The Effects of Processing on the Nutritional Characteristics of Oat Fibre

by

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ABSTRACT

Many countries have approved health claims regarding ß-glucan soluble fibre from oat. The proposed mechanism for cholesterol reduction from ß-glucan is due to its water soluble property, which can create a high viscous solution at low concentration to attenuate cholesterol absorption. The viscous properties of oat ß-glucan in the gut are influenced not only by β-glucan content, but also solubility and molecular weight. The objective of this study was to determine the effects of oat processing, genotype and growing location on the physicochemical properties of β-glucan. Five oat genotypes (HiFi, Leggett, CDC Dancer, Marion and CDC Morrison) grown in two locations (Saskatoon and Kernen) were dehulled (untreated) and processed in a pilot facility through kilning (kilned/not flaked) and subsequent steaming and flaking (kilned/flaked). Three oat genotypes (HiFi, Leggett and Furlong) went through a commercial processing line including dehulling, kilning and steaming and flaking. Viscosity of oat slurries was determined using a Rapid Visco Analyzer at 37 °C with the addition of enzymes (α-amylase, pepsin and pancreatin) to mimic the rheological behavior of β-glcuan in the human gastrointestinal tract. Molecular weight of the solubilized β-glucan obtained through in vitro digestion was monitored by high performance size exclusion chromatography with Calcofluor detection. Under the pilot scale processing, untreated groats gave a relatively low viscosity (164.30 cP) and a low extractable β-glucan molecular weight (332.44 kDa), but exhibited high β-glucan solubility (90.49 %). Compared to untreated groats, the kilned/not flaked samples had significantly ($p < 0.05$) increased viscosity (313.85 cP) and extractable β-glucan molecular weight (604.71 kDa). Additional processing into kilned/flaked products further increased viscosity (930.75 cP) and β-glucan molecular weight (1221.76 kDa), but β-glucan solubility (63.83 %) was significantly ($p <$

0.05) reduced. Genotype and growing environment also significantly ($p < 0.05$) affected β-glucan viscosity and molecular weight, but no significant interaction effects between processing, genotype and environment were found. Under the commercial scale processing, untreated groats also exhibited low viscosity (52.33 cP) associating with a low extractable β-glucan molecular weight (41.88 kDa). Continuously processing (kilning and then flaking) had consecutive increases in viscosity at 469.00 cP (kilned/not flaked groats) and 717.75 cP (kilned/flaked groats). Both kilned and kilned/flaked groats had relative high molecular weight (1854.89 kDa and 1800.14 kDa) comparing to untreated one, but no further increase was observed in molecular weight from kilned to flaked groats. The solubility of β-glucan was decreased from untreated groats (90.86 %) to kilned groats (37.06 %), and then increased in kilned/flaked groats (46.30 %). Commercial scale processing and genotype had significant effects on viscosity, molecular weight and solubility of β-glucan, and interaction effects of processing-by-genotype were observed in molecular weight and solubility ($p < 0.05$). Results indicate that there is potential for processors to improve the physicochemical/nutritional properties of oat end products through processing of specific oat genotypes from selected growing locations.

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LIST OF ABBREVIATIONS

cP	centipoise
DP	degree of polymerization
d.w.b.	dry weight basis
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion chromatography
kDa	kilodalton
kg	kilogram
μm	micrometer
Mp	most abundant molecular weight in the polymer population
mPa.s	millipascal-second
Mn	number-average of molecular weight
Mw	weight-average of molecular weight mass
nm	nanometer
w/w	ratio of weight by weight

1. Introduction

Oats (*Avena sativa L.*) are well suited to growing areas that consist of cool, moist climatic conditions; they tolerate poor and acidic soils, but require enough water during the growing phase and plenty of sunshine during the harvest phase (Duss & Nyberg, 2004; Welch, 1995). These requirements describe growing environments that are present in Canada. Therefore it is not surprising that Canada is a major producer of oats worldwide. According to a recent market outlook report, Canada is the world largest oat exporter and the third largest oat producer after the European Union and Russia (Agriculture and Agri-Food Canada, 2010). In 2009-2010, total domestic use was 1.17 million tonnes, and exports were 2.10 million tonnes. On average, 92-99% Canadian oats and their products were exported to the United States (US). With an annual average of 1.85 (Saskatoon) and 0.95 (Manitoba) million tonnes of oats produced from 2006 to 2010, Saskatchewan and Manitoba became the major oat producing provinces in Canada.

Initially used mainly as animal feed, oats attracted the attention of the food industry and consumer market in the 1980s, with updated medical research and positive public messages about oat consumption and its effects on consumer health. Most of the health messages have been related to β-glucans present in oat and clinical studies showing cholesterol lowering and blood glucose regulation, which help reduce the risk of coronary heart disease and diabetes (Wood, 1991). The health benefits are due to the unique structure of the soluble fiber present in oat, commonly known as (1-3)(1-4)-β-D-glucan located in the endospermic cell wall (Wood, 1993). β-1,3-linkages existing between β-1,4-linkages contributes to the structure irregularity and disrupts the intramolecular hydrogen bonding (Sakurai, 1991), which makes β-glucans a linear and partially water soluble fibre (Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004).

An earlier study in 1963 showed that healthy human volunteers experienced reduced serum cholesterol levels by including oatmeal in their diets (De Groot, Luyken, & Pikaar, 1963). In 1997, the first health claim of oat β-glucans was approved by US Food and Drug Administration (FDA) submitted by Quaker Oats Co., which required 0.75 gram β-glucans per serving size in oat products to meet the health claim of lowering cholesterol and the risk of coronary heart disease.

To meet the requirements of the health claim for consumer products, the food processing industry requires high quality oats with a high content of β-glucans to work with. Both the oat cultivar and the growing environment can affect the nutritional composition of oat, including β-glucan content. Proper genotype selection, developing new oat germ plasm through plant breeding or identifying specific environmental conditions could result in a desirable oat grain supply with a high level of β-glucans for industrial use and consumer applications. Since harvested oat grain usually undergoes primary processing before milling or processing into food products, processing may also affect the nutritional value and specifically the β-glucan content of oats. There is limited information on how genotype and environment interact with processing conditions and how these interactions may affect the nutritional properties of oat.

A number of studies have used various oat products or concentrated β-glucans to confirm health effects and identify the mechanism of cholesterol and glycemic response reduction by β-glucans. However, not all studies reported a statistically significant lowering effect. The variation in results may be related to the physiochemical properties of oat β-glucan used in the treatments. The beneficial physiological properties of β-glucan are thought to be related to its viscous properties, which depend on the content, solubility and molecular weight of the β-glucan

(Tosh et al., 2010; Tosh, Brummer, Wolever, & Wood, 2008; Wolever et al., 2010; Wood, 2010; Wood, Beer, & Butler, 2000). The extractability (solubility) of β-glucans can be modified during processing operations such as steaming, heating and drying (Johansson, Tuomainen, Anttila, Rita, & Virkki, 2007; Tiwari & Cummins, 2009; Wood, 1993). Steaming, heating, and drying are the common commercial processing techniques used in oat kilning and flaking (Ganssmann & Vorwerck, 1995). Food processing has been considered to be a main contributor to viscosity, solubility and molecular weight of β-glucans (Tiwari & Cummins, 2009; Tosh et al., 2010). Therefore, it is necessary to investigate the physiochemical characteristics of oat β-glucans during typical processing operations to determine if processing, oat cultivar or growing location may influence the properties of β-glucans that confer health benefits.

Therefore, the goal of this research is to determine the effects of processing on the physicochemical characteristics of oat fibre, in order to provide the oat industry (and consumers) with valuable information to guide them in food product development (and selection), for improved consumer health. The specific objectives of this study were as follows:

i. To determine the effects of processing, genotype, environment, processing-by-genotype, genotype-by-environment and processing-by-environment interactions on oat β-glucan characteristics using a pilot scale processing facility located at the Quaker Oats plant in Cedar Rapids, Iowa to simulate commercial operations commonly employed by industry.
ii. To determine and confirm the effects of processing, genotype and processing-by-genotype interactions on oat β-glucan characteristics using a commercial production facility at Richardson Milling in Portage La Prairie, Manitoba.

iii. To determine the effects of processing on other oat fibre characteristics in addition to oat β-glucans.

2. Literature Review

2.1. Oat

The oat grain consists of the kernel and groat which is a soft caryopsis with long and elliptical shape, covered by a fibrous hull comprised of the lemma and palea. Because the hull contains silicate particles and can irritate the mouth, esophagus and gastrointestinal tract, it has limited value for the food industry (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008). Therefore, it is essential to remove the hull (dehull) from the whole oat before any further milling or processing for food use.

Compared to the hull, whole oat groats contain various valuable nutrients including soluble fibre, protein, unsaturated fatty acids, vitamins and minerals (Kirk & Sawyer, 1991). A groat contains about 7.5 % fat (higher than other grains) which can be problematic during storage and processing (Butt et al., 2008). This is because once the kernel is damaged during processing operations such as dehulling, grinding, endogenous lipid-related enzymatic oxidation is initiated and results in rancidity. Hence, a hydrothermic treatment using steam (also known as kilning) is carried out following cleaning and dehulling to stabilize the oat groat to ensure a longer shelf life (Ganssmann & Vorwerck, 1995). The oat groat consists of the bran, endosperm and germ. The pericarp, testa and nucellus are compressed and fused to the surface layers of the bran. The aleurone layer is underneath the surface layers of bran surrounding the endosperm and most of the embryo, also as part of bran. The sub-aleurone layer in the endosperm is located on the surface adjacent to aleurone layer, and it holds the majority of endosperm cell walls. Oat β-glucans are located in the endosperm cell walls which are distributed throughout the endosperm, with the sub-aleurone layer having the highest content of β-glucans (Butt et al., 2008; Fulcher &

Miller, 1993). β-glucans are also found in the cell walls of the aleurone layer (Miller, Fulcher, Sen, & Arnason, 1995).

2.1.1. Genotypic Factors

Oat cultivars share the same characteristics mentioned above, but genotypic and phenotypic differences exist to varying degrees depending on the trait. β-glucan content is one of the traits that plant breeders strive to increase in breeding lines and new cultivars to improve oat product quality. High β-glucan oat genotypes are developed and utilized by breeders and industries to enhance health benefits. However, genotypic differences are able to influence the quality of food products (Lapvetelainen et al., 2001; Molteberg, Solheim, Dimberg, & Frølich, 1996; Rhymer, Ames, Malcolmson, Brown, & Duguid, 2005), and may have potential physiological effects as well. Differences among genotypes in oat β-glucan distribution have also been reported (Fulcher & Miller, 1993). High β-glucan cultivars have a lower concentration of β-glucans detected in the sub-aleurone layer for the central region of the kernel compared to low β-glucan varieties. The concentration of β-glucans may shift to the central endosperm associated with a total β-glucan content increase in the oat groat, or cell wall thickness could be varied due to the reduction of β-glucans in the sub-aleurone layer. High β-glucan varieties are accompanied by a smaller cell size in the endosperm (Fulcher & Miller, 1993). Differences in cell wall thickness, cell size and β-glucan distribution (Miller & Frégeau-Reid, 2009) among genotypes would have diverse effects on commercial food processing, and potentially on the physiochemical properties of oat β-glucans associated with health benefits.

2.1.2. Environmental Factors

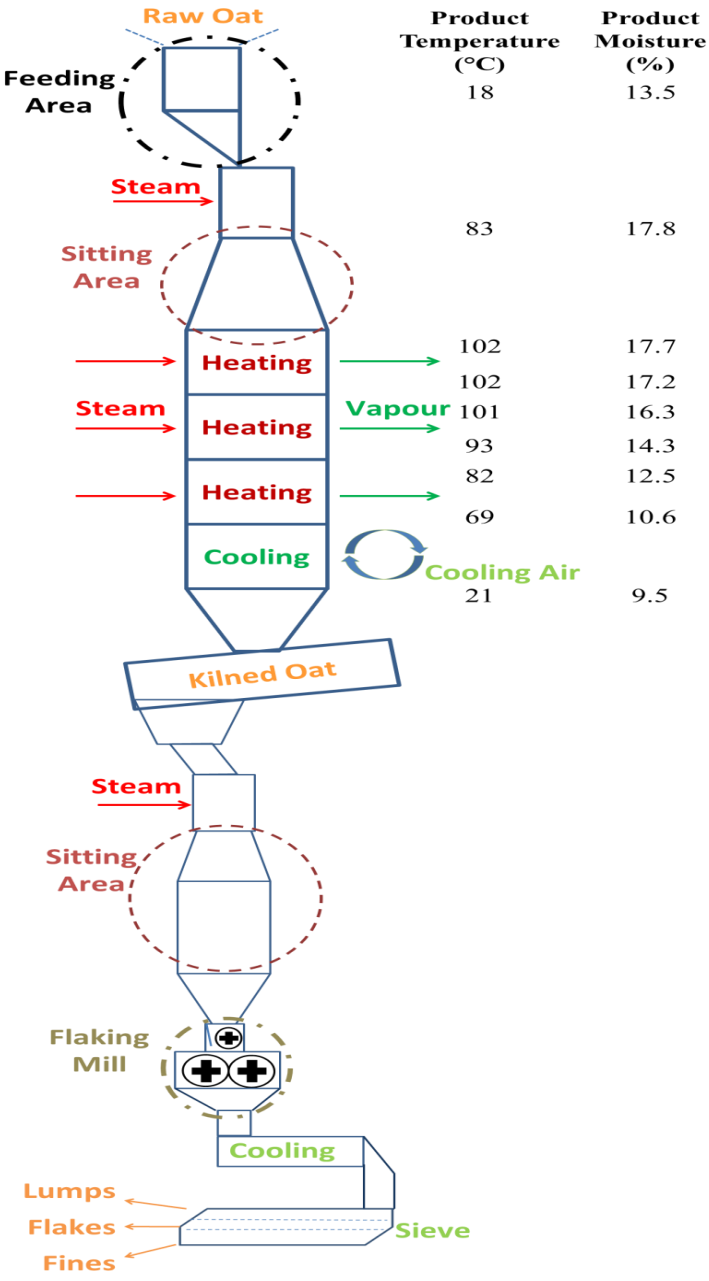
Growing conditions and environmental variation can also influence oat composition and nutritional quality. Climatic conditions, soil moisture, nitrogen concentration and photosynthetic efficiency are among the major factors influencing oat growth during grain filling. Low temperatures (below 10-12 ℃) for several days give an irreversible depression of photosynthesis and carbon dioxide uptake (Larcher, 1981; Lyons, 1973). Low temperatures also cause grain hardening, which modifies the degree of saturation of membrane lipids (Levitt, 1980). On the other hand, high temperatures (35-45 ℃) at the end of the growing cycle can disrupt cell development, photosynthesis and nitrogen metabolism, and result in low kernel weight (Alexandrov & Bernstam, 1977). Drought leads to the reduction of carbon dioxide fixation, photosynthesis and grain yield. Excess water or waterlogging causes decreased grain yield and increased grain weight (Brouwer & Flood, 1995). Nitrogen concentration could be associated with grain protein (Cataldo, Schrader, Peterson, & Smith, 1975). In a Finnish study, results showed that large groat size and low protein content was associated with high β-glucan content (Saastamoinen, Plaami, & Kumpulainen, 1992). Even though other studies showed different relationships between protein and β-glucan content (Welch & Lloyd, 1989; Welch, Leggett, & Lloyd, 1991), these reports show the importance of the role of growing environment on groat size, groat weight and protein content, which are also relevant to the performance of oats during the milling process (Doehlert & McMullen, 2000).

2.2. Oat Processing

There are a wide variety of oat products on the market, such as oatmeal cereal, cereal bars and steel-cut oats. To produce a desirable oat product, milling and processing which involves

dehulling, cleaning, klining, cutting, steaming, flaking, and drying are crucial (figure 2.1). Since this study focuses on the effects of processing on physiochemical properties of oat β-glucans and other fibres, several major critical points of processing relative to physiochemical properties change is discussed as below.

Figure 2.1 Typical commercial scale processing associating with oat products (adapted from Ganssmann & Vorwerck, 1995).



2.2.1. Dehulling and Cleaning

An impact huller is widely used to detach the inedible hulls from whole oat grain (Ganssmann & Vorwerck, 1995). The device has a high-speed rotor attached to the horizontal distribution plate surrounded by an impact ring on horizontal level. A hollow shaft is located on the top of the plate for sample feeding purposes. Once the oat grain is fed into the huller, it drops on the plate and is thrown against the impact ring by centrifugal force created by the rotor. The hull is broken into pieces and the oat groat released.

Hullability is influenced by oat characteristics, such as moisture content, proportion of husk, grain size, grain weight and fragility (Ganssmann & Vorwerck, 1995; Vorwerck & Rohde, 1984). Besides, extreme dehulling would increase the risk of groat breakage. It is preferred to reach a balance stage between hullability and groat breakage (Doehlert & McMullen, 2001; Peltonen-Sainio, Lehtinen, Kontturi, Rajala, & Kirkkari, 2004). Nevertheless, hullability can be reduced by enhancing rotor speed, but fibre concentration in oats has a negative association with rotor speed increasing (Peltonen-Sainio, Kontturi, Rajala, & Kirkkari, 2004), which is due to the fibre loss associated with higher breakage rate.

The mixture of groats, free husks, fines and unhulled groats produced the dehulling process are separated during cleaning. In a closed air circuit, husks and fines from the mixture are drawn upward by an aspirator and collected in a separate place. The cleaning procedure is usually repeated twice to remove the remaining husks and fines on groats. However, over-cleaning would cause the loss of groats in the waste along with husks and light particles. Hulled and unhulled groats are separated by an indented cylinder, table and paddy separator according to the

differences in size, shape, density, surface texture and their response to gravitation (Ganssmann & Vorwerck, 1995).

2.2.2. Kilning and Drying

After dehulling, kilning is employed to denature the activated lipid enzymes and lead to a longer shelf life (Ganssmann & Vorwerck, 1995). The kilning process also reduces the microbial load of oat groats, and enhances flavour (Ganssmann & Vorwerck, 1995). In former times, kilning was initially done in stone ovens on a heated iron plate (Findlay, 1956). Now, radiator kilns are commonly employed and mounted with heating and cooling sections. The heating section uses steam-heated radiators to maintain optimal temperature; the cooling section uses air to vent out excess heat and moisture. Steam flows through the radiator sections without any direct contact with the oat groats.

To enhance the efficiency of enzyme deactivation, a short term steam is added before kilning. According to Ganssmann and Vorwerck (1995), the most effective inactivation can be achieved at 90-100 ℃with minimum moisture content no less than 12%. Furthermore, higher moisture content requires a shorter inactivation time. Therefore, prior to steaming, moisture content is ideally to increase to 17 % and groats are heated to 80 ℃. Once oat groats are fed into the radiator sections, they are heated up to 100 ℃. After the inactivation procedure is completed, groats are cooled to 25 ℃ and dried by air ventilation to 9.5 % of moisture. The whole kilning process which includes steaming and cooling, takes about 90-120 minutes. Since kilning helps unhulled oats to shrink their kernel away from their husks, it is followed by sequential cleaning and flaking.

Endo-β-glucanases are a group of enzymes deactivated during kilning process. They naturally exist in oats and are active during cell growth and development (Sakurai, 1991). Endo-β-glucanases degrade the molecular weight of β-glucans (Sakurai & Masuda, 1979). Depolymerization of β-(1-3)(1-4)-glucans helps cell elongation and synthesis of mRNA and proteins (Sakurai & Masuda, 1979; Sakurai, 1991). Endo-β-glucanase requires 3 hours of incubation in order to release β-glucans from oat cell walls, and it only slowly affects β-glucan depolymerisation up to 60 hours (Huber & Nevins, 1980). Unlike lichenase (one type of β-glucanase enzyme, it will be discussed later in this thesis), endo-β-glucanases requires much longer period of time to depolymerize β-glucan into smaller molecular size. Kilning at 60 ℃ for 16 hours can deactivate the activities of endo-(1-3)(1-4)-β-D-glucanase at 70-80 %; endo-(1-3)-β-D-glucanase at 30 %; while negligible activity occurs when the temperature is increased to 70 ℃ (Ballance & Meredith, 1976; Burkus & Temelli, 1998).

2.2.3. Steaming and Flaking

The process of flaking begins with steaming to create a softer groat with less breakage, which helps to reduce production of fines and flours. After one to two minutes of steaming, the temperature of oat groats will reach about 95 to 104 ℃, with three to five percent of moisture content increase (Ganssmann & Vorwerck, 1995). Steamed oat groats stay in the steamer chamber for another 15 to 25 minutes. The sitting time allows the moisture to be evenly distributed in the groats. Optimal tempering time should minimize the breakage of groats while maintaining levels of vitamins and nutrients in the end product.

Steamed and tempered groats are continuously fed into flake rollers, which consist of a pair of rolls with circumferential speed of 5 to 8 m/s. Flake thickness is controlled by setting the gap

between the rolls. The thickness of whole groats (regular oat meal available on the market) is usually between 0.6 to 0.8 mm (Ganssmann & Vorwerck, 1995). Cut and smaller-cut groats are rolled into thinner flakes. The smaller-cut groats are categorized as instant or quick-cooking flakes with a thickness of 0.25 to 0.35 mm.

Lastly, flaked oats leave the roller at 90 to 95 ℃ and enter a drying and cooling section. In this section, constant air flow enables removal of heat and moisture to prolong shelf life. Oat flakes are cooled to 5 to 10 ℃ above ambient temperature, with moisture content from 10 to 12 %. Another cleaning step is done after flaking to further separate fines or lumps from the flakes before packaging.

2.3. β-Glucans

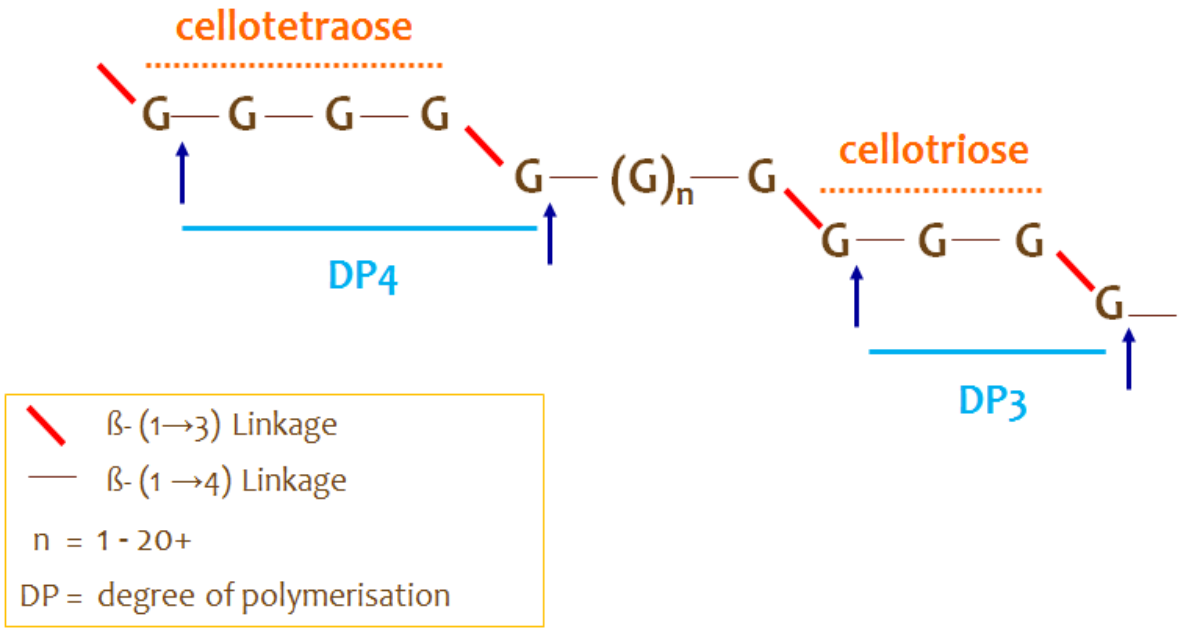
The main soluble fibre found in oat is β-glucans, also known as the mixed-linkage polysaccharide (1-3)(1-4)-β-D-glucan. A rich amount of β-glucans (3-7 %) are found in oat endosperm cell walls (Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003). During the old days, highly viscous β-glucans (Butt et al., 2008) has shown to negatively impact on animal feed and filtration relative processing (Brennan & Cleary, 2005). High β-glucan content in animal feed reduces nutrient uptake and animal weight gain because its viscous nature decreases feed digestibility and nutrient absorption (Annison, 1991; Bergh, Razdan, & Åman, 1999; Classen, 1996; Jeroch & Danicke, 1995). Several observational studies using pig had further validated these phenomena (Knudsen & Canibe, 2000; Baidoo & Liu, 1998; Leterme, Souffrant, & Théwis, 2000; Morel, Padilla, & Ravindran, 2003).

Nonetheless, the high viscosity of β-glucans have been shown to benefit human by lowering cholesterol and glycemic response. The underlying mechanisms behind these beneficial effects

are still under debate, but the commonly acceptable assumption is that β-glucans increase intrinsic viscosity in the gastrointestinal tract and slow the absorption of glucose and cholesterol during digestion (Jenkins et al., 1987; Story & Kritchevsky, 1976; Vahouny, Tombes, Cassidy, Kritchevsky, & Gallo, 1980). Since viscous properties of β-glucan seem to play a major role in delivering the beneficial effects according to most studies, factors that attribute to its optimum level in oat products has caught the attention of the food industry. The rheological behavior (viscosity) of β-glucans can be modified by modifying β-glucan content, molecular weight and solubility (Wood, 2004), which vary depending on the variety of oat and various processing and storage conditions (Beer, Wood, Weisz, & Fillion, 1997; Degutyte-Fomins, Sontag-Strohm, & Salovaara, 2002; Zhang et al., 1992).

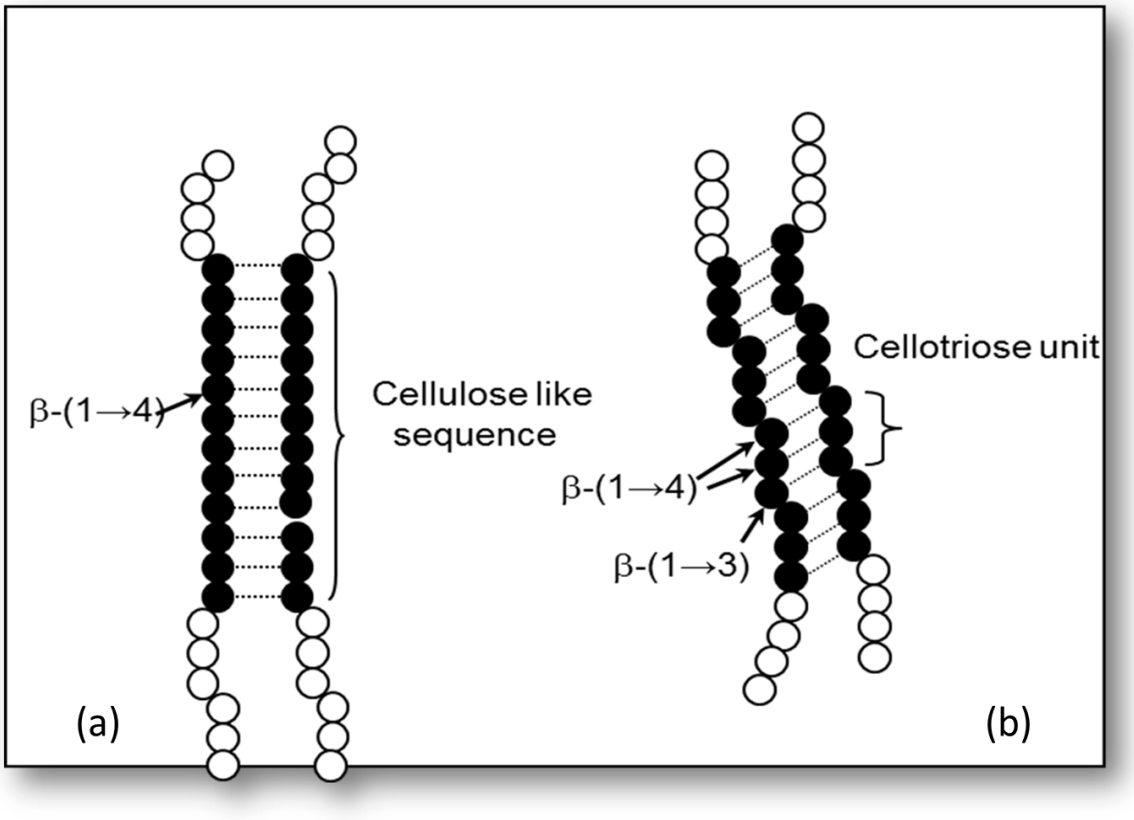
2.3.1. Structure

Figure 2.2 Structure of β-glucan (adapted from Lazaridou & Biliaderis, 2007b).



Oat β-glucans are linear unbranched polysaccharides connected by β-(1-3) and β-(1-4) linkages (figure 2.2). The ratio between β-(1-3) and β-(1-4) linkages is approximately 3 to 7 (Aspinall & Carpenter, 1984; Parrish, Perlin, & Reese, 1960; Perlin & Suzuki, 1962; Wood, Weisz, & Blackwell, 1991). Most of the β-(1-4) linkages exist in groups of two or three, separated by single β-(1-3) linkages. Cellulose on the other hand has only β-(1-4) glycosidic linkages, and its regular shape allows intermolecular hydrogen bonds to form between polymers (BeMiller, 2007). This additional force created by hydrogen bonding makes cellulose a crystalline structure and insoluble in aqueous solutions. The irregular shape of β-glucans gives the chains a more flexible helical structure and prevents close packing and H-bond forming, which allows β-glucans to become partially soluble in water (Buliga, Brant, & Fincher, 1986; Böhm & Kulicke, 1999; Woodward, Fincher, & Stone, 1983). Compared to other soluble fibres in oats, β-glucans have the greatest capacity to increase viscosity by binding water (Wood, 1993).

Figure 2.3 The interactions formed between β-glucan molecules in junction zones: (a) cellulose-like sections of the polymer and (b) cellotriosyl sequential sections of the polymer (adapted from Tosh, Brummer, Wood, Wang, & Weisz, 2004 with permission from Elsevier provided by Copyright Clearance Center).



Depending on the number of the glucose residues, β-(1-4) linked segments are named cellotrioses (DP3, degrees of polymerisation) or cellotetraoses (DP4) (figure 2.2). However, cellulose-like sequences can be present up to DP20, with most of them being DP5, DP6 and DP9. The ratios of DP3 to DP4 vary between different cereal grains (Tosh et al., 2004). The ratio of DP3 to DP4 is used as a "finger print" to identify the source of β-glucans. Two individual β-glucan polysaccharides chains with three consecutive cellotriosyl units (3 units of DP3 linked by β-(1-3)) may form a stable cellulose-like junction zone, or an elastic cellotriosyl sequence junction zone between each other (Tosh et al., 2004; Tvaroska, Ogawa, Deslandes, & Marchessault, 1983) (figure 2.3). Cellulose-like junction zones are stabilized by hydrogen bonds,

which have crystalline-like gel forming properties and thereby decrease the solubility of β-glucans in solution (Vårum & Smidsrød, 1988). On the other hand, cellotriosyl junction zones have a helical structure with more flexibility and solubility compared to cellulose-like ones. Soluble β-glucans have lower ratio of DP3 to DP4 than insoluble ones (Izydorczyk, Macri, & MacGregor, 1998a; Izydorczyk, Macri, & MacGregor, 1998b). Hence, a higher proportion of DP3 in the molecular structure may be associated with lower solubility. According to literature findings, the ratio of DP3 to DP4 of soluble oat β-glucans ranged from 2.1 to 2.4, which is lower than that of barley (2.8 to 3.3), and wheat (3.1 to 4.5) (Cui, Wood, Blackwell, & Nikiforuk, 2000; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Wood, Weisz, & Blackwell, 1994; Wood et al., 1994). Therefore, based on DP3 to DP4 ratios, oat generally has a higher solubility of β-glucans compared to barley and wheat (Cui et al., 2000).

