 with AC Carnduff having the lowest and AC McDuff having the highest. Compared to their UNF and 40F counterparts, the rhamnose to xylose ratios of 60F were considerably higher. When the monosaccharide composition of the seven cultivar composite was compared to the average for the seven cultivars some differences were observed.

The ratio of rhamnose to xylose is often examined because it is indicative of the ratio of acidic to neutral polysaccharides which may be important in predicting its rheological behavior. For each of the three extracts, the variation among the cultivars was determined by calculating the coefficient of variation (C.V.) for the rhamnose to xylose ratio and for each monosaccharide. These values are provided in Tables 4.6 to 4.8. The UNF extract had the highest coefficient of variation (37.8%) for the rhamnose to xylose ratio which was similar to the value reported by Oomah et al. (1995). The coefficient of variation for the rhamnose to xylose ratio of 40F was 19.1% indicating that the variation among the cultivars was less when a smaller amount of ethanol was used for precipitation. In addition, there was a substantial decrease in the coefficient of variation for arabinose, xylose, galactose and galacturonic acid for the 40F extract compared to the UNF extract. The coefficient of

Table 4.8. Monosaccharide composition[a] of mucilage fraction 60F

Cultivar	Rhamnose	Fucose	Arabinose	Xylose	Galactose	Glucose	GalA[b]	R/X[c]
CDC Bethune	37.3 ± 0.9 [b]	10.1 ± 0.4 [a]	5.2 ± 0.1 [ab]	16.9 ± 0.5 [b]	27.7 ± 0.1 [cd]	2.8 ± 0.1 [c]	31.5 ± 1.3 [a]	2.21
AC Carnduff	35.2 ± 0.8 [b]	8.6 ± 0.2 [cd]	5.6 ± 0.1 [a]	18.9 ± 0.2 [a]	27.3 ± 0.2 [d]	4.5 ± 0.0 [a]	28.1 ± 0.0 [b]	1.86
AC Emerson	37.4 ± 0.3 [b]	7.3 ± 0.0 [e]	5.6 ± 0.2 [a]	19.2 ± 0.3 [a]	27.9 ± 0.1 [cd]	2.6 ± 0.1 [d]	32.3 ± 0.3 [a]	1.95
AC Linora	39.8 ± 0.5 [a]	9.7 ± 0.3 [ab]	4.7 ± 0.0 [bc]	14.3 ± 0.0 [d]	28.7 ± 0.3 [b]	2.7 ± 0.0 [cd]	28.0 ± 0.6 [b]	2.78
AC McDuff	42.1 ± 0.8 [a]	8.3 ± 0.1 [d]	4.1 ± 0.2 [d]	10.6 ± 0.4 [e]	32.4 ± 0.0 [a]	2.6 ± 0.1 [d]	33.0 ± 1.0 [a]	3.97
NorLin	40.1 ± 0.0 [a]	9.1 ± 0.1 [bc]	4.6 ± 0.1 [c]	15.7 ± 0.0 [c]	28.0 ± 0.1 [c]	2.5 ± 0.1 [d]	27.7 ± 0.7 [b]	2.55
Vimy	37.4 ± 0.0 [b]	9.3 ± 0.2 [abc]	5.5 ± 0.0 [a]	17.0 ± 0.2 [b]	27.5 ± 0.2 [cd]	3.4 ± 0.1 [b]	32.0 ± 0.2 [a]	2.20
Average	38.5	8.9	5.0	16.1	28.5	3.0	30.4	2.50
C.V. (%)	6.0	10.5	11.6	18.4	6.2	23.9	7.7	28.8
7 Cultivar Composite	32.3 ± 0.2	7.1 ± 0.1	7.6 ± 0.0	25.5 ± 0.1	24.7 ± 0.3	2.8 ± 0.0	24.2 ± 0.1	1.27

[a] Relative monosaccharide composition expressed as mole %, n = 2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[b] Galacturonic acid content determined colorimetrically and reported on a polysaccharide weight basis, n = 2. Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

[c] R/X = rhamnose to xylose ratio

variation for the xylose to rhamnose ratio of 60F (28.8%) was midway between that of 40F and UNF.

Thus, similar to the results reported in Chapter 3, the monosaccharide composition of flaxseed mucilage varies when different levels of ethanol are used for precipitation. Furthermore, results from this study show that within each of the extracts significant differences ($p \leq 0.05$) in monosaccharide composition also exist among the cultivars.

4.4.4. Rheological Measurements

A solution of 1 M NaCl was used for measuring the intrinsic viscosity of the UNF extracts and the values ranged from 3.56 to 5.51 dl/g (Table 4.9) with AC McDuff having the lowest value and AC Emerson having the highest. These values fall within the range obtained by Cui and Mazza (1996) for other flaxseed cultivars when measured under similar conditions. The intrinsic viscosity value depends mainly on the molecular size of a polymer, rigidity of the polymer chain and solvent quality (Launay et al., 1985). Considering the range of intrinsic viscosity values observed for the UNF extract, it is believed that the average molecular weight of polysaccharides extracted with 75% ethanol differed among some of the cultivars. Since the intrinsic viscosity is also dependent on the structure and conformation of the polysaccharide, it is possible that differences in these characteristics may also exist among the cultivars. From the monosaccharide composition results, it is apparent that the UNF extract contains a mixture of both neutral and acidic polysaccharides but the amount of each polysaccharide varies among cultivars. Cui and Mazza (1996) reported that the neutral polysaccharide has a larger molecular size than the acidic polysaccharide, therefore it was

Table 4.9. Intrinsic viscosities of the mucilage extracts (dl/g)

Cultivar	UNF[a]	40F[b]	60F[a]
CDC Bethune	4.58	6.29	4.19
AC Carnduff	3.90	6.67	4.26
AC Emerson	5.51	7.76	4.43
AC Linora	4.82	6.58	4.80
AC McDuff	3.56	4.47	3.97
NorLin	5.04	7.37	4.60
Vimy	4.75	5.26	5.09

[a] 1 M NaCl was used as the solvent for the intrinsic viscosity measurements.
[b] Deionized water was used as the solvent for the intrinsic viscosity measurements.

Table 4.10. Flow behavior index (n) and consistency coefficient (m) for the UNF extracts (1.0% w/w) according to the Power Law model $\eta = m\gamma^{n-1}$ (0.367-116 s^{-1})

Cultivar	n	m
CDC Bethune	0.45	3.27
AC Carnduff	0.51	1.48
AC Emerson	0.45	3.17
AC Linora	0.43	3.04
AC McDuff	0.56	0.85
NorLin	0.48	2.56
Vimy	0.47	3.23

suggested that cultivars having higher amounts of xylose, the main sugar found in the neutral polysaccharide, should have a higher intrinsic viscosity. Cui and Mazza (1996) compared intrinsic viscosity and xylose content for three flaxseed cultivars with yellow seed coats and found a fairly strong relationship between these two parameters. In general there is limited data available on the relationship between intrinsic viscosity and xylose content for both brown and yellow seeded flaxseed cultivars. Figure 4.1 shows the relationship between xylose content and intrinsic viscosity for the UNF extracts collected in this study and the Pearson's correlation coefficient was calculated to be 0.85 ($p = 0.016$). The coefficient of determination (r^2) for this relationship was 0.72.

The steady shear flow curves for UNF extracts are shown in Figure 4.2. All cultivars exhibited a decrease in viscosity with an increase in shear rate (shear thinning), but definite differences were observed in the steady shear flow curves among the cultivars. Throughout the range of shear rates examined, the steady shear flow curves for CDC Bethune and AC Emerson were fairly similar. Both CDC Bethune and AC Emerson had higher apparent viscosities than the remaining five cultivars at the low shear rates, but as the shear rate increased the steady shear flows curves for NorLin, Vimy and AC Linora became more similar to CDC Bethune and AC Emerson. AC McDuff exhibited the lowest apparent viscosity at all shear rates with AC Carnduff being slightly above. Differences in shear thinning behavior among the UNF extracts was quantified using the power law model. Given that polysaccharides solutions with smaller flow behavior index (n) values exhibit a higher degree of shear thinning, the results in Table 4.10 indicate that AC McDuff exhibited the lowest degree of shear thinning followed by AC Carnduff. The low degree of shear thinning

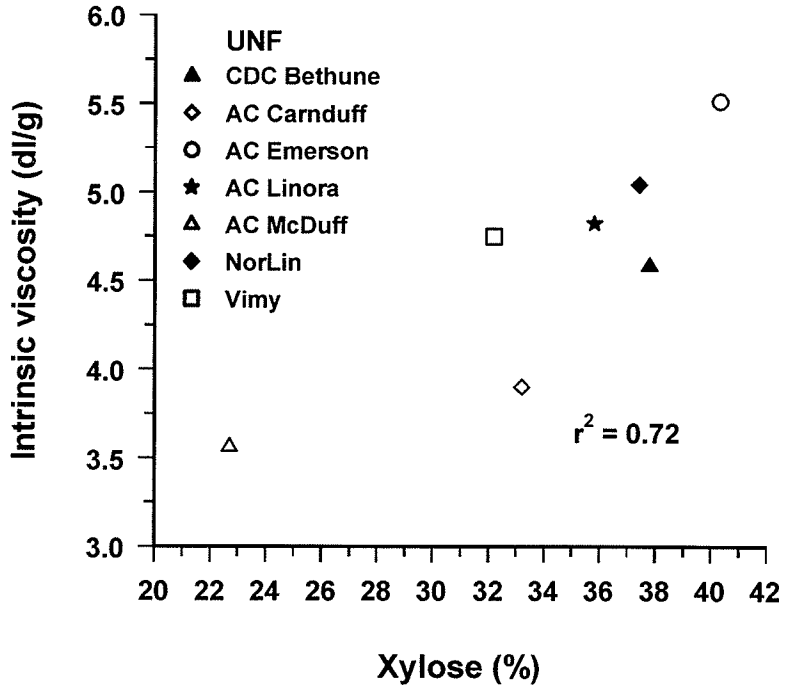

Figure 4.1. The relationship between intrinsic viscosity and xylose content for the UNF extract.

