PRE-HARVEST GLYPHOSATE EFFECTS ON PROPERTIES OF BETA-GLUCAN FROM OAT GROATS

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MASTER OF SCIENCE

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ABSTRACT

Pre-harvest glyphosate is applied to cereal grains to remove weeds. However, it has been claimed that oat compositions are affected by pre-harvest glyphosate. Research was conducted to evaluate differences in properties of β-glucan in the treated versus untreated oat groats. Two oat cultivars (Rockford and Souris) were grown at Minot and Prosper, North Dakota in 2015, and glyphosate was sprayed during the soft dough stage, physiological maturity stage, or not applied. β-Glucan viscosity was not significantly ($p > 0.05$) affected by treatment at soft dough or physiological maturity stages. Use of glyphosate at the soft dough stage significantly ($p < 0.05$) reduced the percentages of β-glucan content and solubility versus untreated samples. Treatment at soft dough and physiological maturity stages significantly ($p < 0.05$) increased β-glucan molecular weights compared to untreated controls. Therefore, glyphosate can be applied at the physiologically mature stage of grain development because β-glucan properties from the groats were not negatively affected.

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DEDICATION

I would like to thank my mother, Norah. I always stay motivated because of her. Mum: keep your head up, and I am on track to make you HAPPY.

I would like to thank my father, Khalid for his unwavering support throughout my education. Dad: you gave me everything; thanks so much.

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LIST OF ABBREVIATIONS

AMPA ..... Aminomethylphosphonic Acid

CED ..... Cohesive Energy Density

cP ..... Centipoise

DV ..... Daily Value

DWB ..... Dry Weight Basis

EPSPS ..... 5-Enolpyruvyl-Shikimate-3-Phosphate Synthase

FDA ..... United States Food and Drug Administration

GEHT ..... Genetically Engineered Herbicide Tolerant

GP ..... Groat Percentage

HD ..... Hard Dough

HPSEC ..... High Performance Size Exclusion Chromatography

1b/bu ..... Pounds/Bushel

LDL ..... Low-Density Lipoprotein

LD_{50} ..... Lethal Dose, 50%

LSD ..... Least Significant Difference

MALS ..... Multi Angle Laser Light Scattering

MWT ..... High or Low Molecular Weight

M_W ..... Weight Average Molecular Weight

M_W/M_n ..... Polydispersity Index

Oz/acra ..... Ounces/Acra

PBGR ..... Peak Blood Glucose Response

PEP ..... Phosphoenolpyruvate

RI ..... Refractive Index

RVA . . . . . Rapid Visco Analyzer

SAS . . . . . Statistical Analysis Software

SD . . . . . Soft Dough

S3P Shikimate-3-Phosphate

W . . . . . Watt

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1. INTRODUCTION

1.1. Glyphosate

Glyphosate is considered a broad spectrum herbicide, which provides control of annual and perennial weeds (Monsanto Company, 2017). It is an enzyme inhibitor that stops the activity of the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase. This enzyme is naturally produced and utilized to synthetize protein for proper growth of plants. Inactivation of this enzyme inhibits the formation of aromatic amino acids (such as Tyrosine), which leads to plant death.

Since humans and animals do not generate the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase by themselves, glyphosate should not be listed as a biological hazard for humans. Thus, glyphosate is classified non-poisonous to mammals, with an LD_{50} greater than 5 g kg $^{-1}$ for rats (Annett, Habibi, et al., 2014, Duke and Powles, 2008). However, some studies suggest that glyphosate use may contribute to environmental toxicity, such as residues persisting in soils, and might be carcinogenic to humans (Bai and Ogbourne, 2016, Myers, Antoniou, et al., 2016).

In addition to weed control, glyphosate may be applied just prior to harvest on crop such as oats, wheat, and barley. Applying this herbicide prior to harvest will combat weeds, which will also reduce moisture content and speed up the harvest (Manthey, Chakraborty, et al., 2004). Researchers have found that pre-harvest glyphosate application improves the physical quality of oats. For instance, pre-harvest glyphosate treatment when oat grain moisture is at 30% or less can reduce the percentage of thin kernels in the samples; this reduction of thin kernels is beneficial to farmers (Guenther, 2017).

1.2. Oats

Around 64 and 50 million bushels of oats were produced in the United States in 2016 and 2017, respectively (United States Department of Agriculture, 2018). Historically, oats grown in the United States are mostly used for livestock feed as nutritious grains and forage (Strychar, 2011). More recently, oat consumption has been rising dramatically in the United States for human nutrition because of health benefits of β-glucan (Mathews, 2011).

To many consumers, the most well-known food commodity made from oats is oatmeal, which is a popular hot cereal breakfast choice due to health benefits via β-glucan. This product

contains 6 grams of dietary fiber per 40 grams of oatmeal (Quaker Oat Company, 2018). β-Glucan is a large compound of dietary fiber found in oatmeal. Oatmeal has become a popular food item due to the benefits of lowering cholesterol levels and controlling blood glucose response. It is accepted that for every 1% decreasing LDL cholesterol levels, the risk of growth of coronary heart disease is lowered by 1 to 2% (Wood, 2011). Ingesting oat β-glucan is beneficial for healthy people and patients suffering from type-two diabetes (Wood, 2011).

These physiological effects of oats are mainly attributed to the elevation of viscosity by the β-glucan, which relies on its solubility and its molecular weight (Liu, Bailey, et al., 2010, Wang, Storsley, et al., 2016). To analyze the physicochemical properties of oat β-glucan, oat grains harvested from the field are first cleaned to remove undesirable and foreign materials (Decker, Rose, et al., 2014). Then, hulls are removed from the kernels, leaving the edible groat behind. Because of the presence of high levels of lipid-digesting enzymes in groats, the groats next need to be steamed and kiln-dried in order to inactivate the enzymes and prevent spoilage (Ovando-Martínez, Whitney, et al., 2013). The heated groats are milled to produce oat flour, which is necessary to provide particle size reduction of the samples for viscosity measurement.

2. LITERATURE REVIEW

2.1. Glyphosate

Glyphosate (*N-phosphonomethyl glycine*) is a phosphonate derivative of the amino acid glycine. It consists of one amino group and three ionizable acidic groups (Figure 2.1). The amount of glyphosate-based herbicides used in the United States has increased approximately 300-fold since the late 1970s (Benbrook, 2016, Myers, Antoniou, et al., 2016). Glyphosate is an effective herbicide, and its use is increasing due to conventional weed control, glyphosate-tolerant crops, and pre-harvest use pattern (Benbrook, 2016, Griffin, Boudreaux, et al., 2010).

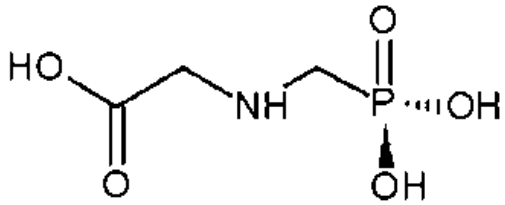

Figure 2.1. Chemical Structure of Glyphosate

Glyphosate was invented in 1950 by the Swiss chemist Dr. Henri Martin who worked for the pharmaceutical company Cilag (Franz, Mao, et al., 1997). The product had no pharmaceutical applications and was not reported in commercial use. Thus, Cilag sold the small amounts of glyphosate to several companies in the 1960s, including Monsanto Company. Monsanto was developing compounds to test as potential water softening agents using aminomethylphosphonic acid (AMPA) analogs. AMPA is considered the major metabolite of glyphosate breakdown.

AMPA analogs were synthesized and were tested for herbicidal activity on perennial weeds via Monsanto chemists. They found that two showed herbicidal activity; however, the unit activity was too low to be a marketable herbicide (Dill, Sammons, et al., 2010). Monsanto chemist Dr. John Franz started to discover the feature of those two compounds and worked on synthesizing analogs and derivatives. Glyphosate was first synthesized and tested by Monsanto in 1970, and then, in 1974, it was introduced as Roundup® herbicide through the company to the market.

2.1.1. Mechanism of Action

Glyphosate is the only herbicide that inhibits the enzyme 5-enolpyruvyl shikimate-3 phosphate synthase (EPSPS) of the shikimate pathway in plants (Fedtke, 2012). Glyphosate attaches to the region of EPSPS that binds the phosphate moiety of phosphoenolpyruvate (PEP). The enzyme catalyzes the transfer of PEP to shikimate-3-phosphate (S3P); this is a key stage in the synthesis of aromatic amino acids of the shikimate pathway (Figure 2.2). That means inhibition of EPSPS prevents the reaction between PEP and S3P to form 5-enolpyruvyl shikimate-3-phosphate synthase, which is detrimental to the plant in depletion of chorismate and increased carbon expenditure.

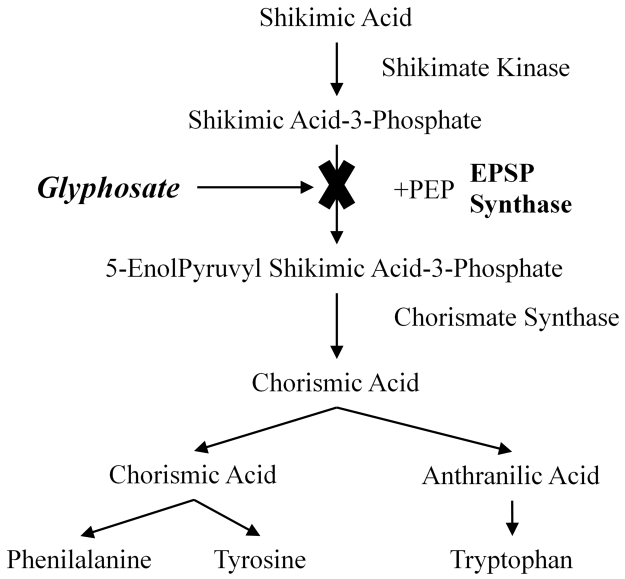

Figure 2.2. The Site of Inhibition of Glyphosate (Dill, Sammons, et al., 2010).

First, the primary effect of disruption of the shikimate pathway is an absence of ability to synthesize the compound chorismate, which is an essential forerunner to create three aromatic amino acids: phenylalanine, tyrosine, and tryptophan. Lacking chorismate, these amino acids necessary for protein synthesis cannot be produced in plants (Figure 2.2).

Second, the inhibition of EPSPS leads to uncontrolled flow of carbon into the shikimate pathway resulting in deregulation of carbon fixation (Duke and Powles, 2008). This is potentially because of the lack of feedback inhibition caused by reductions in the aromatic amino acids.

Thus, large carbon flow to S3P via the shikimate pathway results in shortages of carbon for other important pathways, possibly disrupting other aspects of plant metabolism (Fedtke, 2012).