A low DP3 content in oat β-glucans is associated with low gelling intensity and high β-glucan solubility. However, gelation is also affected by molecular size. Low molecular weight β-glucans exhibit faster gel formation than β-glucans with a high molecular weight (Lazaridou, Biliaderis, & Izydorczyk, 2003; Tosh, Wood, Wang, & Weisz, 2004). In the Lazaridou et al. (2003) study, β-glucans with a molecular weight of 50 kDa gelatinized in less than two hours, while β-glucans with molecular weight of 150 kDa gelatinized over 40 hours, illustrating that the higher mobility of smaller molecular size polymers had reduced the gelatinization period by 20 times (Doublier & Wood, 1995). Besides DP3 concentration and molecular size, the intensity of gelation may also be affected by the food matrix, and other existing monosaccharides and solutes (Lazaridou & Biliaderis, 2007a).

The unique structure of β-glucans was first recognized by Parrish et al. in 1960. Now, methylation analysis is commonly employed to characterize oligosaccharides in β-glucans by using (1-3)(1-4)-β-D-glucan-4-glucanohydrolase (lichenase) (Izydorczyk, Macri, & MacGregor, 1998a; Johansson et al., 2004; Wood, 1994). Lichenase breaks β-glucans into oligosaccharides by cleaving only the (1-4)-linkage next to the (1-3)-linkage. Hence, the oligosaccharides from β-glucan cleavage is made of (1-4)-linked oligosaccharides attached to one (1-3)-linked glucose unit at the reducing end. The characteristics of these oligosaccharides are then analyzed by gel chromatography (Bock, Duus, Norman, & Pedersen, 1991). A dramatic decrease in viscosity was detected when β-glucans were cleaved into oligosaccharides by lichenase (Roubroeks, Andersson, Mastromauro, Christensen, & Åman, 2001). Therefore, lichenase is also used to confirm the viscosity effects of β-glucans in solution, i.e., since lichenase specifically breaks down β-glucan, addition of the enzyme would show the residual viscosity due to the other components in solution (starch, protein).

2.3.2. Viscosity

Health benefits of β-glucans are mainly attributed to the high viscosity created by β-glucans dissolved in aqueous solution (Jenkins et al., 1978; Wood et al., 1994). The level of viscosity is determined by concentration, solubility and molecular weight of β-glucans (Wood, Weisz, & Mahn, 1991; Wood et al., 2000).

A solution of high molecular weight β-glucans with concentration below 0.2 % behaves like a Newtonian liquid (Autio, Myllymäki, & Mälkki, 1987; Doublier & Wood, 1995), in which viscosity is not affected by shear rate change. Once the concentration increases above 0.2 %, the viscosity of the β-glucan solution behaves like a non-Newtonian fluid with shear thinning

characteristics (Doublier & Wood, 1995). β-glucan starts to entangle and form a pseudoplastic solution. The concentration and molecular weight of β-glucans dissolved is positively associated with the viscosity of the solution. With different β-glucan extraction methods, different rheological properties are produced; hence, to make a comparison of viscosities in different products at an equivalent level, the same extraction method should be applied to all samples throughout the entire analysis (Johansson et al., 2007).

The nutritional impact of viscosity was initially recognized by Jenkins et al. in 1978. In a study by Wood et al. (1994), the viscosity of oat gum had a significant effect on the reduction of postprandial glucose and insulin response. The beneficial effects were weakened following acid hydrolysis, which lead to a decrease in viscosity or β-glucan dose (by hydrolyzing β-glucan down to glucose) (Wood et al., 1994). Hence, it is necessary to maintain the viscosity at an optimum level to maximize the nutritional value of β-glucans (Wood, Braaten, Scott, Riedel, & Poste, 1990).

However, a higher concentration of β-glucans does not necessarily warrant a more beneficial effect as noted in Tappy et al. (1996), where three different concentrations of β-glucans (4.0, 6.0, and 8.4 grams) were tested in a clinical trial. Significant declines in glycemic response were observed in all three dosages, but the results were similar between treatment 6.0 and 8.4 grams. Therefore in a given food system there may be an optimum dosage of β-glucan above which cholesterol and/or glycemic response or other health effects stay the same (plateau); in this case it would not be necessary for industry to incorporate more than the optimum amount of β-glucan in food products.

2.3.3. Molecular Weight

Besides structure, the molecular weight of β-glucans also plays a major role in determining their physiological characteristics, such as hydration and conformation in aqueous solution. All physiological characteristics of β-glucans will affect its rheological behavior. Molecular size is heterogeneous; even β-glucans extracted from the same source will vary in molecular size. There are several terms used to describe the polydispersity and distribution of molecular weight, such as M_n, M_w and M_p. M_n is the number-average of molecular weight, which is calculated by dividing total weight of substance by the total number of moles. In this expression of average molecular weight, each molecule is considered to have the same contribution to the physiological behaviors. M_w is the weight-average of molecular weight mass, which is measured by the proportions of all molecular weights distributed in the mixture. The polydispersity of polysaccharides is described by the ratio M_w/M_n. M_p is the molecular weight which is most abundant in the polymer population. All the aforementioned molecular weight values can be determined by size exclusion chromatography (SEC) using a high performance gel column with post-column Calcofluor and fluorescent detection (Wood et al., 1991). Calcofluor has the unique ability to specifically bind and dye β-glucans within a solution (Jørgensen & Aastrup, 1988; Wood, 1980), which allows SEC to detect the peak and makes comparison of molecular weights of β-glucan solutions without prior purification.

$$M_n = \frac{\sum M_i N_i}{\sum N_i} = \frac{\sum W_i}{\sum W_i / M_i}$$

$$M_w = \frac{\sum M_i{}^2 N_i}{\sum M_i N_i} = \frac{\sum W_i M_i}{\sum W_i}$$

Where M_i = molar mass of the component molecules of kind i

N_i = number-fraction of the component molecules i

W_i = weight-fraction of the component molecules i

In Tosh, et al.'s study (2010), extrusion was used to make a ready-to-eat (RTE) cereal from a mixture of oat bran and corn flour, and β-glucan molecular weight significantly decreased during the extrusion procedure at 237 ℃. The viscosity was measured by Beer's in vitro digestion method which aims to mimic human digestion (Beer et al., 1997). When molecular weight (M_p) decreased from 1930 kDa to 251 kDa, the viscosity dramatically dropped from 2900 to 131 mPa.s. Although the solubility of β-glucans increased from 66.8 % at M_p 1930 kDa to 101.7 % at M_p 251 kDa after extrusion, it was not sufficient to make up for the decrease in viscosity due to the loss of molecular weight/size. This further demonstrates that molecular weight plays a key role in determining the rheological behavior of β-glucan solutions. Molecular weight has been shown to be inversely associated with solubility/extractability in β-glucans. For example, molecular weight of β-glucans (M_p) at 2213 kDa had solubility at 61.5% compared to 73.4 % with low molecular weight at 211 kDa measured by in vitro digestion extraction method at 1 % concentration of β-glucan using the Rapid Visco-Analyzer (Gamel, Abdel-Aal, Wood, Ames, & Tosh, 2012).

To expand on the relationship between viscosity and health effects, high molecular weight β-glucans (which have a high viscosity) have specifically been shown to have a greater propensity to lower glycemic response and cholesterol. In a study by Regand et al. (2009), several oat products were examined for their effect on glycemic response. The solubility, viscosity, and molecular weight (Mp) between oat crisp bread and oat pasta were 61.3 %, 12.30 mPa.s, and 197

kDa; versus 28.8 %, 6.50 mPa.s, and 465 kDa; respectively. The results indicated that oat pasta with higher M_p had a higher efficiency in attenuating the peak blood glucose response (PBGR) and incremental area under glucose response curve (AUC). In Brummer et al.'s study (2012), oat β-glucan enriched extruded cereals with various molecular weights of β-glucans were tested for glycemic effects. A significant interaction effect between AUC and $\log_{10}[M_w]$ ($r^2 = 0.96$) was observed; AUC was inversely associated with M_w. The same association was found between AUC and $\log_{10}$ [M_w x Concentration] ($r^2 = 0.96$). This study again strengthens the statement that the molecular weight of β-glucan is one of the determining factors that impacts the nutritional value of oats. In 2010, the same oat enriched extruded cereals in Brummer et al.'s study were utilized in Wolever et al.'s study on β-glucan and its physicochemical effect on serum low-density-lipoprotein (LDL) cholesterol lowering. LDL cholesterol was significantly reduced by 5 % (≈0.2 mmol/L) in participants treated with high molecular weight (M_p = 2210 kDa), or medium molecular weight (M_p = 530 kDa) β-glucans. The efficiency was decreased by 50 % for the low molecular weight (M_p = 210 kDa) β-glucan treatment, which was not statistically different from the control group (wheat-fibre cereal). The LDL cholesterol lowering effect was similar among all clinical participants regardless of age, sex and baseline LDL cholesterol level (Wolever et al., 2010). Hence, it is necessary to maintain the molecular weight of oat β-glucans at a certain level to maximize the physicochemical effects on human health.

2.3.4. Solubility/Extractability

Another factor contributing significantly to viscosity and subsequent nutritional benefits is β-glucan solubility, or the amount of β-glucans in aqueous solution. As mentioned earlier, the amount of β-glucan in solution determines its rheological behavior, exhibiting Newtonian flow

(concentration < 0.2%), or shear-thinning flow (concentration > 0.2%). β-glucan solubility is influenced by its structure directly or indirectly, especially by the ratio of DP3 to DP4 (Cui et al., 2000). Meanwhile, higher content of consecutive cellotriosyl units (DP3) exhibits a tendency of stable conformation, gelation (aggregation) via hydrogen bonding along the cellodextrin (Tvaroska et al., 1983), which causes the loss of solubility (Lazaridou & Biliaderis, 2007b). However, the cellotriosyl units can be degraded or broken down by heat and shear force. In oats, β-glucans from the inner endosperm cell walls are released into aqueous solution more quickly than those from the subaleurone cell walls (Wood & Fulcher, 1978). The solubility of β-glucan shows physiological impacts similar to the molecular weight of β-glucans in terms of influencing viscosity (Cui & Wang, 2009).

Commercial processing and handling are known to modify the solubility (extractability) of oat β-glucans (Johansson et al., 2007; Lan-Pidhainy, Brummer, Tosh, Wolever, & Wood, 2007; Wood et al., 1991). In one study, commercial oat products (instant oats, rolled oats and oat bran) had solubility in the range of 58 to 85 %, with similar molecular weight at 2520-2960 kDa (Wood et al., 1991). In another study, instant and rolled oats had a molecular weight of 2520-2540 kDa, but the solubility of instant oats (58 %) was much lower than that of rolled oats (67 %). Solubility of β-glucans can also be reduced by freeze-thaw cycling (Lan-Pidhainy et al., 2007). In the study of freeze-thaw effect, two freeze-thaw cycles had significantly decreased the solubility from 39.6 % to 28.5 %; another 10.2 % reduction was observed when another two freeze-thaw cycles were applied. On the other hand, boiling and cooking oat flakes consistently increased the solubility of β-glucans compared to the starting material. All samples were analyzed by using three different hydrolysis methods (acid hydrolysis, lichenase hydrolysis and

modified AOAC 995.15 method). However, baking oat bran bread was shown to have a negative effect on β-glucan solubility (Johansson et al., 2007).

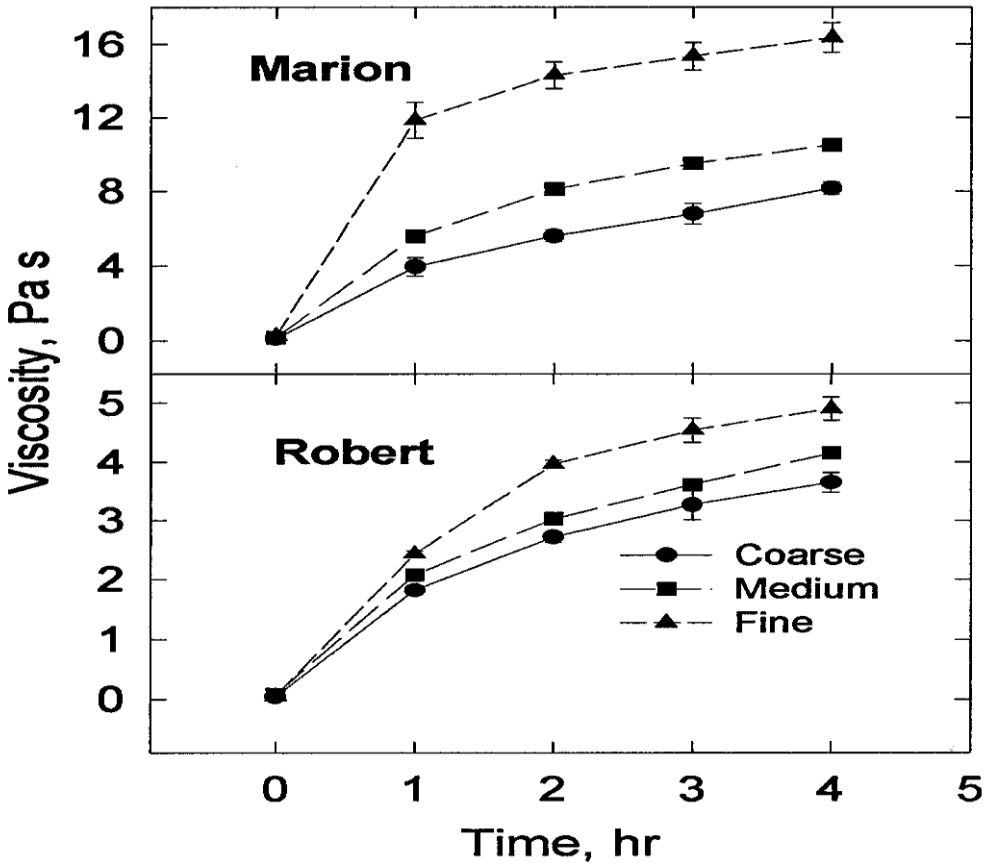
Despite all natural and processing effects, β-glucans with the same solubility may perform differently in the extraction procedure with any changes in parameters, such as initial particle size of material, solvent, temperature and duration of extraction (Wood, 1993). In this study, solubility, viscosity and molecular weight are measured by an in vitro digestion extraction method (Beer et al., 1997). This extraction mimics human digestion by exposing samples to pH changes and enzymes similar to those in the human gut. Oat products containing β-glucans are ground to a certain particle size and "digested" by a-amylase, pepsin and pancreatin at 37 ℃. The amount of β-glucans found in the final extraction solution is measured to determine its solubility.

2.3.5. Effects of Processing on β-glucan

Various processing techniques are employed to produce oat products suitable for the food industry. Thermal and mechanical processing has been observed to modify the viscosity of β-glucans by changing the concentration, molecular weight and solubility of β-glucans (Cenkowski, Ames, & Muir, 2006; Johansson et al., 2007; Tiwari & Cummins, 2009; Tosh et al., 2010; Zhang, Doehlert, & Moore, 1998; Zhang, Doehlert, & Moore, 1997). Since β-glucans in oats are mainly located in the cell walls of endosperm, the milling process can influence β-glucan content and viscosity by removing or adding back fractions from the bran, outer endosperm or inner endosperm (Wikström, Lindahl, Andersson, & Westerlund, 1994). The particle size of oat flour has a negative correlation with β-glucan extractability (solubility) (Regand et al., 2009). Fine

milling (0.2 mm) oat flour increased hydration rate and viscosity compared to coarse (1.0 mm) or medium milling size (0.5 mm) (Zhang et al., 1997) (figure 2.4).

Figure 2.4 Effect of oat flour particle size on viscosity development of steamed oat flour slurries (23.2 %, w/w, dwb). Apparent viscosity was measured with a Brookfield viscometer (RV-6 spindle, 50 rpm) at 30 ℃ (n = 3) (adapted from Zhang et al., 1997 with permission from the American Association of Cereal Chemists (AACC) International).



In a study by Doehlert, et al. (1997), both dry (roast) and wet (steam) thermal processing exerted significant effects on the viscosity of oat flour slurries compared to the raw untreated slurries (Doehlert, Zhang, & Moore, 1997). In addition to achieving the highest viscosity, steaming also allowed the solution to remain viscous for an extended time. This may be partially due to the high efficiency of steaming on inactivation of endo-β-glucanase (Paton & Lenz, 1993; Peterson, 1995). Roasted oat flour slurry viscosity peaked and started to drop after an hour, while

raw oat flour slurries saw a drop in viscosity after about half an hour. The change in viscosity of raw and roasted oat flour slurries was probably due to residual endo-β-glucanase activity (Wood, Siddiqui, & Paton, 1978). Furthermore, a smaller molecular weight of β-glucans was detected (70 kDa) which was associated with reduction of viscosity after three hours extraction at room temperature. Unlike raw and roasted flour, steamed oat flour had maintained a stable molecular weight at 2000 kDa at both one hour and three hours extraction. Initially, all raw, roasted and steamed oat flours had the same molecular weight at 2000 kDa, which indicated no degradation during the first hour of extraction. The endo-β-glucanase required a much longer time than lichenase to degrade β-glucans and reduce the viscosity of the solution at room temperature (Doehlert et al., 1997). Lichenase was employed in the study to ensure that the difference in viscosity at 30 ℃ was caused by the physicochemical change in β-glucans and not by starch gelatinization. Steaming oats for 20 minutes or roasting oats at 104 ℃ for 120 minutes did not gelatinize the starch (figure 2.5) (Doehlert et al., 1997).

Figure 2.5 Measurement of starch gelatinisation (pasting) on Rapid Visco-Analyzer using oat flour slurries prepared from raw, steamed or roasted groats (means, n = 4) (adapted from Doehlert et al., 1997 with permission from John Wiley and Sons provided by Copyright Clearance Center).

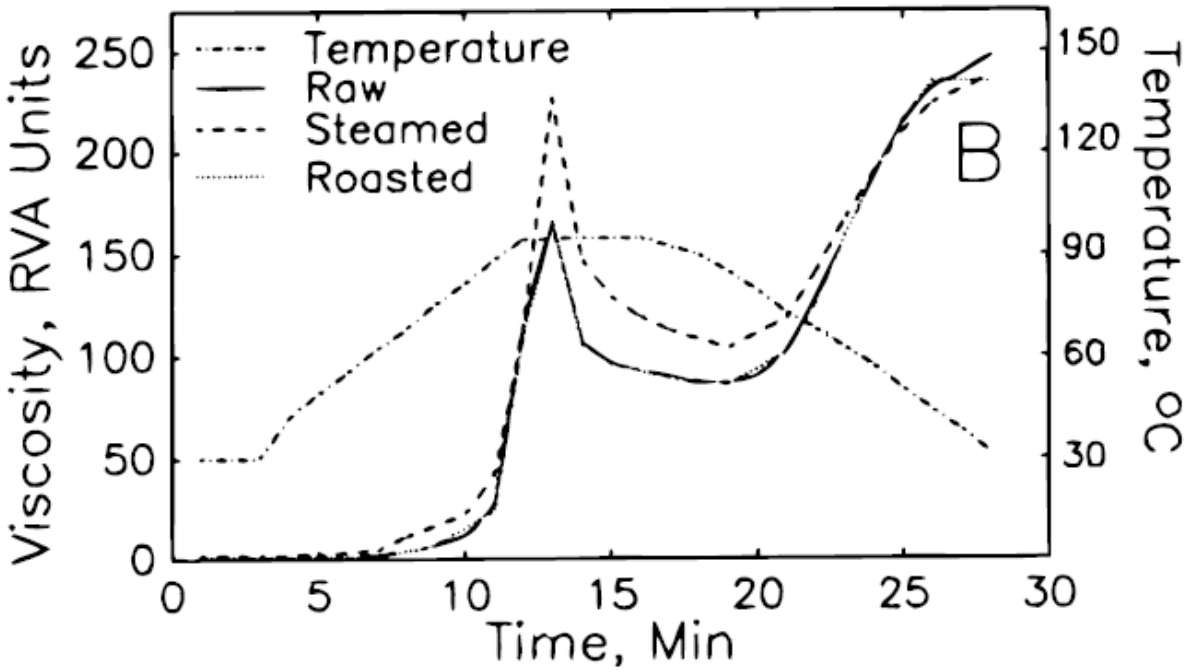


Table 2.1 below showed the apparent viscosity caused by β-glucans measured during the first hour of extraction (before the degradation by endo-β-glucanase). The effects of different heat treatments on viscosity were compared between roasted, steamed, roasted followed by steamed, and steamed then roasted oat flours (table 2.1). Roasted followed by steamed oat flour had similar viscosity as the steamed oat flours, significantly higher than that of raw and roasted oat flours. The viscosity of oat flour which was treated with steaming followed by roasting was statistically higher compared to the raw or roasted oat flour; but significantly lower than that of oat flours treated with just steaming or roasting then steaming. The results indicated that both roast and steam treatments had positive effects on β-glucan viscosity, with steaming having the greatest effect. Nevertheless, a decreased solubility of β-glucans (74 %) was observed in the steamed oat flour slurry compared to the raw (82 %) and roasted (84 %) oat flour slurry (Table

2.2). Doehlert et al. (1997) proposed that steaming had a positive effect on catalyzing the interaction between β-glucans and water molecules, which helped the steamed oats to overcome the reduction in β-glucan solubility, resulting in a high viscosity.

Table 2.1 Effect of heat treatments of three oat cultivars on apparent viscosity (mPa.s) of their respective flour slurries (6.5 g flour in 21.5 g water), one hour after addition of water (n = 3) (adapted from Doehlert et al., 1997).

Treatment	Apparent Viscosity (mPa.s)			
	Robert	Steele	Marion	Average
Raw	880	1310	2780	1656
Steamed	2056	5170	6900	4708
Roasted	1336	2173	3993	2501
Steamed/roasted	1513	4240	4323	3359
Roasted/steamed	1826	5470	8303	5200
Least significant difference (LSD) (α = 0.05)	239	591	1601	531

Table 2.2 Effect of genotype and heat treatment of whole oats on the relative viscosity of water soluble extracts, the concentrations of soluble and total β-glucan, and ratio of soluble and insoluble β-glucan in oat flour (adapted from Doehlert et al., 1997).

	Relative Viscosity	Soluble β-glucan (g/kg)	Total β-glucan (g/kg)	Soluble/total β-glucan Ratio
Treatment				
Raw	2.18	39.8	48.3	0.82
Roasted	2.27	40.0	47.2	0.84
Steamed	3.12	34.4	46.7	0.74
LSD (a = 0.05)	0.21	2.2	NS	0.05
Genotype				
Marion	2.91	46.0	55.5	0.83
Steele	2.45	40.1	47.1	0.85
Robert	2.21	28.0	39.7	0.71
LSD (a = 0.05)	0.21	2.2	1.7	0.05

In a study by Zhang et al. (1997), autoclaving, a hydrothermal processing method, was used to treat the oats; the viscosity of water extractable β-glucans was then analyzed (Zhang et al., 1997). In the one hour aqueous extraction, viscosity increased with increasing duration of autoclaving (up to 40 minutes) (Table 2.3). It was shown that as little as five minutes of autoclaving had produced a significant increase in β-glucan viscosity compared to the raw (as mentioned earlier, the endo-β-glucanase would not break down the molecular weight of β-glucans in raw oats with an extraction period of less than one hour). In the same study, roasting of oats demonstrated a negative correlation between viscosity and increased roasting temperature (Table 2.4). It is possible that heat treatments may impact polymer hydration and chain interaction in β-glucans (Zhang et al., 1997). The water penetration potential of steaming and autoclaving could be the answer for the improvement in viscosity of β-glucans by replacing intramolecular hydrogen bonding with water binding (Zhang et al., 1998; Zhang et al., 1997).

Table 2.3 Effect of oat grain autoclaving time on grain moisture after autoclaving and on apparent viscosity of oat flour slurries of Marion and Robert (adapted from Zhang et al., 1997).

Autoclaving Time (min)	Grain Moisture (%)		Apparent Viscosity* (Pa.sec)	
	Marion	Robert	Marion	Robert
0	9.7^{a}	10.9^{a}	3.51^{c}	0.44^{d}
5	8.0^{c}	8.5^{c}	8.86^{b}	5.75^{c}
10	8.4^{b}	8.5^{c}	11.09^{ab}	6.49^{bc}
20	8.4^{b}	8.7^{bc}	12.49^{a}	6.98^{b}
40	8.5^{b}	9.0^{b}	13.03^{a}	9.49^{a}

*Apparent viscosity was measured at 50 rpm after one hour holding time at 30 °C. Values within a column with the same letter were not significantly different (a = 0.05).

Table 2.4 Effect of oat grain roasting temperature on grain moisture after roasting and the viscosity of oat flour slurries of Marion and Robert (adapted from Zhang et al.,1997).

Roasting Temperature (℃)	Grain Moisture (%)		Apparent Viscosity* (Pa.sec)	
	Marion	Robert	Marion	Robert
Control	9.7[a]	10.9[a]	3.57[a]	0.44[a]
95	6.0[b]	6.0[b]	3.81[a]	0.48[a]
105	5.2[c]	4.8[c]	3.14[b]	0.43[a]
130	3.3[d]	3.4[d]	2.91[b]	0.34[b]
150	1.8[e]	2.4[e]	0.17[c]	0.18[c]

*Apparent viscosity was measured at 50 rpm after one hour holding time at 30 ℃. Values within a column with the same letter were not significantly different (a = 0.05).

In addition, extrusion (combining both mechanical and thermal processing) has the potential to increase the solubility by breaking down the molecular size of β-glucans (Rimsten, Stenberg, Andersson, Andersson, & Åman, 2003; Tosh et al., 2010; Åman, Rimsten, & Andersson, 2004). However, the degradation of β-glucan molecular weight was lessened by elevating the water content from 7 % to 19 %, and lowering the temperature from 237 ℃ to 181 ℃ during the extrusion process (Tosh et al., 2010). Baking has similar effects to extrusion as far as enhancing the extractability of β-glucans by reducing molecular weight (Beer et al., 1997; Regand et al., 2009; Rimsten et al., 2003; Sundberg et al., 1996; Torronen et al., 1992; Tosh et al., 2010; Tosh et al., 2008). Fermentation plays an important role in degrading the molecular weight of β-glucans during the baking process (Degutyte-Fomins et al., 2002; Johansson et al., 2006; Rimsten et al., 2003). Unlike extruding and baking, pasta preparation results in the reduction of both molecular weight and solubility of β-glucans (Regand et al., 2009; Åman et al., 2004). Cooking oat porridge has been shown to increase the extractability without changing the

molecular weight of β-glucans (Johansson et al., 2007; Regand et al., 2009; Åman et al., 2004). Lastly, freezing has exhibited an ability to lower extractability without affecting β-glucan molecular size (Cleary, Andersson, & Brennan, 2007; Kerckhoffs, Hornstra, & Mensink, 2003).

According to the existing literature, processing has a significant impact on the physicochemical properties of β-glucans. Hydrothermal processing seems to have the most positive influences compared to other processing techniques. However, there is limited information on the effect of kilning and flaking on β-glucan solubility, molecular weight, and viscosity. The findings obtained from performing these steps and measuring the associated physicochemical changes in β-glucans will be the key to understanding and optimizing the nutritional value of oat products.

2.4. Processing Effects on Other Non-Starch Polysaccharides

Other than β-glucans, grain also consists of other types of dietary fibre, such as resistant starch (RS) and arabinoxylan, which are not digestible in the human gastrointestinal tract (Collins et al., 2010). Heat processing has potential to generate resistant starch from the digestible starch (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009). Irreversible gelatinization occurs during hydrothermal processing where starch granules are swelling with a loss of crystalline molecular order (Cooke & Gidley, 1992; Hancock & Tarbet, 2000; Ring, Gee, Whittam, Orford, & Johnson, 1988). Once the temperature cools down, starch molecules undergo retrogradation and reform a stronger structure than unprocessed starch (Miles, Morris, Orford, & Ring, 1985), which is thermally stable and resistant to enzymatic digestion (known as RS) (Bird et al., 2009). Heat and moisture are the major components in kilning and flaking (Ganssmann & Vorwerck, 1995), and play an important role in starch gelatinization and

retrogradation (Czuchajowska, Sievert, & Pomeranz, 1991; García-Alonso, Jiménez-Escrig, Martín-Carrón, Bravo, & Saura-Calixto, 1999; Rosin, Lajolo, & Menezes, 2002; Sievert & Pomeranz, 1989; Sievert & Pomeranz, 1990). The concentration of arabinoxylan in oat flakes decreased after steaming from 7.4 % to 6.8 % (Westerlund, Andersson, & Åman, 1993). In another study, hydrothermal treatment at 290 °C increased the water soluble dietary fibre 2.2 times in wheat bran compared to the untreated wheat bran samples (Wakita et al., 2004). The authors made the assumption that the insoluble dietary fibre became soluble, and thus the soluble dietary fibre content increased (Wakita et al., 2004). Therefore, it is valuable to monitor the other dietary components besides β-glucan, and analyze their changes during the hydrothermal process.

2.5. Health Claim in Canada and Other Health Benefits

Health Canada has approved a health claim on oat products and blood cholesterol lowering in 2010 (Health Canada, 2010). Well established research strongly supports the evidence of cholesterol lowering effects following consumption of oat products enriched with β-glucans (Anderson et al., 2009; Andersson & Hellstrand, 2012; Andon & Anderson, 2008; Daou & Zhang, 2012; Othman, Moghadasian, & Jones, 2011), this leads to reduction of the risk of atherosclerosis (Chen, Jiao, & Ma, 2008; Glass & Witztum, 2001). To meet the requirement of Canada`s health claim, the oat products must contain at least 0.75 gram of β-glucans per serving size. In 2011, the European Food Safety Authority (EFSA) had summarized several health claims related to β-glucans from oat and barley: (i) maintaining normal blood LDL-cholesterol concentration (ID 1236, 1299), (ii) increasing satiety and reducing energy intake (ID 851, 852), and (iii) lowering post-prandial glycaemic response (ID 821, 824) (EFSA, 2011). Furthermore, several studies also indicate that oat β-glucan has a great potential to regulate the blood pressure

(Keenan, Pins, Frazel, Moran, & Turnquist, 2002; Pins et al., 2002; Swain, Rouse, Curley, & Sacks, 1990), although a health claim has not yet been approved.

2.5.1. Proposed Mechanisms of Soluble Oat β-Glucan

Even though the underlying mechanisms of oat β-glucan on cholesterol lowering is still undefined (Kritchevsky, 1997), a lot of evidence has shown that the viscosity created by β-glucans in the digestion tract plays an important role (Jensen, Spiller, Gates, Miller, & Whittam, 1993). There are several proposed mechanisms explaining how β-glucans exert their beneficial functions in human metabolism (Drozdowski et al., 2010; Edwards & Parrett, 1996; Ellegård & Andersson, 2007; Kerckhoffs, Brouns, Hornstra, & Mensink, 2002; Queenan et al., 2007; Theuwissen & Mensink, 2008; Würsch & Pi-Sunyer, 1997). These proposed mechanisms are:

i. In the gastrointestinal tract, the soluble β-glucans create a thick viscous unstirred layer surrounding the nutrients as a physical barrier to prevent the absorption of glucose, fat, cholesterol or bile acids
ii. The soluble β-glucans bind bile acids directly or indirectly, which helps in the excretion of bile acids (in the feces). This lowers re-absorption of bile acids in the small intestine, thus encouraging the liver to synthesize new bile acids by using stored cholesterol in the blood
iii. The reduction of glucose intake as mentioned above attenuates the requirement of insulin at the critical period of time. A high level of insulin would lead to increase of hepatic cholesterol biosynthesis

iv. The indigestible β-glucans undergo a fermentation process in the intestine and produce short-chain fatty acids (SCFAs), such as propionate, acetate and butyrate. These SCFAs are shown to help inhibiting the biosynthesis of cholesterol in the liver

2.5.2. Controversial Results of Oat β-Glucan in Clinical Trials

In 1992, Ripsin et al. analyzed twenty existing clinical trials and tried to identify the relationship between oat products and reduction of blood cholesterol. The results showed a great association between oat product intake and cholesterol lowering. Daily intake of approximately three grams of soluble fibre from oat lowered blood cholesterol by about 0.13 to 0.16 mmol/L. Moreover, the cholesterol lowering effect has been shown to be greatest among hypercholesterolemia subjects. Brown, et al. (1999) did a meta-analysis on studies including pectin, oat bran, guar gum and psyllium, which showed a small effect of soluble fibre on cholesterol lowering. However, some other clinical studies had failed to show a significant effect on cholesterol lowering (Beer, Arrigoni, & Amado, 1995; Rieckhoff, Trautwein, Mälkki, & Erbersdobler, 1999; Torronen et al., 1992). Salovaara, et al. (2007) collected 27 studies investigating the effects of dietary fibre on cholesterol and glycemic response since 1996, only six of the studies included viscosity or molecular weight data within their studies. The diverse results of 27 studies would be better if the data on viscosity, molecular weight and solubility in fibre had been collected and analyzed. Therefore, it is critical to monitor the changes in viscosity, molecular weight and solubility of β-glucans. This information not only helps to improve the processing methods of the food industry, it also contributes to the scientific research.