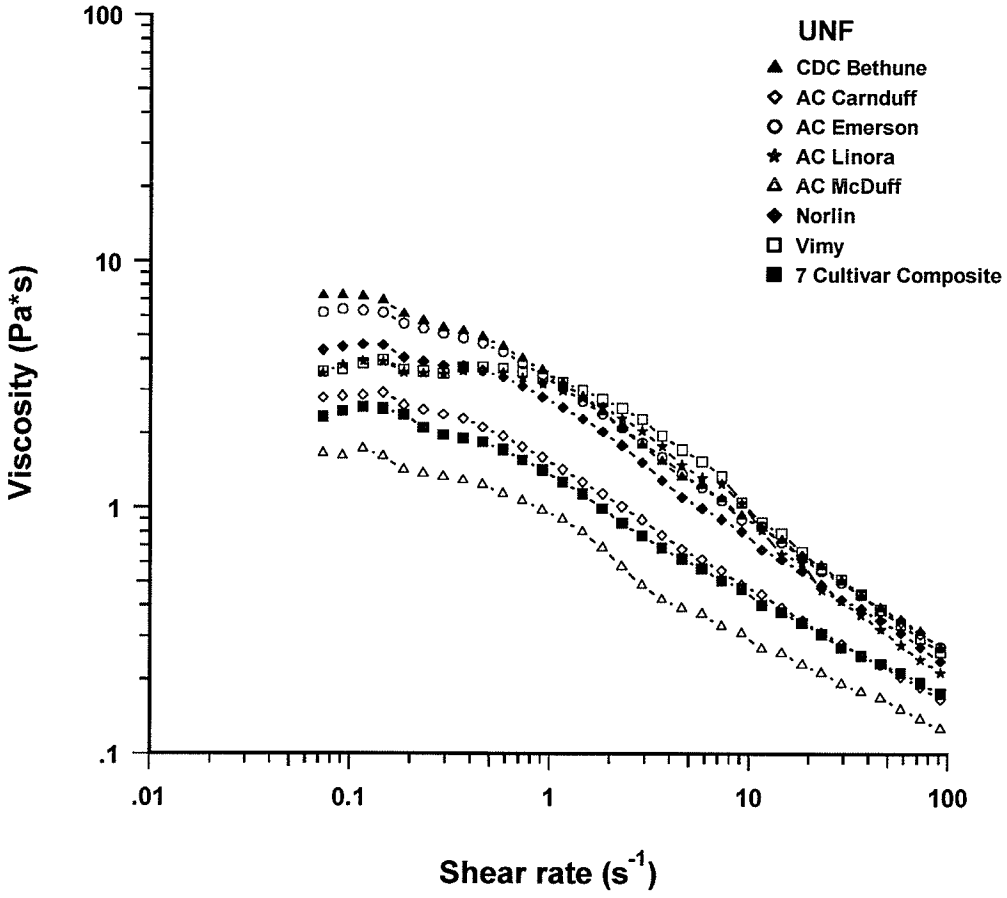

Figure 4.2. Steady shear rheological flow curves for the UNF extract (1.0% w/w).

observed for these two cultivars corresponds well with the low intrinsic viscosity values. Polysaccharide solutions with a higher intrinsic viscosity tend to exhibit greater shear thinning properties (Fedeniuk and Biliaderis, 1994). Overall, AC Linora had the highest degree of shear thinning (0.43) of all the UNF extracts followed by AC Emerson and CDC Bethune (0.45). AC Emerson and CDC Bethune exhibited the same degree of shear thinning which corresponds with the similarity that was observed in the steady shear flow curves.

In concentrated solutions, interactions between molecules contribute to their viscosity. Therefore, the higher viscosity values observed at the low shear rates in Figure 4.2 are caused by coil overlap and entanglement of the polysaccharides. Previous studies have found that mucilage solutions containing primarily neutral polysaccharides are more viscoelastic and shear thinning than their acidic counterparts (Section 3.4.5.; Fedeniuk and Biliaderis, 1994). In addition Cui et al. (1994a) attributed the shear thinning behavior of the neutral polysaccharide to the arabinoxylan component, as a result, a relationship between xylose content and apparent viscosity would be expected. Figure 4.3 shows the relationship between xylose content and apparent viscosity at a shear rate of 0.116 s^{-1} for the UNF extracts and the Pearson's correlation coefficient was calculated to be 0.84 ($p = 0.018$). The coefficient of determination (r^2) for this relationship was 0.70 which is similar to the value observed for the xylose content and intrinsic viscosity relationship (Figure 4.1). From the r^2 values it can be said that 72% of the variation observed in intrinsic viscosity and 70% of the variation observed in apparent viscosity can be explained by the linear relationship with xylose content. These results suggest that the proportion of neutral polysaccharides present in the mucilage extract is a major factor affecting the intrinsic viscosity and apparent viscosity values.

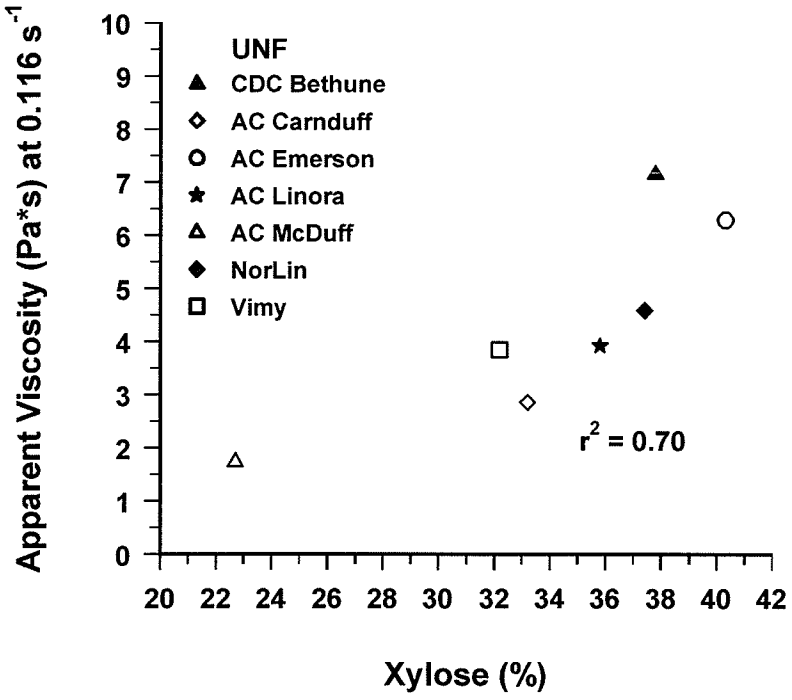

Figure 4.3. The relationship between apparent viscosity at 0.116 s^{-1} and xylose content for the UNF extract (1.0% w/w).

The mechanical spectra for the UNF extracts are shown in Figure 4.4. The gum dispersions (1.5% w/w) of all cultivars except for Vimy exhibited weak gel properties with the storage modulus (G') greater than the loss modulus (G") over the entire frequency range. In the case of Vimy, the G' and G" values were very similar over the entire frequency range suggesting that this cultivar exhibited more liquid-like properties compared to the other cultivars. Figure 4.5 compares the G' and G" values measured at 1 Hz for all cultivars. From this figure it is apparent that at this particular frequency, G' is larger than G" for all cultivars except Vimy, and significant differences were observed among some of the G' values ($p \leq 0.05$). Figure 4.5 shows that CDC Bethune exhibited the largest difference between G' and G" at a frequency of 1 Hz. Another rheological parameter that is often examined is the phase angle or loss tangent which is equal to G"/G'. If the loss tangent is greater than 1 the solution tends to have more liquid-like characteristics, whereas if it is less than 1 the solution has more solid-like characteristics. The loss tangents for each frequency examined are presented in Figure 4.6. Over the entire frequency range, Vimy had a loss tangent value close to 1, while the loss tangent for CDC Bethune ranged from 0.37 to 0.58. Although all cultivars except Vimy exhibited weak gel properties, some cultivars showed more solid-like characteristics than others as indicated by the difference in loss tangent values. Previous reports have suggested that it is the neutral polysaccharide that is responsible for the weak gel-like properties exhibited by some cultivars due to their greater tendency for structure formation in an aqueous environment (Cui et al., 1996; Cui et al., 1994a; Fedeniuk and Biliaderis, 1994). As a result, the relationship between the xylose content and the G' values recorded at 1 Hz was examined for the UNF extracts (Figure 4.7) and the Pearson's correlation coefficient was

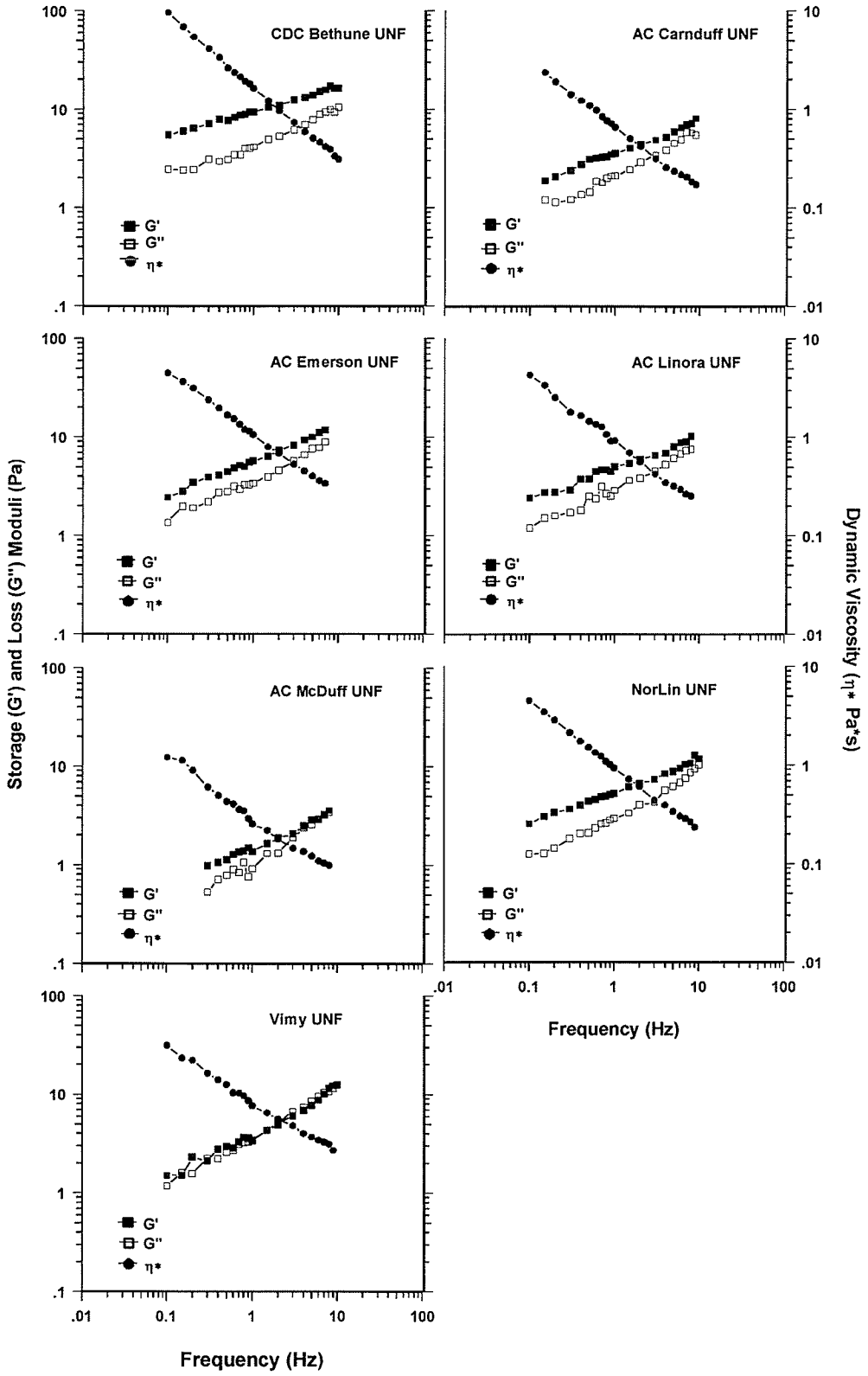

Figure 4.4. Mechanical spectra for the UNF extracts (1.5% w/w).