Inability to synthesize chorismate and reduction of carbon fixation in plants have been explored in previous studies due to applying glyphosate. It is clear that glyphosate has only one site of action (inhibition of EPSPS) in all plants. Hence, glyphosate is a non-selective herbicide because the enzyme EPSPS is conserved and activated on a wide range of plant species. Nevertheless, the enzyme EPSPS is not found in mammals or birds; thus, glyphosate is considered non-toxic with normal doses.

Glyphosate can be sprayed on growing plants, making it beneficial for pre-harvest weed management applications. The dose efficiency of glyphosate applied to the plants is usually dependent on its efficacy of uptake and its extent of translocation. To assist in these two events, glyphosate is taken up gradually through plant (leaves) surfaces then it is penetrated across the cuticle. The amount of glyphosate inside the leaf, not removed by washing, demonstrates the efficacy of uptake (Dill, Sammons, et al., 2010).

After uptake, the herbicide is able to be translocated across the cuticle via distribution in plant species. However, glyphosate can be transported through the phloem from leaves to other plant tissues, causing phytotoxicity in growing tissues (Duke and Powles, 2008). Thus, the diffusion pattern of glyphosate to sink tissues is determined by the efficiency of translocation. Slow mode of action, excellent uptake, and good translocation are the primary factors for effective treatment of glyphosate prior to harvest.

2.1.2. Incremental Usage

Glyphosate has been in the market for four decades, and its total volume used in the agricultural sector from 1974 to 2014 in the United States accounted for 1607 million kg (Benbrook, 2016). Glyphosate was initially registered in 1974; farmers and ranchers applied 0.36 million kg of glyphosate that year (Benbrook, 2016, Myers, Antoniou, et al., 2016). In the 20 years following its commercial registration, glyphosate application steadily rose to 12.5 million kg by 1995, which made it the seventh most applied pesticide.

However, in 1996, genetically engineered herbicide tolerant (GEHT) crops were approved for planting in the United States. In addition, Monsanto Company introduced GEHT under name Roundup Ready® to the market with their first GEHT crops being Roundup Ready® soybean.

Roundup Ready® crops are genetically engineered to be resistant to glyphosate. Thus, glyphosate is an optimal herbicide for improvement of herbicide-resistant crops by transforming a glyphosate-resistant enzyme EPSPS in the plant (Fedtke, 2012). The version of EPSPS generated in a particular strain of Agrobacteria has slightly changed shape. This change prevents glyphosate from binding, allowing that resistant enzyme EPSPS to catalyze the reaction of amino acid synthesis. Consequently, the amount of glyphosate use rose rapidly to 83 million kg in and continued to increase by 2014 with an estimate of 110 million kg (Myers, Antoniou, et al., 2016). This represents a 300-fold increase in usage since its commercial registration in the United States.

2.1.3. Pre-harvest Applications of Glyphosate

Registrations for pre-harvest applications of glyphosate as a harvest aid are constantly active in several countries, such as the United States and Canada. Glyphosate is commonly applied prior to harvest as a method to control weeds, promoting timely and efficient harvest (Manthey, Chakraborty, et al., 2004). This practice will not only kill any weeds found in the field but will also damage the crops, which dehydrates the foliage. Typically, glyphosate can be sprayed to crops when a grain reaches physiological maturity; the kernel is at the hard dough (HD) stage. However, the crops cannot legally be treated with glyphosate at soft dough (SD) stage, which is less physiologically mature than crops treated at the HD. The question now is to see what happens when glyphosate is applied at different moisture contents present at these stages.

There are some advantages and disadvantages of pre-harvest glyphosate applications shown from previous studies. The pre-harvest application provides a few benefits to producers. For example, the pre-harvest glyphosate has demonstrated the ability to reduce the moisture content of harvested crop, promoting effective harvest (Bresnahan, Manthey, et al., 2003, Griffin, Boudreaux, et al., 2010). Additionally, the glyphosate applications can speed up a harvest operation, which allows for an earlier crop harvest (Benbrook, 2016). Delaying crop production could be related to lower yields in soybean (Philbrook and Oplinger, 1989).

Pre-harvest glyphosate treatment has undesirable aspects that could influence crop quality. Pre-harvest glyphosate application on crops grown for seed might lead to a reduction in germination and plant growth (Monsanto Company, 2017). The oats treated with pre-harvest glyphosate at 35% seed moisture has resulted in lower viscosity (Guenther, 2017). This finding in oat flour is not desirable for manufacturing oat products because an elevation of viscosity in the flour is associated

with physiological benefits in the body. Application of glyphosate to oats at 35% seed moisture can diminish kernel size and kernel weight while the treatment at 30% seed moisture leads to producing more plump kernels (Guenther, 2017). It is clear that the pre-harvest application of glyphosate in softer dough, at 35% moisture or higher, produced negative effects on the physical quality of oats.

2.2. Glyphosate Toxicity

2.2.1. Effects in Non-target Plant Species

As a broad-spectrum herbicide, glyphosate has toxic influences on plant species. Impacts on reduction in activity of mycorrhizal fungi and increased susceptibility to plant damages are two serious effects of using glyphosate (Cox, 1995). Mycorrhizal fungi are positive microorganisms living in and around plant roots. The fungi can assist plants to absorb water and protect plants from weather conditions, such as drought. Thus, at high doses, glyphosate is toxic to large species of mycorrhizal fungi and affects the growth of the beneficial fungi.

Glyphosate and AMPA residues are found in non-target crop species after application. Glyphosate residues and its metabolite (AMPA) can have phytotoxic effects on crop species. Phytotoxicity is plant damage, as leaf injury, which can be caused by a chemical spray like glyphosate. Phytotoxicity tends to influence plant performance via reducing absorption of essential nutrients and productivity, especially with a decrease in crop yield and quality (Duke and Powles, 2008). For example, plant biomass has been reduced up to 50% in several non-target plant species following glyphosate treatment (Bai and Ogbourne, 2016).

2.2.2. Environmental Contamination

Glyphosate can move around the plants and will remain in the soil. While glyphosate binds strongly to soil, it can be transferred through groundwater, which leads to environmental contamination (Myers, Antoniou, et al., 2016). After repeat applications, this herbicide is able to become a long-term source of soil and groundwater contamination. In pond water of ground following application, studies resulted in the half-life of glyphosate, which persisted 12 to 60 days (Cox, 1995).

The half-life of glyphosate in soil ranges between several days to a few months, depending on soil composition and location. For instance, half-lives of glyphosate and AMPA have been estimated to be up to 151 and 98 days, respectively (Bai and Ogbourne, 2016). Nevertheless, the wide range in persistence of these compounds is based on soil properties and environmental condition. The

high half-life of glyphosate might increase the long-term risk of environmental pollution. Persistent long-term use of glyphosate could offer a threat to soil health, with possible negative effects on crop productivity.

2.2.3. Human Toxicity

Since the shikimate pathway (glyphosate target) is not found in humans, glyphosate is considered non-toxic to humans. In addition, the US Environmental Protection Agency classifies glyphosate as a "least toxic" category, category IV, component for animals (Williams, Kroes, et al., 2000). Even so, there is a debate discussing the safety of glyphosate with long-term toxicity to humans.

Residues of glyphosate increased an estimated 10 to 30 % in grain samples from 2007 to 2013 (Benbrook, 2016). Because of this increase, glyphosate's residues has been found in kidney, liver, fat and muscle at high levels in animal feeding studies (Myers, Antoniou, et al., 2016). Consuming these kind of animals might pose a risk of liver damage and chronic kidney disease in humans.

Additionally, glyphosate and AMPA can be a factor of cardiorespiratory toxicity through hemolysis and hemoglobin oxidation in human erythrocytes (Bai and Ogbourne, 2016, Kwiatkowska, Huras, et al., 2014). This happens because high concentration of glyphosate and AMPA would only be present in a poisoning solution and will not occur by consuming food treated with glyphosate.

2.3. Oats

2.3.1. Oat Production

Oat (*Avena sativa*) is defined as a cereal crop that yields grain as an edible component. According to Statistics Portal website, planting of oat presently ranks sixth in world grain production behind corn, wheat, barley, sorghum, and millet (2017). Russia is the top producer of oats worldwide with 200 million bushels produced in 2010 (Marshall, Cowan, et al., 2013). The United States of America is one of the global producers of oat and accounts for seven percent of the total world's supply of the grain (Strychar, 2011). However, there was a decrease in the United States oat production over a ten-year period from 2000 to 2009, due to a decline in the demand for oat. The United States harvested 89.5 million bushels of oats in 2015 (United States Department of Agriculture, 2016).

North Dakota was the fourth largest oat producing region in the United States based on production value of oat in 2015, following Wisconsin, South Dakota, and Minnesota. North Dakota

produced 10.3 million bushels of oats in 2015 (United States Department of Agriculture, 2016). Oats planted in North Dakota are usually of high quality, including excellent test weight and great protein levels (Ransom, McMullen, et al., 2018). Some oat cultivars are Deon, Shelby, Rockford, and Souris that are grown at different places (such as Minot and Prosper) in this state.

2.3.2. Composition of Oat Grain

The four major parts of oat grain structure are hull, bran, endosperm, and germ that create more elongated kernel compared to other grains (Figure 2.3). The hull accounts for 25 to 36% of the oat grain weight and mainly consists of cellulose and hemicellulose including small amounts of lignin (Gulvady, Brown, et al., 2013). The function of hull is to protect the oat seed from harsh environments (Gulvady, Brown, et al., 2013). The oat hull covers a groat, which is the component for human consumption (Figure 2.3). The groat contains the remaining parts of the structure including: bran, endosperm, and germ.

The second portion of oat kernel is the bran, which makes up 20 to 25% of the groat by weight (Fulcher and Miller, 2011). The bran is a major source of vitamins and minerals for human consumption. The bran contains a pericarp, seed coat, nucellus, and an aleurone layer. The aleurone layer of bran is comprised of small quantities of protein bodies and dietary fiber, called β-glucan.

Another important part of the oat grain is a starchy endosperm that represents the largest fraction of oat, making up 70% of groat's weight (Fulcher and Miller, 2011). The endosperm is considered the storehouse of polysaccharides because it encompasses a large amount of starch; this portion stores some proteins and lipids as well. Markedly, the starchy endosperm is a primary source of β-glucan.

The last significant fraction of the oat grain is the germ, which accounts for the smallest proportion of the oat groat (Gulvady, Brown, et al., 2013). The germ, or embryo, is made up of the embryonic axis and scutellum. The germ is naturally high in proteins and lipids and only contains a small amount of starch.