3. Materials and Methods

This section includes the materials and methods employed in Experiment One and Two of this study, which are referred as Effect of Pilot Scale Processing on β-Glucan Characteristics (Experiment One) and Effect of Commercial Scale Processing on β-Glucan Characteristics (Experiment Two).

3.1. Materials

3.1.1. Experiment One: Effect of Pilot Scale Processing on β-Glucan Characteristics

Five genotypes HiFi, Leggett, CDC Dancer, Marion and CDC Morrison were grown in two locations Kernen and Saskatoon in 2011 by the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada. All samples were processed by Xin Wang with technical support from Quaker Oats at a pilot scale processing facility located at Quaker Oats in Cedar Rapids, Iowa, United States.

3.1.2. Experiment Two: Effect of Commercial Scale Processing on β-Glucan Characteristics

Three genotypes HiFi, Leggett and Furlong were grown in Manitoba, Canada in 2009. Three samples were processed by Richardson Milling using a commercial production facility at Richardson Milling in Portage La Prairie, Manitoba, Canada.

3.2. Processing (Grain Pretreatment)

3.2.1. Experiment One: Effect of Pilot Scale Processing on β-Glucan Characteristics

Pilot scale samples was processed by myself with technicians at Quaker Oats. Firstly, ten different oat samples (five genotypes by two locations) (10 kg) were dehulled and cleaned at

Quaker Oats using a small custom made dehuller and a small Carter Day cleaner. Then, moisture contents of the samples were measured using a digital moisture analyzer balance (OHAUS MB45). Next, 3.18 kg of freshly dehulled and cleaned raw oat groats were weighed for one time kilning step. The rest of the raw oat was kept in the freezer at -20 ℃ to halt endo-β-glucanase activity. Raw oat groats were initially tempered up to 10 % moisture before kilning. During the tempering process, variable amounts of water were added to each sample depending on the initial moisture content, and mixed thoroughly by using a small custom made rotor mixer for 30 minutes. The final moisture was measured to confirm the efficiency of tempering. Then, the tempered samples were transferred into a small custom made kiln and kilned at 111.7 ℃ for 50 minutes. Finally, kilned oat groats were moved into a bucket, and cooled at room temperature for 16 hours.

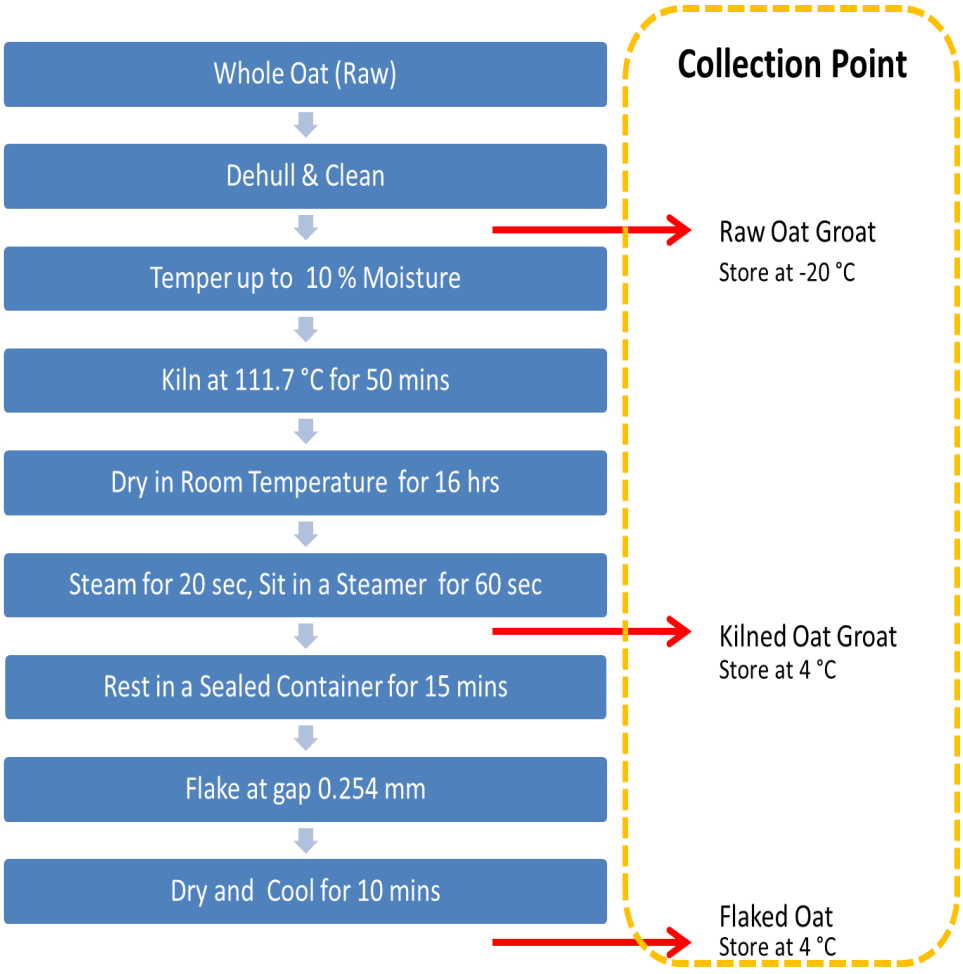
Prior to flaking, 150 grams of kilned oat groats were subsampled and stored in the fridge for laboratory analysis. 1.36 kg of kilned oat groats were steamed using a small custom made steamer. After 20 seconds of steaming, the sample sat in the steamer for another 60 seconds. Then, the sample was transferred into a sealed container for an additional resting time (15 minutes) which was followed by flaking with a small custom made flaker. The flaker was set up with a gap width of 0.254 mm, and the accompanying flake thickness ranged between 0.508 and 0.635 mm. Flaked samples were spread on the cooling pan and placed on an air circulation system for 10 minutes to cool and dry. Lastly, flaked oats were packed in sealed bags and stored in the fridge for further analysis.

Samples were collected separately at each different processing stage (which included raw, kilned and flaked oat groats) and stored in the proper conditions at all times (figure 3.1). All

samples were ground to a particle size of <0.5 mm before analysis using a centrifugal mill (Restch ZM-200, Brinkmann Instruments, Westbury, NY).

One random oat sample (Leggett) with no location information was used to test flaking effect alone, which skipped the kilning procedure on raw oat groat and was directly flaked to flakes. This oat sample also went through the kilning with continuous flaking procedure to compare with oat flakes skipped the kilning procedure. Due to the shortage on time, no duplicate was made in this experiment. Hence, there was not enough data to make any statistical analysis and conclusion.

Figure 3.1 Processing line and collection points in Experiment One using pilot scale processors.



3.2.2. Experiment Two: Effect of Commercial Scale Processing on β-Glucan Characteristics

Three genotypes of oats (HiFi, Leggett and Furlong) were dehulled, cleaned, kilned and flaked carefully by technicians at Richardson Milling in Portage La Prairie, Manitoba, Canada. All the procedures were similar to a typical commercial processing as described in the literature review. Detailed information on the processing procedures is confidential and is not described in this thesis.

The duplicates of raw groats were collected at two different locations in the loading bin. The duplicates of kilned or flaked oats were collected along the production line with one hour apart from two replicates.

HiFi flake was treated with additional wet heating treatments in Dr. Ames' lab at Richardon Centre for Functional Foods and Nutraceuticals, Winnipeg, Manitoba, Canada. The flake was steamed for 30 seconds with a steam bullet and rested for 10 minutes. Half of the flake was then treated again with same steaming procedure. Both processed samples were dried at room temperature for one hour and stored in the fridge for analysis. Both treatments were done in duplicates.

3.3. Moisture Content

Sample (one gram) ground to particle size 0.5 mm was weighed and incubated in the oven at 130 ℃. After an hour, it was transferred into a desiccator and cooled to room temperature for an additional hour. The loss of weight was calculated as the equilibrium moisture content of the sample. This is known as one stage moisture protocol. The moisture contents are calculated as shown below:

$$\text{Moisture Content (\%)} = \frac{(M_S + M_C) - M_D}{M_S} \times 100\%$$

Where M_S = the mass of wet sample

M_C = the mass of the container

M_D = the mass of the dried sample and container

When sample had a high moisture content (≥10 %), two stages moisture protocol was employed. Sample was repeatedly dried using the one stage moisture protocol until no further loss in sample weight.

3.4. β-Glucan Content

The total β-glucan content was determined enzymatically according to AOAC 995.16 by using the Megazyme kit (Megazyme International, Bray, County Wicklow, Ireland). Ground sample at 0.5 mm was firstly defatted by a mixed solution of aqueous ethanol (50 % v/v) and sodium phosphate buffer (20 mM, pH 6.5). Lichenase and β-glucanase were employed to break β-glucan down to D-glucose (figure 3.2). The total β-glucan content was calculated based on D-glucose value measured by the spectrophotometer. The calculation is listed as below:

$$\beta - \text{glucan}\left(\%\,{}^{W}/_{W}\right) = \Delta A \times F \times 94 \times \frac{1}{1000} \times \frac{162}{180} \times \frac{100}{W}$$

Where ΔA = the absorbance of D-glucose minus the blank absorbance

F = the factor to convert the standard curve value of D-glucose absorbance to μg

$$= \frac{100}{\text{Absorbance of D-glucose Standard}}$$

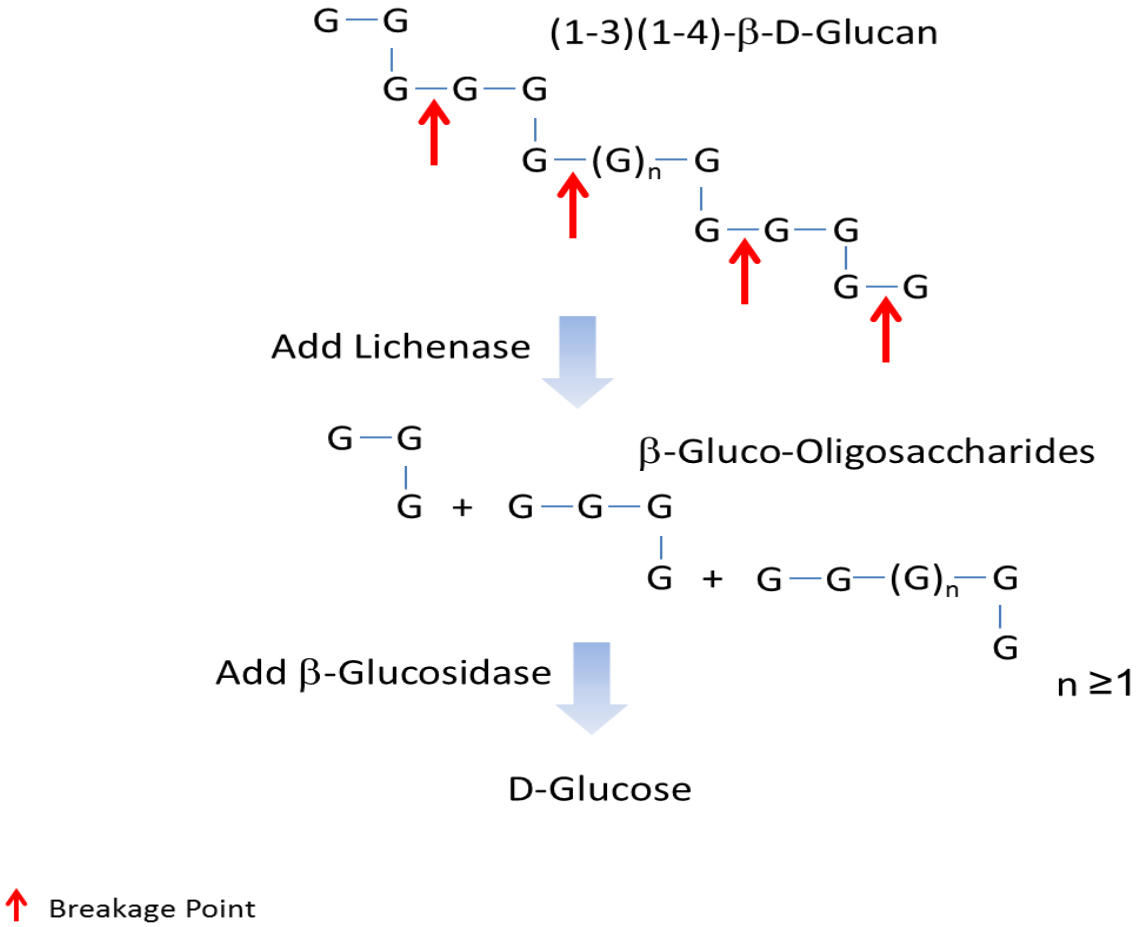
94 = the volume correction factor according to the final extraction solution of 9.4 ml

$\frac{1}{1000}$ = conversion from μg to mg

$\frac{162}{180}$ = the factor to convert the value from free D-glucose to β-glucan

$\frac{100}{W}$ = the factor to express β-glucan content as a percentage of flour weight

Figure 3.2 Principle of β-glucan assay by Megazyme kit.



The content of β-glucan is usually expressed as a percentage of dry flour weight, which is calculated by using flour moisture content:

$$\beta - \text{glucan}\left(\%\ {}^{\text{W}}/_{\text{W}}, \text{dry basis}\right) = \frac{\beta - \text{glucan}\left(\%\ {}^{\text{W}}/_{\text{W}}\right)}{100\% - \text{Moisture Content}\ (\%)} \times 100\%$$

3.5. Viscosity in Vitro Digestion Extraction

Viscosity of β-glucans was measured by Rapid Visco Analyzer (RVA), using a method described in Gamel et al. (2012). Firstly, milled oat flour (0.5 mm) was weighed in a RVA canister to create the slurry at 1 % β-glucan (0.255 g) in the total solution:

$$Weight_{sample} = \frac{0.255\ g}{\beta - glucan\ content\ \left(\%\ {}^{\text{W}}/_{\text{W}}, \text{dry basis}\right)}$$

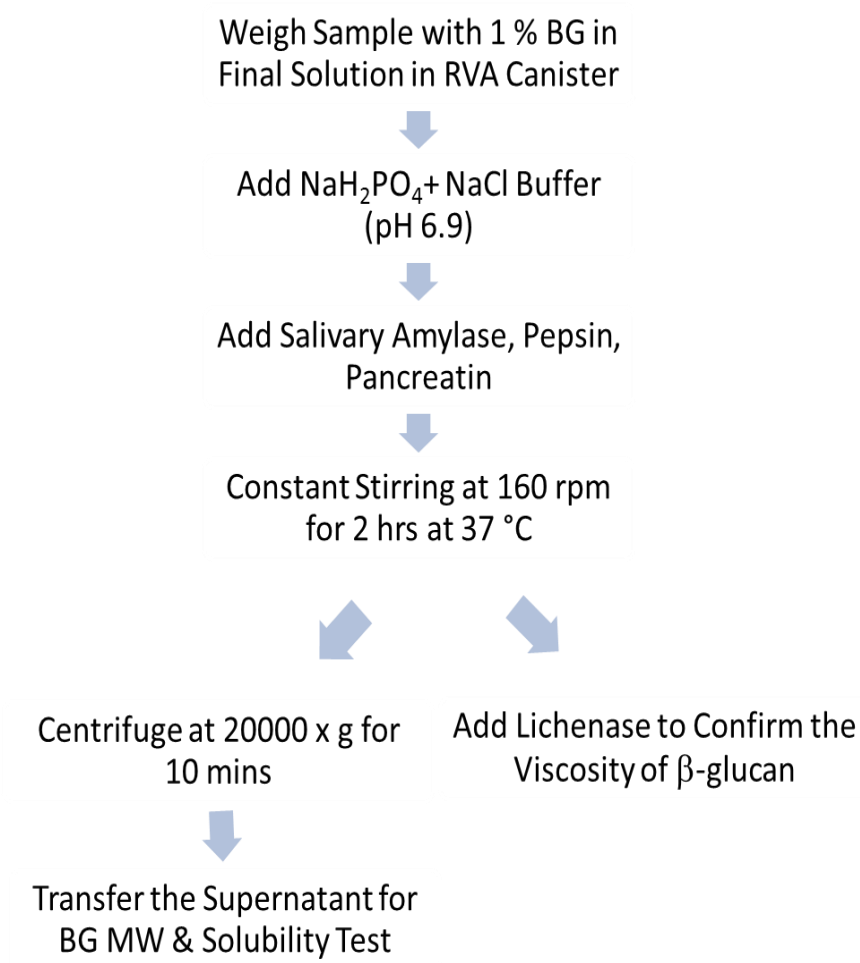
Mixed buffer 100 mM sodium phosphate and 10 mM sodium chloride at pH 6.9 was added into RVA canister with the volume of 25 ml minus moisture existing in the sample:

$$\text{Volume of } 100\text{mM } NaH_2PO_4 + 10\text{mM NaCl}$$
$$= 25\text{ml} - \text{Weight}_{\text{sample}} \times \text{Moisture} \div \text{Density}_{\text{water}}\ ({}^{1.0\text{g}}/_{\text{ml}})$$

Three digestive enzymes, 63 µl of salivary amylase (220 U/ml in 2.5 mM $CaCl_2$), 150 µl of pepsin (1150 U/ml in 0.9% NaCl) and 300 µl of pancreatin (0.5 mg/ml in sodium phosphate buffer, pH 6.9) were then added to the canister (Beer et al., 1997). The extraction started in RVA with constant stirring speed at 480 rpm for 10 seconds, and then the speed was reduced to 160 rpm and stirred for another two hours at 37 ℃. The change of viscosity was monitored at all times during this two-hour period. The final record on the RVA represented the viscosity of β-glucan in the sample. The rheological behavior of β-glucan in the extraction was examined by adding additional lichenase (endo-(1-3)(1-4)-β-D-glucan 4-glucanohydrolase, Megazyme, 1000

U/ml) (Megazyme International, Bray, County Wicklow, Ireland). After the extraction, an extensive reduction was observed with low viscosity at the end stage (Figure 3.3).

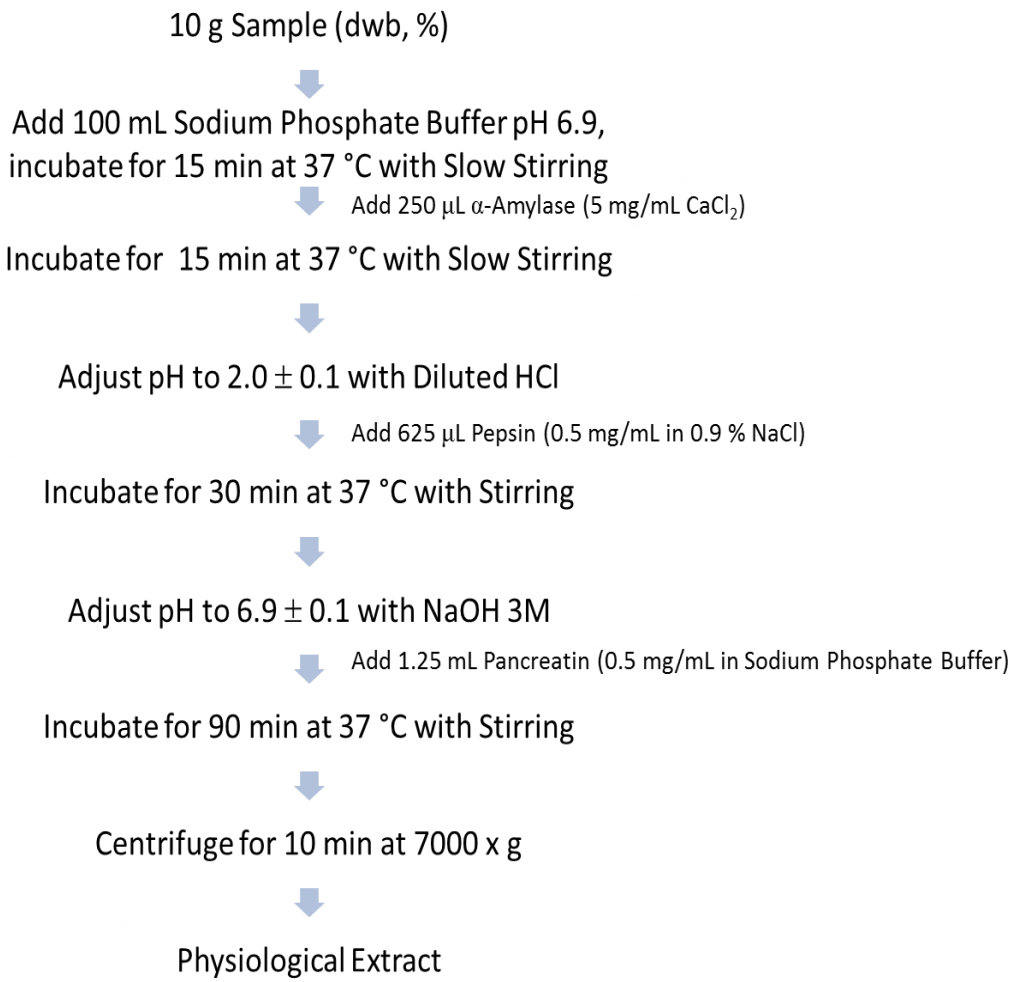
Figure 3.3 Procedure for viscosity measurement in vitro digestion extraction by RVA (Gamel et al., 2012).



In this study, samples in Experiment Two were also analyzed by another in vitro digestion extraction method developed by Beer et al. (1997), which monitored the rheological change according to the weight basis (figure 3.4). The rheological behavior of β-glucan in the extract was measured by a controlled strain rheometer (ARES, TA Instruments, New Castle, DE) in a

shear rate range of 0.1 to 100 s^{-1} at 37 °C with a cone-and-plate geometry (angle of 0.04 radius, diameter of 50 mm). Apparent viscosity was measured at the shear rate 30 s^{-1}.

Figure 3.4 Procedure for viscosity measurement in vitro digestion extraction developed by Beer et al. (1997).



3.6. Molecular Weight

After the measurement of viscosity, the extract was transferred into Eppendorf tubes and centrifuged at 20,000 x g for ten minutes. The supernatant obtained was then transferred into

glass tubes to measure the molecular weight and concentration (this value was used to calculate solubility) of β-glucan. The supernatant was first incubated in boiling water for ten minutes and cooled in room temperature water bath for another ten minutes. The boiled and chilled supernatant was filtered through a 0.45 μm nylon filter before injection and determination of molecular weight distribution of β-glucan via high performance size exclusion chromatography (HPSEC). The supernatant passed through the 0.45 μm filter more readily after boiling; however, filtering hot supernatant gave an unclear solution once filtered and cooled. The molecular weight of β-glucan was analyzed by HPSEC with calcofluor postcolumn detection (Wood et al., 1991). Two columns (Shodex OHpack SB-806M, Showa Denko K.K. and Ultrahydrogel linear 10 μm, 7.8mmX300mm, Waters) were used in this study with a guard column (Shodex OHpack SB-806M, Showa Denko K.K.). The columns were maintained at 40 ℃ and eluted with 0.1 M tris buffer (pH 8.0) at 1 ml/min (Lan-Pidhainy et al., 2007). In the postcolumn, the eluent was mixed with 20 mg/L of calcofluor in 0.1 M tris buffer (pH 8.0) at ratio 1:1 (Lan-Pidhainy et al., 2007). The filtered extract was injected at volume of 2 to 10 μl depending on the concentration of β-glucan present in the solution, and its molecular weight distribution was read using a fluorescence detector with a wavelength of excitation at 415 nm and emission at 445 nm (Rimsten et al., 2003; Suortti, 1993). Each extract was injected and analyzed in triplicate. The oat β-glucan standards were kindly donated by Dr. Susan Tosh (Agriculture and Agri-Food Canada, Guelph Food Research Centre), and were used to calibrate the HPSEC columns. These standards (six in total) had peak molecular weights (Mp) of 90, 250, 300, 490, 670 and 1000 kDa, respectively. The check standard β-glucan (extracted from barley) with Mp at 128 kDa was obtained from Megazyme (Megazyme International, Bray, County Wicklow, Ireland) (catalogue number P-BGBL).

3.7. Solubility

The supernatant obtained from the RVA in vitro digestion method was subsampled (0.4 ml) and measured by a β-glucan measurement kit from Megazyme (Megazyme International, Bray, County Wicklow, Ireland). The procedure was the same as when measuring β-glucan content of a dry oat sample. The β-glucan content measured in the supernatant was used to calculate the β-glucan solubility of raw and treated samples after the in vitro digestion procedure. The solubility of β-glucan was calculated as:

$$Solubility\ of\ \beta-glucan(\%) = \Delta A \times F \times 94 \times \frac{1}{1000} \times \frac{162}{180} \times \frac{1}{1000} \times \frac{V_{total}}{V_{subsample}} \div W_{\beta-glucan} \times 100\%$$

Where ΔA = the absorbance of D-glucose minus the blank absorbance

F = the factor to convert the standard curve value of D-glucose absorbance to μg

$$= \frac{100}{\text{Absorbance of D-glucose Standard}}$$

94 = the volume correction factor according to the final extraction solution of 9.4 ml

$\frac{1}{1000}$ = conversion from μg to mg

$\frac{162}{180}$ = the factor to convert the value from free D-glucose to β-glucan

$\frac{1}{1000}$ = conversion from mg to g

V_{total}= the total volume of extraction

$V_{subsample}$= the subsampled volume being measured

$W_{\beta-glucan}$ = the total weight of β-glucan added into the extraction

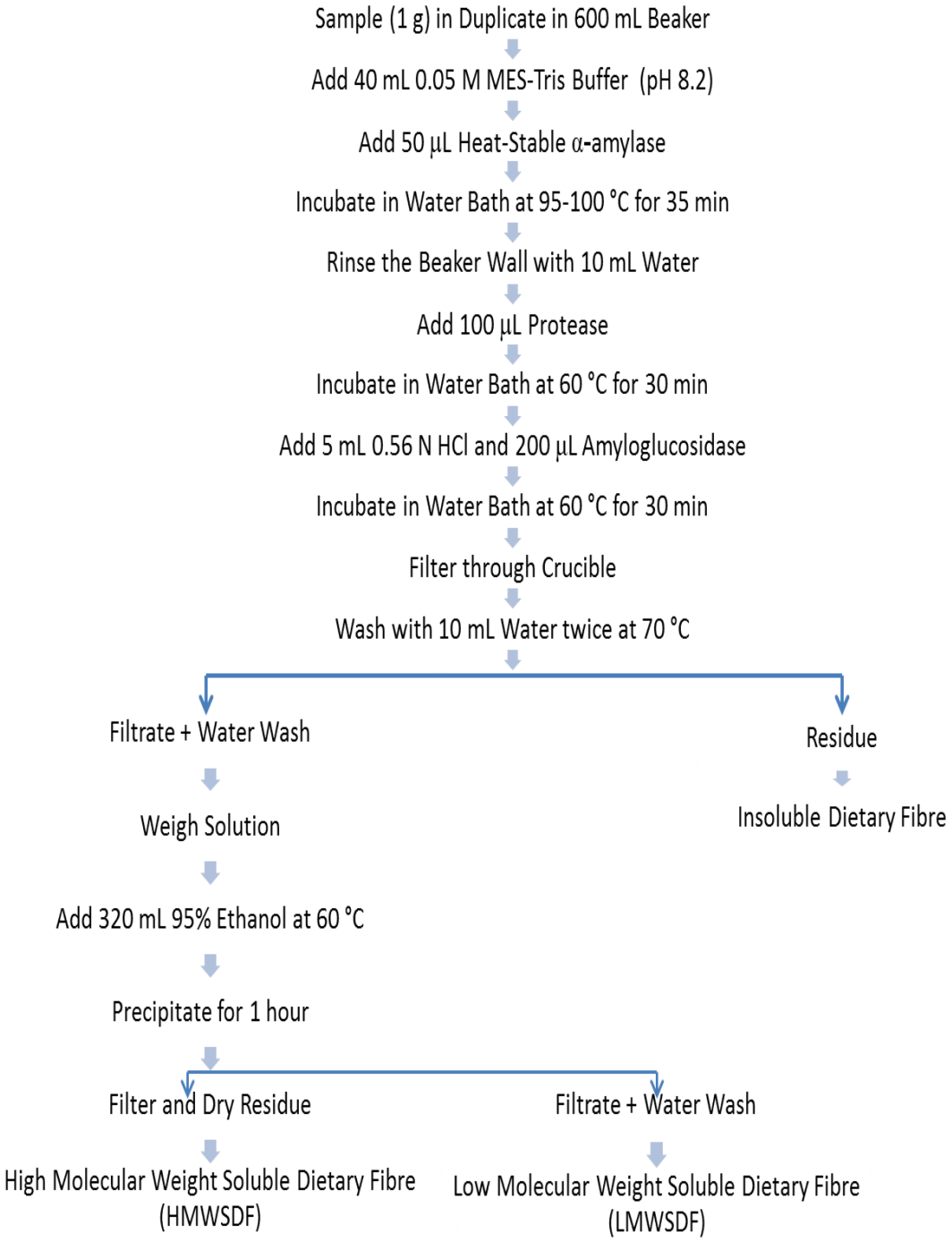
3.8. Arabinoxylan

Arabinoxylan content was measured by using a Megazyme D-xylose (including xylan and arabinnoxylan) assay (Megazyme International, Bray, County Wicklow, Ireland). Samples were milled (<0.5 mm) and hydrolyzed by 1.3 M hydrochloride at 100 ℃ for 60 minutes to release the xylose from the arabinose backbone. Then, samples were cooled to room temperature and subsequently neutralized by 1.3 M sodium hydroxide. D-xylose was oxidized by NAD^+, and the amount of NADH formed was measured at 340 nm using spectrophotometer. Arabinoxylan in oat flour contains 81.4 % xylose residues (Kormelink & Voragen, 1993), and this percentage was used as the conversion factor to calculate the arabinoxylan content based on xylose.

3.9. Total Dietary Fibre

Total dietary fibre was measured enzymatically according to AOAC 2009.01 using a Megazyme kit (Megazyme International, Bray, County Wicklow, Ireland), which measures soluble and insoluble dietary fibre in the sample (figure 3.5). Starch and protein were first hydrolyzed and depolymerized by α-amylase, protease and amyloglucosidase. After the first filtration, insoluble dietary fibre (IDF) was the remaining residue, and soluble dietary fibre (SDF) was washed away along with depolymerized protein and starch. High molecular weight soluble dietary fibre (HMWSDF) was then precipitated using ethanol and remained as a residue in the second filtration; low molecular weight soluble dietary fibre (LMWSDF) in the filtrate was measured by high performance liquid chromatography (HPLC).

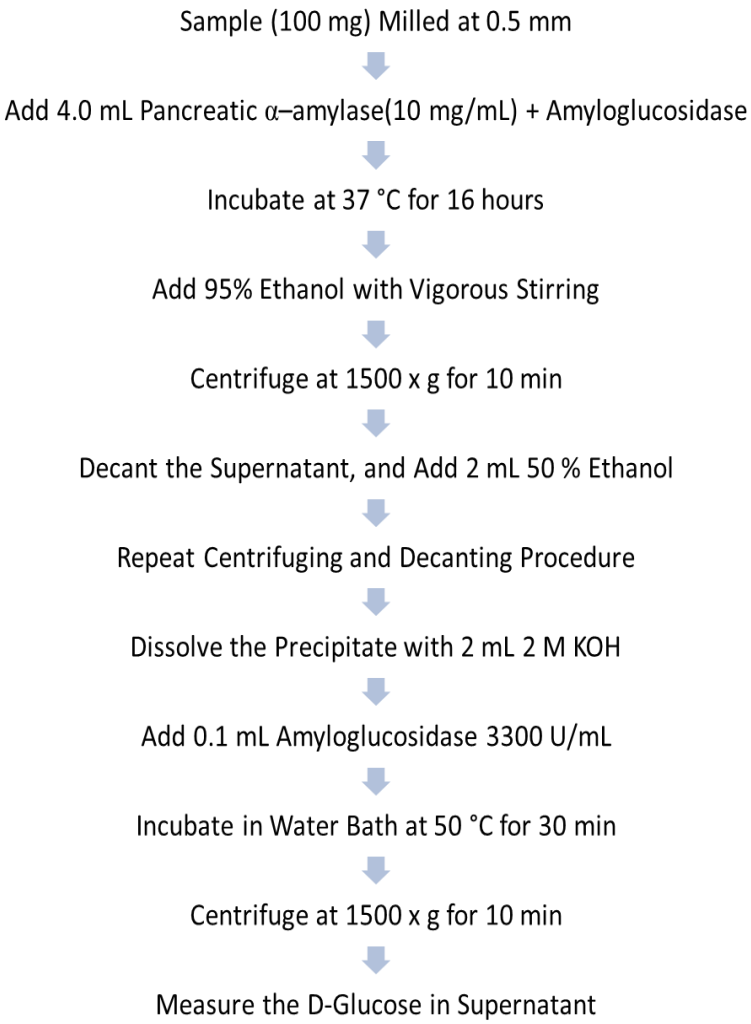
Figure 3.5 The determination procedure of total, soluble and insoluble dietary fibre.