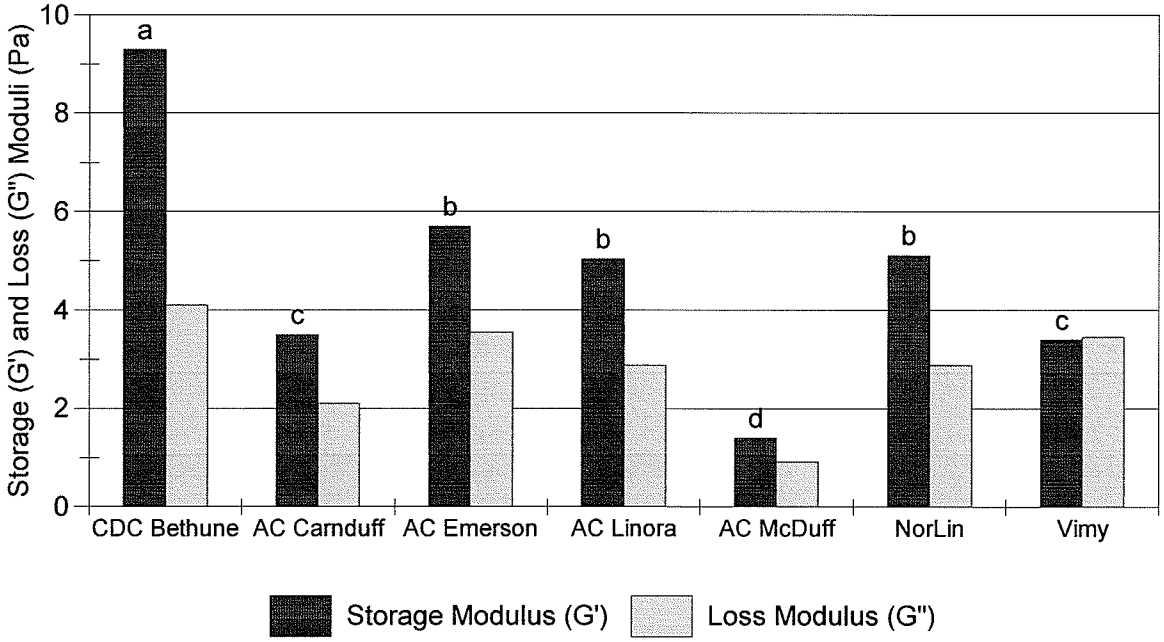

Figure 4.5. Comparison of G' (storage modulus) and G" (loss modulus) values at 1 Hz among the UNF extracts (1.5% w/w) from various cultivars. Bars with different letters are significantly different ($p \leq 0.05$) as determined by the Tukey's studentized range test.

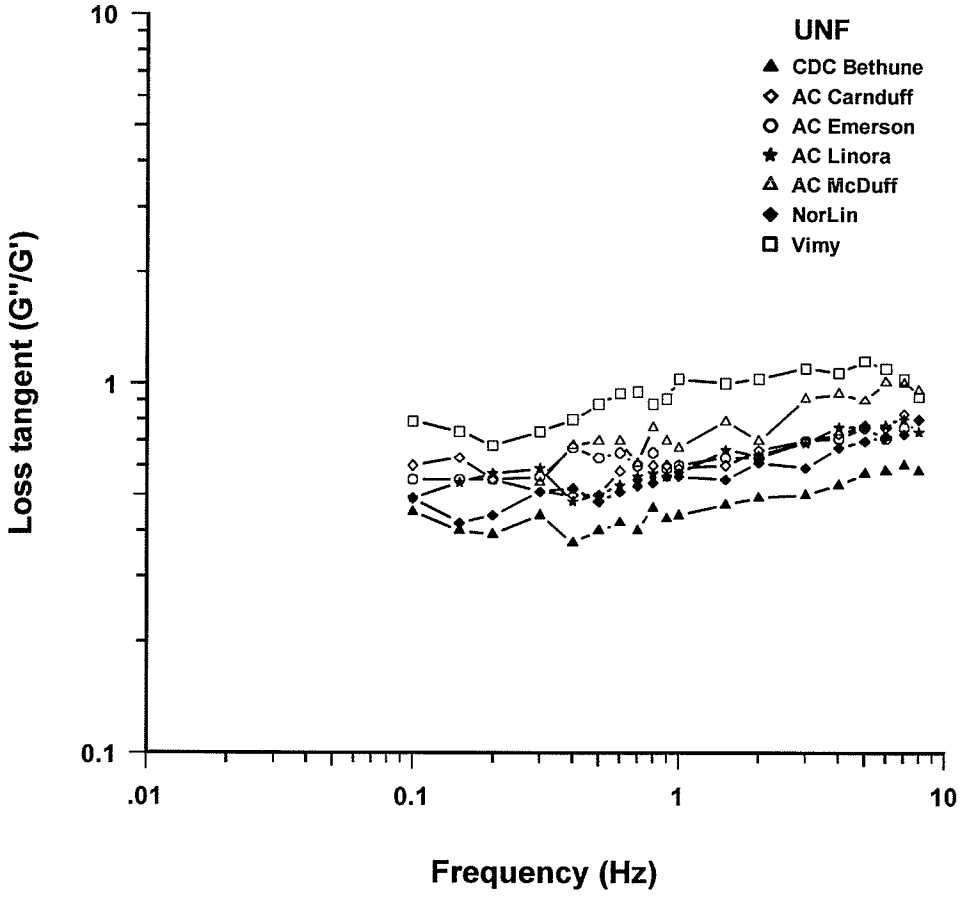

Figure 4.6. Comparison of the loss tangent values among the UNF extracts (1.5% w/w).

calculated to be 0.78 ($p = 0.04$) . The r^2 value for this relationship was 0.60. This result suggests that there is a fairly strong relationship between the storage modulus (G') and the proportion of neutral polysaccharides present in the mucilage extract. However, other factors such as molecular weight and structural conformation of the constituent polysaccharides likely also have an effect on the viscoelastic properties of flaxseed mucilage.

Deionized water was used for measuring the intrinsic viscosity of the 40F extracts and the values ranged from 4.47 to 7.76 dl/g (Table 4.9). Like the UNF extract, AC McDuff exhibited the lowest intrinsic viscosity value and AC Emerson had the highest value. When the remaining cultivars were ranked in order of increasing intrinsic viscosity the overall order within the UNF and 40F extracts differed. Although the 40F extracts were enriched with the neutral polysaccharides, the cultivars had varying amounts of acidic polysaccharides present which may have affected the intrinsic viscosity values. Figure 4.8 shows the relationship between xylose content and intrinsic viscosity for the 40F extracts and the Pearson's correlation coefficient was calculated to be 0.74 ($p = 0.058$). The r^2 value for the linear relationship between the two variables was 0.54. Although this relationship is not as strong as the one observed for the UNF extracts there still appears to be a slight positive relationship. The relationship between intrinsic viscosity and xylose content was more difficult to see for the 40F extracts because all the cultivars contained a significant amount of neutral polysaccharides (xylose). It is believed that the range of intrinsic viscosity values observed among the cultivars was likely caused by differences in the molecular size of the constituent polysaccharides.

The steady shear flow curves for the 40F extracts are shown in Figure 4.9. Each

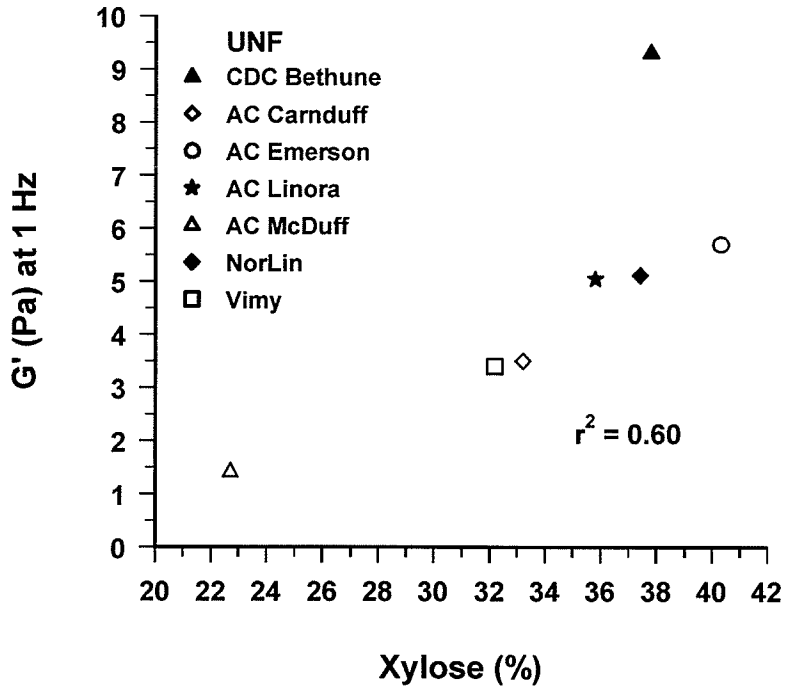

Figure 4.7. The relationship between the G' (storage modulus) value at 1 Hz and xylose content for the UNF extracts (1.5% w/w).

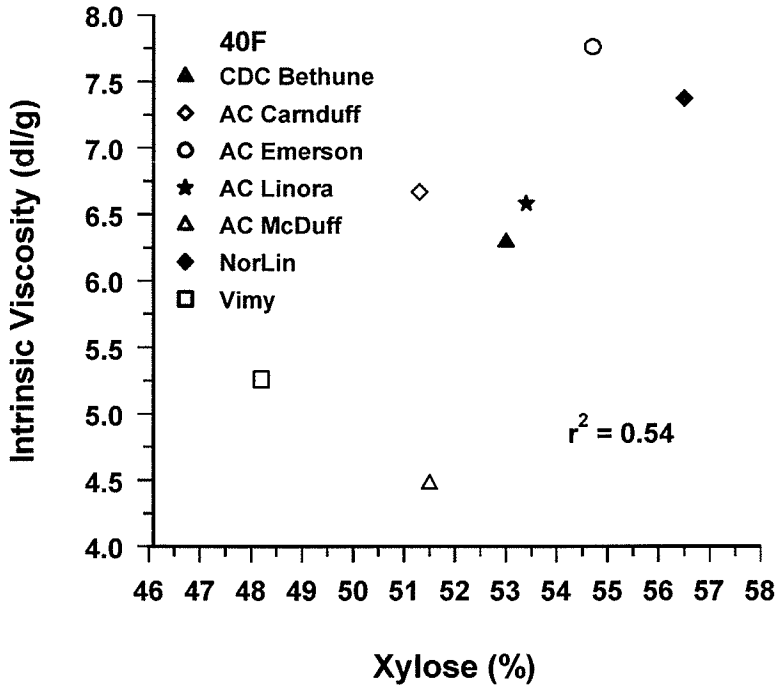

Figure 4.8. The relationship between intrinsic viscosity and xylose content for the 40F extract.