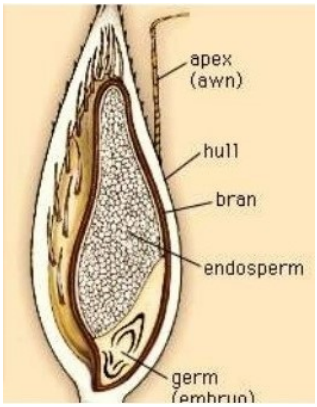

Figure 2.3. Anatomical Section of an Oat Kernel

In terms of human consumption, the groat (including bran, endosperm, and germ) has the majority of nutrients, consisting of approximately 59% carbohydrates, 10% fiber, 15% proteins, 7% lipids, and 4% vitamins plus minerals (Gulvady, Brown, et al., 2013). In addition, β-glucan should be found mainly in two regions of the oat groat: the endosperm and the aleurone layer (Fulcher and Miller, 2011). The β-glucan makes up a high portion of the endosperm and a small part of the aleurone layer.

2.3.3. Human Consumption of Oat

Per person annual consumption of oat products in the United States increased from 4.0 pounds in 1984 to 6.5 pounds in 1990 (Strychar, 2011). Oats planted in this country are mostly harvested for animal feed, and the rest is used in industrial applications, which considered is as the first benefit of oat. Industrial applications create a variety of foods in the United States, such as oatmeal, muffins, and cookies, which are manufactured by using oat in their processing. Quaker Oats Company claimed that soluble fiber (β-glucan) from oatmeal is considered as a part of low saturated fats and low cholesterol diet leading to reduce the risk of heart diseases. This claim was approved by the U.S. Food and Drug Administration in 1998.

Oatmeal is prepared from rolled or steel cut groats. Oatmeal can be prepared in 5 to 7 minutes and has many health benefits. Due to the increasing demand for health food, oatmeal consumption has increased because of its high nutritive value. Carbohydrates are the primary component found in oatmeal at 27 g per 40 g (Quaker Oat Company, 2018). This is followed by protein at 5 g per 40 g of oatmeal, fiber at 6 g per 40 g, and lipid at 3 g per 40 g. Oatmeal is rich

in B vitamins, and a 40 g serving of oatmeal provides 20% of the recommended daily value (DV) of thiamin and 26% of the DV of niacin. Furthermore, oatmeal is high in the minerals, such as phosphorus (11% DV in 40g serving) and iron (8% DV in 40g serving).

Regarding the benefits of oat, oatmeal is rich in protein and contains desirable fat constituents compared to wheat products. The consumption of 90 grams of oatmeal daily provides about 13 to 15% of dietary energy (Welch, 2011). In addition, oatmeal is comprised of β-glucan, which has been found to provide health benefits. High β-glucan is preferred in the human diet because the fiber is able to lower elevated cholesterol and to modulate fluctuations in blood sugar (Welch, 2011).

2.4. Oat β-Glucan

$(1 \rightarrow 3)(1 \rightarrow 4)$ β-D-glucan, known as β-glucan, is an unbranched polysaccharide component of the endosperm and aleurone layers of oat groat (Wood, 2010). Oat contains 3 to 5% of β-glucan in the endosperm of cell walls (Anttila, Sontag-Strom, et al., 2004). Chemically, oat β-glucan refers to the non-starch polysaccharides of cell walls in its grain and includes long linear chains of β-D-glucopyranosyl units. Structure of oat β-glucan is made up of unbranched mixed-linkage $(1 \rightarrow 3)(1 \rightarrow 4)\beta$-D-glucans.

More specifically, the chemical structure of oat β-glucan consists of linear unbranched $(1 \rightarrow 4)$ linkages in groups of two to four units, which are separated by one β-$(1 \rightarrow 3)$ linked glucose unit (Figure 2.4). The structure of oat β-glucan is dominated and controlled via β-$(1 \rightarrow 3)$-linked cellotriosy1 and cellotetraosy1 units, which lead to no mixture between the $(1 \rightarrow 3)$ and $(1 \rightarrow 3)$ linked polysaccharides. Thus, the β-$(1 \rightarrow 3)$ linkages occur mostly in a single unit (Ahmed, Anjum, et al., 2011, Anttila, Sontag-Strom, et al., 2004, Fulcher and Miller, 2011). Because of the successive β-$(1 \rightarrow 4)$ linkages in blocks separated by a β-$(1 \rightarrow 3)$ linked glucose unit, β-glucan forms viscous solutions.

The ratio of β-$(1 \rightarrow 3)$-linked cellotriosy1 to β-$(1 \rightarrow 3)$-linked cellotetraosy1 units in oat β-glucan is about 2:1, which is less than barley β-glucan at 3:1 (Wood, 2011). In short, oat β-glucan is defined as a linear unbranched polysaccharide combined of approximately 70% of 4-linked and 30% of 3-linked glucopyranosyl units. Because of this, the 4-linked glucopyranosyl units occur mainly in attached groups of two to four glucose units while the 3-linked glucopyranosyl units happen alone after two or more of 4-linked glucopyranosyl units.

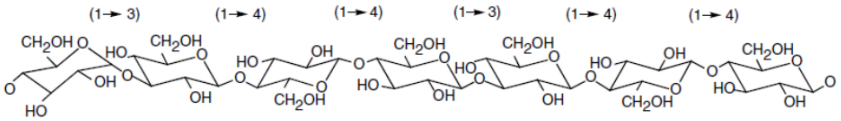

Figure 2.4. Structure of β-D-Glucan (Izydorczyk, Cui, and Wang, 2005)

2.4.1. Molecular Weight of β-Glucan

Molecular weight, or molar mass, (M_W) is a basic characteristic of oat β-glucan, and its determination is important and related to physiological functions. A recent study showed that the high molecular weight of β-glucan is more useful in reducing blood cholesterol levels compared to low M_W forms of the fiber (Wang, Storsley, et al., 2016). The weight-averaged molecular weight of oat β-glucan has been recorded ranging between $6.5x10^4$ and $3.1x10^6$ (Kala, Hamaker, et al., 2013). Oat β-glucan consists of up to 200,000 glucose units, resulting in a high range of average molecular weight (Kala, Hamaker, et al., 2013). Since β-glucans contain large molecules and are polydisperse, the determination and size distribution of the various M_W can be quantified using high performance size exclusion chromatography (HPSEC) (Wood, 2011).

The HPSEC is used with a combination of detectors, such as refractive index (RI) and multi-angle laser light scattering (MALS) (Storsley, Izydorczyk, et al., 2003). The HPSEC includes the two detectors, which determine the average molecular weight (M_W) and polydispersity index, molecular weight size distribution, (M_W/M_n). For each sample, the polydispersity can be calculated from the ratio of average molecular weight to number average molecular weight (Christensen, Ulset, et al., 2001). The HPSEC chromatograph of $(1 \rightarrow 3)(1 \rightarrow 4)$ β-D-glucan in solution is gained from two detection systems: a refractive index detector for size distribution and a light scattering detector for direct molecular weight determination (Wang and Cui, 2005). In one measurement, the molecular weight determination (M_W) and polydispersity index (M_W/M_n)can be calculated at the same time.

In this method, when a polymer solution enters into a HPSEC column, its molecules have different elution rates. The small particles trap into the stationary phase pore system and can enter the gel. The smaller particles consume more volume and elute later. Thus, their retention time is longer to traverse (Wyatt Technology Corporation, 2013). In contrast, the large particles

simply pass through the stationary phase pores, and they cannot enter the gel. The larger particles take less volume and elute sooner. Hence, their retention time is shorter to go across. Therefore, larger particles elute via the column faster than smaller solutes. It is important to know that each size exclusion column contains a range for each molecular weight for separation. When molecular weight of a solute is high, its particles trap into the stationary phase. Molecular weight of a solute is low, the particles enter and pass via the stationary phase.

2.4.2. Solubility (Extractability) of β-Glucan

Molecular weight and chemical structure are the two main factors that determine the solubility of β-glucan (Wang and Cui, 2005). In most cases, an increase in molecular weight of oat β-glucan leads to a decrease in its solubility because of an increase of cohesive energy density (CED). CED is the amount of energy required to entirely remove a unit volume of molecules from their interiors to the surface inside the solubilizing liquid to infinite separation. Larger of β-glucan molecules provide a higher M_W and size, indicating that solvent molecules have to struggle to surround bigger molecules. The greater M_W of β-glucan results in less solubilization of the fiber.

On the other hand, chemical structure of β-glucan promotes its solubility, providing health benefits. Oat β-glucan is more soluble and viscous than cellulose because of the β-$(1 \rightarrow 3)$ linkages, which provide unique physiochemical features in health promotion (Cui and Liu, 2013). A set of $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ linkages for β-glucan disturbs intermolecular hydrogen bonding, resulting in a water soluble fiber molecule (Johansson, Tuomainen, et al., 2007). Therefore, the distinctive structure and high solubility of β-glucan can be responsible for its health benefits.

β-Glucan is classified as a soluble fiber, and it represents the major component of the soluble fiber fraction in oats. Oat contains more soluble β-glucan than other cereals. For example, the percentage of the solubility of β-glucan is estimated at 88% per 100 g of oatmeal, 69% per 100 g of pealed barley, and 40% per 100 g of whole grain wheat (Welch, 2011). One way to increase β-glucan solubility is through processing, especially heating and steaming. Heat treatments increase β-glucan solubility, which plays a major role in its health effects (Grundy, Quint, et al., 2017).

2.4.3. Viscosity of Oat β-Glucan

Viscosity, which refers to the resistance to flow of a fluid, is the key physiochemical feature related to the physiological influence of β-glucan. Attenuating postprandial blood glucose and lowering LDL cholesterol levels are primary attributed to the elevation of β-glucan's viscosity

(Gamel, Abdel-Aal, et al., 2012). The ability to generate high viscosity of β-glucan depends largely on concentration, molecular weight, and solubility of the fiber. In addition, processing affects oat functionalities such as β-glucan's viscosity when oat products have been manufactured.

The high amount of β-glucan in oats can produce the viscosity required for the health benefits (Gamel, Abdel-Aal, et al., 2012). Furthermore, high β-glucan content provides a higher molecular weight for β-glucan thus providing greater viscosity, which is mandatory for the physiological effects as well (Wang, Storsley, et al., 2016). The concentration and molar mass of β-glucan should be at levels high enough to create the viscosity needed for reducing blood glucose and for decreasing LDL cholesterol (Johansson, Tuomainen, et al., 2007). Viscosity is primarily influenced by the concentration and M_W of oat β-glucan, and extractability (solubility) is also essential to developing β-glucan's viscosity. When heat treatments are applied during oat processing, β-glucan's viscosity increases, resulting in greater nutritional value of oats (Decker, Rose, et al., 2014). Thus, heat treatments have clear impact on increasing solubility and viscosity of β-glucan.