3.10. Resistant Starch

The resistant starch was measured enzymatically according to AOAC 2002.02 using a Megazyme kit (Megazyme International, Bray, County Wicklow, Ireland) (figure 3.6). Non-resistant starch was hydrolyzed by pancreatic α-amylase and amyloglucosidase. Resistant starch was precipitated by using ethanol and re-dissolved in potassium hydroxide. Recovered resistant starch was neutralized and hydrolyzed to glucose by adding acetate buffer and amyloglucosidase. Resistant starch was measured by the increase in glucose within the solution.

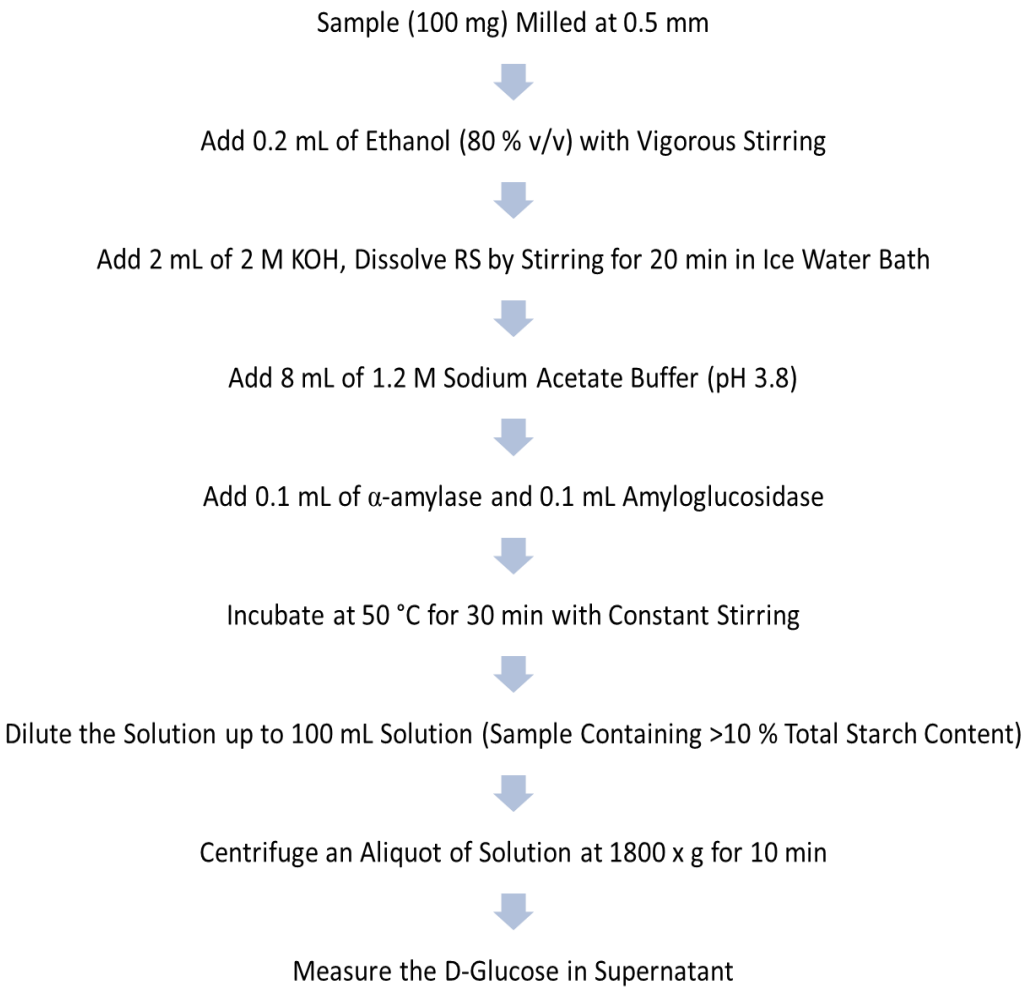
Figure 3.6 The determination procedure of resistant starch.



3.11. Total Starch

The total starch was measured enzymatically according to AOAC 996.11 using the Megazyme kit (Megazyme International, Bray, County Wicklow, Ireland) (figure 3.7). Resistant starch was dissolved in potassium hydroxide (KOH) and neutralized by adding sodium acetate buffer. Total starch including resistant starch was hydrolyzed by pancreatic α-amylase and amyloglucosidase. Total starch was measured by the increase in glucose in the solution.

Figure 3.7 The determination procedure of total starch.



3.12. Statistical Analyses

All statistical analyses were executed using SAS statistical software 9.2 (SAS Institute Inc., Cary, NC). One-way, two-way and three-way analyses of variance (ANOVA) were performed using GLM to test processing, genotype, environment, processing-by-genotype, genotype-by-environment and processing-by-environment effects ($p<0.05$) on original data or transformed data (transformed data = $\log_{10}$(original data)). The Least Square Means with Tukey's test were performed for individual comparisons. In Experiment One, oat samples were processed in a single run due to the limited amount of sample material. . Unprocessed and processed oats associating with five genotypes grown in two locations were separated into three groups (raw, kilned and flaked oats). Genotypes associating with locations in each group were considered as the replicates of one processing parameter. The means of three groups individually were used to test the variability of processing. Raw, kilned and flaked oats associating with five genotypes were divided into two groups according to their locations, which were treated as the replicates of location in each group. The means of two groups individually were used to test the variability of location. All the oat samples grown in two locations were separated into three groups based on their genotypes. Samples in each group were considered as replicates of each genotype. The means of five groups individually were used to test the variability of genotype. With fixed factor levels, three-way ANOVA was employed for analysis assuming three-factor interactions equal zero (Kirk, 1982; Neter, Wasserman, & Kutner, 1996). Experiment Two was carried out in duplicate.

4. EXPERIMENT ONE:

Effect of Pilot Scale Processing on β-Glucan Characteristics

4.1. Results and Discussion

4.1.1. β-glucan Content

According to the ANOVA summary (Table 4.1), overall processing had a significant effect on modifying the β-glucan content of the oat samples tested ($p < 0.05$). However, the statistical differences in concentration were not found between raw, kilned and steamed/flaked oat (Table 4.2). Further investigation would be necessary with more duplicates of one genotype grown in one location to confirm significance. The modification of β-glucan content in the products might be caused by the loss of some materials from oat within processing. A small change of β-glucan content was also observed in Tosh et al.'s study (2010) after extrusion on oat flour. Milling procedures (dehulling and cleaning), which occurs in grain pre-treatment processing, might increase or decrease the level of β-glucan by losing oat fractions that are either poor or rich in β-glucan content (Ganssmann & Vorwerck, 1995; Tiwari & Cummins, 2009). A genotypic effect was observed and found to be significant in this study ($p < 0.05$), but environmental effects were not significant. No interaction effect was found with respect to processing-by-genotype, processing-by-environment, or genotype-by-environment (Table 4.1). The data with more detail are shown in Table 4.3, 4.4 and 4.5. Nonetheless, potential interaction effects were observed through statistical graphs in Figure 4.1, 4.2, 4.3. The statistical graphs showed that there were interaction trends since their trend lines intersected. However the large of standard deviation rendered the interaction effects insignificant. Reducing standard deviation

Table 4.1 ANOVA for effects of processing, genotype and environment on concentration, viscosity, molecular weight (M_p and M_w) and solubility of β-glucan in Experiment One with transformed data.

	P-value					
	T	**G**	**E**	**T × G**	**T × E**	**G × E**
β-glucan content (%, dwb)	0.0457	<0.0001	0.5518	0.3911	0.9078	0.8071
	*	***	NS	NS	NS	NS
Viscosity (cP)	<0.0001	0.0020	<0.0001	0.4592	0.0031	0.4381
	***	**	***	NS	**	NS
Mp (kDa)	<0.0001	0.0407	<0.0001	0.5163	0.0034	0.5511
	***	*	***	NS	**	NS
Mw (kDa)	<0.0001	0.0608	<0.0001	0.5826	0.0053	0.5716
	***	NS	***	NS	**	NS
Solubility (%)	<0.0001	0.0195	0.1510	0.7138	0.3983	0.4697
	***	*	NS	NS	NS	NS

T = Processing, heat treatment, G = Genotype, E = Environment, location
NS $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4.2 Effects of processing, genotype and environment on concentration, viscosity, molecular weight (M_p and M_w) and solubility of β-glucan in Experiment One.

	β-glucan Content (%, dwb)	Viscosity (cP)	M_p (kDa)	M_w (kDa)	Solubility of β-glucan (%)
Treatment					
Raw	5.16^{a}	164.30^{a}	332.44^{a}	370.06^{a}	90.49^{b}
Kiln	5.41^{a}	313.85^{b}	604.71^{b}	628.36^{b}	83.96^{b}
Steam/Flake	5.40^{a}	930.75^{c}	1221.76^{c}	1242.54^{c}	63.83^{a}
Genotype					
HiFi	5.75^{cd}	563.42^{c}	792.57^{b}	813.16^{b}	83.01^{b}
Leggett	5.03^{b}	344.67^{a}	647.30^{a}	690.94^{a}	77.33^{ab}
CDC Dancer	4.23^{a}	435.92^{ab}	746.82^{ab}	777.03^{ab}	70.18^{a}
Marion	5.50^{c}	477.50^{bc}	705.61^{ab}	729.95^{ab}	81.46^{ab}
CDC Morrison	6.10^{d}	526.67^{bc}	705.88^{ab}	723.84^{ab}	85.14^{b}
Environment					
Kernen	5.30^{a}	415.20^{a}	611.76^{a}	646.01^{a}	81.02^{a}
Saskatoon	5.35^{a}	524.07^{b}	827.51^{b}	847.96^{b}	77.83^{a}

Effects of processing/heat treatments were reported as means of all genotypes and environments (n = 10)
Genotype effects were reported as means of all treatments and environments (n = 6)
Environment effects were reported as means of all treatments and genotypes (n = 15)
Values within a column and within a main effect followed by the same letter are not significantly different (P > 0.05)

with more duplicates might result in identification of interaction effects among these three variables (processing, genotype and environment).

Processing did not change the level of β-glucan significantly (Table 4.2); a small increase was observed from 5.16 % (raw) to 5.41 % (kilned), and 5.40 % (flaked). Products made from five oat genotypes grown in two locations had varied content of β-glucan. Morrison and HiFi maintained their β-glucan contents (6.10 % and 5.75 %) at a higher level compared to the others. CDC Dancer had the lowest β-glucan content (4.23 %) of the five genotypes. In barley, genetic factors play a more important role in determining the concentration of β-glucan than environmental factors (Özkara, Basman, Köksel, & Celik, 1998). The same observations were found in this study. The oats grown at two locations, Kernen and Saskatoon, had a similar mean β-glucan content 5.30 % and 5.35 %, respectively, not significantly different from each other.

Table 4.3 Interaction effect of processing-by-genotype on oat β-glucan content (%, dwb) in Experiment One.

Genotype	Treatment	Environment		Mean	Stdev
		Kernen	Saskatoon		
HiFi	Raw	5.70	5.83	5.77	0.09
	Kiln	5.68	5.77	5.73	0.06
	Steam/Flake	5.88	5.62	5.75	0.18
Leggett	Raw	4.93	4.73	4.83	0.14
	Kiln	4.92	5.22	5.07	0.21
	Steam/Flake	5.02	5.38	5.20	0.25
CDC Dancer	Raw	4.15	4.05	4.10	0.07
	Kiln	4.09	4.35	4.22	0.18
	Steam/Flake	4.18	4.53	4.35	0.25
Marion	Raw	5.29	5.53	5.41	0.17
	Kiln	5.65	5.50	5.57	0.10
	Steam/Flake	5.58	5.47	5.52	0.08
CDC Morrison	Raw	5.53	5.84	5.68	0.22
	Kiln	6.53	6.36	6.45	0.12
	Steam/Flake	6.35	6.01	6.18	0.24

Figure 4.1 Interaction effect of processing-by-genotype on oat β-glucan content (%, dwb) in Experiment One.

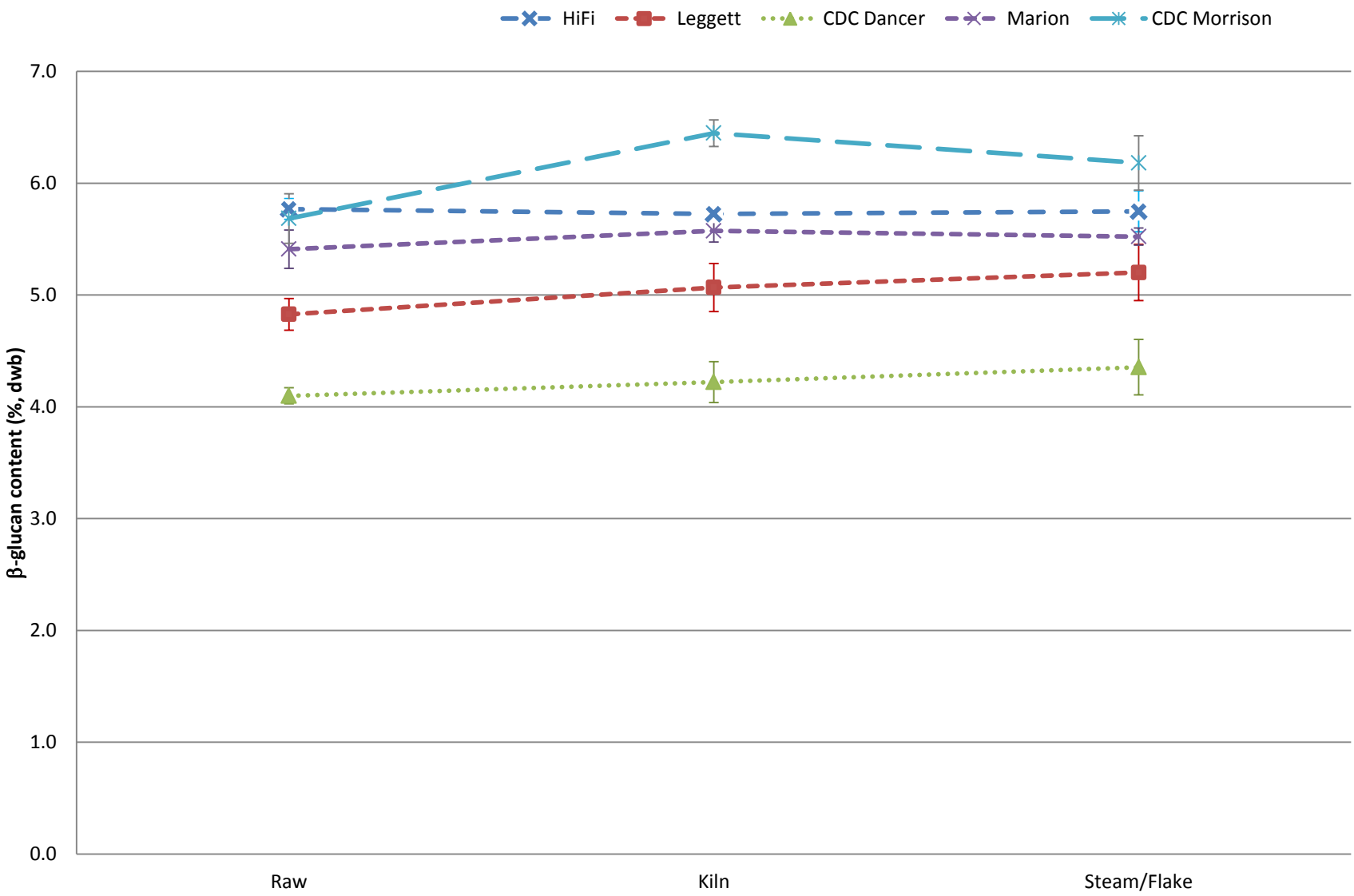


Table 4.4 Interaction effect of processing-by-environment on oat β-glucan content (%, dwb) in Experiment One.

Environment	Treatment	Genotype					Mean	Stdev
		HiFi	Leggett	CDC Dancer	Marion	CDC Morrison		
Kernen	Raw	5.70	4.93	4.15	5.29	5.53	5.12	0.62
	Kiln	5.68	4.92	4.09	5.65	6.53	5.37	0.92
	Steam/Flake	5.88	5.02	4.18	5.58	6.35	5.40	0.84
Saskatoon	Raw	5.83	4.73	4.05	5.53	5.84	5.20	0.79
	Kiln	5.77	5.22	4.35	5.50	6.36	5.44	0.74
	Steam/Flake	5.62	5.38	4.53	5.47	6.01	5.40	0.54

Figure 4.2 Interaction effect of processing-by-environment on oat β-glucan content (%, dwb) in Experiment One.

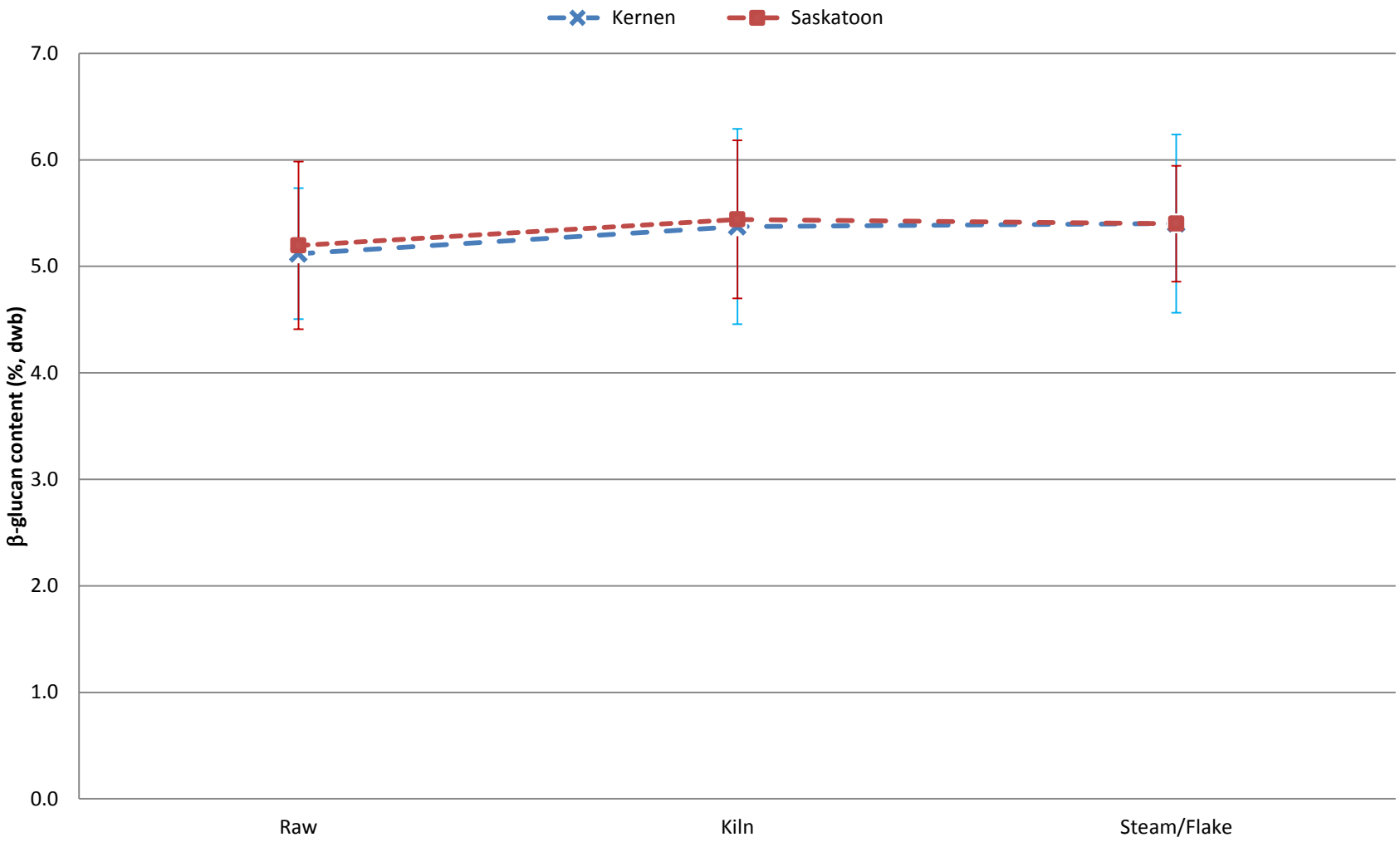
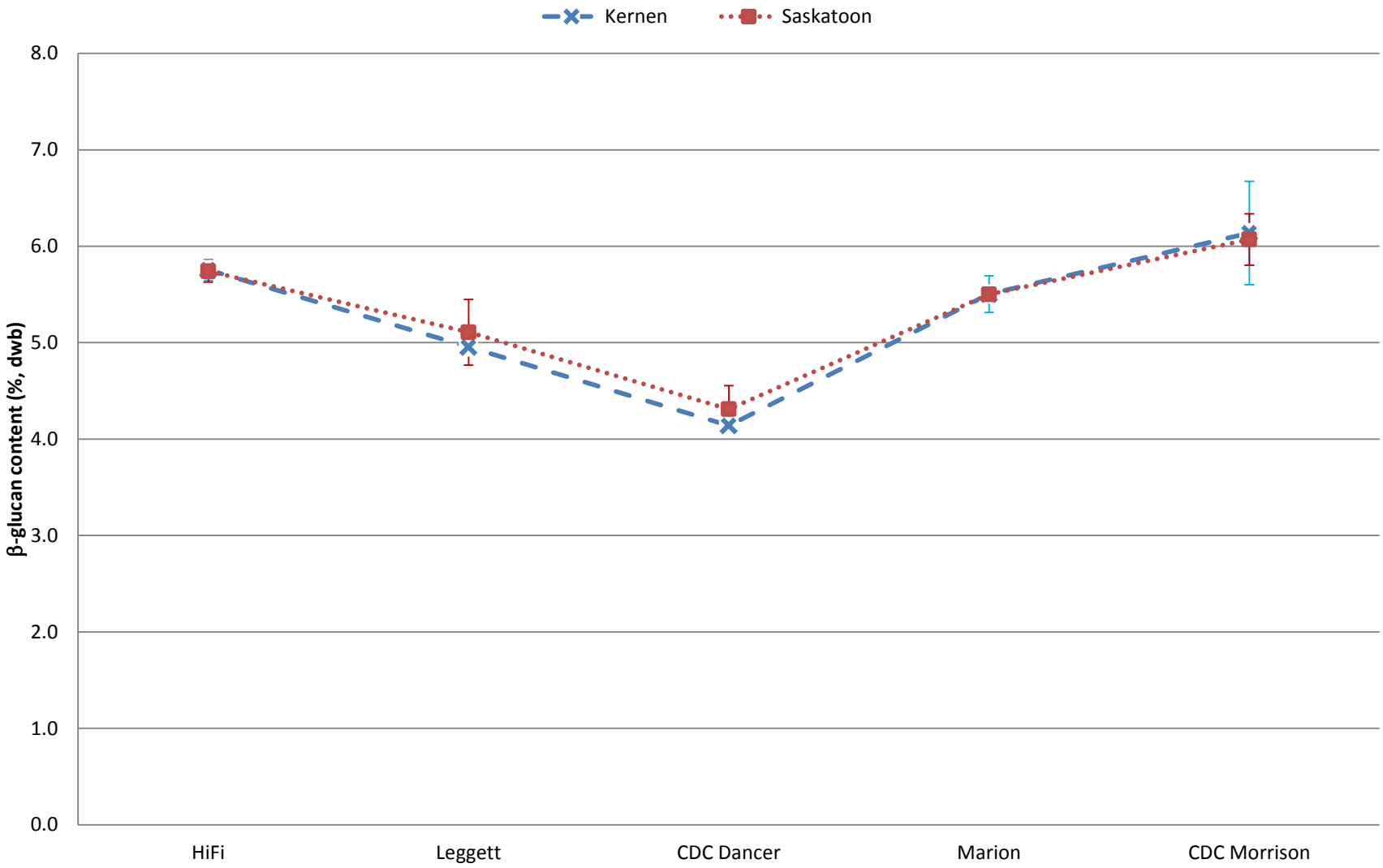


Table 4.5 Interaction effect of genotype-by-environment on β-glucan content (%, dwb) in Experiment One.

Environment	Genotype	Treatment			Mean	Stdev
		Raw	Kiln	Steam/Flake		
Kernen	HiFi	5.70	5.68	5.88	5.76	0.11
	Leggett	4.93	4.92	5.02	4.96	0.06
	CDC Dancer	4.15	4.09	4.18	4.14	0.04
	Marion	5.29	5.65	5.58	5.50	0.19
	CDC Morrison	5.53	6.53	6.35	6.14	0.54
Saskatoon	HiFi	5.83	5.77	5.62	5.74	0.11
	Leggett	4.73	5.22	5.38	5.11	0.34
	CDC Dancer	4.05	4.35	4.53	4.31	0.24
	Marion	5.53	5.50	5.47	5.50	0.03
	CDC Morrison	5.84	6.36	6.01	6.07	0.27

Figure 4.3 Interaction effect of genotype-by-environment on oat β-glucan content (%, dwb) in Experiment One.



4.1.2. Viscosity in Vitro Digestion Extraction

There are many different methods to extract β-glucan, with an extraction rate ranging from 24 to 86 % (Wood, 1993). This study investigated the bioactivity of β-glucan by simulating the human gastrointestinal system. An RVA method established by Gamel et al. (2012) was employed to mimic the rheological behavior of β-glucan in the human gastrointestinal tract. The concentration of β-glucan was controlled at 1 % to allow for direct comparison of viscosity between samples (Gamel et al., 2012). The samples were prepared and milled by a centrifugal mill (Restch ZM-200) equipped with 0.5 mm screen, which eliminated the inconsistency of viscosity measurement caused by the difference in particle size (Zhang et al., 1997). An extensive reduction of viscosity was detected after adding lichenase (an enzyme specific for breaking down β-glucan), which confirmed that the rheological behavior of the sample was due to the presence of β-glucan (rather than starch, for example).

The viscosity of β-glucan was significantly affected by processing, genotype and environment, and the interaction effect of processing-by-environment was significant ($p < 0.05$) (Table 4.1). The data listed in Table 4.6, 4.7 and 4.8, explained the interaction effects in statistical charts (Figure 4.4, 4.5, 4.6). It indicated that the interaction effects actually existed.

The viscosity (on average) increased from 164.30 cP in raw oat to 313.85 cP in kilned oats and was raised to 930.75 cP in flaked oats (final product). The viscosity differences between the raw and processed products could exist for two reasons. First, endo-β-glucanase might survive in raw and kilned oats, which reduces the viscosity by degrading the molecular weight of β-glucan from a large to a small molecular size (Sakurai & Masuda, 1979; Wood et al., 1991; Wood et al., 2000). According to Figure 4.7, the viscosity of flaked oats without the

Table 4.6 Interaction effect of processing-by-genotype on log(viscosity) (cP) of oat β-glucan in Experiment One.

Genotype	Treatment	Environment		Mean	Stdev
		Kernen	Saskatoon		
HiFi	Raw	2.17	2.41	2.29	0.17
	Kiln	2.54	2.74	2.64	0.14
	Steam/Flake	3.01	3.03	3.02	0.01
Leggett	Raw	1.86	2.23	2.05	0.26
	Kiln	2.13	2.46	2.29	0.24
	Steam/Flake	2.80	2.89	2.84	0.07
CDC Dancer	Raw	2.01	2.36	2.19	0.25
	Kiln	2.31	2.53	2.42	0.16
	Steam/Flake	3.01	2.85	2.93	0.11
Marion	Raw	2.08	2.32	2.20	0.17
	Kiln	2.42	2.62	2.52	0.14
	Steam/Flake	2.93	3.00	2.96	0.05
CDC Morrison	Raw	2.10	2.30	2.20	0.14
	Kiln	2.21	2.64	2.42	0.30
	Steam/Flake	3.01	3.08	3.05	0.05

Figure 4.4 Interaction effect of processing-by-genotype on log(viscosity) (cP) of oat β-glucan in Experiment One.

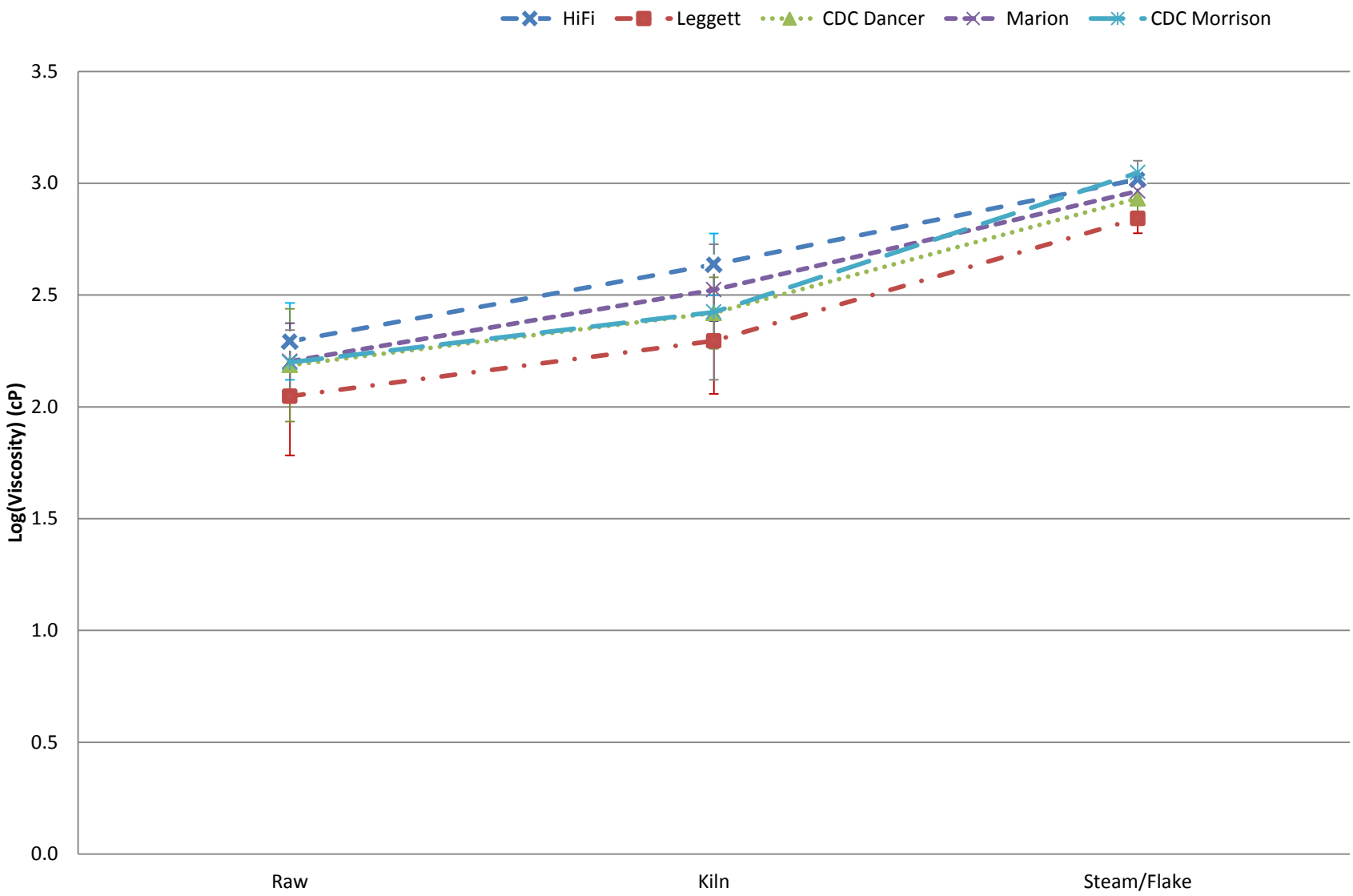


Table 4.7 Interaction effect of processing-by-environment on log(viscosity) (cP) of oat β-glucan in Experiment One.