cultivar exhibited a decrease in viscosity with an increase in shear rate but differences in apparent viscosity were observed. Among the cultivars there was some cross over of the steady shear flow curves but in general CDC Bethune and AC Emerson exhibited the highest apparent viscosity followed by AC Linora, NorLin, Vimy, AC McDuff and AC Carnduff. The steady shear flow curves for the seven and four cultivar composites fell midway between the seven cultivars. Differences in shear thinning behavior among the 40F extracts were quantified using the power law model. Only small differences were observed among the cultivars with n values ranging from 0.40 for NorLin to 0.47 for AC Carnduff and Vimy (Table 4.11). When comparing the steady shear flow curves to the intrinsic viscosity values, it would be expected that the cultivars exhibiting higher apparent viscosities and a greater degree of shear thinning would have higher intrinsic viscosity values. This was true for some cultivars but not for others. In particular, AC Carnduff had a intrinsic viscosity value that was relatively high compared to the remaining cultivars indicating that the constituent polysaccharides were of a relatively high molecular weight and possibly quite rigid, yet it exhibited relatively low apparent viscosity values and a lower degree of shear thinning compared to the other cultivars. These discrepancies could be related to the fact that the intrinsic viscosity values were measured using very dilute concentrations whereas the steady shear flow curves were determined using concentrated solutions. With a concentrated solution, other factors besides molecular size affect the rheological properties of a polysaccharide solution, including entanglement of the polymers and non-covalent interactions among polymers. The degree to which this type of activity occurs varies among the cultivars due to differences in composition (ratio of acidic to neutral polysaccharides), molecular size

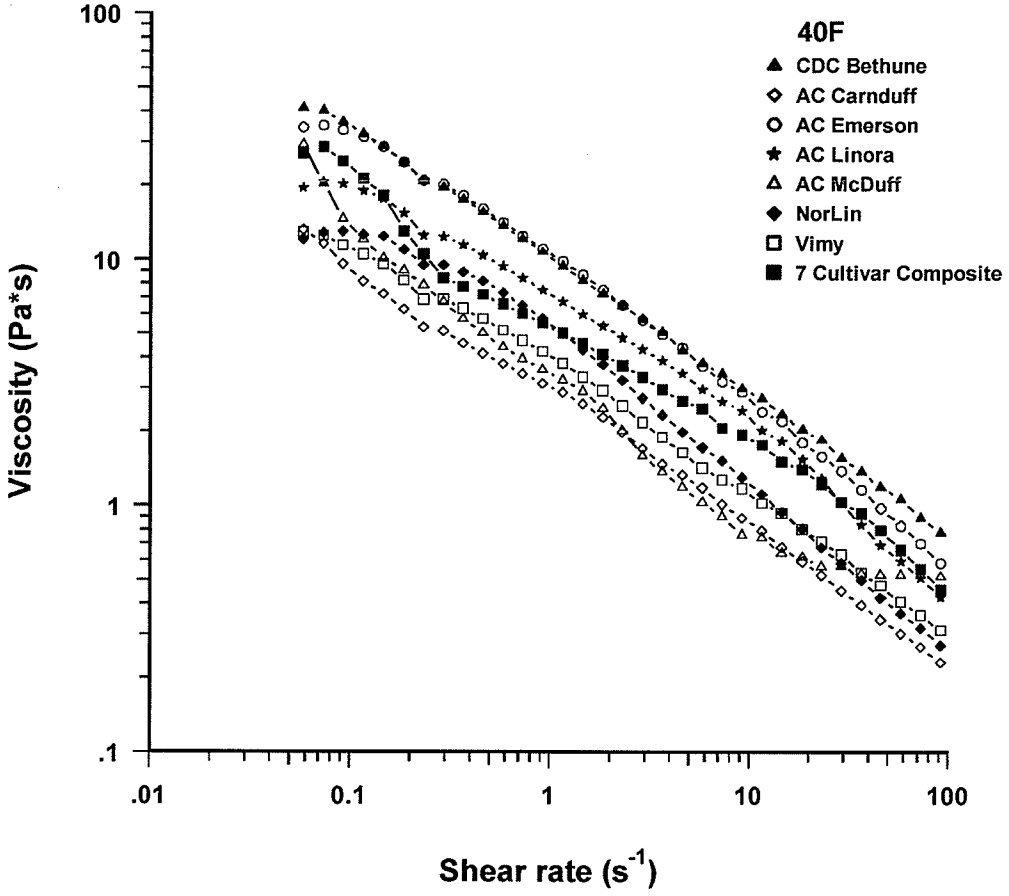

Figure 4.9. Steady shear rheological flow curves for the 40F extract (1.0% w/w).

Table 4.11. Flow behavior index (n) and consistency coefficient (m) for the 40F extracts (1.0% w/w) according to the Power Law model $\eta = m\gamma^{n-1}$ (0.0921-116 s^{-1})

Cultivar	n	m
CDC Bethune	0.45	9.98
AC Carnduff	0.47	2.79
AC Emerson	0.41	9.83
AC Linora	0.44	6.73
AC McDuff	0.42	3.27
NorLin	0.40	4.57
Vimy	0.47	3.65

Table 4.12. Flow behavior index (n) and consistency coefficient (m) for the 60F extracts (2.0% w/w) according to the Power Law model $\eta = m\gamma^{n-1}$ (0.460-116 s^{-1})

Cultivar	n	m
CDC Bethune	0.74	0.82
AC Carnduff	0.71	1.15
AC Emerson	0.74	1.52
AC Linora	0.83	0.54
AC McDuff	0.67	1.43
NorLin	0.84	0.62
Vimy	0.83	1.07

and possibly structural conformation of the constituent polysaccharides.

It is interesting to note that the n values for UNF and 40F for each cultivar did not differ to a great degree and in some cases were identical. This is interesting considering that the intrinsic viscosity values for UNF and 40F were different. As was reported in section 3.4.5, deionized water could not be used for measuring the intrinsic viscosity of the UNF and 60F extracts due to the polyelectrolyte nature of the acidic polysaccharide which was present to a larger degree in these two fractions. Given that very little difference was observed in the degree of shear thinning between UNF and 40F, it can be concluded that the increased amount of acidic polysaccharides in UNF compared to 40F did not have as great of an effect on the shear thinning as it did on the intrinsic viscosity. This helps to understand why cultivars with a higher intrinsic viscosity did not always exhibit a greater degree of shear thinning.

The mechanical spectra for the 40F extracts are shown in Figure 4.10. The gum dispersions (1.5% w/w) of all cultivars exhibited weak gel properties with the storage modulus (G') greater than the loss modulus (G") over the entire frequency range. Figure 4.11 compares the G' and G" values measured at 1 Hz and large differences were observed between the two moduli for all cultivars. In addition, significant differences were observed in the G' values among all the cultivars ($p \leq 0.05$). The loss tangent values were also calculated (Figure 4.12) and each cultivar exhibited solid-like characteristics (loss tangent value < 1). Figure 4.13 is a plot of the G' values recorded at 1 Hz by xylose content. A fairly strong positive relationship was observed with a Pearson's correlation coefficient of 0.87 ($p = 0.01$) and a r^2 value of 0.76 . This result suggests that for the 40F extract 76% of the

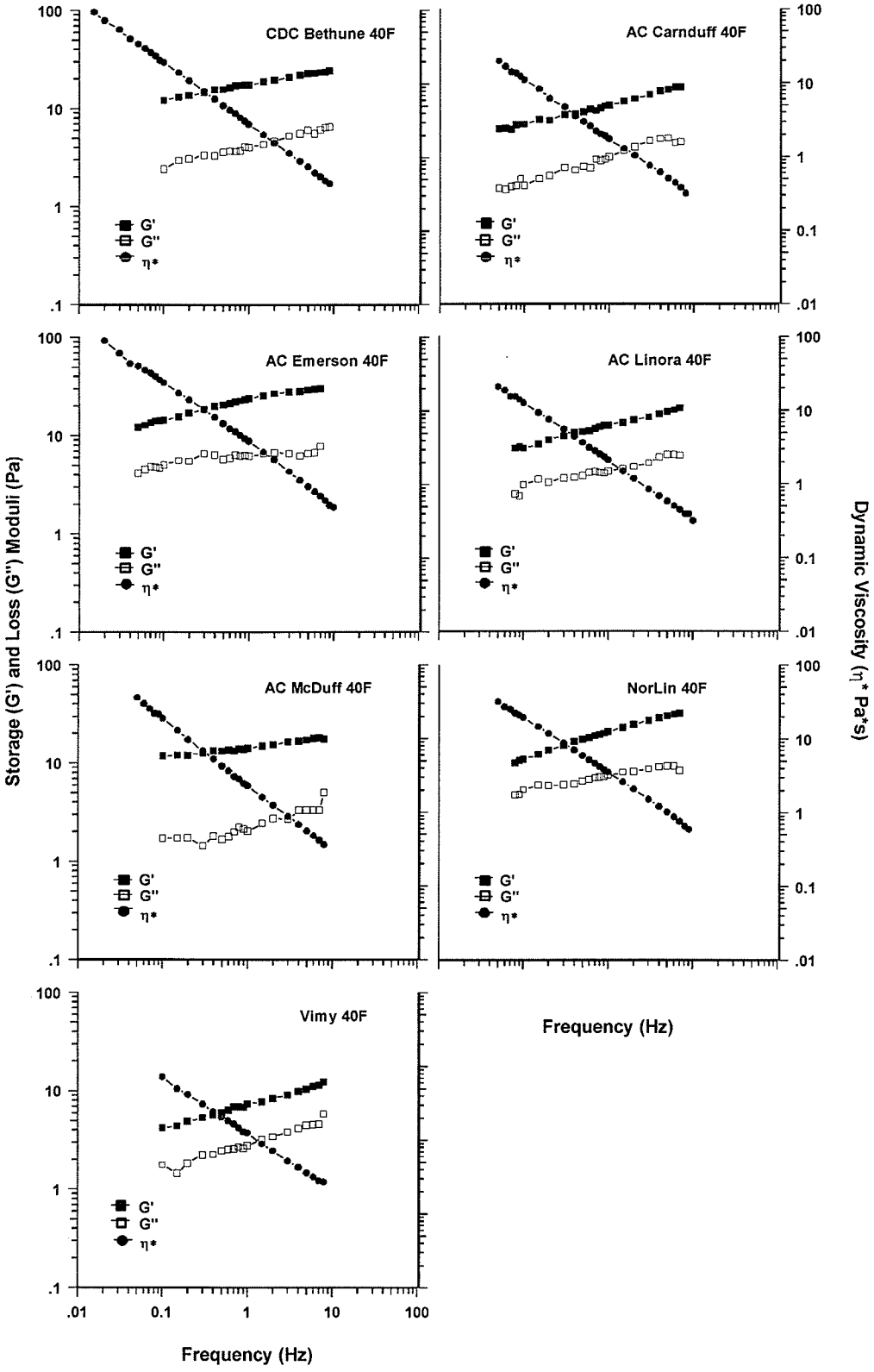

Figure 4.10. Mechanical spectra for the 40F extracts (1.5% w/w).