The Rapid Visco Analyzer (RVA) can be used for the measurement of oat β-glucan viscosity. The RVA is a rotational viscometer that is able to continuously record viscosity of samples under controlled temperature. The RVA attaches a sample in a solvent, which maintains them in suspension throughout the test. The RVA has been used as a standard methods in cereal applications, with a technique to measure pasting properties of oat flours (AACCI, 2010). Furthermore, RVA is utilized to examine the effects of β-glucan molecular weight and solubility on the viscosity of oat flour slurry using specific enzymes (Liu and White, 2011).

A specific in *vitro* extraction protocol for oat β-glucan was established that uses digestive enzymes including human salivary α-amylase, pepsin, and pancreatin in order to analyze viscosity by Beer, Wood, et al., 1997, and Gamel, Abdel-Aal, et al., 2012. The digestive enzymes are added to the RVA canister containing the oat flour with the buffer. The measurement is conducted at body temperature, 37°C. In the RVA assay, it is important to ensure the plateau or peak that are complete before ending the assay. The plateau indicates that starch, protein, and lipids have been digested, therefore the impact of these components on viscosity is limited. Final viscosity from RVA is different among oat samples because oats are diverse in β-glucan content, molecular weight, and solubility.

2.4.4. Mechanism of Cholesterol Reduction by β-Glucan

Oat β-glucan has been found to have many health benefits and is a good addition to the human diet. An increasing β-glucan consumption up to 3 g/day can lead to a reduction in total cholesterol levels (FDA, 1998). It accepted that for every 1% decreasing LDL cholesterol levels, the risk of growth of coronary heart disease is lowered by 1 to 2%. Studies have demonstrated that there is an inverse relationship between viscosity β-glucan and LDL cholesterol levels after four weeks of dietary observation (Kala, Hamaker, et al., 2013).

One of the most commonly proposed mechanism of cholesterol-lowering is that β-glucan forms a viscous layer or increases viscosity in the small intestine. Improved intestinal viscosity reduces the uptake of dietary cholesterol and impairs reabsorption of bile (Daou and Zhang, 2012). Since bile acids are not reabsorbed, the synthesis of bile acids using LDL cholesterol rises, leading to lower LDL and total cholesterol levels (Kala, Hamaker, et al., 2013). Therefore, β-glucan's viscosity plays an important role in the mechanism of cholesterol reduction.

2.4.5. Controlling Blood Sugar by β-Glucan

Another main health advantage of oat β-glucan is to control blood sugar. The viscous and soluble β-glucan modulates the chyme properties of the gastrointestinal tract, influencing gut motility and nutrient absorption, which are reflected in attenuating postprandial blood glucose (Behull, Scholfied, et al., 2006). Hence, ingesting oat β-glucan is beneficial for healthy people and patients suffering from type-2 diabetes. It was reported in literature that an inverse relationship exists between viscosity of β-glucan and postprandial peak blood glucose response (PBGR). High viscous β-glucan lowered postprandial PBGR (Wood, 2011).

When diets--containing β-glucan--leave the stomach, its viscosity starts to have impacts on digestion and absorption of nutrients in the small intestine. Although β-glucans are not digested in the small intestine, β-glucans increase the viscosity found in small intestine (Kala, Hamaker, et al., 2013). As the digesta moves of into the small intestine, the high viscosity might change the flow pattern resulting in a decrease of imbibing nutrients by restraining diffusion of components to the intestinal wall. The reductions of nutrient motion to the wall can affect starch digestibility and glucose absorption to the intestinal wall, which contains mucosal α-glucosidases. This has been

investigated as a mechanism for lowering postprandial PBGR by β-glucan. Moreover, inhibition of α-glucosidases is an approach for medication of type-2 diabetes (Daou and Zhang, 2012).

2.5. Oat Processing

To achieve the recommended level of β-glucan in oat products, processing operations are necessary to transform oat grains into edible foods for human. There are several steps to convert oat grains into forms that are easy to be prepared for consumption. Some essential processing of harvested oats involves cleaning and grading, dehulling, kilning, and then dry milling.

2.5.1. Cleaning and Grading

Before cleaning, it is important to determine test weight of oats and other physical quality characteristics, such as kernel size and shape as well as groat density. Test weight is a measure of the specific volume of an amount of oats, and it is expressed in lb/bu (Ames, Rhymer, et al., 2014). Typically, it is determined using a Dickey John machine by measuring the specific weight in grams of oat grains, and then automatically converting to lb/bu. A greater test weight is desirable because it is related to higher physical quality features. Thus, the milling market demands a high test weight ranging between 36 to 38 lb/bu, which is the minimum test weight for the United States grade No. 1 oats (Ransom, McMullen, et al., 2018).

Cleaning oats is a unit operation designed to take away contaminating substances and undesirable grains from oat shipments. Oat samples are cleaned through a dockage tester, which eliminates foreign materials such as impurities and dust from the oat grains. Another purpose of cleaning is grading based on width that leads to remove unwanted oat grains, such as pin oats, double oats, and light oats.

During cleaning, oats are graded or sorted by passing the grains through a series of perforated cylinders, which include holes of decreasing diameter (Decker, Rose, et al., 2014). Unwanted oats, pin oats are short and thin grains with small groats inside the hulls or without groats. Double oats refers to the grains that include two groats covered by only one hull. Light oats have regular sizes like plump kernels; however, they include very small groats. Hence, unwanted oats need to be removed by the dockage tester because they have lower test weight, while normal plump grain is an indicator of higher test weigh and higher groat percentages (Girardet and Webster, 2011).

2.5.2. Dehulling

Upon maturity, the hull is solid and becomes unsuitable for human consumption; therefore, it needs to be removed. Oats can be dehulled through mechanical dehulling using an air-pressure dehuller that contains two tools: an oat huller laboratory and grain aspirator machine. The oat huller has a spinning disk that throws the grains into impact rings, separating the hull from the groat. When 85 to 90% of the oats are dehulled, the groats with remaining hulls are thrown into the grain aspirator machine to remove the hulls left and broken groats. The mechanical method for dehulling oats is commonly used by oat experts and commercial millers. However, the problem of using mechanical dehulling is the potential groat breakage due to high rotational speeds and high air-pressure in the dehuller (Ames, Rhymer, et al., 2014). Because of this, groat breakage might lead to losing weight of the oat groats weight.

The degree of dehulling can be controlled by moisture content and groat percentage, which are important grain properties due to an effect of dehulling efficiency (Girardet and Webster, 2011). If the moisture content of oat samples is too high, efficient dehulling is low, leading to lower production rates and milling problems. In contrast, if the moisture content of the oat samples is too low, dehulling efficiency is high, resulting in groat breakage. It has been recommended that 12 to 13% of moisture content in oat kernels is an optimum range for dehulling and milling processes (Decker, Rose, et al., 2014). In addition, oat samples with lower hull content have higher dehulling efficiency, which leads to high groat percentage.

2.5.3. Heat Treatments

Oats are rich in lipids compared to other cereals and contain high levels of lipid-digesting enzymes. During oat processing, the groat lipases need to be inactivated through steaming and dry kilning in order to avoid spoilage in samples. The first of three reasons for applying heat, inactivation of the lipases prevents the breakdown of lipids and the resulting unpleasant taste so that the lipids are not oxidized. Second, applying heat to inactivate groat lipases positively affects physicochemical properties of oat fiber by an elevating viscosity and solubility of β-glucan (Grundy, Quint, et al., 2017). Third, the steaming and kilning reduces microbial growth in the groats. That means both steaming and drying kiln operations can decrease groat damages and maintain the nutritional value of the oats. Steaming is accomplished by first passing the oat groats over long

vertical columns and then raising the temperature of the groats with steam (Decker, Rose, et al., 2014). After steaming, the groats are exposed to dry heat, kilning, to reduce the moisture content to 10 to 12% to enhance their storing condition.

2.5.4. Dry Milling

A dry milling process is utilized to create a portion including high β-glucan content and to provide uniform particle size. One of the significant reasons to run dry milling on oat groats is to enrich the β-glucan content, to increase extraction of the dietary fiber (Stevenson and Inglett, 2011). Since dry milling produces an elevated amount of β-glucan, it also generates small oat flour particles. The small particles of the flour can achieve higher viscosity and solubility of β-glucan compared to large particles (Gamel, Abdel-Aal, et al., 2012). The principle of milling by the Udy Mill is that the impeller and centrifugal forces rotate at a high velocity causing a high speed air-flow to push oat groats against the abrasive surface to grind the groats. When oat flour becomes small enough, it moves with air-flow out of the miller into a sample collection bottle.

2.6. Justification, Objectives, and Hypothesis

2.6.1. Justification

Consumer hesitation concerning food ingredients and compositions is growing, especially over the use of pesticides on foods. In 2015, the Canadian firm Grain Millers, Inc. announced they would no longer purchase oats treated with pre-harvest glyphosate, citing performance issues comparable to an early freeze. Since the use of pre-harvest glyphosate allows for a more convenient harvest, it is worth examining whether this practice does, in fact, cause a decrease in oat quality. However, no research has yet been performed on the effect of glyphosate on oat β-glucan properties.

2.6.2. Objectives

- To determine the total β-glucan production in oat groats treated with pre-harvest glyphosate versus untreated oat groats
- To evaluate the differences in properties of the β-glucan in the treated versus untreated oat groats

2.6.3. Hypothesis

Pre-harvest applications of glyphosate will not affect the deposition of β-glucan in oat groats. Early application of glyphosate at a high moisture stage will affect the physiochemical properties of the β-glucan.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Samples

Oat grains utilized in this experiment were grown during the 2015 crop year at two locations: Prosper, ND and Minot, ND. Eighteen samples were cultivated in Prosper and consisted of the cultivars, Souris and Rockford. Each cultivar from Prosper received three treatments of glyphosate: untreated check, soft dough, and physiological maturity (Figure 3.1). Each of the treatments included three replications. Another 18 oat samples were harvested in Minot and consisted of the same treatments and replications that were used in Prosper.