Environment	Treatment	Genotype					Mean	Stdev
		HiFi	Leggett	CDC Dancer	Marion	CDC Morrison		
Kernen	Raw	2.17	1.86	2.01	2.08	2.10	2.04	0.12
	Kiln	2.54	2.13	2.31	2.42	2.21	2.32	0.16
	Steam/Flake	3.01	2.80	3.01	2.93	3.01	2.95	0.09
Saskatoon	Raw	2.41	2.23	2.36	2.32	2.30	2.33	0.07
	Kiln	2.74	2.46	2.53	2.62	2.64	2.60	0.10
	Steam/Flake	3.03	2.89	2.85	3.00	3.08	2.97	0.10

Figure 4.5 Interaction effect of processing-by-environment on log(viscosity) (cP) of oat β-glucan in Experiment One.

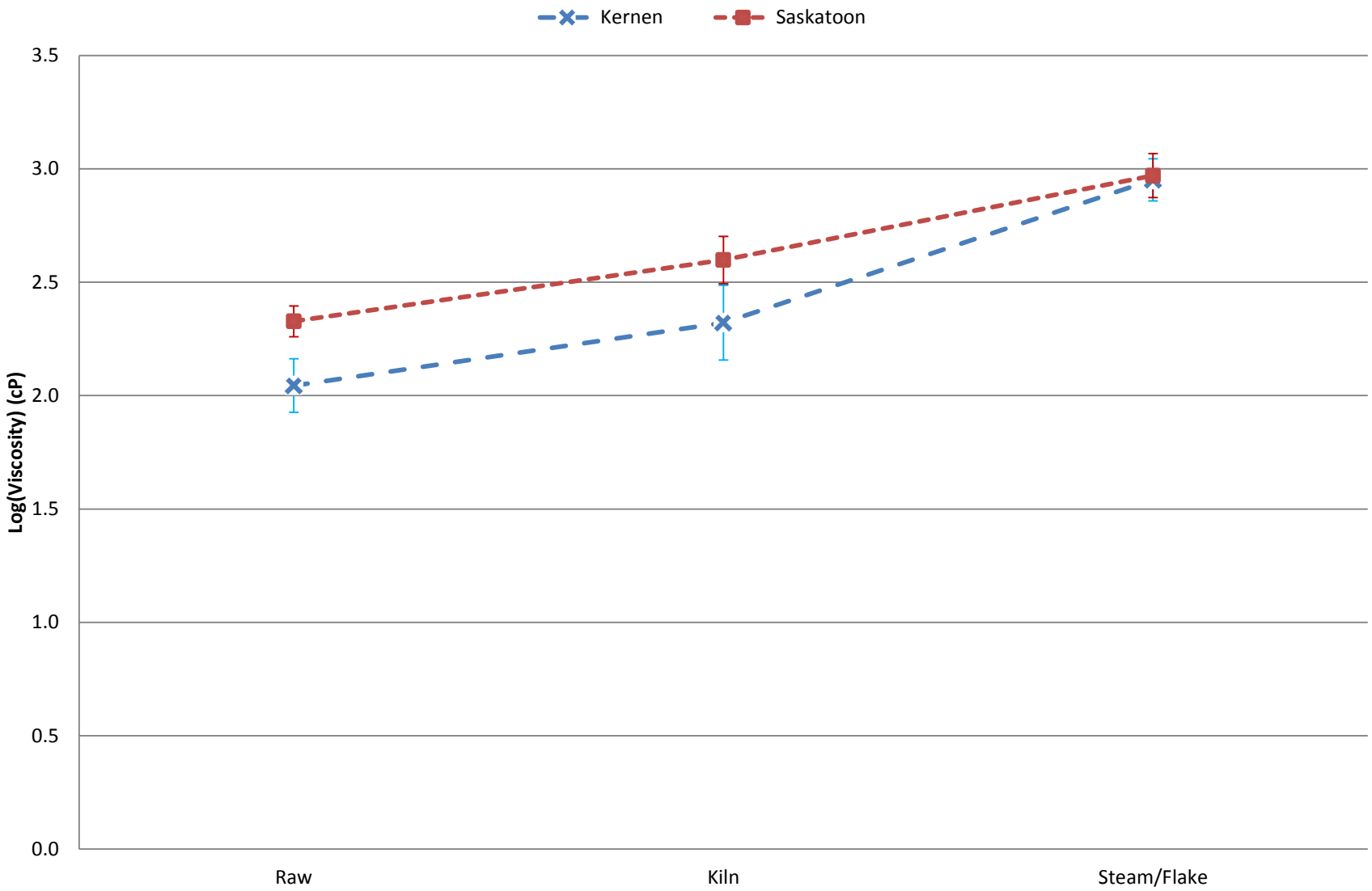


Table 4.8 Interaction effect of genotype-by-environment on log(viscosity) (cP) of oat β-glucan in Experiment One.

Environment	Genotype	Treatment			Mean	Stdev
		Raw	Kiln	Steam/Flake		
Kernen	HiFi	2.17	2.54	3.01	2.57	0.42
	Leggett	1.86	2.13	2.80	2.26	0.48
	CDC Dancer	2.01	2.31	3.01	2.44	0.51
	Marion	2.08	2.42	2.93	2.48	0.43
	CDC Morrison	2.10	2.21	3.01	2.44	0.50
Saskatoon	HiFi	2.41	2.74	3.03	2.73	0.31
	Leggett	2.23	2.46	2.89	2.53	0.33
	CDC Dancer	2.36	2.53	2.85	2.58	0.25
	Marion	2.32	2.62	3.00	2.65	0.34
	CDC Morrison	2.30	2.64	3.08	2.67	0.39

Figure 4.6 Interaction effect of genotype-by-environment on log(viscosity) (cP) of oat β-glucan in Experiment One.

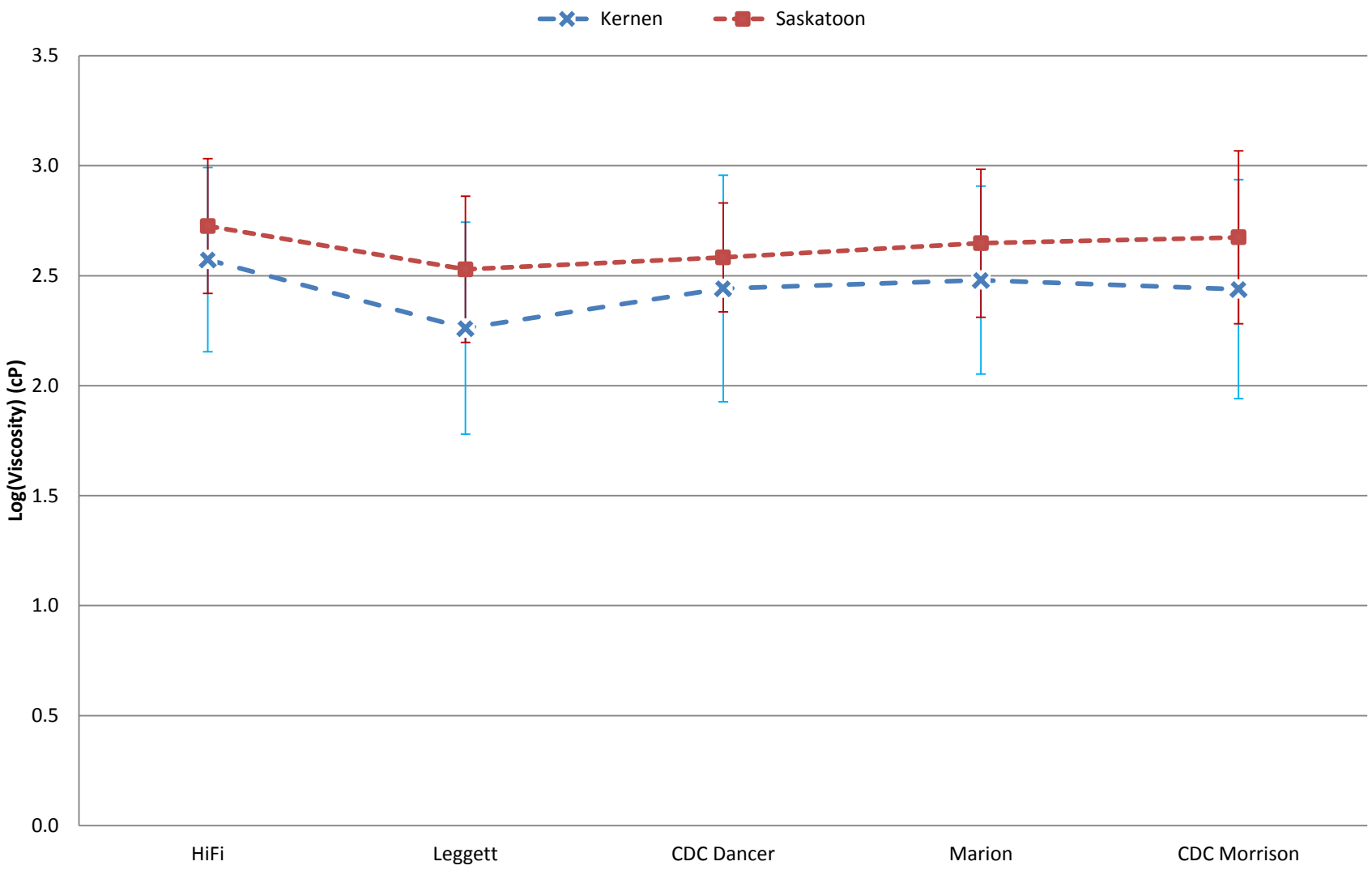
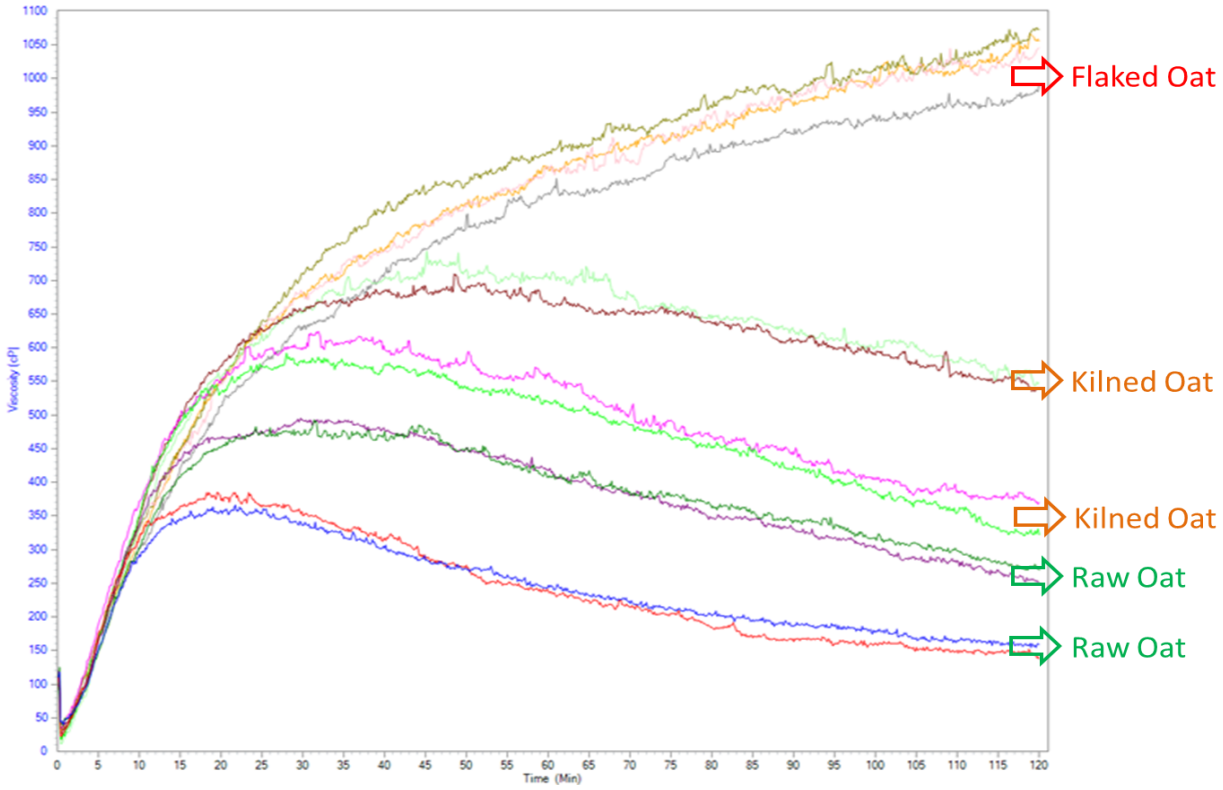


Figure 4.7 Viscosity of 1 % β-glucan in raw, kilned and flaked oat measured on RVA



interference of endo-β-glucanase activity steadily increased in the two hours extraction period and reached a plateau. On the other hand, the viscosity of raw and kilned oat both increased in the first 20 to 30 minutes and then gradually decreased at the later stage of the extraction. These results indicate that endo-β-glucanase activity might be one of the factors involved in reducing viscosity in the raw and kilned oats. Furthermore, as Doehlert et al. (1997) proposed, wet heat may break the intramolecular associations (hydrogen bonds) between β-glucans, which when replaced with water molecules, enhanced the solubility of β-glucan. Interestingly, there was no increase in the solubility of β-glucan from kilned oats to steamed/flaked oats. Therefore, the increase of viscosity in this study was more likely to be associated with the inactivation of endo-β-glucanase. Not every heat treatment has similar effects on the rheological behaviour of β-

glucan. Zhang et al. (1997) illustrated that wet heat was more effective than dry heat on the inactivation of endo-β-glucanase. According to Ganssmann and Vorwerck (1995), higher moisture content (such as wet heat) required a shorter inactivation time. Autoclaving with steam generated 23.3 % higher viscosity compared to autoclaving without steam; while oats roasted at 130 ℃ for two hours had a lower viscosity than raw oat. In this study, kilning followed by steaming/flaking had a positive effect on the rheological properties of β-glucan (Figure 4.7).

The five genotypes tested were found to have different levels of viscosity when measured at an equivalent (1 %) concentration of β-glucan (Table 4.2). HiFi had the highest viscosity over all (563.42 cP), which was significantly higher than Leggett (344.67 cP) and CDC Dancer (435.92 cP). Zhang et al.'s study (1998) also reported that genotype had a significant effect on the viscosity of β-glucan. Unlike their method of viscosity measurement using the same dry weight of samples (and therefore different β-glucan levels depending on the genotype), this study used the same concentration of β-glucan in the extraction slurry (buffer, enzyme and sample mixture), making comparison of β-glucan viscoelastic properties between genotypes possible.

In addition to genotypic differences in viscosity, viscosity differences were also observed in processed oats grown in two different environments. Oats grown in Saskatoon had a significantly higher viscosity of β-glucan than those grown in Kernen ($p < 0.05$). The viscosity differences existing among genotypes and between environments were explained/supported by their diverse molecular weights and solubility of β-glucan (Wood et al., 1991; Wood et al., 2000). Overall, a linear exponential relationship between viscosity and molecular weight (M_p and M_w) or viscosity and solubility of β-glucan was observed (Figure 4.8, 4.9) ($\log_{10}$[viscosity] and M_p*solubility R^2= 0.9457, $\log_{10}$[viscosity] and M_w*solubility R^2 = 0.9495). Since the values for M_p and M_w were

close to each other, it was difficult to determine whether there was a factor responsible for the viscosity differences observed. An inverse linear relationship has been observed between glycemic response and $\log_{10}$[viscosity], $\log_{10}[M_w]$ or $\log_{10}[M_p * \text{solubility}]$ in many clinical studies (Brummer et al., 2012; Regand et al., 2009; Tosh et al., 2008; Wood et al., 2000; Wood et al., 1994). Measurement of viscosity (in vitro) and M_w seem to be a common approach to determine the potential nutritional value of β-glucan in oat products.

Figure 4.8 Relationship between viscosity, Mw and solubility of β-glucan measured by in vitro digestion method on RVA.

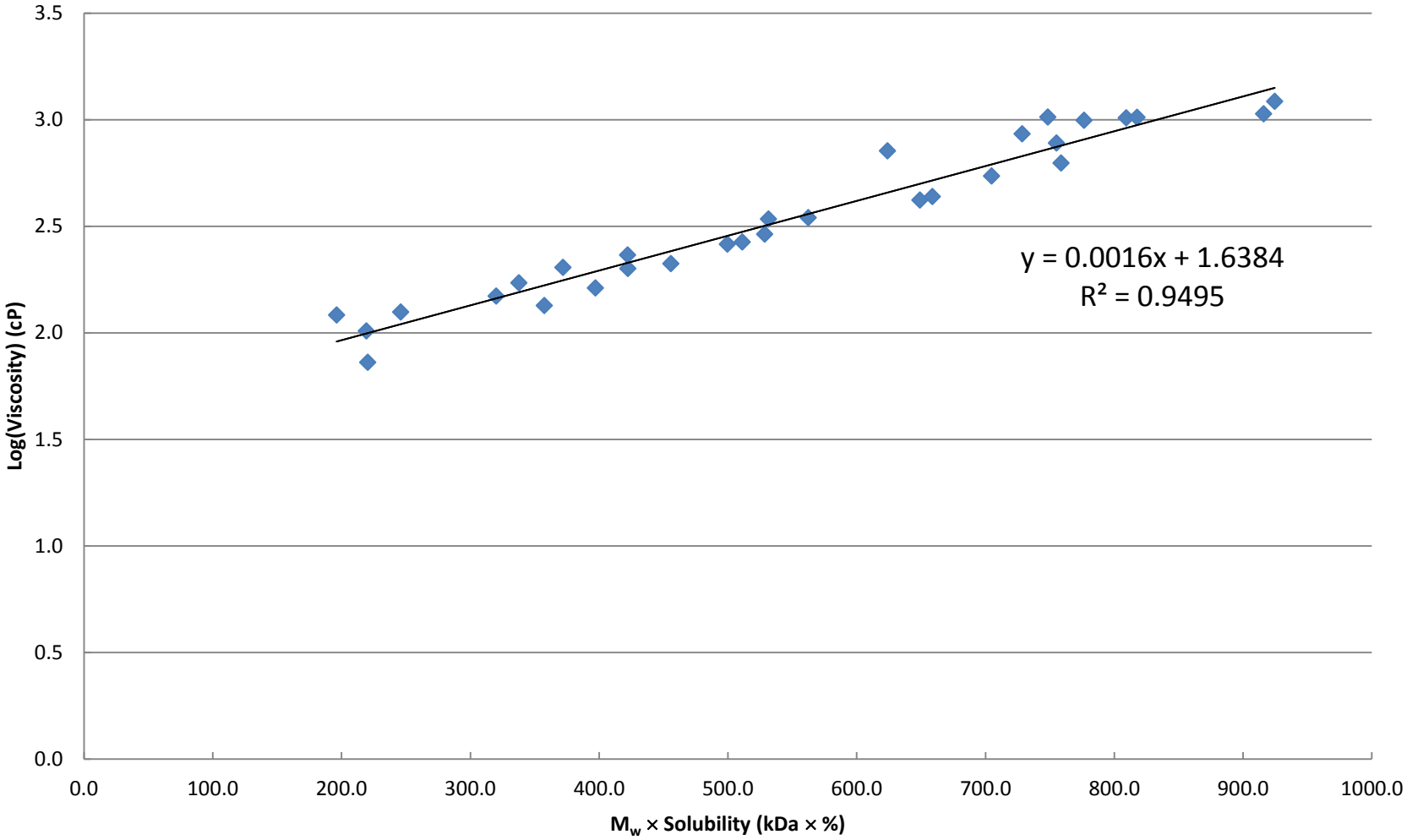
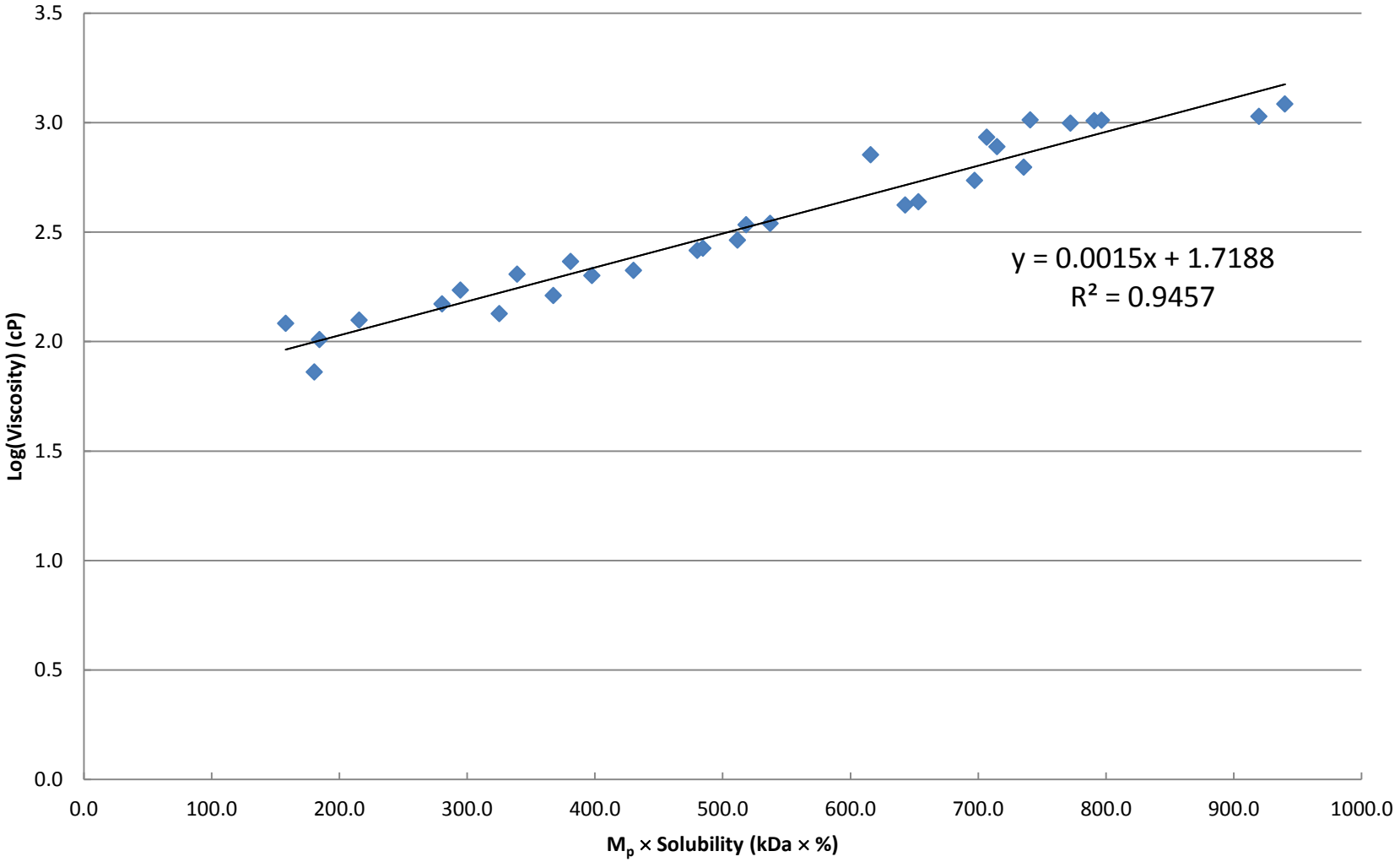


Figure 4.9 Relationship between viscosity, Mp and solubility of β-glucan measured by in vitro digestion method on RVA.



4.1.3. Molecular Weight in Vitro Digestion Extraction

As one of the key factors influencing the physiological behavior of β-glucan (Wood et al., 1991; Wood et al., 2000), the molecular weight (M_p and M_w) of β-glucan was used in this study to help explain the viscosity differences existing in oat products, and to explain how they are influenced by processing, genotype and environment. Processing, genotype and environment all had significant effects on M_p, but only processing and environment showed significant effects on M_w ($p<0.05$) (Table 4.1). No significant interaction effect was found with processing-by-genotype and genotype-by-environment, but a highly significant processing-by-environment interaction effect ($p<0.01$) was found with M_p and M_w. The results with detailed data are shown in Table 4.9-14 with statistical graphs in Figure 4.10-15.

The molecular weight of β-glucan was increased from raw (M_p 332.44 kDa; M_w 370.06 kDa) to kilned (M_p 604.71 kDa; M_w 628.36 kDa), and further increased after steaming/flaking (M_p 1221.76 kDa; M_w 1242.54 kDa) (Table 4.2). This corresponded with an increase in the viscosity of β-glucan. Viscosity levels were positively associated with increased molecular weight. These observations might be due to inactivation of endo-β-glucanase during steaming and heating. With a negligible amount or no endo-β-glucanase activity, β-glucan in processed oats undergoes less depolymerisation. Besides the function of deactivating enzymes, wet heat processing may degrade β-glucan polymers. In a typical commercial extrusion process, tempered oat bran at 181 ℃ and 18.7 % moisture content decreased the Mp of β-glucan from 2484 kDa to 1930 kDa (Tosh et al., 2010). The optimal processing (kilning and flaking) conditions including temperature, water content and processing time while protecting the integrity of the β-glucan molecule is still undefined.

Table 4.9 Interaction effect of processing-by-genotype on log(Mp) (kDa) of oat β-glucan in Experiment One.

Genotype	Treatment	Environment		Mean	Stdev
		Kernen	Saskatoon		
HiFi	Raw	2.48	2.72	2.60	0.16
	Kiln	2.76	2.91	2.83	0.10
	Steam/Flake	3.09	3.12	3.10	0.02
Leggett	Raw	2.32	2.50	2.41	0.12
	Kiln	2.64	2.80	2.72	0.12
	Steam/Flake	2.99	3.12	3.05	0.09
CDC Dancer	Raw	2.35	2.67	2.51	0.23
	Kiln	2.61	2.87	2.74	0.19
	Steam/Flake	3.11	3.14	3.12	0.02
Marion	Raw	2.23	2.68	2.46	0.32
	Kiln	2.72	2.88	2.80	0.11
	Steam/Flake	3.02	3.10	3.06	0.06
CDC Morrison	Raw	2.34	2.62	2.48	0.20
	Kiln	2.64	2.86	2.75	0.16
	Steam/Flake	3.06	3.11	3.08	0.04

Figure 4.10 Interaction effect of processing-by-genotype on log(Mp) (kDa) of oat β-glucan in Experiment One.

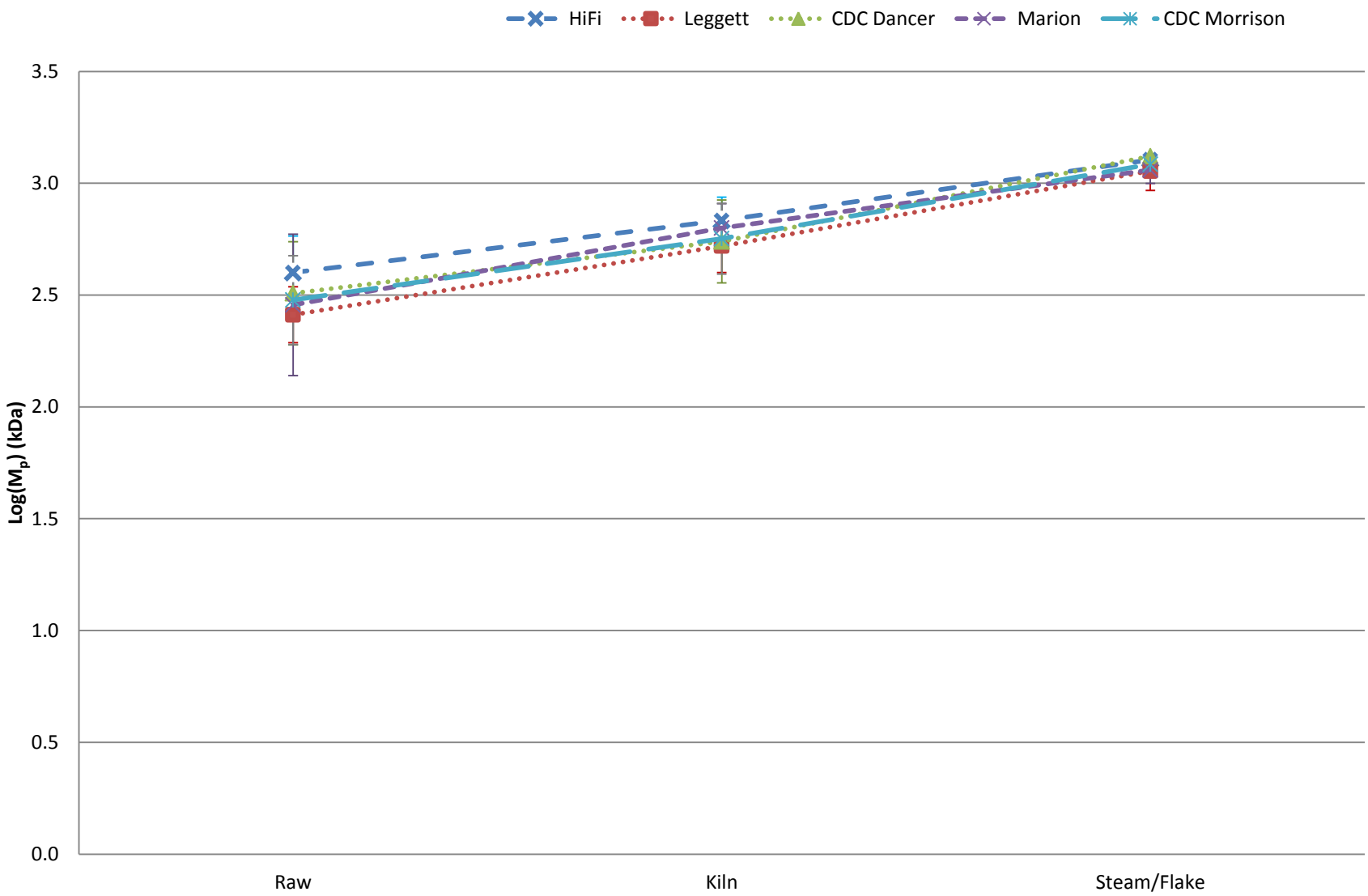


Table 4.10 Interaction effect of processing-by-environment on log(Mp) (kDa) of oat β-glucan in Experiment One.

Environment	Treatment	Genotype					Mean	Stdev
		HiFi	Leggett	CDC Dancer	Marion	CDC Morrison		
Kernen	Raw	2.48	2.32	2.35	2.23	2.34	2.34	0.09
	Kiln	2.76	2.64	2.61	2.72	2.64	2.67	0.06
	Steam/Flake	3.09	2.99	3.11	3.02	3.06	3.05	0.05
Saskatoon	Raw	2.72	2.50	2.67	2.68	2.62	2.64	0.08
	Kiln	2.91	2.80	2.87	2.88	2.86	2.86	0.04
	Steam/Flake	3.12	3.12	3.14	3.10	3.11	3.12	0.01

Figure 4.11 Interaction effect of processing-by-environment on log(Mp) (kDa) of oat β-glucan in Experiment One.