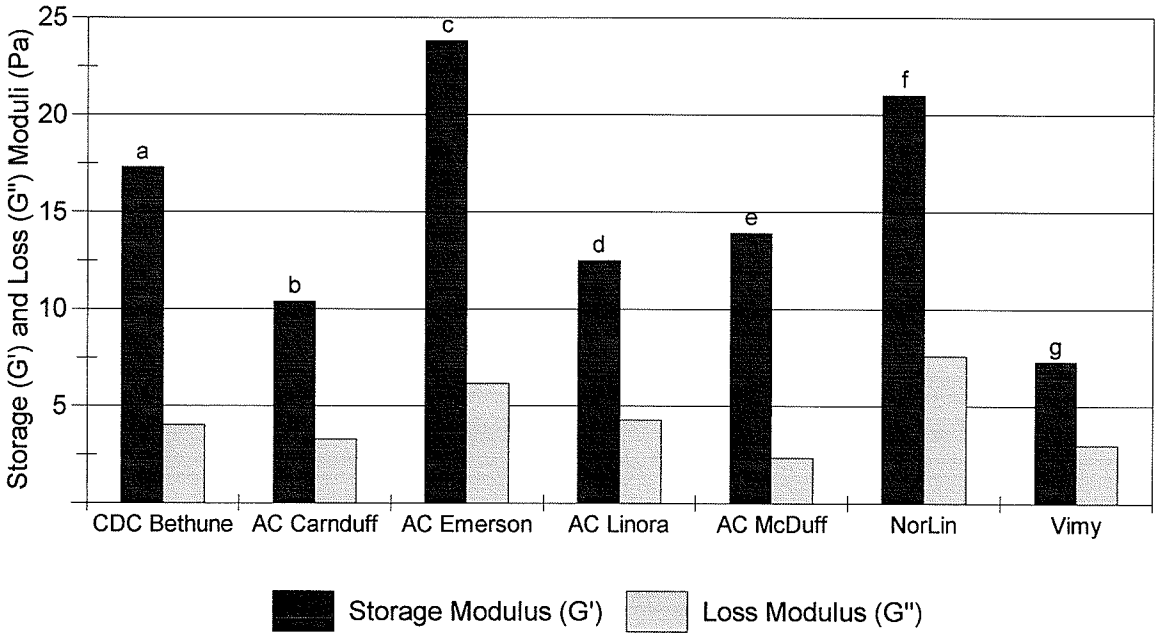

Figure 4.11. Comparison of G' (storage modulus) and G" (loss modulus) values at 1 Hz among the 40F extracts (1.5% w/w) from various cultivars. Bars with different letters are significantly different ($p \leq 0.05$) as determined by the Tukey's studentized range test.

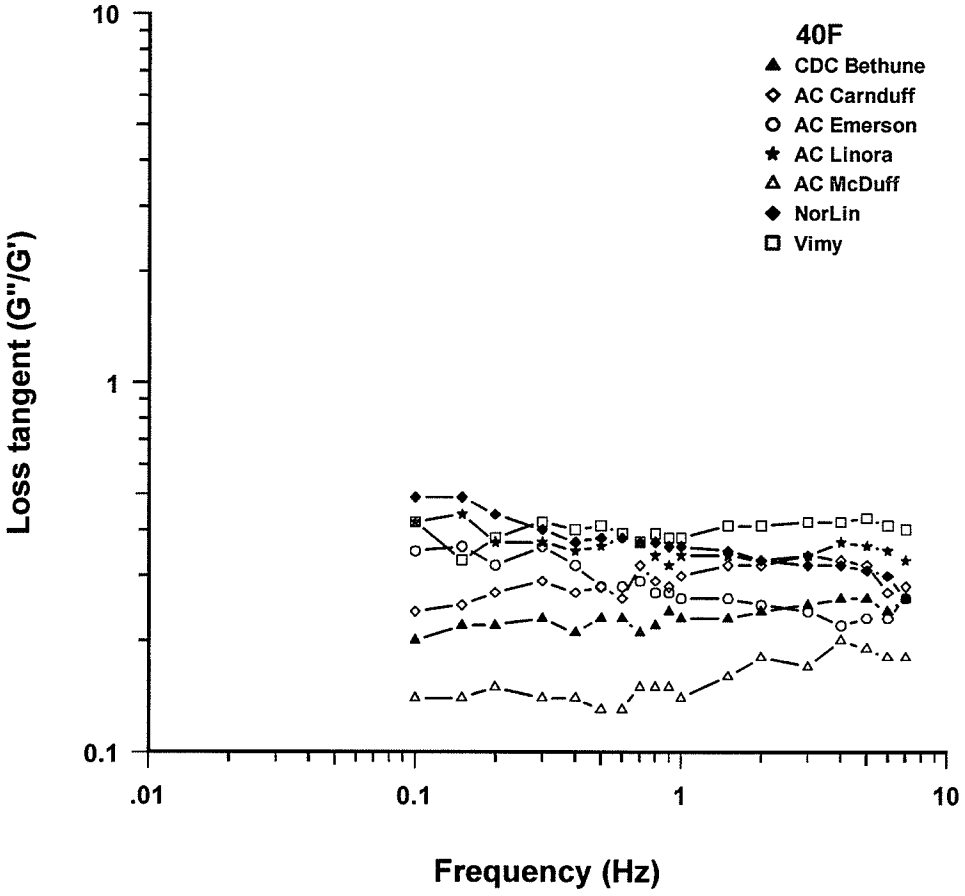

Figure 4.12. Comparison of the loss tangent values among the 40F extracts (1.5% w/w).

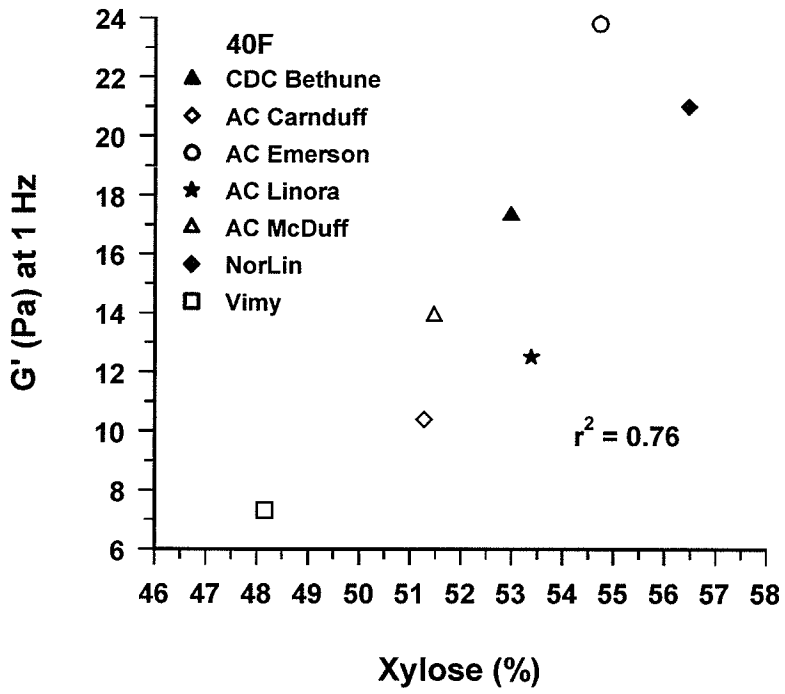

Figure 4.13. The relationship between the G' (storage modulus) value at 1 Hz and xylose content for the 40F extracts (1.5% w/w).

variation observed in the G' values can be explained by the linear relationship with xylose content, which is slightly higher than what was observed for the UNF extract.

The calculated intrinsic viscosity values for the 60F extracts dissolved in 1M NaCl are presented in Table 4.9. The values ranged from 3.97 to 5.09 dl/g with AC McDuff having the lowest value and Vimy the highest value. Although the same solvent was used these values are slightly higher than the intrinsic viscosity value reported by Cui and Mazza (1994) for the acidic fraction of cultivar NorMan (2.48 dl/g) obtained by ion exchange chromatography. On the other hand, the values are comparable to that reported by Fedeniuk and Biliaderis (1994) for the acidic fraction of flaxseed cultivar Linott (4.6 dl/g in 0.2M NaCl) obtained by selective precipitation with cetyltrimethylammonium bromide (CTAB).

The steady shear flow curves for the 60F extracts are shown in Figure 4.14. Some of the cultivars exhibited a slight decrease in viscosity with an increase in shear rate but not to the same degree that was observed for 40F and UNF. Among the seven cultivars, AC Emerson exhibited the highest apparent viscosity while AC Linora exhibited the lowest. The two flaxseed composites appeared to have higher apparent viscosity values than the majority of the cultivars. Figure 4.15 shows the relationship between xylose content and apparent viscosity at a shear rate of 0.581 s^{-1}. With the exception of AC McDuff, which appears to be an outlier, there is a fairly strong positive correlation between the two variables with a Pearson's correlation coefficient of 0.94 ($p = 0.006$). When AC McDuff is included the Pearson's correlation coefficient drops dramatically to 0.15 ($p = 0.75$).

Differences in shear thinning behavior among the 60F extracts were quantified using the power law model (Table 4.12). The n values ranged from 0.67 to 0.84 suggesting that

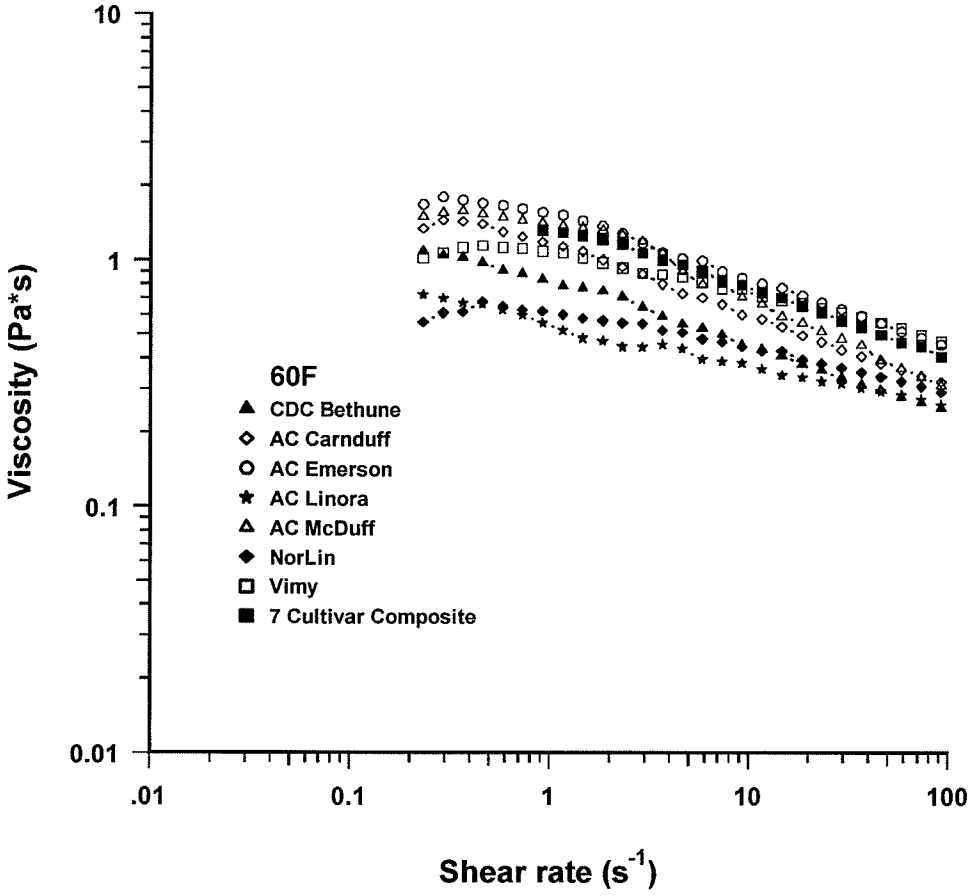

Figure 4.14. Steady shear rheological flow curves for the 60F extract (2.0% w/w).