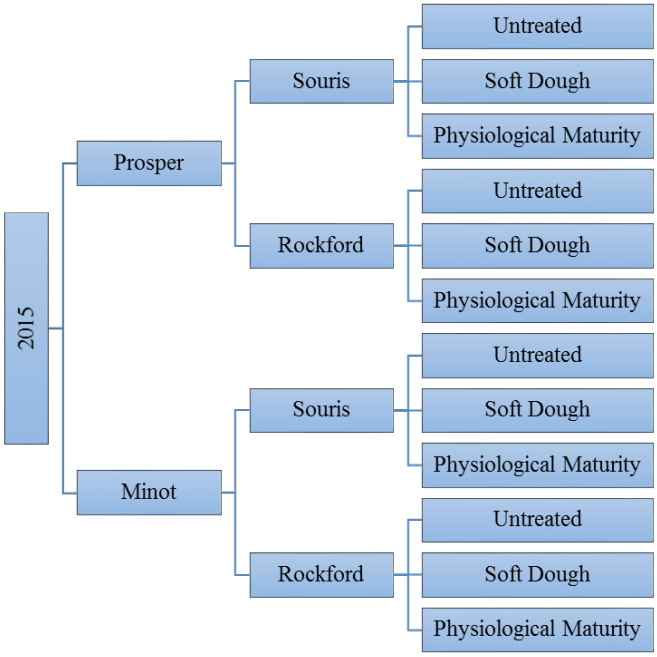

Figure 3.1. Summary of Crop Year 2015 Samples from Prosper and Minot

Glyphosate was applied in the form of Monsanto's Roundup PowerMAX® Herbicide, Registration No. 524-549 at a concentration of 866 grams glyphosate acid/ha. The untreated check treatment had no glyphosate. Soft dough treatment had glyphosate applied at a stage when the oat grain color had changed from green to yellow, and the kernel was no longer liquid but altered

from soft to hard consistency of dough. Physiological maturity treatment had glyphosate applied at a stage in which the oat had become completely yellow and dried, and the kernel had become hard and harsh.

The oat plots in Prosper were planted on May 22, 2015 and harvested on August 26, 2015. Glyphosate was sprayed on August 5 for the soft dough treatment and August 12 for the physiological maturity treatment. The average air temperature was within 2°F degrees of the 30 year average for each month of the planting season (Table 3.1). Rainfall was above average at 2.8 inches in May and below average at almost 1.2 inches in August; however, June and July recorded near average rainfall levels.

Table 3.1. Weather Data for Crop Year 2015 in Prosper, ND

Month	Air Temperature (°F)		Rainfall (inches)	
	Actual	30 Year Average	Actual	30 Year Average
May	54	56	5.85	3.05
June	67	66	4.32	3.95
July	70	70	3.48	1.43
August	67	69	1.43	2.62

North Dakota Agricultural Weather Network, 2018

The oat plots in Minot were planted on May 1, 2015 and harvested on August 19, 2015. Glyphosate was sprayed at the soft dough stage on July 31 and at the physiological maturity stage on August 5. The average air temperature was within 2°F degrees of the 30 year average for each month of the growing season (Table 3.2). Rainfall was within approximately 1 inch of the 30 year average for all months of the planting season, except for June which recorded a rainfall of 2.6 inches above average.

Table 3.2. Weather Data for Crop Year 2015 in Minot, ND

Month	Air Temperature (°F)		Rainfall (inches)	
	Actual	30 Year Average	Actual	30 Year Average
May	53	54	3.12	2.57
June	65	63	6.10	3.49
July	70	69	1.82	2.55
August	68 67	1.09	2.00	

North Dakota Agricultural Weather Network, 2018

3.2. Sample Preparation

3.2.1. Test Weight

Test weight was determined using a Dickey-John GAC 2100b analyzer (Dickey-john, Auburn, IL). When receiving the oats, 400g of each sample was placed into the top-slot of the Dickey John machine to measure its moisture content and test weight. After that, the top right button of the machine was clicked to drop 250g of the sample inside the instrument, and the spillover was dropped out into the machine's drawer to measure test weight (Figure 3.2). The outcomes of moisture content and test weight of the oat sample were recorded. Then, the bottom right button was pressed to flush the sample onto the drawer. The total sample was collected for the cleaning process.

Figure 3.2. Dickey John GAC 2100 2100b Analyzer

3.2.2. Cleaning Standard Operating Procedure

The oats were cleaned using a dockage tester (Figure 3.3) made from Carter-Day International Company, Minneapolis, MN. The oat cleaning eliminated foreign materials, such as impurities and dust from the oat grains. Another purpose of cleaning was to remove pin oats (grains with tiny or no groats). The settings for cleaning were as follows:

- #6 Riddle: foreign material and double oats
- #4 Oblong sieve: thick/plump kernels
- #6 Tringle sieve: thin kernels
- Blank: foreign material
- Air flow: setting #4
- Feed rate: setting #5

Figure 3.3. Dockage Tester

The cleaner device had to be worked before placing a sample and had to also be free of material falling into the pans with free shakes. The sample was then cleaned by placing it into the dockage tester and letting the process continue until all of the sample was in the pans (Figure 3.3).

After that, the thin oat kernels and foreign materials were taken from their pans and their masses were recorded. The sample of plump oat was weighed out too and placed into a plastic bag for the dehulling process.

3.2.3. Dehulling and Groat Separation

After cleaning, the groats were then separated from the hulls by a Codema Laboratory Oat Huller. Each of the oat grain sample was dehulled using an air-pressure dehuller to remove the hull from the grain resulting in two portions: the hulls and the groats. According to Codema Company, 45g of the plump oat sample was placed into the slot of oat huller laboratory. The switch was turned on, and the sample was dehulled in the oat huller in exactly two minutes. During the dehulling process, the oat grains passed into a spinning rotor in the oat device, and the kernels collided with the oat huller's wall. Therefore, the hulls and impurities were separated from the groats, and they were sorted into two collection canisters (Figure 3.4). The first canister was used for gathering hulls and impurities, and another canister was used for dropping mainly groats and some hulls.

Figure 3.4. Codema Laboratory Oat Dehuller

Then, the groats with some hulls left were dropped into the grain aspirator machine. The machine processed in a few seconds resulting in hulls and groats (Figure 3.5). Sequentially, the groats were weighted out and placed in a single plastic bag. The reason of using the grain aspirator was entirely to remove the remaining hulls and broken groats. Whole oat groats were steamed and kilned, and then ground.

Figure 3.5. High Quality Grain Aspirator 63

3.2.4. Heat Treatments

Heat was applied in order to inactivate enzymes such as lipase, lipoxygenase, and β-glucanases, which affect the physicochemical properties of oat and oat β-glucan during oat processing (Ovando-Martínez, Whitney, et al., 2013). To apply heat, 100g samples of the groats were first placed in mesh baskets (Figure 3.6, A). Then, the groats were steamed at 100% humidity, at 100°C for 40 minutes using an Adcraft full size food cooker/warmer 1500W (Admiral Craft, Westbury, NY). During steaming, each basket of groats was stirred via a small stirrer every 10 minutes to prevent the groats from sticking to the basket (Figure 3.6, B). The mesh basket was held in the

Adcraft warmer while stirring to prevent temperature changes. Temperature was monitored by a probe thermometer.

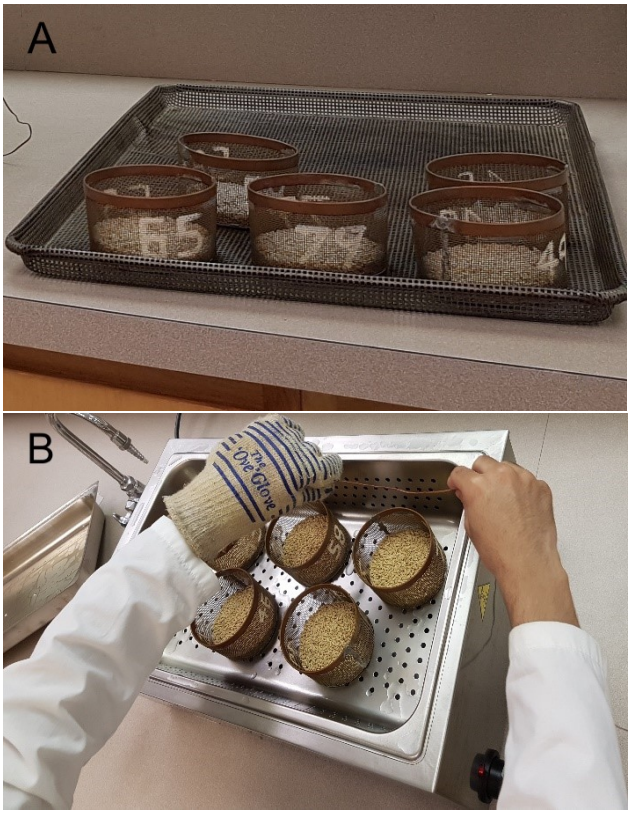

Figure 3.6. Oat Groat Steaming Heat Treatment

After steaming, the oat groats were immediately kilned at 106°C for 2 hours in a convection oven (Ovando-Martínez, Whitney, et al., 2013). The groat samples were kilned in the convection oven, stirring the samples every 20 minutes to prevent the groats sticking inside the basket (Figure 3.7). The mesh baskets were held in the oven during stirring to avoid temperature changes. Afterward, the groats were removed from the oven and left out on the bench top to cool and dry overnight at room temperature to allow the moisture to equilibrate.

Figure 3.7. Oat Groat Heat Kilned in a Convection Oven

3.2.5. Grinding of Oat Groats

The oat groats were ground using an Udy Mill to produce oat flour (Figure 3.8). The 25-gram samples of the steamed and kilned groats were milled in the Udy Mill fitted with a 0.5 mm sieve (Liu, Bailey, et al., 2010). The milling process was mandatory to provide particle size reduction of the samples resulting in an increase of surface area for further analyses.

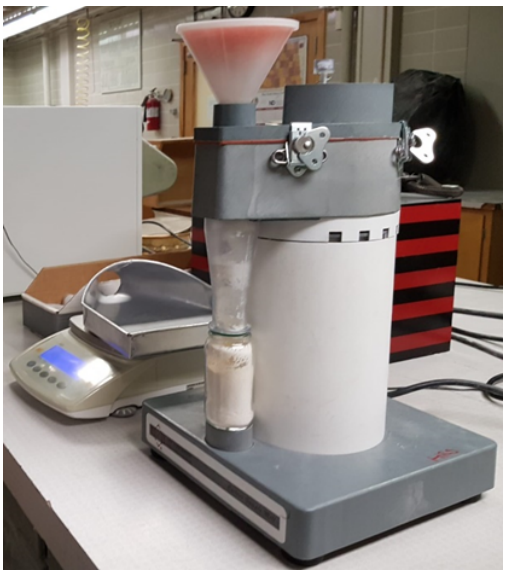

Figure 3.8. Udy Miller Used for Grinding Oat Groats

3.3. Methods

3.3.1. Rheological Measurements of Oat β-Glucan

The purpose of this method was to determine the viscosity of β-glucan in oat flour. Also, this experiment was completed utilizing a *vitro* digestion protocol that included incubating samples with enzymes to hydrolyze the starch and protein components of the oat flour (Beer, Wood, et al., 1997, and Gamel, Abdel-Aal, et al., 2012). Rapid Visco Analyzer (RVA) was used to perform the analysis on the heat treated samples. The weights of flour sample and buffer in grams were determined by the β-glucan content and moisture content of each sample. Hence, 5 to 7g of oat flour was weighed out into a canister, to which 23.00-23.95 mL of buffer containing 20mM sodium phosphate and 10mM sodium chloride with pH 6.9 was poured into the canister (Figures 3.9. a, b, c).