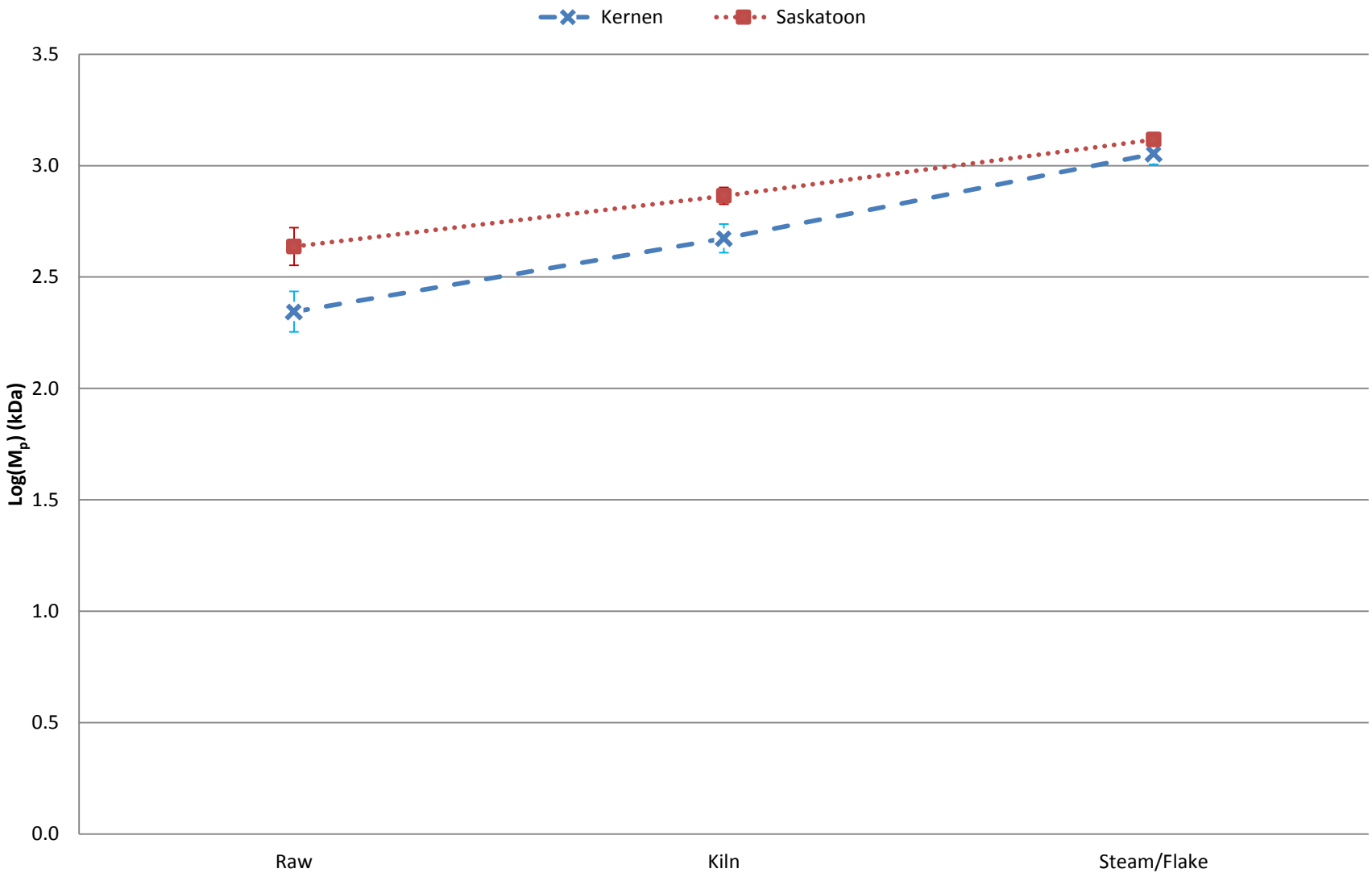


Table 4.11 Interaction effect of genotype-by-environment on log(Mp) (kDa) of oat β-glucan in Experiment One.

Environment	Genotype	Treatment			Mean	Stdev
		Raw	Kiln	Steam/Flake		
Kernen	HiFi	2.48	2.76	3.09	2.78	0.30
	Leggett	2.32	2.64	2.99	2.65	0.34
	CDC Dancer	2.35	2.61	3.11	2.69	0.39
	Marion	2.23	2.72	3.02	2.66	0.40
	CDC Morrison	2.34	2.64	3.06	2.68	0.36
Saskatoon	HiFi	2.72	2.91	3.12	2.91	0.20
	Leggett	2.50	2.80	3.12	2.81	0.31
	CDC Dancer	2.67	2.87	3.14	2.89	0.23
	Marion	2.68	2.88	3.10	2.89	0.21
	CDC Morrison	2.62	2.86	3.11	2.86	0.25

Figure 4.12 Interaction effect of genotype-by-environment on log(Mp) (kDa) of oat β-glucan in Experiment One.

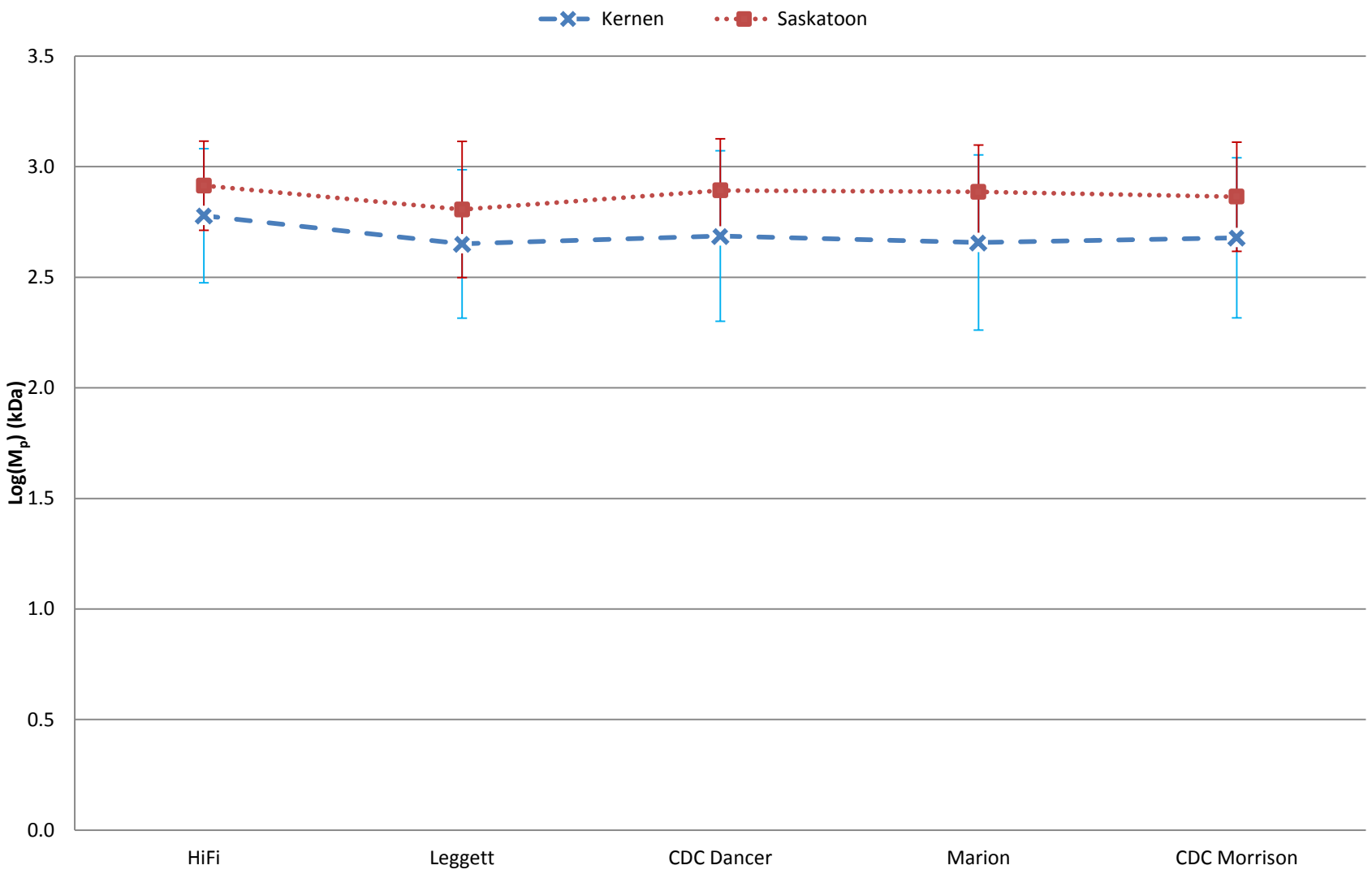


Table 4.12 Interaction effect of processing-by-genotype on log(Mw) (kDa) of oat β-glucan in Experiment One.

Genotype	Treatment	Environment		Mean	Stdev
		Kernen	Saskatoon		
HiFi	Raw	2.54	2.73	2.64	0.14
	Kiln	2.78	2.91	2.85	0.09
	Steam/Flake	3.10	3.12	3.11	0.01
Leggett	Raw	2.41	2.56	2.49	0.11
	Kiln	2.68	2.82	2.75	0.10
	Steam/Flake	3.01	3.14	3.07	0.09
CDC Dancer	Raw	2.42	2.72	2.57	0.21
	Kiln	2.65	2.88	2.77	0.16
	Steam/Flake	3.11	3.14	3.13	0.02
Marion	Raw	2.33	2.70	2.52	0.27
	Kiln	2.75	2.88	2.81	0.10
	Steam/Flake	3.03	3.10	3.07	0.05
CDC Morrison	Raw	2.40	2.64	2.52	0.18
	Kiln	2.67	2.87	2.77	0.14
	Steam/Flake	3.07	3.10	3.09	0.03

Figure 4.13 Interaction effect of processing-by-genotype on log(Mw) (kDa) of oat β-glucan in Experiment One.

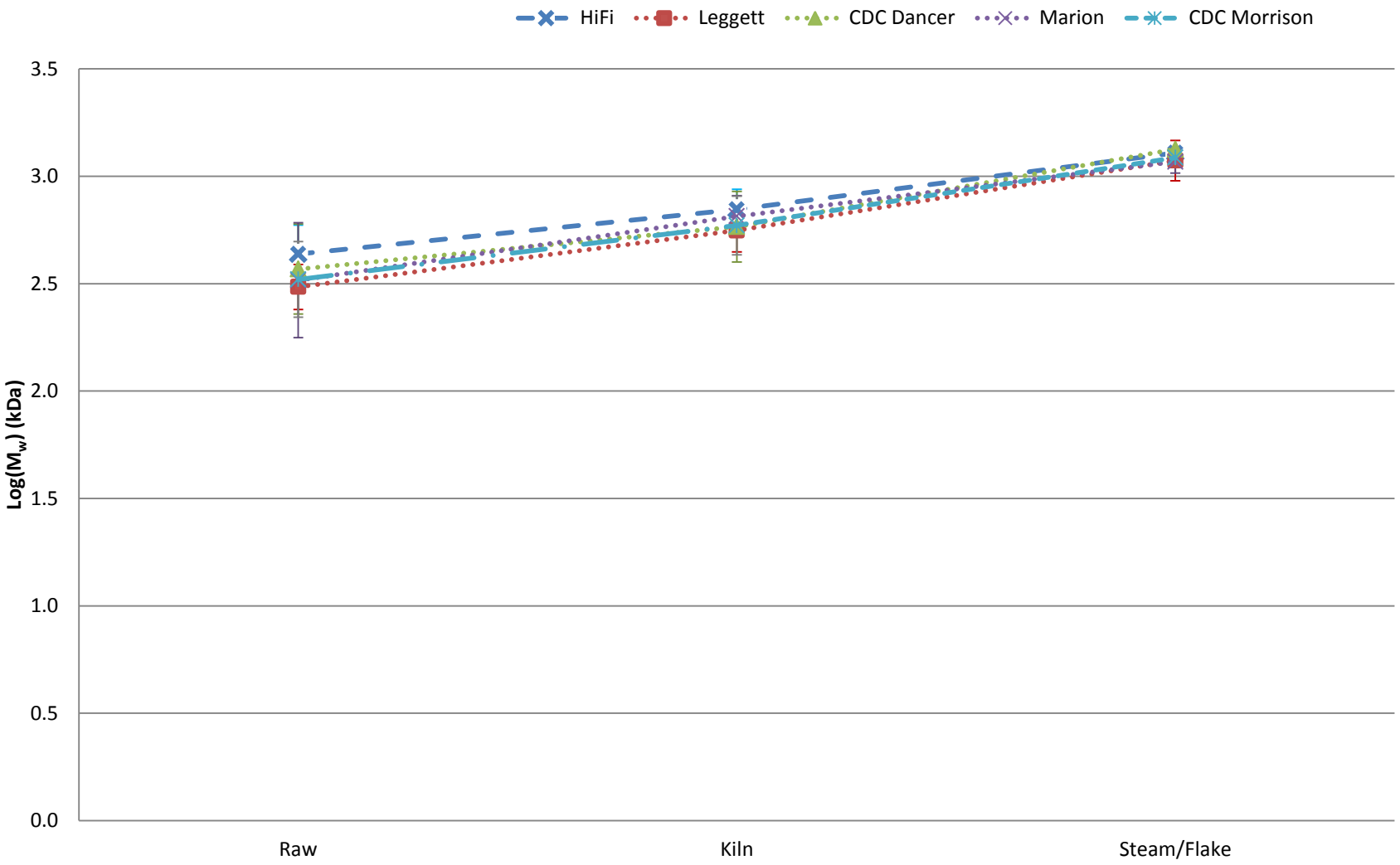


Table 4.13 Interaction effect of processing-by-environment on log(Mw) (kDa) of oat β-glucan in Experiment One.

Environment	Treatment	Genotype					Mean	Stdev
		HiFi	Leggett	CDC Dancer	Marion	CDC Morrison		
Kernen	Raw	2.54	2.41	2.42	2.33	2.40	2.42	0.08
	Kiln	2.78	2.68	2.65	2.75	2.67	2.70	0.05
	Steam/Flake	3.10	3.01	3.11	3.03	3.07	3.06	0.04
Saskatoon	Raw	2.73	2.56	2.72	2.70	2.64	2.67	0.07
	Kiln	2.91	2.82	2.88	2.88	2.87	2.87	0.03
	Steam/Flake	3.12	3.14	3.14	3.10	3.10	3.12	0.02

Figure 4.14 Interaction effect of processing-by-environment on log(Mw) (kDa) of oat β-glucan in Experiment One.

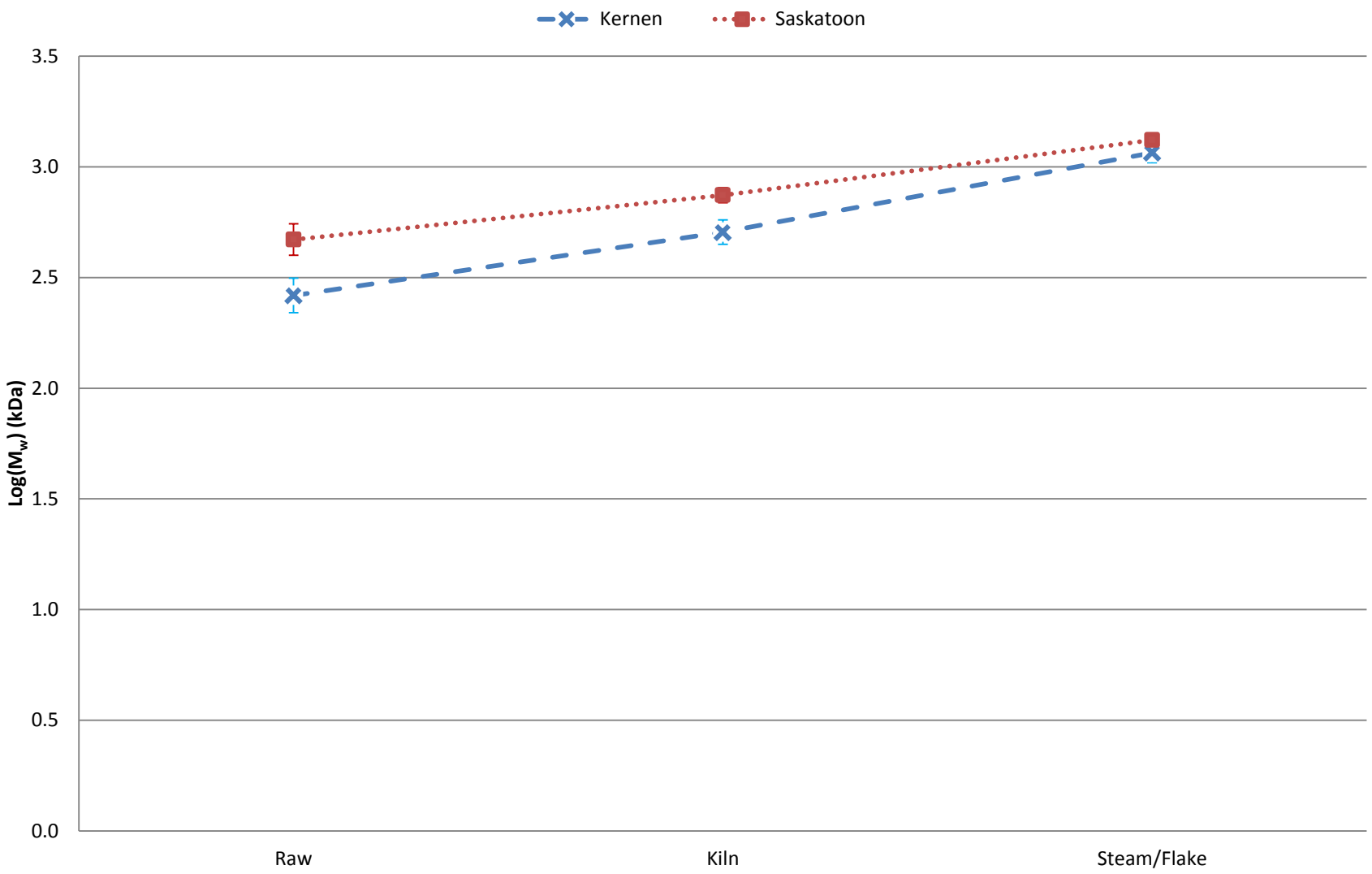
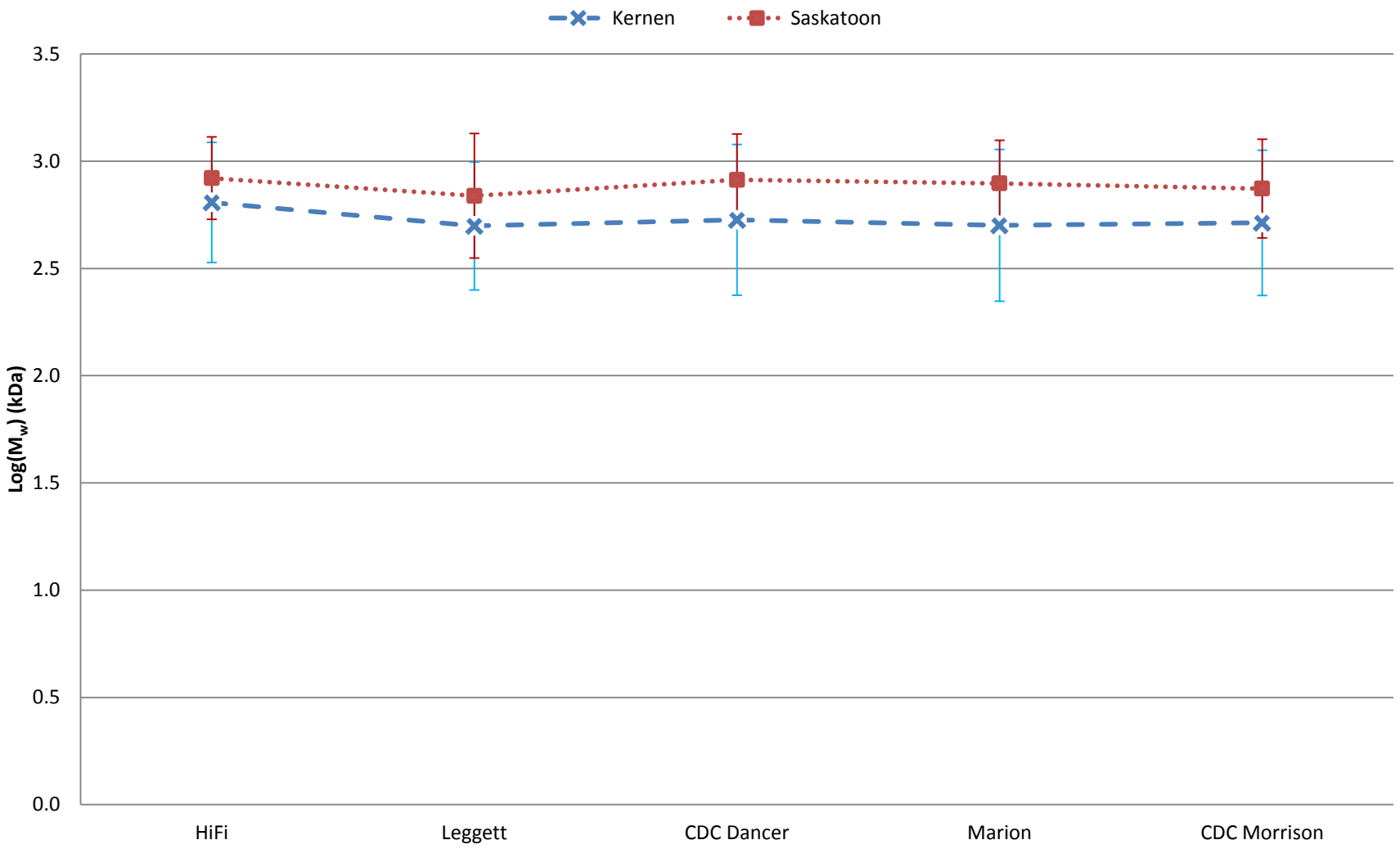


Table 4.14 Interaction effect of genotype-by-environment on log(Mw) (kDa) of oat β-glucan in Experiment One.

Environment	Genotype	Treatment			Mean	Stdev
		Raw	Kiln	Steam/Flake		
Kernen	HiFi	2.54	2.78	3.10	2.81	0.28
	Leggett	2.41	2.68	3.01	2.70	0.30
	CDC Dancer	2.42	2.65	3.11	2.73	0.35
	Marion	2.33	2.75	3.03	2.70	0.35
	CDC Morrison	2.40	2.67	3.07	2.71	0.34
Saskatoon	HiFi	2.73	2.91	3.12	2.92	0.19
	Leggett	2.56	2.82	3.14	2.84	0.29
	CDC Dancer	2.72	2.88	3.14	2.91	0.21
	Marion	2.70	2.88	3.10	2.90	0.20
	CDC Morrison	2.64	2.87	3.10	2.87	0.23

Figure 4.15 Interaction effect of genotype-by-environment on log(Mw) (kDa) of oat β-glucan in Experiment One.



HiFi had the highest M_p (792.57 kDa) and M_w (813.16 kDa) among the five genotypes, which was significantly higher than that of Leggett with M_p 647.30 kDa and M_w 690.94 kDa ($p<0.05$) (Table 4.2). The p-values for genotypic effects were $p=0.0407$ (M_p), and $p=0.0608$ (M_w). Therefore, based on lack of statistical significance for M_w (p-value was slightly greater than 0.05) and a p-value just under 0.05 for M_p, which might conclude that oat genotypes have less contribution to β-glucan molecular weight comparing to β-glucan content and viscosity. However, the molecular weight of β-glucan was highly affected by environment ($p<0.0001$) (Table 4.1). Oats grown in Saskatoon (Mp 827.51 kDa; Mw 847.96 kDa) had a significantly higher β-glucan molecular weight compared to those grown in Kernen (Mp 611.76 kDa; Mw 646.01 kDa) (Table 4.2). Hence, it seemed like that grown environment played a larger role in influencing the molecular weight of β-glucan than genotype. Similar findings were observed in the Ajithkumar et al. study (2005); the molecular weight of β-glucan extracted from 93 Swedish and 41 American unprocessed oats depended more on environmental factors. The molecular weight differences remained unchanged in oat products after pilot scale processing in Experiment One. Underlying reasons for environment affecting the production of long chain or short chain β-glucan are not well defined. Rainfall might be one of the factors responsible for this result (Ajithkumar et al., 2005).

4.1.4. Solubility in Vitro Digestion Extraction

In addition to molecular weight, the solubility of β-glucan also has a significant effect on viscosity (Cui & Wang, 2009; Ren, Ellis, Ross-Murphy, Wang, & Wood, 2003). The percent solubility content of β-glucan in this study was determined based on the amount of β-glucan dissolved in a 1 % concentration (w/v) of β-glucan in the total solution. Processing and genotype

had significant effects on solubility of β-glucan ($p<0.05$), but no significant effect was found with environment (Table 4.1). There was no interaction effect of processing-by-genotype, processing-by-environment or genotype-by-environment on the solubility of β-glucan (Table 4.1). The detailed data are listed in Table 4.15, 4.16, 4.17 with statistical charts (Figure 4.16, 4.17, 4.18).

Opposite to the trend observed with processing and β-glucan molecular weight, the solubility of β-glucan was reduced significantly from raw to processed oat samples. The solubility dropped from 90.49 % (raw) to 83.96 % after kilning, and further reduced with flaking to 63.83 % (Table 4.2). Solubility of oat β-glucan appeared to be highest when endo-β-glucanase was not completely inactivated. The smaller molecular weight was associated with higher solubility of the β-glucan polymer. This inverse relationship was also observed in previous studies (Ajithkumar et al., 2005; Tosh et al., 2010; Tosh et al., 2008; Wood et al., 1991). Nevertheless, this is not the only means by which solubility or extractability of β-glucan can change when exposed to processing. Boiling and cooking oat flakes (wet heat) increased the solubility, but baking bread (dry heat) reduced the solubility (Johansson et al., 2007). The literature shows that insoluble β-glucan becomes soluble by damaging the cellulose-like junction zone formed between two individual β-glucans and replacing the hydrogen bonds with water molecules under wet heat processing (Tosh et al., 2004; Tvaroska et al., 1983; Vasanthan, 2009; Vårum & Smidsrød, 1988; Zhang et al., 1998; Zhang et al., 1997). Dry heat may promote intermolecular hydrogen bonding between two β-glucans, which makes β-glucan less soluble (Vasanthan, 2009). Unfortunately, the inefficiency of endo-β-glucanase inactivation interfered with the determination of processing effects on the solubility of β-glucan in Experiment One due to the

decline of molecular weight in raw/kilned oats causing the increase of solubility during the in vitro digest extraction.

CDC Morrison (85.14 %) and HiFi (83.01 %) exhibited a significantly higher β-glucan solubility than did CDC Dancer (70.18 %) ($p<0.05$) (Table 4.2). Oats grown in Kernen and Saskatoon had a similar solubility of β-glucan. The same findings occurred in the Ajithkumar et al. study (2005); solubility of β-glucan is more affected by genotype than environment. In the current study, oats with a high content of β-glucan seem to have a higher β-glucan solubility (Table 4.2). For example, HiFi had an average solubility at 83.01 % and Mp at 813.16 kDa; while CDC Dancer had average solubility at 70.18 % and Mp at 777.03 kDa. The distribution of β-glucan in oats plays an important role in determining its solubility. Oats with high concentrations of β-glucan tend to have more located in the central endosperm than in the subaleurone layer (Fulcher & Miller, 1993; Miller & Frégeau-Reid, 2009), and it is released faster into aqueous solution from the endosperm than the subaleurone layer (Wood & Fulcher, 1978). Therefore, in the two hours vitro digestion extraction, β-glucan had a better chance to be released into the aqueous solution with samples containing a higher concentration of β-glucan in oat.

Table 4.15 Interaction effect of processing-by-genotype on log(solubility) (%) of oat β-glucan in Experiment One.

Genotype	Treatment	Environment		Mean	Stdev
		Kernen	Saskatoon		
HiFi	Raw	1.96	1.97	1.96	0.00
	Kiln	1.97	1.94	1.95	0.02
	Steam/Flake	1.81	1.84	1.83	0.03
Leggett	Raw	1.93	1.97	1.95	0.03
	Kiln	1.88	1.91	1.89	0.02
	Steam/Flake	1.87	1.74	1.81	0.10
CDC Dancer	Raw	1.92	1.91	1.92	0.01
	Kiln	1.92	1.84	1.88	0.05
	Steam/Flake	1.76	1.65	1.71	0.08
Marion	Raw	1.97	1.95	1.96	0.01
	Kiln	1.96	1.93	1.95	0.02
	Steam/Flake	1.83	1.79	1.81	0.03
CDC Morrison	Raw	2.00	1.98	1.99	0.01
	Kiln	1.92	1.95	1.94	0.02
	Steam/Flake	1.84	1.86	1.85	0.01

Figure 4.16 Interaction effect of processing-by-genotype on log(solubility) (%) of oat β-glucan in Experiment One.

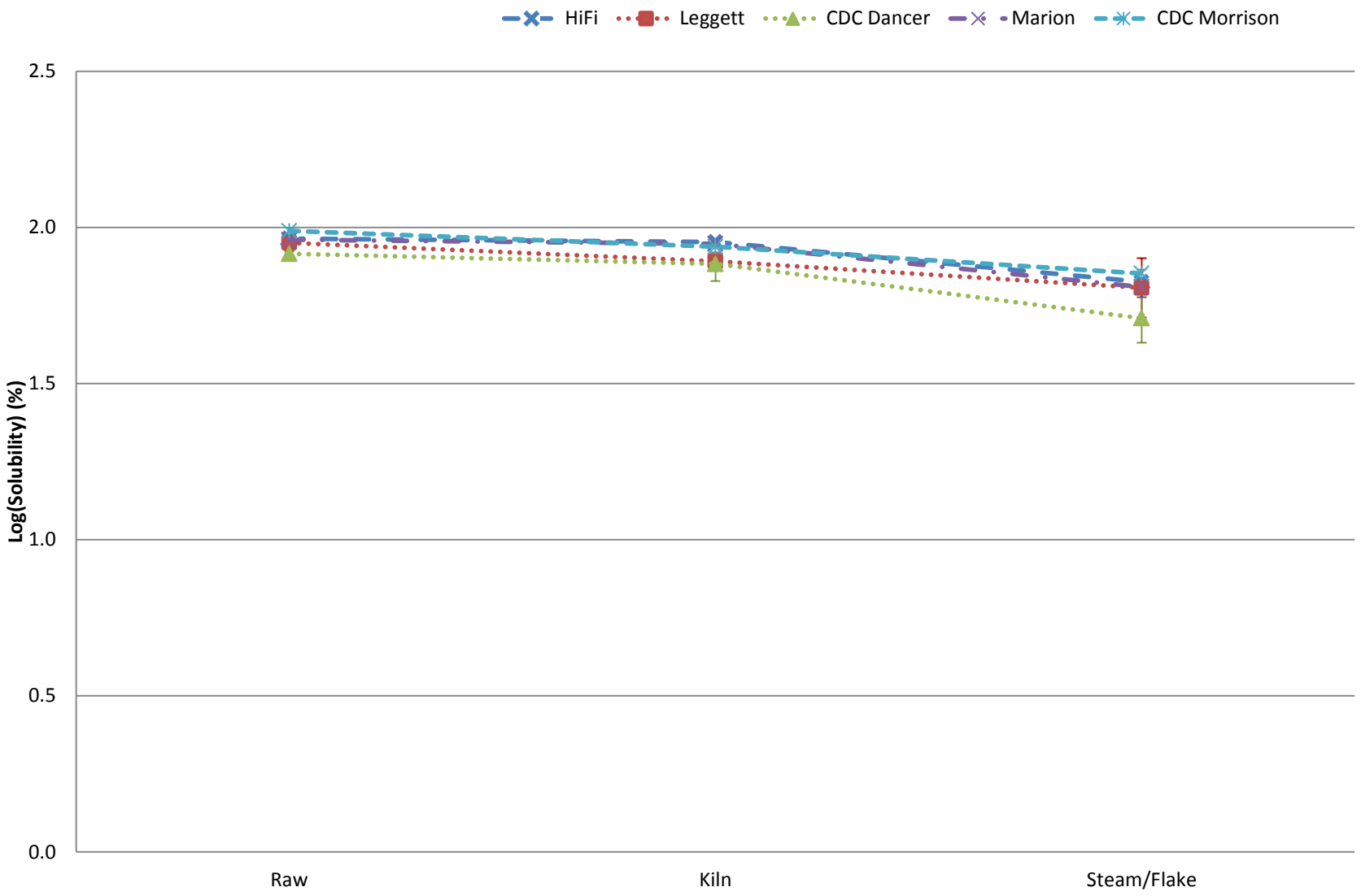


Table 4.16 Interaction effect of processing-by-environment on log(solubility) (%) of oat β-glucan in Experiment One.