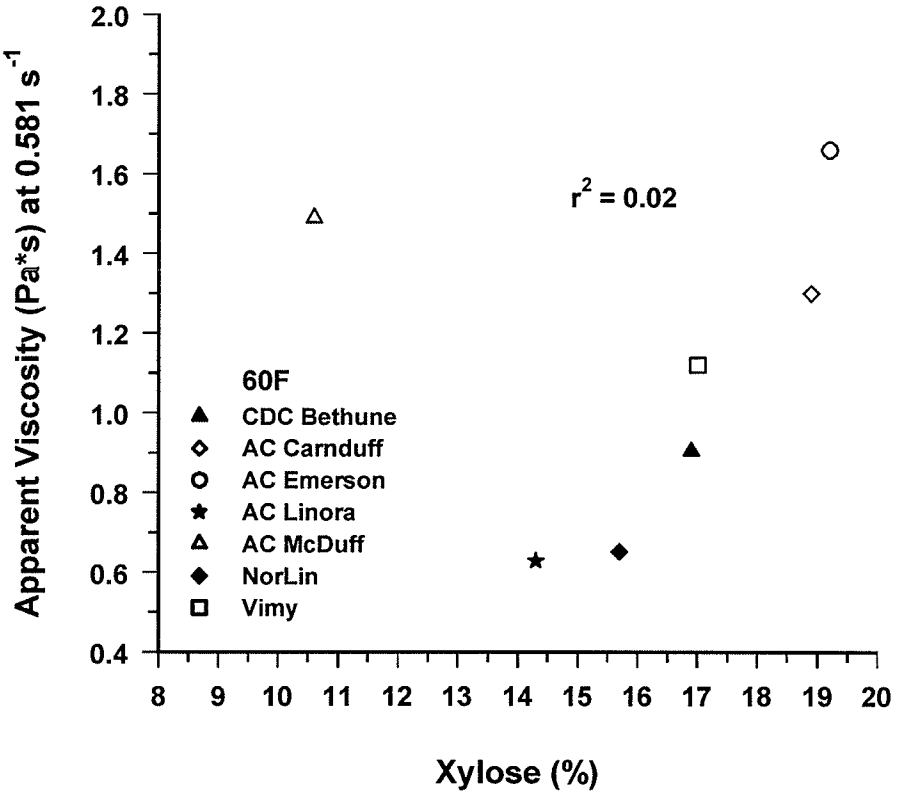

Figure 4.15. The relationship between apparent viscosity at 0.581 s^{-1} and xylose content for the 60F extract (2.0% w/w).

the cultivars varied in their degree of shear thinning. In addition, for each cultivar the n values observed for 60F were higher than those observed for UNF and 40F which indicates that 60F exhibited less shear thinning. This corresponds with the fact that the 60F extract contained a lower proportion of neutral polysaccharides which are believed to be the major factor responsible for the shear thinning behavior.

4.4.5. Evaluation of Emulsifying Ability

The interfacial areas for the UNF extracts ranged from 14.8 x 10^3 to 22.7 x 10^3 m^{-1} (Table 4.13) with AC McDuff having the lowest value and AC Emerson the highest value. Composition and solution viscosity of the mucilage extracts both have an effect on the initial emulsification (Section 3.4.8) and it is apparent from the interfacial area results that the cultivars studied differ in their ability to form good quality emulsions. According to Gaonkar (1991), an increase in the viscosity of the continuous phase facilitates emulsion formation by favoring drop breakage leading to higher turbidity and increased interfacial area values. The interfacial area as a function of time was monitored (Figure 4.16a) in order to get an indication of the polymers ability to stabilize the oil-in-water emulsion. Among the UNF extracts, the emulsion prepared with AC Emerson was the most stable whereas the emulsion prepared with AC McDuff was the least. The rate constant for emulsion decay was also calculated and the results are reported in Table 4.14. The rate constants for the UNF extracts ranged from 0.051 to 0.130 min^{-1} with AC Emerson exhibiting the slowest rate of decay and AC McDuff the fastest. The relationship between the rate constant (k) and the intrinsic viscosity for the UNF extracts is shown in Figure 4.17. A strong negative correlation was

Table 4.13. Interfacial area data for oil-in-water emulsions prepared using flaxseed mucilage (0.25% w/w) from different cultivars

Cultivar	Interfacial area[a] x 10^3 m^{-1}		
	UNF	40F	60F
CDC Bethune	20.2 ± 0.0	21.9 ± 0.1	18.8 ± 0.9
AC Carnduff	19.8 ± 0.1	19.0 ± 0.4	18.7 ± 0.9
AC Emerson	22.7 ± 0.3	22.7 ± 1.1	17.2 ± 0.2
AC Linora	21.3 ± 0.7	20.8 ± 0.6	16.2 ± 0.9
AC McDuff	14.8 ± 0.3	18.7 ± 0.2	13.5 ± 1.2
NorLin	20.8 ± 1.2	19.0 ± 1.8	16.0 ± 0.2
Vimy	20.8 ± 0.0	21.0 ± 0.3	17.7 ± 0.4
7 Cultivar Composite	20.5 ± 1.1	21.6 ± 0.8	18.7 ± 0.7

[a] Means ± SD (n = 2). Interfacial area values were calculated from the absorbance reading immediately after emulsification (t = 0).

Table 4.14. Rate constant (k) for emulsion decay for oil-in-water emulsions prepared using flaxseed mucilage (0.25% w/w) from different cultivars

Cultivar	Rate constant[a] (k) min^{-1}		
	UNF	40F	60F
CDC Bethune	0.076 ± 0.009 ^{b}	0.069 ± 0.008 ^{bcd}	0.103 ± 0.008 ^{bc}
AC Carnduff	0.084 ± 0.019 ^{b}	0.072 ± 0.007 ^{bc}	0.099 ± 0.008 ^{bcd}
AC Emerson	0.051 ± 0.002 ^{b}	0.023 ± 0.000 ^{e}	0.137 ± 0.003 ^{a}
AC Linora	0.068 ± 0.001 ^{b}	0.048 ± 0.004 ^{cde}	0.092 ± 0.001 ^{cd}
AC McDuff	0.130 ± 0.004 ^{a}	0.100 ± 0.003 ^{a}	0.118 ± 0.009 ^{ab}
NorLin	0.053 ± 0.009 ^{b}	0.044 ± 0.006 ^{de}	0.118 ± 0.004 ^{ab}
Vimy	0.066 ± 0.002 ^{b}	0.081 ± 0.012 ^{ab}	0.073 ± 0.005 ^{d}
7 Cultivar Composite	0.061 ± 0.008	0.066 ± 0.007	0.099 ± 0.010

[a] Means ± SD (n = 2). Means followed by a different superscript within a column are significantly different ($p \leq 0.05$) as determined by Tukey's studentized range test.

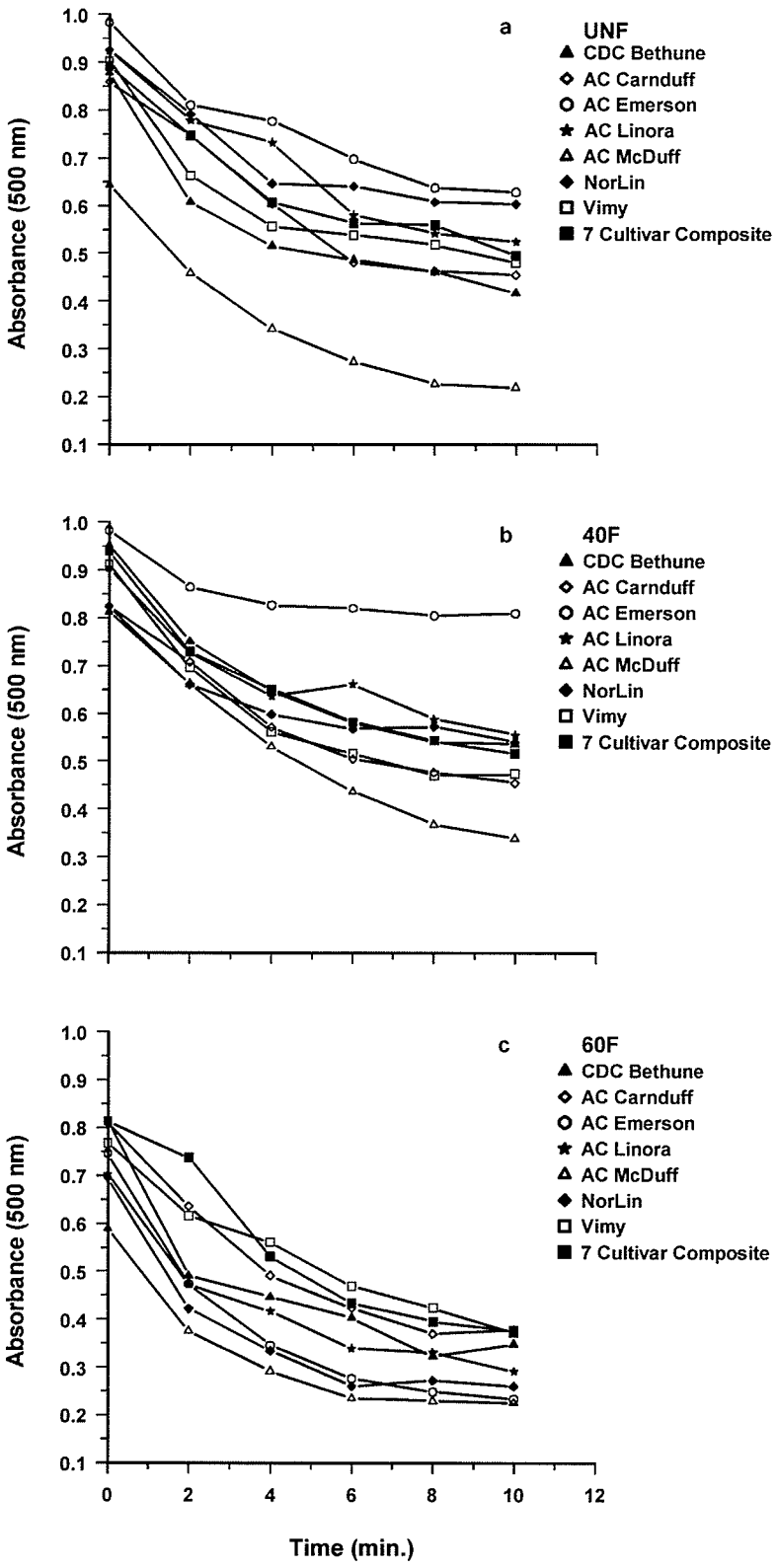

Figure 4.16. Emulsifying ability of mucilage extracts (0.25% w/w): (a) UNF, (b) 40F, (c) 60F. The emulsions were diluted (50-fold) with 0.1% sodium dodecyl sulfate (SDS), and the emulsifying ability was evaluated by measuring the absorbance at 500nm.

observed with a Pearson's correlation coefficient of -0.92 ($p = 0.004$) indicating that cultivars having higher intrinsic viscosity values have a tendency to exhibit slower rates of decay.