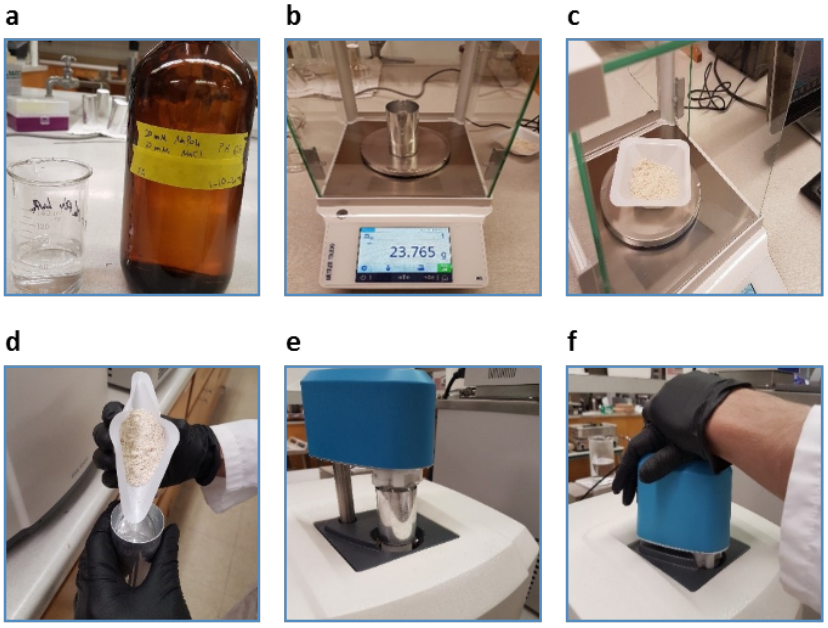

Figure 3.9. Preparation of Sample for Rapid Visco Analyzer Buffer (a), Weighing of Buffer (b), Weighing of Flour (c), Mixture of Buffer, Sample, and Enzymes (d), Insertion of Sample Can into RVA (e) and Starting of Test in RVA (f)

The enzymes added to hydrolyze protein and starch were chosen to mimic human digestion in the laboratory. Three digestive enzymes were placed to the canister in the following amounts:

63 μL of salivary amylase (220 U/mL in 2.5mM $CaCl_2$), 150 μL of pepsin (1,150 U/mL in 0.9% NaCl), and 300 μL of pancreatin (0.5 mg/mL in sodium phosphate buffer, pH 6.9). All the contents of the canister were well-mixed, and the canister was inserted into RVA for analysis of viscosity of β-glucan (Figures 3.9. d, e, f). The RVA test parameters were mixing speed at 480 rpm for 10 seconds, followed by reduction to 160 rpm and stirring for two hours at 37°C (Wang, Storsley, et al., 2016). After 2 hours, the canister was removed from the RVA, and the viscosity of β-glucan was recorded for each oat sample.

Immediately, homogenous aliquots of the slurry was taken and added to 2 mL microcentrifuge tubes with dividing to 6 tubes (Figure 3.10, a). These homogenous aliquots were centrifuged at 13000×g for 15 minutes. Next, the supernatant from all 6 tubes were removed and placed into 4 tubes. Two of the four tubes were put directly into the freezer for determination of soluble β-glucan (Figure 3.10. b, c). Another two of the supernatant's tubes were boiled for 10 minutes in a water bath beaker; then cooled at room temperature (Figure 3.10. d, e, f). Then, they were stored in a freezer at -18°C for molecular weight measurements.

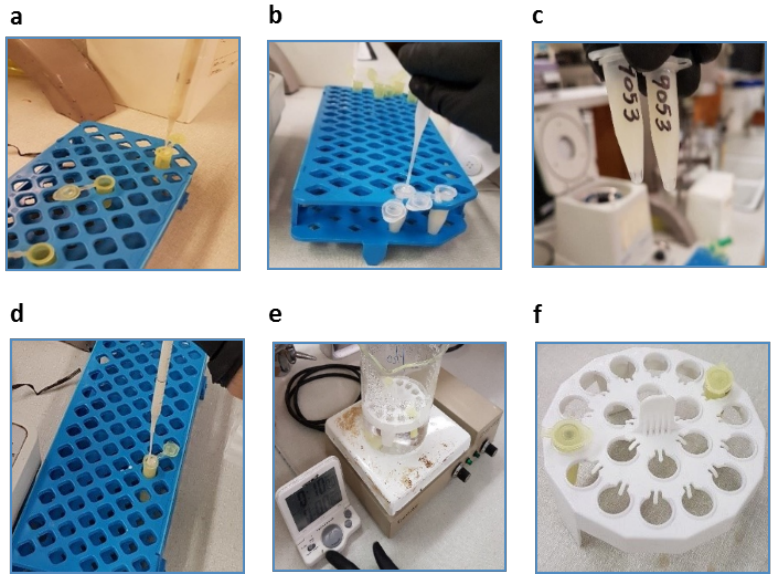

Figure 3.10. Preparation of Solubilized β-Glucan for Measurement of β-Glucan Content and Molecular Weight Aliquots of Samples for Centrifugation (a), Removal of Supernatant after Centrifugation for β-Glucan Determination (b), Tubes to be Frozen for β-Glucan Determination (c), Transfer of Supernatant after Centrifugation for Boiling (d), Boiling Tubes for Molecular Weight Determination (e), Tubes to be Frozen after Boiling (f)

3.3.2. Solubility of Oat β-Glucan

The frozen supernatants that came from the centrifuge of slurry of RVA canister were thawed. After thawing, 0.5g from each thawed sample was weighed into a clean screw cap type test tube. Then, the solubility of β-glucan was determined with a Megazyme kit, AACCI reapproved method 32-23.01 (AACC International, 1999). The principle of this method was to suspend and hydrate samples in a buffer solution of pH 6.5 (20 mM sodium phosphate). The samples were then incubated in purified lichenase enzyme and filtered. The filtrate was hydrolyzed to finalization in purified β-glucosidase. Thus, percentage of β-glucan in the slurry was calculated using the formula from Megazyme kit:

$$\%\,\text{of } \beta\text{-glucan in liquid} = \Delta A \times (F/W) \times FV \times 0.6 \tag{3.1}$$

Where:

ΔA = absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance.

F= factor for the conversion of absorbance values to μg of glucose.

FV = final volume equals 9.4 mL for oat and barley flour in example.

W = factor to express β-glucan content as a percentage of sample weight.

$$\begin{aligned} \%\,\text{of Soluble } \beta\text{-glucan} \;=\; & (\beta\text{-glucan concentration in the mixture after digestion} \\ & \div\ \beta\text{-glucan concentration in the mixture before digestion}) \\ & \times\ 10 \end{aligned} \tag{3.2}$$

3.3.3. Molecular Weight (M_W) Measurements

The slurry derived from the RVA experiment was centrifuged in order to obtain a supernatant. The supernatant was boiled, cooled, and stored into freezer. To prepare for analyzing molecular weight, 150mM sodium nitrate with 0.02% sodium azide buffer (pH 8.0) was filtered through a 0.2 μm nylon syringe filter. After thawing supernatants, the volume samples ranged from 0.74 to 6.81 mg into cap test tube depending upon the concentration of extracting β-glucan

in each sample. Then, the samples and Shodex Standard P-800 Pullulan were dissolved adding 1 mL 0.1um filtered buffer while they were gently vortexed and then heated at 90°C for 3 hours. The amount of water in ranging (0.49 – 4.54 mL) was added to samples, and extra 1.5 mL was placed to all samples to be diluted. The final β-glucan content for all samples was 1.5mg/ml.

The samples were analyzed using an Agilent 1200 high performance liquid chromatograph with refractive index (RI) detector and a Wyatt Technologies multi-angle laser light scattering (MALS). The injection volume was 50 μl and a Shodex Ohpak SB-806M column, which was held at 25°C and was used for separation. 150mM sodium nitrate containing 0.02% sodium azide was used as an eluant and the flow rate of mobile phase was 0.4 ml/min (Wang, Storsley, et al., 2016, Storsley, Izydorczyk, et al., 2003). The calculation of molecular weight was completed using Astra Software v. 6.0.5 and the dn/dc value was 0.145 for β-glucans (Storsley, Izydorczyk, et al., 2003). Normalization was conducted using a pullulan standard with a known molecular weight. The weight average molucaule weight (M_W) and the polydispersity index (M_W/M_n) were quantified using a Debye plot with a fit degree of 2 and a first-order polynomial fit.

3.4. Statistical Analysis

This experiment was designed using a split-plot layout, with location as the main plot and cultivar and glyphosate application as the sub-plots. Statistical analysis was performed with Statistical Analysis Software (SAS for Windows 9.4, SAS Institute, Cary, NC). The least significant difference (p value) was used for mean separation.

4. RESULTS AND DISCUSSION

Oat samples were grown in Minot, ND and Prosper, ND in the 2015 crop year. At each location, Rockford and Souris cultivars were planted. Each cultivar from both locations received three treatments: application of glyphosate at the soft dough stage, application of glyphosate at the physiological maturity stage, and no glyphosate application.

4.1. Physical Quality of Oat Groats

Groat quality measurement can be completed to express the final characteristics of oat quality in advance. For example, greater plump oats indicate to a higher groat percentage (GP) and better test weight, which meet the quality requirements of milling oats. Test weight is a measure of the specific volume of oats that can be included with a standard bushel weight. GP refers to the amount of hull-less grains acquired after dehulling, called as the percentage of the sample weight. GP is calculated through dividing the groat weight by the kernel weight before dehulling. High GP is indicative of a good marker of milling yield in oats.