Environment	Treatment	Genotype					Mean	Stdev
		HiFi	Leggett	CDC Dancer	Marion	CDC Morrison		
Kernen	Raw	1.96	1.93	1.92	1.97	2.00	1.96	0.03
	Kiln	1.97	1.88	1.92	1.96	1.92	1.93	0.04
	Steam/Flake	1.81	1.87	1.76	1.83	1.84	1.82	0.04
Saskatoon	Raw	1.97	1.97	1.91	1.95	1.98	1.96	0.03
	Kiln	1.94	1.91	1.84	1.93	1.95	1.91	0.04
	Steam/Flake	1.84	1.74	1.65	1.79	1.86	1.78	0.08

Figure 4.17 Interaction effect of processing-by-environment on log(solubility) (%) of oat β-glucan in Experiment One.

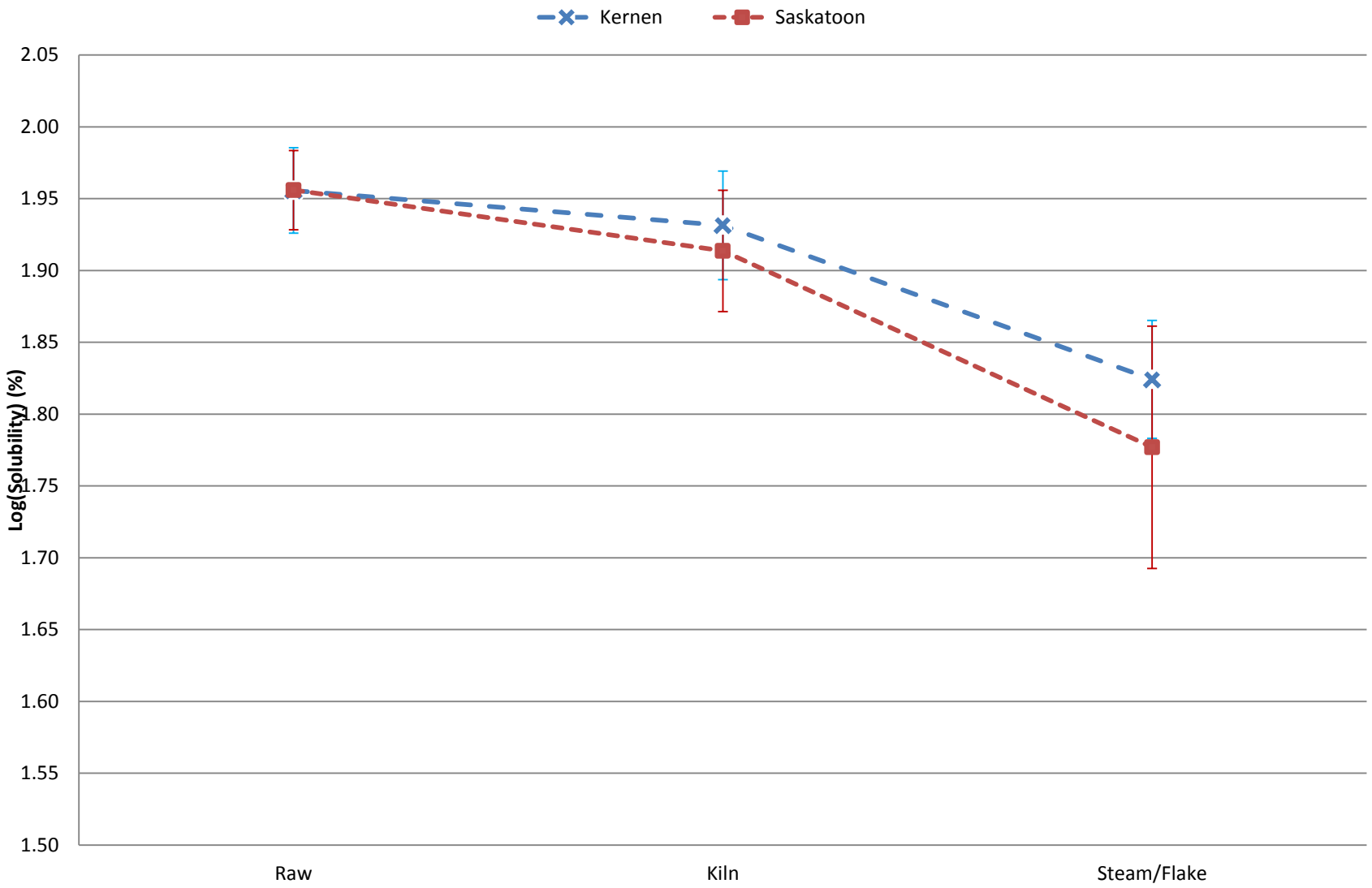
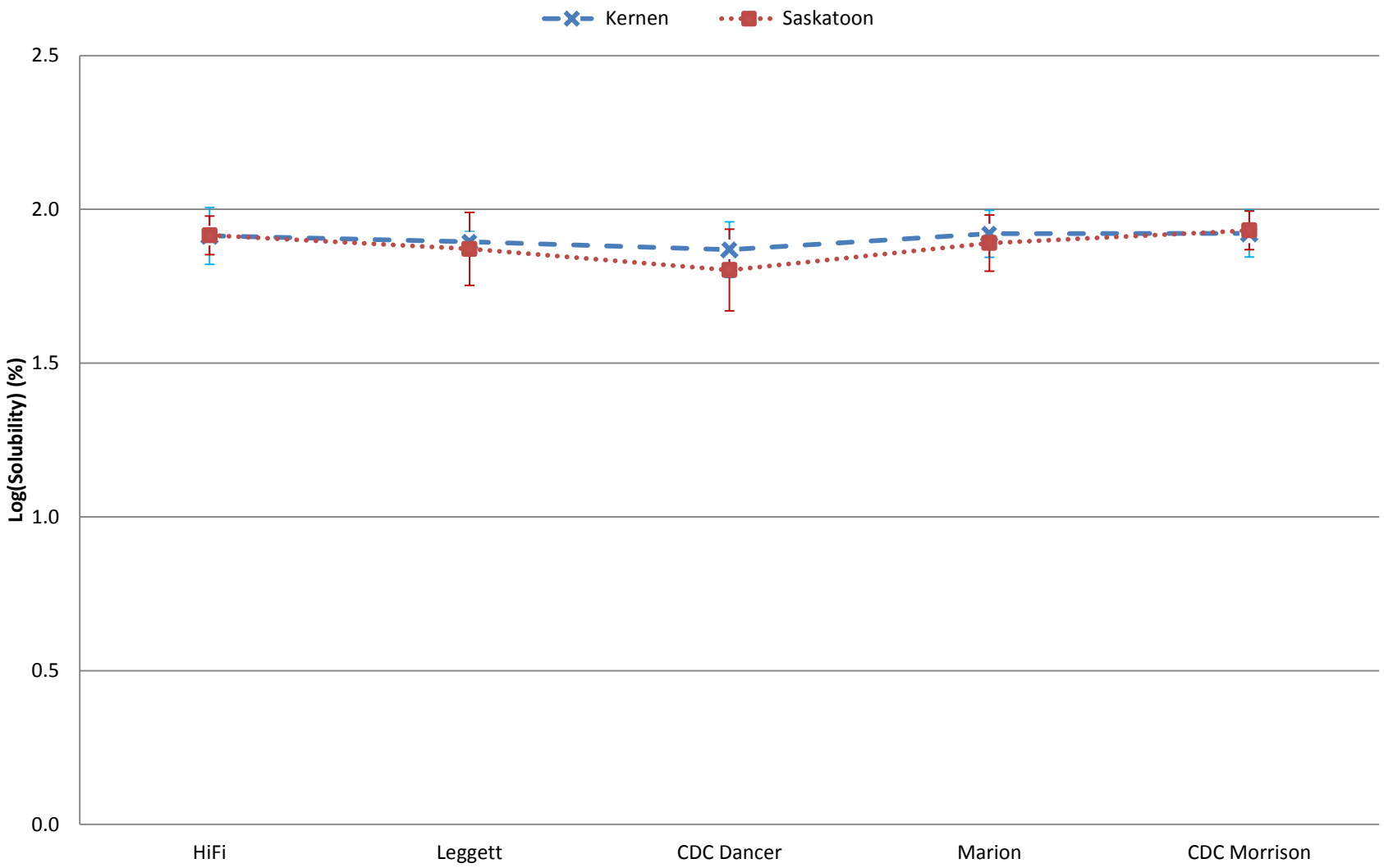


Table 4.17 Interaction effect of genotype-by-environment on log(solubility) (%) of oat β-glucan in Experiment One.

Environment	Genotype	Treatment			Mean	Stdev
		Raw	Kiln	Steam/Flake		
Kernen	HiFi	1.96	1.97	1.81	1.91	0.09
	Leggett	1.93	1.88	1.87	1.89	0.03
	CDC Dancer	1.92	1.92	1.76	1.87	0.09
	Marion	1.97	1.96	1.83	1.92	0.08
	CDC Morrison	2.00	1.92	1.84	1.92	0.08
Saskatoon	HiFi	1.97	1.94	1.84	1.92	0.06
	Leggett	1.97	1.91	1.74	1.87	0.12
	CDC Dancer	1.91	1.84	1.65	1.80	0.13
	Marion	1.95	1.93	1.79	1.89	0.09
	CDC Morrison	1.98	1.95	1.86	1.93	0.06

Figure 4.18 Interaction effect of genotype-by-environment on log(solubility) (%) of oat β-glucan in Experiment One.



4.1.5. Clarifying the Effects of Kilning and Flaking on β-glucan Properties

One random oat sample, Leggett, was used to distinguish the effects of kilning and steaming/flaking on β-glucan properties. One set of Leggett oats was processed through regular commercial processing, kilned and then steamed/flaked. The other set of Leggett was only treated with steaming/flaking. In Table 4.18, Leggett after being kilned and then flaked showed similar β-glucan traits to those samples in Experiment One: sequential increases were observed with each step in terms of viscosity and molecular weight of β-glucan, but with a decrease in solubility. The results of steamed/flaked oats were similar to kilned ones in β-glucan concentration, viscosity, molecular weight and solubility, which suggests that kilning and steaming/flaking might have equal effects on physicochemical properties of β-glucan in oats. Therefore, sequential processing (kilning and then steaming and flaking) will be more effective than kilning or steaming/flaking alone, as demonstrated in this study. Because of the limited amount of sample, each processing step was only performed once. No statistical analysis was done due to lack of duplicates.

Table 4.18 Effects of processing (kiln, steam/flake vs steam/flake only) on concentration, viscosity, molecular weight (Mp and Mw) and solubility of β-glucan in Experiment One.

	β-glucan Content (%, dwb)	Viscosity (cP)	Mp (kDa)	Mw (kDa)	Solubility of β-glcuan (%)
Treatment					
Raw	4.58	115.00	205.64	236.76	89.12
Kiln	4.87	173.50	445.26	488.77	80.68
Kiln & Steam/Flake	4.93	778.50	1238.63	1261.80	66.05
Steam/Flake only (no kilning)	4.85	192.50	498.20	540.53	79.60

4.2. Summary

It is easier and more convenient to use pilot scale processors to monitor the processing, genotypic and environmental effects on the physiochemical properties of oat β-glucan than it is to do it on a large commercial scale. Even though the kilning process was not efficient to deactivate endo-β-glucanase completely on a pilot scale, the positive effects on rheological behavior of β-glucan were still observed in Experiment One. Steaming/flaking resulted in a high viscosity and molecular weight of β-glucan with no activity of endo-β-glucanase. Processing and genotype significantly affected the concentration, viscosity, molecular weight and solubility of β-glucan; environment had a significant impact on viscosity and molecular weight. Furthermore, an interaction effect of processing-by-environment was only found in viscosity and molecular weight in Experiment One. However, the interaction effects of processing-by-genotype, processing-by-environment and genotype-by-environment could still exist. Increasing the amount of duplicates and replicates ($n \geq 3$) might help to identify the interaction effects. Viscosity of β-glucan was strongly positively associated with molecular weight and solubility. High molecular weight was maintained by deactivating endo-β-glucanase after flaking. Residual endo-β-glucanase activity likely depolymerized β-glucan into medium or small chains, which increased the solubility of β-glucan during extraction. Oat samples with low or medium molecular weight but with high solubility exhibited significantly lower viscosity than samples with high molecular weight and lower solubility, which implies that molecular weight played a more important role in affecting the rheological behavior of β-glucan than did solubility in this study. In the oat products, the concentration and solubility of β-glucan was more strongly affected by genotype, and the molecular weight was more influenced by environment. Looking

at genotypic effects, CDC Morrison showed the highest level of β-glucan and solubility, and CDC Dancer had the lowest values. This implies that an oat genotype with a higher concentration of β-glucan might have a higher solubility, because a greater concentration of β-glucan typically results in more β-glucan located in the endosperm and hence β-glucan is released more easily into solution. Considering environment effects, Saskatoon overall grew oats with a higher molecular weight of β-glucan than did Kernen. Consecutive processing (kilning followed by steaming/flaking) had positive effects on enhancing the rheological behavior of β-glucan.

5. EXPERIMENT TWO:

Effect of Commercial Scale Processing on β-glucan Characteristics

5.1. Results and Discussion

5.1.1. β-glucan Content

In Experiment Two, raw oats underwent commercial scale processing, which included dehulling, cleaning, kilning, steaming, flaking and drying. The concentration of β-glucan in oats was significantly affected by both processing and genotype (Table 5.1) ($p<0.05$). The interaction effect of processing-by-genotype was significant as well, a finding which differed from the results of Experiment One. This suggests that the interaction effects may potentially exist in Experiment One. β-glucan levels significantly decreased from raw oats (5.03 %) to flakes (4.84 %) ($p<0.05$) (Table 5.2). Kilned oats had a similar content of β-glucan as raw oats. This indicates that a loss of β-glucan may occur in the flaking process. For example, during the cleaning step after flaking, a small portion of β-glucan may be lost through separating and discarding the fines or lumps of flakes. Due to genetic differences, HiFi had a significantly higher concentration of β-glucan (6.07 %) than Leggett (4.84 %), and Furlong (4.09 %) ($p<0.05$). Detailed information regarding β-glucan concentration in oat genotypes is listed in Table 5.3 and Figure 5.1.

Table 5.1 ANOVA for effects of processing and genotype on concentration, viscosity, molecular weight (M_p and M_w) and solubility of β-glucan in Experiment Two with transformed data.

	P-value		
	T	**G**	**T × G**
β-glucan content (%, dwb)	0.0012	<0.0001	0.0240
	**	***	*
Viscosity[a] (cP)	<0.0001	0.0461	0.4667
	***	*	NS
Mp (kDa)	<0.0001	<0.0001	<0.0001
	***	***	***
Mw (kDa)	<0.0001	<0.0001	<0.0001
	***	***	***
Solubility (%)	<0.0001	<0.0001	0.0062
	***	***	**
Viscosity[b] (cP)	< 0.0001	< 0.0001	< 0.0001
	***	***	***

T = Processing, heat treatment, G = Genotype
NS P ≥ 0.05; * P < 0.05, ** P < 0.01, *** P < 0.001
[a] Viscosity measured by Gamel Method
[b] Viscosity measured by Beer Method

Table 5.2 Effects of processing and genotype on concentration, viscosity, molecular weight (M_p and M_w) and solubility of β-glucan in Experiment Two.

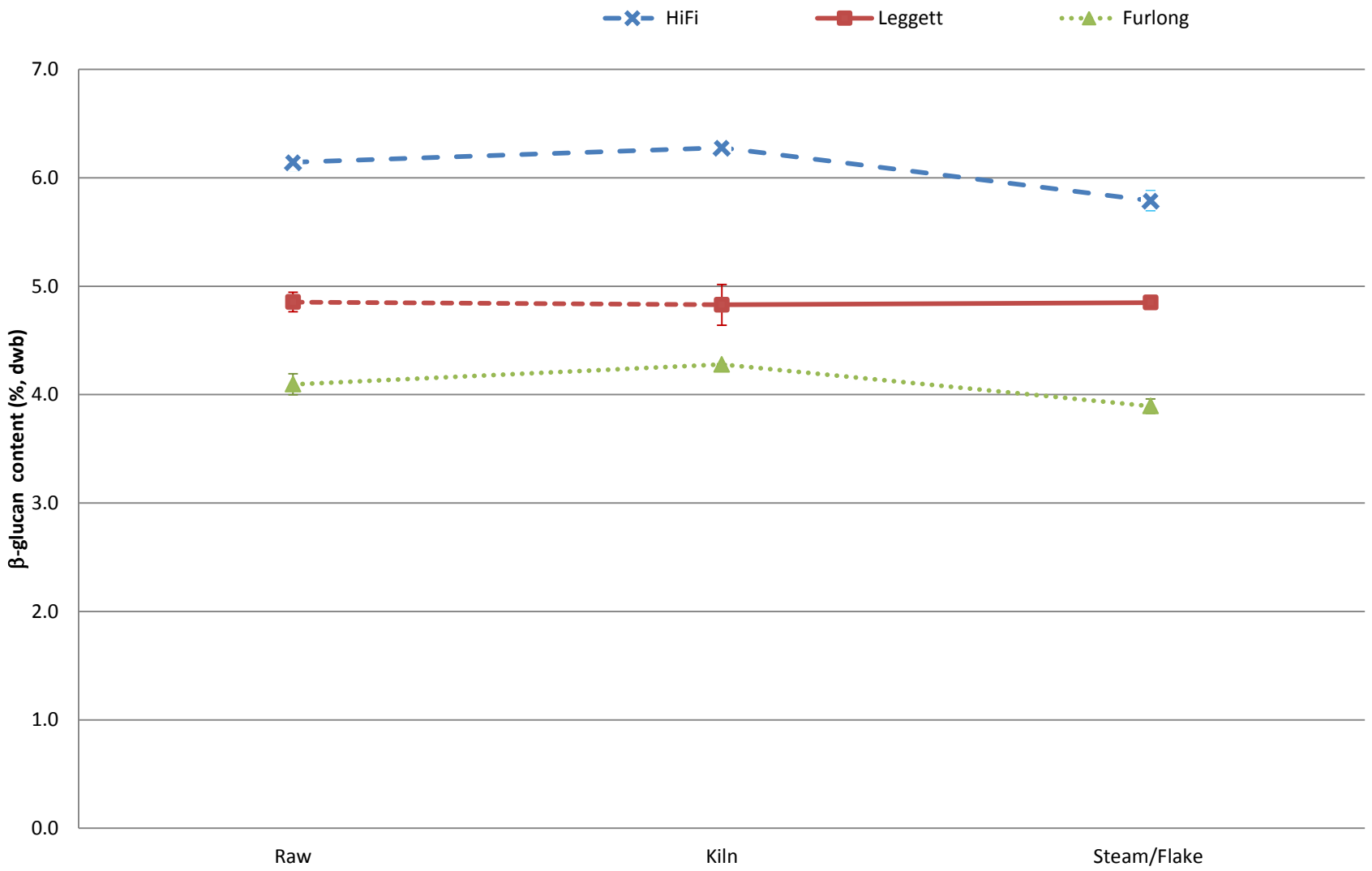
	β-glucan Content (%, dwb)	Viscosity (cP)	M_p (kDa)	M_w (kDa)	Solubility of β-glucan (%)
Treatment					
Raw	5.03[b]	52.33[a]	41.88[a]	52.91[a]	90.86[c]
Kiln	5.13[b]	469.00[b]	1854.89[b]	1869.71[b]	37.06[a]
Steam/Flake	4.84[a]	717.75[c]	1800.14[b]	1859.04[b]	46.30[b]
Genotype					
HiFi	6.07[c]	449.33[b]	1345.93[b]	1334.33[b]	61.59[c]
Leggett	4.84[b]	432.75[ab]	1229.34[a]	1286.70[a]	57.98[b]
Furlong	4.09[a]	357.00[a]	1121.64[a]	1160.63[a]	54.65[a]

Effects of processing/heat treatments were reported as means of all genotypes (n = 6)
Genotype effects were reported as means of all treatments (n = 6)
Values within a column and within a main effect followed by the same letter are not significantly different (P>0.05).

Table 5.3 The effects of processing-by-genotype on oat β-glucan content (%, dwb) in Experiment Two.

Treatment	Genotype			Mean	Stdev
	HiFi	Leggett	Furlong		
Raw	6.14	4.85	4.09	5.03[b]	1.04
Kiln	6.28	4.83	4.28	5.13[b]	1.03
Flake	5.79	4.85	3.89	4.84[a]	0.95
Mean	6.07[c]	4.84[b]	4.09[a]		
Stdev	0.25	0.01	0.19		

Figure 5.1 Interaction effect of processing-by-genotype on oat β-glucan content (%, dwb) in Experiment Two.



5.1.2. Viscosity and Extraction of β-glucan Through In Vitro Digestion

The viscosity of β-glucan in oats was measured through an in vitro digestion extraction using an RVA (Gamel et al., 2012) or rheometer (Beer et al., 1997). Processing was shown to enhance the viscosity of β-glucan (Table 5.2, 5.4 and 5.5, Figure 5.2). In the Gamel et al. method (2012), viscosity analyzed by RVA showed a stable increase in commercially processed kilned and flaked oats (Experiment Two), with no reduction in viscosity over time (Figure 5.3), which implied that the commercial kilning process successfully deactivated all endo-β-glucanase in the oat samples. For oats processed using the pilot scale facility (Experiment One), the viscosity was increased with flaking but kilning did not appear to eliminate the activity of beta-glucanase (Figure 5.3). The pilot scale processing used in this study failed to represent the quality of kilned oat at commercial scale. Sequential processing (kilning and then flaking) at pilot scale eventually stabilize the rheological property of oat beta-glucan. With no interruption by endo-β-glucanase, flaked oats exhibited a higher viscosity than kilned oats when measured using a 1 % concentration of β-glucan in solution; this would be caused by the differences in molecular weight and solubility of β-glucan in the oat products. The RVA extraction method resulted in a low viscosity for raw oats, 52.33 cP (Table 5.2). In Experiment One, the viscosity of β-glucan in raw oats was first noticed to increase, and then started to drop after 30 minutes of extraction (Figure 4.7). Following this pattern, at least a slight increase in viscosity at the beginning of the extraction period was expected to be seen in raw oats in Experiment Two. However, raw oats were observed to exhibit low viscosity throughout the entire extraction procedure (Figure 5.3). It is possible that β-glucan in the raw oat samples was degraded during the storage period after processing of the other samples. The procedure of kilning appeared to stabilize the β-glucan in

oat products for a longer shelf life. HiFi had the highest viscosity at 449.33 cP, which was significantly higher than that of Furlong (357.00 cP) ($p<0.05$) (Table 5.2). According to Table 5.1, the viscosity of β-glucan was significantly affected by processing and genotype, but no interaction effects of processing-by-genotype were found. Linear exponential relationship between $\log_{10}$[viscosity] and M_p*solubility ($R^2 = 0.9365$) and M_w*solubility ($R^2 = 0.9598$) was also found observed in Experiment Two (Figure 5.4 and 5.5).

Table 5.4 The effects of processing-by-genotype on log(viscosity)* (cP) of oat β-glucan in Experiment Two.

Treatment	Genotype			Mean	Stdev
	HiFi	Leggett	Furlong		
Raw	1.73	1.72	1.71	1.72^{a}	0.01
Kiln	2.74	2.67	2.59	2.67^{b}	0.08
Flake	2.87	2.89	2.80	2.85^{c}	0.05
Mean	2.45^{b}	2.43^{ab}	2.37^{a}		
Stdev	0.62	0.62	0.58		

* Viscosity was measured by RVA in vitro digestion method developed by Gamel et al. (2012).

Table 5.5 The effects of processing-by-genotype on log(viscosity)* (cP) of oat β-glucan in Experiment Two.

Treatment	Genotype			Mean	Stdev
	HiFi	Leggett	Furlong		
Raw	1.13	1.06	1.09	1.10[a]	0.03
Kiln	2.04	1.55	1.41	1.67[b]	0.33
Flake	2.08	1.77	1.39	1.75[c]	0.35
Mean	1.75[c]	1.46[b]	1.30[a]		
Stdev	0.54	0.36	0.18		

* Viscosity was measured by rheometer in vitro digestion method developed by Beer et al. (1997), shear rate range of 0.1-400 sec^{-1} at 37 °C with a cone-and-plate geometry (angle of 0.04 radians, diameter of 50 mm); apparent viscosity was recorded at the shear rate of 30 sec^{-1}.

Figure 5.2 Interaction effect of processing-by-genotype on log(viscosity) (cP) of oat β-glucan in Experiment Two (Gamel et al., 2012)

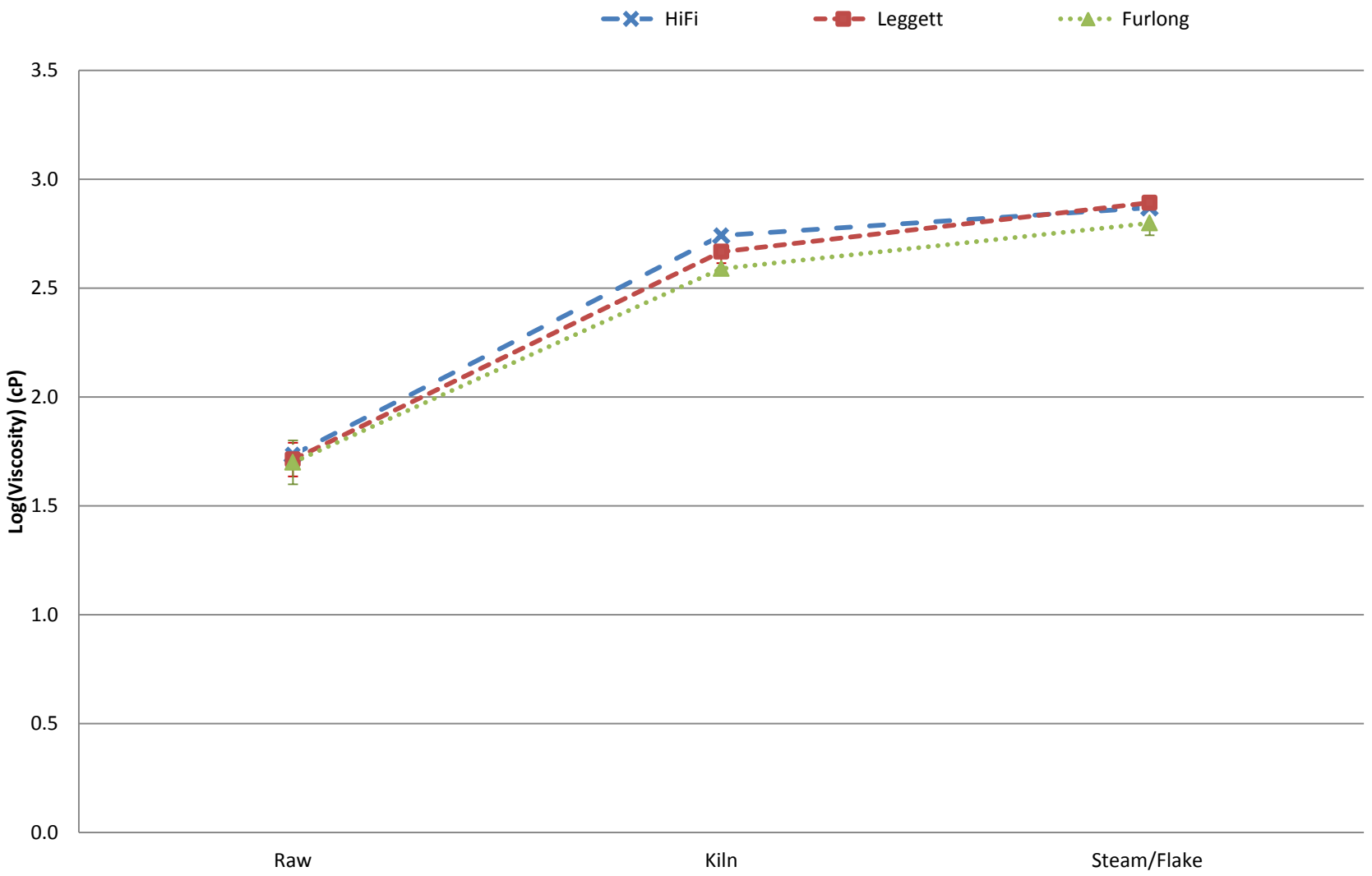


Figure 5.3 Viscosity of 1 % β-glucan in raw, kilned and flaked oat measured on RVA

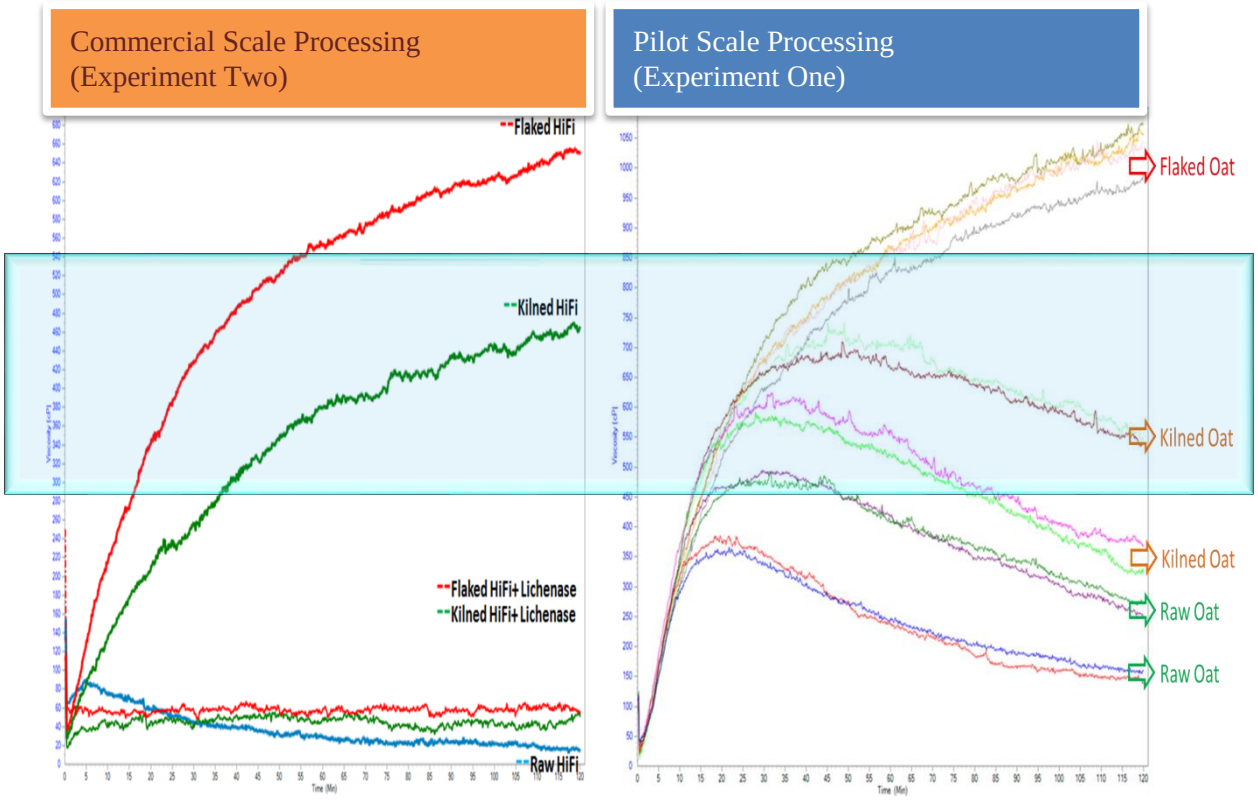


Figure 5.4 Relationship between viscosity, M_p and solubility of β-glucan measured by in vitro digestion method on RVA