The interfacial areas for the 40F extracts ranged from 18.7 x 10^3 to 22.7 x 10^3 m^{-1} (Table 4.13) and like the UNF extracts, AC McDuff exhibited the lowest interfacial area while AC Emerson had the highest. For the majority of the cultivars, the interfacial area value for the 40F extract was very similar to the value obtained for the UNF extract, with the one exception being AC McDuff. The interfacial area as a function of time was also monitored and is presented in Figure 4.16b. It is apparent that the cultivars varied in their ability to stabilize the emulsion. Among the 40F extracts, the emulsion prepared with AC Emerson was the most stable whereas the emulsion prepared with AC McDuff was the least stable. Further comparisons among the cultivars can be made by examining the rate constant for emulsion decay (Table 4.14). The rate constants for the 40F extracts ranged from 0.023 to 0.100 min^{-1} with AC Emerson exhibiting the slowest rate of decay and AC McDuff the fastest. For all cultivars, except Vimy, the 40F extract formed a more stable emulsion than the UNF extract. The relationship between the rate constant (k) and the intrinsic viscosity for the 40F extracts is shown in Figure 4.18 . A strong negative correlation was observed with a Pearson's correlation coefficient of -0.94 ($p = 0.002$) indicating that cultivars having higher intrinsic viscosity tended to exhibit slower rates of decay.

The interfacial areas for the 60F extracts ranged from 13.5 x 10^3 to 18.8 x 10^3 m^{-1} (Table 4.13) with AC McDuff having the lowest value and CDC Bethune having the highest value. The interfacial area was monitored as a function of time (Figure 4.16c) and the rate constant for emulsion decay was calculated (Table 4.14). The rate constants for the 60F

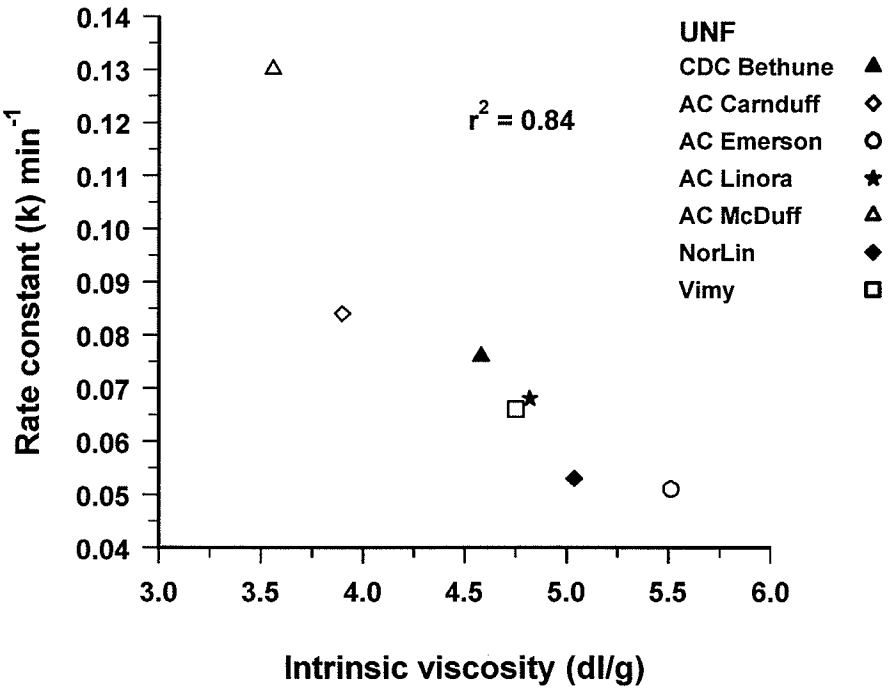

Figure 4.17. The relationship between the rates of emulsion decay and intrinsic viscosity for the UNF extract.

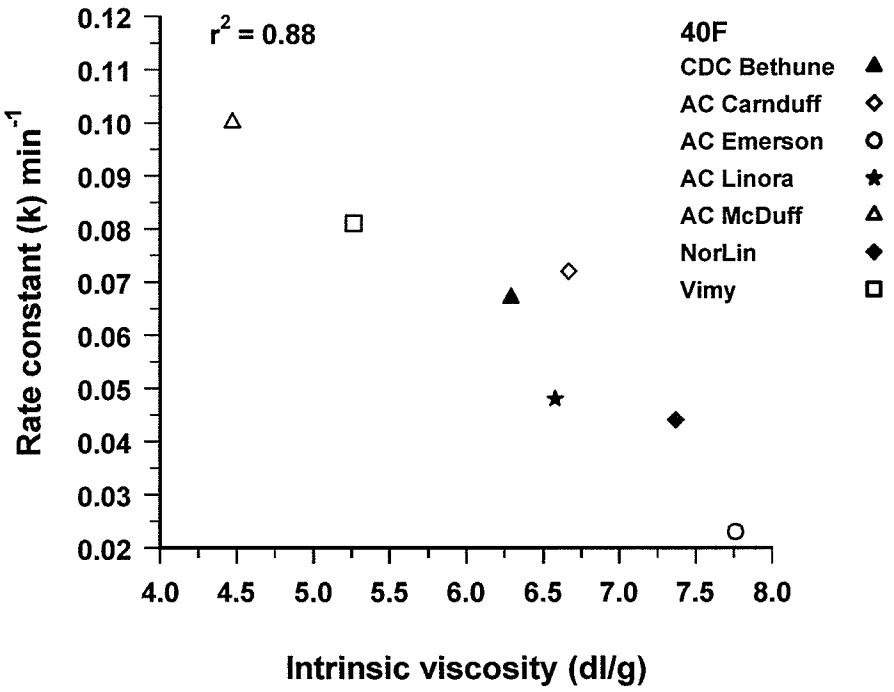

Figure 4.18. The relationship between the rates of emulsion decay and intrinsic viscosity for the 40F extract.

extracts ranged from 0.073 to 0.137 min^{-1} with Vimy exhibiting the slowest rate of decay and AC Emerson the fastest. Overall the interfacial area results for 60F were lower than the corresponding UNF and 40F extracts, and 60F had the fastest rate of decay for all cultivars except Vimy. Unlike UNF and 40F, a weaker relationship was observed between intrinsic viscosity and rate of decay for 60F (Figure 4.19) with a Pearson's correlation coefficient of -0.59 ($p = 0.16$). This most likely occurred because the 60F extracts were enriched with acidic polysaccharides which exhibit weaker rheological characteristics than the neutral polysaccharides which were found in greater amounts in the UNF and 40F extracts. When examining the interfacial area results, it appears as though both the UNF and 40F extracts produced similar quality emulsions immediately after emulsification. It was not until the emulsion was monitored over time that differences in the performance of these two extracts as an emulsion stabilizer were observed as indicated by the different rates of emulsion decay. Although all of the extracts possessed some ability to stabilize the oil-in-water emulsion, for each cultivar (except Vimy), the 40F extract out performed the UNF and 60F extracts with AC Emerson 40F having the most superior stabilizing characteristics. In general, the interfacial area and rate constant results for the two cultivar composites were fairly close to the average for all cultivars as would be expected.

4.5. Conclusions

Differences were found among the seven cultivars in the monosaccharide composition, rheological and emulsifying properties for the three extracts. Although the cultivars which contained a larger quantity of neutral polysaccharides (higher xylose content) tended to also

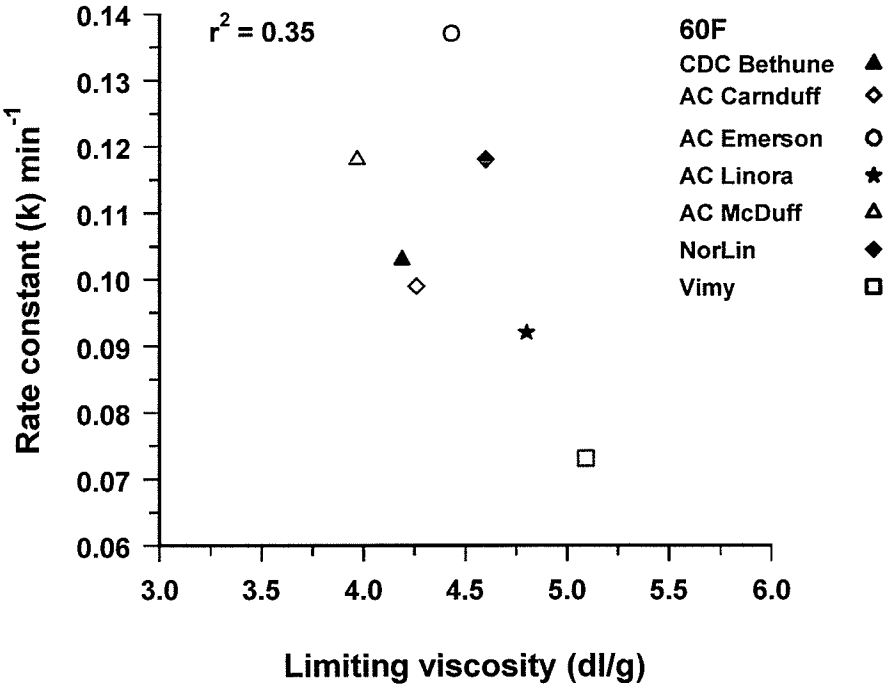

Figure 4.19. The relationship between the rates of emulsion decay and intrinsic viscosity for the 60F extract.

exhibit increased viscosity and weak-gel properties, the variation in the rheological behavior observed among cultivars could not be fully accounted for by the difference in xylose content. Differences in intrinsic viscosity and rates of shear thinning were also observed which suggested that the molecular weight and structural conformation of the extracted polysaccharides differed among the cultivars. It was concluded that the diverse rheological properties observed among the cultivars was caused by differences in the proportion of neutral and acidic polysaccharides as well as differences in molecular weight and structural conformation of the extracted polysaccharides. For the UNF and 40F extracts, a strong relationship between intrinsic viscosity and rate of decay was observed indicating that cultivars which had higher intrinsic viscosity values tended to exhibit slower rates of decay.

5. CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

Sequential ethanol precipitation was investigated for two reasons, to find out whether it could be used to separate the neutral and acidic polysaccharides present in flaxseed mucilage and to determine whether this technique would be useful for investigating the effect of cultivar on the physicochemical properties of flaxseed mucilage. Among the seven cultivars, the three extracts (UNF, 40F, and 60F) differed in monosaccharide composition, rheological and emulsifying properties. Further examination of 40F and 60F for AC Emerson and Vimy revealed that the polysaccharides present in the two extracts also differed in molecular weight, rheological properties and functional characteristics (emulsifying ability and foam stabilization). From these results it was concluded that the amount, composition, and molecular characteristics of the mucilage present in flaxseed was influenced by cultivar.