4.1.1. Plump Oats

The results from the grading experiment showed a general change that plump oat percentages ranged from 90.56-92.68% for the crop 2015 year. Significant ($p < 0.05$) differences were found among the glyphosate treatments (Table 3). Treatment at the soft dough stage led to a significantly ($p < 0.05$) lower percentage of plump oats by 90.56% versus untreated samples with 92.32% plump. This is potentially because the oats treated at the soft dough stage did not completely mature or develop, resulting in smaller grains. Application of glyphosate creates disruption of the shikimic acid pathway leading to high carbon outflow in the pathway. Thus, glyphosate application during seed development with less carbon available can reduce the seed production, resulting in smaller oat grains (Griffin, Boudreaux, et al., 2010).

4.1.2. Oat Groats

The findings from the dehulling experiment demonstrated a general trend that groat percentages ranged from 68.90% for soft dough treatment to 70.28% for untreated controls. Groat percentage was significantly ($p < 0.05$) lower in the soft dough treatment oats compared to other treatment samples (Table 3). Similarly, the groats treated at the soft dough stage did not entirely

mature or develop (by contrast, the treatment at physiological maturity stage), resulting in smaller groats. Application of glyphosate during seed development might result in a lower production of seed, leading to smaller groats. Early glyphosate application led to a lower groat percentage because groat was smaller, which leaded to a higher hull proportion being in the kernel weight.

4.1.3. Test Weight

Test weight was not significantly different ($p > 0.05$) among the three treatments. Test weight for the variety of treatments ranged from 36.24-36.90 1b/bu, which is within the range for the United States grade. No 1 oats (Table 3). The lack of significant difference in test weight among the three treatments is surprising as there was a significant difference in plump oat percentage, which is related to test weight (Ames, Rhymer, et al., 2014). Test weight was not influenced from glyphosate applied at soft dough or physiological maturity stages.

Table 4.1. 2015 Physical Quality of Oat Groats

Glyphosate Treatment	Plump (%)	Groat Percentage (%)	Test Weight (1b/bu)
Untreated	92.32	70.28	36.24
Soft Dough	90.56	68.90	36.90
Physiological Maturity	92.68	70.00	36.61

Values are averages of all location/cultivars. Values with the same superscript level are not significantly different ($p > 0.05$). Least significant difference was used for mean separation. 1b/bu: pounds/bushel.

Overall, glyphosate applied at the soft dough stage reduced the percentage of plump grains and groat percentage, when compared to the untreated samples. However, the plump grains and groat percentages were not significantly affected at the physiological maturity stage; it is possible that the oat kernels were fully developed before glyphosate killed the oat plant. Lower percentage of plump oats indicates to a lower groat percentage, which decreases the milling quality of oats. Consequently, the pre-harvest application of glyphosate at the lower moisture content stage did not have negative effects on the quality of oat groats.

4.2. Physicochemical Properties of Oat β-Glucan

Physicochemical characteristics of β-glucan can be measured to better understand the health benefits of oat products. For instance, higher oat β-glucan concentration is indicative of elevated β-glucan viscosity and higher molecular weight of the fiber (Kim, White, et al., 2013). In addition, higher soluble β-glucan indicates an improved viscosity of β-glucan while higher β-glucan solubility leads to a lower molecular weight of the molecule. β-glucan is a viscous fiber based on its content and its solubility is based on its molecular weight. The viscous and soluble β-glucans found in oats are associated with two major health promoting effects the attenuation of postprandial blood glucose and the control of LDL cholesterol levels (Kala, Hamaker, et al., 2013). Due to health benefits of oat β-glucan and an increase of glyphosate application on cereal crops, it is important to analyze β-glucan concentration, viscosity, solubility, and molecular weight to insure glyphosate does not adversely affect health benefits of β-glucan.

4.2.1. β-Glucan Content

The 2015 samples showed some changes in physicochemical properties of β-glucan due to glyphosate treatment (Table 4). β-Glucan content for the three treatments ranged from 4.35-4.65%. This measurement is within the range reported by other studies without use of glyphosate, which indicated the β-glucan contents to be approximately 3-5% in oat groats (Anttila, Sontag-Strom, et al., 2004). The concentrations of β-glucan from oats treated at both stages, soft dough and physiological maturity, had significantly ($p < 0.05$) lower contents compared to untreated oat groats with 4.65% β-glucan. The lowest concentration of β-glucan was in samples that were treated at the soft dough stage. Lower β-glucan concentration is indicative of a decrease in viscosity, solubility, and molecular weight of the fiber, leading to reducing its health benefits.

As mentioned above in physical quality results, the groat is a primary source of β-glucan content in oat, and groat percentage of oats was reduced following glyphosate treatment at the soft dough stage (Table 3). Thus, it is possible that the concentration of β-glucan could decrease in oats treated at the soft dough stage because of the reduction in the percentage of groats. Another point, the maximum amount of β-glucan is reached after the soft dough stage when seed growth is almost completed. Hence, the glyphosate application during the soft dough stage might affect the maximization of β-glucan concentration. Reduction of β-glucan content by glyphosate treatment,

especially at the soft dough stage, may cause a significant change in the nutritional value of oat products.

Table 4.2. Mean of β-Glucan: Concentration, Final Viscosity, Solubility, Weight Average Molecular Weight (M_W), and Polydispersity Index (M_W/M_n)

	β-Glucan						
Glyphosate Treatment	Content* (%)	Viscosity (cP)	Solubility (%)	M_W High MWT ($\times 10^6$)	M_W/M_n of High MWT	M_W Low MWT ($\times 10^5$)	M_W/M_n of Low MWT
Untreated	4.65[a]	1149.67[a]	60.59[a]	3.5[a]	1.04[a]	3.0[a]	2.36[a]
Soft Dough	4.35[b]	1082.17[a]	52.08[b]	4.4[b]	1.03[ab]	5.5[b]	2.27[a]
Physiological Maturity	4.43[b]	1165.75[a]	57.17[a]	3.8[c]	1.02[b]	3.3[c]	2.18[a]

*dwb: dry weight basis. cP: centipoise. M_W: weight average molecular weight. MWT: molecular weight. M_W/M_n: polydispersity index

4.2.2. Viscosity of β-Glucan

The differences in β-glucan viscosity were not statistically significant ($p > 0.05$) among the untreated samples and treated oats at soft dough and physiological maturity stages. Even though the β-glucan viscosity was not significantly different, a slightly higher viscosity of the fiber was observed in oats treated at the physiological maturity stage by 1165.75 cP compared to the untreated samples 1149.67 cP (Table 4). Viscosity for soft dough-treated oats was estimated by 1082.17 cP, which was lower than the viscosity of β-glucan in the untreated controls but was not significantly different.

The reason for the decrease of viscosity at the soft dough treatment samples could be because of low concentration of β-glucan at this treatment (Table 4). The higher concentration of β-glucan creates a higher viscosity required for the health benefits such as attenuating postprandial blood glucose and lowering LDL cholesterol levels (Gamel, Abdel-Aal, et al., 2012). Glyphosate application possibly interferes with the grain filling period that occurs in the soft dough stage, leading to a smaller β-glucan content, resulting in a lower β-glucan viscosity.

One reason for the increase in β-glucan viscosity among three treatments especially in untreated and physiological maturity stage samples is due to heat processing, which owes to the

inactivation of endo-β-glucanase (Liu, Bailey, et al., 2010). High viscosities of some samples from both cultivars are related to no active endo-β-glucanase within 2 hours kilning and 40 minutes steaming. In addition, polysaccharide components, such as starch, have an effect on oat viscosity (Anttila, Sontag-Strom, et al., 2004). It is possible that application of glyphosate has an effect on starch content, which was not investigated in this study.

4.2.3. Solubility of β-Glucan

The β-glucan of oats treated at the soft dough stage contained a significantly ($p < 0.05$) lower solubility than either β-glucan of untreated groats or β-glucan groats treated at the physiological maturity stage. In this study, solubility was determined based on the concentration of β-glucan dissolved when a sample was weighed and mixed, including a buffer to obtain a 1% total β-glucan content in the slurry. The solubility of β-glucan showed a significant reduction at the soft dough treatment samples, which dropped from 60.59% to 57.17% by the physiological maturity stage and then to 52.08% in the soft dough stage (Table 4). However, β-glucan solubility of the untreated controls was not significantly different in comparison to the β-glucan solubility of oat groats treated at the physiological maturity stage.

High β-glucan content in oat groats is potentially a factor causing an increase of its solubility. Thus, low quantity of β-glucan led to less solubility of β-glucan, which reduces the health benefits of oat consumption. Oats with high concentrations of β-glucan tend to include more soluble β-glucan located in the groats (Wang, Storsley, et al., 2016). In this study, higher β-glucan concentration appeared to be related to higher β-glucan solubility, independent of its molecular weight (Table 4). Soft dough-treated groats negatively affected the concentration and solubility of β-glucan. Physiological maturity treatments could not considerably have an influence on oat β-glucan concentration and the solubility. Presumably, application at the hard dough stage is a prime time in the grain growth that glyphosate might not interfere with the grain filling, or other component development that may affect β-glucan solubility.

4.2.4. β-Glucan Molecular Weight

The weight average molecular weight of high and low MWT oat β-glucan was determined from the peak retention time of the HPSEC chromatograms. When a polymer solution enters into a HPSEC column, its molecules have different elution rates. For example, the small particles trap into the stationary phase pore system and can enter the gel. The smaller particles consume

more volume and elute later. Thus, their retention time is longer to traverse (Wyatt Technology Corporation, 2013). In contrast, the large particles have different elution rates. There are two fractions of β-glucan molecular weight: high MWT and low MWT (Figure 4.1). The column is used to separate the molecules based on size. RI detector indicates the concentration of sample using dn/dc in each fraction, and light scattering signal shows the weight of β-glucan in each fraction. That explains peaks including high light scattering signal and low refractive index signal contain a high MWT (Figure 4.2). However, peaks with low light scattering signal and high refractive index signal include a low MWT.

The high MWT β-glucan between glyphosate treatments significantly differed ($p < 0.05$). The high MWT of β-glucan decreased in the following order: the soft dough treatment (high MWT, 4.4×10^6), physiological maturity treatment (high MWT, 3.8×10^6), and untreated (high MWT, 3.5×10^6) oat samples (Table 4). Similarly, the low MWT of β-glucan between glyphosate treatments was significantly different ($p < 0.05$). The low MWT of β-glucan declined in following order: the soft dough treatment (low MWT, 5.5×10^5), physiological maturity treatment (low MWT, 3.3×10^5), and untreated (low MWT, 3.0×10^5) samples.