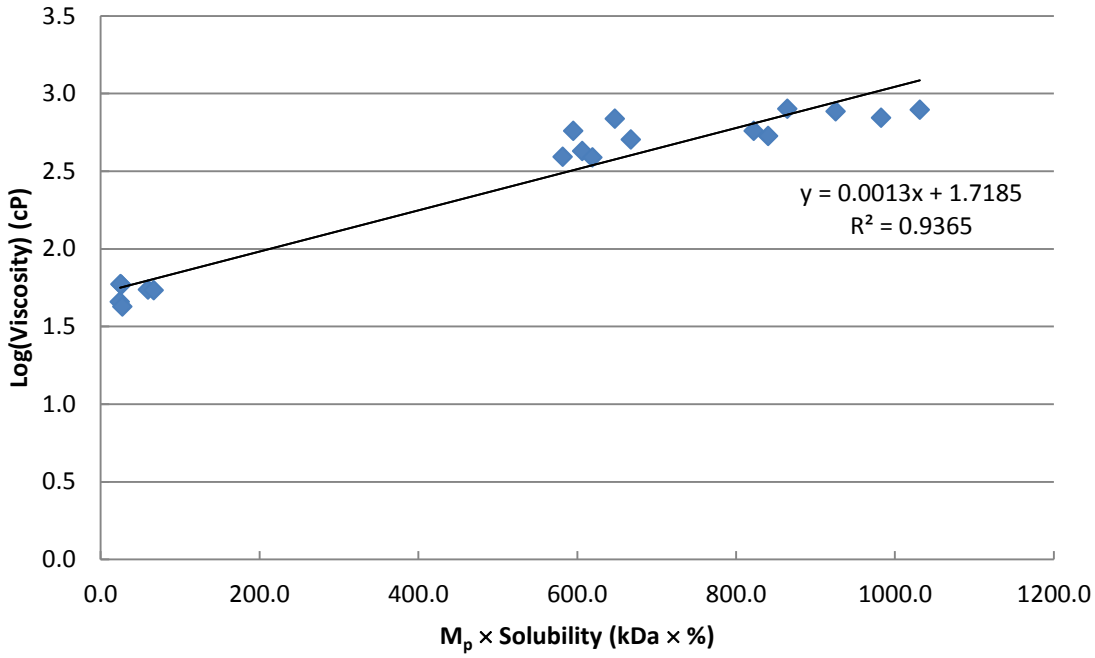
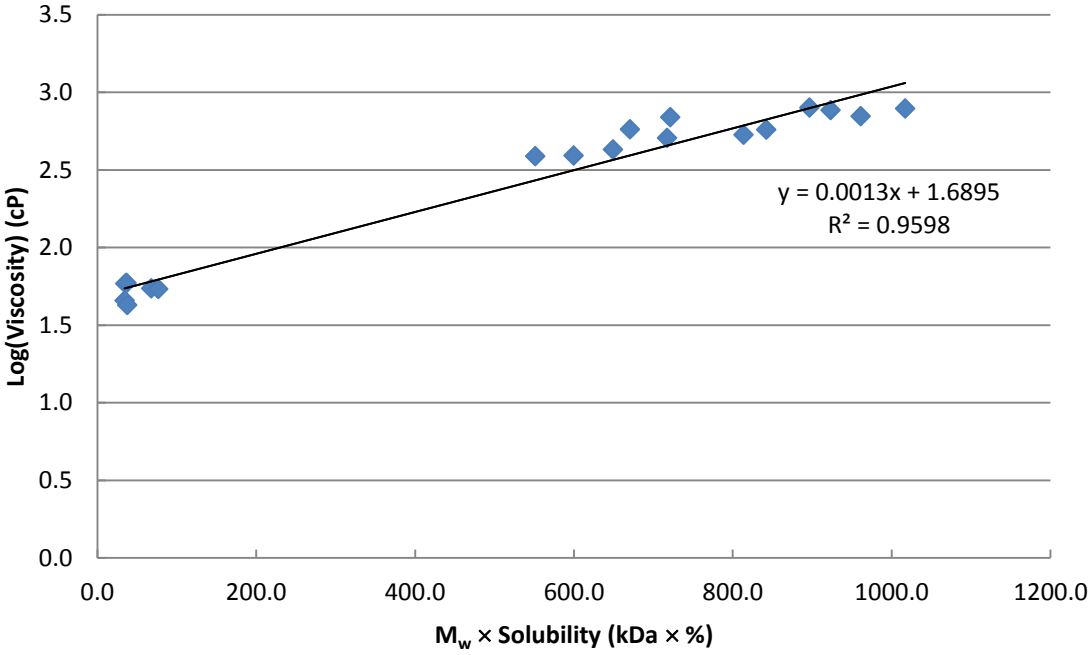


Figure 5.5 Relationship between viscosity, M_w and solubility of β-glucan measured by in vitro digestion method on RVA



In a study by Beer et al. (1997), the viscosity of β-glucan was measured based on a 10 grams (dry basis) oat sample i.e. not on a percent-β-glucan basis, which allowed investigators to observe the rheological behavior of β-glucan from a different point of view. As shown in Table 5.5, a continuous and significant increase in viscosity from raw (12.49 cP) to kilned (57.16 cP), and then from kilned to flaked (120.62 cP) was seen. HiFi again was observed to have the highest viscosity at 81.51 cP, which was significantly higher than that of Leggett (35.34 cP), and Furlong (20.90 cP) (Table 5.5). HiFi oat flakes using 10 grams dry weight created a viscosity at 120.62 cP in the extraction solution, and Furlong 24.61 cP. These results showed that by consuming the same amount of oat flakes, HiFi may work more efficiently than Furlong. The effects of processing, genotype and processing-by-genotype were significant in the viscosity of β-glucan measured by the Beer et al method (1997) (Table 5.1).

These two extraction methods were both good indicators to identify the viscosity change in processing. The Gamel et al. method (2012) was more suitable for Experiment One and Two to compare the rheological change of β-glucan at an equal level of β-glucan and identify the solubility differences with more accuracy. In the Beer et al. method (1997), commercially processed samples based on 10 grams sample size had different concentrations (0.4~0.6%) of β-glucan in the final extraction solution, which interference the observations of viscosity differences associating with the change of molecular weight and solubility of β-glucan.

5.1.3. Molecular Weight in Vitro Digestion Extraction

Molecular weights of β-glucan (M_p and M_w) were significantly affected by processing and genotype ($p<0.05$) (Table 5.1). The interaction effects of processing-by-genotype were significant for both M_p and M_w. Detailed data are listed in Tables 5.6 and 5.7, and Figures 5.6

and 5.7. According to Figure 5.3, no reduction of viscosity occurred during the two hour extraction, indicating that β-glucan was not damaged by endo-β-glucanase in kilned and flaked oat samples. In Table 5.2, kilned and flaked oats had no significant difference in molecular weight (both M_p and M_w), which implied that processing had no effect on increasing the molecular weight of β-glucan in oats, but rather prevented the β-glucan polymers from depolymerization. A slight decrease was observed from kilned oat (M_p 1854.89 kDa, M_w 1869.71 kDa) to flaked oat (M_p 1800.14 kDa, M_w 1859.04 kDa) (Table 5.2), which indicated that the molecular weight of β-glucan might have decreased due to over-processing, such as over-heating (Tosh et al., 2010). Low molecular weights of M_p 41.88 kDa and M_w 52.91 kDa were found in raw oats exhibiting low viscosity. Based on genotype, HiFi showed a significantly higher molecular weight of β-glucan than Leggett and Furlong (Table 5.2) following in vitro digestion. Although environment played a critically important role in determining the molecular weight of β-glucan in Experiment One, genotype still had effects on molecular weight in both Experiment One and Two.

Table 5.6 The effects of processing-by-genotype on log(Mp) (kDa) of oat β-glucan in Experiment Two.

Treatment	Genotype			Mean	Stdev
	HiFi	Leggett	Furlong		
Raw	1.84	1.44	1.46	1.58[a]	0.22
Kiln	3.29	3.25	3.26	3.27[b]	0.02
Flake	3.31	3.27	3.17	3.25[b]	0.07
Mean	2.81[b]	2.66[a]	2.63[a]		
Stdev	0.84	1.05	1.01		

Figure 5.6 Interaction effect of processing-by-genotype on log(Mp) (kDa) of oat β-glucan in Experiment Two.

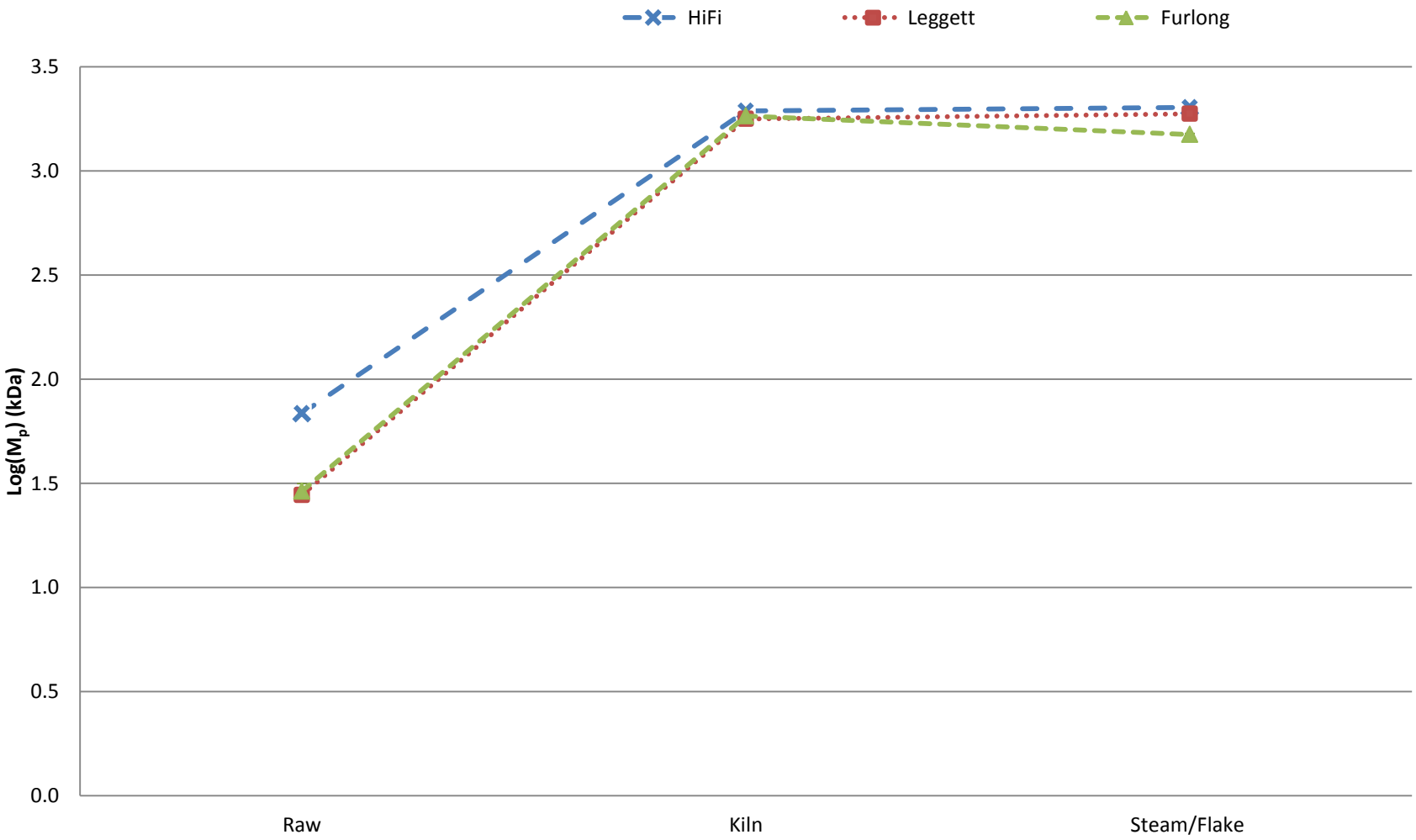
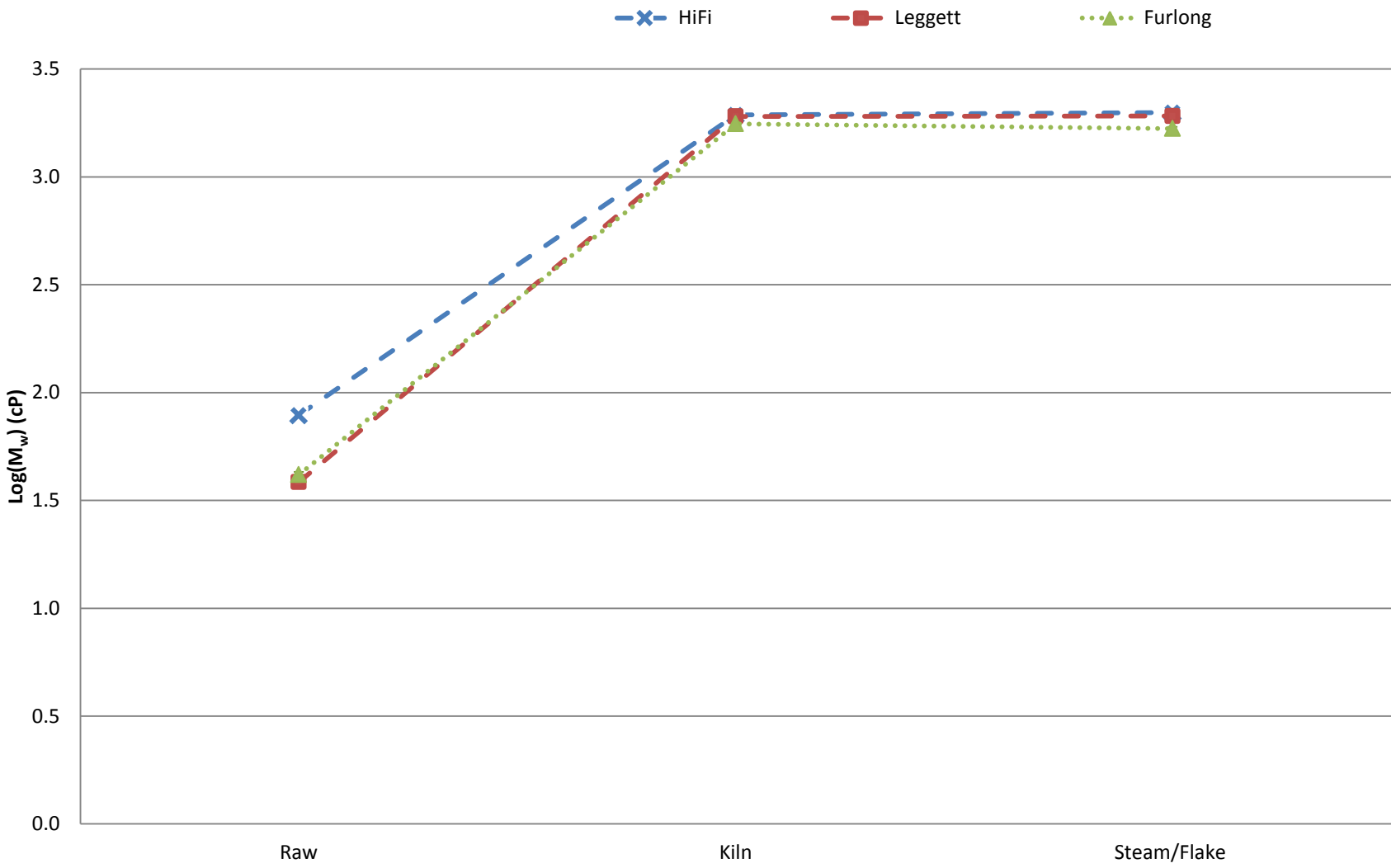


Table 5.7 The effects of processing-by-genotype on log(Mw) (kDa) of oat β-glucan in Experiment Two.

Treatment	Genotype			Mean	Stdev
	HiFi	Leggett	Furlong		
Raw	1.90	1.58	1.62	1.70[a]	0.17
Kiln	3.29	3.28	3.25	3.27[b]	0.02
Flake	3.30	3.28	3.22	3.27[b]	0.04
Mean	2.83[b]	2.72[a]	2.70[a]		
Stdev	0.81	0.98	0.93		

Figure 5.7 Interaction effect of processing-by-genotype on log(Mw) (kDa) of oat β-glucan in Experiment Two.



5.1.4. Solubility in Vitro Digestion Extraction

Solubility was significantly affected by processing, genotype and processing-by-genotype (Table 5.1). In Table 5.2, the solubility of β-glucan in oat decreased from raw (90.86 %) to kilned (37.06 %), and increased from kilned to flaked oat (46.30 %), which supported the idea raised in Experiment One that steam/heat may increase the solubility of β-glucan. The high solubility of β-glucan in raw oats still corresponded with a small molecular size of β-glucan (Table 5.2). The high molecular weight of β-glucan found in kilned and flaked oats, as in Experiment One, corresponded with a significantly lower solubility compared to raw oats ($p<0.05$). Despite a similar molecular weight of β-glucan, flaked oat had significant higher solubility than kilned oat, which further proved that steaming and heating enhanced the solubility of β-glucan. As the degree of steaming and heating increased during processing (kilning, and then steaming/flaking), more insoluble β-glucan was converted into soluble by replacing hydrogen bonds in cellulose-like junction zones with water molecules (Tosh et al., 2004; Tvaroska et al., 1983; Vasanthan, 2009; Vårum & Smidsrød, 1988; Zhang et al., 1998; Zhang et al., 1997). Again, when looking at genotypic effects, oats with a high concentration of β-glucan were shown to have a higher solubility; this observation was also found in Experiment One. HiFi had a solubility of 61.59 % at 6.07 % β-glucan, and Furlong had a solubility of 54.65 % at 4.09 % β-gluan (Table 5.2). The solubility of β-glucan in Leggett (57.98 %) was significantly lower than that in HiFi, but significantly higher than in Furlong with its β-gluan content at 4.84 % ($p<0.05$). All the detailed data are shown in Table 5.8 and Figure 5.8. Because there was no difference in molecular weight of β-glucan between kilned and flaked oat, solubility determined the levels of

viscosity in processed oat products. As the solubility increased, the viscosity was significantly increased from kilned to flaked oats.

5.1.5. Processing Effects on Other Non-Starch Polysaccharides

Resistant starch, arabinoxylan, insoluble dietary fibre (IDF), soluble dietary fibre (SDF), low molecular weight soluble dietary fibre (LMWSDF) and total dietary fibre (TDF) were measured to identify processing effects on the content of other oat non-starch polysaccharides besides β-glucan. These measurements were carried out using HiFi oat samples processed on a commercial scale. Resistant starch was minimal (< 0.5 % w/w) in processed oat (data not included), and it is not thought to contribute to the fibre content in oats (Lan-Pidhainy et al., 2007). Total starch was monitored to investigate processing effects on starch content. As seen in Table 5.9, total starch levels were significantly increased from raw oat (55.93 %) or kilned oat (55.98 %) to flaked oat (58.42 %) ($p<0.05$). It indicated that processing enhanced the non-resistant starch level in oat products, which might be caused by depolymerizing non-starch polysaccharides and producing oligosaccharides. Arabinoxylan, one form of dietary fibre, showed a significant reduction after processing, from 1.95 % in raw oats to 1.73 % in flaked oats (Table 5.9).

TDF remained the same before and after processing, but a shift in IDF and LMWSDF was noticed. IDF was increased significantly from 4.87 % (raw oat) to 6.99 % (kilned oat), and was maintained at 6.59 % in flaked oat. LMWSDF was significantly reduced from 2.02 % (raw oat) to 0.33 % (kilned oat), and stayed at 0.33 % in flaked oat. SDF was not significantly affected by processing. There have been no other studies demonstrating an increase in IDF but a decrease in LMWSDF with processing. The change in IDF and LMWSDF might be caused by one or both of

Table 5.8 The effects of processing-by-genotype on log(solubility) (%) of oat β-glucan in Experiment Two.

Treatment	Genotype				
	HiFi	Leggett	Furlong	Mean	Stdev
Raw	1.96	1.96	1.95	1.96[c]	0.01
Kiln	1.63	1.55	1.51	1.57[a]	0.06
Flake	1.70	1.68	1.62	1.66[b]	0.04
Mean	1.76[c]	1.73[b]	1.70[a]		
Stdev	0.18	0.21	0.23		

Figure 5.8 Interaction effect of processing-by-genotype on log(solubility) (%) of oat β-glucan in Experiment Two.

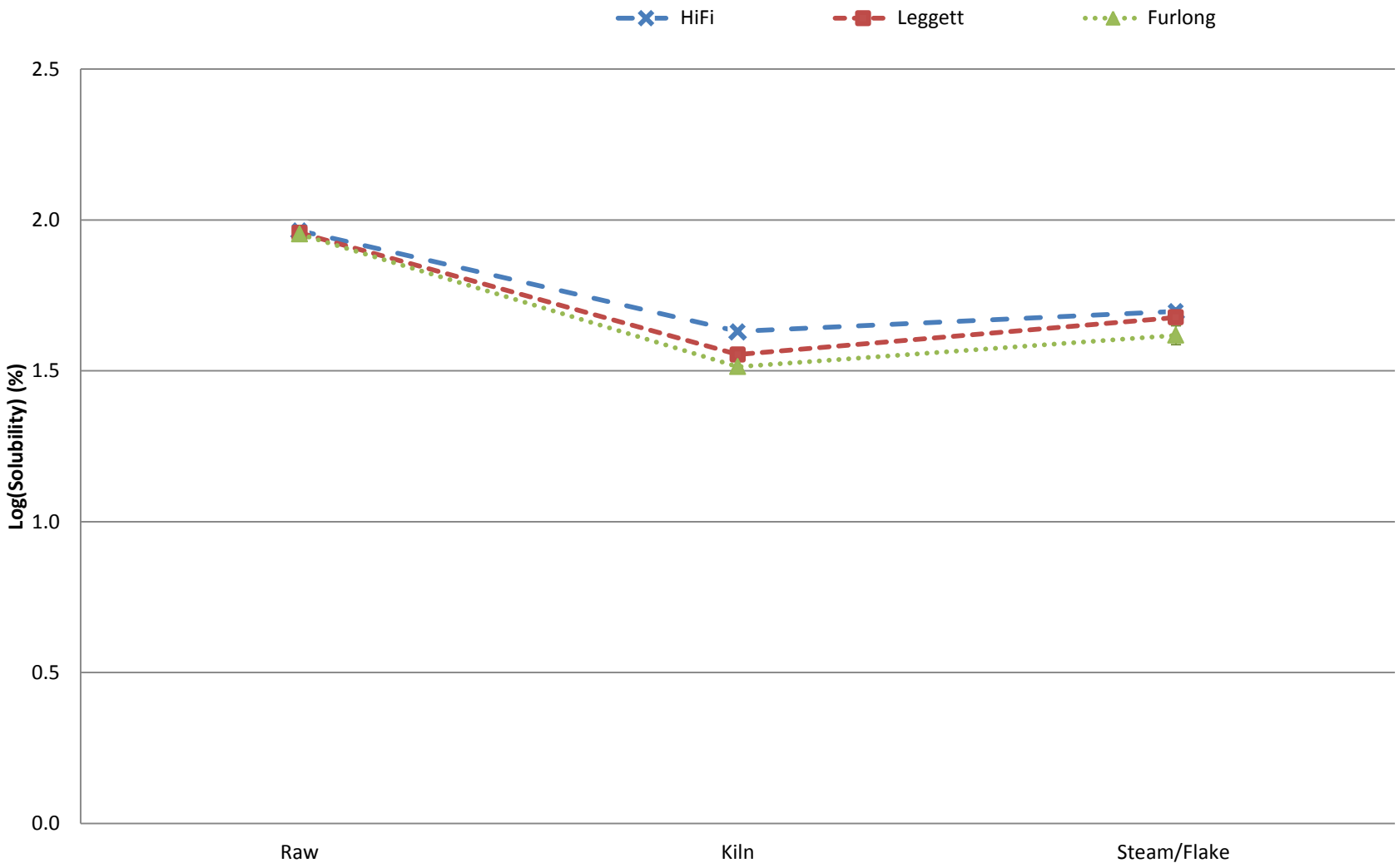


Table 5.9 Effects of processing on content of total starch, arabinoxylan, IDF, SDF, LMWSDF and TDF in HiFi oat in Experiment Two.

	Total Starch (%, dwb)	Arabinoxylan (%, dwb)	IDF (%, dwb)	SDF (%, dwb)	LMWSDF (%, dwb)	TDF (%, dwb)
Treatment						
Raw	55.93[a]	1.95[b]	4.87[a]	5.33[a]	2.02[b]	12.22[a]
Kiln	55.98[a]	1.84[ab]	6.99[b]	5.80[a]	0.33[a]	13.12[a]
Steam/Flake	58.42[b]	1.73[a]	6.59[b]	5.53[a]	0.33[a]	12.44[a]

Values within a column followed by the same letter are not significantly different (P>0.05).

the following: first, there may be some endo-enzymes naturally existing in raw oat, which could break IDF and SDF into LMWSDF or oligosaccharides. Those natural endo-enzymes may have been later inactivated during the kilning process. No further effects on IDF and LMWSDF were observed in steamed/flaked oats, which might be explained by no or little activity of endo-enzymes after kilning (Table 5.9). Therefore, in kilned and flaked oats, more IDF remained intact, and a negligible amount of LMWSDF was found. Second, some of the nutrient components may have been lost during the processing. For an example, LMWSDF was broken down into oligosaccharides. The material lost in processing would result in a concentrating of IDF.

Although measurements were only based on HiFi oat, processing effects on several non-starch polysaccharides in this oat material was observed. Unlike the existing literature (Bird et al., 2009; Wakita et al., 2004; Wakita et al., 2004), commercial processing did not produce more non-starch polysaccharides. The reduction of arabinoxylan by steaming was also found in the Westerlund et al. study (1993).

5.1.6. Effects of Additional Steaming on Oat Flake

The reduction in arabinoxylan content observed indicated depolymerisation of non-starch polysaccharides during processing. Further steaming of flakes was tested in Experiment Two to identify the optimal level of wet heating on the physiochemical properties of oat β-glucan. To mimic heating procedure in kilning and flaking (injecting steam for a short period of time), commercial flaked oat was steamed for 30 seconds or 60 seconds and rest for 10 minutes. According to Table 5.10, steaming for 30 and 60 seconds had no significant effect on the

Table 5.10 Effects of processing with additional steaming processes on concentration, viscosity, molecular weight (M_p and M_w) and solubility of β-glucan in HiFi oat in Experiment Two.

	β-glucan Content (%, dwb)	Viscosity (cP)	M_p (kDa)	M_w (kDa)	Solubility of β-glucan (%)
Treatment					
Raw	6.14[bc]	54.25[a]	68.71[a]	78.67[a]	92.23[d]
Kiln	6.28[c]	552.00[b]	1947.12[c]	1938.22[c]	42.73[a]
Steam/Flake	5.79[a]	741.75[c]	2021.95[c]	1986.12[c]	49.80[bc]
1st Steam[a]	5.91[ab]	791.50[c]	1588.95[b]	1290.06[b]	53.09[c]
2nd Steam[b]	5.79[a]	762.25[c]	1673.84[bc]	1384.74[b]	46.81[ab]

Values within a column followed by the same letter are not significantly different (P>0.05).
[a] 1st steam = steaming oat flake for 30 seconds
[b] 2nd steam = steaming oat flake for 60 seconds

concentration, viscosity and solubility of β-glucan. However, the molecular weight (M_p and M_w) of β-glucan decreased significantly by the steaming process ($p<0.05$). The degree of depolymerisation was found to be higher in M_w than that of M_p. Observing the difference between M_w and M_p may be used as an initial indicator for identifying the breakdown of molecular weight of β-glucan during processing. The equation of correlation between $\log_{10}$[viscosity] and molecular weight * solubility developed in Experiment One might not be applicable to this data after depolymerisation has occurred. There is an upper limit for processing (wet heat) on enhancing the nutritional properties of β-glucan. Over processing may have adverse effects including depolymerizing β-glucan, which might reduce the efficacy of β-glucan on attenuating glycemic response in humans (Brummer et al., 2012).

5.2. Summary

Commercial scale processing showed a negative effect on β-glucan levels in oat products. The loss of β-glucan might due to milling and cleaning procedures. Oat fractions high in β-glucan produced in the milling process (from kilning to flaking) may be lost along with fines or lumps. There was no loss of β-glucan during processing in Experiment One. Therefore, the concentration of β-glucan could be modified during processing (Vasanthan & Temelli, 2008). With more milling steps involved in commercial scale processing, there is a greater chance to observe changes in nutritional value, such as β-glucan levels. The viscosity of β-glucan in oats was increased significantly and continuously from raw to kilned, and then to flaked, which was seen in Experiment One. However, the commercial kilning procedure seemed like successfully deactivating the endo-β-glucanase more effectively comparing to the pilot scale, with no reduction of viscosity observed in kilned oats during a two hours extraction period (Figure 5.3).

Using a 1 % concentration of β-glucan in vitro digestion extraction method (Gamel method), the increase in solubility of β-glucan contributed to an increase in viscosity from kilned to flaked oat, because no difference in molecular weight of β-glucan was detected. The improvement of solubility of β-glucan without decreasing the molecular weight might be due to replacement of the intramolecular hydrogen bond between β-glucans with water molecules (Vasanthan, 2009; Zhang et al., 1998). Even though genotype had significant effects on viscosity and molecular weight of β-glucan, it played a larger role in determining the concentration and solubility of β-glucan in oats. Interaction effects of processing-by-genotype were found in the concentration, molecular weight and solubility of β-glucan, except viscosity. This indicates that potential interactions among processing, genotype and environment would have existed in Experiment One given a sufficient sample size. The Gamel method was suitable for this study to compare the rheological properties of β-glucan consistently, and the Beer method could be used to provide a simple guideline to consumers regarding serving size and nutritional properties.

Further steaming of oat flakes had no additional effects on the rheological behavior and concentration of β-glucan. The solubility of β-glucan was not increased by continuous steaming, but a reduction in molecular weight of β-glucan was shown. Hence, oversteaming might have an adverse effect on physicochemical properties of β-glucan. Besides the effects on β-glucan, commercial scale processing had no positive effect on enhancing resistant starch, arabinoxylan, SDF, LMWSDF and TDF. However, IDF might be protected from endo-enzymes by inactivating them in the kilning procedure.

6. General Conclusions

The present study has shown that processing (pilot scale and commercial scale), genotype and environment have significant effects on the physicochemical properties of β-glucan. Processing involving steam and heat may effectively protect the integrity of β-glucan from the damage by deactivating endo-β-glucanase. However, over steaming and heating has no additional effect on increasing the solubility of β-glucan, but a reduction in molecular weight might occur due to depolymerisation. Higher integrity of molecular weight and solubility of β-glucan have positive effects on its rheological behavior. Genotype is a greater contributor to concentration and solubility of β-glucan in oat, while environment has a greater contribution to molecular. A high concentration of β-glucan in oats would be accompanied by a higher solubility. The interaction effects of processing-by-genotype, processing-by-environment and genotype-by-environment failed to show in this study under the pilot scale processing, but processing-by-genotype interaction effects on β-glucan properties were exhibited under commercial scale processing. The statistical graphs indicated the interaction effects potentially existing in the study under pilot scale processing. An extended study with more duplicates will help to identify the interaction effects among processing, genotype and environment. A strong exponential relationship between viscosity and molecular weight*solubility of β-glucan was found, which helped to explain the association between them by using mathematical equations. Unlike for other grains described in the literature, no positive effect on enhancing the amount other non-starch polysaccharides, such as resistant starch, arabinoxylan, SDF, and TDF was observed in kilned and flaked oat comparing to raw oat. The pilot scale processing was not effective as the commercial scale processing especially in kilning. Adjustment of pilot scale processing would be required to re-create an optimal simulation of commercial scale processing.

Overall, sequential processing (kilning and then flaking) continuously increased the viscosity of oat products associating with nutritional values comparing to the raw oat. The wise selection of genotype and environment by industry would help to enhance the therapeutic potential of oat with the same amount of β-glucan content. The oat genotype with high content of β-glucan may be a good choice to be picked up first by breeders. Besides β-glucan content, the viscosity and molecular weight of β-glucan should be included on the nutritional label of oat products. Hence, consumers are not only aware of the concentration of β-glucan in the products, but also know the efficiency of this natural lifestyle medication is.

7. Future Work

Since this study mainly focused on typical commercial processing (kilning and steaming/flaking) in oat pre-treatments, future research should focus more on other commercial processing, such as toasting, extruding and baking. The exponential relationship between viscosity, molecular weight and solubility needs to be defined under those processing techniques with more genotypes grown in more different environments. The optimal level of steaming and heating should be identified to maximize the nutritional values of β-glucan in oats. All extended studies would help to promote oats in commercial products as a good source β-glucan, and increase the nutritional benefits by optimizing the efficacy of β-glucan.

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