Compositional analysis of the mucilage extracts revealed that they contained a considerable amount of protein. It is believed that the majority of the protein present in the mucilage was not chemically bound to the polysaccharides but was most likely present due to contamination from the endosperm. Cui et al. (1994b) found that the protein content of extracted mucilage tends to increase as the water to seed ratio and temperature used in the extraction process increases. A temperature of around 80°C has commonly been used for the extraction of mucilage from flaxseed, therefore a similar extraction temperature was used in this study. With respect to the water to seed ratio, the present study used a slightly higher water to seed ratio for the extraction process since preliminary experiments revealed that as the water to seed ratio was reduced the aqueous extracts became extremely viscous and very

difficult to work with, particularly when it came to filtering. Using a lower water to seed ratio may have helped to reduce the protein content but difficulties in handling the material would have existed. The protein content also could have been reduced or eliminated by treatment with vega clay or a protease but this would have reduced the yield of mucilage and may have contaminated the mucilage. At this time it is not known whether the presence of large quantities of protein had a positive or negative effect on the performance of the mucilage, but it is possible that an additional purification step, which would inevitably lengthen and complicate the extraction procedure, may not be necessary. Given the strong rheological characteristics that were observed for the majority of the cultivars studied, it is believed that the presence of proteins did not have a negative impact on the viscosity of the mucilage. Although the stabilizing ability of flaxseed mucilage is primarily due to its viscosity building effect, the impact of protein on the stabilizing properties of flaxseed mucilage in a foaming system or an oil-in-water emulsion remains unresolved at this time. Given the amphoteric nature of proteins their presence in the mucilage extracts may have been beneficial in these two model systems. In order to gain a better understanding of whether a purification step would be required in a commercialized extraction process, it is recommended that the rheological and functional characteristics of mucilage treated with a protease be compared to those of untreated mucilage.

It is evident from the monosaccharide analysis results that sequential ethanol precipitation was able to separate the neutral and acidic polysaccharides to some degree, such that the neutral polymers were concentrated in 40F while the 60F extract was enriched with the acidic polymers, but complete separation was not achieved in the two fractions. Although

the original purpose for employing sequential ethanol precipitation was to try to separate the neutral and acidic polysaccharides, the results from this study suggest that complete separation with this method would likely not be possible. It is believed that sequential ethanol precipitation fractionates polymers mainly on the basis of molecular weight, but reports on whether high or low molecular weight polysaccharides precipitate first have been inconsistent. This is likely due to the fact that polysaccharides extracted from different sources normally differ in structural conformation, proportion of charged polysaccharides as well as molecular weight, all factors which inevitably have an effect on the order of precipitation. The size exclusion chromatography profiles obtained for the 40F and 60F extracts of AC Emerson and Vimy revealed that both extracts were complex having either two or three distinguishable polymer populations. Each population was found to be fairly polydisperse indicating that the populations contained polysaccharides having a range of molecular weights. Overall, for both AC Emerson and Vimy the average molecular weight of the 40F extract was greater than that of the 60F extract suggesting that higher molecular weight polysaccharides tend to precipitate at lower ethanol concentrations. Results from molecular weight analysis and intrinsic viscosity measurements revealed that the polysaccharides in the two extracts must have also differed in structure and conformation. Differences in the molecular characteristics of the extracts were reflected in the rheological behavior with 40F exhibiting very high apparent viscosity and gel-like properties. Given that sequential ethanol precipitation appears to be an effective and relatively simple method for obtaining polysaccharides with different physicochemical characteristics, it is believed that further investigation into this technique is warranted.

While investigating the effects of sequential ethanol precipitation, it was determined that no additional mucilage precipitated after the aqueous extract was adjusted to 60% ethanol (approximately 2 volumes), therefore it was concluded that 4 volumes of ethanol were not necessary for complete mucilage precipitation. This is an important finding because it dramatically reduces the amount of ethanol that would be required if the extraction process were to become commercialized. In addition, if a higher viscosity extract was required results from this study show that even less ethanol (adjustment to 40% ethanol) could be used for precipitation. Sequential ethanol precipitation was carried out on the aqueous extract immediately after the extraction procedure therefore the solution was highly concentrated with mucilage. It is believed that a more finite separation could have been obtained if sequential ethanol precipitation was carried out on mucilage solutions of lower concentrations. It is recommended that sequential ethanol precipitation be carried out on solutions of unfractionated mucilage of a known concentration, where the unfractionated mucilage was obtained by an aqueous extraction procedure followed by ethanol precipitation at a level of 60%. If additional fractions are obtained under these circumstances, molecular characterization of this material may provide further insight into the complex and polydisperse nature of flaxseed mucilage.

The proportion of neutral and acidic polysaccharides present in the mucilage extracts also varied among the cultivars. Although cultivars containing a larger quantity of neutral polysaccharides tended to exhibit increased viscosity and weak-gel properties, the variation in the rheological behavior observed among the cultivars could not be fully accounted for by the differences in xylose content. This finding is not surprising because the proportion of

neutral polysaccharides may be greater in one cultivar, but the polysaccharides may be of a lower molecular weight or have a different structural conformation, both being factors which have an effect on rheological characteristics. Molecular weight analysis of the 40F and 60F extracts from AC Emerson and Vimy did confirm that the average molecular weight of the polymers differed among these two cultivars. Thus, it would be beneficial to analyze the molecular weight for several other cultivars to establish how diverse their molecular weight distributions are.

In addition to molecular weight, it is recommended that more detailed studies concentrating on the structure of the polysaccharides from different cultivars be carried out. It is possible that the degree of branching for both the neutral and acidic polysaccharides may vary among cultivars. If this is the case, this type of variability in the structure would help to explain some of the diversity observed in the rheological properties. For each of the extracts, the differences in intrinsic viscosity observed among the various cultivars suggests that they differed in both structural conformation and molecular weight but how the structure may have varied among the cultivars is not yet known. The fact that the mucilage extracts contained varying proportions of neutral and acidic polysaccharides, in addition to some protein, made it impossible to obtain the intrinsic viscosity of a pure polymer. Therefore, it is important to point out that the complex nature of the mucilage extracts likely had an effect on the intrinsic viscosity measurements making it difficult to interpret some of the results.

The investigation into the stabilizing properties of the mucilage extracts in a foaming system were not as successful as hoped since it was not possible to obtain a method for measuring foam stability which gave reproducible results. From the measurements that were

made on AC Emerson and Vimy, the mucilage extracts which exhibited higher solution viscosity were more effective than the lower viscosity solutions in stabilizing the foam during heating. Since the foam stability test was highly subjective, it was not possible to determine whether the differences in the rheological characteristics observed among the cultivars contributed to the ability to stabilize a foam.

The ability of the mucilage extracts to stabilize an oil-in-water emulsion varied among the three extracts within a cultivar as well as among the cultivars. For the UNF and 40F extracts, a strong relationship between intrinsic viscosity and rate of decay was observed indicating that cultivars which had higher intrinsic viscosity values tended to exhibit slower rates of decay. Overall, it appears that there is potential for flaxseed mucilage to be used as an emulsion stabilizer but further testing of the various extracts in actual food systems is required in order to fully understand and elucidate its functional role as a food ingredient.

Variability in the oil and protein content of the seed did exist among the cultivars but no strong relationships (Appendix) were observed between seed composition and the mucilage's composition, rheological or functional properties for the various cultivars. The composition, rheological properties and emulsion stabilizing ability of the mucilage extracts did vary among the cultivars and some fairly strong correlations among these parameters were observed. Due to the complex nature of flaxseed mucilage no single parameter explained the variability observed among the seven cultivars, however, the diversity could be explained by the proportion of neutral and acidic polysaccharides, molecular weight and structural conformation of the extracted polysaccharides. For the UNF extract, AC Emerson and AC McDuff showed the greatest differences among the cultivars tested. Even though differences

were observed among the cultivars for the 40F and 60F extracts, the variation was not as great as what was observed for the UNF extracts. For the 40F and 60F extracts, no consistent trends were observed among the various cultivars for the parameters evaluated, however, the 40F extracts of AC Emerson and AC McDuff did show the greatest differences for intrinsic viscosity, interfacial area, and rate of emulsion decay. Nevertheless, cultivar diversity was observed among the seven cultivars which indicates that genetic variability exists in the physicochemical properties of mucilage. However the effect of growing location and environment remains to be examined. The genetic variability observed in this study may allow plant breeders to develop cultivars that contain mucilage with rheological and functional characteristics for specific end uses.

Research has shown that gums, pectins and some hemicelluloses can promote beneficial physiological effects. In particular, the presence of these viscous compounds in the small intestine tends to slow the digestion and absorption process of carbohydrates and reduces the rate of glucose flowing into the bloodstream (Olson et al., 1987; Coultate, 1989). In order to take advantage of such beneficial characteristics it has been suggested that water-soluble fibers be incorporated into the diets of people with Type 2 diabetes. Previous work by Cunnane et al. (1993) on the nutritional benefits of flaxseed and flaxseed mucilage found that both had a positive effect in lowering the blood glucose response of normal subjects due to its highly viscous nature. Whether this beneficial effect is true for people with Type 2 diabetes and whether it is the mucilage that is responsible for this lowering effect remains unresolved at the present time. In order to determine which component or components of flaxseed have the greatest effect on the glycemic control in people with Type 2 diabetes, it is

recommended that a nutritional study which examines the effect of milled flaxseed, flaxseed mucilage, and flax oil on the blood glucose and insulin response of healthy subjects and people with Type 2 diabetes be carried out.

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Table 1. Pearson's correlation coefficients for selected relationships between seed composition and mucilage characteristics

Variable 1	Variable 2	Correlation[a]	Significant Probability
UNF			
Protein content in seed	% CHO[b] in mucilage	-0.49	0.268
Oil content in seed	% CHO in mucilage	-0.43	0.345
Protein content in seed	% xylose in mucilage	-0.36	0.431
Oil content in seed	% xylose in mucilage	-0.55	0.196
Protein content in seed	Intrinsic viscosity	-0.45	0.309
Oil content in seed	Intrinsic viscosity	-0.25	0.593
Protein content in seed	Rate of decay	0.47	0.282
Oil content in seed	Rate of decay	0.52	0.233
40F			
Protein content in seed	% CHO in mucilage	-0.50	0.252
Oil content in seed	% CHO in mucilage	-0.78	0.038
Protein content in seed	% xylose in mucilage	-0.48	0.272
Oil content in seed	% xylose in mucilage	-0.38	0.399
Protein content in seed	Intrinsic viscosity	-0.68	0.094
Oil content in seed	Intrinsic viscosity	-0.70	0.082
Protein content in seed	Rate of decay	0.65	0.115
Oil content in seed	Rate of decay	0.42	0.344
60F			
Protein content in seed	% CHO in mucilage	-0.79	0.035
Oil content in seed	% CHO in mucilage	-0.66	0.109
Protein content in seed	% xylose in mucilage	-0.41	0.365
Oil content in seed	% xylose in mucilage	-0.73	0.063
Protein content in seed	Intrinsic viscosity	-0.13	0.774
Oil content in seed	Intrinsic viscosity	-0.04	0.926
Protein content in seed	Rate of decay	-0.54	0.216
Oil content in seed	Rate of decay	-0.22	0.637

[a] n = 7

[b] CHO = carbohydrate