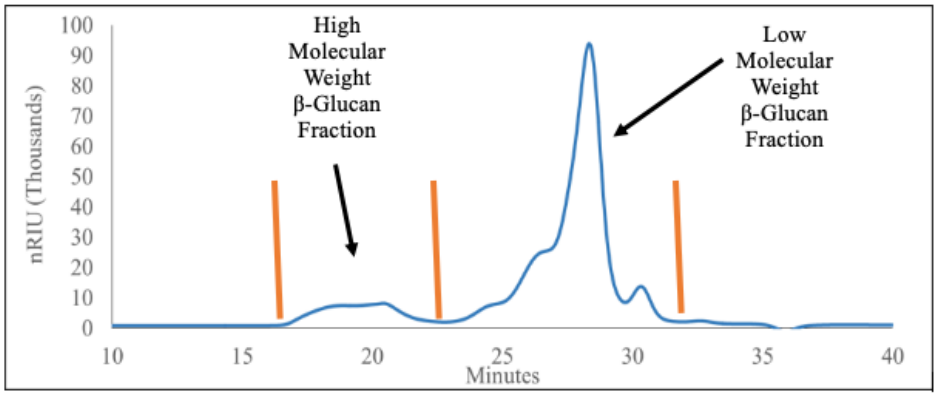

Figure 4.1. Two Fractions of β-Glucan Molecular Weight: High and Low MWT

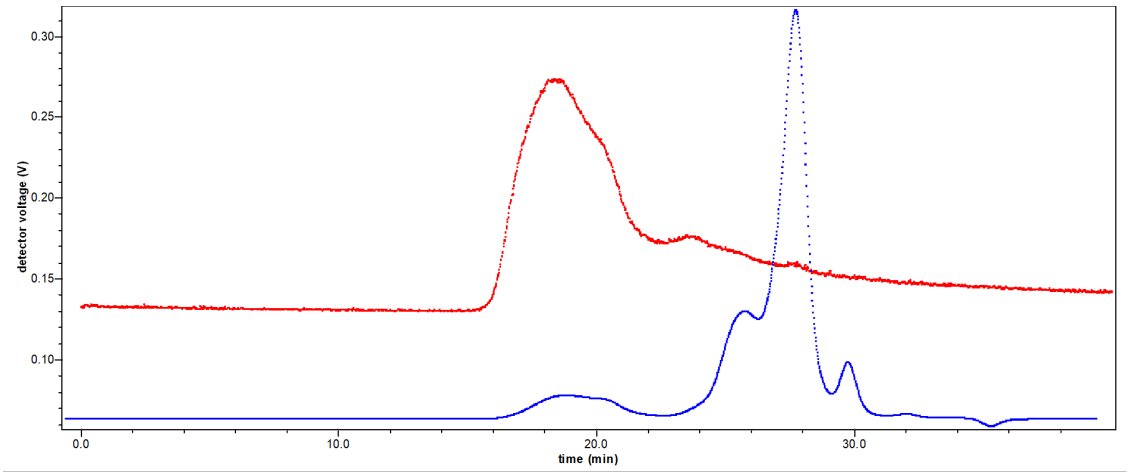

Figure 4.2. RI Detector Determining the Concentration of the Sample and Light Scattering Signal Showing the Weight of β-Glucan in Each Fraction

The polydispersity index (M_W/M_n)--molecular weight size distribution--of the high and low MWT β-glucan was obtained by the peak retention time of the HPSEC chromatograms. To note, there was no significant differences ($p > 0.05$) among the three treatments in records to M_W/M_n in the low MWT. However, when considering M_W/M_n in the high MWT at the physiological maturity stage, significant difference ($p < 0.05$) has been found compared to the untreated samples. A higher M_W/M_n was found in the low MWT relative to the high MWT, indicating that the polymers within each low MWT of the β-glucan fraction generally contained a wider range of molecular weights than those of high MWT β-glucan as shown in table 4.

Table 4 summarizes the relationship between the soluble β-glucan and molecular weights of β-glucan. The solubility, high MWT, and low MWT of β-glucan in the soft dough treated-samples were 52.08%, 4.4×10^6, and 5.5×10^5, respectively. The solubility, high MWT, and low MWT of β-glucan in the physiological maturity treated-oats were 57.17%, 3.8×10^6, and 3.3×10^5, individually. The solubility, high MWT, and low MWT of β-glucan in untreated controls were 60.59%, 3.5×10^6, and 3.0×10^5, respectively. The differences in high MWT and low MWT of β-glucan seem to imply that lower solubility of β-glucan was associated with their higher molecular weights.

These results support the concept that a higher MWT of β-glucan leads to less solubilization (Grundy, Quint, et al., 2017). Glyphosate applications indirectly affected high MWT and low MWT of β-glucan by reducing its solubility. Increased solubility of β-glucan is a desirable property into

foods, resulting in enhanced physiological activities. High molecular weight of β-glucan is more useful in health benefits compared to low M_W forms of the fiber, in case, the solubility is not reduced. Another point, the increase in molecular weights of oat β-glucan with the soft and hard dough treatments of pre-harvest glyphosate application might be related to the inactivation of endo-β-glucanase during steaming and kilning (Wang, Storsley, et al., 2016).

Overall, glyphosate applied at the soft dough stage reduced the percentage of β-glucan concentration, when compared to the untreated samples. This might lead to a reduction of β-glucan viscosity in groats treated at the soft dough stage as well. It is possible that the oat kernels were not fully developed when glyphosate was applied at a higher moisture content stage. Viscosity and solubility of β-glucan were not significantly affected at the physiological maturity stage. However, the solubility significantly decreased at the soft dough stage because molecular weights of β-glucan significantly increased.

5. CONCLUSIONS AND FUTURE STUDIES

Application of glyphosate at the soft dough stage had impacts on physicochemical characteristics of β-glucan. Use of glyphosate at the soft dough stage decreased the percentage of β-glucan concentration, which is possibly due to the reduction in the percentage of oat groats. Pre-harvest glyphosate during the soft dough stage resulted in low solubility and low viscosity of β-glucan. Thus, decreasing the physicochemical properties of oat β-glucan negatively impacted the health benefits of β-glucan. Application of glyphosate at the soft dough stage reduced the β-glucan solubility and increased its molecular weight compared to the untreated controls. Pre-harvest glyphosate at the soft dough stage significantly affected β-glucan content, solubility, and molecular weights while treatment at the physiological maturity stage only affected β-glucan concentration and its molecular weights. In conclusion, early pre-harvest application of glyphosate can influence development of β-glucan oat groats.

In particular, application of glyphosate at the high moisture stage is related to a decrease in physiochemical features of the β-glucan. Therefore, in order to maintain viscosity and solubility of oat β-glucan, it is recommended farmers apply glyphosate at the low moisture stage (hard dough). Future research can be performed to determine the cause of the decrease in physiochemical properties of the β-glucan observed, especially in groats treated at the soft dough stage. Since oat is rich in protein, it will be interesting to investigate whether glyphosate application affects the essential amino acids in oat groats. Sensory evaluation of end use products can be a future study to insure pre-harvest glyphosate does not adversely affect sensory properties, such as flavor, of oat productions.

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APPENDIX

Table A.1. 2015 Physical Quality of Oat Groats by Location and Cultivar

Location	Cultivar	Glyphosate Treatment	Plump (%)	Groat Percentage (%)	Test Weight (1b/bu)
Minot	Rockford	Untreated	97.26	72.54	41.10
		Soft Dough	96.87	71.78	41.70
		Physiological Maturity	97.29	71.54	41.30
	Souris	Untreated	97.85	71.27	40.90
		Soft Dough	97.57	70.27	40.77
		Physiological Maturity	98.12	70.80	41.50
Prosper	Rockford	Untreated	87.27	68.37	31.67
		Soft Dough	81.18	64.72	32.63
		Physiological Maturity	86.85	68.27	32.13
	Souris	Untreated	86.92	68.93	31.30
		Soft Dough	86.62	68.84	32.50
		Physiological Maturity	88.44	69.36	31.50
LSD within location			1.09	1.64	1.40
LSD between locations			1.24	1.54	1.51

Table A.2. 2015 Physical Quality of Oat Groats by Location

Location	Plump (%)	Groat Percentage (%)	Test Weight (1b/bu)
Minot	97.49^{a}	71.37^{a}	41.21^{a}
Prosper	86.21^{b}	68.09^{b}	31.96^{b}

Values are averages of all location/cultivars/treatments. Values with the same superscript level are not significantly different ($p>0.05$) Least significant difference was used for mean separation. 1b/bu: pounds/bushel.

Table A.3. Mean of β-Glucan: Concentration, Final Viscosity, Solubility by Location and Cultivar

			β-Glucan		
Location	Cultivar	Glyphosate Treatment	Content* (%)	Viscosity (cP)	Solubility (%)
Minot	Rockford	Untreated	4.5	909.7	51.9
		Soft Dough	4.3	913.7	44.8
		Physiological Maturity	4.0	887.0	47.7
	Souris	Untreated	3.9	1325.3	59.8
		Soft Dough	4.1	1060.7	54.9
		Physiological Maturity	4.1	1138.3	57.0
Prosper	Rockford	Untreated	5.3	1214.7	67.3
		Soft Dough	4.6	1187.7	53.5
		Physiological Maturity	4.9	1390.0	64.7
	Souris	Untreated	5.0	1149.0	63.3
		Soft Dough	4.4	1166.7	55.1
		Physiological Maturity	4.7	1247.7	59.3
LSD Within Location			0.4	335.5	9.0
LSD Between Locations			0.5	339.2	8.9

Table A.4. Mean of β-Glucan: M_W High MWT, M_W Low MWT, M_W/M_n of High MWT, and M_W/M_n of Low MWT by Location and Cultivar

			β-Glucan			
Location	Cultivar	Glyphosate Treatment	M_W High MWT ($\times 10^6$)	M_W/M_n of High MWT	M_W Low MWT ($\times 10^5$)	M_W/M_n of Low MWT
Minot	Rockford	Untreated	3.3	1.05	2.7	2.66
		Soft Dough	4.3	1.02	6.0	2.57
		Physiological Maturity	4.0	1.01	2.8	2.35
	Souris	Untreated	4.6	1.06	4.3	2.30
		Soft Dough	5.4	1.04	6.0	2.28
		Physiological Maturity	4.9	1.02	4.4	2.14
Prosper	Rockford	Untreated	3.0	1.03	2.0	2.08
		Soft Dough	4.2	1.03	5.5	2.06
		Physiological Maturity	3.2	1.01	2.9	2.14
	Souris	Untreated	3.0	1.04	3.0	2.39
		Soft Dough	3.6	1.04	4.6	2.17
		Physiological Maturity	3.1	1.02	3.0	2.10
LSD Within Location			3.3×10^5	0.04	1.5×10^4	0.40
LSD Between Locations			3.0×10^5	0.04	1.5×10^4	0.47

M_W: weight average molecular weight. MWT: molecular weight. M_W/M_n: polydispersity index.